THE EFFECT OF HEAT PROCESSING CONDITIONS UPON CHOLESTEROL CONTENT OF A RESTRUCTURED PORK/SOY HULL PRODUCT

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THE EFFECT OF HEAT PROCESSING CONDITIONS UPON CHOLESTEROL CONTENT OF A RESTRUCTURED PORK/SOY HULL PRODUCT

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ABSTRACT

The effect of convective heat processing upon cholesterol and cholesterol oxides in a restructured pork/soy hull model system was studied. Thirty different time-temperatures relationships were studied. Quadratic equations were determined for all time-temperature relationships. The integral (area) and second derivative (acceleration) were determined. Two GC-MS instruments were used to quantify and qualify cholesterol and cholesterol oxides in the product. Results indicated that no cholesterol oxides were developed in the product with all 30 time-temperature profiles, ranging from 19 - 89.6 min, and 9 - 97°C final product temperatures. The mean cholesterol content of heat processed samples ranged from 34 - 64 mg/100 gm (wet basis). Three plant sterols were identified in the product. Positive relationships were found between final temperature, fat, and cholesterol content. Negative relationships were found between the integral of the time-temperature curve, moisture, and product yield.

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CHAPTER ONE

INTRODUCTION

1.1 Purpose of research

Cooking is a process which, due to thermal treatment, chemical, physical, and microbial changes take place in the product, leading finally to a condition in which food is edible. Heat processing of food is, therefore, a classical food engineering process that can be comprehensively characterized only by combining engineering with other disciplines such as chemistry, physics, and biology (Paulus, 1984).

The current interest in nutritional labeling of food products has drawn increased attention to the question of what happens to food components during thermal processing. This interest has increased because of public health awareness (Tannebaum, 1979). The food industry and government agencies are spending significant amounts of time and money to update information on the nutrient as well as non-nutrient components of food.

The American diet has undergone marked changes in the last 70 years. Data compiled by Friend (1967), Gartner (1975) and Page and Friend (1978) indicated that the level of available protein has remained constant, but the ratio of animal protein to vegetable protein has more than

doubled. The consumption of carbohydrates has fallen by 21% (from 492 to 388 g/person/day). This latter trend was believed to suggest consumer resistance to high sugar prices. Data reported by Anon (1988) indicated that American's fat consumption has now increased by a third over the 1965 level. The per capita consumption of fat by Americans in 1965 was 144 g/day (Worthington, 1981). Increasing consumption of fat, and reducing consumption of complex carbohydrates and dietary fiber is found to be associated with many diseases of western societies (Burkitt, 1973; Toma and Curtis, 1986).

Dietary cholesterol and fat have been implicated as causes of coronary artery disease. The Framingham study in the United States was one of the first to describe cholesterol as a risk factor for coronary artery disease that includes hypercholesterolemia (Kennel and Gordon, 1974). Studies have shown (Anon, 1984; Hegsted et al., 1965) that a drastic reduction of these in the diet may cause a gradual reduction in serum cholesterol and some regression of cardiovascular diseases.

The propensity of cholesterol to oxidation has been amply documented and well over 60 products resulting from autoxidation, photoxidation, and enzymatic action have been described (Smith, 1981). Several of the cholesterol oxidation products have been implicated in adverse human

health effects (Chen et al., 1974; Streuli et al., 1979; Yachnin and Hsu, 1980; and Streuli et al., 1981) and current knowledge of their biological and biochemical activities has been summarized (Imai et al., 1976; Taylor et al., 1979; Peng et al., 1979; Spangrude et al., 1982; Peng et al., 1978, Peng et al., 1985; Chan and Black, 1974; and Smith and Kulig, 1975). These and other studies provide ample evidence that certain cholesterol oxides show angiotoxicity, cytotoxicity, antherogenicity, mutagenicity, and/or carcinogicity effects.

Some cholesterol-containing foods are subject to oxidizing conditions during various stages of processing or preparation. Conditions such as exposure to elevated temperatures in contact with air for even a brief period, or prolonged storage in air ambient temperatures, are likely to lead to the formation of cholesterol oxides (Maerker and Unruh, 1986).

The relationship of diet to health is one of the factors that influences food purchasing decisions (Bursey, 1983). This stimulates food industry scientists to modify existing food products or develop new food products that are low in cholesterol. To meet dietary goals, modification of cholesterol in a diet can be attained by formulating a meat product that has a high dietary fiber (Anon, 1979; Spiller et al., 1978).

Food process engineers need tools to help them design process systems that perform predictably. They also need tools to help improve or optimize existing processes and develop control systems for processing operations (Thompson, 1982).

Mathematical models of chemical changes occurring in the processing of food are of substantial value to process development; such kinetic models are essential to develop optimization procedures for such processes (Evan, 1982). The development of kinetic models for degradation of food components during thermal processing has been receiving attention in recent years and has been aided by the increasing availability of appropriate computer software (Mishkim et al., 1984).

1.2 Objectives of Research:

Based upon these changes, there is a need to develop meat products which have a fiber content and lower cholesterol content than meat alone. Further, there is a need to determine if cholesterol oxides form during the heat processing and storage conditions of that product. Thus, three research objectives were established:

1. To formulate a high fiber pork product.
2. To study the impact of heat processing conditions on the moisture, fat and cholesterol content of the restructured pork/soy hull product.

3. To determine cholesterol by-products, oxidized cholesterol derivatives (OCDs), resulting from exposure of high fiber pork product to different times and temperatures.

CHAPTER TWO

REVIEW OF LITERATURE

The literature was reviewed to determine how: (1) the science of food processing affects food quality, (2) cholesterol and cholesterol oxides affect human health, (3) dietary fiber is important, (4) to develop a new pork product, "re-structured meat", (5) to chemically analyze cholesterol and cholesterol oxides, and (6) heat processing affects cholesterol and cholesterol oxides.

2.1 Food Processing: The Science

Food processing is a chain of events initiated by harvesting and ending by service of food to people. Cooking of meat is a process in which, due to thermal treatment, chemical, physical, and microbial changes take place in the meat product leading finally to a condition where meat is palatable, acceptable, and safe for human consumption.

Today, health conscious consumers are advised to reduce or eliminate the consumption of a growing list of foods and food ingredients. This list might include additives, alcohol, artificial colors and flavors, caffeine, fats, meat, and cholesterol (Bursey, 1983).

Food processing has overall beneficial effects since it decreases food spoilage and enables a wide variety of seasonal foods that are appealing, nutritious, and safe. In some cases food processing may cause unfavorable reactions in food, resulting in a decrease in the content of a nutrient or formation of antinutritional and possible toxic derivatives, (Fennema, 1985).

2.2. How Cholesterol and Cholesterol Oxides Affect Human Health

Cholesterol is the most familiar of all sterols. The structure of cholesterol is composed of four interlocking rings, of which three contain six carbon atoms each and the fourth contains five carbon atoms. An alcohol, hydroxy group (OH) is attached to carbon number three of the first ring and a chain of eight carbon atoms is attached to carbon number seventeen of the fourth ring (Fig. 2.1). Another way of illustrating the chemical structure is given in Fig. 2.2.

Cholesterol has figured in extensive controversies regarding its possible contribution to the etiology of atherosclerosis and coronary heart diseases (Matison et al., 1972; Anderson et al., 1976; Whyte and Havenstein, 1976; and Buzzard et al., 1983).

Although the role of cholesterol in disease is being debated, there is no question about the necessity for this

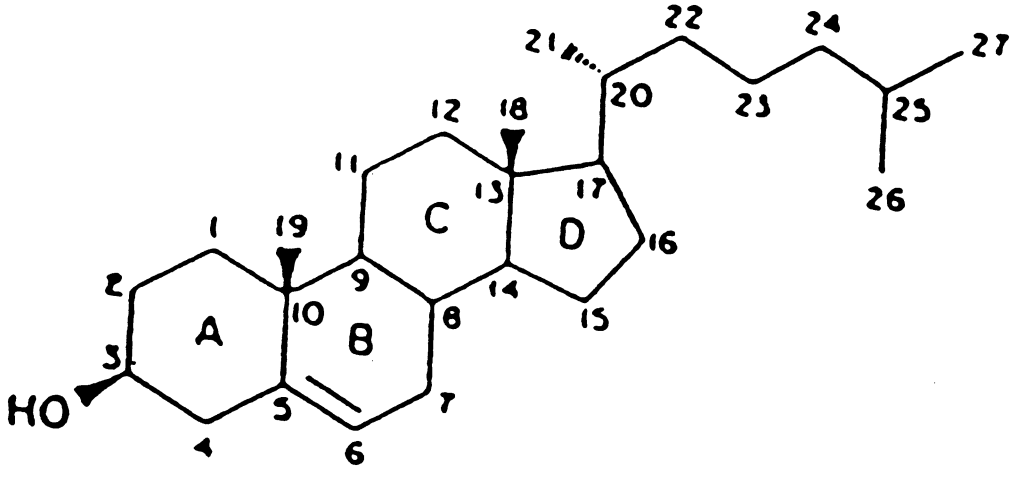


Fig. 2.1. Cholesterol (5-Cholesten-3B-OL; 3B hydroxy-5-cholestene)

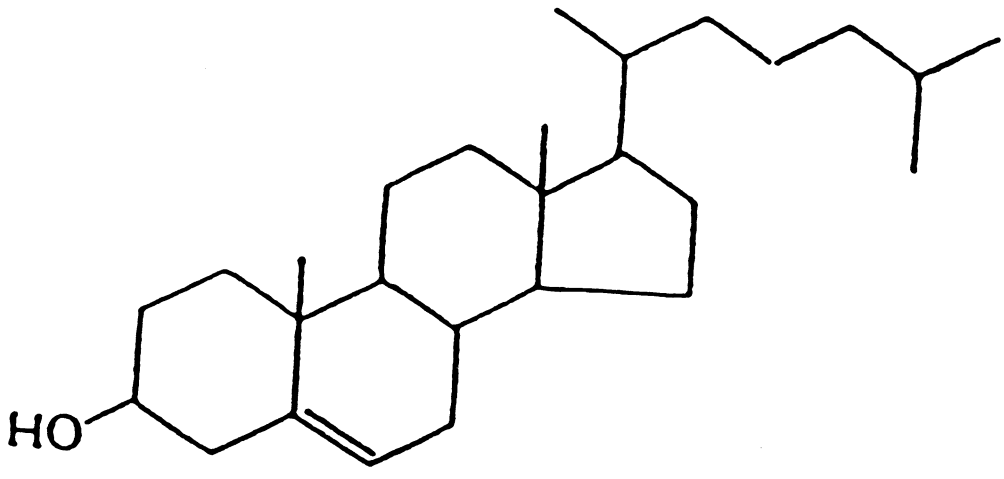


Fig. 2.2 Cholesterol (5-Cholesten-3B-OL; 3B hydroxy-5-cholestene)

lipid in human bodies. Cholesterol is essential as a pre-cursor of several important compounds, particularly the steroid hormones (which include cortisone and testosterone). Cholesterol is also required for the body's production of vitamin D and bile salts (Kreutler, 1980).

Cholesterol is needed metabolically but is not an essential nutrient. The liver manufactures cholesterol at the rate, perhaps, of (50^{15}) molecules per second. The raw material that the liver uses to make cholesterol is acetoacetate which can be taken from glucose or saturated fatty acids (Hamilton and Whiney, 1981).

After manufacturing, cholesterol either is transformed into related compounds like the hormones just mentioned or it leaves the liver. The cholesterol that leaves the liver has three possible destinations: (1) it may be excreted, (2) it may be deposited in body tissue or (3) it may end up accumulating in arteries.

Cholesterol can be found in a wide range of foodstuffs of animal origin and total daily intake can be varied greatly by altering the amounts of cholesterol-rich foods consumed (Sabine, 1977). According to the Select Committee on Nutrition and Human Needs (1977), cholesterol consumption should be reduced to about 300 mg/day.

The tendency of cholesterol to undergo autoxidation in air has been extensively recorded. More than 60 products have been reported to result from cholesterol autoxidation

(Smith, 1981). Many of the cholesterol oxides are extensively documented to possess undesirable biological activities, these include: cytotoxicity, antherogenicity, mutagenicity, and carcinogenicity (Roff, 1938; Roff, 1942; Roff, 1944; Chen et al., 1974; and Peng and Taylor, 1984).

A justification for the cholesterol oxides and their impact on biological activities of cells was given by Nourooz-Zadeh and Appelqvist (1987). They stated that cholesterol oxides or a single pure compound can effect the feedback inhibition of cholesterol biosynthesis at the 3-hydroxy-3-methyl glutamyl-Co enzyme A (HMG-CoA) reductase level. More or less inhibition of this enzyme in the aorta cells will cause a defect in the aortic surface. Also a high reduction (HMG-CoA) reductase will lead to a reduction in cholesterol biosynthesis, causing cell death because of membrane dysfunction (cytotoxicity). The dead cells can be a primary area for lipid filtration, leading to atherosclerosis.

Most of cholesterol oxidation involves the A and B rings (Fig. 2.1) (Finocchiaro and Richardson, 1983). The formation of cholesterol oxides varies from product to product. Park and Addis (1985) isolated five oxidized cholesterol compounds from heated tallows. These oxides included: 7α-hydroxy, 7B hydroxy and ketocholesterol, and the α and B-epoxide of cholesterol (Figure 2.3). The cholesterol oxidation products in pork meat include: 7

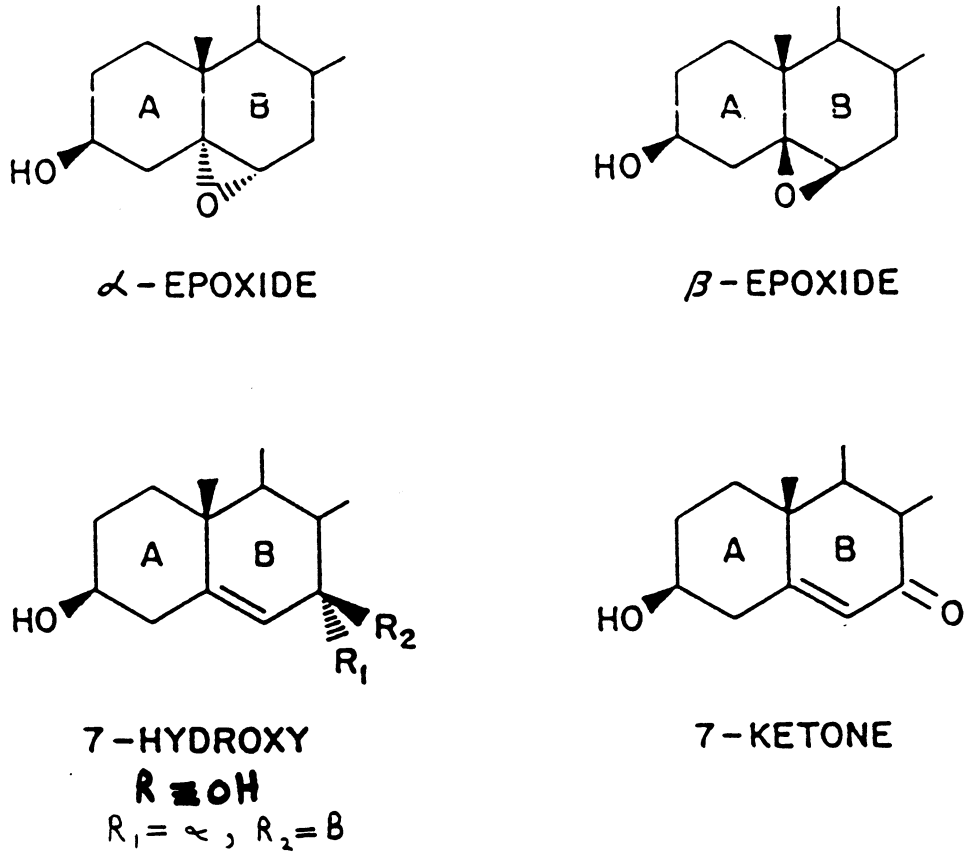


Fig. 2.3. Cholesterol oxides.

ketocholesterol (Williams and Pearson, 1965), 5-cholesten-3B-oL-22one, 5-cholesten-3B-oL-7one (7 keto), and cholestane 3B, 5α, 6B-Triol (Higley et al., 1986). The latter two oxides as well as 7α-, 7B-hydroxy cholesterol and α and B epoxide were identified in freeze dried pork meat (Park and Addis, 1987).

2.3. Importance of Dietary Fiber

Dietary fiber is that portion of plants that reaches the large intestine, unmetabolized. There are many different forms of dietary fibers, these include: cellulose, hemicellulose, pectin, gums, mucilages, lignin, and storage polysaccharides (Anderson, 1979). Dietary fiber components may be broadly divided into two categories, soluble and insoluble, on the basis of their solubility characteristics under simulated digestive tract conditions. Soluble dietary fibers include: pectin, some hemicelluloses, gums, mucilage, and storage polysaccharides. Insoluble dietary fibers include: cellulose, lignin, and many hemicelluloses.

Benefits of dietary fiber have been reported (Spiller et al., 1978; Anon, 1979; and Gerich, 1989). These benefits include: relieving constipation problems; treating or preventing diverticular diseases; preventing a variety of disorders such as colon-rectal cancer, diabetes, obesity, ischemic heart disease; and reducing blood cholesterol.

Coronary heart disease (CHD) is the leading cause of death in affluent countries (McGill and Mott, 1976; Kritchevsky, 1979). Kritchevsky and Tepper (1965) and Moore (1967) were among the first scientists to show the influence of dietary fiber on serum cholesterol levels in cholesterol-fed rats. Walker and Arvidsson (1954) suggested that the lack of coronary heart diseases in the African population may be due to the high level of fiber in their diets. Dietary recommendations of several prominent scientific groups, including the American Diabetes Association (1979) and the National Cancer Institute (1979) recommended that the American public increase their intake of fiber.

Although numerous benefits of dietary fiber have been reported, it has been suggested that too great of a consumption of dietary fiber may cause diarrhea and a high incidence of mineral loss, particularly zinc, iron, calcium, copper, and magnesium (Kelsay et al., 1979; Hallfrisch et al., 1987).

In 1982 over ninety percent of the world supply of soybeans was produced in the United States (World Food Institute, 1984). In the United States, Missouri is a major soybean producer. Missouri soybean production in 1985 was 1.8 million bushels (Anon, 1986). Soybean hulls, considered to be the least valuable portion of the bean, account for about 1/2 of the 6% of fiber which is present in the

unhulled, defatted meal (Allan and Sidney, 1972). The total amount of soy seed coat or hull which enters the U.S. soybean processing plant annually, at the present level of processing, is about 2.4 million tons.

Analytical results reported about the composition of soybean hulls from different laboratories do not agree. According to Nelson et al. (1950), the principal components of the hull were: cellulosic type materials, 49.3%; pentosans, 22.6%; lignin 4.5%; ash 5.7%; and nitrogen, 1.6%. In another investigation, Sanella and Whistler (1962) reported the partial analysis of soybean hulls and gave the composition as: alpha cellulose 49.8%; lignin 7.8%; hemicellulose A 10.6%; hemicellulose B 6.0%; crude protein 13.6%; and ash 4.9%. Rasper (1979) and Melissa and Zabik (1979) reported that soybean hulls contained 41% cellulose and 3% lignin. Total dietary fiber in soybean hulls is 88%, Dintzis et al. (1979). Soybean hulls are a concentrated source of iron, containing 32% of the total seed iron (Levine et al., 1982). Soybean hulls have a low fat content, about 1.0% (Mitaru et al., 1984). Three forms of sterols have been identified in soybeans, these include: 24-methyl-cholesterol, stigmasterol, and sitosterol (Fenner et al., 1986). B-sitosterol is the major sterol in soybean. This sterol has a hypocholesterolemic effect on animals and man (Pollak et al., 1981).

As a fiber source, soybean hulls are used to reduce energy intake for animal feeds. Hale and Utley (1985) reported that replacing high energy corn and soybean meal for pigs with 20% soybean hulls resulted in a high crude fiber diet which reduced body weight and increased carcass leanness. Similar findings were reported by Muir et al. (1985) and Tsantir and Gorman (1973).

It is well known that dietary fiber and phytic acid may affect bioavailability of minerals in animal and humans (Vanderaav et al., 1983; Gordon et al., 1983; and Davis et al., 1977). When soybean hulls were added to a rat's diet, they had no significant effect upon the bioavailability of zinc or calcium (Weingartner et al., 1979; Erdman and Weingartner, 1980; and Laszlo, 1987). This has been attributed to the fact that soybean hulls contain over half of the fiber found in soybeans, but a negligible proportion of the phytic acid. On the other hand, Meyer et al. (1983) reported that the bioavailability of zinc from an animal source is greater than from plant sources. There is some evidence that an addition of animal products to a meal based on plant protein enhances the absorption of zinc from the meal (Sandstorm et al., 1980). Johnson et al. (1985) reported that soybean hulls were an inexpensive source of available iron, suitable for enriching flour in baked products. Also it was found that consuming soybean hulls improved some measures of glucose tolerance for diabetic patients (Mahalko et al., 1984).

In summary, soybean hulls are an excellent source of iron. They are low in oil; rancidity should not be a concern. They contain a negligible amount of phytic acid, a frequent plant component associated with poor mineral bioavailability. These findings make soybean hulls valuable. Food scientists should find the means to maximize the utilization of this inexpensive product.

2.4. Development of a New Pork Product "Restructure"

Interest in the development of meat restructuring technology first emerged in the early 1940's. However, at that time the process was discarded as it was found to be uneconomical (Campbell et al., 1977). Restructuring was not successful at the time primarily due to a lack of suitable equipment with which to comminute and form the meat. In the early 1970's comminuting and forming machinery was developed which was suitable for the restructuring of meat (Ashton, 1973).

Pork products have not enjoyed wide acceptance in the hotel, restaurant, and industrial markets. Several factors are responsible for this poor showing: (1) religious and medical reasons; (2) prestige and status; (3) lack of uniform portion control; (4) lack of uniform quality, including juiciness, tenderness, and flavor; and (5) lack of adequate preparation to preserve quality (Anon, 1974). In an effort to provide more suitable pork products,

techniques for production of restructured pork products have been developed (Popenhagen et al., 1973; Belohavy and Mandigo, 1974).

The concept of restructuring pork was to provide the consumer with a highly palatable product at a reasonable cost by using less valuable carcass cuts. Restructuring pork involves conversion of raw material into fractions which can be recombined with regard to structure, composition, size, and shape. Several procedures for restructured meat were described by Garner, (1975); Pearson and Tauber, (1984); and Mandigo, (1974). These include: (1) chunking and forming, (2) flaking and forming, and (3) tearing and forming. In restructured pork formulations, precautions in the selection of raw materials for restructured pork should be applied. Only fresh meat that is microbiologically sound and free from flavor defects should be utilized in producing restructured pork. The total concept in processing restructured pork involves the intact pork muscles, boneless pork and other lean masses. The cuts are removed from the carcass, deboned and trimmed of excess fat, frozen, tempered to -4°C to -3°C, pressed into uniform logs and cut into uniform portions.

Several factors can influence the quality of restructured pork. The effect of flaking size and temperature was reported by Popenhagen and Mandigo (1978). They used three flaking-head sizes (3.0, 6.9, and 12.7 mm)

and two different temperatures (5.6°C and 2.2°C) for restructured pork trimmings. The smallest head size had lower cooking losses than the others. Mandigo et al. (1972) found that an addition of 1.0% NaCl to restructured pork was preferred by sensory panelists. Schwartz and Mandigo (1974) reported that an addition of salt at the 2.25% level and sodium triphosphate (STP) at the 0.37% level, resulted in significantly decreased cooking losses. Similar findings were reported by Huffman and Cordray (1979); Pepper and Schmidt (1975); and Neer and Mandigo (1977). As these investigators had shown that the use of salt and STP improved the characteristics of restructured pork, Schwartz and Mandigo (1976) studied their effect on the storage characteristics of restructured pork. They found that added salt and STP increased thiobarbituric acid (TBA) values, and packaging losses. The latter effect was due to the fact that, as the salt content of the product increased, the product stickiness increased during heat processing and packaging, causing more meat particles to adhere to the side of the package. Also they found that added salt and STP improved cooked color, aroma, flavor, and eating texture, and decreased cooking loss. Among several combination levels, 0.75% salt and 0.125% STP were most desirable for the production of restructured pork. Similar findings were reported by Huffman et al. (1981). In contrast, Ande et al. (1985) reported that TBA values

for frozen beef pork nuggets increased over storage time, but the addition of STP significantly lowered these values.

The influence of the fat content of restructured pork was reported by Stanley et al. (1984). They evaluated five mean fat percentages (9.4, 12.4, 16.7, 19.3 and 19.9) of restructured pork. They found that there were no differences in percentage cooking losses among different fat levels, but as the fat content increased, frozen storage and shelf life time decreased. Water holding capacity differed among fat levels treatments, with the higher fat treatments having lower water holding capacity. Also, they found that lower fat level treatments required more pressure to shear and had a lower TBA value than the higher fat level treatment.

Ideas for the prevention of restructured pork from changing during frozen storage were suggested by Miles et al. (1984). They recommended placing the restructured pork in a polyvinyl package under vacuum and using 0.5% NaCl and antioxidants [butylated hydroxanisole (BHA), butylated-hydroxytoluene (BHT), and citric acid]. A similar finding was reported by Chastain et al. (1982). They found that antioxidants lowered TBA values of restructured pork. Also, they found that BHA was very effective in protecting the color of that pork.

The effect of cooking on restructured pork was reported by Hamouz et al., (1985). They formulated restructured pork with 50% trimmed fresh ham and 44% pork trim (flaked, 2-3 mm head), then cooked with a moist and a dry heat system at 107°C to an internal temperature of 76.7°C. Although the heat processing system was not described, their results indicated that cooking time was greater for roasts cooked in a dry heat environment and these roasts were also lighter in color.

Campbell and Mandigo (1978) recommended cooking and reheating structured pork with convection ovens. This procedure gave an acceptable product with lower cooking losses than an electric grill followed by reheating in a microwave oven.

Restructuring pork involves conversion of raw material into fractions which can be combined into appropriate proportions. Modifications of this process can be used to introduce dietary fiber to pork meat, providing the consumer with a highly platable, nutritious product at a reasonable cost.

2.5. Determination of Cholesterol and Cholesterol Oxides

Cholesterol in animal tissues is not found in a free form. Three steps are involved in cholesterol detection and analysis: extraction, isolation and separation, and quantitation (Gibbon et al., 1982).

Several methods for lipid extraction have been published (AOAC, 1984). Variations of the method of Folch et al. (1957) remain the standard and preferred method for extraction of the total lipid from biological material (Kritchevsky et al., 1961; Tu et al., 1967; and Rhee et al., 1988). In this method a ground or dispersed material is exposed to chloroform/methanol (2:1, v/v) to readily remove all lipids plus some water-soluble materials. The cholesterol and related compounds can be removed by treating the biological material with an alcoholic alkaline (e.g. potassium hydroxide) under heat treatment (Rhee et al., 1982a; and Rayan and Gray, 1984). A possible problem with hot saponification is the development of cholesterol oxides (Vanlier et al., 1975). The nonsaponifiable lipid fraction containing cholesterol can be extracted with petroleum ether.

A wide range of chromatographic methods and systems (column, paper, thin layer, gas-liquid) have been proposed as an ideal method for the separation of lipid mixtures (Rayan and Gray, 1984; and Adams et al., 1986). Although precipitation of cholesterol as digitonin was an indispensable technique in earlier studies (Strain, 1950), the method has several drawbacks in quantitative analysis, including low rates of recoveries (Gibbon et al., 1982).

Quantitation of cholesterol comes from extraction, separation, precipitation, and weighing of the cholesterol.

As an alternative, a number of colorimetric procedures have been used (Searcy and Berquist, 1960; Sabine, 1977; Kregel et al., 1986; Reitmeier and Prusa, 1987; and Singer, 1986). These methods are based on the fact that cholesterol reacts with a variety of compounds to produce measurable color. Within colorimetric methods, inaccuracies can arise from a number of causes including particular losses during extraction, different color intensities from cholesterol and cholesterol esters, interference of other compounds, and instability of reagents.

The use of gas chromatography (GC) in quantitative analysis has been described (Burchfield and Storrs, 1962; Giddings and Keller, 1970; Karger et al., 1973; and Meloan and Pomeranz, 1987). Although this type of instrumentation involves large capital investment, it provides rapid, sensitive, and specific assays for cholesterol.

Several methods have been proposed for cholesterol oxides analysis by thin-layer chromatography (TLC) (Smith et al., 1967); high pressure liquid chromatography (HPLC) (Tsai et al., 1980; Finocchiaro et al., 1984; Park and Addis, 1985a; Higley et al., 1986; and Maerker et al., 1988) and gas chromatography (GC) (Fioriti and Sims, 1967; Maerker and Unruh, 1986; and Nourooz-Zadeh and Appelqvist, 1987). Although all of these several methods can be used for the analysis of cholesterol oxides, the analysis of these oxides in foods is difficult, primarily due to their

trace concentration and close structural similarity (Finocchiaro and Richardson, 1983; and Park and Addis, 1987). Among all methods, capillary gas chromatography was found superior for the analysis of cholesterol oxides, and enables the simultaneous quantitation of all oxides (Korahani et al., 1982; Park and Addis, 1985b; Park and Addis, 1986a; Park and Addis, 1986b; and Park and Addis, 1987). For sophisticated qualitative analysis, gas chromatography must be coupled to other analytical systems (McFadden, 1973; Gudzinowicz et al., 1977). Mass spectrometry has proven to be the most effective ancillary device. However, no single technique provides all necessary analytical data. Therefore, a combination of GC-MS is recommended for the analysis of cholesterol and cholesterol oxides (Park and Addis, 1986).

2.6. Effect of Heat Processing on Cholesterol and Cholesterol Oxides

Literature was reviewed to determine the influence of heat processing on cholesterol in different meat products.

Larsen and Morris (1943) heated lard at several temperatures between 200-250°C, for periods of 30-150 min. Cholesterol analysis revealed that approximately 40% of the original sterol was no longer precipitable with digitonin after lard had been heated at 300°C for thirty minutes.

After two hours at 300°C, 75% of the sterol had been rendered inactive to digitionin. Heating at 350°C for thirty minutes reduced the precipitable cholesterol to zero. A similar finding was reported by Kriby (1943). Cholesterol heated to 270-300°C for 30 minutes in air led to decomposition of cholesterol and the formation of a substance having a blue fluorescence in the ultra violet beam.

Foriti and Sims (1967) used thin layer chromatography and gas-liquid chromatography to separate and identify products from the autoxidation of cholesterol. Both cholesterol~~<~~ epoxide and 1,3 cholestadien-3-one were found. They found that autoxidation in bulk was quite slow at 82°C, requiring several weeks for development of detectable quantities of decomposition products. The reaction could be accelerated by UV light or by heating sterol above its melting point.

Janicki and Appeldorf (1974) reported the effect of broiling, grilling, frying, and microwave cooking on the cholesterol content in beef patties. They used a colorimetric method for cholesterol analysis. Although no internal product temperature was given, results indicated that decreases in total cholesterol content were observed in all cooking treatments, except microwave, when compared to the raw patties.

Rayan et al. (1981) used thin layer and gas chromatography to evaluate cholesterol in heated tallow. Data indicated that tallow cholesterol was oxidized when tallow was exposed to high temperature (180°C) for 75 h. They did not quantified the amount of the sterols. However, they observed that the size of the cholesterol peak decreased as the number of the oxidation products increased.

Rhee et al. (1982b) used the colorimetric method to evaluate cholesterol content in steak cooked to an internal temperature of 60°C and 75°C. Their results indicated that the cholesterol content of cooked steaks was 22-48% higher than a raw steak when cooked to 60°C, and 38-50% higher, when cooked to 75°C. A similar finding about higher cholesterol contents of cooked meat was reported by Rhee et al. (1983) and Moss et al. (1983). The same colorimetric method was used by Rhee and Smith (1983) to evaluate cholesterol in ground beef patties. The beef patties were prepared to contain 0, 10, 20, or 30% rehydrated textured soy protein and 8, 16, or 27% fat. Patties were cooked from the frozen state on a rack in a preheated oven at 177°C to an endpoint internal temperature of 75°C. The cholesterol content of raw ground beef patties decreased as the amount of textured soy protein increased. In the cooked samples at initial fat levels of 8 and 16%, the amount of cholesterol in 100 g or in an entire patty tended to decrease as the amount of textured

soy protein increased. However, this decrease in cholesterol content was not proportional to the increase in the amount of textured soy protein, as was the case in the raw patties. At an initial fat level of 27%, however, the amount of cholesterol per cooked patty did not decrease with an increase in the amount of textured soy protein.

Prusa and Hughes (1986a) applied the colorimetric method to measure the cholesterol content of turkey breast roasts that were heat processed by three oven types (conventional, convection, and microwave) to two internal endpoint heating temperatures (77°C and 82°C). When comparing raw to cooked samples, heating increased the cholesterol content of the turkey. The cholesterol content of the heated roasts was not affected by the type of the oven; however, heating to the higher internal end point temperature increased the cholesterol content. Cholesterol retention was 96% when averaged over the three oven types.

Kruegel et al. (1986) used the colorimetric method for evaluating cholesterol in ground beef patties that had three fat levels (8%, 18% and 28%), and were broiled to two end point temperatures (71°C and 77°C). Results indicated that different levels of fat content of the raw products did not effect the final cholesterol content of the ground beef patties. Also, they found that cholesterol increased with higher internal end point temperature. This latter trend can be attributed to the high evaporation rates

associated with the high temperatures. Park and Addis (1986a) used gas chromatography to study the oxidation of cholesterol in tallow at various frying temperatures: 135°C, 150°C, and 180°C. Their results indicated that the formation of 7 ketocholesterol increased with the heating times. There were no significant differences in the rate of formation of this oxide among these temperatures. More oxides were detected by Park and Addis (1986b). When they heated tallow for 300 h at 155°C, they found: 7α-hydroxy, 7 B-hydroxy, 7 ketocholesterol, and α-epoxide.

Prusa and Hughes (1986b) used a colorimetric method for the determination of cholesterol in pork tenderloin steaks that were heat processed by three oven types (conventional, convection, and microwave) to two internal endpoint temperatures (71°C and 77°C). Results indicated that cholesterol content (wet weight basis) of pork steak was not affected by oven type; however, heating to the higher internal endpoint temperature (77°C) increased the cholesterol. On a dry weight basis, microwave-heated steaks contained more cholesterol when compared to conventional and convection-heated samples. Similar endpoint temperatures were used by Reitmeier and Prusa (1987) to evaluate cholesterol content in broiled ground pork that had different fat levels (4%, 9%, 18%, and 23%). Results indicated that the cooked patty cholesterol content

(wet weight) did not differ among the four fat levels. High fat ground pork retained less cholesterol than low fat ground pork. A similar finding was reported by Hoelscher et al. (1987) that cholesterol content of broiled beef patties was not related to the initial fat content.

From the above it is clear that there is controversy regarding the effect of heat processing on cholesterol. This can be partially attributed to the fact that the endpoint temperature is not shown in most of the studies. When the method of cooking and the final product temperatures are described, many researchers did not specify the way for monitoring these temperatures. An effective method for monitoring food product temperatures was described by Unklesbay et al. (1987). A precise monitoring temperature system is required to study the impact of heat processing on cholesterol, in order to describe subsequent changes as a function of time and/or temperature. Since sterol oxidation is a free-radical process any sterol-containing food exposed to pro-oxidant should be suspected as a possible source of oxidized, dietary sterol (Finocchiaro and Richardson, 1983). Particular emphasis should be placed on developing better isolation and quantification techniques. A systematic study to determine food processing and environmental factors which might facilitate sterol oxidation is also needed.

CHAPTER THREE

EXPERIMENTAL PROCEDURES

Research methods were designed to meet the project objectives and to be compatible with recommended procedures and available instrumentation.

3.1 Procurement of Product

Pork shoulder (27.3 kg) was obtained from the same lot of hogs that were fed a regular diet (Meat Lab., University of Missouri, Columbia). The meat tissue and visible fat were separated. Meat was ground with a Hobart grinder (Model K5-A, Troy, Ohio) with a screen size of 6.0 mm. Ground tissue and fat were added so that the total mixture would contain approximately 17% fat. This fat content was determined by using the Rapid Babcock Method with slightly modifications (See Appendix A). Ground meat was mixed by hand and divided into equal amounts (520 gm). Each portion was wrapped with freezer paper and stored at -12°C for not more than three weeks. Each portion was tempered at 5°C for 24 h before formulation.

3.2 Restructured Pork Formulation

Several preliminary trials were performed to formulate the product. The product formula is shown in Table 1.

TABLE 3.1. Formula for restructured pork meat with soyhulls.

Product	Wt. (gm)	%
Pork shoulder, ground	100	64
Milk, 2%	40	26
Soyhulls, crushed	10	6
Seasoning, mild BBQ rub and sauce mix (S-16403)	4.8	3
Salt	1	1
	155.8	100

The milk, seasoning (Milwaukee Seasoning Laboratories, Inc., Germantown, WI), salt and soy hulls (Archer-Daniel-Midland Soybean Processing Plant, Mexico, MO) were mixed together and set for 15 min. to enable soyhulls to absorb all of the liquid. Ground meat was added, and the formula was mixed in the Hobart mixer (Model K5-A, Troy, Ohio) at speed #2 for 5 min.

3.3 Shaping of the Pork Model

Procedures were developed for having 5 layers of product placed on top of one another in a metal box.

The mixture was placed on a smooth stainless steel surface on which two strips (1 cm thick) were positioned, 18 cm apart. By using a roller (24 x 5 cm) length and diameter respectively, the formula was spread and cut with a sharp knife. Each layer was (10 x 10 x 1 cm). They were weighed on a Sartorius balance (1204 Mp, Division of Brinkmann Instrument, Inc., Westbury, NY). The mass of each layer ranged from 100-120 gm.

A box was constructed from galvanized steel sheet metal. Joints were soldered with high temperature solder. On one side of the box 5 holes (1 cm apart, and 0.3 cm diameter) were drilled for insertion of thermocouple wires. In the bottom of the box a galvanized steel wire screen was placed to enable collection of drip loss. The screen

dimensions were (10 x 10). The screen was located 1 cm above the bottom of the box. On the screen a fabric mesh (Illusion, 100% nylon, 9.5 x 9.5 cm) was placed, then a type K thermocouple (aluminum chromel) was inserted through the hole in the side of the box. The edge of a thermocouple was bent up 0.5 cm, and located 5 cm from each side of the box. The first layer was carefully placed so that the bent thermocouple was located exactly in the geometric center of the layer. Next, another fabric mesh was placed on the layer, followed by the insertion of the thermocouple and a second meat layer. This was repeated for all the five layers with the exception that the fifth layer on top was not covered with fabric mesh. Each box with the five meat layers and five thermocouple wires was covered with aluminum foil and placed at 5°C overnight. The next day the box was placed in a large box (26 x 26 x 14 cm) that was filled from the bottom to a level of 8 cm with a vermiculite (Grace Zonolite Construction Product, Cambridge, Mass.). The R value was 2.7. The insulation was added so the small box was insulated from all sides with the exception of the top. The thickness of the insulation from the five sides of the small box to the outside walls and bottom was 8 cm. The top of the large box was covered with an aluminum cover (26.5 x 26.5 cm) which had an opening in the center (10 x 10 cm) to enable the surface of the meat layers to remain uncovered. This procedure was

done to have a precise method for temperature monitoring. In addition, the direction of the heat transferred by conduction could be controlled. The surface of the product was very smooth. Heat was transferred from the top to the bottom of the layers by conduction.

3.4 Heat Processing of Layers

The large box with the sample was placed in an electric non-preheated forced air convection oven (Lang Co-20). The oven temperature was 141.9°C. The surface of the meat layers was located in the geometric center of the oven cavity. This was accomplished by placing the box on three metal rods that were connected together in a triangle shape.

The five thermocouple wires were connected to a data logger (Omega Model OM-500) and the temperatures of the oven and the five meat layers were recorded every two minutes. The surface temperature of the upper layer was monitored. This was facilitated by placement of an infrared pyrometer (Cole Parmer Model No. 8158-5) in the front of the oven. A hole (12 cm x 13.5 cm) was made in the front of the oven. This hole was covered with a heavy aluminum flap which was attached to one side of the cavity with electrical tape. Thus, it could be opened and closed quickly when pyrometer readings were required, every 2 min throughout heat processing.

By using this physical arrangement, it was possible to heat process the product to many different temperatures. The layers were numbered one through five, with the fifth one being in the top position. To achieve a wide variety of product temperatures and times, a decision was made to heat process the product until the fourth layer from the bottom reached one of six temperatures: 20, 32, 44, 56, 68 or 77°C. Five replications were performed for each of these temperatures. By using this scheme, it was possible, for each temperature, to have one layer reach a higher temperature, as well as for the three lower levels to be removed at lower temperatures. Thus, the time-temperature conditions simulated all of those which would be encountered in a heat processing situation.

After heat processing, each layer was separated, weighed and placed in a vacuum bag. They were vacuum-packaged on a vacuum packaging unit (Multivac AC 800, Wolfertschwenden, W.G.) and stored at -45°C in covered aluminum foil pans until analysis.

3.5 Sampling

After heat processing 150 samples with known time-temperature heating patterns were available for analysis. The temperature profiles were statistically analyzed to determine how representative samples could be selected for analysis.

General linear models (GLM) procedures (Sas, 1985) were used to analyze the time and temperature data. The 150 temperature profiles were identified as quadratic equations. Next, the coefficients were used as inputs for statistical cluster (CLUSTER) analysis (SAS, 1985). Results from the CLUSTER analysis revealed that these coefficients could not be used to separate the samples into representative groups. In other words, there were not enough distinct patterns of time-temperature curves which enabled sample selection.

Based on these results, the 150 samples were grouped into 30 samples by combining them according to layer position and internal product temperature achieved by the fourth layer. As an example, for the five replications which reached an internal product temperature of 20°C in layer four, five composite samples were selected:

Composite Sample 1: Layer 1, Reps 1, 2, 3, 4, 5

Composite Sample 2: Layer 2, Reps 1, 2, 3, 4, 5

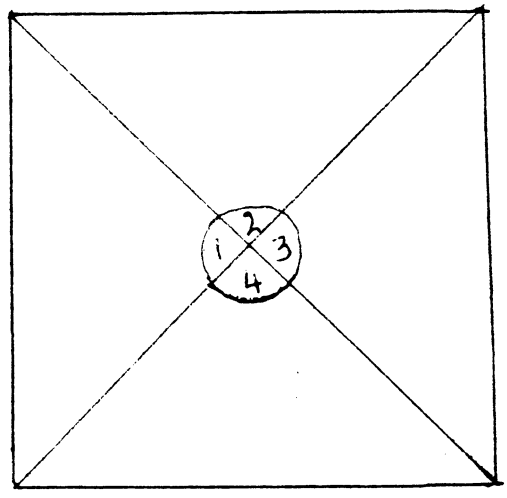
Composite Sample 3: Layer 3, Reps 1, 2, 3, 4, 5

Composite Sample 4: Layer 4, Reps 1, 2, 3, 4, 5

Composite Sample 5: Layer 5, Reps 1, 2, 3, 4, 5.

These procedures were repeated for the other five temperatures. An advantage to this scheme was that the mean time-temperature relationships for each layer could be included in the analysis.

In order to physically take a representative sample from each layer, the layer was kept in its vacuum packaging. The center was determined on the surface. A circle (5 cm diameter) was drawn around the center point. A sharp knife was used to cut out the circle. Each circle was cut into precis ly four equal parts as follows:



At random, one portion from each of the five replications was selected for cholesterol analysis. At the same time, two portions were selected for moisture analysis. The remaining fourth portion was designated for a further study beyond the scope of the current investigation. All replications of the same temperature and layer position were placed in one small polyethylene bag, thoroughly mixed, manually, sealed and frozen (-45°C) until analysis.

3.6 Sample Analyses

3.6.1 Layer Product Yields

Weighing procedures were used for determining the yield of the layers, as recommended by Matthews (1976).

3.6.2 Moisture Content

Moisture content was measured for samples by using the vacuum oven method 7.003 (AOAC,1984). Five grams of the thawed sample were weighed in an aluminum dish by using a Mettler balance (Model H31AR, Mettler Instrument Corp., Princeton, New Jersey 08540). The pan was then placed in a Fisher Isotemp Vacuum Oven (Model No. 281). The temperature was set for 99°C, and the oven was turned on and a vacuum was drawn (-25 psig). The samples were allowed to dry for 24 hours until the weights were constant. Samples were placed in a lab-line desicator (Model no. 1477) to prevent samples from absorbing moisture. The loss in weight and percentage of moisture were determined.

3.6.3 Fat Determination

Total fat content was determined on the raw and cooked samples by using the Folch procedure (Folch et al., 1957). Five grams of the sample were blended with 100 ml of chloroform/methanol (2:1) for three minutes in an air powered Waring blender (Model 31BL4T, Waring Product Division, New Hartford, Conn.). This

blender was used since the chloroform/methanol solution is highly volatile and inflammable. Its use prevented the occurrence of an explosion caused by a spark in the presence of this solution.

After blending, the slurry was transferred to a 100 ml volumetric flask. The blender, cover and jar, were carefully rinsed with $CHCl_3/CH_3OH$ to get as much of the tissue as possible into the flask. The flask was then brought to volume with the $CHCL_3/CH_3OH$ solution.

The samples were vacuum filtered using Whatman #1 paper and this step was repeated until a clear solution was obtained. This clear solution was then poured into a 100 ml graduated cylinder; the volume was measured and recorded. The samples were washed with distilled water (0.2 x volume in the graduated cylinder). The cylinders were covered with aluminum foil and refrigerated (6°C) overnight to enable the layers to separate.

When the graduated cylinders were removed from the refrigerator, the volume of the interface between the layers was recorded. Using a water aspirator and a pipet, any residue floating on top of the lipid solution was removed until only a clear solution remained. Two 5 ml aliquots were transferred to two 50 ml beakers that had been preweighed and the fat was evaporated to dryness under a stream of purified nitrogen gas for about 25 minutes or until completely dry. The beakers were then reweighed and

the difference in mass was due to the amount of fat in the tissue. This procedure was chosen as recommended by Rhee et al. (1988).

3.6.4. Cold Saponification and Extraction

From the same extract, a sample contained 0.2 gm fat was taken and placed in a test tube (25 x 200 mm) with a teflon lined screw cap. A teflon lined cap was used, because Teflon is inert and will not interact with the extract. The extract was evaporated to dryness under a stream of purified nitrogen. Cholesterol and its oxidized derivatives were extracted from saponified fat as described by Park and Addis (1986). First, 40 ug of 5 ∝-cholestane (Sigma Chemical Company, St. Louis, MO) was added as an internal standard to the 0.2 gm fat in the test tube. Then 10 ml of 2N KOH in methanol was added. The mixture was shaken vigorously by using a MaxiMix (Model No. M16715, Thermolyne Corporation, Dubuque, IA) until it become free of dispersed fat particles. Saponification was conducted at room temperature overnight according to Chicoye et al., (1968). On the second day, a 10 ml portion of distilled water was added to the saponified mixture. Nonsaponifi-ables were extracted three times, by using separatory funnels each time with 10 ml of diethy ether (HPLC grade, Curtin Matheson Scientific Inc., Houston, TX). Pooled diethyl ether extracts were washed once with 5 ml of 0.5 N KOH in

methanol, and three times with 5 ml of distilled water and dried over anhydrous sodium sulphate (Sigma Chemical Company, St. Louis, MO). The dried extracts were filtered with Whatman #1 filter paper and re-extracted with another 10 ml of diethyl ether and filtered again. The combined filtrates were concentrated to ca. 1 ml in a rotary vacuum evaporator (Buchi, Model No. 28555, Glasapparatefabrik, Flawil, Switzerland). The filtrates were transferred into a small amber vial. Then the solvent was freed under nitrogen flash until dryness. The vials were then sealed and kept in a freezer at -40°C.

In preliminary trials some samples were prepared, and injected into the GC-MS. Results indicated that some of the free fatty acid remained with the unsaponifiable material (see chromatogram, Appendix B). Thus, a decision was made to clean up the sample before injection.

3.6.5. Sample Clean-Up

Ten grams of alumina basic (Brockman activity 1, Fisher Scientific, Fair Lawn, NJ) were made into a slurry with 100 ml 9:1 chloroform/methanol (v/v) and packed into a glass column (10.5 mm internal diameter x 25 cm length) that was obtained from ACE glass, Inc., Vineland, NJ) Silanized glass wool (Baker Chemical Co., Phillipsburg, NJ) was placed at the bottom. Lipid extract was redissolved in a minimal amount of solvent, and the

sample was eluted with 100 ml $CHCL_3/CH_3OH$ 9:1. The elution was evaporated to ca 1 ml by using the rotary vacuum evaporator, with the water bath temperature between 19°C and 22°C. The extract was transferred into a small amber vial, and excess solvent was evaporated to dryness under a stream of purified nitrogen.

3.6.6. Sample Derivatization

Several reasons for performing a chemical reaction on a sample to form derivatives were discussed by Miller (1988). The high molecular weight of steroid compounds and the presence of polar groups in their molecule, which are often not stable under chromatographic conditions, are the properties that make the direct GC analysis of free steroids difficult. In this procedure, derivatization was performed to form nonpolar volatile compounds which were more thermal stable for GC analysis (Drozd, 1981). Derivatization of cholesterol to trimethyl (TMS) ether was done by dissolving the dried sample in 100 ul pyridine (Fisher Scientific Company, Fair Lawn, NJ) to which 50 ul Sylon BTZ (Supelco, Inc., Bellefonte, PA) was added. This reaction was held for 30 min. at R.T. Then after derivatization, the derivatizing agents were evaporated to dryness under a stream of nitrogen.

3.6.7. Preparation of Sterol Standards

Sterols used in the study were: cholesterol;, 7 ketocholesterol; cholestane-3B,5A,6B-

Triol; 7-B-hydroxycholesterol;, 25 hydroxycholesterol; and 5 ∝ cholestane and cholesterol 5A,6A epoxide (Sigma Chemical Company, St. Louis, MO). These sterols were chosen because they were found in freeze dried pork (Park and Addis, 1987). All sterols were dissolved in ethylacetate and stored at -40°C. Standard solutions were made to permit injection of 40 ug of 5 ∝ cholestane, with cholesterol oxides ranged from 10 to 150 ng, and cholesterol from 50 to 1000 ng, respectively. This high ratio of cholesterol to the oxides was recommended by Park and Addis (1985). Calculations for standard preparations are given in Appendix C.

The response linearity was examined by plotting the area response ratio of each sterol over the internal standard, against the weight ratio of each sterol over the internal standard.

For determining the recovery of the sterols through extraction and saponification procedures, spiked samples were prepared included the raw pork formula, and 40 ug 5 -cholestane, cholesterol, and cholesterol oxides in the same concentration range as the standards.

Standards and spiked samples were treated in the same way as the raw formula and heat processed samples, for GC-MS analyses.

3.6.8 Gas Chromatography - Mass Spectrometry

Two gas chromatography - mass spectrometry instruments were utilized during the course of the study. Hewlett Packard gas chromatograph - Mass spectrometer system (Model 5890 and 5970B respectively, Palo Alto, CA) was used with a DB-1 Capillary Column (15 m x 0.25 mm i.d., 0.1 um film thickness, (J and W Scientific, Inc., Rancho Cordova, CA). For confirmation of the peaks of cholesterol and cholesterol oxides, the mass spectometer was directly coupled to the GC. Helium was used as a carrier gas and the oven temperature was programmed from 100 to 265°C at 5°/min rise. The mass spectra of TMS ether sterols were obtained by using a scanning mass-to-charge (mle) range = 100-600. Scanning rate was 1 second. The electron energy was 65 electron volts (ev).

The derivatized extract of the raw pork formula, and heat processed samples were placed in an autosampler tray (Hewlett Packard Model 7673A, Palo Alto, CA). After every ten samples four spiked samples were run. One microliter was injected from each sample.

For further confirmation of results, standards, spiked samples, and selected samples were rerun on a gas chromatograph (Model Sigma 3B, Newark, CO), Mass spectrometer (Finnigan OWA-30B, San Jose, CA). This system is equipped with a combination EI/CI (Electron impact/

Chemical impact ionization) source. The absolute sensitivity of this system is approximately 5 times higher than the Hewlett Packard GC-MS system. Cholesterol and cholesterol oxides were calculated in terms of mg/100 gm sample (See Appendix D).

3.7. Statistical Methods

To achieve the research objectives regarding moisture, fat and cholesterol, several statistical analyses were performed.

Composite samples were selected for analyses as described in Section 3.5. In addition, samples of the raw restructured pork/soy hull formula were selected for analysis. Each composite sample represented five heat processing replications, according to layer position and heat processing time. The 150 layers were composited into 30 samples.

The heat processing time (min) and internal product temperature (°C) data were used to determine the heat penetration equation using GLM (SAS, 1985) procedures. Once the equations were determined, the data was analyzed to determine the integral and second derivative. The purpose of these analyses were to precisely define the 30 heat processing conditions.

Each of the raw sample and composite heat processed samples, were analyzed for moisture, fat, cholesterol, and

cholesterol-oxides. Two-way analysis of variance procedures for completely randomized designs with unequal cell sizes were performed on the food components with either layer or temperature range as the independent variable (SAS, 1985). The least square means were analyzed for significant differences. Finally, Pearson product moment correlations were determined among the experimental variables (time, temperatures, integral, acceleration) and food components.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

In order to study the effect of heat processing on the cholesterol content of restructured pork, findings from the statistical analysis (Section 3.7) will be discussed.

4.1. Heat Processing Conditions

Heat processing conditions (time, temperature, and layer position) are given in Tables 4.1-4.4. In this system the heat is transferred through the oven by convection and some radiation. It was transferred through the product by conduction. The conduction heat transfer equation is:

$$q = \frac{kA}{L} (T_1 - T_2)$$

where:

q = heat flow (W/hr)

A = cross sectional area for heat flow (m^2)

L = length of heat flow path (m)

T_1, T_2 = high and low temperature (°C)

k = the property of material and, called thermal conductivity, (W/hr - m - °C)

In Table 4.1 it is clear that in each group layer #5 attained the highest final temperature followed by layer

TABLE 4.1. Heat Processing Conditions (Time, Temperature, Layer Position and Food Components).

Conditions			Food Components			
					Cholesterol mg/100 gm	
Time (min)	Final Internal Temp. (°C)	Layer Position[1]	Moisture (%)	Fat (%)	Wet Basis	Dry Basis
19	8.6	2	64.63	13.9	40.20	113.66
	10.5	3	65.62	12.0	34.87	101.43
	10.6	1	63.66	11.2	36.83	101.35
	20.3	4	63.10	13.0	36.60	99.19
	49.5	5	62.08	12.1	44.73	117.96
31.4	15.3	2	63.40	13.1	37.03	101.18
	19.2	1	63.35	12.4	32.37	88.32
	20.2	3	64.06	14.7	52.13	145.05
	32.2	4	63.49	13.4	50.13	137.31
	65.7	5	61.02	16.0	43.33	111.16
43.6	24.0	2	63.43	13.2	42.93	117.39
	26.5	1	64.05	12.4	36.37	101.17
	28.6	3	63.48	12.2	51.13	104.95
	44.2	4	63.03	13.7	42.43	114.77
	73.4	5	58.83	14.8	59.63	144.84
56.2	35.5	2	64.91	13.4	52.60	149.90
	40.2	1	63.84	13.0	64.35	177.96
	41.3	3	63.71	13.9	58.13	115.82
	56.1	4	61.69	14.0	55.47	144.79
	86.3	5	56.58	14.0	66.47	153.09
71.2	48.2	2	63.80	12.7	41.30	114.09
	52.7	3	63.27	12.5	40.43	110.07
	53.8	1	63.65	11.9	40.67	111.88
	68.1	4	61.43	14.4	33.93	87.97
	94.3	5	56.72	15.2	45.43	103.89
89.6	61.0	2	61.71	17.6	50.77	132.59
	62.8	1	61.54	13.0	47.33	123.06
	64.8	3	60.65	20.0	56.00	142.31
	77.0	4	58.21	16.9	46.53	111.34
	97.0	5	52.90	21.0	53.05	112.63

[1]Refers to position of the layer in the box (1 = bottom layer; 5 = top layer).

#4. This is due to the fact that as the length increases the quantity of heat flow decreases, i.e. as L in the above equation increases then q will decrease. In this way one would expect layer #3 to obtain higher temperatures than layers #1 and #2. Among layers #1, #2 and #3 there is no systematic order or pattern in the final temperature. This can be attributed to small temperature differences i.e. T_1, and T_2. Also this can be affected by the thermal conductivity of the layer which can be influenced by the specific heat of the product. The latter property can be affected by the constituents of the product such as the moisture and fat contents. Also from Table 4.1 it is clear that the experimental procedure enabled the study of a wide range of time and temperatures. Although the time for the experiment just varied by 70.6 min, the final internal temperatures varied by 88.4°C. On the other hand, focusing on heat processing time, and the variation in the internal product temperature within this time, it is clear that: at 19 min, internal product temperature varied by 40.9°C; at 31.4 min, by 50.4°C· at 43.6 min, by 49.4°C; at 56.2 min, by 50.8°C; at 71.2 min, by 46.1°C; at 89.6 min, by 36°C. From the above it is clear that less variation in internal product temperature is found with the lowest and highest heat processing times used. The greatest variation in internal product temperature is found in heat processing times ranging from 31.4 to 71.2 min.

Table 4.2 gives the results from the curve fits for the quadratic equation of the mean heat processing time versus mean internal product temperature for each of the 30 composite samples. Note that for the majority of the equations, R^2 or the goodness of fit exceeds 95%. The data in Table 4.2 indicate that layers #1, #2, and #3 always have the same sloped curve. Layer #5 has a different slope, as it attains a higher internal product temperature more rapidly than the other layers.

Figure 4.1 - 4.5 give the heat penetration curves with confidence intervals for layers #1 to #5, respectively, when layer #4 was heat processed to 77°C. Note the curve direction differs for the top two layers.

Table 4.3 gives the heat processing time, and the integrals of the heat penetration curves. The integral is the area (°C min) under the heat penetration curves that describes the time and temperature relationships. This experimental procedure produced a range of integrals from 155.81 to 6318.05°C min, a difference of 6162.24°C min between them. Note that after 89.6 min of heat processing the integrals of the heat penetration curves ranged from 2850.78 to 6318.05°C min. This finding was illustrated in Figure 4.1 - 4.5, with layer #5 (Figure 4.5) having a much greater area under the curve than layer #1 (Figure 4.1). This finding is clearly illustrated in Figure 4.6 when all heat penetration curves are shown.

TABLE 4.2. Results of Curve Fit of Heat Processing Time (min) and Final Internal Product Temperature (°C)

$$T(t) = at^2 + bt + c$$

Composite sample		a	b	c	R^2
20[1]	1[2]	0.01057546	0.07271964	5.74102189	0.918935
	2	0.00725097	0.02341939	5.22620125	0.806799
	3	0.01180683	0.05035858	5.18116595	0.918297
	4	0.00449200	0.63241139	4.82223640	0.863582
	5	-0.06462770	3.33502859	6.97043088	0.819622
32	1	0.00793660	0.17375119	6.05369036	0.958694
	2	0.01140930	-0.06435216	5.79762222	0.976504
	3	0.01561394	-0.02227985	5.66558431	0.947527
	4	0.00699680	0.64811488	4.33956466	0.953176
	5	-0.03571635	2.92958768	8.14433141	0.958416
44	1	0.00900352	-0.01652222	6.84211860	0.591946
	2	0.01017885	-0.02597287	6.03073835	0.992084
	3	0.01037427	0.09497802	5.22256476	0.986085
	4	0.00321209	0.79063949	4.38306624	0.979739
	5	-0.01853462	2.28361270	9.12628609	0.982856
56	1	0.01024659	0.04451260	6.50713822	0.973999
	2	0.00948682	0.01039133	5.37409507	0.990541
	3	0.00768781	0.25690523	4.02250793	0.981912
	4	-0.00235713	1.07357889	2.62664103	0.974890
	5	-0.02078146	2.41852810	13.63082204	0.987800
68	1	0.00723308	0.15683486	4.76291944	0.995764
	2	0.00702283	0.13197105	3.98294671	0.995013
	3	0.00377714	0.44651935	2.37074314	0.988966
	4	-0.00438899	1.20513796	2.41454729	0.962050
	5	-0.01743067	2.29438421	13.74060344	0.943204
77	1	0.00249677	0.47439230	4.83837768	0.975668
	2	0.00396199	0.31619045	4.51193793	0.971337
	3	0.00215263	0.51852619	3.14925389	0.990954
	4	-0.00182564	0.97593420	4.09063[illegible]3	0.991771
	5	-0.00900699	1.68819436	15.402181[illegible]0	0.970081

[1]Refers to final product temperature of layer 4
[2]Refers to layer position (1 = bottom; 5 = top)

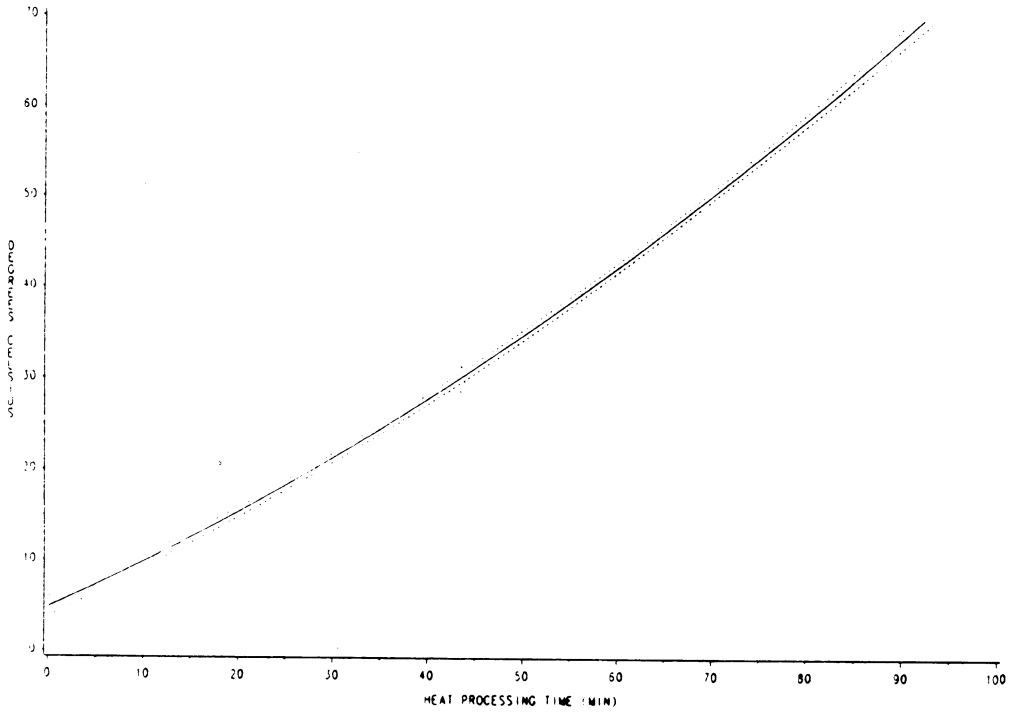


Fig. 4.1. Heat Penetration Curve of Layer #1 When Layer #4 Heat Processed to 77°C.

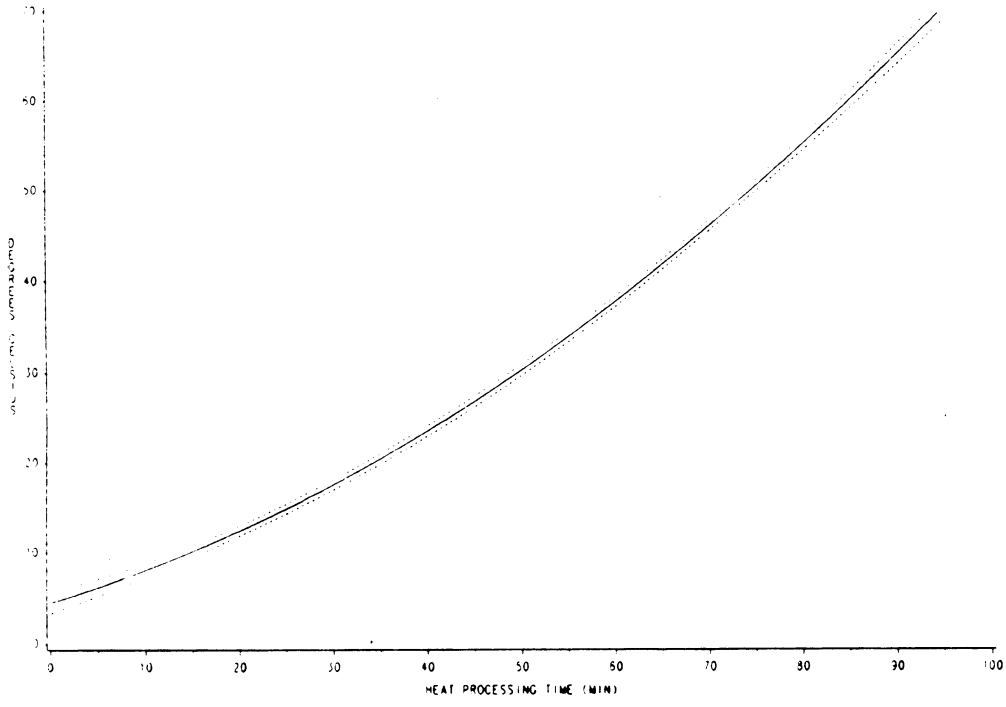


Fig. 4.2. Heat Penetration Curve of Layer #2 When Layer #4 Heat Processed to 77°C.

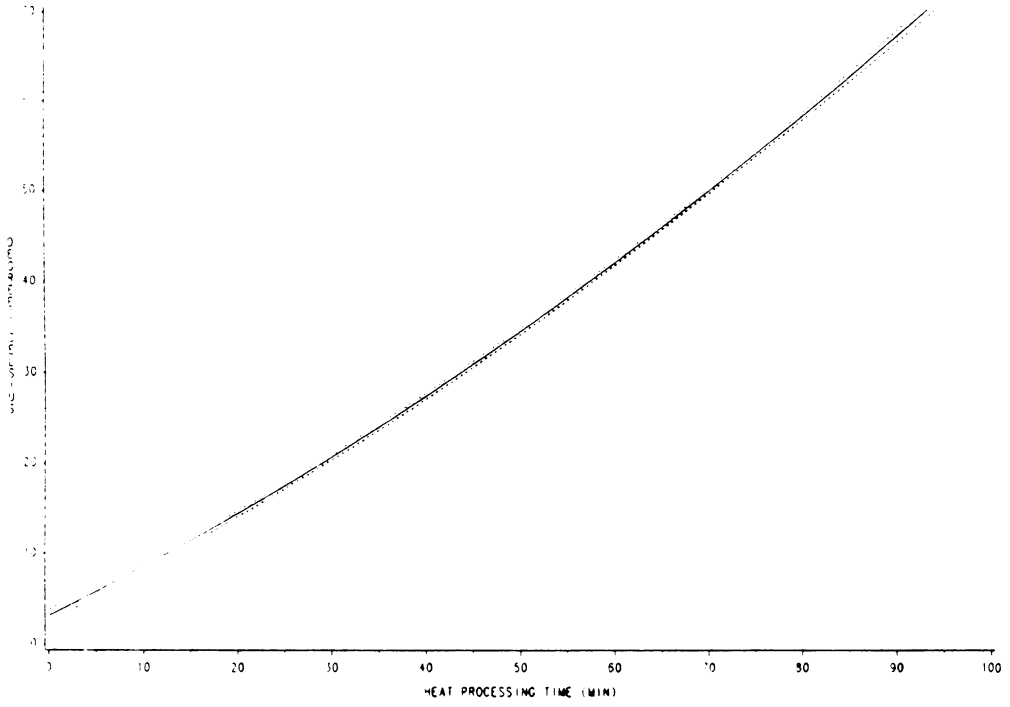


FIG. 4.3. Heat Penetration Curve of Layer #3 When Layer #4 Heat Processed to 77°C.

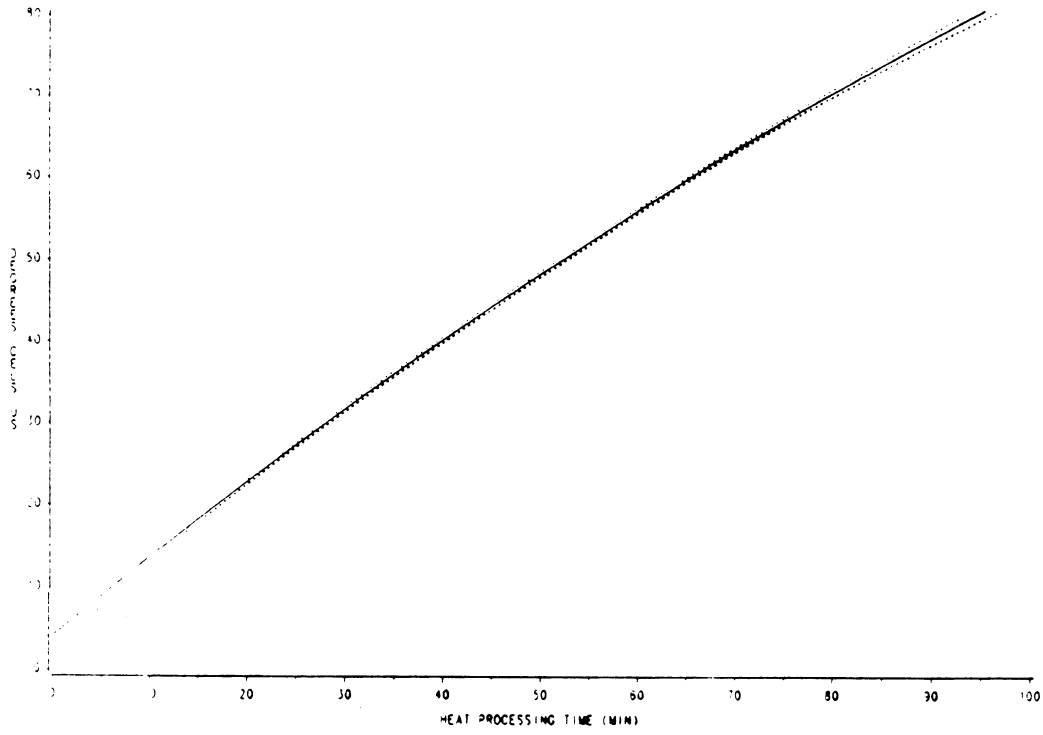


FIG. 4.4. Heat Penetration Curve of Layer #4 When Heat Processed to 77°C.

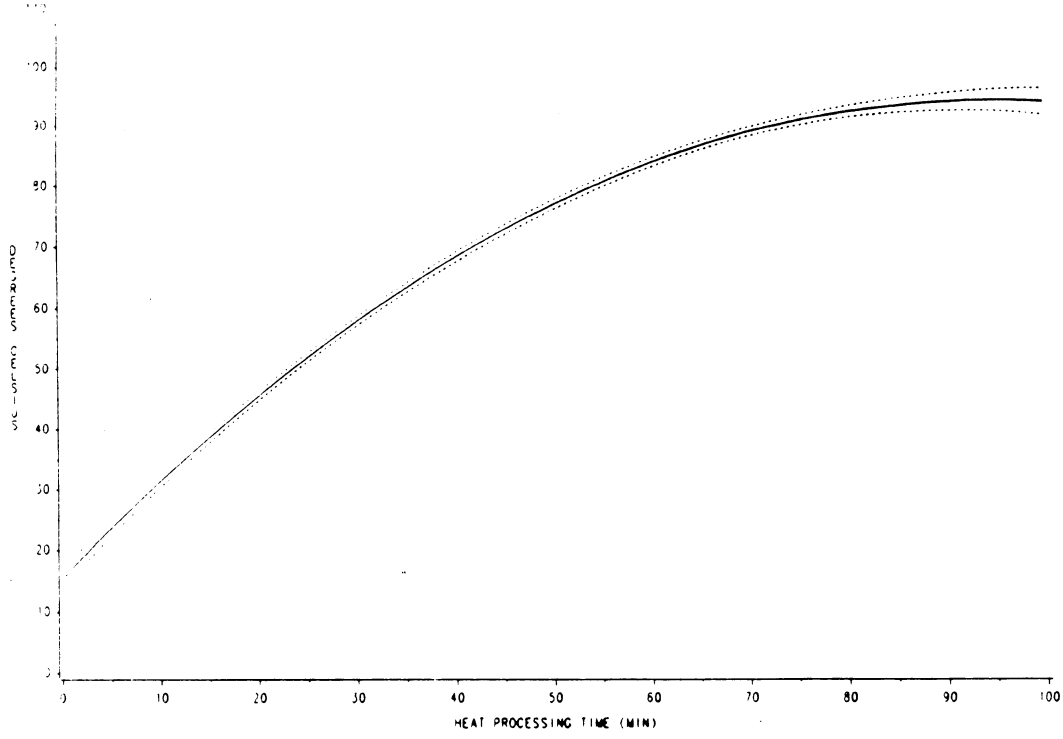


Fig. 4.5. Heat Penetration Curve of Lyer #5 When Layer #4 Heat Processed to 77°C.

TABLE 4.3. Heat Processing Conditions (Time, Integral and Food Components).

Condition		Components			
H.P. time (min)	Integral (°C min)	Moisture (%)	Fat (%)	Cholesterol mg/100 gm Wet	Cholesterol mg/100 gm Dry
19	155.81	64.63	13.9	40.2	113.66
	180.37	65.62	12.0	34.87	101.43
	194.17	63.66	11.2	36.83	101.35
	296.40	63.10	13.0	36.60	99.19
	780.33	62.08	12.1	44.73	117.96
31.4	309.40	63.40	13.1	37.03	101.18
	384.32	64.06	14.7	53.13	145.05
	408.36	63.35	12.4	32.37	88.32
	613.82	63.49	13.4	50.13	137.31
	1520.28	61.02	16.0	43.33	111.16
43.6	580.19	63.43	13.2	42.93	117.39
	589.38	64.05	12.4	36.37	101.17
	677.32	63.48	12.2	51.13	140.01
	1142.33	63.03	13.7	42.43	114.78
	2234.51	58.83	14.8	59.63	144.84
56.2	1106.82	64.91	13.4	52.60	149.90
	1303.01	63.84	13.0	64.35	177.96
	1353.91	63.71	13.9	58.13	160.18
	2039.01	61.69	14.0	55.47	144.79
	3842.59	56.58	14.0	66.47	153.08
71.2	1880.64	63.80	12.7	41.30	114.09
	2054.40	63.65	11.9	40.67	111.88
	2201.41	63.27	12.5	40.43	110.07
	3230.07	61.43	14.4	33.93	87.97
	5380.47	56.27	15.2	45.43	103.89
89.6	2850.28	61.71	17.6	50.77	132.59
	3112.41	60.65	20.0	56.00	142.31
	3170.91	61.54	13.0	47.33	123.06
	4111.47	58.21	16.9	46.53	111.34
	6318.05	52.90	21.0	53.05	112.63

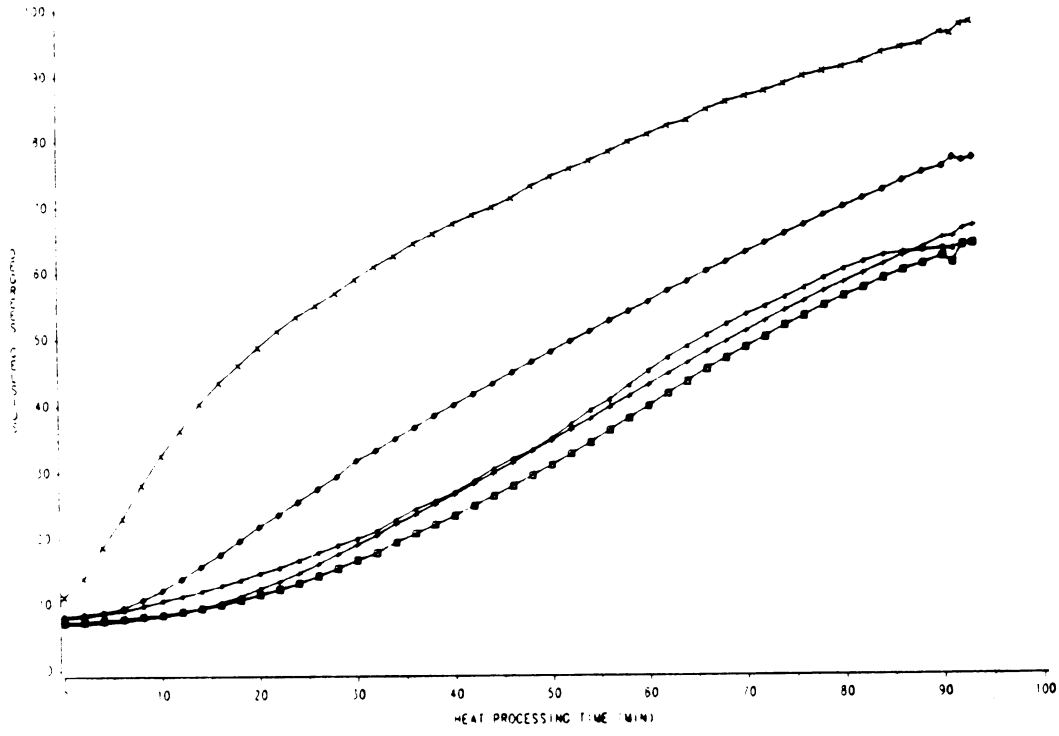


Fig. 4.6. Heat Penetration Curve of the Five Layers Restructured Pork When Layer #4 Reached 77°C. (Star = layer 1, Box = layer 2, Asterisk = layer 3, Diamond = layer 4, X = layer 5).

Table 4.4 gives the second derivative of the quadratic equation. This is the acceleration of the heat penetration curve. Note, that with the exception of layer #1 and sometimes layer #2, the acceleration is positive. Again this can be observed in the curve fits for 89.6 min of heat processing (Figure 4.1 - 4.5).

The mean internal temperature and mean heat processing time for the six sets of temperatures are given in Figure 4.7. This wide spectrum of data points illustrates the wide scattering of the data listed in Table 4.1. Figure 4.8 shows a scatter plot of the acceleration ($°C/min^2$) versus heat processing time. It is clear that the majority of these second derivatives are positive ones, as shown in Table 4.4.

No previous study has examined the impact of all of these heat processing conditions on cholesterol. By having all of these parameters, it was feasible to justify the cholesterol data. Furthermore, if cholesterol oxides were to develop under these conditions, heat processing methods for their elimination could be recommended.

TABLE 4.4. Heat Processing Conditions (Time, Acceleration and Food Components).

Conditions		Components			
Time (min)	Acceleration ($°C/min^2$)	Moisture (%)	Fat (%)	Cholesterol mg/100 gm Wet	Cholesterol mg/100 gm Dry
19	-0.12926	62.08	12.1	44.73	117.96
	0.00898	63.10	13.0	36.60	99.19
	0.01450	64.63	13.9	40.2	113.66
	0.021151	63.66	11.2	36.83	101.35
	0.02361	65.62	12.0	34.87	101.43
31.4	-0.07143	61.02	16.0	43.33	111.16
	0.01399	63.49	13.4	50.13	137.31
	0.015587	63.35	14.4	32.37	88.32
	0.02282	63.40	13.1	37.03	101.18
	0.03123	64.06	14.7	52.13	145.05
43.6	-0.03707	58.83	14.8	59.63	144.84
	0.00642	63.03	13.7	42.43	144.77
	0.01801	64.05	12.4	36.37	101.17
	0.02036	63.43	13.2	42.93	117.39
	0.02075	63.48	12.2	51.13	140.01
56.2	-0.04156	56.58	14.0	66.47	153.09
	-0.00471	61.69	14.0	55.47	144.79
	0.01538	63.71	13.9	58.13	160.18
	0.01897	64.91	13.4	52.60	149.90
	0.02049	63.84	13.0	64.35	177.96
71.2	-0.03486	56.27	15.2	45.43	103.89
	-0.00878	61.43	14.4	33.93	89.97
	0.00755	63.27	12.5	40.43	110.09
	0.01405	63.80	12.7	41.30	114.09
	0.01447	63.65	11.9	40.67	111.88
89.6	-0.01801	52.90	21.0	53.05	112.63
	-0.00365	58.21	16.9	45.53	111.34
	0.00431	60.65	20.0	56.00	142.31
	0.00499	61.54	13.0	47.33	123.06
	0.00792	61.71	17.6	50.77	132.59

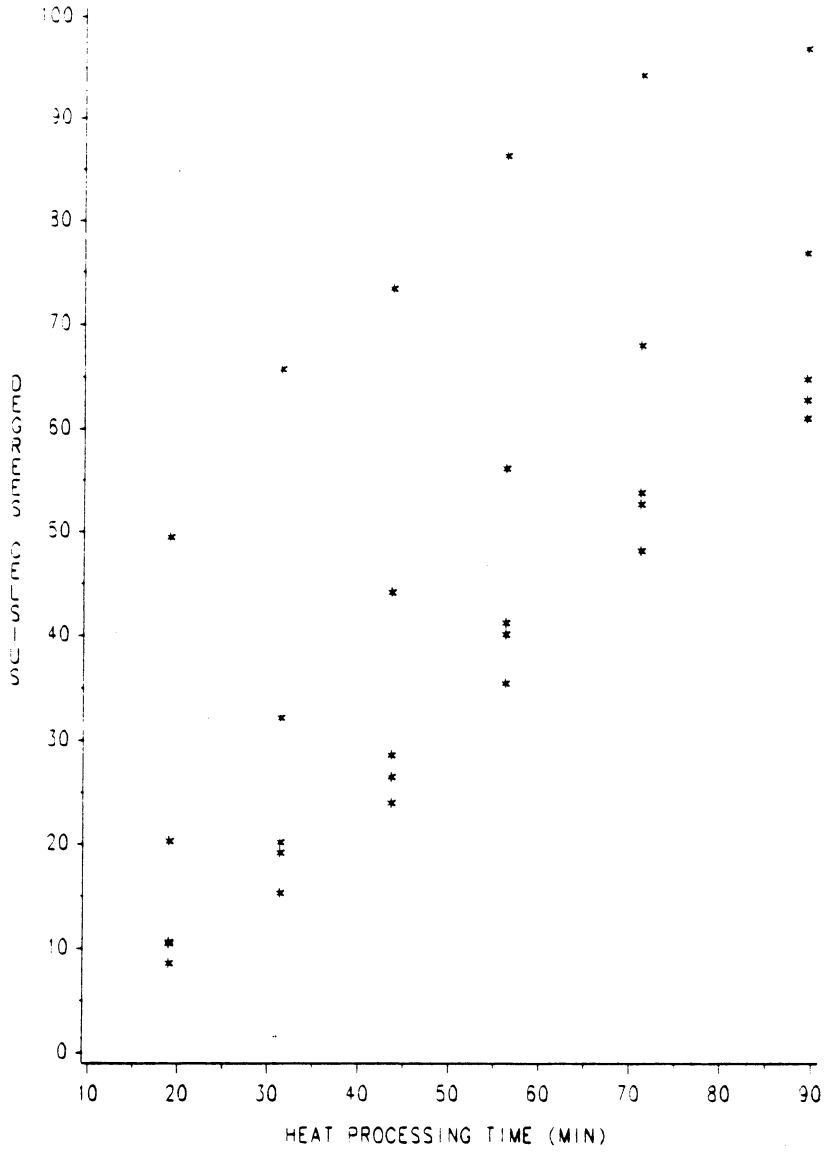


FIG. 4.7. The Mean Internal Temperatures and the Mean Heat Processing Time for the Six Temperature Sets of Restructured Pork.

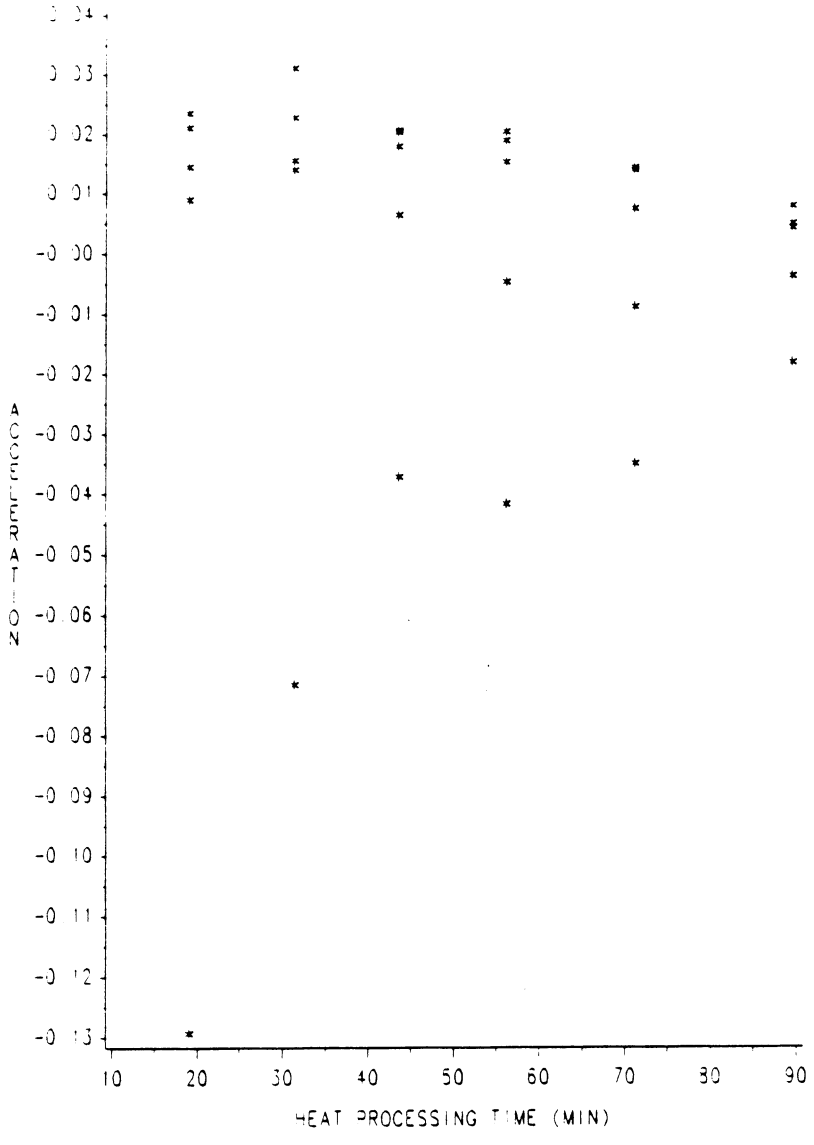


FIG. 4.8. Acceleration vs. Heat Processing Time of Restructured Pork.

4.2. Gas chromatography - mass spectrometry

Figures (4.9-4.16) show the chromatograms of sterol standards and the mass spectrum of each peak. These peaks are identified as: peak at 1079 is 5⍺cholestane, that at 1250 is cholesterol, at 1301 is expoxide, at 1302 is 7B hydroxy, at 1363 is Triol, followed by 7 keto-cholesterol, and 25 hydroxycholesterol, respectively. Note that expoxide and 7B hydroxy are found under the area 1306, and can be separated by narrowing a window in the mass spectrum at this area.

4.2.1. Response linearity of TMS Sterols Standards

The response linearities of cholesterol and 7B-hydroxycholesterol are given in Figures 4.17 and 4.18. These values were obtained by plotting the normalized response vs concentration. The normalized response for each point was calculated by this equation:

$$\text{Normalized response} = \frac{\text{Area of sterol}}{\text{Area of standard}} \times \text{average of std. area}$$

These figures show the instrument response to the different levels of sterol concentrations and the fixed level of 5⍺cholestane.

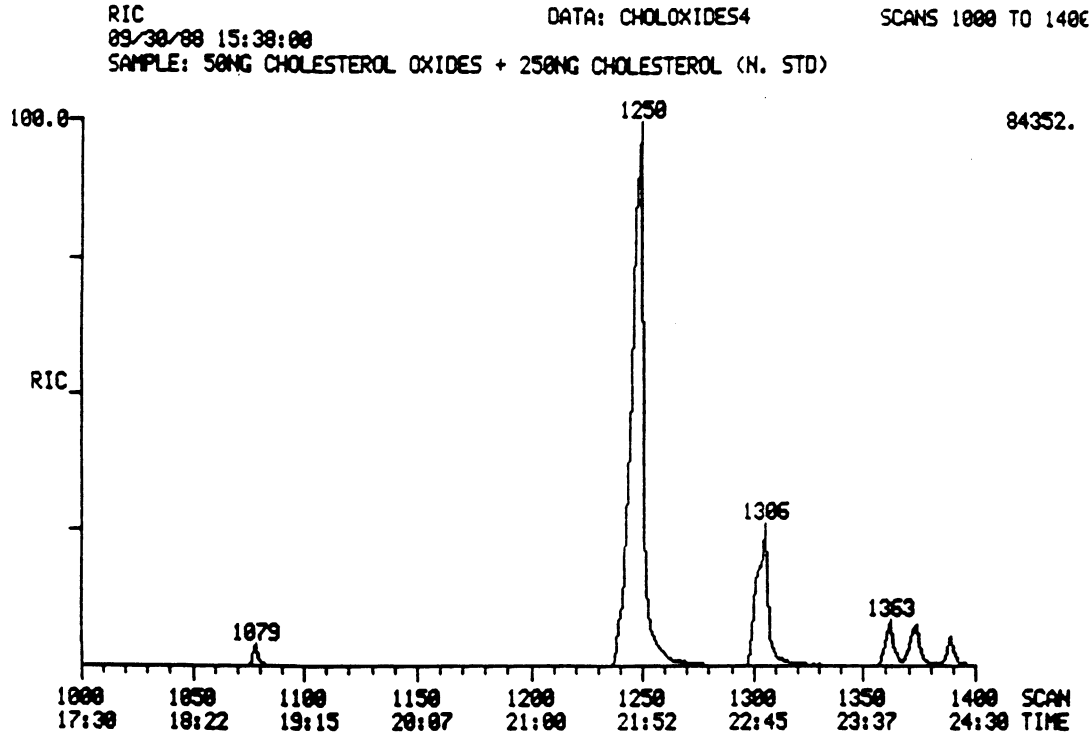


FIG. 4.9. Chromatogram of Sterol Standards.

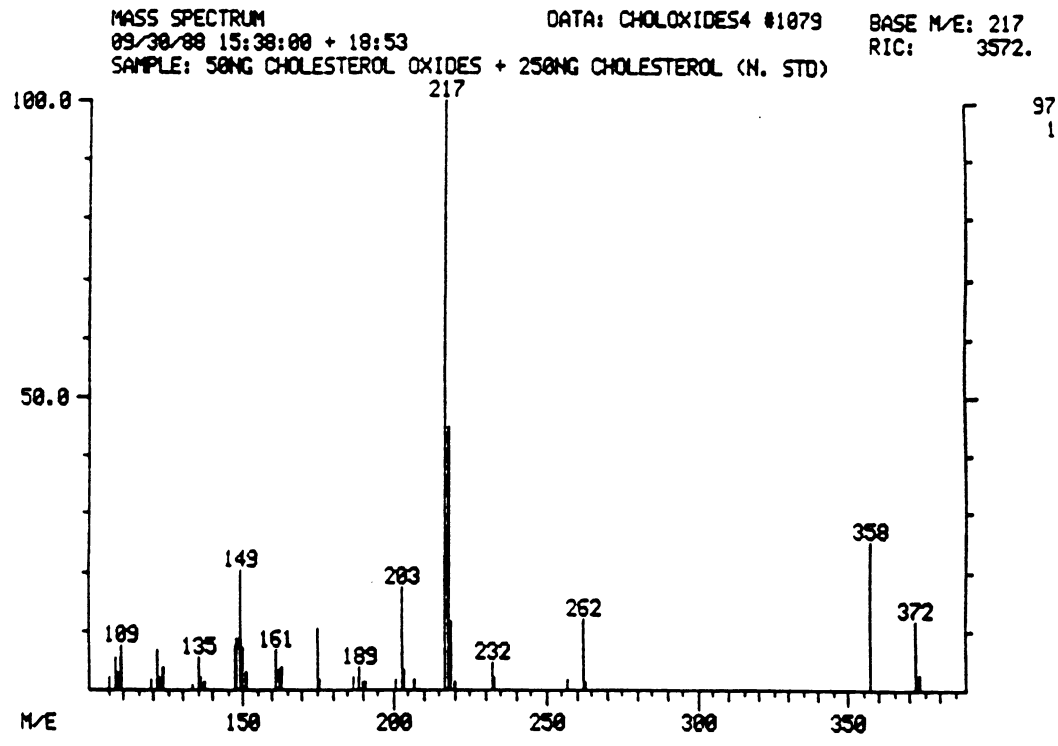


FIG. 4.10. Mass Spectrum of 5A Cholestane.

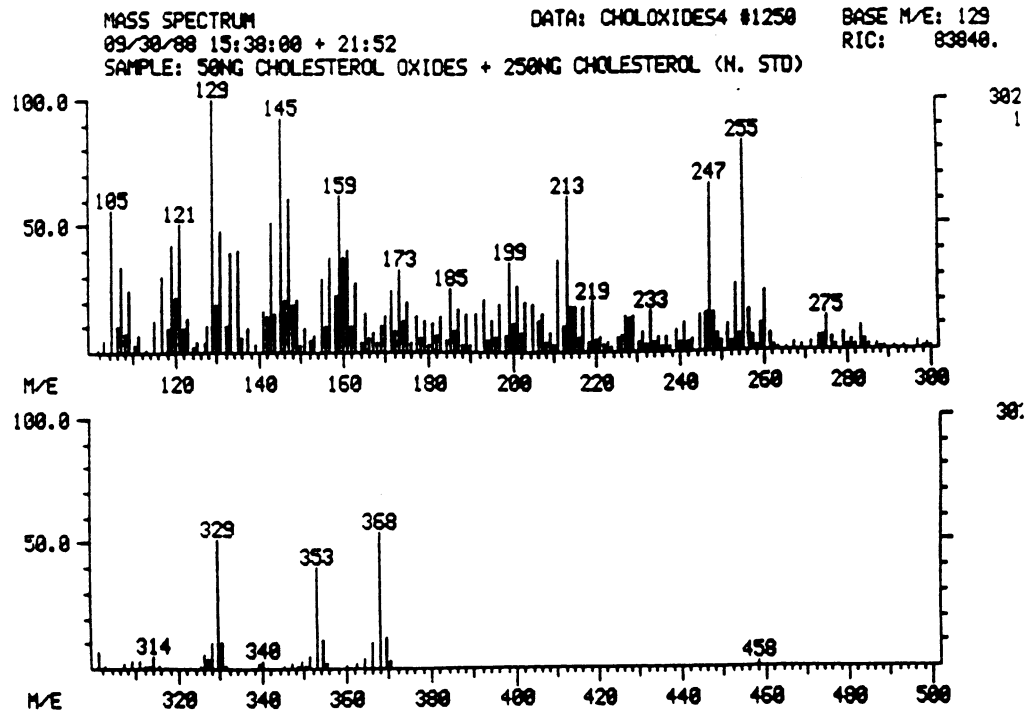


FIG. 4.11. Mass Spectrum of Cholesterol.

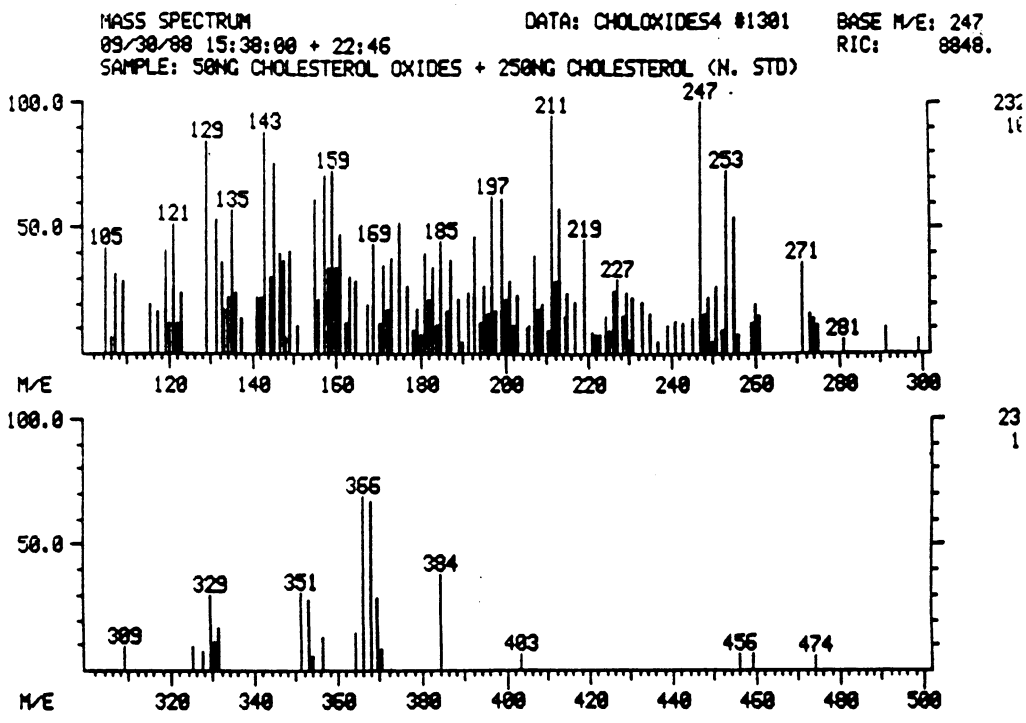


FIG. 4.12. Mass Spectrum of 5A,6A Cholesterol Epoxide.

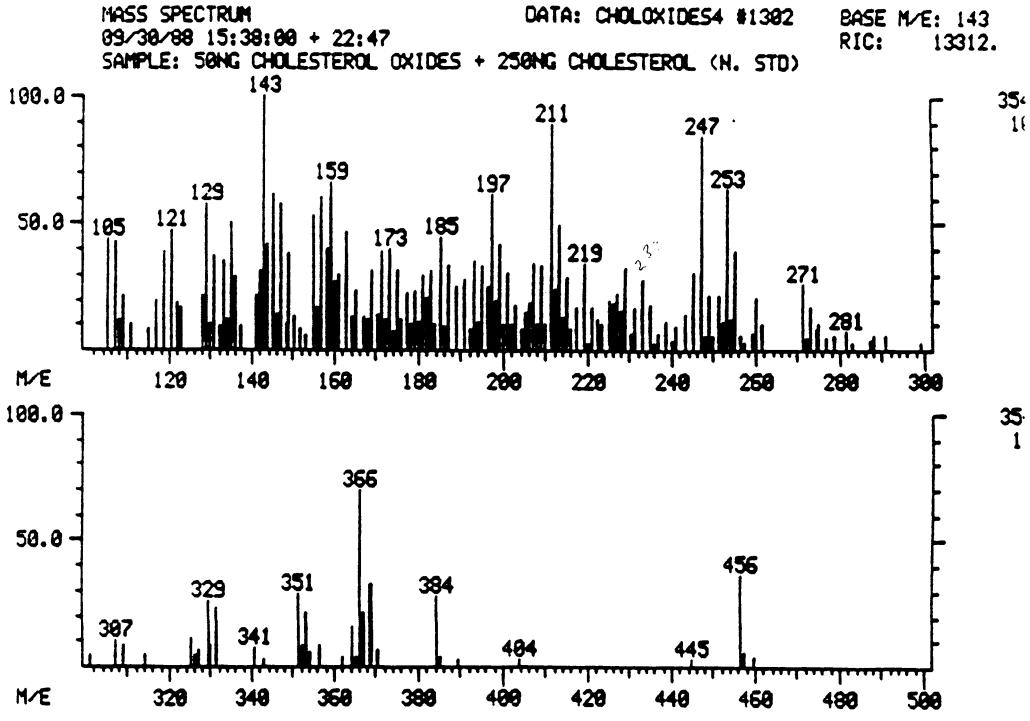


FIG. 4.13. Mass Spectrum of 7B Hydroxycholesterol.

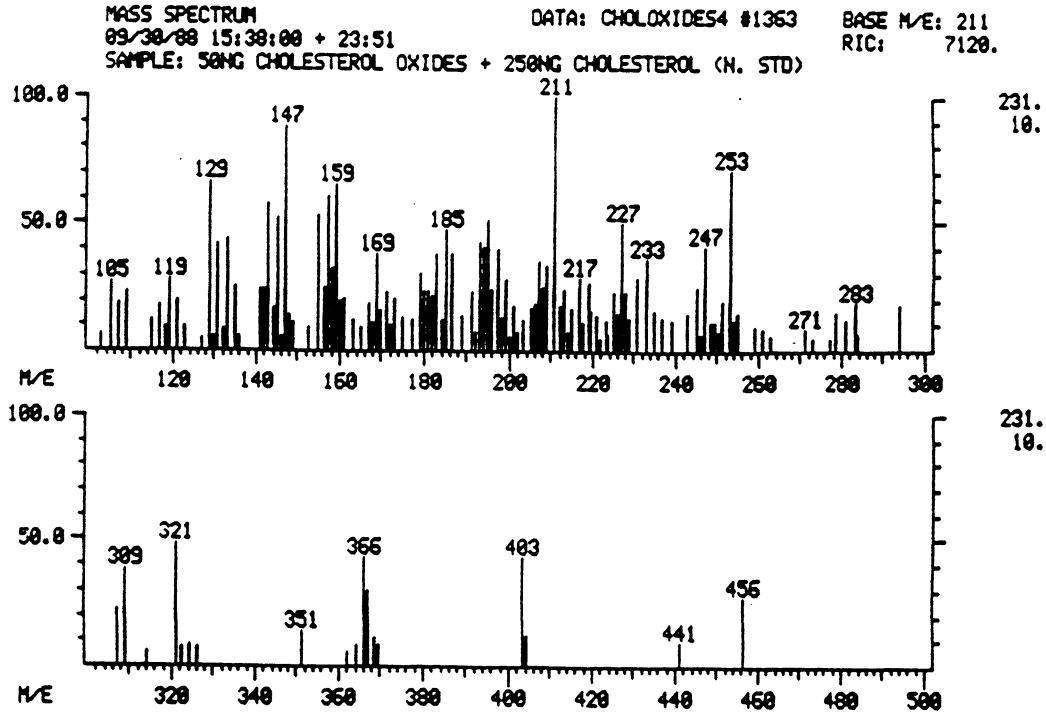


Fig. 4.14. Mass Spectrum of Cholestane-3B,5A,6B-triol.

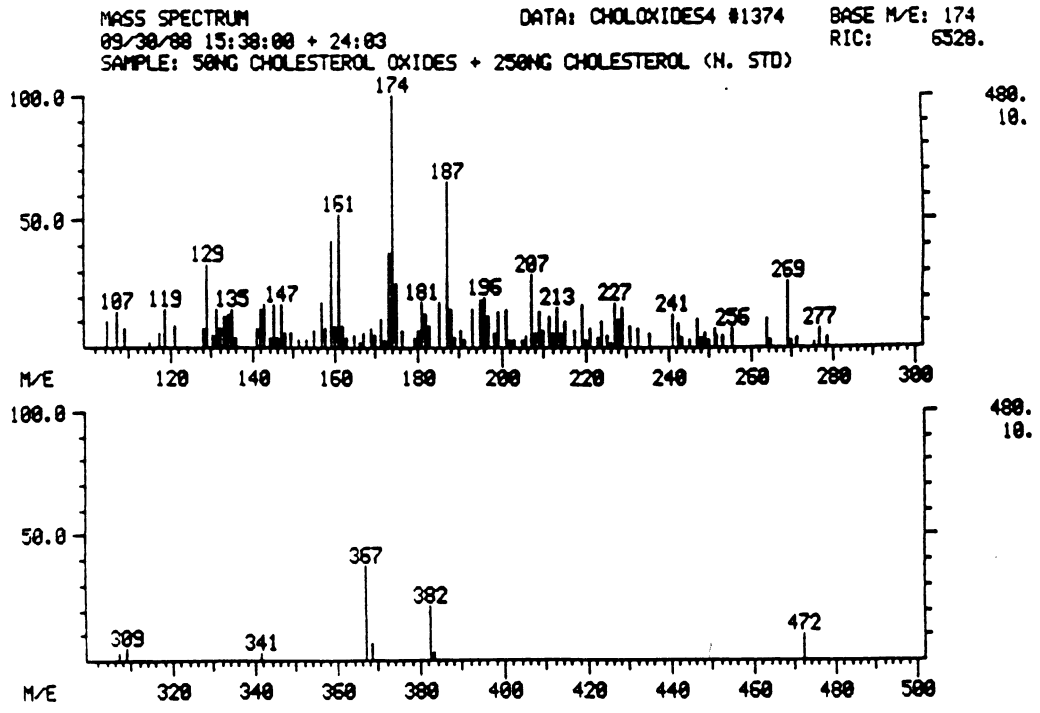


FIG. 4.15. Mass Spectrum of 7 ketocholesterol.

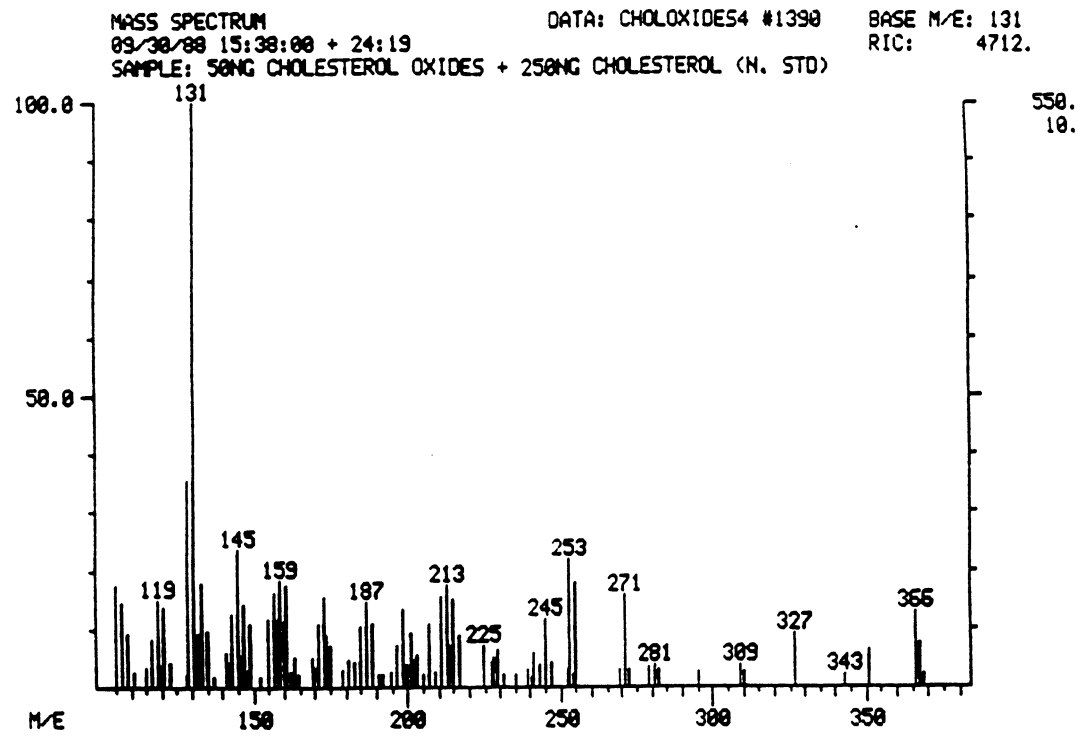


Fig. 4.16. Mass Spectrum of 25 Hydroxycholesterol.

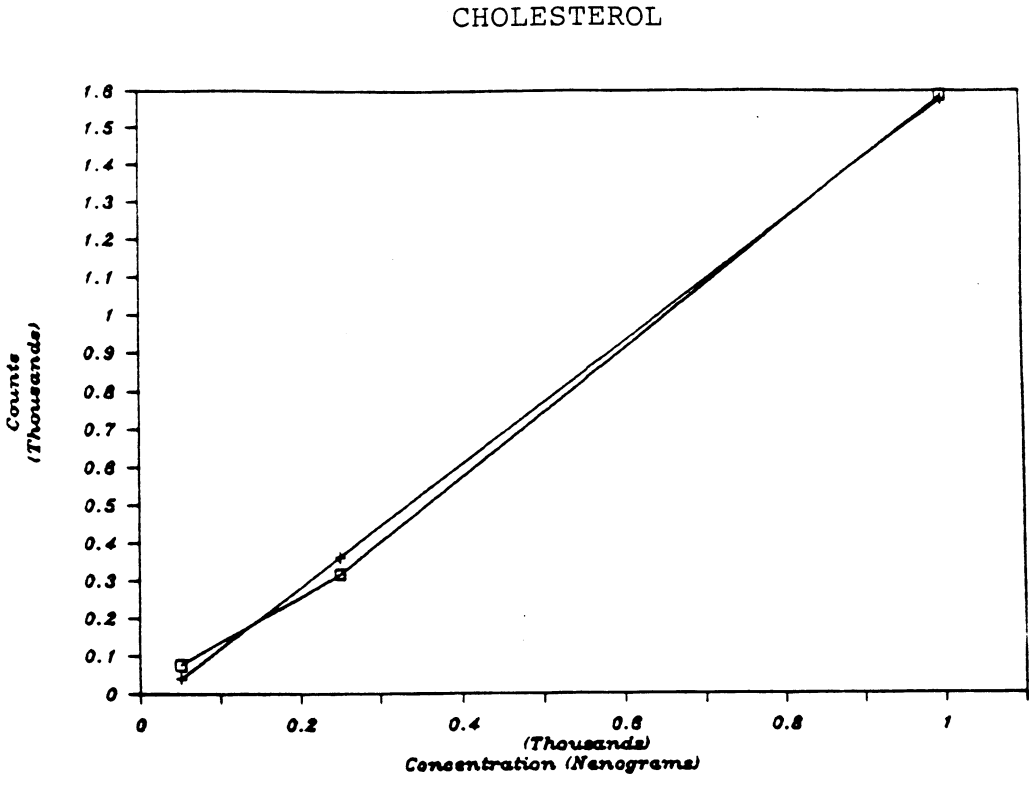


FIG. 4.17. Response Linearity of Cholesterol With Various Amounts and Fixed Amount of 5A Cholestane.

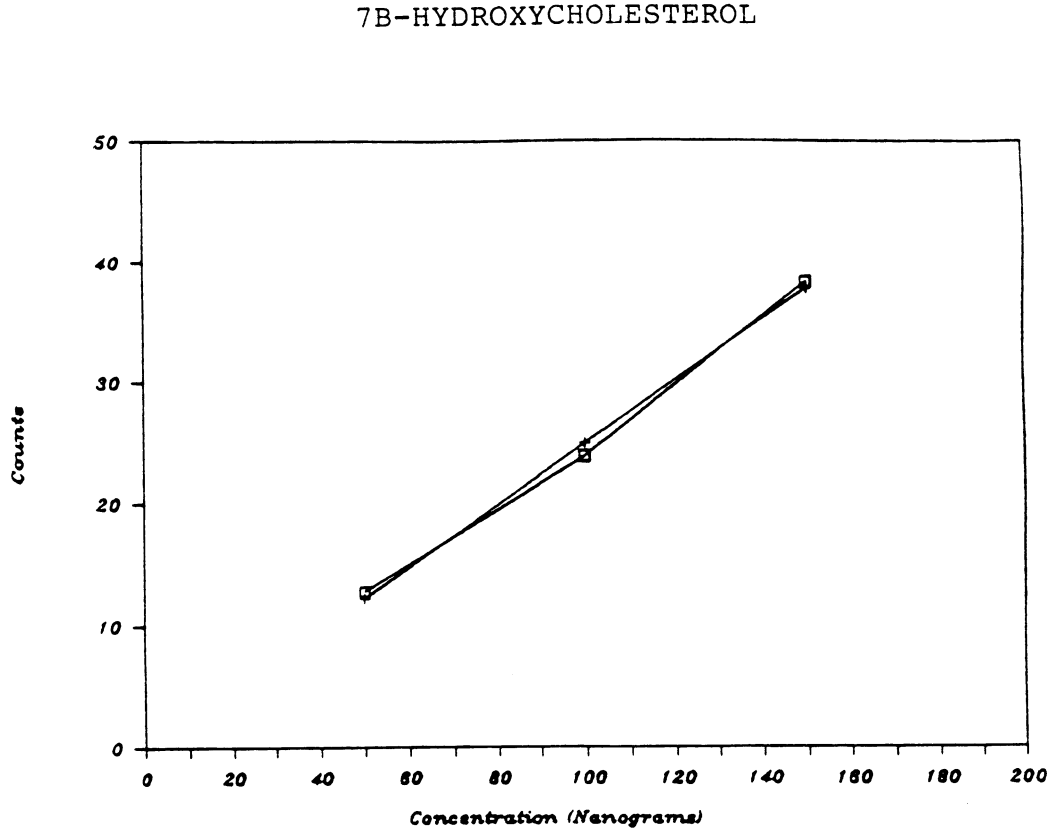


FIG. 4.18. Response Linearity of 7B Hydroxycholesterol With Various Amounts of Fixed Amount of 5A Cholestane.

4.2.2 Recovery and precision of sterol spiked samples

The quantitative data for cholesterol and cholesterol oxides prepared through cold saponification and liquid extraction of the nonsaponifiable fraction is given in Table 4.5. This table shows the amount of sterols recovered through the sample preparation used in this study. The percent recovery was determined as follows:

$$\% \text{ recovery} = \frac{\text{Determined value in spiked sample}}{\text{Determined value in sample + spiked amount}} \times 100$$

The percent recovery for cholesterol was 92%, and the recovery for cholesterol oxides ranged from 98% to 128% with an exception of 7 ketocholesterol which had a recovery of 80%. Since the sample recovery for most of the sterols was not less than 90%, the sample preparation described in the procedures (Section 3.64 - 3.67) was considered acceptable. No correction was necessary.

4.2.3. Gas chromatography - mass spectrometry of heat processed sample

Figure 4.19 shows the chromatogram of composite heat processed sample, layer #2 when layer #4 was heat processed to 56°C. The peaks were identified as 5 α cholestane at 1076, cholesterol at 1248, campesterol at 1286, followed by stigmasterol, and sitosterol at 1331. The latter three peaks are plant sterols from the soybean hulls.

TABLE 4.5. Quantitative Data of Cholesterol and Cholesterol Oxides in Spiked Sample (50 ng/each oxide + 250 ng cholesterol)

Compound Name	Determined Value ng/ul	Total Amount ng/ul	% Recovery
Cholesterol	663.4	718	92
Epoxide	49.19	50	98
7B hydroxycholesterol	63.93	50	128
Triol	52.17	50	104
7 ketocholesterol	40.17	50	80
25 hydroxycholesterol	55.95	50	112

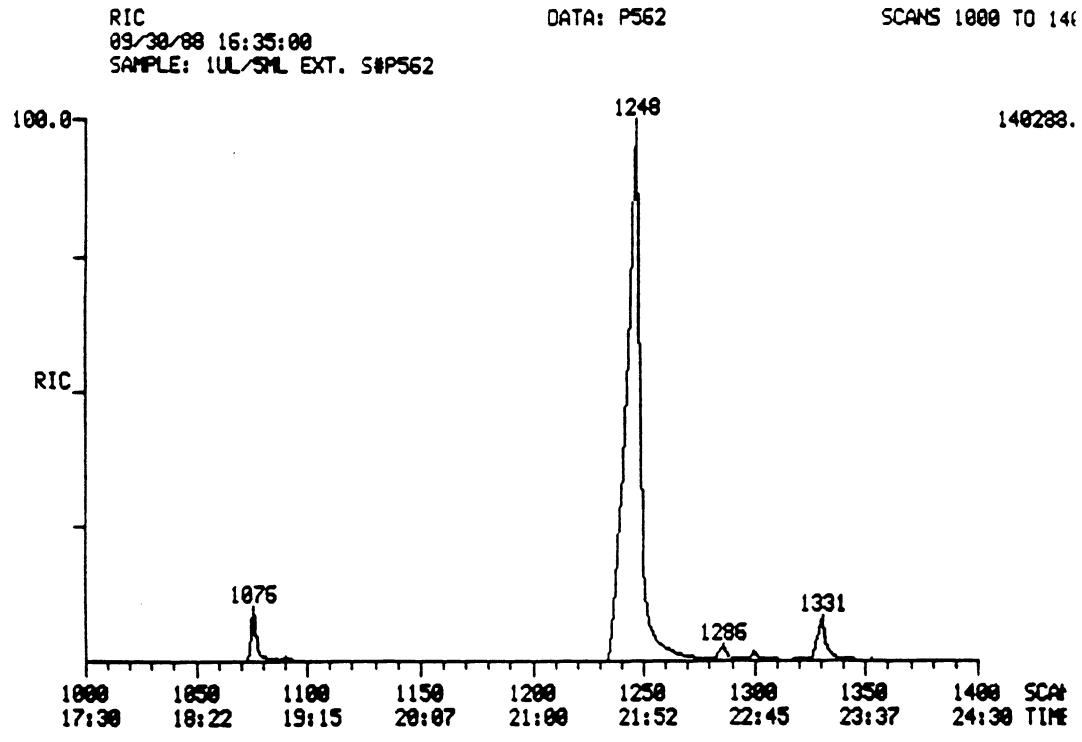


FIG. 4.19. Chromatogram of Composite Heat Processed Sample for Layer #2 When Layer #4 Was Heat Processed to 56°C.

Although these sterols have been identified in soybeans, the area of the plant sterols in this sample was 1.7%, 1.0%, and 6.1% of the area of cholesterol for campesterol, stigmasterol, and sitosterol, respectively. Sitosterol is the major sterol among these plant sterols. This sterol has a hypo-cholesterolemic effect on animals and man (Clarkson et al., 1981).

All of the composite samples shown in Table 4.1 were analyzed for the five cholesterol oxides: Epoxide, 7B hydroxy, Triol, 7 ketocholesterol, and 25 hydroxy-cholesterol, according to the procedures given in Section 3.6.7. As illustrated by the chromatogram (Figure 4.19), no cholesterol oxides were identified in any of the heat processed samples. The detection limit for the instrument was 16 ppm, i.e. any amount above this level can easily be detected. This finding occurred for all of the composite samples ranging in heat processing time from 19 to 89.6 min; internal temperature from 8.6 to 97.0°C (Table 4.1), and area under the curve from 155.81 to 6318.05°C min (Table 4.3). In addition to internal product temperature, the surface or crust temperature of layer #5 reached a mean temperature of 128°C. These conditions simulated practically all conceivable time-temperature relationships that would be encountered during the thermal processing of a restructured pork product. Therefore, the results proved that the formation of cholesterol oxides does not occur

under these conditions of thermal processing and subsequent storage as described in Section 3.4.

4.3. Cholesterol Content of Raw Restructured Pork/Soy Hull Product

In Table 4.6, the percent moisture, product yield, and percent fat were calculated as described in Chapter Three. The percent fat retention and percent cholesterol retention were calculated as described by Renk et al. (1985) as follows:

$$\% \text{ fat retention} = \frac{\% \text{ fat in cooked product} \times \text{product yield}}{\% \text{ fat in the raw formula}} \times 100$$

$$\% \text{ cholesterol retention} = \frac{\% \text{ cholesterol in cooked product} \times \text{product yield}}{\% \text{ cholesterol in the raw formula}} \times 100$$

From Table 4.6 note that the cholesterol content in the raw formula was 46.3 mg/100 gm on a wet weight basis. This value is lower than values reported in the literature for ground pork: i.e. 59.1 mg/100 gm (Punwar and Derse, 1978), 60.0 mg/100 gm (Feeley et al., 1972); 61.6 mg/100 gm (Tue et al., 1967) and 70.0 mg/100 gm (Ockerman, 1980). According to Feeley et al. (1972) several factors can affect the cholesterol content of the animal. These include the age, breed, sex, kind of the muscle, part of carcass, and degree of marbling. In this formula the lower cholesterol content can be attributed to the presence of the soybean hulls. As shown in Table 3.1 the rehydrated soy

TABLE 4.6. Moisture, Fat, Cholesterol Content in Raw Formula and Their Percent Retention in Heat Processed Restructured Pork/Soy Hull Product.

Samples		Moisture (%)	Fat (%)	Cholesterol Wet (mg/100 gm)	Fat Retention (%)	Cholesterol Retention (%)	Yield (%)
Formula, raw		66.90	12.0	46.30	-----	-----	-----
20[a]	1[b]	63.66	11.2	36.83	92.61	78.93	99.22
	2	64.63	13.9	40.20	115.03	86.23	99.31
	3	65.62	12.0	34.87	99.63	75.03	99.63
	4	63.10	13.0	36.60	107.81	78.67	99.52
	5	62.08	12.1	44.73	93.52	89.60	92.75
32	1	63.35	12.4	32.37	101.39	68.60	98.12
	2	63.40	13.1	37.03	108.23	79.29	99.14
	3	64.06	14.7	52.13	121.41	111.59	99.11
	4	63.49	13.4	50.13	110.28	106.93	98.76
	5	61.02	16.0	43.33	113.77	79.86	85.33
44	1	64.05	12.4	36.37	100.86	76.68	97.61
	2	63.43	13.2	42.93	108.60	91.54	98.73
	3	63.48	12.2	51.13	100.33	108.99	98.69
	4	63.06	13.7	42.43	111.39	89.41	97.57
	5	58.83	14.8	59.63	100.32	104.76	81.34
56	1	63.84	13.0	64.35	103.84	133.23	95.86
	2	64.91	13.4	52.60	108.25	110.13	96.94
	3	63.71	13.9	58.13	112.06	121.46	96.74
	4	61.69	14.0	55.47	107.65	110.54	92.27
	5	56.58	14.0	66.47	89.72	110.40	76.90
68	1	63.65	11.9	40.67	91.84	81.35	92.61
	2	63.80	12.7	41.30	100.69	84.87	95.14
	3	63.27	12.5	40.43	98.44	82.52	99.50
	4	61.43	14.4	33.93	105.00	64.12	87.50
	5	56.27	15.2	45.43	93.24	72.23	73.61
77	1	61.54	13.0	47.33	97.79	92.28	90.27
	2	61.71	17.6	50.77	135.92	101.62	92.67
	3	60.65	20.0	56.00	150.85	109.47	90.51
	4	58.21	16.9	46.53	116.41	83.08	82.66
	5	52.38	21.0	53.05	119.19	78.04	68.11

[a]Refers to final product temperature (°C) of layer #4.
[b]Refers to layer position (1 = bottom; 5 = top).1

hulls (soy hulls and milk) represented 32% of the total formula. Thus it is reasonable that cholesterol content could be lowered by ca. 25%.

4.4 Effect of Heat Processing on Cholesterol and Other Food Components of Restructured Pork

In order to find the impact of heat processing on cholesterol, this discussion will focus upon: (1) layer positions, (2) temperature ranges, (3) correlation coefficients among experimental variables for each group set, and (4) correlation coefficients among experimental variables and heat processing conditions.

4.4.1. Effect of heat processing on restructured pork according to layer positions

From the data analysis in Table 4.7 it is clear that there were no significant differences in initial temperature among layers #1-4. Only the initial temperature of layer #5 was different. This difference had no practical significance for the findings in this study. The mean final temperature of layer #5 differed from the other four layers; no significant differences were found among the mean final temperatures for layers #1-4. This same pattern was obtained for moisture content and percent yield. The samples from layer #1 had the lowest mean percent fat content. This could be attributed to the drip loss as they were positioned directly above the wire mesh screen. Note

TABLE 4.7. Heat Processing Conditions and Food Components According to Layer Position for Restructured Pork/Soy Hull Product[1,2]

Layer #	Conditions: Initial Temp. (°C)	Conditions: Final Temp. (°C)	Food Components: Yield (%)	Moisture (%)	Fat (%)	Cholesterol mg/100 gm: Wet basis	Cholesterol mg/100 gm: Dry basis	Fat Retention (%)	Cholesterol Retention (%)
1	6.3^{a} ±0.34	35.5^{a} ±8.30	95.6^{a} ±1.42	63.3^{a} ±0.37	12.3^{a} ±0.28	43.0±4.74	117.3±13.03	98.06^{a}±2.01	88.51±9.48
2	5.7^{a} ±0.30	32.1^{a} ±8.17	97.0^{a} ±1.08	63.6^{a} ±0.46	14.0^{ab} ±0.74	44.1±2.52	121.5±7.01	112.79^{b}±4.98	92.28±4.71
3	5.6^{a} ±0.28	36.4^{a} ±8.35	96.5^{a} ±1.43	63.4^{a} ±0.66	14.2^{ab} ±0.74	48.8±3.71	133.2±9.21	113.79^{b}±8.27	101.51±7.48
4	6.5^{a} ±0.37	49.7^{a} ±8.80	93.0^{a} ±2.79	61.8^{a} ±0.80	14.2^{ab} ±0.57	44.2±3.34	115.9±8.90	109.76^{ba}±1.61	88.79±7.18
5	8.9^{b} ±0.41	77.7^{b} ±7.48	79.7^{b} ±3.58	57.9^{b} ±1.28	15.5^{b} ±1.22	52.1±3.83	123.9±8.20	101.63^{ba}±4.95	89.15±6.30

[1] Mean value ± standard error of mean.
[2] Where superscripts differ vertically, mean values differ significantly ($P < 0.05$).

that the fat content on both a percent basis, and percent retention basis, was lowest for layer #1. Layer #5 had the highest fat content, attributed to the surface evaporation loss. Although layer #5 differed in final temperature, percent yield and percent moisture the from other four layers, no significant ($P < 0.05$) differences were found in cholesterol content, on a wet or dry basis, or for cholesterol retention.

4.4.2. Effect of heat processing on restructured pork according to final temperature ranges

Table 4.8 shows that percent yield ranged from 72.8 to 99.0 percent. This is slightly higher than values in the literature (59 to 67 percent) for restructured pork (Renk et al., 1985). This can be attributed to the presence of the soybean hulls in the product as well as to the wide range of heat processing temperatures (8.6°C to 97.0°C) as given in Table 4.1. Also data in Table 4.8 shows that percent yield decreased as final temperature increased. This agreed with the data reported by Reitmeier and Prusa (1987). They found that heating pork patties to higher internal endpoint temperatures increased cooking losses. As expected, there were significant ($P < 0.05$) differences in final product temperatures. However, there was no difference in cholesterol content on a wet basis, with the exception of the two lower temperature ranges. When comparing the lowest temperature with the

TABLE 4.8. Heat Processing Conditions and Food Components According to Range of Final Product Temperatures[1,2]

Conditions					Food Components						
								Cholesterol mg/100 gm			
N	Temp. (°C)	Initial Temp. (°C)	Final Temp. (°C)	Time (min)	Yield (%)	Moisture (%)	Fat (%)	Wet basis	Dry basis	Fat Retention (%)	Cholesterol Retention (%)
5	19	5.54^{a} ±0.22	12.84^{a} ±1.94	23.9^{a} ±3.04	99.08^{a} ±0.25	64.13^{a} ±0.44	12.52^{a}±0.46	36.26^{a}±1.29	101.19^{a}±4.01	103.38^{a}±3.83	77.62^{a}±2.89
7	20-39	5.76^{a} ±0.13	26.76^{b} ±2.19	38.4^{ac} ±4.56	98.48^{a} ±0.34	63.79^{a} ±0.23	13.19^{a}±0.31	45.98^{ab}±2.74	127.14^{b}±7.96	108.22^{a}±2.65	97.79^{b}±5.78
8	40-59	6.00^{a} ±0.34	48.25^{c} ±2.08	55.6^{bc} ±6.29	94.68^{b} ±0.71	63.13^{a} ±0.29	12.98^{a}±0.29	48.44^{b}±3.33	131.46^{b}±9.23	102.43^{a}±2.72	99.12^{b}±7.04
7	60-79	7.62^{b} ±0.31	67.54^{d} ±2.18	72.1^{b} ±9.38	87.18^{c} ±1.61	60.48^{b} ±0.53	16.10^{b}±0.88	48.22^{b}±3.20	121.90^{ab}±2.72	117.15^{ab}±7.41	90.74^{ab}±6.08
3	80	9.53^{c} ±0.64	92.53^{e} ±3.21	72.3^{b} ±9.66	72.87^{d} ±2.56	55.25^{c} ±1.18	16.73^{b}±2.16	54.98^{b}±6.15	123.20^{ab}±15.15	100.72^{a}±9.29	86.89^{ab}±11.87

[1] Mean value ± standard error of mean.
[2] Where superscripts differ vertically, mean values differ significantly ($P < 0.05$) from each other.

highest temperature (Table 4.7), it is clear that heating increased the cholesterol content of the restructured pork, on a wet basis. This finding agreed with the results reported by Rhee et al. (1982) and Prusa and Hughes (1986). The increased moisture losses at higher temperatures accounted for this apparent increase in cholesterol content (mg/100 g). Although there was no significant difference in the fat content among the three lower temperature ranges, the highest temperature range had the highest fat content. This is due to the loss of moisture at these high temperatures. This finding agreed with the data reported by Retmeier and Prusa (1987). They found that the fat content of ground pork increased as the moisture content decreased.

4.4.3. Correlation coefficient among experimental variables for each group set

Tables 4.9 to 4.14 show the correlation coefficients among experimental variables for the different groups of composite samples when layer #4 was heat processed to either 20°C, 32°C, 44°C, 56°C, 68°C, or 77°C. Among all these groups sets, there was a significant ($P < 0.05$) positive relationship between moisture content and product yield with an exception when layer #4 only reached 20°C (Table 4.9). This was due to the lower temperatures attained (Table 4.1). Also among all these temperatures sets there was a negative relationship between the percent

TABLE 4.9. Correlation Coefficients Among Experimental Variables for Composite Samples When Layer #4 Was Heat Processed to 20°C[a]

Variable	Yield	Fat	Chol. Wet	Chol. Dry	Acceleration	Integral
Moisture	0.725	0.137	-0.672	-0.371	0.750	-0.802
Yield		0.187	-0.886**	-0.755	0.995*	-0.974*
Fat			0.183	0.315	0.123	-0.177
Chol. Wet				0.937**	-0.885**	0.817
Chol. Dry					-0.744	0.633
Acceleration						-0.987*

[a] N = 5
*Significant at 1% level
**Significant at 5% level.

TABLE 4.10. Correlation Coefficients Among Experimental Variables for Composite Samples When Layer #4 was Heat Processed to 32°C[a]

Variable	Yield	Fat	Chol. Wet	Chol. Dry	Acceleration	Integral
Moisture	0.977*	-0.654	0.162	0.310	0.990*	-0.950**
Yield		-0.782	0.016	0.165	0.992*	-0.975*
Fat			0.523	0.406	-0.726	0.791
Chol. Wet				0.988*	0.042	0.132
Chol. Dry					0.193	-0.018
Acceleration						-0.983*

[a] N = 5
*Significant at 1% level
**Significant at 5% level.

TABLE 4.11. Correlation Coefficients Among Experimental Variables for Composite Samples When Layer #4 was Heat Processed to 44°C[a]

Variable	Yield	Fat	Chol. Wet	Chol. Dry	Acceleration	Integral
Moisture	0.981*	-0.880*	-0.848	-0.690	0.982*	-0.974*
Yield		-0.828	-0.781	-0.605	0.982*	0.955**
Fat			0.591	0.404	-0.897**	0.905**
Chol. Wet				0.969*	-0.756	0.779
Chol. Dry				-0.574	0.611	
Acceleration					0.992*	

[a] N = 5
*Significant at 1% level
**Significant at 5% level.

TABLE 4.12. Correlation Coefficients Among Experimental Variables for Composite Samples When Layer #4 was Heat Processed to 56°C[a]

Variable	Yield	Fat	Chol. Wet	Chol. Dry	Acceleration	Integral
Moisture	0.987*	-0.593	-0.641	0.282	0.989*	-0.998*
Yield		0.511	-0.642	0.267	0.980*	-0.993*
Fat			-0.070	-0.728	-0.653	0.587
Chol. Wet				0.556	-0.524	0.617
Chol. Dry					0.416	-0.309
Acceleration						-0.993*

[a] N = 5
*Significant at 1% level

TABLE 4.13. Correlation Coefficients Among Experimental Variables for Composite Samples When Layer #4 was Heat Processed to 68°C[a]

Variable	Yield	Fat	Chol. Wet	Chol. Dry	Acceleration	Integral
Moisture	0.992*	-0.897**	-0.442	0.382	0.986*	-0.998*
Yield		0.882**	-0.415	0.403	0.974*	-0.993*
Fat			0.066	-0.682	-0.947**	0.913**
Chol. Wet				0.660	-0.297	0.382
Chol. Dry					0.520	-0.442
Acceleration						-0.993*

[a] N = 5
*Significant at 1% level
**Significant at 5% level.

TABLE 4.14. Correlation Coefficients Among Experimental Variables for Composite Samples When Layer #4 was Heat Processed to 77°C[a]

Variable	Yield	Fat	Chol. Wet	Chol. Dry	Acceleration	Integral
Moisture	0.995*	-0.607	-0.183	0.669	0.996*	-0.994*
Yield		-0.530	-0.112	0.719	0.999*	-0.999*
Fat			0.794	0.111	-0.542	0.535
Chol. Wet				0.608	-0.123	0.133
Chol. Dry					0.712	-0.702
Acceleration						-0.996*

[a] N = 5
*Significant at 1% level
**Significant at 5% level.

yield and the integral (°C min). This was due to the fact that, as time and temperature increased, percent yield decreased due to the loss of moisture and other volatiles. All sets showed a negative relationship between yield and fat content as well as between acceleration and the integral.

4.4.4 Correlation coefficients among experimental variables and heat processing conditions

Correlation coefficients among experimental variables and heat processing conditions are given in Tables 4.15 and 4.16. In Table 4.15 there is an inverse relationship between moisture content and fat. This can be attributed to the fact that 1 BTU is required to raise 1 lb. water 1°F, and 0.4 BTU, required to raise 1 lb. fat 1°F (Ockerman, 1980). The total energy or BTU = specific heat X weight X temperature differences. This means that, as the fat content increases the evaporation rate increases, resulting in a lower moisture content. Also in Table 4.15 there is an inverse relationship between cholesterol content (wet basis) and moisture content. This can be explained by the positive relationship between moisture content and product yield. As yield increases, this leads to less cholesterol concentration in terms of mg/100 gm. In Table 4.15 there is a positive relationship between cholesterol and fat content, i.e. as the fat content increases the cholesterol content increases. This

TABLE 4.15. Correlation Coefficients Among Experimental Variables for Heat Processed Restructured Pork[a]

Variable	Fat	Chol. Wet	Chol. Dry	Yield	Final Temp.
Moisture	-0.687*	-0.383**	-0.004	0.962*	-0.865*
Fat		0.381**	0.143	-0.632*	0.622*
Chol. Wet			0.923*	-0.375**	0.454**
Chol. Dry				-0.014**	0.141
Yield					-0.920*

[a]N = 30
*Significant at 1% level
**Significant at 5% level

TABLE 4.16. Correlation Coefficients Among Heat Processing Conditions and Food Components[a]

Variable	Yield	Fat	Chol. Wet	Chol. Dry	Acceleration	Integral
Moisture	0.961*	-0.687*	-0.383**	-0.004	0.489*	-0.889*
Yield		-0.632*	-0.375**	-0.014	0.534*	-0.915*
Fat			0.381**	0.143	-0.714	0.687*
Chol. Wet				0.923*	0.157	0.353
Chol. Dry					0.038	0.029
Acceleration						-0.307

[a] N = 30
*Significant at 1% level
**Significant at 5% level.

agrees with the data reported by Slover et al. (1987). Also there is a negative relationship between fat content and yield, due to the negative relationship between fat and moisture. As the temperature increases cholesterol content increases. This is due to the same reason explained earlier in this chapter. Table 4.16 shows the negative relationships between integral and moisture, integral and yield, and integral and acceleration. Justification for these trends was given in Section 4.4.3.

CHAPTER FIVE

CONCLUSION, RECOMMENDATION AND SUMMARY

Following the analysis of data and discussion of results presented in Chapter Four, conclusions were made, recommendations were given for further research and the research was summarized.

5.1. Conclusions

The experimental procedures enabled a wide variety of heat penetration curves. Composite samples ranged in heat processing time from 19 to 89.6 min; internal temperature, from 8.6 to 97.0°C. In addition to internal product temperature, the surface temperature of layer #5 reached a mean temperature of 128°C. By applying the methods of extraction, isolation, and quantification for cholesterol in heat processed restructured pork, a conclusion was made about the stability of cholesterol in heat processed products. Although no previous research had been performed about describing the impact of heat processing conditions on cholesterol, this study showed that, when heating restructured pork/soy hulls under the 30 conditions described, and up to a surface temperature of 128°C, cholesterol oxides were not developed.

5.2. Recommendations for Further Research

Based upon findings of the study the following recommendations were made:

(1) Apply the same method of isolation and quantification to quantify and qualify cholesterol oxides in other food products.

(2) Heat processing parameters must be studied carefully as mentioned above to explain how cholesterol oxides can develop.

(3) Further studies are needed in pork meat without the addition of the soybean hulls to find out if the cholesterol oxides can develop in the absence of this dietary fiber, under these heat processing conditions.

(4) More studies are required to find out how much or at what level cholesterol oxides can affect human health. This can be done by nutritional, biological and pathological studies.

(5) More studies are needed to find out whether plant sterols can provide sterol oxides, because of their structural similarities to cholesterol, and whether those oxides can produce any negative effects on human health.

5.3. Summary

The purpose of the research was to: (1) study the impact of heat processing conditions on the moisture, fat and cholesterol content of a restructured pork/soy hull

product, and (2) determine the formation of any cholesterol by products, oxidized cholesterol derivatives, resulting from exposure of the high fiber pork product to different times and temperatures. An experimental procedure was developed for obtaining 30 different time-temperature relationships, simulating those that would occur during thermal processing.

Heat processing parameters were established and performed for layers (10 cm x 10 cm x 1 cm) of restructured pork/soy hulls. During convective heat processing (141.9°C), five layers were placed one on another. The internal product temperatures were taken every 2 min. The product was heat processed to six end temperatures when layer #4 (second layer from the top) reached temperatures: 20°C, 32°C, 44°C, 56°C, 68°C, or 77°C. The mean surface temperature of the top layer when a temperature of 77°C was achieved was 128°C. A composite sample from the five layers for each of the six temperatures was used for fat analysis, cholesterol and cholesterol oxide determination, and moisture content.

Two gas chromatography-mass spectrometry instruments were utilized during the course of the study to quantify and qualify cholesterol and cholesterol oxides in the product. The mass spectrometer was directly coupled to gas chromatography. A capillary column DB-1 was used.

Results indicated that within all the 30 time-temperature ranges no cholesterol oxides were developed in the restructured pork/soy hull product. No significant differences were found in cholesterol content and cholesterol retention (%) due to any of the heat processing conditions. The mean cholesterol content for heat processed samples ranged from 34 to 64 mg/100 gm on a wet basis; from 87.97 to 149.70, mg/100 gm on a dry basis. This value is 25% less than reported values for pure ground pork after heat processing. Three plant serols were identified in this restructured pork/soy hull product; campesterol, stigmasterol, and sitosterol. These three sterols together contributed less than 10% of the cholesterol found in each sample. Correlation coefficients between experimental variables and heat processing conditions revealed that: (1) Negative relationships were found between final product temperature and moisture content of the product, (2) Positive relationships were found between final product temperature and fat content, (3) Negative relationships were found between final product temperature and product yield, (4) Positive relationships were found between the fat and cholesterol content of the product, and (5) Negative relationships were found between the integral or area (°C min) and moisture (%), yield (%), and acceleration (°C min^2). Several recommendations were made for further research.

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APPENDICES

APPENDIX A

Modified Babcock for Rapid Fat Determination

Objective:

To determine the approx. fat content of:

(1) raw pork

(2) formula

(3) heat processed pork (temp. 66)

Method:

(1) Each sample was placed in a beaker and mixed very well by using spoon.

(2) 9 gram of each sample was placed into poley bottle (2 replications from each sample).

(3) 10 ml of warm water was added into paley bottle through wide opening.

(4) Wide opening was stoppered and bottle was shaked to disperse meat

(5) Carefully and slowly, 17 ml of con. H_2SO_4 was added through reading tube. Sample was mixed, until digested (dark purplish-black color).

(6) Hot water was added to bring fat to 45% mark.

(7) A preheated centrifuge was balanced and sample was centrifuged for 3 minutes.

(8) Bottle was removed and by using caliper a fat content was determined.

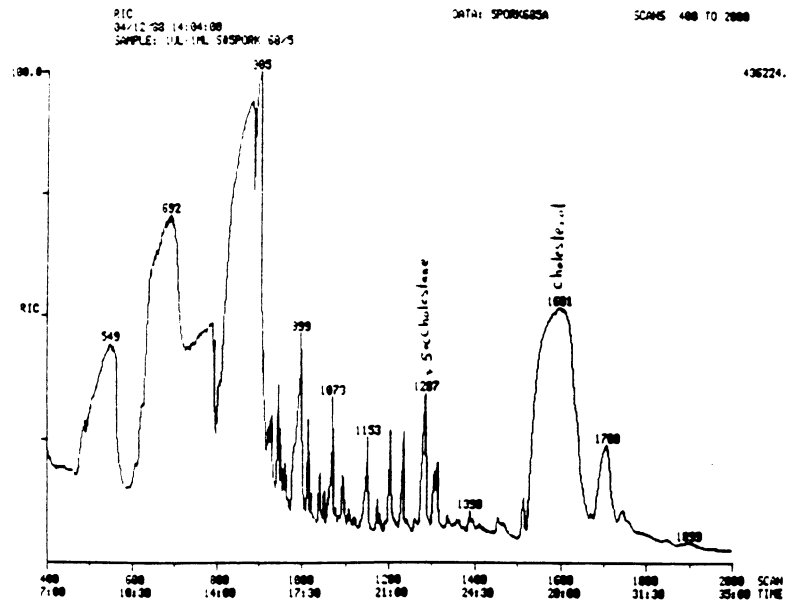
Result:

		(% Fat)	(Approx. fat content, %)
Raw pork sample	(1)	16.5	16.7
	(2)	17.0	
Formula	(1)	8.0	8.0
	(2)	8.0	
Cooked pork	(1)	10.5	10.7
	(2)	11.0	

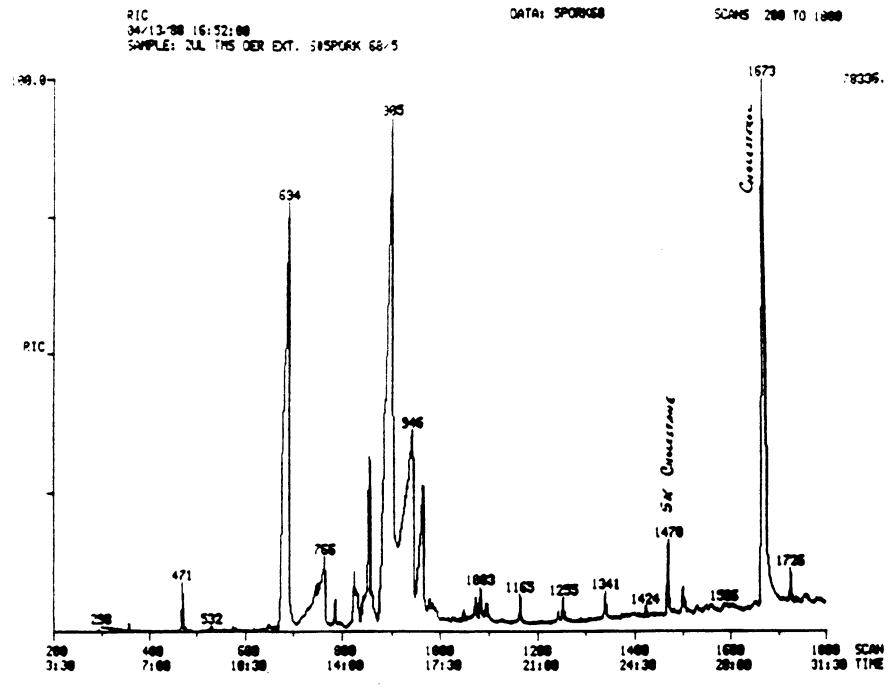
APPENDIX B

Appendix B.1. Chromatogram of Heat Processed Sample Obtained from Layer #5 when Layer #4 Heat Processed to 68°C (uncleaned sample).

Appendix B.2. Chromatogram of Heat Processed Sample Obtained from Layer #5 When Layer #4 Heat Processed to 68°C (sample cleaned with basic alumina column).



APPENDIX B.1. Chromatogram of Heat Processed Sample Obtained from Layer #5 when Layer #4 Heat Processed to 68^{o}C (uncleaned sample).



APPENDIX B.2. Chromatogram of Heat Processed Sample Obtained from Layer #5 when Layer #4 Heat Processed to 68°C (sample cleaned with basic alumina column).

APPENDIX C

Preparation of the Standards

A. Preparation of 5∝ -cholestane:

(1) Weigh 40 mg (0.040 gm) of 5∝ cholestane in 10 ml volumetric flask

(2) Add 3-4 ml ethylacetate and shake it very well.

(3) Complete the volume to 10 ml

(4) From the above take one ml and dilute it to 10 ml (i.e. to the 1 ml you will add 9 ml ethylacetate).

(5) From the final dilution

100 ul $\longrightarrow$ 40 ug of 5∝ cholestane

B. Preparation of cholesterol:

Cholesterol stock contain 50 mg/5 ml = 10 mg/ml = 10 ug/ul

The stock was prepared by weighing 50 mg cholesterol which was dissolved in 5 ml ethyl acetate.

Since the stock contain 10 ug/ul

$(ug)_1\ (ul)_1 = (ug)_2\ (ul)_2$

$(c_1)_{stock}\ (v)_{stock} = (c)_{dilnew}\ (v)_{new\ volume}$

(1) Preparation of 50 ng/ul cholesterol:

10,000 ng/ul xv = (50 ng/ul) (5 ml)

$$v = \frac{50 \times 5}{10,000} = 0.025 \text{ ml}$$

We can add 0.025 ml from the stock.

(2) Preparation of 250 ng/ul cholesterol:

10,000 ng/ul xv = (250 ng/ul) (5 ml)

$\frac{250 \times 5}{10,000}$ = 0.125 ml

0.125 ml from stock can be taken

(3) Preparation of 750 ng/ul cholesterol:

10,000 ng/ul xv = 750 ng/ul x 5

$\frac{750 \times 5}{10,000}$ = 0.375 ml

0.375 ml from the stock can be taken

(4) Preparation of 1000 ng/ul cholesterol:

10,000 xv = 1000 x 5

$\frac{5 \times 1000}{10,000}$ = 0.5 ml

0.5 ml from the stock can be taken

C. Preparation of the oxides:

Each oxide stock was prepared as follows:

10 mg/5 ml = 2 mg/ml = 2 ug/ul

i.e. 10 mg of the oxide was dissolved in 5 ml of ethylacetate

(1) Preparation of 10 ng/ul of the oxide standard:

2 ug/ul

2000 ng/ul x v = 10 ng/ul x 5

$$v = \frac{10 \times 5}{2000} = 0.025$$

0.025 ml of the stock can be taken

(2) Preparation of 50 ng/ul of the oxide standard:

2000 x v = 50 x 5

$$v = \frac{50 \times 5}{2000} = 0.125 \text{ ml}$$

0.125 ml can be taken from the stock

(3) Preparation of 100 ng/ul of the oxide:

2000 ng/ul x v = 100 x 5

$$v = \frac{5 \times 100}{2000} = 0.25 \text{ ml}$$

0.25 ml can be taken from the oxide stock.

(4) Preparation of 150 ng/ul of the oxide:

2000 ng/ul x v = 150 x 5

$$v = \frac{150 \times 5}{2000} = 0.375 \text{ ml}$$

0.375 ml of the oxide stock can be taken.

D. Preparation of cholesterol OCD + IS

(1) 10 ng of each oxide + 50 ng chol. + 40 ug I.S.:

(This was labeled as long standard)

1.e 25 uL of chol stock

25 ul = each oxide stock

100 ul of 5 ∝ -cholestane

After evaporation the volume was dissolved in 5 ml ethylacetate to give the right concentration.

(2) 50 ng of each oxide + 250 chol. + 40 ug I.S.

(This was labeled as 50 ng standard)

125 ul of chol. stock

125 ul = each of the oxide

100 ul of 5 ∝ cholestane

After evaporation volume was dissolved in 5 ml ethylacetate to give the right concentration.

(3) 100 ng oxide + 750 ng chol. + 40 ug I.S.:

(This was labeled as 100 ng standard)

375 ul of chol. stock

250 ul of each oxide stock

100 ul of 5 ∝ cholestane stock

Final volume was completed to 5 ml to obtain the right concentration.

(4) 150 ng oxide + 1000 ng chol. + 40 ug I.S.:

(This was labeled as 150 ng standard)

0.5 ml from chol. stock

375 ul = each oxide stock

100 ul of 5 ✂ cholestane stock

Final volume was completed to 5 ml to obtain the right concentration.

APPENDIX D

Calculation of Cholesterol Amount mg/100 gm sample

One ul was injected into the GC. The amount of cholesterol was given in term of ng/ul. To calculate the amount of chol. this procedure can be followed:

e.g. p. 564 ⟶ 158.5 ng/ul

But the final volume was 5 ml

158.5 ng/ul x 5000 = 792500 ng/5000 ul

Since this was obtained from sample that 0.2 gm fat

792500 ng/0.2 gm

= 792500 ng/100 mg

396250 ng/100 mg

3962500 ng/1000 mg

3962500 ng/g

3.9625 mg/g

Since the fat content in this sample was 14%

Amount of cholesterol = 3.9625 x 14 = <u>55.48 mg/100 gm sample</u>

VITA

Niama Ibrahim was born [redacted] in Malakal, Sudan. She spent formative years and received her elementary and high school education in Khartoum, Sudan. In 1977, she entered the University of Khartoum and received her B.S. degree from Faculty of Education, Dept. of Home Science, Nutrition and Dietetics in 1981. She was then employed in the same department as Teaching Assistant. She enrolled at the University of Missouri in Winter 1982. She was awarded the M.S. degree in Food Science in May 1984. She continued her research in the same department at the University of Missouri. She is currently completing the requirements for the degree of Doctor of Philosophy in Food Science and Nutrition.

She is married to Ahmed B. Ali, M.D. and has four children.

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