TOTAL CHOLESTEROL DETERMINATION - EVALUATION OF ANALYTICAL METHODS AND SURVEY OF MEAT PRODUCTS

N.J. ENGESETH AND J.I. GRAY

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824, USA

INTRODUCTION

Several methods for the determination of the total cholesterol content in foods have been reported in the literature. These methods, which include enzymatic, colorimetric, gas chromatographic and high performance liquid chromatographic procedures, have been applied to a variety of foods with a high degree of variation in the results reported. Sweeny & Weihrauch (1976) reviewed the methods for cholesterol determination and pointed out the lack of available and reproducible analytical data. Although several improvements in analytical methodology have been made since, the problem of variation still exists.

Variation in the reported total cholesterol content of meat products may be the result of several factors including diet, sex and age of the animals, and analytical methodology, including the poor reporting of the procedures applied (Sweeny & Weihrauch, 1976). The problem of obtaining accurate and reproducible cholesterol information is intensified with the increased public awareness and interest in the cholesterol content of food (Karkalas et al., 1982).

The primary objective of this study was to evaluate some existing methods for total cholesterol determination for accuracy, efficiency and repeatability, and to apply the chosen method in surveying a variety of meats for cholesterol content and changes in total cholesterol with cooking.

MATERIALS AND METHODS

Materials

Meat and seafood samples were purchased from a local supermarket on the day of analysis. Cholesterol and 5α-cholestane standards were purchased from Steraloids Inc., Wilton, NH.

Methods

Moisture content of the meat samples was determined by the A.O.A.C official method (1984), while the total lipid content was determined by the method of Folch et al. (1957).

Evaluation of methods for total cholesterol content:

The first phase of the study was an evaluation of several methods of determining the total cholesterol content of ground raw beef bottom round. Enzymatic cholesterol determination was carried out using a kit obtained from Boehringer IN. Corp., Indianapolis, to Cholesterol was oxidized cholestenone by cholesterol oxidase with production of hydrogen peroxide. Catalase was added to facilitate the oxidation of methanol to formaldehyde, which was then reacted with acetylacetone to form a yellow lutidine dye in the presence of ammonium ions. Color was determined spectrophotometrically at 405 nm.

A colorimetric method (Rhee et al., 1982b) was also evaluated in which a Folch lipid extract of the ground beef sample was saponified, followed by a hexane extraction of the unsaponifiable fraction. The cholesterol content was determined by the colorimetric procedure of Searcy & Berquist (1960) which involved the reaction of $FeSO_4$ - acetic acid and concentrated H_2SO_4

to produce color to be analyzed at 490 nm.

The major focus of the study was on gas chromatographic (GLC) analyses. Two methods, a direct saponification procedure (Adams et al., 1986) and the official method (A.O.A.C., 1984), were evaluated. Extracts prepared by each method were analyzed in either derivatized (silylated) or underivatized form. GLC analysis was carried out using capillary (polydimethylsiloxane-15m x 0.25 mm) and packed (1% SE-30 on 100/120 Gas Chrom Q) columns. A Hewlett Packard 5890A GC, at 190°C for 9.5 min and programmed at 20°C/min to 260°C for 30 min, was used for the capillary analyses. Packed column determinations were achieved with a HP 5840A GC under isothemal conditions (230°C for 20 min).

Survey of meat products

The second phase of the study involved the application of the selected analytical method in Phase 1 to a variety of meat and seafood products. Cholesterol content was determined before and after cooking. Samples of ground beef, pork loin chops, lamb shoulder blade chops, and veal shoulder blade steak were broiled in a conventional electric oven to an internal temperature of 170°F. Chicken breasts and leg quarters were baked at 350°F to an internal temperature of 170°F. Salmon was baked under similar conditions, while shrimp was peeled and boiled for 5 min.

RESULTS AND DISCUSSION

Evaluation of methodology

The first phase of the study was designed to evaluate methods for total cholesterol determination. Beef bottom round samples were analyzed by the colorimetric method of Rhee et al. (1982b). This method was found to be time-consuming and quite difficult, with low reproducibility. Extreme care must be taken in such colorimetric procedures, especially consistency in mixing after the addition of sulfuric acid to the cholesterol extract. Inconsistent temperatures may provide variable results (Tonks, 1967). Bubble formation must also be avoided as this will interfere with spectrophotometer readings. Sweeny & Weihrauch (1976) have expressed other concerns with such colorimetric procedures including color reactions with other sterols and possibly other compounds, time dependence, and moisture effects.

The enzymatic procedure provided reproducible data, but it was somewhat inconvenient and expensive. Karkalas et al. (1982) compared this procedure to GLC analysis and found the results to be very similar, especially with animal products. Results of our analyses confirmed these observations. Karkalas et al. also noted that the enzymatic procedure will not be as accurate for foods containing vegetable oils as there may be interference from the phytosterols in the vegetable oils.

The main emphasis of the study was thus placed on GLC analysis. Based on the results obtained for the beef bottom round samples, the method chosen as the most efficient and reproducible was direct saponification (Adams et al., 1986) without derivatization, followed by analysis on the packed column system. Results from the samples of both extraction techniques, with or without derivatization, and packed versus capillary systems were quite similar. The greatest difference was observed as a result of the extraction technique, where the A.O.A.C. method which involves chloroform/methanol extraction of the beef lipids gave cholesterol values (49-56 mg/100g) which were somewhat lower than those obtained

by direct saponification (62-69 mg/100g).

Kovacs et al. (1979) discussed the benefits of direct saponification over saponification of a lipid extract as in the A.O.A.C. official method. Not only is it less time consuming, there also is the advantage that the cholesterol may be more efficiently extracted since cholesterol in muscle membranes is bound amongst phospholipids and proteins and may not be completely extracted in a chloroform-methanol lipid extraction.

The silylation process is undesirable as it involves an additional reaction step and is therefore more time-consuming. Also, the chromatograms from the packed column system were less complex and easier to interpret, with less interfering peaks.

Survey of meat products

Phase two involved application of the direct saponification method without derivatization to meat and seafood samples for analysis by packed column GLC. Total cholesterol, as well as lipid and moisture contents were determined before and after cooking. These values are presented in Table 1. Lipid and moisture values and cook loss percentage are given in order to relate cholesterol to dry weight and fat content.

Cholesterol values obtained in this study generally agree with the values reported by a number of investigators including Kritchevsky & Tepper (1961), Tu et al. (1967), Karkalas et al. (1982), Rhee et al. (1982a,b) and Prusa & Hughes, (1986). These literature values were obtained by a number of methods including those investigated in the present study.

Total cholesterol in the cooked products increased in all cases. This increase was expected, as there would be concentration of cholesterol after drip loss. Only a few reports of changes in total cholesterol with cooking have been published (Karkalas et al., 1982; Rhee et al., 1982a,b; Kregel et al., 1986; Prusa & Hughes, 1986). Karkalas et al. (1982) investigated changes in the cholesterol content of chicken with cooking. Based on fresh weight, they observed an increase in the cholesterol content of white meat (67 mg/100g raw meat; 80 mg/100g cooked meat) and a decrease in dark meat (107 mg/100g to 92 mg/100g). Data obtained in this study showed similar trends when corrected for cook loss (white meat 86 mg/100g to 90 mg/100g; dark meat 123 mg/100g to 119 mg/100g).

Prusa & Hughes (1986) compared cholesterol levels in pork tenderloin steaks upon cooking by different methods. The change in cholesterol content upon conventional cooking to an internal temperature of 77°C was from 45 mg/100g to 78 mg/100g. This change was comparable to that obtained in this study (60 mg/100g to 87 mg/100g).

CONCLUSIONS

Several methods for determining the total cholesterol content of muscle foods were evaluated. The method chosen as most desirable was a direct saponification procedure without derivatization (silylation), followed by gas chromatographic analysis with a packed column (1% SE-30 on 100/120 mesh Gas Chrom Q). This method was direct, gave reproducible results, and was simple to perform. This method was used to survey a range of meat products for total cholesterol content before and after cooking. Total cholesterol values were higher in the cooked products. Values in general were comparable to those reported in the

literature.

ACKNOWLEDGMENTS

Michigan Agricultural Experiment Station Journal No. 13041. This work was supported in part by a grant from the National Live Stock and Meat Board.

REFERENCES

A.O.A.C. 1984:
"Official Methods of Analysis of the Association of Official Analytical Chemists," 14th ed. Association of Official Analytical Chemists, Washington, D.C.

Adams, M.L., Sullivan, D.M., Smith, R.L. & Richter, E.F. 1986:
Evaluation of direct saponification method for determination of cholesterol in meats. J. Assoc. Off. Anal. Chem. 69:844.

Folch, J., Lees, M. & Sloane-Stanley, G.A. 1957:
Simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497.

Karkalas, J., Donald, A.E. & Clegg, K.M. 1982:
Cholesterol content of poultry meat and cheese determined by enzymic and gas-liquid chromatography methods. J. Food Technol. 17:281.

Kovacs, M.I.P., Anderson, W.E. & Ackman, R.G. 1979:
A simple method for the determination of cholesterol and some plant sterols in fishery-based food products. J. Food Sci. 44:1299.

Kregel, K.K., Prusa, K.J. & Hughes, K.V. (1986):
Cholesterol content and sensory analysis of ground beef as influenced by fat level, heating and storage. J. Food Sci. 51:1162.

Kritchevsky, D. & Tepper, S.A. 1961:
The free and ester sterol content of various foodstuffs. J. Nutr. 74:441.

Prusa, K.J. & Hughes, K.V. 1986:
Cholesterol and selected attributes of pork tenderloin steaks heated by conventional, convection, and microwave ovens to two internal endpoint temperatures. J. Food Sci. 51:1139.

Rhee, K.S., Dutson, T.R. & Smith, G.C. 1982a:
Effect of changes in intermuscular and subcutaneous fat levels on cholesterol content of raw and cooked beef steaks. J. Food Sci. 47:1638.

Rhee, K.S., Dutson, T.R., Smith, G.C., Hostetler, R.L. & Reiser, R. 1982b:
Cholesterol content of raw and cooked beef longissimus muscles with different degrees of marbeling. J. Food Sci. 47:716.

Searcy, R.L. & Berquist, L.M. 1960:
A new color reaction for the quantitation of serum cholesterol. Clin. Chim. Acta 5:192.

Sweeny, J.P. & Weihrauch, J.L. 1976:
Summary of available data for cholesterol in foods and methods for its determination. In "Critical Reviews in Food Science and Nutrition," p. 131, Ed. Furia, T.E. CRC Press, Inc., Cleveland, OH.

Tonks, D.B. 1967:
The estimation of cholesterol in serum: a classification and critical review of methods. Clin. Biochem. 1:12.

Tu, C., Powrie, W.D. & Fenneman, O. 1967:
Free and esterified cholesterol content of animal muscles and meat products. J. Food Sci. 32:30.

Table 1. Cholesterol contents of various muscle foods as determined by the direct saponification - gas chromatographic procedure.

Food	Moisture (%)		Cook Loss (%)	Lipid (%)		Cholesterol (mg/100g)	
	Raw	Cooked		Raw	Cooked	Raw	Cooked
Beef bottom round	70.0			6.59		62.16	—
Ground beef	69.97	57.4	33.7	8.60	11.23	59.14	109.55 188.78
Veal, shoulder blade steaks	74.05	68.3	21.6	6.06	5.52	183.90	87.00
Pork, loin chops	71.16	59.22	25.27	6.63	14.26	59.96	139.41
Lamb, shoulder blade chops	61.92	51.27	24.62	21.80	28.47	99.21	138.12
Chicken, dark	71.91	64.55	13.56	11.48	17.79	123.05	105.04
Chicken white	70.70	65.90	14.72	8.13	11.34	86.18	269.70 150.05
Shrimp	78.89	73.17	30.28	1.19	1.94	262.05	
Salmon	66.20	65.07	15.65	7.30	17.90	100.06	

Values represent the mean of four determinations for each meat sample.