AN ABSTRACT OF THE THESIS OF

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in FOODS AND NUTRITION (Major) presented on May 3, 1972 (Date)

Title: THE STEROLS AND OTHER LIPIDS OF CRAB MEAT

Abstract approved: Elisabeth S. Yearick

The moisture, nitrogen, and lipid constituents of frozen and canned Dungeness crabmeat have been determined. Moisture accounted for 78% of the weight of frozen crab, and 75% of the weight of the canned crab. On a wet weight basis, frozen crab contained 18.98% protein (Kjeldahl N x 6.25), while canned crab contained 22.43% protein. The total extractable lipid accounted for 1.20% of the wet weight of frozen crabmeat and 1.29% of the wet weight of canned crab. On the dry weight basis, the frozen and canned crabmeat contained 5.5% and 5.2% extractable lipid, respectively. The major lipid component was phospholipid (lipid P x 25). In the frozen crab, 76.3% of the total lipids was phospholipid. In the canned crab, phospholipid accounted for 81.7% of the total lipids. Triglyceride constituted only a small percentage of the lipids of crabmeat: 1.5% of the frozen crab lipids and 0.9% of the canned crab lipids. Frozen crab contained 94.5 mg cholesterol per 100 gm wet weight, of which 85% was in the free form.

In canned crab, there were 102.0 mg cholesterol per 100 gm wet tissue, and 91% was in the free form. There were considerably more non-esterified fatty acids in canned crab than in frozen crab (2.6% vs. 0.9% of the total lipid). It is suggested that the high concentration of non-esterified fatty acids in canned crab may have resulted from hydrolysis of fatty acid esters during the prolonged heat treatment which occurs in the canning process.

The nonsaponifiable matter of frozen crab and canned crab lipids was examined by argentation thin-layer chromatography and by gas-liquid chromatography. Cholesterol was the only sterol identified in Dungeness crab by either chromatographic procedure.

The Sterols and Other Lipids of Crab Meat

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1972

APPROVED:

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in charge of major

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Date thesis is presented May 3, 1972

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ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my advisor Dr. Elisabeth S. Yearick, for her continuous guidance and encouragement during the period of my study, research, and preparation of this manuscript.

I am grateful to Dr. Andrea C. Mackey for allowing me to use the gas chromatograph for part of my experiment.

Many thanks are given to Dr. Clara A. Storvick and other faculty and staff members of Foods and Nutrition Research for their interest and helpfulness, which made my laboratory experience at Oregon State University a pleasant and valuable one.

Special thanks are due to my family for their encouragement, comfort, and financial support, which made my studies in this country possible.

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THE STEROLS AND OTHER LIPIDS OF CRAB MEAT

INTRODUCTION

The incidence of atherosclerotic disease, with the clinical phenomena of hyperlipemia and hypercholesterolemia, is high in the western world. The American Heart Association, in order to decrease the risk of coronary disease which has been correlated with the high incidence of atherosclerosis, has recommended a decrease in the dietary intake of total lipids, saturated fat, and cholesterol, and an increase in the intake of polyunsaturated fat (Alfin-Slater, 1969).

Animal food is a good source of protein, both in quantity and quality, but it also contains abundant lipids, especially of the saturated variety, and moderate amounts of cholesterol (Watt and Merrill, 1963). The flesh of aquatic animals also supplies liberal amounts of protein with the same amino acid pattern as others in the animal kingdom (Beach et al., 1943). In a diet aimed at the prevention of atherosclerosis, fish has been preferred because of its relatively low lipid content. Also, the lipids of fish contain a high percentage of unsaturated fatty acids (Hilditch and Lovern, 1936). However, shellfish have a relatively high concentration of cholesterol (Kritchevsky and Tepper, 1961).

The popular procedures of cholesterol determination, including digitonin precipitation and color production with strong acids, are not

specific to cholesterol. Digitonin will precipitate a variety of 3β-ols (Fernholz, 1935), and strong acids will produce color with a number of sterols (Idler and Baumann, 1953; Avigan et al., 1960; Kritchevsky and Tepper, 1961). It has been shown that the sterol component of shellfish may not be entirely cholesterol, although it has been reported as cholesterol. Furthermore, these other sterols, such as 24-methylene-cholesterol in butter clam (Reiner et al., 1960) and in scallop (Idler et al., 1970) appear not to be atherogenic. Thus, further identification of shellfish sterols, heretofore reported as cholesterol, is necessary. The characterization of individual sterols is now possible by use of the techniques of TLC and GLC (DiTullio et al., 1965; Kammereck et al., 1967; Feldman and Kuck, 1966).

In western United States, both frozen and canned Dungeness crabs are common on the market. Considering the possible atherogenic effect of Dungeness crab, it seems to be worthwhile to determine their lipid composition and to identify the types of sterols which they contain.

REVIEW OF LITERATURE

Relation of Sterols to Atherogenesis

Connor (1968) estimated that the average American diet provided about 730 mg of sterol daily. Of this total sterol, 500 mg was cholesterol, 180 mg consisted of plant sterols such as sitosterol, stigmasterol, and campesterol, and 50 mg of the total sterols were from seafood sterols other than cholesterol, such as desmosterol and 24-methylene-cholesterol (Idler and Wiseman, 1968). The extent to which these various sterols are hypercholesteremic, and the degree to which they may be atherogenic is still under investigation.

Cholesterol is readily absorbed from the intestinal lumen, although the extent of absorption depends on many factors. The amount absorbed is enhanced by the presence of fat in the diet (Swell et al., 1955), and by the presence of bile salts (Gallo-Torres et al., 1971). It also depends on the amount of cholesterol in the diet. Keys et al. (1965) demonstrated that small but significant increases in serum cholesterol accompanied increases in the dietary cholesterol of normal subjects. Dietary plant sterols, on the other hand, do not appear in any great concentration in plasma (Katz et al., 1970; Salen et al., 1970), and it is generally stated that the plant sterols are poorly absorbed (Alfin-Slater and Aftergood, 1968). In fact, certain plant sterols have been reported to inhibit cholesterol absorption. Grundy

et al. (1969) demonstrated that the addition of large amounts of plant sterols (chiefly β-sitosterol) to a moderate-cholesterol formula reduced the intestinal absorption of labeled cholesterol in humans. A number of hypotheses have been advanced to explain these differences. Glover and Green (1957) observed the rapid equilibration of 7-dehydrocholesterol between the lipoprotein constituents of intestinal cells after cholesterol or 7-dehydrocholesterol administration, and concluded that absorption of sterols across the mucosa was accomplished by a rapid exchange between the various lipoproteins of the cell membrane, organelles, and groundplasm. They suggested that the plant sterols might not fit onto the lipoprotein as readily as cholesterol, and thus would enter into exchanges more slowly than cholesterol. This would account for the observation that plant sterols were absorbed poorly. On the other hand, if the lipoproteins originally available to cholesterol were occupied by plant sterols, the absorption of cholesterol would be inhibited and a hypocholesteremic effect would be seen. A second hypothesis is that the plant sterols may increase cholesterol turnover. Gerson et al. (1965) found that injected β-sitosterol increased the synthesis of cholesterol, fatty acids, and CO_2 from acetate in rats. Because the tissue concentrations of cholesterol decreased after β-sitosterol administration, they concluded that the rate of oxidative degradation exceeded the rate of biosynthesis. The hypothesis that the plant sterols may produce increased degradation of cholesterol is

supported by the observations of Katz et al. (1970). These investigators reported that a number of plant sterols, fed with cholesterol to mice, prevented the cholesterol-induced rise in liver cholesterol, although there was no apparent hypocholesteremic effect.

Despite the hypocholesteremic effect of β-sitosterol, Curran and Costello (1956) found atheromatous lesions in aorta of rabbit which had received 9 gm soy sterol (mainly as β-sitosterol) per week for 4 weeks. For rats, however, the soy sterols were not atherogenic. The rats absorbed little of the soy sterols and had no deposition of the soy sterols in liver.

Not all of the plant sterols show the same cholesterol-depressing activity, nor are they absorbed to the same degree. Subbiah et al. (1971) noticed a greater absorption of campesterol than of β-sitosterol in a human subject. Konlande and Fisher (1969) also found that more campesterol than β-sitosterol was absorbed by the chicken. They noted that the anti-hypercholesteremic effect of soy sterols, as contrasted to that of wheat germ oil sterols, was correlated with the relatively higher concentration of campesterol in the soy sterols. However, when Katz et al. (1970) fed the individual sterols along with cholesterol at a 1% level to mice, campesterol and ergosterol were less efficient in decreasing liver cholesterol than stigmasterol or β-sitosterol, even though campesterol was the only detectable plant sterol (over 1.0% of total sterol) in both plasma and liver.

Daskalakis and Chaikoff (1955) observed that dihydrocholesterol decreased the cholesterol recovered in lymph to the extent of 80% when they fed rats 3 mg of labeled cholesterol and 3 mg of dihydrocholesterol by stomach tube. Also, Curran and Costello (1956) noted a lowered cholesterol in the serum of rats fed 1% dihydrocholesterol. Dihydrocholesterol accumulated in the liver, although none was found in the serum. Cook et al. (1954) reported that dietary cholestanol was deposited in the tissues of rabbits. They attributed the atherogenic effect of cholestanol to its accumulation in aorta. However, this idea was not supported by the findings of Fumagalli et al. (1971) who analyzed human aortic tissue from 7 males. The tissue was either normal, infiltered with fat, or atheromatous. The cholestanol concentration of the three types of tissue was essentially the same, and no correlation existed between the aortic content of cholestanol and the presence or degree of atherosclerosis. The atherosclerotic lesion did show an increased level of 26-hydroxy-cholesterol. The difference between the two studies might be due to differences in cholestanol absorption by humans and by rabbits.

Daskalakis and Chaikoff (1955) also tested the effects of 7-dehydrocholesterol, brassicasterol and stigmasterol in depressing the absorption of dietary cholesterol by rats. At the low levels administered (3 to 12 mg for each individual sterol), no effects were observed.

More hypocholesteremic sterols have been found recently, such as fucosterol and Sagassum sterols from marine algae (Reiner et al., 1962). Also, the sterols of butter clam, consisting principally of 24-methylene-cholesterol, proved to be hypocholesteremic for chicks (Reiner et al., 1960). Idler et al. (1970) discovered that scallop sterols, containing 25% cholesterol, 12% 22-dehydrocholesterol, 19% brassicasterol, and 20% 24-methylene-cholesterol, also produced a hypocholesteremic effect in chicks. Whether this effect was due mainly to the large proportion of 24-methylene-cholesterol, to the lowered proportion of cholesterol, or to the effects of the other sterol forms, needs further study.

Besides cholesterol, and possibly cholestanol, other sterols such as lathosterol and 7-dehydrocholesterol might be converted to cholesterol thus becoming potentially atherogenic (Cook et al., 1954). When Avigan and Steinberg (1962) fed rabbits a diet containing cholesterol, desmosterol and triparenol for 112 days, desmosterol accounted for about one third of the sterols of serum, liver, normal aorta, and the aortic lesion. Thus, the total sterol in the diet may be more important to atherogenesis than the cholesterol content alone.

Composition of Crab Meat

The moisture, protein and total lipid contents of Dungeness crab have been reported by a number of authors (Table 1), but few analyses

Table 1. The composition of crab meat (percent).

Reference	Species	Sample preparation	Moisture	Protein	Lipid
1. Watt and Merrill, 1963	Blue, Dungeness, rock and king crab	cooked and steamed	78.5	17.3	1.9
	Blue, Dungeness, rock and king crab	canned	77.2	17.4	2.5
2. Thompson, 1964	Dungeness crab, body meat	raw	81.0		1.2
	Dungeness crab, claw meat	raw	80.3		1.0
3. Farragut and Thompson,[a] 1966	Male Dungeness crab				
	body meat	frozen raw	79.0-80.3	16.6-17.8	0.9-1.6
	claw meat	frozen raw	78.5-82.3	15.2-18.6	0.7-1.7
	whole meat	cooked, canned with brine	76.9-78.5	16.8-18.9	0.9-1.4
	whole meat	cooked, canned without brine	76.6-82.9	14.7-21.3	1.1-1.2
4. Allen, 1971	Male Dungeness crab skeletal muscle	heated at 50°C for 20 minutes	78.5±3.2	16.4±1.6[b]	0.9±0.1

[a] Crabs were obtained in January, March, June and August.

[b] Tissue proteins were analyzed by using the Folin phenol reagent.

of individual lipids of crab meat have been done up to present time. Munn (cited by Huggins and Munday, 1968) reported that the lipids of Carcinus maenus muscle were distributed as follows: 73.6% phospholipids, 4.2% triglyceride, 4.0% free fatty acids, 3.5% monoglyceride, 1.3% sterol ester and 0.7% free sterol. Huggins and Munday failed to report the amount of total lipid in this species of crab, however. According to Allen (1971) over half of the fatty acids in Dungeness crab are polyunsaturated.

The amount of sterol in different species of crab has been investigated by many workers and has generally been reported as cholesterol. Further identification of the specific sterols was achieved after the newly improved techniques such as thin-layer chromatography and gas-liquid chromatography were introduced (Table 2). Kritchevsky et al. (1967) analyzed a crab sample of unknown species and found that cholesterol accounted for less than 60% of the total sterols; nearly 37% of the sterol appeared to be brassicasterol. On the other hand, Idler and Wiseman (1968) reported that the sterols of Alaskan king crab were largely cholesterol (62%), and desmosterol (31%). These investigators suspected that the brassicasterol reported by Kritchevsky et al. (1967) was actually desmosterol because the two sterols had similar retention times on GLC under the conditions applied by the Kritchevsky group.

Table 2. Sterol composition of crab meat (mg/100 gm wet weight).

Reference	Species	Cholesterol[a]	NSM[b]	GLC Results	
				Total sterol	Individual sterol as % of total sterol
Okey, 1945	Unknown	145			
Watt and Merrill, 1963	Unknown	125			
Thompson, 1964	Blue crab, Southern (Callinectes sapidus)	76			
	Blue crab, Eastern (Callinectes sapidus)	98			
	Dungeness crab, body meat (Cancer magister)	63			
	Dungeness crab, claw meat (Cancer magister)	52			
Kritchevsky et al., 1967	Unknown	140	142		57.4 Cholesterol 36.7 Brassicasterol 3.9 22-dehydrocholesterol 2.0 24-methylene cholesterol
Idler and Wiseman, 1968	Alaskan king crab (Paralithodes camtschatica)			24[c]	62.3 Cholesterol 31.1 Desmosterol 3.1 22-dehydrocholesterol 1.7 Brassicasterol also 24-methylene cholesterol β-sitosterol Fucosterol

Table 2. Continued.

Reference	Species	Cholesterol[a]	NSM[b]	GLC Results		
				Total sterol	Individual sterol as % of total sterol	
Idler and Wiseman, 1968	North Atlantic queen crab (Chionoecetes opilio)			50[c]	93.8	Cholesterol
					6.1	Desmosterol
					also	22-dehydrocholesterol
						24-methylene cholesterol
Teshima and Kanazawa, 1971	Crab (Portunus tribuberculatus)		150	17	100.0	Cholesterol
	Mantis crab:					
	(Gonodactylus chiragra)		520	21	100.0	Cholesterol
	(Gonodactylus falcatus)		690	7	100.0	Cholesterol
	(Odontodactylus scayllarus)		490	3	100.0	Cholesterol

[a]Apparent cholesterol content based on cholesterol standard.

[b]Nonsaponifiable matter.

[c]Calculated from dry weight data assuming 80% moisture in the tissue.

The components of crabmeat vary in both quantity and quality among the different species. There are also variations in the composition of crabs within the same species, due to such factors as the molting cycle (cited by Vonk, 1960), sexual cycle, season of the year (Farragut, 1965; Farragut and Thompson, 1966), and geographical location (Thompson, 1964). The latter two factors especially affect the crab's diet, which may in turn, alter the lipid composition of crab (Jezyk and Penicnak, 1966). In fact, Van Den Oord (1964) indicated that the sterol of crab may be totally dependent on exogenous sources. Further variations in reported values may derive from differences in sampling, preparation and analytical methods.

General Cholesterol Methods

The commonly used methods of determining cholesterol are far from specific. Generally, the sterol is isolated as the digitonide and then recovered by solution in pyridine or acetic acid. Most of the sterols that have a 3-β-hydroxy group can be precipitated by digitonin (Fernholz, 1935). Thus, not only cholesterol, but also a number of other sterols may be included in the isolate. Measurement of the sterols which were isolated by digitonin precipitation has been accomplished by a variety of methods: gravimetric, nephelometric, manometric, and titrimetric procedures. However, colorimetric procedures have largely replaced the older methods, because they are fast-reacting

and sensitive to small quantities of sample. Here again, error may be introduced because the colorimetric methods are not specific to cholesterol. Idler and Baumann (1953) tested a series of sterols reacting with the Liebermann-Burchard reagent, and found that all Δ^5-, Δ^6-, Δ^7-, $\Delta^{8(9)}$-, $\Delta^{8(14)}$-, and $\Delta^{14(15)}$- cholestenols gave the same color reaction, differing only in speed and intensity of the color formation. They also found that plant sterols, such as ergosterol, α-spinosterol, and sitosterol reacted more rapidly with the Liebermann-Burchard reagent than did cholesterol. Later, Kritchevsky and Tepper (1961) observed that cholesterol and β-sitosterol showed almost the same absorption spectra when they reacted with the ferric chloride-acid reagent. Avigan et al. (1960) found that desmosterol gave some color with the Liebermann-Burchard reagent at 635 mu, although the color was less intense than the color produced by cholesterol. Thus, if a lipid extract contains several sterols, the colorimetric methods of analysis do not give an accurate picture of the cholesterol content.

The advent of chromatographic methods has made it possible to separate closely-related sterols on the basis of their differences in solubility, adsorption or volatility in the stationary and moving phases.

Thin-layer Chromatography (TLC)

Thin-layer chromatography is a simple and convenient way to separate a small amount of sample in a relatively short time. It is

based on the principles of adsorption and partition chromatography. Adsorption chromatography is experimentally easier and more sensitive to those solutes with steric or spatial differences. Partition chromatography is quite sensitive to those solutes with small differences in molecular weight (Bobbitt et al., 1968).

The choice of adsorbent for the stationary phase depends upon the properties of the molecules to be separated, and on the chosen chromatographic method. For example, acidic silica gel is used for separating non-polar acidic and neutral mixtures in both adsorption and partition chromatography, while basic alumina is generally used for separating the non-polar basic or neutral mixtures in adsorption chromatography. Neutral Kieselguhr is a good solid support for partition chromatography, although it has the least capacity to separate a mixture quantitatively (Bobbitt, 1964). Alumina has not been used as extensively as silica gel, possibly because of its more reactive properties, such as the tendency to catalyze ester hydrolysis and isomerization of double bonds (Reichstein and Shoppee, 1949; Johns and Jerina, 1963). Furthermore, alumina will not accommodate as large a sample as can be separated on a silica gel layer (Bobbitt, 1964). Adsorbents may be modified to change their properties. For example, silver nitrate solution, incorporated into silica gel or alumina, improved the separation of some unsaturated lipids (Morris, 1966).

The choice of the solvent system for the moving phase in TLC depends mainly on the chosen chromatographic method. In adsorption chromatography, the solvents are completely miscible in each other, and their ability to move a sample follows the normal elutropic series. In partition chromatography, there must be a two-phase system with a desirable partition coefficient to separate the components of the particular sample. In normal partition chromatography, the stationary phase is polar; in reversed phase partition chromatography, the stationary phase is non-polar (Bobbitt, 1964).

According to Idler and Wiseman (1968), the sterols found in crab are mainly cholesterol and desmosterol. The only difference between these two sterols is that desmosterol has one more double bond at carbon 24. Consequently, they are not separated by most TLC systems. However, reversed phase TLC has been used successfully to separate cholesterol from desmosterol. Wolfman and Sachs (1964) accomplished the separation on an undecane-coated silica gel layer, with acetic acid-acetonitrile as the mobile phase. De Souza and Nes (1969) also separated these two sterols on a Kieselguhr layer covered with paraffin oil, with aqueous acetone, saturated with paraffin oil, as the moving phase. Using this procedure, they found straight-line relationships of Rm versus carbon number and Rm versus number of double bonds in the sterols, where $Rm = \log \frac{1}{R_f} - 1$, and $R_f = \frac{\text{distance sample moved}}{\text{distance of solvent front.}}$

The most convenient and commonly-used system for sterol separation is argentation TLC. Between the silver ions and the double bonds of the unsaturated constituents there are weak interactions, and π-complexes are formed reversibly (Winstein and Lucas, 1938). Unsaturated compounds with different number or geometry of the double bonds can be separated because of the differences in the stabilities and polarities of the π-complexes (Morris, 1962). The R_f values of all sterols are increased in argentation chromatography, probably because the silver nitrate reduces the surface of silica available for adsorption (Klein et al., 1966). Argentation TLC has been used by a number of workers to separate the sterol acetates (Avigan et al., 1963; Copius-Peereboom and Beekes, 1965; Klein et al., 1966). However, Klein and his coworkers (1966) found that the silver nitrate impregnated plate did not give the same degree of selectivity for the unsaturated sterols as did a narrow pore diameter silica gel. In 1961 and 1962, Klein had reported that silica gels which have a small pore diameter are able 1) to distinguish between sterols with Δ^{24} double bonds and those with nuclear double bonds, 2) to show the number of different substituents, such as methyl or ethyl groups, on the unsaturated side chain of sterols, 3) to separate most isomers with single and double nuclear double bonds, and 4) to differentiate the C27, C28, C29, and C30 sterols of the cholesterol-lanosterol variety. Since separation of the saturated sterols was also enhanced on silver nitrate

impregnated plates, Klein et al. (1966) suggested that the silver-olefin complex was less important to sterol separations than the geometric changes in pore diameter.

All of the workers cited above converted the free sterols to their acetate derivatives before using argentation TLC. The free sterols, differing only in side-chain unsaturation, have very similar mobilities on the silver nitrate impregnated plate, but their separation can be achieved through the use of their less polar derivatives such as acetates (Klein, 1961 and 1962). However, to convert sterols into their acetates is a time-consuming and inconvenient procedure, especially when working with a small amount of sample. More recently, both DiTullio et al. (1965) and Tu et al. (1970) were able to separate the free forms of cholesterol and desmosterol on silver nitrate impregnated silica gel with chloroform:acetone (95:5, v/v) as the developing agent. In 1967 Kammereck et al. reported the separation of cholesterol and desmosterol on a silver nitrate impregnated neutral alumina plate, using a more polar developing mixture of chloroform:acetone, 65:35.

Gas-liquid Chromatography (GLC)

Gas-liquid chromatography (GLC) is a rapid way to separate minute amounts of sample which may contain up to 50 or more components, and to give information on both quantity and quality aspects

simultaneously. Another advantage is that the column is usually continuously regenerated.

The whole system of GLC involves the choice of carrier gas (moving phase), operating temperature, and stationary phase. Carrier gas, usually inert gas, flows through the column to facilitate the separation of samples and to protect against oxidative breakdown. The choice of carrier gas is dependent on the detector used in the system. For example, any inert gas is suitable for the flame ionization detector, while helium or argon would be the better choice for the ionization detector. In GLC, the temperature of the column must be high enough to volatilize the sample so that it can pass through the detector, but not so high as to cause decomposition of the sample. The non-volatile liquid phase coating the solid support of the stationary phase is more important than the type and mesh of the solid support, or the diameter, length and type of tubing. The components in a sample can be separated by their various degrees of solubility in the liquid phase, or by their different boiling points. Components which have similar boiling points but different polar natures can be effectively separated on either a non-polar or a highly stationary phase (Bobbitt, et al., 1968). The polar nature of a compound is a function of the molecular weight, the number of functional groups, and the molecular configuration.

The GLC operating conditions, used by several workers to separate sterols, are shown in Table 3. All except Kritchevsky et al.

Table 3. Operating conditions of GLC.

	Reference	Column Packing	Column Length	Temperature (°C)	Sample
I.	Teshima and Kanazawa, 1971	1) 1.0% XE-60 on 60/80 mesh Chromosorb W	2.0m x 4mm I.D.	column: 205	sterol acetates from marine crustaceans
		2) 1.0% NGS: 1.0% XE-60 (1:1, v/v) on 60/80 mesh Chromosorb W	2.5m x 4mm I.D.	column: 215	trimethyl silylated sterols from marine crustaceans
II.	Tu et al., 1970	1.0% NGS on 80/100 mesh Gas Chrom Q	6 ft x 4mm I.D.	column: 211 flash: 280 detector: 255	elution of the nonsaponifiable matter in the egg yolk from $AgNO_3$ plate
III.	Idler and Wiseman, 1968	3% XE-60: 3% NGS (1:1) on 100/120 mesh Gas Chrom Q	14 ft x 4mm I.D.	column: 212 inlet: 258 detector: 253	steryl methyl ether of Alaskan king crab and the North Atlantic queen crab
IV.	Kritchevsky et al., 1967	4.1% SE-52 on 80/100 mesh Gas Chrom P	4 ft	column: 231	nonsaponifiable matter in seafoods
V.	Feldman and Kuck, 1966	3% XE-60 on 100/120 mesh Gas Chrom Q	6 ft x 4mm I.D.	column: 215	free form of the commercial sterols

(1967), and Idler and Wiseman (1968), used the flame ionization detector which is characterized by its great stability and resistance to contamination. Nitrogen was the carrier gas. In most cases, the column temperatures were within a very close range (205^{o}-215^{o} C).

Solid supports used by these investigators include several forms of treated diatomaceous earth (Gas Chrom and Chromosorb), in particle sizes ranging from 60 to 120 mesh. Various liquid coatings were employed. A nitrile silicone, XE-60, which has the properties of double bond selectivity and thermal stability, served to separate the free forms of cholesterol, desmosterol, stigmasterol, and β-sitosterol in only fifteen minutes (Feldman and Kuck, 1966). However, Teshima and Kanazawa (1971) found that the acetates of desmosterol and brassicasterol had almost identical retention times on XE-60. Kritchevsky et al. (1967) used SE-52, a phenyl-substituted, moderately polar silicone, to separate the free sterols in the nonsaponifiable matter of seafood lipids. Again, desmosterol and brassicasterol showed the same retention time. In 1962, Vandenheuvel and Horning had reported that NGS, a neopentyl glycol succinate, had a selective retention effect for carbon-carbon unsaturation. The NGS coating was used by Tu et al. (1970) to separate the free forms of cholesterol and desmosterol in the nonsaponifiable matter of egg yolk. Idler and Wiseman (1968) used equal parts of XE-60 and NGS as the liquid phase, and successfully separated the steryl methyl ethers of desmosterol and

brassicasterol. More recently, Teshima and Kanazawa (1971), using a slight modification of the Idler and Wiseman procedure, were able to separate the trimethyl silylated derivatives of desmosterol and brassicasterol.

PROCEDURE

Food Samples

Two specimens of crabmeat were selected for analysis in this project: frozen crabmeat and canned crabmeat. Both of them were purchased at a local supermarket.

The canned crabmeat was Pacific Pearl Brand, packed from Dungeness crab whole body meat, and distributed by Ivar Wendt, Seattle, Washington. The frozen crabmeat was also the Dungeness variety, and a mixture of body meat and claw meat.

Each specimen was drained and patted dry on paper toweling and was kept in a sealed container at -10° C.

Moisture Determination

Moisture was determined according to the air drying method 24.003 outlined in Methods of Analysis (A. O. A. C., 1970). A 2-gm sample of flaked crabmeat was dried for three hours in a circulating hot air oven maintained at 125° C. After drying, the sample was covered with aluminum foil, cooled in a desiccator, and reweighed. The moisture was calculated as the loss in percent of wet weight. Each specimen was analyzed in triplicate. Weights were determined to the nearest 0.0001 gm on a Mettler electronic balance.

Protein Determination

The nitrogen content of the crabmeat was determined according to the Micro-Kjeldahl method 42.016 described in Methods of Analysis (A.O.A.C., 1970). A 100-mg sample of crabmeat was oxidized in hot concentrated sulfuric acid with a catalyst mixture of HgO and K_2SO_4. The ammonium sulfate in the digest was then decomposed in $NaOH-Na_2S_2O_3$ solution, and the ammonia was distilled into 20 ml 0.4% boric acid containing methyl red-methylene blue solution as the indicator. The nitrogen content was determined by back titration with 0.02N HCl solution, and the crude protein concentration was calculated by multiplying the amount of nitrogen by 6.25. Protein was expressed as percent of wet weight. Four samples of each specimen were analyzed.

Extraction and Purification of Lipids

The lipids of the crabmeat specimens were extracted by a modification of the Folch et al. (1957) procedure, described by Chou (1972). Twenty grams of crabmeat were homogenized with 140 ml of chloroform:methanol:0.018% $CaCl_2$ (3:3:1), and the lipids were isolated in the chloroform phase. In this procedure, the methanol denatured and precipitated tissue protein, water washed out the water-soluble substances, while the added $CaCl_2$ altered the distribution of lipids and decreased

the layer of interfacial fluff between the aqueous and chloroform phases (Folch et al., 1957).

The chloroform phase was filtered, separated, and dried down in a tared 25 ml Erlenmeyer flask under a stream of nitrogen. The flask with crude lipid was kept in the desiccator (P_2O_5) until a constant weight was obtained. The purification of lipids was finished by re-extracting the crude lipids with chloroform, transferring the extract into another tared 25 ml volumetric flask, and repeating the drying and weighing procedure until a constant weight was again obtained. The amount of lipids was expressed as percent of wet weight. The purified lipids were dissolved in chloroform, made up to a volume of 25 ml, and stored at -10° C.

To determine the recovery of added lipids, 200 mg lecithin, 40 mg cholesterol and 10 mg tripalmitin were added to 20 gm of canned crabmeat, and were extracted and purified as described above. For checking the accuracy of experimental methods and possible technical errors, a solution of pure lipids containing 200 mg lecithin, 40 mg cholesterol and 10 mg tripalmitin in 25 ml chloroform was analyzed.

For each of the following analyses, the volume of pure lipid solution was the same as that of the crab lipid extract. One half volume of the recovery sample was used.

Lipid Phosphorus Determination

The microprocedure of Lowry et al. (1954), as modified by Hawthorne, Smith, and Pescador (1963), was used to determine the lipid phosphorus of crabmeat.

In this procedure the lipid phosphorus was oxidized to inorganic phosphate by perchloric acid, converted to phosphomolybdic acid, and reduced to molybdenum blue complex by ascorbic acid. The absorbance of the molybdenum blue was measured in a Beckman DU Spectrophotometer at 820 mμ. One milliliter of the extract was brought to 20 ml with chloroform. Ten microliters of each diluted sample was analyzed in triplicate. The phospholipid concentration was calculated by multiplying the phosphorus concentration by 25.0 (based on analyzed P in lecithin), and was expressed as percent of wet weight.

Triglyceride Determination

The triglyceride method of Carlson and Wadström (1959), as modified by Henry (1964), was adjusted for use with crabmeat.

According to this method, phospholipids were removed from the lipid extract by adsorption onto silicic acid. Glycerol was then released from the glycerides by saponification, and oxidized to formaldehyde. The color produced by the reaction of formaldehyde with chromotropic acid was measured photometrically.

The method described by Henry (1964) was devised for use with serum, which contains phospholipids and triglycerides in a ratio of about 3:1. Since preliminary studies indicated that the ratio of phospholipids to triglycerides in crab lipids is about 20:1, it was necessary to increase the size of the silicic acid column. Therefore, a 3-gm. column of silicic acid:celite (2:1) was used to separate 5 ml of the crab lipid extract. Two hundred milliliters of the chloroform eluate were collected and the glycerol content of 20 ml of eluate was determined according to the procedure of Henry (1964). All analyses were performed in duplicate. A column blank was analyzed. A tripalmitin solution was used as the standard, and triglycerides were expressed as mg. tripalmitin per 100 gm. crabmeat.

Non-esterified Fatty Acid Determination

Dole's titrimetric method (1956) as modified by Trout, Estes and Friedberg (1960) was used for the determination of the non-esterified fatty acids of crabmeat.

One milliliter of the lipid extract was re-extracted with isopropanol-heptane, containing sulfuric acid. Aliquots of the heptane phase were titrated with sodium hydroxide, and Nile blue was the indicator. The non-esterified fatty acids were expressed as mEq of palmitic acid per 100 gm of crabmeat.

Cholesterol Determination

Free and total cholesterol were determined essentially by the Schoenheimer-Sperry procedure described by Oser (1965). Slight modifications of the method included: the use of a water bath instead of sand jar to control the temperature, the use of a vibrator instead of a stirring rod to mix the sample, substitution of a 0.5% solution of digitonin for 0.4% solution, and the use of alcoholic acetic acid instead of the aqueous solution for neutralization.

Duplicate 200 µl samples of the lipid extracts were used for analysis. Except for the free cholesterol, the samples were saponified and neutralized prior to precipitation with digitonin. After purification, the digitonide was treated with the Liebermann-Burchard color reagent. Forty-five minutes later, the absorbance was measured in a Beckman Model DU Spectrophotometer at 635 mµ. Cholesterol was expressed as mg per 100 gm of crabmeat.

Extraction of Nonsaponifiable Matter (NSM)

The nonsaponifiable matter in the crab lipids was extracted by the procedure of Williams (1966). Approximately 0.25 gm of the purified lipids from 20 gm of crabmeat were saponified by refluxing for 1 hour with 8 ml of 2% alcoholic KOH. The cooled soap solution was extracted three times with ethyl ether. The combined ether extracts

were washed first with 2.5% KOH solution to remove any remaining fatty acids, then several times with water to obtain neutrality. The ether extracts were dried under nitrogen and held in a desiccator until a constant weight was obtained. The NSM was expressed as mg per 100 gm of crabmeat.

The NSM was dissolved in 10 ml chloroform, and kept at -10° C for further identification of the sterols of crabmeat by TLC and GLC.

Thin-layer Chromatography (TLC)

Argentation TLC was used in an attempt to separate and identify the sterols of the nonsaponifiable matter (NSM) from the lipid extract. Clean glass plates (20 x 20 cm) were spread with a slurry of the stationary phase to a thickness of 0.25 cm, by use of a leucite applicator.[1] Three different stationary phases were used:

1. Aluminum oxide with silver nitrate (Kammereck et al., 1967)

   Thirty-five grams Aluminum Oxide GA with 10% $CaSO_4$ binder[1] were mixed with 25 ml redistilled water for 1 minute. To the mixture was added a solution of 10.5 gm $AgNO_3$ in 10 ml redistilled water, and the slurry was mixed for another minute. The slurry was spread on the glass plates and the plates were air-dried for 2 to 3 hours.

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[1] Applied Science Laboratories, Inc., State College, Pa.

2. Silica gel with silver nitrate (Tu et al., 1970)

   Adsorbosil-1-ADN with 10% $CaSO_4$ binder and impregnated with 25% $AgNO_3$[2] was mixed for 1 minute with redistilled water in the amounts of 33.3 gm to 45 ml. The slurry was spread on the glass plates and the plates were air-dried in a dark cabinet.

3. Silica gel with silver nitrate and fluorescein (DiTullio et al., 1965; Bobbitt et al., 1968)

   Fifteen grams of Silica Gel H[3] were mixed with 85 mg sodium fluorescein until an evenly distributed pink powder was obtained. A solution of 5.3 gm $AgNO_3$ in 42.5 ml redistilled water was stirred into the silica gel mixture for 1 minute. The slurry was spread on glass plates and air-dried in a dark cabinet.

All plates were activated at 110°C for 1 hr before application of the sample. Three samples were applied to each plate: a 40 µl sample of NSM in chloroform (estimated to contain about 100 µg sterol), 20 µl of a 0.2% solution of cholesterol in chloroform and 20 µl of a 0.2% solution of desmosterol in chloroform.

The plates were developed in a filter paper-lined tank containing 100 ml chloroform:acetone (95:5, v/v), until the solvent front had moved a distance of 17 cm on the plate.

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[2] Applied Science Laboratories, Inc., State College, Pa.

[3] Brinkmann Instruments, Inc., Westbury, L.I., N.Y.

The plates to which no fluorescein had been added were then sprayed with a 0.04% solution of sodium fluorescein. The sterols appeared as yellow spots against a pink background after the plates had dried. Under ultraviolet light they appeared as green spots on a dull background.

Gas-liquid Chromatography (GLC)

For identification of sterols in the NSM of crab lipid extract, the GLC procedure described by Feldman and Kuck (1966) was used with minor temperature adjustments. By this procedure, Feldman and Kuck had been able to separate cholesterol and desmosterol, applied in the free form rather than as the derivative. For this study, the following procedure was used:

1. Instrument: Barber-Colman Model 10 with hydrogen flame ionization detector
2. Column: Gas Chrom Q[4], 100/120 mesh, was coated with 3% w/w Silicone GE XE-60[4], and packed into a 6 ft x 5 mm I.D. Pyrex column. The Gas Chrom Q is a specially purified diatomaceous earth. Silicone GE XE-60 is a nitrile and cyanoethyl-substituted silicone. The column was chosen to provide double bond selectivity and thermal stability.

[4]Applied Science Laboratories, Inc., State College, Pa.

3. Operating conditions:

	Temperature	Voltage	Pressure	
column	218° C	78	N_2	14 psi
cell	268° C	69	air	10 psi
flash heater	290° C	42	H_2	50 psi

4. Injected samples:

Known concentrations of pure cholestane, cholesterol and desmosterol were prepared in chloroform. Known concentrations of the NSM of the lipid extract from frozen crab and canned crab were prepared similarly. Appropriate volumes of these were combined so that the injected sample would contain no more than 50 μg of any one sterol. In each case, cholestane was used as the internal standard. Exactly 5 μl of each of the following sterol mixtures were chromatographed:

a. Mixed standards: Five microliters of the mixture containing 50 μg each of cholestane, cholesterol and desmosterol

b. NSM of crab lipid extract: Five microliters of the mixture containing 50 μg of cholestane and 50 μg of NSM from either frozen crab or canned crab lipid

c. Recovery samples: Five microliters containing 50 μg cholestane, and 25 μg each of cholesterol, desmosterol, and the NSM from either frozen crab lipid or canned crab lipid.

5. Retention times of the standard sterols and the NSM sterols were computed relative to the retention time of cholestane.

RESULTS AND DISCUSSION

Moisture

The percentage composition of frozen and canned male Dungeness crabmeat is presented in Table 4. The frozen crabmeat contained 78% moisture. Thompson (1964) had reported 80.3 - 81.0% moisture in raw Dungeness crabmeat, and Farragut and Thompson (1966) had found 78.5 - 82.3% moisture in raw Dungeness crabmeat (Table 1). The somewhat lower moisture content of the frozen crabmeat used in the present study may be ascribed to heat treatment. In commercial processing, the crab is heated in the shell before the meat is extracted, washed, and frozen. Heat denatures the protein and expresses some water, thus decreasing the moisture content of the tissues. Allen (1971) first heated the crab in the shell at 50° C for 20 minutes and then dried the sample in a desiccator to constant weight. Analyzed by this method, the crabmeat contained 78.5% moisture, a value which is comparable to that determined for frozen crabmeat in the present study.

The canned crabmeat had only 75% moisture (Table 4). Canning entails a prolonged heat treatment; the crabmeat is heated in a sealed can under 3 lb. pressure at 105° C for 90 minutes (Tanikawa and Doha, 1965). Under these conditions, more water may be expressed. The canned Dungeness crabmeat analyzed by Farragut and Thompson

Table 4. Percentage composition of two kinds of crab.

	Moisture	Protein[a]	Total Lipids	Phospho-lipids[b]	Total Cholesterol	Free Cholesterol	Trigly-ceride[c]	NEFA[d]	NSM[e]
Wet weight basis:									
Frozen crab	78	18.98	1.1985	0.9150	0.0945	0.0800	0.0180	0.0102	0.1090
Canned crab	75	22.43	1.2910	1.0550	0.1020	0.0930	0.0115	0.0332	0.1015
Dry weight basis:									
Frozen crab	0	86.27	5.4477	4.1591	0.4295	0.3636	0.0818	0.0464	0.4955
Canned crab	0	89.72	5.1640	4.2200	0.4080	0.3720	0.0460	0.1328	0.4060

[a]Kjeldahl N x 6.25.

[b]Calculated as lecithin (P x 25).

[c]Expressed as tripalmitin.

[d]Non-esterified fatty acids calculated as palmitic acid (1 mEq = 255.4 mg).

[e]Nonsaponifiable matter.

(1966) contained 76.6 - 82.9% moisture. Average values, cited by Watt and Merrill (1963) are 78.5% for cooked crab and 77.2% for canned crab (Table 1).

Farragut and Thompson (1966) observed that the moisture content of crabmeat was higher in March and June than in August and January. The frozen crabmeat used in the present study was caught in the winter. It was impossible to determine the season of catch of the canned crabmeat.

Protein

There were 18.98 gm protein per 100 gm wet weight of the frozen crabmeat (Table 4). This value exceeded the range of 15.2 - 18.6% protein reported by Farragut and Thompson (1966) for raw Dungeness crabmeat (Table 1). Allen (1971), using the Folin phenol reagent to determine protein, reported that 16.4% of the crab muscle was protein (76.3% of the dry weight). On the basis of dry weight, the frozen crabmeat of the present study contained 86.27% protein (Table 4).

In contrast to the frozen crab, the wet tissue of canned crab contained 22.43% protein, or 89.72% of dry weight (Table 4). This concentration was considerably higher than the 17.4% protein in canned crabmeat, cited by Watt and Merrill (1963). Farragut and Thompson (1966) found that the protein of canned crab ranged from 14.7 to 21.3% of the wet weight (Table 1). These investigators found

small but insignificant differences between the protein content of heat-processed and raw crab, and between crab cooked with or without brine. Calculated on the basis of dry weight, there was 86 - 92% protein in the canned crab which they analyzed. The long, moist heat treatment used in canning may have coagulated soluble proteins within the tissue thus increasing the protein content of the solids. Another factor causing differences in protein content may have been the season of catch. Farragut and Thompson (1966) reported that the nitrogen content of claw meat decreased significantly during the months of March to June, when eggs and spermatozoa were maturing. Body meat, on the other hand, remained quite constant in nitrogen concentration.

Total Lipids

There was little difference between the two kinds of crabmeat with respect to total lipids (Tables 4 and 5). In terms of wet weight, the frozen crab had 1.2% lipids while the canned crab had 1.3%. On the dry weight basis, the frozen crab had 5.5% and the canned crab had 5.2%. Farragut and Thompson (1966) reported no significant change in lipid concentration due to cooking, canning, or brine treatment. Sparre (1965) suggested that oxidation and polymerization of fat during storage resulted in reduced concentrations of extractable lipids of fish

Table 5. Lipid composition of 100 gm crab meat (wet weight).

	Total Lipids	Lipid P	Total Cholesterol	Free Cholesterol	Triglyceride[a]	Non-esterified Fatty acids[b]	Non-saponifiable Matter
	gm	mg	mg	mg	mg	mEq	mg
Frozen crab	1.1985	36.6	94.5	80.0	18.0	0.040	109.0
Canned crab	1.2910	42.2	102.0	93.0	11.5	0.130	101.5

[a]Expressed as tripalmitin.

[b]Expressed as palmitic acid.

meal. This may have accounted for the slightly lower lipid content of the canned crab used in this study.

The lipid values for frozen and canned crab are within the ranges which Farragut and Thompson (1966) reported for fresh and canned Dungeness crabmeat (Table 1). But the commonly used food tables of Watt and Merrill (1963) give lipid values for four types of crabmeat which are almost twice as high as those found in this study (Table 1). Undoubtedly, there are species differences in lipid content; seasonal differences have also been reported (Farragut and Thompson, 1966). Furthermore, various extraction procedures differ in their degree of efficiency.

In the present study, a modified Folch extraction procedure was used (Chou, 1972). The recovery of pure lipids added to the canned crabmeat before extraction and purification was 92.5% (Table 6). When pure lipids alone were dissolved in chloroform, transferred, dried, and reweighed, the recovery was 96.1%. This would suggest that less than 8% of the lipids was lost during the extraction and purification procedure, and that less than 4% was lost during the purification step.

Phospholipids

The frozen crabmeat contained 36.6 mg lipid phosphorus per 100 gm (Table 5), or 0.915% phospholipid, expressed as lecithin (Table 4). There were 42.2 mg lipid phosphorus in 100 gm of canned crabmeat,

Table 6. Percentage recovery of pure lipids[a] added to canned crab and extracted, and of pure lipids[a] dissolved in chloroform.

	Total Lipids	Lecithin	Total Cholesterol	Free Cholesterol	Tripal-mitin	Average of Individual Recoveries[b]
Pure lipids in canned crab	92.5	92.6	109.0	104.8	100.1	98.6
Pure lipids only	96.1	92.9	97.8	84.2	97.4	96.1

[a]200 mg lecithin, 40 mg cholesterol, 10 mg tripalmitin, added to 20 gm of crab or dissolved in 25 ml chloroform.

[b]Average of four recovery values: Total lipids, lecithin, tripalmitin, total cholesterol.

equivalent to 1.055% phospholipid, expressed as lecithin. On a dry weight basis, the phospholipid content of the two specimens was almost identical - 4.16% of frozen crab, and 4.22% of the canned crab (Table 4). It would appear that little hydrolysis of phospholipids occurred during the canning process.

Phospholipids constituted the major fraction of the crabmeat lipids (Table 7). In the frozen crab, 76.3% of the total lipids were phospholipid; in the canned crab, phospholipids accounted for 81.7% of the total lipid. Munn, cited by Huggins and Munday (1968), found a somewhat lower proportion of phospholipid in the muscle of Carcinus maenus - 73.6% of the total lipids. Species differences and differences in analytical methods probably account for the variations in reported phospholipid content.

Recovery of pure lecithin, added to the canned crabmeat before extraction and purification, was 92.6% (Table 6). Since recovery of lipid phosphorus from lecithin in a chloroform solution of pure lipids was 92.9%, there appeared to be little loss of lipid phosphorus during the extraction procedure.

Triglycerides

Expressed as tripalmitin, the triglycerides constituted 0.08% of the dry weight of frozen Dungeness crab and 0.05% of the dry weight of the canned Dungeness crab (Table 4). Triglycerides accounted for only a small percentage of the total lipids of the crab muscle - 1.5% of frozen crab lipids, and 0.9% of the canned crab lipids (Table 7). These values are far lower than Munn's finding of 4.2% triglyceride

Table 7. Lipid fractions of crab meat expressed as percent of total lipid present.

	Phospholipids[a]	Total Cholesterol	Free Cholesterol	Triglyceride[a]	Non-esterified Fatty Acids[b]
Frozen crab	76.3	7.9	6.7	1.5	0.9
Canned crab	81.7	7.9	7.2	0.9	2.6

[a]Expressed as tripalmitin.

[b]Expressed as palmitic acid.

and 3.5% monoglyceride in the muscle lipids of Carcinus maenus (Huggins and Munday, 1968). Here again, species, analytical method and sample preparation may have been responsible for the differences.

The triglyceride content of canned crab was markedly lower than that of frozen crab. It is possible that the triglycerides may have been hydrolyzed during the prolonged heating process, or during the subsequent storage at room temperature. In this study, the triglycerides were determined by measurement of their glycerol content. If hydrolysis did occur, the free glycerol would not have been extracted into heptane, and thus would have escaped measurement. The hydrolysis hypothesis is supported by the finding of greater amounts of non-esterified fatty acids in the canned crab than in the frozen crab.

Recovery of pure tripalmitin, added to the canned crab before extraction and analysis was 100.1% (Table 6). The recovery of tripalmitin from a chloroform solution of pure lipids was 97.4%.

Non-esterified Fatty Acids (NEFA)

There were 0.04 mEq NEFA per 100 gm frozen crabmeat and 0.13 mEq in 100 gm of canned crabmeat (Table 5). Expressed as palmitic acid, the NEFA fraction constituted 0.05% and 0.13% of the dry weight of the frozen and canned crab (Table 4). Munn (cited by Huggins and Munday, 1968) had found that 4.0% of the total lipids of Carcinus maenus muscle was free fatty acid. In the Dungeness crab

of the present study, NEFA constituted only 0.9% and 2.6% of the total lipids in frozen and canned crabmeat, respectively (Table 7).

The possibility that the considerably greater concentration of NEFA in canned crab might be directly related to the lower triglyceride and cholesterol ester content was explored, but no direct molar relationship could be established. Nevertheless, hydrolysis of fatty acid esters seems to be a reasonable explanation for the high NEFA content of canned crab. Non-esterified fatty acids, amounting to 0.095 mEq/gm lipid, were found in the chloroform solution of pure lipids, which contained only lecithin, tripalmitin, and free cholesterol. Examination of each pure lipid individually revealed that the cholesterol and lecithin specimens were both contaminated with non-esterified fatty acids. This would account for the increased titer of NEFA in the recovery sample of canned crab, to which lecithin, tripalmitin, and free cholesterol had been added. In this case, the increase in NEFA was 0.11 mEq/gm added lipid.

Cholesterol

The total sterol content of the frozen crab, measured as cholesterol, was 94.5 mg/100 gm tissue (Table 5). The canned crab had 102.0 mg sterol per 100 gm. In both types of crabmeat, the cholesterol constituted 7.9% of the total lipids (Table 7). These concentrations fall within the range of values reported by other

investigators (Table 2). Okey (1945) and Kritchevsky et al. (1967) found 145 and 140 mg cholesterol in 100 gm crabmeat of unstated species. On the other hand, Thompson (1964) reported 52-63 mg cholesterol in 100 gm Dungeness crab, and 76-98 mg/100 gm Eastern Blue crab. Kritchevsky et al. (1967) suspected that the short extraction time was the main reason for the low concentration of cholesterol determined by Thompson. However, Thompson's extraction time of 32 minutes was over 4 times as long as the 7-minute extraction time used in the present study. Despite this, she obtained lower cholesterol concentrations in Dungeness crab than are reported here. Since Thompson's samples were obtained from Washington waters in January, and the frozen crabmeat used in the present study was obtained later in the season from Oregon waters, seasonal and geographical factors may have affected the cholesterol content of the crab muscle. The species and origin of the crabmeat analyzed by Kritchevsky et al. (1967) are not stated, but Thompson (1964) demonstrated that species differences exist.

Kritchevsky and Tepper (1961) stated that most of the cholesterol in seafoods is in the free form. This observation is supported by the findings of the present study. About 85% of the cholesterol in frozen crab, and 91% of the cholesterol in canned crab was in the free form. The higher percentage of free cholesterol in canned crab may have been the result of hydrolysis during the canning and storage process.

Fatty acids, released by hydrolysis may have contributed to the higher non-esterified fatty acid concentrations in canned crab. The extent to which cholesterol is esterified must also be a function of the species. For example, Munn (cited by Huggins and Munday, 1968) found that only 35% of the cholesterol in Carcinus maenus muscle was in the free form.

When free cholesterol was added to the canned crab before extraction and analysis, the recovery was 104.8% (Table 6). A low recovery (84.2%) of free cholesterol from the solution of pure lipids suggests that the cholesterol, which was presumed to be pure, may have been contaminated with some cholesterol ester.. This is supported by the high recoveries of added cholesterol (109.0% and 97.8%) obtained when the samples were saponified prior to analysis, according to the procedure for total cholesterol.

Characterization of Sterols in Nonsaponifiable Matter (NSM)

Nonsaponifiable matter, isolated from the frozen and canned crab lipids, amounted to 109.0 and 101.5 mg/100 gm crabmeat (Table 4). The NSM consists chiefly of sterols, with some hydrocarbons and higher alcohols (Williams, 1966). Kritchevsky et al. (1967) isolated 142 mg NSM from 100 gm crabmeat, of unstated species, and reported that 140 gm appeared to be cholesterol (Table 2). Teshima and Kanazawa (1971) found that the NSM varied with the species of crab;

in four types of Japanese crab, the NSM ranged from 150 to 690 mg/100 gm wet weight (Table 2).

The results of thin-layer chromatography of the pure sterols are shown in Table 8. With all three stationary phases, cholestanol was completely separated from cholesterol, and the separation of cholesterol from desmosterol was nearly complete. On all three stationary phases, the NSM of frozen and canned crab produced a single spot with an R_f corresponding to that of cholesterol.

Of the three stationary phases which were used in this study, the most convenient was Phase 3, in which the fluorescein and silver nitrate were incorporated into the silica gel before the layer was spread. This procedure eliminated the necessity of spraying the plate at the end of the run, thus avoiding the hazard that the silica layer would be scattered.

The retention times of sterols, separated by gas-liquid chromatography, are presented in Table 9. Retention times are given relative to cholestane which was used as an internal standard. The column satisfactorily separated cholestane, cholesterol, desmosterol, and stigmasterol; cholestanol had the same retention time as cholesterol. Application of 50 μg of NSM from either the frozen or canned crab lipids produced only a single peak with a relative retention time close to that of cholesterol. When the NSM was applied in combination with standard cholesterol and desmosterol, there were only two peaks,

Table 8. Thin-layer chromatography of sterols.[a]

	R_f		
	Stationary phase 1	Stationary phase 2	Stationary phase 3
Cholestanol	0.23	0.26	0.38
Cholesterol	0.17	0.24	0.21
Desmosterol	0.13	0.21	0.12
NSM[b] of frozen crab	0.17	0.24	0.20
NSM[b] of canned crab	0.17	0.24	0.21

[a]Stationary phase 1. Aluminum Oxide with silver nitrate.
Stationary phase 2. Silica gel with silver nitrate.
Stationary phase 3. Silica gel with silver nitrate and fluorescein.
Developing solvents: Chloroform:acetone (95:5, v/v) moving 17 cm on the plate.

[b]Nonsaponifiable matter.

Table 9. Gas-liquid chromatography of sterols.[a]

	Relative retention time[b]				
	Standard sterol mixture	NSM from frozen crab	NSM from frozen crab plus standard sterol mixture	NSM from canned crab	NSM from canned crab plus standard sterol mixture
Cholesterol	4.38	4.46	4.24	4.25	4.32
Desmosterol	5.39		5.03		5.12

[a]Operating conditions: Column = 3% Silicone GE XE-60 on Gas Chrom Q, 100/120 mesh, temperature = 218° C in column, 268° C in cell, and 290° C in flash heater.

[b]Relative to internal cholestane with retention time of 1.6 min.

with retention times close to those of cholesterol and desmosterol. Only the cholesterol peak was enhanced when NSM was added to the standard mixture. Thus, cholesterol was the only sterol found in Dungeness crab by the procedure used in this study.

There is a possibility that the concentration of desmosterol or other sterols was too low to be detectable by the procedure used. Kritchevsky et al. (1967) reported that the NSM of crabmeat consisted of 57% cholesterol, 37% brassicasterol, plus small amounts of other sterols. And Idler and Wiseman (1968), using the steryl methyl ethers, were able to detect substantial amounts of cholesterol and desmosterol, as well as 22-dehydrocholesterol in Alaskan king crab and North Atlantic queen crab. On the other hand, Teshima and Kanazawa (1971) found only cholesterol in four types of Japanese crab (Table 2). Although the analytical procedures used by these groups were different, the main explanation for the variations in observed sterols seems to be the difference in species.

SUMMARY

Two forms of Dungeness crabmeat, frozen and canned, have been analyzed to determine the moisture, protein, total lipid and lipid components. Both samples were purchased on the retail market. The composition of the two forms of crabmeat was quite similar. Seventy-eight percent of the frozen crabmeat was moisture, as compared with 75% of the canned crabmeat. The protein content was equivalent to that of other animal tissue: 18.98% of the frozen crab, and 22.43% of the canned crabmeat. The slightly greater protein content and the slightly lower water content of canned crabmeat may be attributable to coagulation of the tissue protein associated with the prolonged heat treatment.

Total extractable lipids of crabmeat were low, accounting for only 1.20% of the weight of frozen crabmeat and 1.29% of the canned crabmeat. Most of the lipid was in the form of phospholipids, which constituted 76% of the frozen crab lipids and 82% of the canned crab lipids. Digitonin-precipitable sterol composed 7.6% of the lipids in both frozen and canned crabmeat. Most of the sterol was in the free form: 85% of the frozen crab sterol and 91% of the canned crab sterol. The lipids of frozen crabmeat contained 1.5% triglyceride and 0.9% non-esterified fatty acids. In canned crabmeat, 0.9% of the total lipid was triglyceride and 2.6% was non-esterified fatty acid. The

higher concentration of non-esterified fatty acids in canned crab, and the lower concentration of triglyceride and cholesterol ester suggest that ester hydrolysis may have occurred during the canning process.

In order to characterize the sterol in the crabmeat specimens, nonsaponifiable matter was isolated from the lipid extracts and subjected to analysis by argentation thin-layer and by gas-liquid chromatography. Neither analytical method revealed more than one sterol in the crab lipids. That sterol was identified as cholesterol.

The analysis showed that Dungeness crabmeat is a high-protein, low-fat food. The lipid consists chiefly of phospholipid with a moderate amount of cholesterol.

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