COMMUNICATIONS

Caloric Densities of Shellfish Meat and Meat Fats

The gross energy content of meats of crab, lobster, three species of shrimp, crawfish, oyster, and squid was analyzed by oxygen bomb calorimetry. The whole meat of lobster yielded the lowest energies and that of squid the highest energies. However, the meat fats of squid and lobster had relatively lower energies than those of the other shellfish compared. Percent fat content and nitrogen content of meats differed significantly; oyster meats had more fat and crawfish very low fat. The gross energy content of the extracted fat differed distinctly among the species. On a unit weight basis, the fats of squid and lobster showed very low gross energies while that of oyster, blue crab, and pink shrimp showed high energies. Gross energy values determined for 19 commercial lipid compounds showed an increase in energies as the percent hydrogen content of the compound increased. Column-fractionated lipid classes of the oyster gave different gross energies, substantiating that sterols give greater energies, while phospholipids and monoglycerides yield low energies. It is suggested that the nature of the lipid classes contributes more toward caloric density of the tissue than the total lipid content.

The caloric density of an organism is dependent on the contents of organic matter (Golley, 1961; Cummins, 1967; Brawn et al., 1968; Cummins and Wuycheck, 1971; Thayer et al., 1973) and ash and lipid (Platt et al., 1969; Prus, 1970; Tyler, 1973). The lipids yield more combustible energy (Brody, 1945) than any other constituents of organic matter. However, caloric densities of animal lipids have not been attempted except for a few foods like lard, oils, butter, etc. (Watt and Merrill, 1963; Burton, 1976). Caloric densities of animal lipids are of interest not only from a nutritional point of view but also in the thermodynamic sense of ecological energetics. This paper considers the gross energy analyses of some shellfish meats and the lipids extracted from them.

MATERIALS AND METHODS

Adult oysters (*Crassostrea virginica*), white shrimp (*Penaeus setiferus*), pink shrimp (*Penaeus duorarum*), brown shrimp (*Penaeus aztecus*), blue crab (*Callinectes sapidus*), squid (*Loligo pealei*), spiny lobster (*Panulirus argus*), and crawfish (*Procambarus clarki*) were used for the experiments. Both sexes were selected for analyses. Crabs and white and brown shrimp were trawled from Davis Bayou in Ocean Springs, Mississippi, during October 1977. Oysters were collected from the natural reefs at Bay St. Louis, Mississippi, during February 1978. The rest were purchased from local seafood suppliers. The pink shrimp, lobster, and squid were collected offshore in the Gulf waters of Florida in March 1978. The crawfish were obtained from Louisiana in April 1978.

The edible muscle tissue from 10–12 animals of each species was taken for analyses. The samples were weighed, homogenized, and dried for 12 h in a ventilated oven at 70 °C, and dried to a constant weight. The water content was calculated from the difference between wet and dry weights. The dried material was ground and pelletized; the pellets were dried to constant weight before burning in a Parr Adiabatic 1103 oxygen bomb calorimeter, with appropriate corrections (Cummins and Wuycheck, 1971). Gross energy values (heat of combustion) were calculated according to the standard methods (ASTM, 1966). Replicate or triplicate analyses were always made and the values of the first two pellets differed not more than 0.5%.

Desiccated subsamples of each species were oxidized in a muffle furnace at 500 °C for 3 h to obtain the total organic and ash content (Raymont et al., 1964).

The total fat of the dried meat was extracted in a Soxhlet apparatus for 6 h with 2:1 chloroform–methanol mixture (Folch et al., 1957) and determined gravimetrically.

For calorimetry, the fats were extracted from wet meat by the procedure based upon that of Bligh and Dyer (1959) with 1% ammonia replacing the water of that system for the final extraction. The meat was sliced and homogenized with the extracting solution (3:1 chloroform–methanol with ammonia water) in a tissue grinder for 15 min at 2000 rpm. The resultant suspension was filtered through a Buchner funnel into a ice-chilled receiver, the filter cake being reextracted twice, after which the total extract was reduced in volume under nitrogen at 25 °C. The extract was then stored under nitrogen at −20 °C, and any ice crystals that formed were removed. Polar and neutral lipids were further fractionated by adsorbant column chromatography (Carrol, 1963) on Florisil (60–100 mesh, Sigma) into the following classes: hydrocarbons, steryl esters, triglycerides, free sterols, diglycerides, monoglycerides, and free fatty acids. The elution was performed with hexane–ether combinations (Teshima and Kanazawa, 1976). The identification of lipid classes in elutants was carried out by thin-layer chromatography on silica gel G with petroleum ether–ethanol–acetic acid (87.5:12.5:1) (Guary and Kanazawa, 1973). Chemically pure lipids were purchased from Sigma Chemical Co.

The total nitrogen content in the dried meat was estimated by micro-Kjeldahl method (AOAC, 1975).

RESULTS

Table I presents the gross energy (heat of combustion) values for the whole meat and meat fats of shellfish. Whole meat of squid yielded the highest gross energies of any other shellfish considered while lobster meat seemed to have the lowest gross energy.

Meat fats showed greater gross energy values than the whole meats. Among the meat fats compared, the lobster and squid fats had relatively lower gross energies than the fats of other shellfish.

0021-8561/79/1427-1125$01.00/0 © 1979 American Chemical Society

Table I. Proximate Composition and Gross Energy Values for the Meat of Some Shellfish[a]

meat source	calories/g dry wt ($\bar{X}$ ± SD)[b] whole meat (4)	meat fat (4)	% water (1)	total lipid, % dry wt (4)	ash content, % dry wt (4)	total nitrogen, % dry wt (4)
crab (claw)	5028 ± 16	7945 ± 27	76.73	5.74 ± 0.92	6.09	11.94 ± 0.91
crab (flake)	4911 ± 11	7812 ± 11	79.32	8.63 ± 0.31	5.79	11.86 ± 0.76
lobster	4230 ± 15	6894 ± 29	79.26	10.16 ± 1.06	4.50	12.46 ± 0.57
white shrimp	4909 ± 10	7612 ± 12	71.78	15.92 ± 1.21	3.04	10.36 ± 0.83
pink shrimp	4957 ± 31	7868 ± 39	72.51	7.95 ± 0.82	3.02	12.68 ± 0.69
brown shrimp	4855 ± 48	7749 ± 46	74.46	8.98 ± 0.32	2.98	12.01 ± 0.93
crawfish	4698 ± 11	7142 ± 41	72.64	1.31 ± 0.12	1.96	13.38 ± 0.58
squid	5259 ± 26	6922 ± 26	80.46	8.18 ± 0.96	3.12	8.04 ± 0.98
oyster[c]	4721 ± 138	7891 ± 198	80.74	16.86 ± 2.31	6.87	8.36 ± 0.81

[a] Number in parentheses is the sample size. [b] Joules = calories × (4.183 × 10^3). [c] These are "fat" oysters collected during February 1978 and weighing 8-10 g without shells. The term "fat" refers to the glycogen content, not to true fat (lipids and sterols). Oysters from the Gulf of Mexico are "fat" during the winter and early spring.

Table II. Gross Energy Values Obtained for Purified Lipids and Related Compounds in the Laboratory

compound	mol wt	percent hydrogen	cal/g[a]
acetylacetone	100.11	8.06	6 018
glycerol	92.09	8.75	4 267
cholic acid (Na salt)	430.56	9.13	6 938
ethyl acetate	88.10	9.15	5 227
retinol acetate	328.49	9.81	9 030
β-carotene	536.85	10.51	10 491
α-tocopherol acetate	430.10	10.69	9 252
ergosterol	396.63	11.18	11 462
cholesterol acetate	428.01	11.21	9 879
7-dehydrocholesterol	384.61	11.53	9 859
stigmasterol	412.67	11.72	13 240
triolein	885.21	11.84	8 846
dihydrocholesterol	386.60	11.90	10 164
cholesterol	386.64	11.99	10 183
oleic acid	282.46	12.13	8 555
β-sitosterol	414.69	12.15	10 226
tripalmitin	807.29	12.24	9 646
palmitic acid	256.42	12.58	9 584
α-lysolecithin (from egg)			7 764

[a] Joules = calories × (4.183 × 10^3).

Table III. Gross Energy Values for Lipid Classes in the Oyster *Crassostrea virginica*[a]

lipid class	cal/g of lipid[b]
phospholipid	6 985
neutral lipid	
hydrocarbons	10 192
steryl esters	8 567
triglycerides	9 632
free sterols	10 354
diglycerides	6 729
monoglycerides	5 242
free fatty acids	8 019

[a] Total lipid was extracted from "fat" oysters (February) of 4-8 g meat weight. [b] Joules = calories × (4.183 × 10^3).

The proximate composition of meats varied with species. The molluscan meats had lower nitrogen content than shrimp and crab meats. Among shrimp, the white shrimp showed low nitrogen content in the meat. Crawfish meat was rich in nitrogen content. Squid and oyster meats showed greater water content than those of crustaceans. Greater ash content was obtained with the meats of oyster, crab, and lobster. The fat content varied significantly among the shellfish. The meats of oyster, white shrimp, and lobster were rich in fat content, whereas crawfish meat had a very low fat content (Table I).

Pure lipid compounds were purchased and their gross energies were estimated by bomb calorimetry in the laboratory (Table II). The molecular weight did not show a significant relationship with the gross energies (correlation coeffiecient = 0.4945) whereas the hydrogen content percentage of the compound showed a relatively significant correlation (r = 0.7415), with gross energy increasing with the hydrogen content of the compound. Even among the compounds compared the sterols showed greater energies than the other fats containing the same percentage of hydrogen.

Table III summarizes the data on the gross energy values of the various lipid classes fractionated from oyster fat. Fractions of hydrocarbons, free sterols, and triglycerides showed greater gross energies than the other classes compared. The monoglyceride and phospholipid fractions gave low gross energy values.

DISCUSSION

Thayer et al. (1973) observed significant differences in the caloric values of species grouped by phylum and an evolutionary trend toward increasing energy content per gram live weight. Their analyses were, however, based on the total organic matter of the species including the gut contents. The present data dealt exclusively with the edible muscle tissue of the shellfish. Dame (1972) reported calorific energies of oysters, varying with season. Many ecological energetic studies are carried out in terms of indirect calorimetry using caloric conversions for the estimates of fat, carbohydrate, and protein content in tissues. Direct calorimetry would reflect gross energy values from all organic constituents present, including different classes of lipids.

Baldwin (1967) indicated that the combustibility of organic compounds in general is related to the hydrogen content. As lipids contain relatively more hydrogen than protein or carbohydrate, it is conceivable that they carry more physiologic energy (calories) (Brody, 1945). Recently, this concept was extended to crustaceans by Childress and Nygaard (1974), who showed that the ash-free calories increase along with an increase in percentage lipid content of the organism. Present data (Table I) showed that the lipid content of the shellfish meat is not closely related to the gross energies of the whole meat (r = −0.1090) or the meat fat (r = 0.2629). The hydrogen content percentage of the lipid is fairly correlated (r = 0.7415) with the gross energies (Table II). This shows obviously that total lipid content is not the main contributing factor for the gross energies of the shellfish meats.

Skinner (1969) and Wadso (1969) emphasized that the heat of combustion is also dependent on the capacity of the compound to become combustible or be ignited. The present data (Table II) offer evidence that among fats, the sterols are highly combustible. Probably for this reason, the squid has high gross energies and lobster meat has low

fat gross energies even though they have a fairly high fat content. Lipids in shellfish meat contribute to the total caloric value of the shellfish meat energy, but at their low level in relation to other sources of energy, especially protein, the lipid would not be the dominant source of calories.

Two conclusions can be drawn from the results: (1) fat contributes toward the heat of combustion even in shellfish and (2) the kind of lipid as well as the quantity contributes to the combustibility of the lipid.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed, Washington, DC, 1975.

American Society for Testing and Materials, "Method D2015-66, Method of Test for Gross Calorific Value of Solid Fuel by the Adiabatic Bomb Calorimeter", 1966.

Baldwin, E., "Dynamic Aspects of Biochemistry", 5th ed, Cambridge University Press, Cambridge, 1967, p 417.

Bligh, E. G., Dyer, W. J., *Can. J. Biochem. Physiol.* **37**, 911 (1959).

Brawn, V. M., Peer, D. L., Bently, R. J., *J. Fish. Res. Board Can.* **25**, 1803 (1968).

Brody, S., "Bioenergetics and Growth", Reinhold, New York, 1945, p 826.

Burton, B. T., "Human Nutrition", McGraw-Hill, New York, 1976, p 163.

Carrol, K. K., *J. Am. Oil Chem. Soc.* **40**, 413 (1963).

Childress, J. J., Nygaard, M., *Mar. Biol.*, *27*, 225 (1974).

Cummins, K. W., "Calorific Equivalents for Studies in Ecological Energetics", University of Pittsburg Press, Pittsburg, PA, 1967.

Cummins, K. W., Wuycheck, J. C., *Int. Assoc. Theoret. Appl. Limnol.* 18, 1 (1971).

Dame, R. F., *Proc. Natl. Shellfish. Assoc.* **62**, 86 (1972).

Folch, J., Lees, M., Sloane-Stanley, G. H., *J. Biol. Chem.* **226**, 497 (1957).

Golley, F. B., *Ecology* **42**, 581 (1961).

Guary, J. C., Kanazawa, A., *Comp. Biochem. Physiol.* **46A**, 5 (1973).

Platt, T., Brawn, V. M., Irwin, B., *J. Fish. Res. Board Can.* **26**, 2345 (1969).

Prus, T., *Polskie Archwm. Hydrobiol.* 17, 183 (1970).

Raymont, J. E. G., Austin, J., Linford, E., *J. Cons. Perm. Int. Explor. Mer.* **28**, 364 (1964).

Skinner, H. A., in "Biochemical Microcalorimetry", Brown, H. D., Ed., Academic Press, New York, 1969.

Teshima, S., Kanazawa, A., *Bull. Jpn. Soc. Sci. Fish.* **42**, 1129 (1976).

Thayer, G. W., Schaaf, W. E., Angelovic, J. W., LaCroix, M. W., *Fish. Bull.* 71, 289 (1973).

Tyler, A. V., *Mar. Biol.* **19**, 258 (1973).

Wadso, I., in "Biochemical Microcalorimetry", Brown, H. D., Ed., Academic Press, New York, 1969.

Watt, B. K., Merrill, A. L., "Composition of Foods", Handbook No. 8, Agricultural Research Service, Washington, DC, 1963.

R. V. Krishnamoorthy
A. Venkataramiah*
G. J. Lakshmi
Patricia Biesiot

Gulf Coast Research Laboratory
Ocean Springs, Mississippi 39564

Received for review June 29, 1978. Accepted April 10, 1979.

Hydroxylation of Monochloroaniline Pesticide Residues by *Fusarium oxysporum* Schlecht

Metabolism of 2-, 3-, and 4-chloroanilines by isolated cultures of the soil fungus *Fusarium oxysporum* Schlecht, and chlorpropham in soil was investigated. Hydroxylated (phenolic) products were detected colorimetrically, extracted, and characterized by thin-layer and gas chromatography and mass spectrometry. The presence of ortho-hydroxylated chloroanilines in *F. oxysporum* culture solutions was established by a characteristic ortho-elimination process which resulted in the formation of chlorinated benzoxazolines during mass spectral analysis of acylated derivatives. 2-Amino-4-chlorophenol and 2-amino-5-chlorophenol were positively identified as metabolites of 3- and 4-chloroaniline, respectively, in *F. oxysporum* culture solutions by comparison of the mass fragmentation pattern with acylated reference standards. Chloroaminophenols were also detected as metabolites of 2-chloroaniline in *F. oxysporum* culture solutions and from chlorpropham-treated soil.

Chloroaniline-based pesticides may be degraded in soils by a variety of processes. Hydrolysis of several chloroaniline-based pesticides by soil microorganisms yields the chloroaniline moiety as the leaving aromatic group. The aniline moiety has been the subject of intensive investigation in soils and with isolated microbial cultures. Metabolism of 4-chloroaniline by cultures of *Fusarium oxysporum* results in oxidation of the amino group to a nitro group, and the formation of 4-chloronitrobenzene (Kaufman et al., 1973). Aromatic ring hydroxylation and acetylation or formylation of the amino and hydroxyl groups can also occur (Kaufman et al., 1972). A soil bacterium, *Bacillus firmus*, converted 4-chloroaniline to 4-chloroacetanilide, 4-chloropropionanilide, and 2-amino-7-chloro-3-hydroxy-3*H*-phenoxazine (Englehardt et al., 1977). Briggs and Walker (1973) tentatively identified a phenoxazinone from a soil bacterium which metabolized 4-chloroaniline and suggested a hydroxylation occurred ortho to the amino group yielding 2-amino-5-chlorophenol, although the phenol was not isolated. Ambrosi et al. (1977) also reported the detection of phenoxazinones in soil.

It has been suggested that microbial peroxidases in soils are reponsible for the transformation of chloroanilines to chloroazobenzenes (Bartha and Bordeleau, 1969; Kaufman et al., 1972). The subject of this paper is the identification and characterization of some of the hydroxylation products formed during metabolism of 2-, 3-, and 4-chloroanilines by cultures of *Fusarium oxysporum*. 2-Chloroaniline is a potential degradation product of the fungicide Dyrene [2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine], whereas 3- and

0021-8561/79/1427-1127$01.00/0 © 1979 American Chemical Society