PROCEDURE

Weigh 15.0 ± 0.1 g of sample into a beaker, dissolve in chloroform, and transfer to 500-ml glass stoppered graduated cylinder.

Rinse beaker thoroughly with chloroform and add rinsing to the graduated cylinder. Add chloroform to cylinder until volume is 200 ml.

Add 200 ml of water, stopper and shake vigorously for one minute. Set aside until phases separate.

When 125 ml or more of the water phase has separated pipet 100 ml of the aqueous phase into an Erlenmeyer flask.

Add 1 ml of phenolphthalein indicator and titrate with 0.5*N* NaOH to end-point.

CALCULATIONS

$$\% \text{ Free Lactic Acid} = \frac{S \times N \times 9.008}{W}$$

S = Titration of sample.
N = Normality of NaOH solution.
W = 7.5 when 15.0 g is weighed for analysis.

Precision

A series of 15 samples of lactylated monoglycerides (10–25% lactic acid) and a series of 12 samples of shortening containing lactylated monoglyceride (approximately 1% lactic acid) were analyzed in two laboratories for total lactic acid by the proposed method.

The 95% probability limits for the difference between single analyses in the two laboratories were 0.36% for the low level and 1.46% for the high level. The standard deviation was 0.13% for the low level and 0.51% for the high level.

REFERENCES

1. Official and Tentative Methods, Assoc. of Official Agri. Chemists—8th Ed., pp. 244–46, 1955.
2. Barker, S. B., and W. H. Summerson, J. Biol. Chem., *138*, 535 (1941).
3. Bonting, S. T., Arch. Biochem. & Biophys., *56*, 307 (1955).
4. Presented by R. J. Buswell at the AOCS meeting in Chicago, Ill., 1958.

[Received July 27, 1962—Accepted March 29, 1963]

Caloric Availability and Digestibility of New-Type Fats

A. N. BOOTH, Western Regional Research Laboratory,[1] Albany, California, and A. T. GROS, Southern Regional Research Laboratory,[1] New Orleans, Louisiana

Abstract

Digestibility and caloric availability of new-type fats were determined by feeding these products to young rats while on a restricted caloric intake. Amylose stearate, amylose palmitate, amylose oleate, distearin adipate, and glycerol adipate were low in both categories. Diolein fumarate was completely digested but poorly utilized. These materials were compared with common oils and fatty acids. These new-type fats have potential use as pan greases and surface coatings for foods.

Introduction

ALTERED FAT PRODUCTS including amylose esters (1), diglyceride esters of succinic, fumaric, and adipic acids (2,3,4), and polymeric fats from stearic, oleic, and short-chain dibasic acids (5), have been prepared at the Southern Regional Research Laboratory. The amylose-containing esters may be useful as dip-type coatings on foods. The dibasic acid containing polyesters and diglyceride esters are also potentially edible fats and may be used as pan greases, slab dressings, or surface coatings for foods.

Little work has been done to determine the extent to which these products are digested and utilized in the animal body. Shull et al. (6) have reported that two types of adipic acid esters of glycerides have high digestibility coefficients and that the rate of oxidation of the stearic acid is greater when fed as the diglyceride adipate than as the polyester. In the present report, use has been made of the caloric availability assay devised by Rice et al. (7) to measure digestibility and utilizability of several new-type fats.

Experimental

Preparation and properties of the modified fats used in this work have been described previously (1–5). Cottonseed oil, corn oil, glucose, amylose, and palmitic, stearic, oleic, and adipic acids were included for comparative purposes.

The biological evaluation described by Rice et al. (7) measures the available energy of a test substance in terms of 1-week gains in weight of young rats fed calorically restricted diets under carefully controlled conditions. Young albino rats of either sex were housed in individual wire-bottom cages and fed a complete diet, the daily intake of which was restricted so that very little gain in weight was possible unless a supplement (caloric source) was added. The basal diet consisted of the following ingredients in per cent: sucrose 50, crude casein 40, cystine 0.3, salts USP XIV 4, corn oil 3, and a complete vitamin mixture 2.7. After an adjustment period of 5–7 days on 5 or 5.5 g of diet per day, all rats weighing 75-85 g were divided into uniform groups of 5. Then for a period of 7 days each rat received daily the basal diet or the basal diet plus a test supplement in an amount shown in Table I. A test group's gain in weight over that of the control group is a measure of the caloric availability of the supplement.

Since a number of the fat derivatives were found to be poorly digested, a modification of the Rice procedure was introduced in order to adjust the 7-day weight gain for 1) undigested intestinal residues of the supplement fed, and 2) abnormal hydration of either the body tissues or the intestinal contents (8). This modification was accomplished by changing all rats to the basal diet for a period of 48 hr at the end of the 7-day test period. During the 48-hr period, any loss in weight greater than that of the control group was interpreted as being a false-positive gain, for the reasons cited above, and was deducted from the 7-day weight gain in order to arrive at the actual gain from utilization of the supplement.

A sheet of filter paper (9 × 11 in.) backed by a thin plastic sheet was placed under each cage in order to collect the urine and feces. Collection was started the second day of the test period and continued to the

[1] Laboratories of the W. & So. Utiliz. Res. & Dev. Div., ARS, U.S.D.A.

TABLE I

Caloric Availability and Digestibility of Various New-Type Fats and Related Materials

Supplement	Amount g/rat/day	Body weight change in grams			Fecal wt increase (5 rats/7 days)	Digestibility [c] %
		Test period (5 rats/7 days)	Post-assay period [a] (5 rats/2 days)	Net change on supplement [b]		
					g, dry wt	
(Trial 1)						
Basal only	5.0	− 6	−11	(base)	(base)	....
+ corn oil	0.5	+37	− 9	+45	0	100
+ cottonseed oil	0.5	+37	− 7	+47	0	100
+ stearic acid	0.5	+ 3	− 5	+15	11.7	33
+ palmitic acid	0.5	+15	−10	+22	6.9	61
+ oleic acid	0.5	+28	−10	+35	0	100
+ amylose	0.5	+14	−14	+17	2	88
+ amylose stearate	0.5	+ 6	−15	+ 8	14.6	17
+ amylose palmitate #23	0.5	+11	−15	+13	14.2	19
+ amylose oleate	0.5	+ 2	−14	+ 5	12.5	29
(Trial 2)						
Based only	5.0	+ 6	+ 5	(base)	(base)	....
+ corn oil	0.5	+44	+ 4	+37	0.5	97
+ amylose palmitate #23	0.28	+13	+ 1	+ 3	6.7	32
+ amylose palmitate #34	0.5	+12	0	+ 1	15.2	13
+ amylose stearate	0.5	+14	+ 4	+ 7	13.9	21
+ succinostearin polymer	0.5	+28	+ 4	+21	10.4	41
+ glycerol adipate	0.5	+35	− 4	+20	diarrhea	?
+ distearin adipate	0.28	+ 6	+ 8	+ 3	10.1	0
(Trial 3)						
Basal only	5.5	+16	+ 8	(base)	(base)	....
+ cottonseed oil	0.28	+32	+ 7	+15	0.6	94
+ succino-olein	0.5	+36	+ 3	+15	3.2	82
+ adipostearin	0.5	+33	+ 6	+15	6.3	64
+ diolein fumarate	0.5	+22	+ 5	+ 3	0.1	100
+ distearin adipate	0.5	+20	+ 2	− 2	14.5	17
+ glycerol adipate	0.5	+22	+ 5	+ 3	diarrhea	?
(Trial 4)						
Basal only	5.0	+ 3	− 2	(base)	(base)	....
+ D-glucose	0.5	+15	− 4	+10	0	100
+ D-glucose	1.0	+25	− 5	+19	0	100
+ adipic acid	0.5	(wt. loss)	....	....	....	....
+ cottonseed oil	0.75	+59	− 3	+55	0.1	100
+ adipostearin	0.75	+33	−17	+15	11.0	58
+ adipo-oleostearin	0.75	+49	− 4	+44	2.1	92
+ adipo-olein	0.75	+57	−12	+44	diarrhea	?

[a] All rats were on basal diet during post-assay period.
[b] Net change equals change during test period plus change during post-assay period minus change in groups on basal diet during the same periods.
[c] Digestibility = I−F/I×100 where I = group total intake of supplement and F = fecal weight in excess of output by control groups.

end of the 48-hr post-assay period. This allowed sufficient time for any non-utilized material to be excreted. At the end of the collection period, the feces were removed from each filter paper, pooled by groups, and dried at 100C to constant weight. The urine-containing sheets were dried 24 hr in a vacuum oven at 70C prior to weighing. From the weight of moisture-free feces excreted, the digestibility of the supplement could be calculated by the equation: $I - F/I \times 100$ where I = group total intake of supplement and F = fecal weight in excess of output by control groups.

Results

Four separate trials are reported in Table I. In the first trial, stearic, palmitic, and oleic acids and their corresponding amylose esters are compared. The 3 amylose esters were poorly digested (17–29%), although utilization (weight gain) of that portion which was digested was quite good, except for amylose oleate (+5).

In the second trial, amylose palmitate #23 and amylose stearate were repeated. Also included was a third amylose ester, amylose palmitate #34, which contained only 64% palmitoyl groups, compared with 75.2% for ester #23. The results indicate that all 3 of these esters were poorly digested, and that ester #34 was the least well utilized for growth. The succinostearin polymer that was digested (41%) was well utilized for growth (+21). The digestibility of glycerol adipate could not be determined due to diarrhea, however, it was well utilized. The entire amount of bis[1-(hydroxymethyl)-2-(stearoyloxy) ethyl] adipate was accounted for in the stool.

In the third trial, female rats were used, and the growth response to glycerol adipate was greatly reduced in comparison with the result obtained in the second trial (+3 vs. +20). However, the observed diarrhea could affect responses when growth is used as the criterion of activity. The distearin adipate tested in trial 3 was actually bis [1-(stearoyloxymethyl)-2-(stearoyloxy)ethyl] adipate and contained 4 stearoyl groups rather than 2 as was the case in trial 2. All but 17% of this compound was accounted for in the feces and no evidence of utilization was detected (−2). Both succino-olein and adipostearin at the 0.5-g levels of intake produced weight gains equal to the weight gain obtained from 0.28 g of cottonseed oil. Their digestibility values were lower than that of cottonseed oil, however. Bis [1-(oleoyloxymethyl)-2-oleoyloxy) ethyl] fumarate (diolein fumarate) was completely digested, yet the weight gain was indicative of poor utilization. A better understanding as to the metabolic fate of this latter compound will require further study.

In trial 4, three diglyceride esters of adipic acid were evaluated, namely adipostearin, adipo-oleostearin, and adipo-olein. Only 58% of the adipostearin was digested and the caloric availability was comparable to an equal weight of D-glucose. Adipo-olein and adipo-oleostearin were well digested and utilized, although diarrhea was noted in the case of adipo-olein.

Weights of the urine solids varied only slightly, and no increases were noted between supplemented and unsupplemented groups. The data are not presented.

Discussion

Generalizations based on these preliminary data would be premature. Several of the new-type fats, including amylose esters, succinostearin and adipostearin, might meet a current demand for low caloric foods because of their low digestibility values. On the other hand, there is little doubt that the portion of the modified fat that is digested is capable of being

utilized for growth to some extent. Eventually it may be possible to calculate the percentage utilization of these products when the caloric availability of the component parts of the new-type fat is determined. Thus about 20% of the amylose stearate containing 18% amylose was digested. If amylose provides 4 calories per g and stearic acid 9 cal per g, then 20% of a 0.5-g supplement of amylose stearate could actually yield only 0.8 cal of energy to the animals which would be equivalent to 0.2 g of glucose.

The data emphasize the importance of the modification in the caloric availability assay that was introduced to detect false-positive weight gains for certain supplements. This was especially true for the 0.75-g adipostearin-supplemented group (−17) and the 0.75-g adipo-olein group (−12).

These data agree well with digestibility values reported by Shull et al. (6) for the diglyceride adipate, adipo-oleostearin.

REFERENCES

1. Gros, A. T., and R. O. Feuge, JAOCS, *39*, 19–24 (1962).
2. Feuge, R. O., and T. L. Ward,, J. Am. Chem. Soc., *80*, 6338–6341 (1958).
3. Ward, T. L., A. T. Gros, and R. O. Feuge, JAOCS, *36*, 667-671 (1959).
4. Feuge, R. O., and T. L. Ward, *Ibid.*, *37*, 291–294 (1960).
5. Feuge, R. O., and A. T Gros, Ind. Engr. Chem., *51*, 1019–1022 (1959).
6. Shull, R. L., L. A. Gayle, R. L. Coleman, R. B. Alfin-Slater, A. T. Gros, and R. O. Feuge, JAOCS, *38*, 84–86 (1961).
7. Rice, E. E., W. D. Warner, P. E. Mone, and C. E. Poling, J. Nutr., *61*, 253–265 (1957).
8. Booth, A. N., Fed. Proc. *21*, 90 (1962).

[Received January 24, 1963—Accepted April 1, 1963]

Cis-Trans Isomerization of Oleic, Linoleic and Linolenic Acids[1]

CARTER LITCHFIELD, MRS. J. E. LORD, A. F. ISBELL,[2] and RAYMOND REISER, Department of Biochemistry & Nutrition, Texas Agricultural Experiment Station, College Station, Texas

Abstract

The equilibrium composition of *cis* and *trans* isomers obtained by isomerizing oleic, linoleic, and linolenic acids with selenium or nitrous acid has been studied using gas chromatography and infrared spectroscopy. The oleic/elaidic equilibrium mixture was found to contain 75–80% elaidic acid instead of the generally accepted 66% value. It is felt that the greater accuracy of gas chromatography and infrared analyses over older methods allows this equilibrium to be defined with greater precision.

Similar studies on the *cis-trans* isomerization of linoleic and linolenic acids indicated that their equilibrium mixtures also contained 75–80% *trans* double bonds. With linoleic acid, these *trans* bonds were shown to be randomly distributed among the double bonds present.

Cis-trans isomerization of linoleic or linolenic acids with selenium produced by-products having elution times equivalent to 18:2, 18:1, and 18:0 on a gas chromatograph. No such by-products were observed when oleic acid was isomerized. Apparently some type of hydrogen-transfer reaction accompanies the *cis-trans* isomerization of polyunsaturated acids with selenium.

Introduction

DURING RECENT WORK in this laboratory on the *cis-trans* isomerization of natural fats, it became necessary to know the maximum amount of *trans* bonds which such a process could introduce into the naturally-occurring *cis* fatty acids. It has long been recognized (1,2) that the *cis-trans* isomerization of unsaturated fatty acids is an equilibrium reaction, and that the complete conversion of all *cis* bonds to *trans* bonds in one reaction is impossible. Therefore, we set out to define the equilibrium concentration of geometric isomers for the three most common unsaturated fatty acids: oleic, linoleic, and linolenic.

Griffiths and Hilditch (1) studied the *cis-trans* isomerization of oleic, elaidic, petroselinic, and erucic acids using nitrous acid and sulfur as catalysts. They reported that the maximum amount of *trans* acid present at equilibrium was 66%. Bertram (2) reached similar conclusions after studying the isomerization of oleic and elaidic acids with selenium. It is now generally accepted that the equilibrium ratio of elaidic to oleic acid is 2:1, and that these equilibrium concentrations are independent of catalyst and processing conditions. However, the analytical techniques available to these workers 25–30 years ago were considerably less sophisticated than those available today; and, as Harwood (3) has recently pointed out, a reinvestigation of the oleic/elaidic equilibrium has been long overdue. Modern techniques such as gas-liquid chromatography (GLC) (4,5,6) and infrared spectroscopy (7) can now give a far more accurate picture of the geometric isomers present in *cis-trans* isomerization reaction mixtures.

The literature does not provide precise information as to the equilibrium ratio of *cis* and *trans* isomers for linoleic acid. MacGee, Mattson, and Beck (8) isomerized ethyl linoleate with SO_2 and were able to reduce the content of the 9-*cis*, 12-*cis* isomer to as low as 7%. They reported an 87% conversion of *cis* bonds to *trans* bonds based on infrared analysis of their reaction product. Subrahmanyam and Quackenbush (9) recently reported that approximately a 2:1 *trans* to *cis* ratio was achieved after the isomerization of ethyl linoleate with selenium. However, neither MacGee et al. (8) nor Subrahmanyam and Quackenbush (9) corrected their values for all the non-9, 12-octadecadienoate by-products present in their reaction mixtures, so that their equilibrium values are only approximate. *Cis-trans* isomerization is known to be accompanied by conjugation (8,9), polymerization (9), and catalyst addition products (1,10,19). On the basis of theoretical steric considerations, Blekkingh (11) predicted that the equilibrium ratio of *cis* and *trans* isomers for isomerized linoleic acid would be[3]: 17.6% 18:2—*9c,12c;* 17.6% 18:2—*9c,12t;* 17.6%

[1] Presented at the AOCS meeting in Toronto, Canada, 1962.
[2] Dept. of Chemistry, A & M College of Texas, College Station, Texas.

[3] For brevity, the geometric isomers of oleic, linoleic, and linolenic acids are referred to in this paper by a shorthand designation. For example, 18:2—*9t,12c* refers to 9-*trans*, 12-*cis*-octadecadienoic acid. See Table I for a complete listing of such shorthand designations.