carp oil composition, whereas that obtained from $F_1 \times M$ exhibited a typical F_1 hybrid oil composition. The report claimed that this would appear to account for mesocarp unsaturation in accordance with the laws of Mendelian inheritance. Actually, the crosses performed by McFarlane et al. (3) are backcrosses and the law of Mendelian inheritance is Mendel's Second Law of Independent Assortment. What McFarlane et al. obtained for the backcrosses are at variance with our results and their interpretations contradict with co-dominance, which they had suggested. Our results show that the backcross ratio of 1:1 (F_1:G or F_1:M) as predicted by the Co-Dominance Theory is obtained (Table IV, A and B, and Table VI, A and B, for saturated and unsaturated palmitate and oleate, respectively), and not one which exhibited a typical *E. guineensis* (G) mesocarp oil composition and one which displayed a typical F_1 hybrid oil composition.

It would appear that the Co-Dominance Theory of this paper is the genetic equivalent to Gunstone's theory 1 of the Restricted Random Distribution Theory. The predictions of the Co-Dominance Theory are supported very well by the intermediate nature of fatty acids and triglycerides; the co-dominance ratio prediction is supported in almost all the results, as well as in the backcross ratios between F_1 and the 2 parentals.

This Co-Dominance Theory may be more generally applicable for most vegetable oils and fats (especially *Elaeis* palm kernel oils) in light of its successful predictions for mesocarp oils from *Elaeis* palms.

ACKNOWLEDGMENT

We thank the Science University of Malaysia for support of this project and the plantation companies—Harrison and Crosfield (Malaysia) Ltd., Socfin Ltd., Chemara Research Station and the United Plantation Ltd. for supply of oil samples. Also, thanks to F.D. Gunstone for helpful discussion.

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[Received July 6, 1979]

Study on Neutral Lipid Composition of Malagasy Zebu Fats: I. Quantitative Analysis of Fatty Acids and Sterols

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ABSTRACT

The fatty acid and sterol compositions of different parts of Malagasy zebu (*Bos indicus*) were evaluated. Investigation by gas liquid chromatography using Carbowax 20 M revealed 35 fatty acids, mainly palmitic (24-27%), stearic (13-24%), and oleic (25-37%) acids. Odd-numbered, iso and anteiso fatty acids were also detected. Small differences in composition were observed between the hump and the kidney fats of *B. indicus*. Comparison between industrial tallow of *B. indicus* and *B. taurus* revealed slight differences in the stearic/oleic acid ratio (0.83 and 0.46, respectively). An OV 17 column was used to separate 9 sterols, mainly cholesterol (89-98%). β-Sitosterol was also found at lower concentrations in all the samples.

INTRODUCTION

With a livestock of more than 10 million animals, meat consumption is rather high in Madagascar, in comparison with some African and Asian countries (1). Madagascar also exports chilled, frozen and canned meat (corned beef). The livestock essentially comprises bovines since there are only 230,000 caprines and 200,000 ovines. The Malagasy zebu (*Bos indicus*) is characterized by its small size (averaging 350 kg weight when being slaughtered). Withers are topped by humps of various volume, depending on sex (bigger for male) and the fattening stage of the animal. The zebu hump tissue is essentially adipose (60-85%).

There has been no previous work on fatty acid and sterol patterns of zebu lipids. We studied the neutral lipid composition of several samples of different parts of malagasy zebu (hump, kidney fat, industrial tallow) and compared the obtained results to those better known of *B. taurus* (2). The first published results cover the quantitative analysis of fatty acids and sterols of those samples.

EXPERIMENTAL PROCEDURES

Four samples of zebu hump tissue and one sample of kidney fat were collected from a local market in Antananarivo. Industrial tallow samples of *B. taurus* (Laboratoire Interrégional de la Repression des Fraudes et du Contrôle de la Qualité, Marseilles, France) and industrial bone tallow samples of *B. indicus* (Prochimad Co., Madagascar) were obtained.

Fat tissues were extracted with hexane in a Soxhlet apparatus for 12 hr and the solvent was removed using a rotary vacuum evaporator. In some cases, melted lipid samples of zebu hump tissues were obtained in a drying

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oven at 113 C.

Extraction of unsaponifiable matter was carried out according to published methods (2,3).

Fatty acid methyl esters were prepared by saponification of triglycerides and acid-catalyzed methylation using BF_3-CH_3OH according to Metcalfe and Schmitz (4). Purification of the methyl esters was done by depositing 100 μL of raw product on a Silica Gel 60 Chromatoplate (0.25 mm gel thickness, 20 x 20 cm, E. Merck, Darmstadt, W. Germany) using a hexane/ethyl ether mixture (80:20, v/v) as developing solvent. The plate was visualized with Rhodamine-B. The spot corresponding to methyl esters was scraped off and extracted with hexane. For the identification of methyl esters, vegetable oils (e.g., olive, peanut, sunflower) were used as standards and also commercial, saturated even-numbered methyl esters (Fluka, Buchs, Switzerland), unsaturated and polyunsaturated methyl esters (Sigma, St. Louis, MO).

A Girdel Model 30 gas chromatograph (92 800 Puteaux, France) equipped with a flame ionization detector was used for the analysis. The column employed was a 50 m long, 0.25 mm id glass capillary column coated with Carbowax 20 M. Temperatures used were: 180 C for column and 270 C for inlet and detector ovens. Inlet pressure of hydrogen used as carrier gas was 0.8 bar (split: 5/100). Peak areas were integrated by an LTT ICAP 5 electronic integrator (Girdel, 92 800 Puteaux, France).

Sterols were separated from other unsaponifiable matter by thin layer chromatography (TLC) (5). Unsaponifiable matter in isopropyl ether was washed with water to neutrality. The organic phase was dried and evaporated under a stream of nitrogen with a rotary vacuum evaporator. The residue was dissolved in carbon tetrachloride to give a 5% solution. Testing was done by depositing 150 μL of the solution on a 0.25 mm thick, 60 F254 silica gel plate (E. Merck) and developing using an ethyl ether/chloroform (10:90, v/v) solvent system. Cholesterol used as standard was spotted for the identification of sterols. The developed plate was sprayed with Rhodamine-B and bands were examined under 366 nm ultraviolet (UV) light. The sterol band was traced and sterols were scraped off and extracted with dichloromethane. Trimethylsilyl ether derivatives of components (50 μL of a solution of 0.5 mL pyridine, 0.45 mL of hexamethyldisilazane (HMDS) and 0.3 mL of trimethylchlorosilane (TMCS)) were then injected at 265 C into a 40-m glass capillary column filled with OV 17 (1.5%). Temperatures used were 280 C for inlet and 270 C for detector ovens. The injection averaged about 3-5 μL. Relative retention time (RRT) was expressed by the ratio of the retention time for the trimethylsilyl ether derivative of substance under examination to the retention time for trimethylsilyl ether derivative of cholesterol.

RESULTS AND DISCUSSION

The neutral lipids investigated were obtained by melting or by extracting with hexane. The fatty acid composition was determined by GLC using a glass capillary column coated with Carbowax 20 M. The corresponding equivalent chain length (ECL) (6,7) was calculated for each methyl ester peak. Identification of the peaks was made using known vegetable oils as standards, commercial methyl esters, and comparing our results with those obtained by Flanzy et al. (8), Mordret et al. (9) and Jeager et al. (10). As shown in Table I, our ECL values for iso 18, 18:1ω9, 18:1ω7, 18:2ω6 . . . are in good agreement with those obtained by Mordret et al. (9), but in some cases, doubts remain about the identification of a fatty acid (see Table II). The peculiarity of the fatty acid composition of ruminant fat is that it contains *trans*-isomers (formed from *cis*-fatty acids during hydrogenation by ruminal microorganisms). The infrared (IR) spectra of animal fats showed an absorption band at 967 cm^{-1} due to isolated *trans* unsaturation (11). However, the separation of *cis* and *trans* isomers is generally poor on Carbowax columns (8).

The fatty acid composition of the neutral lipids of Malagasy zebu hump tissues, kidney fat and industrial tallows of *B. indicus* and *B. taurus* is reported in Table II. We investigated 35 fatty acids, but focused on palmitic (24-26%), stearic (16-29%) and oleic (23-36%) acids. These results agree with other previously studied beef fats (2,8, 12-14). We found also odd-numbered (C_{15}, C_{17}), iso (C_{14}, C_{15}, C_{16}, C_{17}, C_{18}) and anteiso (C_{15}, C_{17}) fatty acids. Nonadecanoic acid was not observed in all samples. Small differences were observed in the composition of stearic and oleic acids of melted and extracted lipid samples. The differences between raw and purified methyl esters were insignificant.

In Table II, industrial bone tallow of *B. indicus* and *B. taurus* were compared. A higher percentage of stearic acid and a lower percentage of oleic acid were observed in the case of *B. indicus*. The ratios of 18:0/18:1ω9 were, respectively, 0.815 for industrial zebu bone tallow and 0.457 for industrial beef tallow.

Nine sterols were investigated using an OV 17 glass capillary column. The RRT was expressed against cholesterol (Table III). Identification was made using mixtures of known sterols as standards (5,15). The most important was cholesterol (89-98%), which is in agreement with the literature (2,9,16). An unknown substance (RRT=1.25) was detected in all the samples, at lower concentrations (0.4-

TABLE I

Equivalent Chain Lengths of Some Fatty Acids of the C_{18} Series on Various Phases

	Type of capillary column					
	Glass FFAP (10)	Steel Carbowax 20 M	Glass Carbowax 20 M (9)[a]			Glass Carbowax 20 M
Fatty acid		AT (8)	1	2	3	(this work)
Iso 18	17.50	17.53		17.51		17.50
18:1ω9*c*	18.10	18.20	18.20	18.13	18.11	18.14
18:1ω9*t*	18.14	–	–	–	18.16	–
18:1ω7	16.18	18.26	18.28	–	–	18.20
18:1ω6	–	18.33	18.34	–	–	18.31
18:2ω6	18.50	18.63	–	18.58	–	18.58
18:3ω6	18.76	18.91	–	18.90	–	18.93
18:3ω3	19.10	19.25	–	19.23	–	19.11
18:4ω3		19.53				19.37

[a]Results obtained on 3 different columns.

TABLE II

Fatty Acid Analysis (% by wt) of Hump and Kidney Neutral Lipids and Industrial Tallows of *Bos indicus* and *Bos taurus*

Fatty acid	ECL[a]	Hump[b]		Kidney fat[b]	Industrial tallows[b]	
		Melted[c]	Extracted[d]	Melted	*Bos indicus*	*Bos taurus*
< Iso 14		0.6	0.9	0.4	0.2	0.7
Iso 14	13.55	0.2	0.3	0.2	0.2	0.2
14:0		3.2	3.5	3.9	2.2	3.5
14:1ω5	14.35	0.8	0.6	0.3	0.2	0.7
Iso 15	14.56	0.5	0.5	0.6	0.5	0.3
Anteiso 15	14.70	0.4	0.5	0.6	0.5	0.3
15:0		0.7	0.7	0.9	0.9	0.5
Iso 16	15.53	0.4	0.4	0.4	0.5	0.3
16:0		25.0	25.6	26.3	22.8	24.3
16:1ω9	16.19	0.1	0.3	0.2	0.3	0.2
16:1ω7	16.24	3.2	2.3	1.4	1.8	3.0
16:1ω5	16.36	0.2	0.4	tr	tr	0.1
Iso 17	16.52	0.6	0.7	0.5	0.7	0.4
Anteiso 17	16.66	0.9	0.8	0.9	0.8	0.7
17:0		1.4	1.6	1.9	1.9	1.2
17:1ω8	17.18	0.9	0.7	0.5	0.7	0.7
Iso 18	17.50	0.2	0.2	0.2	0.3	0.2
18:0		17.2	21.5	29.0	25.1	16.5
18:1ω9	18.14	33.2	29.0	22.7	30.8	36.0
18:1ω7	18.20	4.8	4.9	4.2	5.0	5.0
X_1	18.28	0.4	0.3	0.8	0.5	0.7
18:1ω6	18.31	0.3	0.3	–	–	0.2
18:1ω5	18.37	0.2	0.1	0.3	0.2	0.2
X_2	18.42	0.3	0.2	0.2	0.2	0.2
18:1ω4?	18.48	0.3	0.4	0.6	0.1	3.0
18:1ω3?	18.53	0.8	0.7	0.9	1.7	–
18:2ω6	18.58	0.4	0.3	0.2	0.3	0.2
18:2ω5?	18.72	0.4	0.5	0.4	0.3	0.1
18:3ω6	18.93	0.3	0.2	0.1	0.3	0.2
X_3	19.06	0.3	0.2	0.3	0.2	0.3
18:3ω3	19.11	0.7	0.5	0.2	0.4	0.5
18:4ω3	19.37	0.9	0.5	0.4	0.1	0.3
X_4	19.90	0.2	0.2	0.2	0.3	0.1
20:0		0.2	0.3	0.4	tr	0.2
20:1ω9	20.05	0.1	0.1			
Ratio 18:0/18:1ω9					0.815	0.458

[a]ECL:equivalent chain lengths of fatty acid methyl esters on Carbowax 20 M glass capillary column.
[b]Raw methyl esters.
[c]Mean of 4 tissues.
[d]Mean of 2 tissues.

TABLE III

Sterol Analysis of Various Fat Samples of *Bos indicus* (% by wt)

Sterol	RRT[a]	Hump (4 tissues)				Kidney fat	Industrial tallow
		1	2	3	4		
Cholesterol	1.00	89.2	97.9	91.9	89.3	95.9	98.2
Brassicasterol	1.16	–	–	–	–	–	0.2
?	1.25	6.0	0.9	2.9	1.9	1.4	0.4
Campesterol	1.29	1.3	tr	0.5	1.0	tr	0.4
Stigmasterol	1.39	–	–	–	0.8	–	–
β-Sitosterol	1.59	3.5	1.2	4.7	6.0	2.7	0.8
Δ5-Avenasterol	1.76	–	–	–	0.4	–	–
Δ7-Stigmastenol	1.86	–	–	–	0.4	–	–
Δ7-Avenasterol	2.06	–	–	–	0.2	–	–

[a]RRT is expressed by the ratio of the retention time for the trimethylsilyl ether derivative of substance under examination to the retention time for trimethylsilyl ether derivative of cholesterol.

6.0%). β-Sitosterol (0.8-0.6%) and traces of campesterol were found in all the samples. Δ7-Stigmasterol, Δ5- and Δ7-avenasterol were found in only one tissue of zebu hump.

ACKNOWLEDGMENTS

The "Laboratoire Interregional de la Repression des Fraudes et du Contrôle de la Qualité de Marseille" helped supply samples of various genuine vegetable oils and tallow samples of *Bos taurus*, and Prochimad Co. (Madagascar) supplied industrial samples of zebu bone tallow. Also, we acknowledge the financial assistance of "Fonds d'Aide et de Coopération" (France) which made R. Ramananarivo's stay possible.

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[Received January 14, 1981]

Desolventizing-Toasting of Extracted Soybean Flakes: Development of Pilot Plant Equipment and Operational Procedure

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ABSTRACT

A pilot plant batch desolventizer-toaster (D-T) was designed and built with the intent of producing soybean meals of varied composition, as well as to simulate meals produced in a continuous commercial D-T unit. Trial runs were made first to determine workable loading levels, temperature control and sparge steam generation. Moisture levels after the steam sparge were influenced by the residual hexane content of defatted hexane-wet flakes reaching the D-T. Two moisture levels were used in testing the effectiveness of the toasting operations in producing flakes with low urease activity and trypsin inhibitor levels. The trial runs reported here also provide basic data for current work designed to optimize toasting procedures to produce suitable meals for ongoing animal nutrition studies.

INTRODUCTION

In the desolventizing-toasting process for extracted soybean flakes, the final removal of the last traces of solvent, inactivation of enzymes and destruction of antinutritional factors occur to produce an animal feed with good nutritional quality. The process has been adopted worldwide by most processors of soybeans. Design and operation of a desolventizer-toaster (D-T) have been reported by Kruse (1,2), Cravens and Sipos (3), Sipos and Witte (4) and Milligan (5). In a commercial D-T, hexane-wet defatted flakes are rapidly heated, both indirectly and by sparge steam, in the top section of the D-T where most of the hexane is evaporated and steam is partly condensed on the flakes. Steam sparging, in addition to adding moisture to the flakes, minimizes dust carryover to the condenser, and results in a combination of solvent removal and meal toasting. Meal toasting is continued on the lower trays of the D-T where meal moisture is reduced by indirect steam heating.

Here we report on a pilot plant batch D-T that was intended to prepare meals with varied composition as well as to simulate the conditions and product of a continuous commercial D-T. To determine the effectiveness of the equipment, range of parameters and mode of operation to produce the meals of varied quality, 51 pilot plant runs were made. Four typically significant runs from this series are reported. Since the control methods developed in this report were reliable, a study (6) involving 48 meals of varied predetermined composition was conducted relating these compositions to their nutritional qualities.

[1] Presented at the ISF-AOCS World Congress, New York City, April 1980.

EXPERIMENTAL

Materials and Methods

Beeson variety seed-grade soybeans (1978 crop) and Skellysolve B petroleum naptha (hexane) were used for all runs. To prepare full-fat flakes for extraction, soybeans were cracked, dehulled, heat-tempered and flaked in pilot plant equipment to produce full-fat, dehulled soy flakes of 0.2540-0.3048 mm thickness. The flakes were extracted in a Kennedy pilot-plant extractor with a hexane:flake ratio of 2:1. Defatted flakes were drained on a slotted inclined drag-line conveyor before being discharged from the extractor.

Solvent concentration of the hexane-wet defatted flakes was determined by weighing the flakes before and after solvent vaporization under ambient conditions until odorless. A Brabender Moisture Tester (Brabender Corp., Haake, Inc., Saddle Brook, NJ) was used to measure moisture of defatted hexane-free flakes and meal products by drying the samples at 120 C for 1 hr. Moisture of the wet flakes after sparging was determined by drying the flakes in a forced draft oven at 105 C according to AACC Method 44-15A (72 hr) (7). This method was more accurate than the Brabender Moisture Tester for the partially agglomerated flakes. Urease activity for processed meal at various stages of toasting, reported as pH increase, was determined by AOCS Method Ba 99-58 (8). Trypsin inhibitor values for the meal product were determined according to the procedure of Hamerstrand et al. (9).

Equipment

Typical commercial D-T. A diagrammatic sketch of a com-