Triglyceride Characteristics of Cocoa Butter from Cacao Fruit Matured in a Microclimate of Elevated Temperature[1]

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ABSTRACT

Generally, the melting characteristic of cocoa butter is relatively constant. However, softer than normal butter is sometimes encountered. In Brazil the occurrence of soft butter has been correlated with mean daily temperature during the cropping season. The temperature effect was, therefore, studied more fully by positioning heat lamps near fruit of cacao to create a microclimate of elevated temperature during the most active period of lipid biosynthesis. As determined by differential scanning calorimetry, cocoa butter from these fruits, contained more solid fat at 16 C, and 20 C and 24 C than butter from control fruit matured in a normally lower temperature climate. The temperature effect on softness was in accordance with differences found in triglyceride types. S_2U and SU_2 triglycerides from heat-exposed samples were 88.5% and 8.9%, respectively, compared to 79.3% and 18.3% for cocoa butter from control fruit. Additional evidence of differences due to growth temperature was obtained by analysis of triglyceride fractions separated by high-performance liquid chromatography.

INTRODUCTION

Cocao butter melts just below body temperature within a narrow range. Approximately 90% of the triglycerides are S_2U and SU_2 (S, saturated fatty acid; U, unsaturated fatty acid), with the former predominating. Overwhelmingly, they are glyceride-2 unsaturated (1-4). Obviously, these triglyceride types are especially important relative to the melting characteristics of cocoa butter.

The manufacturing process for chocolate requires cocoa butter that does not vary greatly in melting behavior. Cocoa butter too soft to be easily utilized by confectioners is sometimes found, especially in crops from Bahia. Although the cause and effect relationship must be complex, evidence suggests that environmental temperature during development of cacao fruit is a factor. A survey of several locations in Bahia (Brazil) revealed slightly higher iodine values for cocoa butters from crops grown in regions where mean daily temperature was lowest (5).

Both cropping seasons in Bahia were included in a study of the compositional changes in the lipids of seeds during the growth and ripening of cacao fruit (6). Comparison between seasons was intended, in part, to determine if environmental temperature influenced chemical parameters and the melting behavior of cocoa butter. Unfortunately, expected temperature differences during the growth cycles did not occur, due to unusual weather conditions and logistical problems relative to the timing of pollinations to maximize temperature differences. Therefore, a controlled microclimate temperature study was carried out by focusing heat lamps or resistance heaters towards selected pods (fruit) on trees to raise environmental temperature during the latter stages of growth and ripening.

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MATERIALS AND METHODS

Test Plot Trials

The site of the study was Itabuna, Bahia, at the research center for cacao (CEPEC) of CEPLAC, the Brazilian agency charged with the rejuvenation and economic development of the cacao-growing region. Two trees in a test plot of Comum cacao were used in the trial. Heat lamps were focused on a portion of one tree, resistance heaters on the other. Pods, subjected to an elevated microclimate temperature, were 4 months old at the start of the trial, and they were located about 3 feet above the ground on a horizontal, main limb. At weekly intervals, the focus and distance of the heaters from the pods were adjusted to maintain a 9 C differential at the pod wall nearest the heat source. Adjustments were necessary, since the fruits grew and expanded towards the heaters over the 6-8 week period of the trial. Pods from the same trees, but distant enough to be unaffected by the heaters, served as controls.

Heat treatment was initiated mid-March, 1976, and was

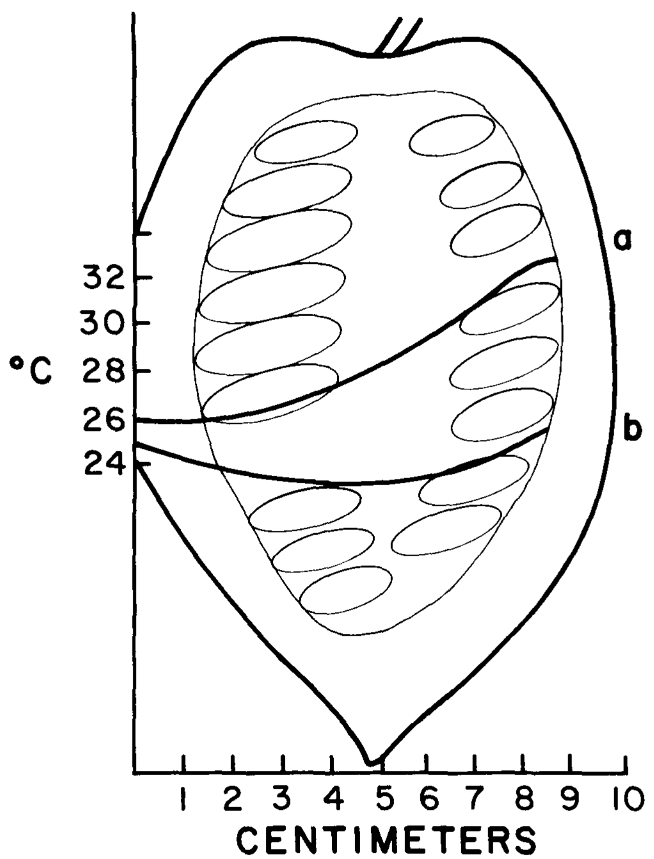

FIG. 1. Temperature gradient across fruit of cacao matured in a microclimate of elevated temperature compared to unheated (control) fruit: (a) Heat-exposed; (b) Control.

continued until the fruit ripened. Just before harvesting, the temperature gradient across the pods was determined by inserting a thermocouple probe at the midsection. Temperatures were recorded as the probe was backed out of the pod. Two samples were collected from each pod. One represented the seeds closest to the heat source; the other was from the cooler backside. The seeds were dried at 60 C for 3 days in a laboratory oven.

Analytical Methods

Preparation of Cocoa Butter Samples. Following the peeling away of shell material (testa), the cotyledon (nib) portion was pulverized and compressed between felt pads in a hydraulic laboratory press (F.S. Carver, Inc., Menomonee Falls, WI) equipped with a 5.7-cm (i.d.) stainless steel cylinder, previously heated to 200 C. Cocoa butter, pressed from the nibs (15,000 psig for 30 min), was collected in a stainless steel pan.

Solid Fat Determination. Melting characteristics of the cocoa butters were determined by differential scanning calorimetry (DSC). Samples (10 mg) in aluminum pans were tempered through a standardized heating and cooling regime, and heated to melt at 5 C/min in the DSC instrument (Perkin Elmer DSC-2). The thermogram was integrated at various temperatures to determine the percentage of fat remaining solid at a particular temperature (7 and H. Adams, personal communication, 1978).

Fatty Acid Composition. Boron trifluoride-methanol (14% w/v) was used to prepare fatty acid methyl esters (FAME), which were separated by gas liquid chromatography (Hewlett Packard Model 5830, flame ionization) on a 6' x 1/8" stainless steel column packed with 15% diethylene glycol succinate on acid-washed Chromosorb W (Supelco, Inc., Bellefonte, PA). Fatty acid composition was quantified using pentadecanoate as an internal standard or by comparison to a standard FAME mixture (K-108, Applied Science Laboratories, State College, PA).

Separation Into Triglyceride Fractions. Cocoa butter was dissolved in tetrahydrofuran, (180 mg/ml) after treatment to remove nonlipid contaminants (8). Separation of 25 μl into triglyceride fractions was accomplished by high performance liquid chromatography (HPLC). Effluent from the column (Waters Associates Triglyceride Analysis Column in a Waters Associates Model ALB/GPC 201 liquid chromatograph, Medford, MA) was monitored with a differential refractometer. Mobile phase was 85:15 (v/v) acetonitrile/tetrahydrofuran pumped at 2.0 ml/min. Each triglyceride fraction was quantified (triangulation method) and recorded as a percentage of the sum of peak areas for all fractions. Fractions were isolated for further analysis using the collection mode of the HPLC instrument.

Analysis for Triglyceride Types. Fatty acid distribution at the 2-position of glycerol was determined by subjecting cocoa butter triglycerides to lipolysis (9). These data were used in the calculation of triglyceride types for cocoa butter (2).

RESULTS AND DISCUSSION

Test Plot Trials

Dimensions and weights of cacao fruit, matured and ripened in a microclimate of elevated temperature, were similar to those for control pods. Likewise, the seeds and adhering mucilaginous pulp were indistinguishable. However, appearances of the fruits were different. Portions of those matured in the microclimate environment remained green and did not change to the yellow color that characterized the ripening phase of control fruits. Also, the pod wall closest to the heat source was thinner. These differences may reflect cellular damage and/or water stress in the pod wall caused by the heat treatment.

TABLE I

Solid Fat Content of Cocoa Butter at Various Temperatures by DSC Analysis. Comparison between Seeds from Fruit Matured in an Elevated Temperature Environment and Seeds from Control Fruit.

Sample treatment	% Solid fat at 16 C	20 C	24 C
Heated			
1	92	70	28
2	94	74	33
3	92	65	23
4	93	78	47
5	93	74	39
6	95	82	48
Control			
1	82	51	17
2	80	43	10
3	80	40	9
4	90	50	16
5	78	54	31
6	78	41	10

Mean temperature elevations (compared to controls), maintained for 8 fruits during the two months of treatment, were 9.5 C and 2.8 C for the exposed and backside surfaces, respectively. Temperature variation at the surface closest to the heat source was 6.5 C to 12.0 C above ambient. These surface temperatures were needed to maintain elevated temperature conditions throughout the fruits. Temperature gradients across heated and control ripe fruits, measured with an electronic thermometer and thermocouple, are shown in Figure 1. Temperature elevations were 7 C and 3 C for exposed and backside interior seed areas, respectively.

Melting Characteristics

The design of the microclimate trials was based on the supposition that the effect of growth temperature on melting behavior might be revealed by comparing the seeds from the exposed and backsides of individual pods. However, cocoa butters of seeds taken from both sides of six pods yielded DSC thermograms which were very similar. Solid fat contents, measured at various temperatures, were about the same as African butter. Apparently, even the 3 C increase for the seeds on the cooler side of the fruit was sufficient to induce a change in butter hardness. No differences were found between the use of heat lamps or resistance heaters to raise temperatures relative to cocoa butter characteristics.

When butters from cacao seeds taken from either side of heated pods were compared to control cocoa butters (no heat treatment), DSC thermogram differences were readily evident (Table I). Included are three butters from seeds on the hot side of fruits (heated 1, 2, and 3), and three butters from the cooler side (heated 4, 5, and 6). At each DSC measurement temperature, solid fat contents of butters from the heated pods were higher than for control samples.

The data in Table I demonstrate that environmental temperature had a pronounced effect on the physical properties of cocoa butter deposited during maturation and ripening of the seeds of cacao. Fruits used in the experiment were 4 months old (postpollination) when the temperature was increased around them. Data, previously presented (6), showed that most of the cocoa butter would by synthesized in the seeds during this heat treatment period. Thus, differences found could logically be attributed to the experimental treatment.

Fatty Acid and Triglyceride Type Comparisons

Lipids of tropical plants generally are more saturated than those synthesized by plants growing in cooler climates. Unsaturated fatty acids of rapeseed, sunflower, and flax increased markedly when growth temperature was reduced (10). They also increased in castor bean and safflower, but the change was much less pronounced. The data for cacao lipid in Table II show modestly more palmitate and stearate, and less oleate and linoleate for butter extracted from seeds of heated fruits compared to unheated controls. Linolenate actually increased slightly, but this may have been due to arachidate, which cochromatographed with linolenate. The temperature effect on cocoa butter was not of the magnitude reported for other seed oils. Sunflower grown at 22 C, compared to 27 C, contained 9% less linoleate, and an even greater decrease (12%) was noted for linolenate in flax (10).

DSC and FAME data (Tables I and II) were supportive in that greater saturation and "harder" butters resulted when fruits were matured under elevated temperature conditions, but more distinct differences in individual fatty acid contents had been expected. However, contrasts were pronounced when triglycerides, isolated by TLC, were subjected to lipolysis for 2-monoglyceride analysis (9), and calculation of triglyceride types (11). Table III shows a 9.2% increase in monounsaturated triglycerides (S_2U), with a concomitant decrease in diunsaturated types (SU_2) for heat-treated samples compared to controls. Data shown are averages for butters from five heat-treated and six control pods.

Triglyceride Fractionation by HPLC

To determine if differences might be revealed between heat-exposed and control cocoa butters, triglycerides were separated into fractions by HPLC (Fig. 2). Peak area distributions among the five HPLC triglyceride fractions are shown in Table IV. Compared to control samples, cocoa butter from pods matured in an elevated temperature environment tended to have reduced amounts of Fractions 1 and 2, more of Fractions 4 and 5, and about the same quantity of Fraction 3. Although trends were relatively consistent among several samples analyzed, statistical significance (5% level) was not established.

Table IV shows mean fatty acid compositions for triglycerides and 2-monoglycerides of each HPLC fraction. Fraction 1, a minor component, was a diverse mixture of triglycerides rich in polyunsaturated fatty acids. Fraction 2 contained about one-fourth of all triglycerides; especially prominant were POP and PLP (P, O, and L are palmitic, oleic, and linoleic acids, respectively). POS (S is stearic acid) dominate Fraction 3, which contributed 46% of the sum of all peak areas. SOS was concentrated in Fraction 4, which was quantitatively about the same as Fraction 2. Fraction 5, another minor component, contained SOA (A is arachidate) and a mixture of trisaturated triglycerides. As demonstrated by the data, the HPLC column separated the triglycerides in a mixed mode, with smaller carbon number, more unsaturated triglycerides eluting first, while least mobile were the highest molecular weight, most saturated triglycerides.

Interesting differences are evident in the lipolysis data (2-monoglyceride, Table IV). For most fractions, heat-exposure of pods resulted in cocoa butter with less linoleic and oleic acid deposition at the 2-glycerol position. In Fraction 2, for example, linoleate at the 2-position was 13.4% for control butter, compared to 9.1% for butter from heat-exposed pods. The cumulative effect of this and other differences among fractions (totaling 9.4% more SU_2 in control butter, Table III) would influence the crystalline packing of cocoa butter, and, thus, melting behavior. Relative amounts of triglyceride types in control butter were similar to those reported for Brazilian butter in another study (11), which also showed greater proportions for the high melting triglycerides in African cocoa butter.

The effect of added unsaturated triglycerides (10% olive oil) on the solid fat content of cocoa butter has been reported (12). Olive oil, which contains equal amounts of SU_2 and U_3 triglycerides, caused a shift in the DSC peak of the highest melting polymorph from 33.5 C to 31.5 C. Thus, alterations in the levels of unsaturated triglyceride types, as influenced by environmental growth temperature, appears to account for the variable melting behavior observed among cocoa butters. Since Bahia is farther from

TABLE II

Fatty Acid Composition (mg %) of Cocoa Butter from Fruit Subjected to a Microclimate of Elevated Temperature during Growth and Ripening.

Sample	% Fatty acid composition[a]				
	16:0	18:0	18:1	18:2	18:3[b] + 20:0
Heat-treated	26.9	33.2	31.8	4.4	3.9
Control	25.3	31.9	34.9	5.0	3.2

[a]Mean of 6 replications.
[b]18:3 and 20:0 not clearly separated.

TABLE III

Effect of Elevated Microclimate Temperature on Triglyceride types during Growth and Ripening of Cocoa Fruit

Sample treatment	Glyceride type (% of Total)			
	S_3	S_2U	SU_2	U_3
Heated	2.4	88.5	8.9	0.2
Control	1.2	79.3	18.3	1.1

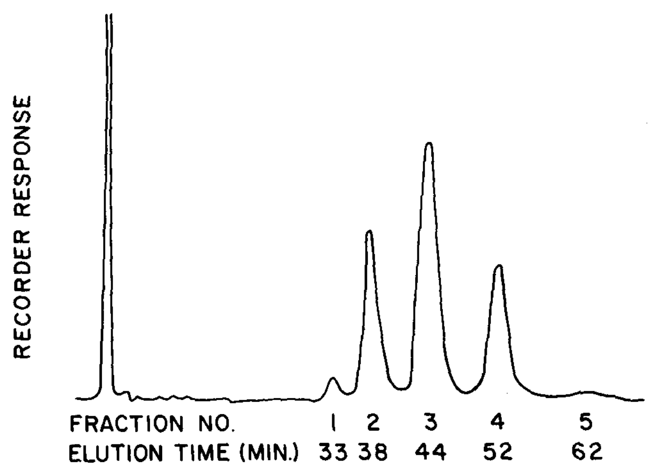

FIG. 2. Typical HPLC elution pattern of cocoa butter triglycerides (Waters Associates Triglycerides Analysis Column, 85:15, (v/v), acetonitrile/tetrahydrofuran mobile phase).

TABLE IV

2-Monoglyceride Fatty Acids and Fatty Acid Composition of Triglycerides Separated by HPLC from Seeds of Control Pods and Pods Matured in an Elevated Temperature Environment

HPLC Peak No.	% of Total peak area	Fatty acid composition (percent by weight)						
		16:0	16:1	18:0	18:1	18:2	18:3	20:0
Whole triglycerides								
1-Control	3.4	46.9	4.5	5.6	16.6	25.9	0.5	—
1-Heated	2.0	49.5	4.6	8.1	14.8	21.3	1.7	—
2-Control	25.7	47.9	—	6.7	40.2	5.2	—	—
2-Heated	23.7	55.6	—	5.7	34.6	4.1	—	—
3-Control	45.8	26.3	—	35.7	36.4	1.6	—	—
3-Heated	46.3	30.1	—	33.9	34.9	1.1	—	—
4-Control	23.7	2.4	—	62.7	32.7	—	—	2.2
4-Heated	26.2	3.4	—	60.5	33.4	—	—	2.7
5-Control	1.4	11.1	—	36.8	32.1	—	—	20.0
5-Heated	1.7	7.2	—	36.9	31.3	—	—	22.6
2-Monoglycerides								
1-Control		8.3	1.8	9.5	21.9	56.6	1.9	—
1-Heated		11.9	4.2	11.6	20.7	47.9	3.7	—
2-Control		1.3	—	1.0	84.3	13.4	—	—
2-Heated		2.1	—	1.9	86.9	9.1	—	—
3-Control		0.9	—	1.1	94.5	3.5	—	—
3-Heated		1.6	—	1.5	94.5	2.4	—	—
4-Control		1.3	—	2.3	96.4	—	—	—
4-Heated		1.8	—	3.0	95.2	—	—	—
5-Control		13.2	—	16.4	70.4	—	—	—
5-Heated		13.7	—	16.7	67.6	—	—	—

the Equator than most cocao-growing regions, softer cocoa butter would be a logical consequence of cooler seasonal temperatures.

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