Lipid Content and Fatty Acid Composition of Free and Bound Lipids in Pearl Millets[1]

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ABSTRACT

Lipid content and fatty acid composition of pearl millets were studied. Free lipid content ranged from 5.55 to 7.08%, bound lipid content from 0.57 to 0.90%. Unsaturated acids averaged 70.3% of the free and 51.7% of the bound lipid fractions. Linoleic, oleic, and palmitic were the principal fatty acids in both free and bound lipids. Trace levels of myristic and behenic acids were found in the free lipids. Of the total bound lipids, 1.34–2.16% had an odd number of carbon atoms ($C_{13:0}$, $C_{15:0}$, and $C_{17:0}$). The total percentage of long chain fatty acids ($C_{20:0}$ and above) was greater for the bound than for the free lipids.

Pearl millet (*Pennisetum americanum* (L.) Leeke) according to Terrell 1976) is a staple food of people in Africa and Asia. Within the past 10 years, considerable emphasis has been placed on its nutritional components (Casey and Lorenz 1977; Dendy 1977). Limited amounts of data have been reported on the lipid composition of different cultivars, however, (Jellum and Powell 1971, Pruthi and Bhatia 1970). Furthermore, the fatty acid composition of free lipids (extractable with ether) and bound lipids (extractable with water-saturated butanol) has not been reported. This article compares the lipid content in different random-mating populations and S_1 lines of pearl millet and describes qualitative differences in fatty acids between free and bound lipids.

MATERIALS AND METHODS

Materials

Pearl millets were grown near Hays, KS. Seventeen HMP550 S_1 lines (Tift 23DB_1/*2PI185642) harvested in 1977 were studied. In addition, four bulk populations were studied: HMP550, a combination of 110 S_1 lines; HMP1700 (PI263540/Tift 23 DB_1/2/Tift 239DB_2/2*Serere 3A); RMPI (S)CI (parentage from Serere 3A, Serere 17, and Tift 239DB_2); and Serere 3A, developed by Serere Experiment Station, Uganda, Africa. The last cultivar was harvested in 1975.

Methods

Location of Lipids. Whole kernels from each millet population were soaked in distilled water overnight at 4° C. Kernels were then hand-sectioned and stained with Oil Red O (Lillie and Ashburn 1943) or Nile Blue (Cain 1947). Light photomicrographs were taken on a Reichert (Austria) light microscope; a Wild Heerbrugg stereoscopic microscope was used to record images of stained half-kernels.

Lipid Extraction. Millet was ground on a Wiley mill with a 40-mesh screen. Free lipids were extracted with petroleum ether (bp 38–55° C) in a Goldfish apparatus for 14 hr. Bound lipids were then extracted from the residue twice with water-saturated *n*-butanol in a Stein mill. In the first extraction, 50 ml of solvent was added and the residue and solvent were mixed for 4, 2, and 2 min, with 1-min intervals between extractions. The second extraction was done three times, 2 min each at 1-min intervals. The rest of the drying and reextraction procedure was done as described by Daftary and Pomeranz (1965) on wheat samples. All lipid extractions were done in duplicate.

Thin-Layer Chromatography. The free and bound lipid fractions from the bulk populations and four S_1 lines were characterized by thin-layer chromatography (TLC) on 0.25-mm silica gel G (Brinkmann Instruments) with chloroform/methanol/water (65:25:4, v/v/v) to separate polar components and with chloroform to separate nonpolar components. Lipid samples were applied at 200 μg per spot. After separation, the spots were made visible by spraying the plates with a chromic H_2SO_4 reagent; a ninhydrin reagent for detection of free amino groups (Lepage 1964); α-naphthol containing reagent specific for compounds containing sugar (Feldman et al 1965); or a molybdenum blue spray specific for compounds containing phosphorous (Dittmer and Lester 1964). Lipids were tentatively identified by cochromatography with known lipid compounds (Sigma Chemical Co.), by comparison of relative Rf values, and by reactions of the specific sprays.

Gas Chromatography. Esterification of fatty acids from bulk populations was carried out by placing a 5-ml aliquot of lipid extract in a teflon-stoppered culture tube and evaporating to dryness under a stream of N_2. Two milliliters of 0.5*N* methanolic NaOH were added, and the vial was sealed. The contents were heated in a boiling water bath for about 2 min, after which 5 ml of BF_3-methanol (14% w/v, Supelco) was added and the mixture

TABLE I
Free and Bound Lipids of Pearl Millet Samples

Sample	Free Lipid (%)	Bound Lipid (%)	Free/Bound Ratio
Bulk populations[a]			
Serere 3A	6.20 a	0.59 a	10.5
RMPI(S)CI	6.80 b	0.90 bd	7.6
HMP1700	6.92 bc	0.74 c	9.4
HMP550	7.08 c	0.82 d	8.6
average	6.75	0.76	...
HMP550 S_1 lines[b]			
114	6.33	0.88	7.2
120	6.21	0.70	8.9
134	6.45	0.65	9.9
149	6.40	0.77	8.3
155	6.46	0.73	8.9
160	6.14	0.58	10.6
179	6.50	0.75	8.7
180	6.32	0.74	8.5
189	5.87	0.76	7.7
191	6.19	0.78	7.9
193	6.07	0.64	9.5
196	5.66	0.57	9.9
200	5.78	0.70	8.3
201	5.95	0.73	8.1
218	5.55	0.68	8.2
220	7.03	0.64	11.0
240	6.56	0.67	9.8
average	6.20	0.70	...

[a] Within each fatty acid, values with different letters differ significantly ($P < 0.05$).
[b] Least significant differences ($P < 0.05$) for S_1 free and bound lipids are 0.24 and 0.09, respectively.

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heated for another 2 min. The contents of the tube were then transferred to a 50-ml separatory funnel with 20 ml of water. Fatty acid methyl esters were extracted with hexane and analyzed on the gas chromatograph. Duplicate analyses were run on each sample.

Fatty acid methyl esters were identified on a Hewlett Packard 5750 gas chromatograph with a flame ionization detector. The column was 6 ft × 1/8 in. and was packed with 10% Sp-2330 on 100/120 Chromosorb W AW (Supelco, Inc.). Temperature at injection port was 250° C; column temperature was 195° C, and N_2 carrier gas flow was 40 ml/min. Relative peak areas were determined by multiplying the peak height by the width of the peak at half height. Weight percentage compositions were calculated by applying correction factors obtained from chromatograms of known mixtures (Supelco, Inc.). Peaks were tentatively identified by comparing the relative retention times with those from the reference standard mixtures run on the same column under the same conditions.

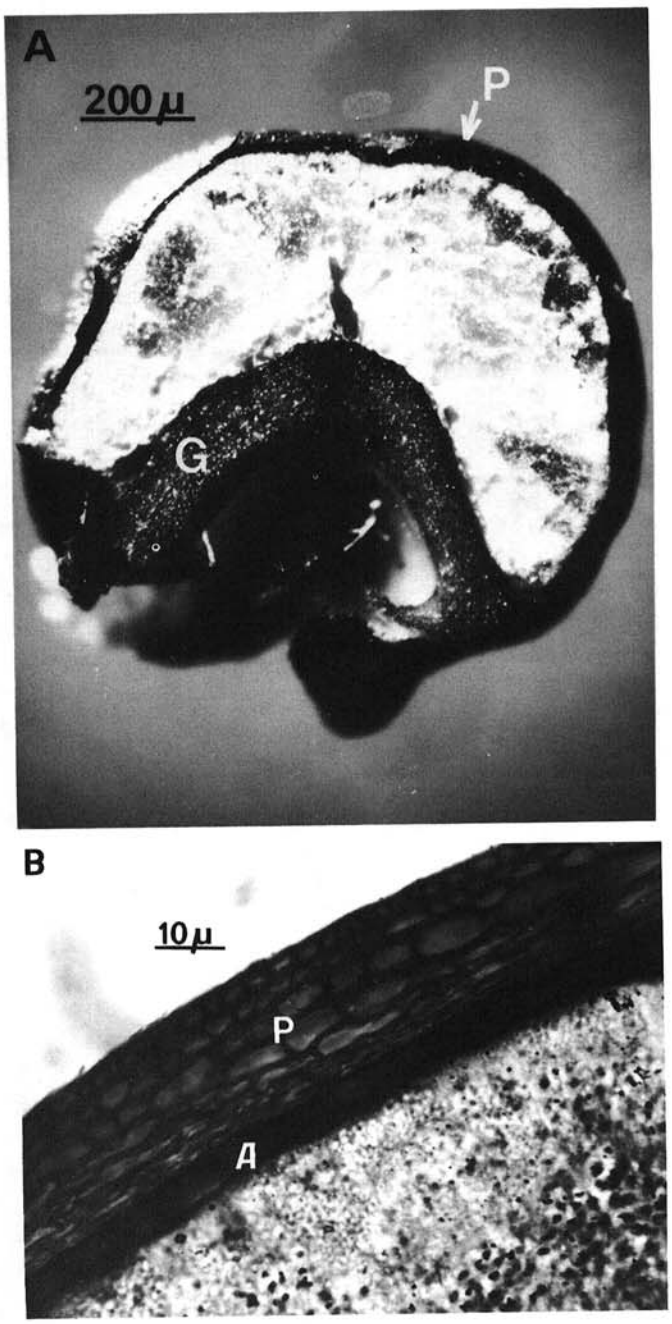

Fig. 1. Cross section of pearl millet kernel (HMP550) stained with Nile blue. **A,** coloration of the germ (G) and pericarp (P); **B,** high lipid concentration in the aleurone layer (A).

Statistical Analysis. Standard deviation of means or least significant difference tests were determined on all data (Steel and Torrie 1960).

RESULTS AND DISCUSSION

Lipid Location

Histochemical studies on half-kernels showed that most lipids were concentrated in the germ and pericarp of the grain (Fig. 1A). Futher observations on thin sections indicated that although the endosperm absorbed small quantities of lipid stain, most of the lipids were concentrated in the pericarp and aleurone layers (Fig. 1B).

Free and Bound Lipids

Lipid contents varied among populations but averaged 6.75 free and 0.76% bound lipids (Table I). Serere 3A contained significantly ($P < 0.05$) less free and bound lipids than other populations did. In addition, the ratio of free to bound lipids was the highest for Serere 3A.

Variability in free and bound lipids also existed among S_1 lines; values ranged from 5.55 to 7.03% for free lipids and from 0.57 to 0.88% for bound lipids (Table I). The average for S_1 lines was 6.20 free and 0.70% bound lipids. Pruthi and Bhatia (1970) reported free and bound lipid contents of 5.0 and 0.5%, respectively, for two pearl millet cultivars. The S_1 lines were similar to bulk samples in ratios of both free and bound lipids.

Thin-Layer Chromatography

Lipid components of free and bound fractions were separated by TLC (Figs. 2 and 3). Although four bulk populations and four S_1 lines were studied, only the results of three samples are shown because they all had similar TLC patterns. Nonpolar lipid components (Fig. 2), both free and bound, were tentatively identified as: hydrocarbons and steryl esters, triacylglycerols, free fatty acids, sitosterol, and partial acylglycerols. Sitosterol spots

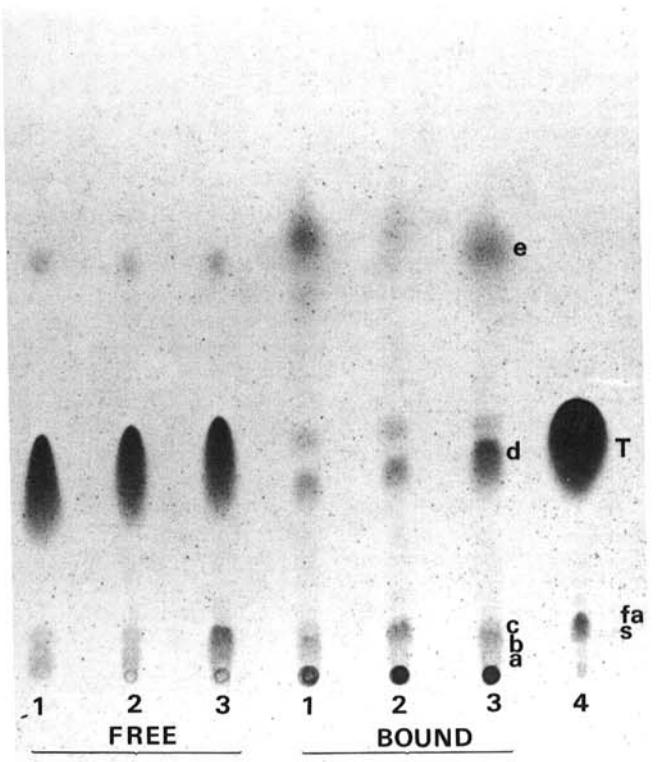

Fig. 2. Thin-layer chromatograms of nonpolar components of free and bound lipids from: **1,** HMP550; **2,** RMPI(S)CI; **3,** Serere 3A; **4,** nonpolar reference standards. **fa** = free fatty acids, **S** = sitosterol, **T** = triacyglycerols. Nonpolar lipids are tentatively identified as **a,** partial glycerides; **b,** sitosterol; **c,** free fatty acids; **d,** triacylglycerols; and **e,** hydrocarbons and steryl esters.

were differentiated from free fatty acids by the appearance of a pink-mauve color after the spots were sprayed with chromic H_2SO_4 reagent. The nonpolar components of the free lipid fraction were similar to those identified by Pruthi and Bhatia (1970). Triacylglycerols were the major free nonpolar lipids.

Like Badi et al (1976), we found that the bound nonpolar lipids contained the same components as the free nonpolar lipids. However, bound nonpolar lipids showed two spots corresponding to the Rf of the triacylglycerol standard (Fig. 2). This subfractionation may be due to the presence of triacylglycerols with fatty acids of different chain lengths (Malins and Mangold 1960).

Polar lipids were chromatographed (Fig. 3), and the fractionated components were tentatively identified by their reactions to specific sprays and by comparison of Rf values with those of the standards (Table II). They were lysophosphatidyl choline, phosphatidyl choline, digalactosyl diglycerides, phosphatidyl ethanolamine, cerebrosides, and free fatty acids. The sitosterol standard had the same Rf as spot i on the chromatogram in Fig. 3. That unknown spot did not turn pink-mauve during the initial charring period after spraying with chromic H_2SO_4, however, so it was tentatively identified as free fatty acids. Badi et al (1976) identified monogalactosyl diglyceride (MGDG) in the bound polar lipids of pearl millet. We were unable to detect MGDG on our chromatograms, however; free fatty acids were probably misidentified as MGDG by Badi et al (1976).

The free lipids of our pearl millet samples contained no polar components (Fig. 3), which agrees with the results of Badi et al (1976).

Gas Chromatography

Fatty acid compositions of free and bound lipids of pearl millet populations are presented in Tables III and IV. Pearl millets were high in unsaturated acids, averaging 70.3% of the fatty acids of the free lipids and 51.7% of the fatty acids of the bound lipids. Significant differences ($P < 0.05$) in some fatty acid contents were observed across populations.

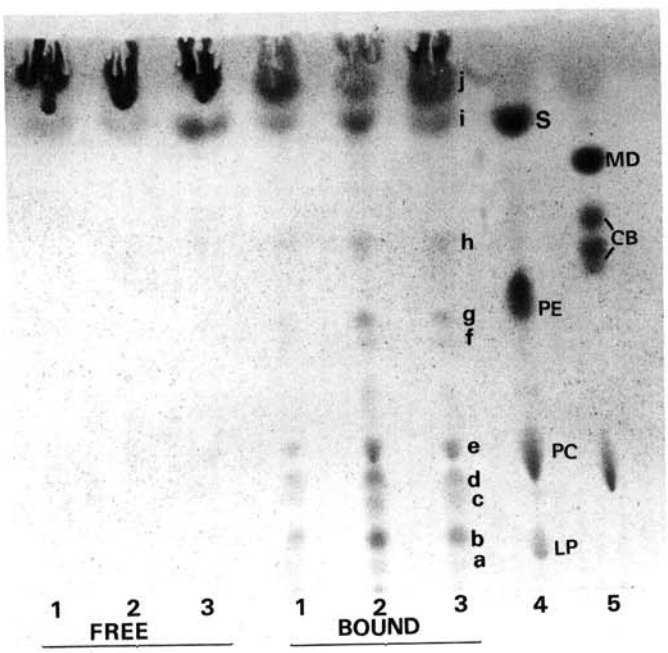

Fig. 3. Thin-layer chromatograms of polar components of free and bound lipids from: **1,** HMP550; **2,** RMPI(S)CI; **3,** Serere 3A; **4,** and **5,** polar references standards. **LP** = lysophosphatidyl choline, **PC** = phosphatidyl choline, **PE** = phosphatidyl ethanolamine, **S** = sitosterol, **CB** = cerebrosides, **MD** = monogalactosyl diglyceride. Polar lipids are tentatively identified as **b,** lysophosphatidyl choline; **e,** phosphatidyl choline; **f,** digalactosyl diglyceride; **g,** phosphatidyl ethanolamine; **h,** cerebrosides; and **i,** free fatty acids. **a, c, d** = unknown; **j,** = unresolved nonpolar lipids.

Linoleic ($C_{18:2}$), oleic ($C_{18:1}$), and palmitic ($C_{16:0}$) acids were the principal acids in free lipids (Fig. 4), which agrees with the results previously reported (Jellum and Powell 1971, Rooney 1978) for the fatty acid composition of millet free lipids. However, Agarwal and Sinha (1964), using the urea adduct method, found that oleic acid (53.84%) was the major acid in their millet samples. Jellum and Powell (1971), using a combination of methanol and petroleum ether as an extractant, reported levels of palmitic acid ($\bar{x} = 21\%$), linolenic acid ($\bar{x} = 3.7\%$), and oleic acid ($\bar{x} = 26\%$) similar to those we found in our Kansas millets. However, they reported an average of 45% linoleic acid, compared to 38% in our samples (Table III). Rooney (1978) also reported a linoleic acid content of 46% for pearl millet, but he gave no details of the lipid extractant or chromatographic conditions. Jellum and Powell (1971) have clearly shown that differences in lipid extraction procedures, as well as genetic variablility, contribute to differences in reported fatty acid contents of pearl millets.

Jellum and Powell (1971) reported that other unidentified fatty acids represented less than 0.5% of the total fatty acids in pearl millet. We tentatively identified trace levels of $C_{14:0}$ and $C_{22:0}$ in free lipids of our Kansas samples (Table III).

Qualitatively, fatty acids present in free lipids, except $C_{22:0}$, were also found in bound lipids (Fig. 5). As in the free lipids, linoleic, oleic and palmitic acids were the major fatty acids in the bound lipids. However, $C_{18:1}$ and $C_{18:2}$ were present in lower weight percentages in bound than in free lipids (Table IV). In addition, six other fatty acids were tentatively identified in bound lipids ($C_{10:0}$, $C_{12:0}$, $C_{13:0}$, $C_{15:0}$, $C_{17:0}$, and $C_{24:0}$), and of the total bound fatty acids,

TABLE II
Polar Lipid Components of Pearl Millets Shown in Fig. 3[a]

Spot	Rf	Tentative Identification	Spray Reagents: Chromic H_2SO_4	Ninhydrin[b]	Molybdenum[c]	α-Naphthol[d]
j	0.93	Unresolved nonpolar lipids	+	−	−	−
i	0.83	Free fatty acids	+	−	−	−
h	0.62	Cerebrosides	+	−	−	+
g	0.48	Phosphatidyl ethanolamine	+	+	+	−
f	0.41	Digalactosyl diglyceride	+	−	−	+
e	0.24	Phosphatidyl choline	+	−	+	−
d	0.16	Unknown	+	+	+	−
c	0.12	Unknown	+	+	−	−
b	0.07	Lysophosphatidyl choline	+	−	+	−
a	0.03	Unknown	+	+	+	+

[a] + = positive reaction, − = negative reaction.
[b] Indicates free amino groups.
[c] Indicates compounds containing sugar.
[d] Indicates compounds containing phosphorus.

TABLE III
Fatty Acid Composition (%) of Free Lipids from Pearl Millet Populations

Fatty Acid	Populations[a]: Serere 3A	RMPI(S)CI	HMP1700	HMP550
$C_{14:0}$	0.13	trace	0.21	trace
$C_{16:0}$	20.01 a	20.71 a	21.62 a	21.56 a
$C_{16:1}$	1.10 a	1.10 a	0.93 a	0.96 a
$C_{18:0}$	8.83 a	10.10 b	6.09 c	6.76 c
$C_{18:1}$	27.23 a	28.04 a	28.21 a	28.20 a
$C_{18:2}$	37.83 ab	36.72 b	38.02 ab	39.78 a
$C_{18:3}$	4.14 a	2.17 b	4.19 a	2.36 b
$C_{20:0}$	0.75 a	1.19 b	0.75 a	1.04 b
$C_{22:0}$	trace	trace	trace	trace

[a] Within each fatty acid, values with different letters differ significantly ($P < 0.05$).

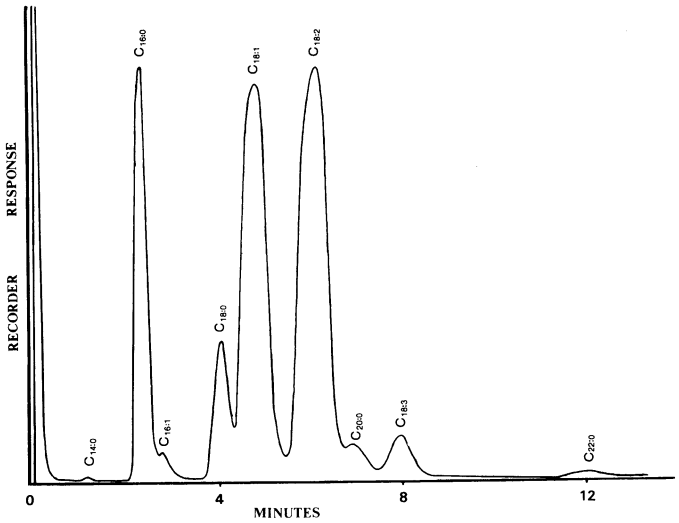

Fig. 4. Gas chromatogram of fatty acid methyl esters of the free lipid fraction from pearl millet meal (HMP550).

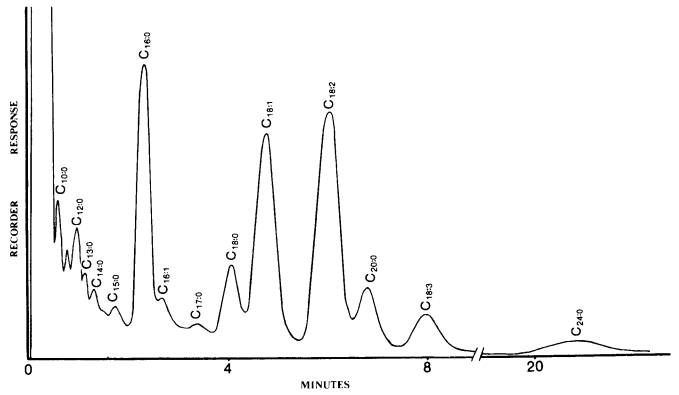

Fig. 5. Gas chromatogram of fatty acid methyl esters of the bound lipid fraction from pearl millet meal (HMP550).

1.34–2.16% had an odd number of carbon atoms. The total percentage of long chain fatty acids ($C_{20:0}$ and above) was also greater for bound than for free lipids.

TABLE IV
Fatty Acid Composition (%) of Bound Lipids from Pearl Millet Populations

Fatty Acid	Populations[a]			
	Serere 3A	RMPI(S)CI	HMP1700	HMP550
$C_{10:0}$	3.41 a	0.73 b	4.0 a	1.18 b
Unknown	0.56 a	1.32 b	0.91 b	0.85 b
$C_{12:0}$	1.90 a	0.71 b	3.16 c	1.05 b
$C_{13:0}$	0.33 a	0.51 a	0.52 a	0.62 a
$C_{14:0}$	0.53 a	0.79 a	0.54 a	0.42 a
$C_{15:0}$	0.98 a	0.32 bc	1.31 a	0.73 ac
$C_{16:0}$	22.12 ab	23.21 a	20.80 b	21.11 ab
$C_{16:1}$	0.91 a	1.36 b	0.78 a	0.96 a
$C_{17:0}$	0.33 a	0.51 a	0.33 a	0.23 a
$C_{18:0}$	6.47 a	7.91 b	4.79 c	5.12 c
$C_{18:1}$	19.36 a	18.76 a	16.62 b	19.49 a
$C_{18:2}$	26.62 a	29.02 b	32.29 c	29.37 b
$C_{18:3}$	3.25 a	2.98 a	1.89 b	3.16 a
$C_{20:0}$	5.12 a	5.09 a	7.10 b	7.81 b
$C_{24:0}$	7.96 a	7.68 a	5.00 b	7.87 a

[a]Within each fatty acid, values with different letters differ significantly ($P < 0.05$).

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