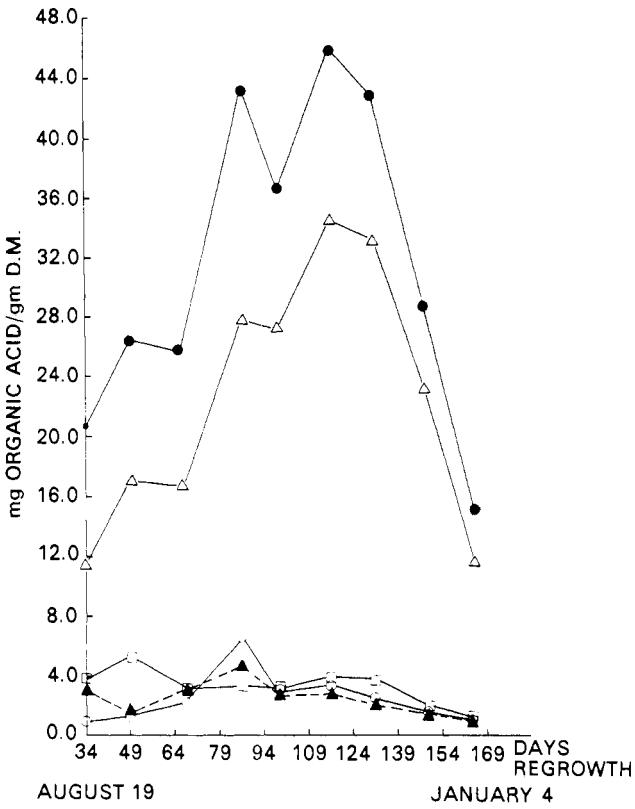


**Figure 5.** Seasonal variation of organic acids in tall fescue. (△——△) malic; (□——□) citric; (○——○) quinic; (▲——▲) shikimic; (●——●) total.

desirable. Malic and citric acid concentrations were different between bluestem (*Andropogon caucasica*) and switchgrass (*Panicum virigatum*) and two genotypes of tall fescue (*Festuca arundinacea*). The method has been applied to a more complete genotypical study of tall fescue to be reported elsewhere.

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# Free Fatty Acid Content of Cacao Beans Infested with Storage Fungi

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Cacao beans from four sources allowed to mold with surface-contaminating fungi and cacao beans (Bahia source) inoculated with *Aspergillus amstelodami, A. flavus, A. niger, A. repens, A. ruber,* a species of *Penicillium,* and a species of *Paecilomyces* had increases in free fatty acid (FFA) content and changes in the FFA composition of the lipids. In beans molded by contaminating fungi (naturally molded), the FFA content of the lipid increased from 1.0–1.5 to 7.4–41.3%. The FFA content of lipid from beans molded by single species of storage fungi (pure culture-molded) increased from 1.4 to 28.0–62.1%. The major FFA present were palmitic, stearic, and oleic and these three fatty acids comprised 36.7–62.5, 49.2–92.4, and 92.0–95.4% of the total FFA for nonmoldy, naturally molded, and pure culture-molded cacao beans, respectively. Linoleic acid comprised 13.8–17.4% of the total FFA in nonmoldy and naturally molded Bahia beans. The fungi increased the acidity of the cocoa lipid through liberation of fatty acids from glycerides.

Cacao beans are produced in tropical regions where storage fungi can be troublesome. Following natural fermentation the moisture content (MC) of the whole bean is reduced from about 60 to 8% or below for sale or storage. Beans are dried in the sun or artificially (Rohan, 1963). If drying is too slow or stopped above 8% MC, storage fungi will invade and grow in the bean. Dry cacao beans are hygroscopic and readily absorb moisture from the humid atmosphere in the tropics. Scott (1963) investigated molds associated with stored cacao beans and concluded that conditions above 75% relative humidity (RH) and 10° fa-

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vored mold growth. Theimer (1958) confirmed this and reported the minimum MC for mold growth to be 8% (wet-weight basis). Bunting (1963) observed, during fermentation, that fungi penetrate the bean through the micopylar end, particularly in damaged or partially germinated beans. Kaden (1954) observed MC between 7 and 1 in cacao beans stored in inland sheds.

Hansen and Keeney (1969) showed fungal activity on cacao beans altered the flavor components of chocolate, especially in pressed cocoa cake. Also, during roasting, flavor components in the free cocoa butter do not increase as high in moldy cacao beans. Hansen and Keeney (1970) also reported fungal activity altered total carbonyls and monocarbonyls in cocoa butter. During this work they observed an abnormal elution pattern on the defatting column (Celite 545–Sea Sorb 43, 1:1, w/w), apparently caused by the increase in free fatty acids (FFA) which changed the polarity of the column. This abnormal behavior of the moldy bean extract led to a study of fungi associated with moldy and nonmoldy cacao beans (Hansen and Welty, 1971) and to this study on the FFA content of naturally molded and pure culture-molded cacao beans.

Hutchinson (1961) reported fat acidity increases in wheat due to fungal growth and natural lipase in the wheat. Dorworth and Christensen (1968) reported that increases in fat acidity in stored grains or seeds depend upon the fungus involved and MC of the grain or seeds. While this work was in progress, Kavanagh *et al.* (1970) published a paper dealing with changes in cacao lipids due to mold growth. Kavanagh utilized moldy cacao beans, extracted the lipids and separated the FFA on a silicic acid column impregnated with KOH. Hansen and Shen also utilized this procedure in their original pure culture studies and found the neutral lipid fraction of the moldy beans contaminated with FFA. Hansen and Shen modified the procedure and solvents and still found the neutral lipid sample contaminated with FFA. This was due to the high concentration of FFA in moldy cacao beans (25–62% FFA of the total lipid).

A new method was developed by Hansen and Shen (1972) using ion exchange resins and was tested quantitatively with C-14 labeled FFA and triglycerides. The method gave excellent separation and recovery of the FFA and was used with gas chromatography (gc) to analyze the fatty acid mixtures.

## MATERIALS AND METHODS

**Sample Preparation.** Nonmoldy cacao beans were obtained from members of the Chocolate Manufacturers' Association of the United States of America. Two thousand grams of Accra, Arriba, Bahia, and Sanchez were divided into two portions, one was used as a control and the other was allowed to mold naturally in a chamber above distilled water to provide 100% RH. After 8 weeks at 24–26°, the beans were removed and air dried.

In another experiment, 20-g samples of nonmoldy Bahia beans were sterilized for 24 hr in a chamber containing propylene oxide (2 ml/l.) to yield bean samples for inoculating with pure cultures. The Bahia variety was selected randomly from the above four varieties for a more in-depth study of the individual fatty acids. One sample served as a control; the others were inoculated by placing them on the surface of a sporulating culture, growing on Czapek's agar, of either *Aspergillus amstelodami* (Mangin) Thom & Church, *A. flavus* Link, *A. niger* Van Tiegham, *A. repens* de Bary, *A. ruber* (K. S. & B.) Thom & Church, a *Penicillium* sp. or a *Paecilomyces* sp. These pure cultures were utilized since they were previously isolated from nonmoldy cacao beans as reported by Hansen and Welty (1971). After inoculation, samples were incubated in a Chamber at 100% RH and 30° for 6 weeks and then removed from the chamber and air dried.

**Lipid Extraction.** Beans were shelled and 10 g was homogenized for 10 min in a Waring Blendor with 200 ml of an ethyl ether–petroleum ether mixture (1:1, v/v). The supernatant was decanted and the residue reextracted with 100 ml of the ethers for an additional 10 min. The mixture and the first extract were transferred to a Buchner funnel, precoated with *ca.* 15 g of Celite 545, and filtered under light vacuum to remove pigments and extraneous material. The residue was washed thoroughly with another 50 ml of the ether mixture. The lipid extract was washed in a separatory funnel with 0.2 times its volume of 0.04% $CaCl_2$ solution to remove nonlipid impurities (Folch *et al.*, 1957). The washed extract was taken to dryness under $N_2$ gas and then held in a vacuum desiccator until reaching constant weight.

**Table I. Comparison of Titration and Ion-Exchange Chromatography for Determination of Free Fatty Acid Content of Lipids from Nonmoldy, Naturally Molded, and Pure Culture-Molded Cacao Beans**

| Bahia cacao beans | FFA as oleic, %[a] | FFA, %[b] |
|---|---|---|
| Nonmoldy | 1.4 | 2.0 |
| Naturally molded | 41.3 | 42.1 |
| *A. amstelodami* | 38.5 | 39.9 |
| *A. flavus* | 43.2 | 43.5 |
| *A. niger* | 62.1 | 67.1 |
| *A. repens* | 28.0 | 29.5 |
| *A. ruber* | 51.5 | 51.9 |
| *Paecilomyces* sp. | 25.0 | 25.0 |
| *Penicillium* sp. | 40.9 | |

[a] Free fatty acids titrated with standard alkali, calculated as oleic acid, and expressed as a percent of total lipids. [b] Free fatty acids separated by ion-exchange chromatography, measured gravimetrically, and expressed as a percent of total lipids.

**Free Fatty Acid Content.** FFA content of the lipid from control and fungus-infested cacao beans was determined by titration. Samples of 0.5–2.0 g of lipid were dissolved in 50 ml of benzene–ethanol mixture. This reagent was prepared by mixing equal portions of 95% ethanol and benzene and was neutralized with alkali immediately prior to use. A half milliliter of phenolphthalein indicator solution was added. The mixture was titrated with a standard 0.1 *N* sodium hydroxide solution until a faint pink color appeared and persisted at least 1 min. The acid value is expressed as the percentage of FFA calculated as oleic acid. Thus, 1% FFA means 1 g of FFA as oleic acid per 100 g of total lipid.

FFA content also was determined gravimetrically as reported by Hansen and Shen (1972). FFA were separated from glycerides and other lipids by ion-exchange chromatography. The eluate containing the FFA was collected and the solvents were evaporated under $N_2$ gas at 25°. The residue was dried further in a vacuum desiccator at room temperature to constant weight.

**Separation.** The FFA of the control and fungus-infested cacao beans was obtained by anion exchange as outlined by Hansen and Shen (1972). Purity of the FFA fraction was verified by thin-layer chromatography (tlc) before methylation. Tlc plates, 0.5-mm thick, were prepared from silica gel G. A solvent mixture of heptane, isopropyl ether, and glacial acetic acid (6:4:0.2 v/v) was used. Individual spots were made visible by either spraying with 0.2% of 2,7-dichlorofluorescein in ethanol and then viewed under ultraviolet light, or by spraying with sulfuric dichromate, and then charred.

After the purity of each fraction was verified, the FFA was methylated (Patton *et al.*, 1964). Methyl esters of the fatty acids (1.0 $\mu$l) were separated on a 0.6 (o.d.) × 160 cm aluminum column packed with 10% EGSS-X on 100–120 mesh Gas Chrom P (Applied Science Laboratories, State College, Pa.). The separation was carried out isothermally at 180° with a helium flow rate of 38 ml/min. Fatty acid was identified by comparing the retention dis-

**Table II. Composition of Free Fatty Acids from Nonmoldy and Naturally Molded Cacao Beans of the Varieties Accra, Arriba, Bahia, and Sanchez**

| | Accra | | Arriba | | Bahia | | Sanchez | |
|---|---|---|---|---|---|---|---|---|
| | Nonmoldy[b] | Naturally molded[c] | Nonmoldy[d] | Naturally molded[e] | Nonmoldy[f] | Naturally molded[g] | Nonmoldy[h] | Naturally molded[i] |
| Fatty acid[a] | % | | % | | % | | % | |
| Lauric | 5.8 | 3.9 | 8.0 | 7.2 | 3.5 | 1.8 | 6.5 | 0.6 |
| Myristic | 6.8 | 2.8 | 6.4 | 4.7 | 4.4 | 0.9 | 6.6 | 0.7 |
| Palmitic | 18.7 | 26.3 | 15.5 | 24.8 | 17.1 | 11.6 | 17.2 | 23.0 |
| Stearic | 20.1 | 30.1 | 13.5 | 27.7 | 21.5 | 14.2 | 15.0 | 33.8 |
| Oleic | 9.8 | 12.8 | 7.7 | 2.7 | 23.9 | 23.4 | 12.8 | 31.6 |
| Linoleic | 3.0 | 1.2 | 3.4 | 1.4 | 13.8 | 17.4 | 6.9 | 4.0 |
| Arachidic | 1.8 | 2.3 | 2.7 | 2.6 | 1.9 | 3.5 | 3.4 | 1.4 |

[a] Twenty-four fatty acids with carbon number greater than 11 were calculated for composition. Only the above acids were tabulated because others were present in relatively low concentrations. [b–i] Free fatty acid content (oleic acid): [b] 1.5%; [c] 10.1%; [d] 1.0%; [e] 7.4%; [f] 1.4%; [g] 41.3%; [h] 1.2%; [i] 21.6%.

**Table III. Composition of Free Fatty Acids from Bahia Cacao Beans Inoculated with Storage Fungi**

| | % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid[a] | Control[b] | *A. amstelodami*[c] | *A. flovus*[d] | *A. niger*[e] | *A. repens*[f] | *A. ruber*[g] | *Penicillium* sp.[h] | *Paecilomyces* sp.[i] |
| Lauric | 3.5 | Trace | Trace | Trace | Trace | Trace | 0.6 | Trace |
| Myristic | 4.4 | 0.5 | 0.8 | 0.7 | 0.5 | 0.6 | 0.6 | 0.6 |
| Palmitic | 17.1 | 22.3 | 28.8 | 25.2 | 24.1 | 24.0 | 29.2 | 21.5 |
| Stearic | 21.5 | 31.3 | 49.7 | 43.6 | 29.4 | 36.8 | 38.1 | 31.2 |
| Oleic | 23.9 | 40.0 | 16.3 | 25.6 | 38.5 | 33.4 | 28.1 | 39.6 |
| Linoleic | 13.8 | 3.4 | 1.6 | 2.3 | 3.8 | 2.2 | 0.7 | 3.4 |
| Arachidic | 1.9 | 1.1 | 1.3 | 1.5 | 1.5 | 1.6 | 1.2 | 2.2 |

[a] See footnote "a," Table II. [b–i] Free fatty acid content (as oleic acid): [b] 1.4%; [c] 38.5%; [d] 43.2%; [e] 62.1%; [f] 28.0%; [g] 51.5%; [h] 25.0%; [i] 40.9%.

tance on the chromatogram from the point of injection to the peak maximum with a standard fatty acid methyl ester mixture and also by running an internal standard. Peak areas were determined by an automatic integrator (like brand) and percent composition was calculated. Mean value of these analyses was reported.

## RESULTS AND DISCUSSION

All fungi tested increased the FFA content of the cacao beans. The melting point of chocolate is an important factor in the dipping and coating of candies, and if the FFA content increases, the resultant lower melting point reduces the coating ability of the chocolate. In initial studies of 100 samples of nonmoldy cacao beans, the FFA content of the lipid was 0.6–1.8% and averaged 1.2%. Most samples contained about 0.8–1.2% FFA. In 20 samples of naturally molded cacao beans, the FFA content of the lipid was 7.4–41.3%. Table I indicates that the FFA content of the lipid in pure culture-molded cacao beans was 25.0–62.1%. This high content of FFA in fungus-infested cacao beans apparently renders the beans unsuitable for candy manufacturing (Mikelos, 1973). The quantity of FFA produced varied among species of fungi. Species of *Paecilomyces* and *Aspergillus repens* yielded the least FFA (25.0–28.0%); *A. amstelodami, Penicillium* sp., and *A. flavus* yielded amounts (38.5–43.2%) similar to those in naturally molded beans (41.3%). *Aspergillus ruber* and *A. niger* gave the highest yields of FFA (51.5–62.1%) in the conditions of this test.

Table I also indicates that percentages of FFA determined by titration were comparable to those determined gravimetrically. Since, in beans inoculated with pure cultures (Table III), 92.0–95.4% of the total FFA were pamitic, stearic, and oleic acids, FFA calculated as aleic acid probably gives a value close to the total. These three fatty acids constituted 36.7–62.5 and 49.2–88.4% of the total FFA in lipids from nonmoldy and naturally molded cacao beans (Table II), respectively. Therefore, the titration procedure, which is simple and fast, probably could be used in routine analysis of FFA in cacao beans and chocolate products. On the other hand, the ion-exchange method, which gives 92.4–106.0% recovery of FFA (Hansen and Shen, 1972), could be used to collect the FFA fraction for gc analysis.

Table II compares composition of seven FFA of naturally molded cacao beans with FFA of nommoldy beans of the same variety. The FFA percentages for molded varieties were 10.1 for Accra, 7.4 for Arriba, 41.3 for Bahia, and 21.6 for Sanchez; for nonmoldy beans of these varieties percentages were 1.5, 1.0, 1.4, and 1.2, respectively. Activities of fungi and other microorganisms increased the fat acidity of the cocoa butter, apparently through liberation of more fatty acids by hydrolysis of the glycerides. Of the four varieties of cacao beans analyzed, the Bahia variety was highest in oleic and linoleic, both in the nonmoldy control and the naturally molded beans. In the control sample, the fatty acid content was 1.4% of the total lipid (Table I). Therefore, there was approximately 29.5 times more of the representative fatty acid in the moldy sample. For example, the oleic acid percentage was very close to that of the control for Bahia (0.5% lower), but yet there was approximately 29 times more of this as free oleic acid in the cocoa butter. With this content of free fatty acid in the molded cacao bean, it would be almost impossible to use moldy beans in the manufacture of chocolate. The percentage of lauric, myristic, and arachidic was observed to be at lower concentration in the naturally molded cacao beans. This was due to the dilution by the acids liberated from the triglyceride portion.

Stokoe (1928) showed that fatty acids liberated from coconut oil by mold lipase did not accumulate but were broken down into methyl ketones. The molds are probably

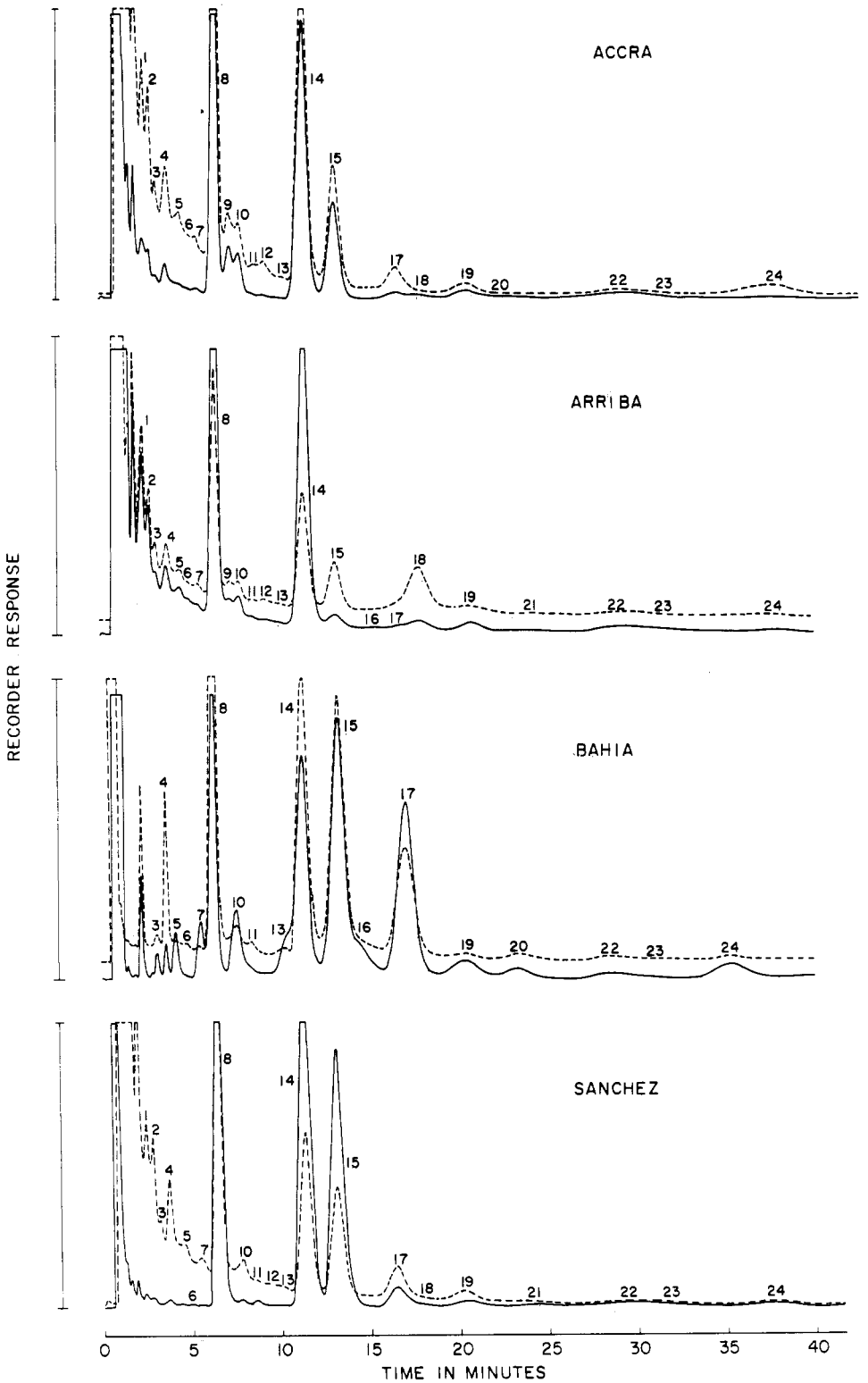


**Figure 1.** Analysis of the free fatty acid methyl esters from lipids in naturally molded and nonmoldy cacao beans of Accra, Arriba, Bahia, and Sanchez varieties. Solid lines show naturally molded and dotted lines show nonmoldy. Ordinate represents recorder's response and abscissa represents time. Fatty acid denoted by number of carbons:number of double bonds. (1) 12:0. (2) 12:1. (3) 14:0 iso. (4) 14:0. (5) 14:1. (6) 15:0. (7) 16:0 iso. (8) 16:0. (9) Unknown. (10) 16:1. (11) 17:0. (12) Unknown. (13) 18:0 iso. (14) 18:0. (15) 18:1. (16) 19:0. (17) 18:2. (18) 20:0 iso. (19) 20:0. (20) 18:3. (21–24) Unknown.

consuming the FFA for energy and cellular building material. Hansen and Keeney (1970) showed that moldy cacao beans had approximately twice the amount of monocarbonyls than nonmoldy beans. Wilcox *et al.* (1955) observed that microbial lipases differed in ability to release volatile fatty acids from butterfat. Cocoa butter is higher in palmitic, stearic, and oleic acids, 92.2–96.6%, as compared to cocoa nibs, 36.7–88.4% (Iverson *et al.*, 1969; Wijngaarden van *et al.*, 1968). If cocoa butter was made from moldy beans, more of these acids would be free as compared to nonmoldy beans (triglyceride). This is probably due to the difference in polarity of the FFA. The shorter chain fatty acids will tend to stay bound to the cocoa or nonfat portion, causing a higher amount of the longer chain acids in

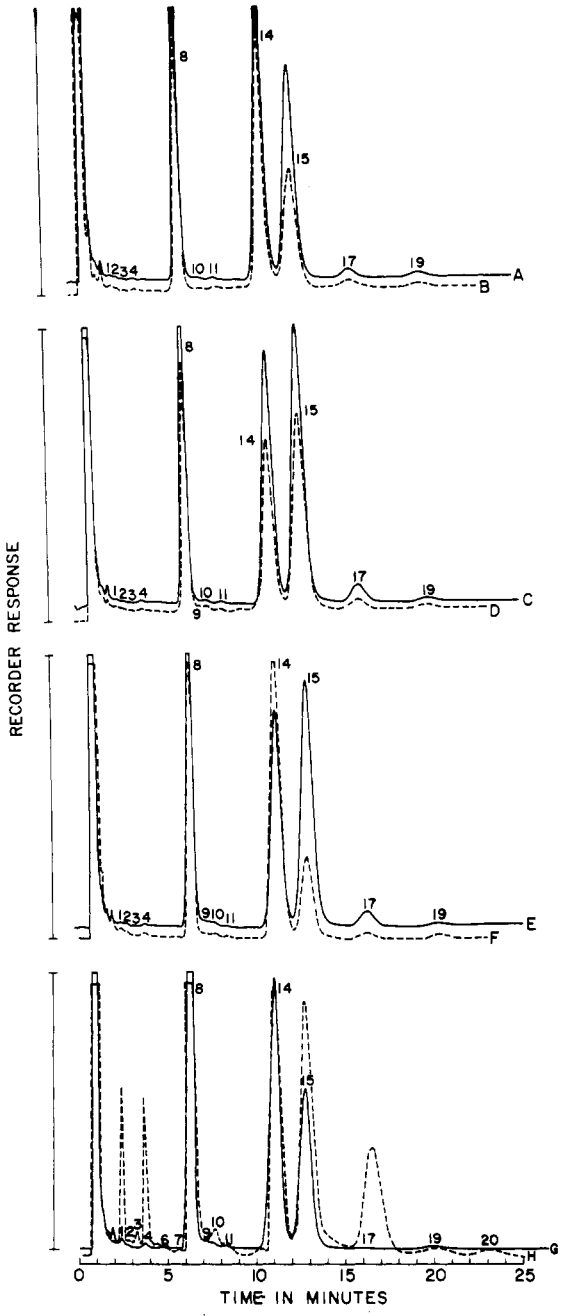


**Figure 2.** Analysis of the free fatty acid methyl esters from lipids in Bahia beans inoculated with (A) *A. ruber*, (B) *A. niger*, (C) *A. amstelodami*, (D) *Paecilomyces* sp., (E) *A. repens*, (F) *A. flavus*, (G) *Penicillium* sp., (H) control (nonmoldy Bahia).

the butter. It is possible that the amounts and kinds of FFA in moldy cacao beans are determined by two factors. One is the substrate specificity of the individual microbial lipases and the other is the utilization of the liberated fatty acids for further microbial breakdown for energy and other carbonyl compounds.

Table III shows the composition of seven FFA from Bahia beans inoculated with single species of storage fungi isolated from cacao beans. The pure culture molded beans yielded higher proportions of palmitic, stearic, and oleic acids than did the naturally molded beans (Table II). One must also consider the amount of FFA produced by the various pure cultures. The naturally molded sample contained 41.3% FFA, whereas pure culture mold samples yielded 25.0–62.1%. Each of the pure culture molded samples showed higher percentages of palmitic, stearic, and oleic then the naturally molded samples (92.0–95.4 *vs.* 49.2%).

From data shown in Tables II and III, it appears that the pure cultures of molds tend to utilize lauric, myristic, linoleic, and arachidic as compared to the naturally molded Bahia (Table II).

The percentages of individual fatty acids in a sample vary according to the species of fungus growing in the beans. Depending on the fungus (Table III), palmitic acid varied from 21.5 to 29.2%, stearic acid from 31.2 to 49.7%, and oleic acid from 16.3 to 40.0%. Apparently the mold lipases exhibit individual specificity in ability to release fatty acids from the glycerides of cocoa butter, as suggested by Dorworth and Christensen (1968) for soybeans. However, all the mold lipases showed a similar pattern on Bahia beans; *i.e.*, only palmitic, stearic, and oleic acids are selectively released. The Accra, Arriba, and Sanchez cacao beans which were molded by natural flora also showed similar trends even though quantitative data were slightly different.

Figure 1 shows the total FFA composition in nonmoldy and naturally molded cacao beans. As many as 24 FFA with carbon numbers greater than 11 were observed in the nonmoldy and naturally molded beans, while only 19 were observed in the pure culture molded beans (Figure 2). This could be due to the fact that various mold cultures preferred utilization of these specific acids. The most important factor in Figure 1 is the reduction in quantity of the shorter chain fatty acids. Accra and Sanchez showed the most drastic reduction, especially in the peak areas of 1–13. This same factor was evident in the pure culture study comparing F, the control, to the letters A–G (Figure 2). The reduction in the minor peaks was due to the dilution by the tremendous increase in FFA and by the utilization of these fatty acids for metabolism by the specific mold culture.

From this study and previous investigations in this laboratory, it is definite that FFA contents can be used as a guide to mold activity on the cacao beans or related products. The amount of FFA found in fungus-damaged beans will depend on storage time, temperature, and relative humidity. Naturally molded cacao beans show results more typical of what is to be expected in the industry. Beans inoculated with *A. niger* and *A. ruber* had higher yields of FFA than beans naturally molded or beans inoculated with the other storage fungi. Competition for the substrate between fungal species and other microorganisms could account for the differences in FFA content.

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# Several Compounds in Golden Delicious Apples as Possible Parameters of Acceptability

Natalio Gorin

Golden Delicious apples stored in a controlled atmosphere at 4° were subjected to enzymatic analyses of soluble sugars (D-glucose, D-fructose, and sucrose) and L-malic acid, determination of protein patterns by disk electrophoresis, and a palatability test. The content of sucrose and L-malic acid decreased, whereas D-glucose and D-fructose remained constant during storage. The protein pattern varied during storage. The contents of sucrose and L-malic acid and protein patterns could be useful parameters of quality (acceptability for marketing).

The purpose of this work was to determine objective criteria (chemical or biochemical) for acceptability and quality of apples.

Golden Delicious apples stored in a controlled atmosphere have an attractive appearance when they reach the market but their aroma and taste are unacceptable. Therefore an attempt was made to find parameters related to good flavor of fruit.

This study was confined to the natural aging of the apples in storage. Problems related to physiological disorders and deterioration due to infection were ignored. Golden Delicious apple was shown to be a suitable variety for this type of research (Faust *et al.*, 1969; Knee, 1971).

Some parameters were studied during storage in a controlled atmosphere (CA) at 4°. D-Glucose, D-fructose, sucrose, and L-malic acid were estimated enzymatically. The changes in protein patterns were determined.

It is known that sugar and acid content are related to taste (Smock and Neubert, 1950a). The problem is that the content of sugars is strongly influenced by climatic and geographic conditions. This can be seen clearly from the work of Rotstein *et al.* (1969), Kvåle (1969), and Kenworthy and Harris (1963). The present study considers the changes in protein pattern in apples during storage. Apparently they are independent of the said factors (Adriaanse *et al.*, 1969) but are related to the genetic makeup of the apple.

## MATERIALS AND METHODS

Golden Delicious apples (200 kg) of uniform color and size (70–75 mm) were purchased from a fruit grower in Puifluik (Netherlands) in 1971. They were stored at 4° in two tanks, each containing 100 kg under an atmosphere of 7–8% $CO_2$ and 3–4% $O_2$ (Stenvers, 1969, 1970). The equipment and conditions used (Figure 1) were as follows. Five boxes (length 56.5 cm, width 36.5 cm, height 30.5 cm), each containing 20 kg of apples, were placed in one tank which was hermetically closed with a round cover (RC) surrounded by a bicycle tire (BT). Once the tire had been inflated, nitrogen (10 l./min) was purged for 90 min through stopcock $S_2$. $S_1$ and orifices (OR) constituted the outlet. Afterwards $S_1$, $S_2$, and orifices OR were closed. When the $CO_2$, produced by respiration, had increased to the desired level of *ca.* 7–8% (after 1 week), the aquarium pump (P) was switched on in order to scrub $CO_2$. This gas was measured every 2 days with a Fyriter $CO_2$ Analyzer (Bacharach Instrument Co., Pittsburgh, Pa., USA). Oxygen was measured every 2 days with a Servomex Oxygen Analyser, Type OA 150 (Servomex Controls Ltd., Crowborough Sussex, England). If the concentration was lower than 3%, some orifices of OR were opened. The required concentration was attained within a day.

After storage for 0, 68, 103, 146, 195, and 216 days, respectively, 1-kg lots of sound samples were removed from the five boxes in each of the two tanks. Rotten fruit was discarded. Rotting had reached serious proportions (30%) after 7 months storage. Of the total mixed sample of 10 kg, 2 kg was used for estimating soluble sugars, L-malic acid, nitrogen, dry matter, and ash, 5 kg was for protein patterns, and 2–3 kg was for a palatability test.

**Estimation of Soluble Sugars (D-Glucose, D-Fructose, Sucrose) and of L-Malic Acid.** These substances were estimated enzymatically as described by Boehringer Mannheim GmbH (1971) with slight modifications. The estimates made with model systems (*i.e.*, the respective compounds dissolved in distilled water) proved satisfactory (coefficients of variability 2–6%). The recovery factors of these substances, added to apples at the start of the procedure described below, were 97–103% (Gorin, 1970, 1971).

Apples without pedicel (2000 g) were homogenized with distilled water (1000 g) in a large Waring Blendor for *ca.* 3–4 min at room temperature. The suspension was stirred at the lowest and medium blending settings. This constitutes *suspension WB*.

A sample of suspension WB (100 g) was promptly pasteurized at 81–82° for 4 min and immediately cooled by placing it at −12° for 25 min. Pasteurization avoided degradation of glucose (Table I) probably by bacterial contamination or conversion by enzymes naturally present in the apple. This preparation was then poured into a vol-

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