MICROFLORA OF COCOA BEANS BEFORE AND AFTER ROASTING AT 150 C[1]

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Abstract

Cocoa beans representative of different fermentation practices, flavor types, and geographic origins were collected and analyzed microbiologically before and after roasting under laboratory conditions which simulated commercial practices. The detectable number of microorganisms per gram of bean before roasting ranged from 3×10^5 to 4.7×10^7. After roasting the beans for 40 min at 150 C the counts ranged from 10^3 to 1.6×10^5 per gram. The genus *Bacillus* accounted for 91% of the 277 isolates. Only bacilli were found in roasted beans and *Bacillus stearothermophilus* and *Bacillus coagulans* were the predominant species. Microorganisms identified in unroasted beans were nineteen species of *Bacillus*, two of *Streptococcus*, two of *Micrococcus*, two of *Escherichia*, and single species of *Aerobacter*, *Flavobacterium*, and *Microbacterium*.

Cocoa beans are exported primarily from West central Africa and Brazil with smaller, but still significant, quantities being harvested in Ecuador north through the southern part of Mexico, the islands of the Carribean Sea, and the Southwest Pacific Ocean. The variability in quality of this agricultural commodity is of great concern to chocolate manufacturers. Much is known about the chemistry of roasted cocoa beans. However, published information on microbiological aspects is quite limited. The objective of the present study was to more fully characterize the microflora of raw and roasted cocoa beans.

After harvesting, cocoa beans usually undergo a fermentation which facilitates development of flavor precursors and helps to remove mucilage from the beans. At the start of fermentation, the predominant microflora are yeasts which are then replaced by lactic acid bacteria, acetic acid bacteria, and finally spore-forming bacilli as the fermentation is completed (3). In Jamaican beans, *Bacillus subtilis* was identified as the predominant organism after fermentation (7). During this process, the internal temperatures in the bean heap may reach 50 C (3). Moisture in beans is then reduced to 6% or less, most commonly by sun drying, but mechanical drying is finding increasing acceptance. Dried beans are exported in bags and are usually stored in silos at the processing plant until roasted.

One of the most important processes affecting quality involves the roasting of cocoa beans in the chocolate factory. During roasting, flavor is developed, moisture is removed, the microbial population is reduced, and the shell is loosened from the nib. Roasting time and temperature combinations may vary from 15 min to 2 hr and from 105 to 150 C (2).

Materials And Methods

Bean samples

Four chocolate manufacturers supplied the following beans representing different fermentation patterns, flavor types, and geographic origins: Arriba (Ecuador), Bahia (Brazil), Ghana (Africa), New Guinea, Samoa, Sanchez (Dominican Republic), Trinidad, and a mixture of Bahia and Arriba.

Temperature measurement and roasting procedure

Raw beans were laboratory-roasted in a forced air STABILTHERM constant temperature cabinet (Blue M Electric Co.). The mixture of Bahia and Arriba beans was used in a preliminary study to determine the temperature gradient in beans at various air temperatures. Thermocouples inserted in the geometric center of the beans were attached to a Honeywell-Brown Electronik potentiometer and temperature recordings were taken periodically for 40 min. At 135 150, 160, and 180 C oven air temperature equilibrium was reached after exposure periods of approximately 10, 15, 15, and 20 min, respectively.

The effect of roasting variables on the reduction in microbial populations was assessed by roasting beans for 30 min at the above mentioned air temperatures and by varying the exposure time (20, 30, and 40 min) at constant roasting temperature (150 C). All identification work involved beans roasted at 150 C. Organoleptic evaluations and chemical comparisons between samples roasted in the laboratory and roasted cocoa beans supplied by chocolate manufacturers indicated that time-temperature combinations employed in this study were representative of commercial practices.

Microbiological analysis

Immediately after roasting, 30 g of beans were blended for 2 min with 270 ml 0.1% sterile peptone water in a sterile Waring Blendor. Decimal dilutions were prepared using 0.1% sterile peptone water and were plated in duplicate on plate count agar (Difco) with 0.1% K_2HPO_4 added. After 48 hr incubation at 37 C the total number of organisms per gram was determined. Unroasted beans were tested for coliforms using the three-tube MPN method and lauryl tryptose broth (Difco). Samples from tubes showing gas

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TABLE 1 TOTAL NUMBER OF MICROORGANISMS IN COCOA BEFORE AND AFTER ROASTING FOR 30 MIN AT VARIOUS TEMPERATURES.

Roast temperature (C)	Number of organisms/g[1]
Unroasted	1.5×10^7
135	2.6×10^6
150	2.7×10^5
165	2.8×10^3
180	0

[1]Average of three trials.

TABLE 2. NUMBER OF MICROORGANISMS PER GRAM OF COCOA BEAN BEFORE AND AFTER ROASTING AT 150 C FOR VARIOUS PERIODS OF TIME.

Bean type	Roast time (min) 0	20	30	40
	(Number of microorganisms/g)[1]			
Trinidad	4.7×10^7	2.2×10^6	4.2×10^5	6×10^4
Mixed Bahia and Arriba	1.5×10^7	1.3×10^6	5.8×10^5	1.6×10^5
Sanchez	5.0×10^6	2.6×10^4	3.8×10^4	2.7×10^3
Samoan	2.8×10^6	1.1×10^5	1.5×10^4	3.1×10^3
New Guinea	2.0×10^6	8.1×10^4	5.9×10^4	1.4×10^4
Ghana	1.7×10^6	1.7×10^5	4.4×10^4	6.4×10^3
Arriba	8.9×10^5	6.3×10^4	3.9×10^4	6.0×10^3
Bahia	3.0×10^5	1.1×10^5	2.7×10^3	8.4×10^2

[1]Average of three trials.

TABLE 3. THE MOST COMMONLY ISOLATED MICROORGANISMS IN COCOA BEANS BEFORE AND AFTER ROASTING FOR VARYING TIMES AT 150 C.

Organisms	Time Roasted (min) 0	20	30	40
	(% of isolates)			
B. stearothermophilus	18.4	23.2	18.6	20.5
B. licheniformis	14.9	5.4	14.0	9.1
B. circulans	11.5	21.4	11.6	15.9
B. coagulans	8.0	10.7	3.6	22.7
B. megaterium	3.4	5.4	7.0	4.5
B. brevis	10.3	5.4	3.6	4.5

production after 48 hr incubation at 37 C were transferred to 2% brilliant green bile broth (Difco) and incubated at 37 C for 48 hr. Positive tubes were then streaked on eosin methylene blue agar for confirmation of coliforms. To determine the number of yeasts and molds on unroasted beans, dilutions of 10^{-3} and 10^{-4} were plated on potato dextrose agar [(Difco), pH adjusted to 3.5 with 10% tartaric acid], and incubated for 72 hr at 25 C.

Individual colonies were isolated from plates having 30-300 colonies. Each isolate was cultured on tryptic soy agar slants (Difco) and classified according to *Bergey's Manual (1)* using procedures described in *Laboratory Methods in Microbiology (6)* and *Manual of Microbiological Methods (10)*, except for sugar fermentation tests in which the microtiter method described by Fung and Miller *(4)* was employed.

RESULTS AND DISCUSSION

The numbers of microorganisms per gram of mixed beans before and after roasting for 30 min at 135, 150, 165, and 180 C are shown in Table 1. As evidenced, roasting at 180 C and 165 C for 30 min had an extreme bactericidal effect, but these temperatures also imparted scorched flavor characteristics.

The number of organisms found before and after roasting for various periods of time at 150 C are shown in Table 2. Microorganisms in unroasted beans ranged from approximately 3×10^5/g for Bahia beans to 4.7×10^7/g for Trinidad beans. Yeast and mold counts were not significant, being less than 10^3/g in unroasted beans. Only one sample, unroasted Trinidad, was positive for coliforms (110-MPN/g). After roasting for 40 min, microbial counts varied from 8.4×10^2/g for Bahia beans to 1.6×10^5/g for the mixed bean sample. As might be expected, the number of microorganisms which survived roasting was highest in the most heavily contaminated samples.

A total of 277 isolates from unroasted and roasted beans were identified. The majority of these microorganisms belonged to the genus *Bacillus* (91%). Bacilli commonly found in unroasted beans but not in roasted beans were *Bacillus cereus* var *mycoides*, *Bacillus firmus*, *Bacillus laterosporus*, *Bacillus macerans*, *Bacillus polymyxa*, and *Bacillus pumilus*. Microorganisms other than bacilli detected in unroasted beans were *Enterobacter (Aerobacter) aerogenes*, *Escherichia coli*, *Escherichia freundii*, *Flavobacterium lactis*, *Microbacterium flavum*, *Micrococcus candidus*, *Micrococcus conglomeratus*, *Streptococcus lactis*, and *Streptococcus thermophilus*. With the exception of *M. conglomeratus*, these organisms were isolated from 1:10 dilutions and were not considered a part of the predominant microflora. They were not detected in roasted beans and presumably were destroyed during roasting.

Bacillus stearothermophilus was the most frequently identified isolate in both roasted and unroasted beans. Other commonly occurring species were *Bacillus brevis*, *Bacillus circulans*, *Bacillus coagulans*, *Bacillus licheniformis*, and *Bacillus megaterium*.

All microorganisms isolated from roasted cocoa beans belonged to the genus *Bacillus*, of which nineteen species were identified. As shown in Table 3, *B. stearothermophilus*, which is extremely heat resistant, and *B. coagulans* accounted for nearly one-half of the isolates from beans roasted for 40 min at 150 C. *Bacillus stearothermophilus*, *B. coagulans*, and *B. circulans* showed more resistance to roasting than other species.

Cocoa beans analyzed in this investigation represented different fermentation practices, flavor types, and geographic areas. Despite these differences, the microflora of the samples were remarkably similar. Results are in general agreement with those of Ostovar who studied the fermentation patterns in Trinidad beans (9). He found that the genus *Bacillus* appeared at the fifth day of fermentation and predominated thereafter. The fact that all of the isolates from roasted beans were *Bacillus* is consistent with the report of Mundt (8) who found *B. cereus* and its variants as the principal microorganism in finished cocoa powders. Results from this investigation are also congruent with the isolation of bacilli and micrococci from 36 samples of cocoa powder reported by Gabis et al. (5).

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BOOK REVIEW

BYPRODUCTS FROM MILK, Second Edition, *Edited by B. H. Webb and E. O. Whittier*, Published by the AVI Publishing Company, Inc., Westport, Connecticut, 1970, 428 p., Price $18.00.

The detailed addition of progress in byproduct research and development during the 20 years between editions of this text has made it very current and worthwhile. The theme, "Gather up the fragments that remain that nothing be lost." (John 6:12), has been the apparent goal of the contributing authors; both to assemble available fragmentary references and to deal with a subject of special concern in our ecology-conscious society. The quality of the work is reflected in the selection of contributors. The majority represent the Eastern Utilization Research and Development Division, Agricultural Research Service, USDA, most responsible for the utilization and development of new products from milk. In addition, W. S. Arbuckle, M. F. Brink, E. H. Marth, and T. A. Nickerson strengthen coverage of some of these complex subjects.

New chapters are included describing utilization of milk fat, not generally considered a byproduct in the past. There also are new contributions reviewing nutritional characteristics and waste disposal.

The text is very readable and adequately reflects the purpose of the editors to assemble the various methods into one volume. Authors have combined bulletins, patents, and journal contributions making it easy to review the state of the art.

My criticisms of the text are minor. The title of chapter two, "Fermentation Products from Skimmilk," doesn't adequately cover inclusion of some whole and partially defatted milk products. Centigrade and Fahrenheit temperatures were used without consistent policy. Summaries of current practices did not always reflect their relative merits. Preparation of some of the lesser-known products such as milk crumb, casein glue, and lipolyzed milkfat products were inadequately described; reflecting the lack of published reference material.

In general, this text represents an excellent compilation by competent experts. It is certainly an indispensable reference work for anyone involved in the research and development of dairy products. It also is a valuable source work for those in practical plant operations and for students of dairy technology.

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