STUDIES ON CACAO

I. ACTINOMYCETES ON CACAO BEANS

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A paper on Actinomycetes or ray-fungi which causes the "mould odor" or "ground odor", or, according to Fellers (**11**), "Actinomyces odor" on dry cacao beans was published in the German Language (**2**), and later new observations were published in the Italian language (**3**). These two papers will be briefly summarized at this time in order to make this series of studies more complete.

In spite of the fact that arthrospores, or true spores, or fragments of the mycelium of Actinomycetes are normally present on dry fermented and unfermented cacao beans, the peculiar taste is not perceptible until the full development of the ray-fungi, e. g., after 52–76 hours of incubation in moist environment. The presence of the microorganisms may be revealed also by the presence of a superficial white dust. Its development is active at 20–25° C., maximum from about 30° C. to 40° C., but in the last case, the taste is mixed musty and rancid, in the other being from musty to musty-ground. Cultures from washing water of cacao seeds in carrot agar and in Czapek-Waksman agar revealed from 1110 to 3760 Actinomycetes for each gram of cacao beans, with an average of 2063 Actinomycetes on eight determinations.

The species most frequently isolated is the *Actinomyces albus* Krainsky emend. Waksman and Curtis, according the description of Waksman (**28**), in a slightly distinct variety (var. *alpha* Cif.). *Actinomyces griseus* Kr. and three other not identified species or forms were present, but scarcer.

In the opinion of the writer, this microorganism is passively carried from the soil to the shell of the cacao pod by the winds and the rainfall. Washing the bark of healthy pods the writer obtained from 36 to 408 colonies of Actinomycetes per gram of fresh pod, with an average of 195 colonies (six determinations). From the air of a cacao estate Actinomycetes were present in the proportions of 3, 9, 8, and 14 per cent of the colonies developed. But the most active agents for carrying spores of Actinomycetes are the men who break the shells of the cacao pods, extracting the masses of cacao beans and isolate the seeds from the rachis. If the beans are fermented, another source

of contamination is the fermenting boxes, from which Actinomycetes were isolated. The Actinomycetes are diffused also by the fermentation liquor, containing from 5 to 11 ray-fungi for each gram of the liquor, with an average of 7 from four determinations. The fermentation humour was inoculated until 4906 colonies of *Actinomyces albus* were obtained for each c. c.; samples were taken each three days and the colonies enumerated. On the 15th day the number decreased to 3550. The conclusion was that the *Actinomyces* live but in an inactive form in the fermentation liquor. The same humour, freshly taken from the fermentation boxes, sterilized and inoculated with 13780 germs per c. c. at the 15th day contained 14070 ray-fungi per c. c.

Previously the writer (**4**) studied the mouldy taste of the wine, finding that the ray-fungi are living on rotten wood of the casks and tuns (less well on unaltered wood), and may communicate easily to the wine the peculiar taste *. According to Fellers (**11**), the bitter taste and the Actinomyces odor of the milk is caused by the *Actinomyces griseus* Kr. Zedfield attributed the same taste of the eggs to the ray-fungi. The mouldy taste of Javanese tobacco sent from Java to Europe in good condition, according to Gandrup (**13**) is caused by unidentified species of Actinomycetes.

The complete prevention of the contamination of cacao beans is impossible, from the practical standpoint, but may be reduced to a minimum by: (1) avoiding the contamination by soil; (2) isolating rotten or wounded pods, chiefly the pods damaged by the bird (*Picumnus* [*Centurus*] *striatus*) and by fruit-eating bats, from healthy pods; (3) avoiding the introduction in the mass of cacao beans of leaves, small branches, and portions of shell of the cacao tree or pod; (4) rigorous cleaning and disinfection of the fermentation boxes; (5) avoiding the mixture of cacao beans from rotten or wounded pods with beans from healthy cacao pods.

Also it may be noted that unfermented cacao beans are poorer in Actinomycetes than the fermented ones (from 320 to 1230 colonies for each c. c. of washing water, with an average of 776 per c. c., as obtained from six determinations), and, of course, unfermented cacao is less susceptible to the mouldy taste than the fermented.

From the technologic standpoint, this moulding is of course, very distinct from the true moulding, but of a very reduced importance, as the contamination is limited to the external surface of the cacao

* The Actinomycetes causing the mouldy taste of the wine was identified as the *Streptothryx Sanninü* Cif., but according to the most accepted generic nomenclature, it is a species of *Actinomyces* and very similar to *A. albus*.

beans. From the commercial standpoint, it may be observed that the responsibility of the farmer is very limited, and the growth in number of the Actinomycetes, as well as the development from the arthrospores or resting spores being effected during shipment.

II. FUNGOUS FLORA OF DRY CACAO BEANS

Two types of cacao mouldings are recognizable on dry fermented and unfermented Dominican cacao, namely: (1) Cacao moulding which gives a peculiar and disagreeable odor known as "mould odor" or "Actinomyces odor". This odor is caused by many species of the genus *Actinomyces*. Colonies of such common moulds as Mucorales and Hyphales may or may not be present. Strongly contaminated cacao frequently appears superficially covered with a whitish delicate dust which is composed of colonies and more or less fragments of the ray-fungi. (2) Cacao moulding without any particular odor, but showing a more or less luxuriant growth of mould fungi (Mucorales and Hyphales), composed of colonies which are blackish, brownish or green-bluish, rarely whitish to yellowish or reddish in color. Very frequently, the two types of moulding are associated, as the ray-fungi are almost constantly present on dry cacao beans, but the "Actinomyces odor" is clearly perceptible only if cacao seeds are intensively contaminated.

In a previous paper (**2**), the writer referred on the first type of cacao moulding. The most frequent and important ray-fungus is the *Actynomyces albus* Krainsky emend. Waksm. & Curt., with a local but scarcely distinguished variety *alpha* Cif.; also *A. griseus* Krainsky is present. The following moulds were isolated: *Rhizopus arrhizus* Fisch., *R. nigricans* Ehrenb., *Macrosporium commune* Rabenh., *Coniothecium effusum* Corda, *Botrytis vulgare* Fries, *Aspergillus fumigatus* Fres., *A. niger* V. Tiegh., *A. elegans* Gasp., *Fusarium* sp., *Spicaria lateritia* Cif.

The purpose of the present investigation, carried out during the years 1926 and 1927 was: (1) the study of numerical distribution of the mould spores on dry fermented and unfermented healthy cacao beans, as well as on moulded cacao beans; (2) the identification of isolated moulds; (3) the study of the comparative growth of the most frequent moulds on pasteurized fermented and unfermented cacao beans.

Scattered notices about moulds of cacao and cacao moulding are found in the literature on cacao, but the first satisfactory determinations of moulds were obtained from the studies of Dr. Thom of

cultures made by Dr. Schwarz from Gold Coast (Accra type) cacao (**26**). He found that several forms of *Aspergillus niger* and *A. flavus* group, were present in practically every phase of the culture work; *A. tamarii* was encountered in the beans taken from near the surface of the mass. He found also abundant Mucoraceace, chiefly species of *Rhizopus,* a few yeasts, and aerobic spore-forming bacteria, including bacteria of the *mesenteric group.**

Reinke (**23**) isolate 142 strains of Aspergilli from cacao, of which the most frequent were *Aspergillus flavus* and *A. niger* (75 per cent), *A. Sydowii* and *A. tamarii* (50 per cent); also *A. repens, A. terreus, A. carbonarius, A. versicolor* var. *flavipes, A. candidus, A. giganteus, A. ochraceus* and *A. versicolor.* From Haitian samples, he isolated *A. niger, A. tamarii, A. carbonarius* and *A. flavus.*

Green, brown, and black *Aspergilli,* six *Mucorales,* seven *Penicilli,* three *Cladosporium, Cephalothecium, Fusarium, Botrytis* and a Sphaeropsidaceous fungus were isolated from cacao beans of many African and American countries by Busse, Henneberg and Zeller (**1 bis**).

EXPERIMENTAL PROCEDURE

Samples of fermented, unfermented and mixed (fermented and unfermented) cacao beans, of about one pound each, were taken from the store houses of the cacao farmers and traders of Moca, Santiago, La Vega, San Francisco de Macorís, Sánchez and Samaná, and carefully packed. As soon as possible, 25 beans from each sample were washed with 100 c.c. of sterile, distilled water. Holding firmly with a sterile forceps, each cacao bean was thoroughly scrubbed with a sterile stiff stencil brush. Duplicate plate cultures on Bacto prume agar were made, using five, ten, fifteen and twenty drops and one c.c. of thoroughly agitated wash water. To inhibit the growth of Schizomycetes, the substratun was mercurized to 1/10.000 (adding 1 c.c of one per cent solution of mercury chloride to 100 c.c. of prune agar), following the method of De Rossi (**10**). Each set of ten plates was incubated in the laboratory at ordinary room temperature (24–32° C.) during three days, and all apparently different colonies of fungi were isolated for later identification. Numbered stock cultures were subdivided in five groups, according

* During the publication of this paper, I knew of the isolation and study of many other fungi isolated from cacao beans. Sartory A., Sartory R. and Meyer J. (Ann. Mycol. 28 (5–6): 362–362. 1930) isolated a species of *Aspergillus (A. halophilus)* from sample of cacao damaged by the contact with sea water and subsequently stored under warm condition I was informed of a few papers on this subject published in the English colonies of Africa, but without knowledge of the content, as well as of the bibliographical reference.

to the results of a preliminary identification, namely: (1) Aspergilli forms, (2) Penicilli forms, (3) species of Mucorales, (4) mould fungi other than the preceding, (5) Saccharomycales and Torulopsidaceae (ascosporic and anascosporic yeasts).

The cacao used was the common cacao (so called Sánchez type), chiefly derivated from Calabacillo variety-group crosses. Samples marked *A* to *G* were taken from 1926 crop, and samples marked *H* to *L* from 1927 crop. The locality and quality of samples was the follow:

Sample A: mixed healthy cacao from Moca
Sample B: unfermented healthy cacao from Moca.
Sample C: fermented healthy cacao San Francisco de Macorís.
Sample D: mixed healthy cacao from San Francisco de Macorís.
Sample E: mixed healthy cacao from La Vega.
Sample F: mixed healthy cacao from Samaná.
Sample G: mixed moulded cacao from Moca.
Sample H: mixed healthy cacao from Santiago.
Sample I: unfermented healthy cacao from San Francisco de Macorís.
Sample J: fermented healthy cacao from San Francisco de Macorís.
Sample K: mixed moulded cacao from Samaná.
Sample L: mixed moulded cacao from Sánchez.

NUMERICAL DISTRIBUTION OF MOULD FUNGI

The numerical distribution of spores of mould fungi per cacao bean (colony counts) * was as follows:

Sample	Per cent Mucorales of	Per cent of Aspergilli	Per cent of Penicilli	Per cent of other moulds	Total number of colonies
A	32	41	21	6	700
B	46	39	7	8	1,400
C	17	66	15	2	1,300
D	56	38	5	1	1,700
E	39	44	13	4	1,900
F	54	41	5	0	4,200
G	48	36	15	1	89,500
H	39	47	13	1	3,000
I	60	21	16	3	4,700
J	40	54	5	1	600
K	67	18	15	0	67,100
L	74	24	2	0	38,900
Average	48	39	10	3	17,917

AVERAGES

Average of mould spores on mixed healthy cacao (samples A, D, E, F and H): 2300.
Average of mould spores on fermented healthy cacao (samples C and J) : 950.
Average of mould spores on unfermented healthy cacao (samples B and I): 3050.
Average of mould spores on mixed moulded cacao (samples G, K, and L): 65270.

* True and anascosporic yeasts, besides as scarce as 0, 1 per cent or less, will be studied in another section of this paper.

IDENTIFICATION OF THE MOULD FUNGI

Group (1) Aspergilli. The systematic and nomenclature of Thom and Church (1) has been followed. Colonies, growing on Czapeck's solution agar, were incubated in the Laboratory at room temperature. In view of the fact that the isolated *Aspergillus* strains, are not specialized on cacao, but are more or less saprophytic forms, the identification was frequently limited to the forms-group only.

Strains N. 12, 15, 31, 56 and 60 belong to the *Aspergillus niger* group, and probably, should be determined as *A. niger* V. Tiegh., an exceedingly variable form. This species is present in almost all cultures in Petri dishes, and is frequently the most abundant mould.

Strains N. 17, 26, 29, 34, 40 and 48 belong to the *A. fumigatus* group, but I have never seen the perithecial forms. This mould is as frequent as the preceding group-species.

Strains 18, 19, 42, 46 and 59 belong to the *A. glaucus* group, and this species is very frequent, but only in certain samples (A, B, D, G, K, L).

Strain N. 38 and 39 belong to the *A. tamarii* group, and is perhaps identic with *A. tamarii.* As in the preceding, this form is frequent only in certain samples (samples *F, K* and *L*).

Strains 22, 24 and 52 belong to the *A. nidulans* group; strains N. 22 and 24, isolated from the sample G, are probably identical with the true *A. nidulans.*

Strains N. 30, 36, 44 and 68 belong to the *A. flavus* group. Strains of this group are as frequent as the *A. niger* and *A. fumigatus.*

Strain N. 67 belong to the *A. versicolor* group. A rare form isolated only from the sample *D.*

Strain N. 85 belong to the group of *A. candidus.* As in the preceding, but from the sample *J.*

Group (2) Penicilli. The study published by Biourge has been followed, and the cultures grown on Raulin-Diercky and on Hayduk solutions, etc., are made according the procedure described in Biourge's monograph and at room temperature.

Strains 32, 54 bis, 58 and 82.—*Penicillium leucopus* (Pers.) Biourge. This form, probably corresponding to *Penicillium glaucum* or *P. crustaceum* Auct. pl., is the one most frequently seen on cultures in Petri dishes, but never as abundant as the blackish Aspergilli.

Strains N. 43 and 64.—*P. notatum* West. An unfrequent form, isolated from the samples D and G.

Strain N. 51.—A form allied with *P. luteum* (Zuk.) Thom (fide Biourge) isolated from the sample H, and here very frequent.

Strain N. 33.—This strain is definite, but very doubtfully, as the *P. roseum* Link, a classic but from the modern point of view, not clearly definite species. It is not considered in Biourge's monograph. It was isolated from the sample E only once.

Strains N. 77 and 80.—These strains are identical with or allied to *P. candidum* Roger (nec Link, fide Biourge), and were isolated from samples J and K. Found also as indefinite, small, arachnoid colonies on moulded cacao beans.

Group (3) Mucorales. Species of this order were studied and classified following the technic and the systematic and nomenclature of Lendner (**18**).

Strains N. 14, 20, 24, 53 and 65.—These strains, isolated from the samples A, C, D, G, H and K, were referable to *Rhizopus nigricans* Ehr.

Strains N. 35, 65 and 83.—*Rhizopus arrhizus* Fisch, isolated from the samples G, K, and L.

Strains N. 22 bis, 75 and 87.—These strains must be referred to *Mucor mucedo* L., a mould as frequent as the *Rhizopus nigricans* in all moulded cacao beans.

Strain N. 74.—This strain, isolated from the sample I, is closely allied or, probably, identic with *Mucor racemosus* Fres.

Group (4) Mould fungi other than Aspergilli, Penicilli and Mucorales. The species of Hyphales isolated from cacao beans were identified following the systematic treatment of this group by Lindau (**20**) and Ferraris (**12**). Cultures were made using many solid media, chiefly Bacto prune agar, peptonized potato agar and carrot agar.

Strains 22, 30, 58 and 71.—All these strains must be referred to *Spicaria lateritia* Cif., one of the saprophytic fungi most universally distributed in the Dominican Republic. It is, also, one of the most frequent contaminaters of cultures. Conidia are present in the air, in water, in soil, etc. It forms large and beautiful colonies, orange or lateritic-red in color, on partially burned wood during the rainy seasons or in moist places.

Strains N. 13, 21, 74 and 79.—All these strains are referable to *Cephalosporium acremonium* Corda, a mould almost as frequent as *Spicaria lateritia.*

Strains N. 39 and 50.—Another largely distributed mould, referred to *Trichothecium roseum* Link.

Strain N. 25.—This strains must be referred to a new species of *Helminthosporium*, a saprophyte described as *H. cacaophilum* Cif., very distinct from *H. theobromicolum* Cif. and *H. theobromae* Turc. Found only in the sample B.

Strain N. 45.—Belongs to *Macrosporium commune* (Rabenh.) Sacc., as conventionally understood. This is an unfrequent mould, isolated twice from sample A.

Strains N. 33, 63 and 73.—This mould was isolated three times from samples B, E, and G, but is probably more common. It is not different from *Pullularia pullulans* (De By.) Berkh., commonly called *Dematium pullulans* De Bary.

Strains N. 37 and 47.—Belongs to *Alternaria tenuis* Nees, and was isolated twice from samples G. and H. About the 50 per cent of the colonies isolated from the sample H are composed of this fungus.

Strain N. 41.—*Catenularia fuliginea* Saito, a rare species found only in the sample F.

Strain N. 65.—For this strain I am describing a new species of the genus *Dendryphium* (*D. congestum* Cif., n. sp.). It was found in the sample L, growing in a single colony.

Strain N. 38.—This strain is of doubtful identification; apparently it belongs to *Coniothecium effusum* Corda. It was found in a single colony from the sample C.

Strain N. 66.—Found in a single colony from cultures derivated from sample K. It was referred to a new genus and new species, *Blastoconium tropicum* Cif., n. gen. et n. sp.

Strain N. 79.—Isolated from sample B and doubtfully referred to *Hormodendron pallidum* Oudem.

Strain N. 55.—*Fusarium sarcochroum* (Desm.) Sacc., or an allied form, isolated from sample K.

Strain N. 78.—This strain must be referred to a slightly different variety of *Fusarium zonatum* (Sherb.) Wollenw., but its systematic position is doubtful. It was isolated from sample G.

GROWTH OF MOULDS ON CACAO BEANS

With a few exceptions, the isolation of a mould from cacao beans does not demonstrate the possibility of a luxuriant growth on the same, or, in other words, that the fungus must be considered as one of the causes of cacao moulding.

The inoculations were made by spraying a suspension of the conidia (from cultures in Petri dishes) in distilled sterilized water with a small atomizer on: (1) fermented uncut cacao beans, (2) fermented cut beans, (3) unfermented uncut cacao beans, (4) unfermented cut beans. Before the inoculation, the four samples of cacao beans were washed with 0.2 per cent solution of mercury bichloride and repeatedly re-washed with sterilized destilled water, and then enclosed in four ample dessiccators partially filled with water, during four days. After the water imbibition by cacao beans, each sample was divided in twenty small samples, all of which was inoculated with one of the isolate moulds, and enclosed in a common sterilized drinking-glass, closed by a photographic plate glass and sealed with paraffine. The set of eighty glasses were kept during thirty days in incubation at the laboratory temperature, and then opened and the growths of moulds observed as appearing to the naked eye. Of the isolated moulds, only twenty forms, appearing as the most frequent, were inoculated. The results are summarized as follows:

(The sign O signifiy no growth; ? doubtful growth; + scarce development; + + abundant development; + + + very abundant development).

Mould	Fermented cacao		Unfermented cacao	
	Cut beans	Uncut beans	Uncut beans	Uncut beans
Aspergillus niger	+ + +	+ + +	+ + +	+ + +
Aspergillus fumigatus	+ + +	+ + +	+ + +	+ + +
Aspergillus glaucus	+	+	+ +	+ +
Aspergillus tamarii	+	?	+	?
Aspergillus nidulans	0	0	+	+
Aspergillus flavus	+	+	+ +	+ +
Aspergillus versicolor	0	?	?	?
Aspergillus candidus	0	0	0	?
Penicillium leucopus	+	+	+	+
Penicillium notatum	0	0	0	0
Penicillum luteum	0	0	?	+
Penicillium roseum	0	0	?	?
Penicillium candidum	0	?	+	+
Spicaria lateritia	0	0	0	?
Cephalosporium acremonium	0	0	0	0
Trichothecium roseum	0	0	?	0
Pullularia pullulans	0	0	0	0
Alternaria tenuis	0	0	?	+
Rhizopus nigricans	+ +	+ +	+ + +	+ + +
Rhizopus arrhizus	+	+	+	+
Mucor mucedo	+ +	+ + +	+ +	+ + +
Mucor racemosus	0	0	?	?

DISCUSSION OF THE RESULTS

The number of strains and mould forms isolated from cacao beans should be summarized as follows:

(1) Aspergilli: 27 strains, 8 forms.

(2) Penicilli: 10 strains, 5 forms.
(3) Mucorales: 12 strains, 4 forms.
(4) Mould others than the preceding: 24 strains, 14 forms.
These strains derived from the following cacao beans samples:

Sample A: 10 forms.	Sample G: 16 forms.
Sample B: 11 forms.	Sample H: 10 forms.
Sample C: 9 forms.	Sample I: 8 forms.
Sample D: 11 forms.	Sample J: 9 forms.
Sample E: 8 forms.	Sample K: 16 forms.
Sample F: 10 forms.	Sample L: 12 forms.

Or, in relation to the quality of cacao, an average of:

Mixed healthy cacao: 10 forms.
Unfermented healthy cacao: 10 forms.
Fermented health cacao: 9 forms.
Mixed moulded cacao: 15 forms.

As one may expect, the fermented healthy cacao beans are the poorest in forms of moulds, and moulded cacao the richest. Mixed and unfermented cacao are equally rich in forms of moulds, and intermediary between the preceding samples.

Aspergilli are the most frequent moulds, and the group is the richest in forms. Penicilli and Mucorales are almost of the same importance. The following species are normally present in samples of cacao beans: *Aspergillus niger; A. fumigatus; A. flavus; A. glaucus; Penicillium leucopus; Rhizopus nigricans; Mucor mucedo; Spicaria lateritia; Cephalosporium acremonium.* Mucorales are the most abundant as to number of spores, then Aspergilli, Penicilli and, last, other Hyphales.

These results agree well with the observation made by Schwarz in Gold Coast on Accra cacao beans, and in relation to the isolated forms, with the preliminary experiments of the writer.

SUMMARY

The writer refers to the results of isolation from and inoculation of fermented and unfermented cacao beans of mould fungi. A new genus and two new species of Dematiaceae are described.

MYCOLOGICAL OBSERVATIONS AND DESCRIPTIONS OF STRAINS

Strain N. 25.—*Helminthosporium cacaophilum* Cif., n. sp. (Description from cultures.)

At the room temperature, the colonies develop easily; colony 5 days old is from 20 to 45 mm. in diameter. It is composed of a well developed system

of myceliar hyphae, without clearly differentiated conidiophores, and a few scattered conidia. The colony is, at first, white and tufted, then sub-lanose and developed in concentric but not well defined rings, varying from light-gray to dull-gray, with black shades. Mycelium abundant, composed of densely but irregularly branched hyphae, septate, containing droplets and refringent corpuscula, generally from 2 to 3.5 mmm. in diameter. Short lateral sub-erected myceliar branches 15–25 mmm. in length, may produce a few conidia, acrogenous or pleuro-acrogenous, inserted on small teeth. Conidia brown to grayish, from 5 to 13-septate, generally 7–10-septate, ellipsoid to ovoid, free and more or less rounded, basal end sub-acuminate, 65 to 94 mmm. in length, 10 to 15.5 mmm. width. Isolated from unfermented healthy cacao beans from Moca, Dominican Republic.

Strain N. 63.—*Dendryphium (Brachycladium) congestum* Cif., n. sp. (Description from cultures).

At room temperature, this fungus grows readily covering almost all the surface of the Petri dish. The colonies are flattened, at first black-greenish, then blackish, smooth, opaque, with a very poor aerial development. The mycelium is brownish, septate, densely branched, and developed abundantly only under the surface of the solid substratum, 2–3 mmm. in thickness. The conidiophores emerge from the substrata, but are sub-erect to prostrate, more or less straight, with a few septa, unbranched or scarcely branched, 20–50 mmm. by 2–3.5 mmm. The conidia are normally acrogenus, very rarely acro-pleurogenous, isolated or, more frequently, from 2 to 6-chained, 3–5-septate, smoky, clearly narrowed at the septa, from elliptic to ovoid, 14–33 by 4.58 mmm. Isolated from mixed moulded cacao beans from Sánchez, Dominican Republic.

Strain N. 66.—*Blastoconium* Cif., n. gen. (Hyphales, Dematiaceae, Phaerdictyae, Coniothecieae).

Similar to the genus *Coniothecium* Corda, but with sterile hyphae torulose-moniliform, from which each single isolated element (chlamydospore) may reproduce itself by budding. As in the genus *Coniothecium*, true conidiophores are absent, and the conidia are transversally and longitudinally septate.

Blastoconium tropicum Cif., n. sp. (Description from cultures).

Develop easily at room temperature. The colonies are, at first very similar to the colonies of the *Pullularia pullulans*, repeating the *Dematium*-like stage of most of the Dematiaceae; the growth of the underground part is more active than the superficial growth. The colonies are smoky to blackish, then black, smooth, humid. The mycelium is composed of branched hyphae, densely septate, at first narrowed at the septa, then producing by budding one or two lateral daughter cells. The daughter cells may separate and reproduce by budding or not, in the last case forming two cells more or less of the same size. Successively, the number of cells constituting the chain increases and branches, and at the same time a number of blastospores become deeper in color while the membrane increases in thickness. The final result is the presence of monilioid or torulose chains, composed of spheric or sphered, elliptic, ovoid, cylindric or irregularly-shaped elements, normally unseptated, rarely transversally septate, very variable in size, from 4

to 15 mmm. in diameter or in length. A few monilioid hyphae may be produced only on the surface of the solid substratum (exceptionally on liquid media) one isolated conidium, apparently generated from one aerial bud of the terminal chlamydospore, clearly distinct from the mother cell. The conidium is irregular in shape, more frequently elliptic or sub-cylindric, from 1-septate to 3-septate, but in many cases with a longitudinal irregular septum; the size of the conidia is 6–17 by 4–12 mmm. Isolated from mixed moulded cacao beans from Samaná, Dominican Republic.

This genus, like *Dematium* or *Pullularia,* probably represents the most primitive and imperfect stage of development of unknown Dematioid fungi.

According the deeseription, *Coniothecium glumarum* Sacc. (Syll. fung., Vol. XIV, p. 1092, 1899) found on *Phragmites communis* in Hungary, must be referred to this genus as BLASTOCONIUM GLUMARUM (Sacc.) Cif., n. comb. This species was described as having torulose-septate hyphae, and the microconidia are probably not other than the young bud-cells of the chlamydospores.

Strain N. 55.—*Fusarium ? sarcochroum* (Desm.) Sacc. Sect. *Latcritium* Woll. Microconidia trichothecioid, 1–3 septate, 12–25 by 4–10 mmm.; macroconidia 3–6 septate, 25–52 by 3–6 mmm.

Strain N. 78.—*Fusarium ? zonatum* (Sherb.) Woll. Sect. *Elegans* Woll., Subsect. *Oxysporum* Woll., Ser. *Pallens* Woll. Sporodochial confluent gelatinous stroma; microconidia very abundant, 4–10 by 2–4 mmm.; macroconidia 32–64 by 3–6 mmm. Probably a form of the species.

III. CACAO MOULDING

This study, made in combination with previous reports on this same subject, was performed in order to determine: (1) the environmental factors and conditions influencing the development of mould fungi, chiefly in relation to (*a*) moisture content of cacao beans; (*b*) moisture absorption and loss of the same; (*c*) temperature; (2) the determination of the critical point of moulding; (3) the prevention of moulding.

As in the preceding, the studies were made both on fermented and unfermented Dominican cacao, Sánchez type.

Preliminary observation having indicated that the most important role is played by moisture content of cacao beans, this factor was studied most accurately. At the same time, a few observation were made for determination of many constants of Dominican cacao, such as average dry weight, specific weight and size of beans.

ORIGIN OF THE SAMPLES

The origin of the samples and the type of cacao beans was the following:

Sample N. 1.—Mixed cacao from Moca (marked S. B.).
Sample N. 2.—Mixed cacao from Moca (marked E. P.).
Sample N. 3.—Fermented cacao from Moca (marked E. N. A.).
Sample N. 4.—Fermented cacao from Moca (marked E. N. A.).
Sample N. 5.—Unfermented cacao from Moca (marked E. N. A.).
Sample N. 6.—Unfermented cacao from Moca (marked E. N. A.).
Sample N. 7.—Fermented cacao from La Vega (marked E. G. G.).
Sample N. 8.—Fermented cacao from La Vega (marked E. G. G.).
Sample N. 9.—Unfermented cacao from La Vega (marked E. G. G.).
Sample N. 10.—Unfermented cacao from La Vega (marked E. G. G.).
Sample N. 11.—Unfermented cacao from Bonao (marked J. J.).
Sample N. 12.—Unfermented cacao from Bonao (marked J. J.).
Sample N. 13.—Unfermented cacao from San Cristóbal.
Sample N. 14.—Mixed cacao from San Cristóbal.
Sample N. 15.—Fermented cacao from San Francisco de Macorís (marked J. M. A.).
Sample N. 16.—Fermented cacao from San Francisco de Macorís (marked B. J. R.).
Sample N. 17—Unfermented cacao from San Francisco de Macorís.
Sample N. 18.—Unfermented cacao from San Francisco de Macorís.
Sample N. 19.—Unfermented cacao from Pimentel.
Sample N. 20.—Mixed cacao from Pimentel.
Sample N. 21.—Unfermented cacao from Villa Rivas.
Sample N. 22.—Mixed cacao from Villa Rivas.
Sample N. 23.—Unfermented cacao from Samaná.
Sample N. 24.—Mixed cacao from Samaná.

NORMAL MOISTURE CONTENT AND DRY WEIGHT OF CACAO BEANS

This constant was determined by taking the actual weight of samples of 100–500 cacao beans, and drying at 100–105°C.

ACTUAL WEIGHT, DRY WEIGHT AND MOISTURE CONTENT OF CACAO BEANS

Sample No.	Average of	Actual weight gr.	Moisture content per cent	Dry weight gr.	Observations
1.....	100 seeds....	1, 068	19	0, 865	
2.....	100 seeds....	1, 059	21	0, 837	
3.....	500 seeds....	0, 949	15	0, 807	
4.....	500 seeds....	0, 936	14	0, 805	
5.....	500 seeds....	1, 020	18	0, 836	
6.....	500 seeds....	1, 059	19	0, 858	
7.....	200 seeds....	0, 970	16	0, 815	
8.....	200 seeds....	0, 945	15	0, 803	
9.....	200 seeds....	1, 081	20	0, 865	
Average....	2, 800 seeds..	1, 010	19	0, 832	General average
Average....	200 seeds....	1, 064	20	0, 851	Average of mixed cacao beans
Average....	1, 400 seeds..	0, 922	15	0, 808	Average of fermented cacao beans
Average....	1, 200 seeds..	1, 053	19	0, 820	Average of unfermented cacao beans

SPECIFIC WEIGHT OF CACAO BEANS

This constant was determined using the pycnometric method. First observations were made by filling the pycnometer with mercury, but the great difference in the specific weight of mercury and that of cacao beans gave many difficulties. The following measurements were made using graduate cylinders filled with distilled water, having observed that the absorbed water does not affect the determinations, if they are made sufficiently rapid. Of course, the precision of this specific weight is reduced.

SPECIFIC WEIGHT OF CACAO BEANS (SAMPLE OF 6 BEANS EACH)

	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 5
	0, 991	0, 974	0, 891	0, 890
	0, 983	0, 972	0, 890	0, 890
	0, 986	0, 968	0, 890	0, 893
	0, 987	0, 966	0, 887	0, 895
	0, 988	0, 973	0, 884	0, 894
	0, 986	0, 974	0, 883	0, 890
	0, 990	0, 974	0, 884	0, 885
	0, 984	0, 975	0, 887	0, 886
	0, 988	0, 976	0, 888	0, 887
	0, 986	0, 972	0, 886	0, 890
Average....	0, 987	0, 971	0, 887	0, 890

Average of mixed cacao.....	0, 979
Average of fermented cacao.....	0, 887
Average of unfermented cacao.....	0, 890

The differences between the specific weight of samples Nos. 1 and 2 composed of mixed cacao, are probably caused by the different contents of moisture (undetermined).

SIZE OF CACAO BEANS

Fifty unselected cacao beans from each sample were measured; the figures of table express the maximum length, maximum breadth and the maximum thickness.

SIZE OF CACAO BEANS

Sample N. 1 (mixed cacao).

Length mm.:	16	17	18	19	*20*	21	22	23	24	25	
Frequency N.:	1	1	7	8	10	7	8	5	1	2	= 50

Breadth mm.:	10	11	*12*	13	14	15	16	
Frequency N.:	3	4	17	13	9	3	1	= 50

Thickness mm.:	4	5	6	7	8	*9*	10	11	12	13	
Frequency N.:	1	2	9	8	11	12	3	3	0	1	= 50

Sample N. 2 (mixed cacao)

Length mm.:	16	17	18	19	20	*21*	22	23	24	25	
Frequency N.:	1	0	2	2	8	13	10	5	7	2	=50

Breadth mm.:	10	11	*12*	13	14	15	16	17	18	19	
Frequency N.:	2	5	15	14	10	3	0	0	0	1	=50

Thickness mm.:	4	5	6	*7*	8	9	10	11
Frequency N.:	2	4	6	16	14	5	2	1 =50

Sample N. 3 (fermented cacao)

Length mm.:	16	17	18	19	*20*	21	22	23	24	25	26	
Frequency N.:	1	3	7	10	19	17	17	13	6	4	3	=100

Breadth mm.:	7	8	9	10	11	*12*	13	14	15	16	17	
Frequency N.:	1	0	0	4	10	30	24	27	7	0	2	=100

Thickness mm.:	3	4	5	6	*7*	8	9	10	11	12	13	
Frequency N.:	1	2	14	22	32	13	4	6	3	2	1	=100

Sample N. 5 (unfermented cacao)

Length mm.:	16	17	18	19	*20*	21	22	23	24	25	26	27	28	29	30
Frequency N.:	2	4	2	13	19	14	9	9	13	11	1	2	0	0	1 =100

Breadth mm.:	9	10	11	12	*13*	14	15	16	17	18
Frequency N.:	2	2	11	22	27	18	11	4	1	2 =100

Thickness mm.:	4	5	6	*7*	8	9	10	11	12
Frequency N.:	1	5	22	29	22	11	8	1	1 =100

Average of the samples N. 1 and 2 (mixed cacao)

Length mm.:	16	17	18	19	20	*21*	22	23	24	25	
Frequency N.:	2	1	9	10	18	20	18	10	8	4	=100

Breadth mm.:	10	11	*12*	13	14	15	16	17	18	19	
Frequency N.:	5	9	32	27	19	6	1	0	0	1	=100

Thickness mm.:	4	5	6	7	*8*	9	10	11	12	13	
Frequency N.:	3	6	15	24	25	17	5	4	0	1	=100

These figures may be summarized as follows:

Mixed cacao: 16–25 by 16–19 by 4–13 mm.. most frequently 21 by 12 by 8 mm.

Fermented cacao: 16–26 by 7–17 by 3–13 mm., most frequently 20 by 12 by 7 mm.

Unfermented cacao: 16–22 by 9–18 by 4–12 mm., most frequently 20 by 13 by 7 mm.

WATER IMBIBITION OF CACAO BEANS

Three samples of 120 cacao beans were submersed in distilled water at 40° C., superficially dried using bibulous paper and weighed after 24 and 48 hours of immersion.

Sample N. 14 (mixed cacao)

Initial weight	gr. 1, 311 = 100
After 24 hours	gr. 1, 550 = 118
After 48 hours	gr. 1, 550 = 118

Sample N. 15 (fermented cacao)

Initial weight	gr. 1, 139 = 100
After 24 hours	gr. 1, 306 = 115
After 48 hours	gr. 1, 306 = 115

Sample N. 17 (unfermented cacao)

Initial weight	gr. 1, 228 = 100
After 24 hours	gr. 1, 478 = 120
After 48 hours	gr. 1, 478 = 120

LOSS IN WEIGHT OF CACAO BEANS EXPOSED TO THE SUNSHINE

Five samples of fermented cacao and five samples of unfermented cacao, varying from 10 to 25 kg. each, were exposed to the direct sunshine 11 hours; 7 hours the first day (from 9 A. M. to 4 P. M. August 26, 1927) and 4 hours the second day (from 8 A. M. to 11 A. M. August 27, 1927). These days were very warm, the temperature varying from 24 to 35° C. and from 24 to 34° C. Losses in weight are expressed in percentages.

PER CENT OF LOSS IN WEIGHT OF CACAO BEANS EXPOSED TO THE SUNSHINE

Hours	Sample No. 3	Sample No. 4	Sample No. 7	Sample No. 8	Sample No. 5	Sample No. 5	Sample No. 6	Sample No. 9	Sample No. 10	Sample No. 24	Average for hour	
											Fermented beans	Unfermented beans
1st	0, 37	0, 28	0, 33	0, 45	0, 38	0, 38	0, 79	0, 48	0, 80	1, 10	0, 36	0, 71
2nd	0, 35	0, 14	0, 14	0, 26	0, 16	0, 16	0, 32	0, 35	0, 49	0, 68	0, 21	0, 40
3rd	0, 35	0, 20	0, 04	0, 27	0, 19	0, 13	0, 29	0, 25	0, 31	0, 48	0, 20	0, 29
4th	0, 29	0, 10	0, 21	0, 24	0, 17	0, 17	0, 24	0, 25	0, 35	0, 41	0, 21	0, 28
5th	0, 27	0, 12	0, 07	0, 25	0, 12	0, 06	0, 20	0, 14	0, 18	0, 10	0, 15	0, 13
6th	0, 16	0, 05	0, 02	0, 08	0, 09	0, 10	0, 08	0, 08	0, 07	0, 00	0, 04	0, 06
7th	0, 00	0, 00	0, 00	0, 04	0, 00	0, 04	0, 00	0, 00	0, 03	0, 00	0, 00	0, 01
(Night)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)	(0, 00)
8th	0, 00	0, 00	0, 18	0, 08	0, 11	0, 14	0, 10	0, 06	0, 05	0, 00	0, 13	0, 07
9th	0, 28	0, 18	0, 16	0, 23	0, 15	0, 14	0, 27	0, 12	0, 28	0, 52	0, 20	0, 27
10th	0, 20	0, 13	0, 10	0, 19	0, 13	0, 08	0, 07	0, 12	0, 12	0, 26	0, 13	0, 13
11th	0, 14	0, 10	0, 10	0, 11	0, 14	0, 10	0, 05	0, 15	0, 12	0, 11	0, 10	0, 11
Total	2, 42	1, 31	1, 34	2, 19	1, 64	1, 50	1, 50	1, 99	2, 71	3, 66	1, 78	2, 27

(The increase in weight during the night, if any, was not considered, and the weight was calculated on the basis of the 7th hour).

These results are graphically expressed in the diagram of plate XXVIII.

WATER VAPOR IMBIBITION OF CACAO BEANS

Ten samples of cacao beans (five of fermented cacao and five of unfermented cacao), composed of 20 seeds each, were exposed to saturated water vapor (100 per cent, or absolute humidity) at the temperature of the Laboratory (about 24 to 32° C.) during five days. For this purpose, the samples were enclosed in two moist chambers. The increase in weight is expressed in per cent of the initial weight.

PER CENT OF INCREASE IN WEIGHT OF CACAO BEANS EXPOSED TO THE SATURATED WATER VAPOR

Sample No.	First day	Second day	Third day	Fourth day	Fifth day	Total
4	0, 00	0, 47	0, 32	0, 01	0, 00	0, 80
7	0, 00	0, 24	0, 25	0, 06	0, 01	0, 56
8	0, 00	0, 26	0, 27	0, 01	0, 00	0, 62
15	0, 00	0, 46	0, 34	0, 06	0, 00	0, 63
16	0, 00	0, 41	0, 34	0, 01	0, 00	0, 56
Average of fermented beans	0, 00	0, 37	0, 30	0, 03	0, 01	0, 63
17	0, 00	0, 22	0, 35	0, 00	0, 00	0, 56
18	0, 00	2, 12	0, 65	0, 00	0, 00	2, 77
19	0, 00	0, 20	0, 37	0, 00	0, 00	0, 57
21	0, 00	0, 33	0, 65	0, 01	0, 00	1, 00
23	0, 00	2, 38	0, 59	0, 00	0, 00	2, 95
Average unfermented	0, 00	1, 07	0, 52	0, 00	0, 00	1, 57

These figures are represented in the graph of the plate XXIX.

WATER VAPOR ABSORTION OF CACAO BEANS DRIED AT DIFFERENT TEMPERATURES

Two samples of cacao beans (one of fermented and one of unfermented cacao), composed of 20 beans each, were dried until of constant weight at different temperatures, ranging from 40° C. to 100° C., then exposed to the saturated water vapor (in a moist chamber) during ten days, and weighted. The moist chamber was situated, as in the preceding experiment, in the laboratory. The increase in weight at the end of the experiment is expressed in per cent as related to dry weight.

PER CENT OF INCREASE IN WEIGHT OF CACAO BEANS DRIED TO DIFFERENT TEMPERATURES AND EXPOSED TO SATURATED WATER VAPOR

FERMENTED CACAO (SAMPLE NO. 15)

Day	Drying temperature degrees C.							Total
	40	50	60	70	80	90	100	
First	0, 51	0, 49	0, 28	0, 11	0, 13	0, 09	0, 08	1, 69
Second	0, 29	0, 27	0, 19	0, 18	0, 09	0, 11	0, 09	1, 22
Third	0, 28	0, 13	0, 12	0, 09	0, 10	0, 10	0, 09	0, 91
Fourth	0, 01	0, 04	0, 05	0, 02	0, 06	0, 03	0, 05	0, 26
Fifth	0, 00	0, 01	0, 00	0, 02	0, 01	0, 05	0, 02	0, 11
Sixth	0, 01	0, 00	0, 02	0, 01	0, 00	0, 02	0, 05	0, 10
Seventh	0, 00	0, 02	0, 00	0, 02	0, 01	0, 02	0, 03	0, 10
Eighth	0, 00	0, 00	0, 00	0, 00	0, 01	0, 01	0, 01	0, 03
Ninth	0, 00	0, 00	0, 00	0, 02	0, 00	0, 00	0, 01	0, 03
Tenth	0, 00	0, 01	0, 00	0, 00	0, 01	0, 00	0, 01	0, 03
Total	1, 10	0, 97	0, 66	0, 47	0, 42	0, 43	0, 44	4, 48

UNFERMENTED CACAO (SAMPLE NO. 17)

Day	Drying temperatures degrees C.							Total
	40	50	60	70	80	90	100	
First	0, 23	0, 20	0, 21	0, 18	0, 15	0, 12	0, 13	1, 22
Second	0, 12	0, 10	0, 20	0, 12	0, 15	0, 16	0, 10	0, 95
Third	0, 09	0, 11	0, 19	0, 13	0, 09	0, 10	0, 09	0, 80
Fourth	0, 11	0, 12	0, 09	0, 06	0, 08	0, 10	0, 09	0, 65
Fifth	0, 07	0, 05	0, 10	0, 08	0, 08	0, 11	0, 14	0, 73
Sixth	0, 02	0, 06	0, 09	0, 09	0, 07	0, 12	0, 17	0, 62
Seventh	0, 05	0, 07	0, 08	0, 10	0, 07	0, 03	0, 12	0, 52
Eigth	0, 06	0, 05	0, 11	0, 09	0, 08	0, 06	0, 08	0, 53
Ninth	0, 04	0, 08	0, 09	0, 03	0, 01	0, 05	0, 05	0, 35
Tenth	0, 08	0, 04	0, 05	0, 07	0, 08	0, 05	0, 00	0, 35
Total	0, 88	0, 88	1, 21	0, 95	0, 86	0, 90	0, 97	6, 62

These results referred also in the clivoids of plate XXX.

DAILY VARIATION OF WEIGHT OF CACAO BEANS AS RELATED TO THE AVERAGE OF AIR HUMIDITY

Specimens of 2 kg. each of both fermented and unfermented cacao beans were taken from the samples N. 3 and 5, and weighted three times per day, at 7 A. M., at 1 P. M. and at 5 P. M. The average of weights were divided in classes of 10 gr. each, from 2000 gr. to 2080 gr. These averages were compared with the average of daily relative humidity, taken three times at the same hours. The results were the following:

Unfermented cacao (Sample N. 5)

Weight gr.																
2000 to 2010	Humidity %	69	75	77	80	81	82	83	84	85	87	88	90	91
	Frequency N.	1	1	2	3	2	2	4	5	1	1	5	2	7
	Humidity %	92	94	95	96	97	100							
	Frequency N.	7	1	3	4	2	1							

Weight gr.

2011 to 2020	Humidity %	63	69	72	76	80	83	87	88	91	95	96
	Frequency N.	1	1	1	3	1	1	3	1	5	2	3
2021 to 2030	Humidity %	72										
	Frequency N.	1										
2031 to 2040	Humidity %	60	62	64	69	83						
	Frequency N.	1	1	1	1	2						
2041 to 2050	Humidity %	64	69	76	87							
	Frequency N.	1	1	1	1							
2051 to 2060	Humidity %	75	76	83	87	91						
	Frequency N.	1	1	1	1	2						
2061 to 2070	Humidity %	62	69	73	83	96						
	Frequency N.	1	1	1	1	1						
2071 to 2080	Humidity %	76										
	Frequency N.	1										

Fermented cacao (Sample N. 3)

2000 to 2010	Humidity %	60	63	69	72	75	76	77	80	81	82	83	85	87
	Frequency N.	1	3	2	3	1	1	4	1	3	4	1	1	5
	Humidity %	88	90	91	92	94	95	96	97	100				
	Frequency N.	4	2	3	1	3	1	1	2	1				
2011 to 2020	Humidity %	69	72	76	80	83	87	91	95	96	97			
	Frequency N.	1	2	1	1	3	1	2	2	4	2			
2021 to 2030	Humidity %	76	77	91	95									
	Frequency N.	1	2	1	1									
2031 to 2040	Humidity %	62	64	69	76	80	87							
	Frequency N.	1	2	3	1	1	2							
2041 to 2050	Humidity %	62	64	69	72	76	77	91						
	Frequency N.	1	2	1	2	1	1	1						
2051 to 2060	Humidity %	69	73	76	77	83	95	96	97					
	Frequency N.	1	2	1	1	1	1	1	1					

It should be noted that the cacao beans were exposed in the open air, during several days and nights.

These results are represented in the diagram of plate XXXI.

HOURLY RECORD OF THE VARIATIONS IN WEIGHT OF CACAO BEANS

Two samples of 500 gr. each of fermented and unfermented cacao beans were taken and left to the open air. During 24 consecutive

hours, and each 15 minutes, the samples were weighted and the relative humidity recorded. The results were as follows:

HOURLY RECORDS OF VARIATIONS IN WEIGHT OF CACAO BEANS AND VARIATIONS OF RELATIVE HUMIDITY

Fermented Cacao Sample No. 3				Unfermented Cacao Sample No. 5			
Hour	Weight gr.	Per cent variations	Relative humid.	Hour	Weight gr.	Per cent variations	Relative humid.
6 p. m.	500, 0	+ 0, 00	91	6 p. m.	500, 0	+ 0, 00	92
6, 15 p. m.	500, 2	.....	96	6, 15 p. m.	500, 1	.....	92
6, 30 p. m.	500, 4	.....	96	6, 30 p. m.	500, 1	.....	91
6, 45 p. m.	500, 6	.....	96	6, 45 p. m.	500, 2	.....	91
7 p. m.	500, 8	+ 0, 16	96	7 p. m.	500, 3	+ 0, 06	91
7, 15 p. m.	501, 0	.....	96	7, 15 p. m.	500, 4	.....	91
7, 30 p. m.	501, 3	.....	96	7, 30 p. m.	500, 5	.....	91
7, 45 p. m.	501, 5	.....	96	7, 45 p. m.	500, 5	.....	91
8 p. m.	501, 7	+ 0, 34	96	8 p. m.	501, 2	+ 0, 24	91
8, 15 p. m.	501, 8	.....	96	8, 15 p. m.	501, 7	.....	96
8, 30 p. m.	501, 9	.....	96	8, 30 p. m.	502, 1	.....	96
8, 45 p. m.	502, 0	.....	96	8, 45 p. m.	502, 2	.....	96
9 p. m.	502, 3	+ 0, 46	96	9 p. m.	502, 4	+ 0, 48	96
9, 15 p. m.	502, 3	.....	96	9, 15 p. m.	502, 5	.....	96
9, 30 p. m.	502, 4	.....	96	9, 30 p. m.	502, 6	.....	95
9, 45 p. m.	502, 5	.....	96	9, 45 p. m.	502, 8	.....	95
10 p. m.	502, 6	+ 0, 52	96	10 p. m.	502, 9	+ 0, 58	95
10, 15 p. m.	502, 7	.....	96	10, 15 p. m.	503, 1	.....	95
10, 30 p. m.	503, 0	.....	95	10, 30 p m.	503, 4	.....	95
10, 45 p. m.	503, 0	.....	95	10, 45 p. m.	503, 9	.....	95
11 p. m.	503, 2	+0, 64	95	11 p. m.	504, 5	+ 0, 90	95
11, 15 p. m.	503, 4	.....	95	11, 15 p. m.	504, 7	.....	95
11, 30 p. m.	503, 6	.....	95	11, 30 p. m.	504, 9	.....	91
11, 45 p. m.	503, 7	.....	95	11, 45 p. m.	505, 7	.....	95
12 p. m.	503, 8	+ 0, 76	95	12 p. m.	505, 9	+ 1, 18	95
12, 15 p. m.	504, 1	.....	91	12, 15 p. m.	506, 1	.....	95
12, 30 p. m.	504, 2	.....	91	12, 30 p. m.	506, 3	.....	95
12, 45 p. m.	504, 3	.....	91	12, 45 p. m.	506, 5	.....	95
1 p. m.	504, 5	+ 0, 86	96	1 a. m.	506, 7	+ 1, 34	95
1, 15 p. m.	504, 7	.....	91	1, 15 a. m.	506, 9	.....	95
1, 30 p. m.	505, 1	.....	82	1, 30 a. m.	507, 2	.....	95
1, 45 p. m.	505, 1	.....	91	1, 45 a. m.	507, 3	.....	95
2, p. m.	505, 1	+ 1, 02	95	2 a. m.	507, 7	+ 1, 54	95
2, 15 p. m.	505, 1	.....	95	2, 15 a. m.	508, 0	.....	95
2, 30 p. m.	505, 1	.....	96	2, 30 a. m.	508, 1	.....	95
2, 45 p. m.	505, 3	.....	95	2, 45 a. m.	508, 1	.....	95
3 p. m.	505, 3	+ 1, 06	95	3 a. m.	508, 7	+ 1, 74	90
3, 15 p. m.	505, 3	.....	95	3, 15 a. m.	508, 7	.....	90
3, 30 p. m.	505, 3	.....	95	3, 30 a. m.	508, 8	.....	90
3, 45 p. m.	505, 4	.....	95	3, 45 a. m.	508, 9	.....	90
4 p. m.	505, 4	+ 1, 08	95	4 a. m.	509, 0	+ 1, 80	90
4, 15 p. m.	505, 5	.....	95	4, 15 a. m.	509, 1	.....	90
4, 30 p. m.	505, 5	.....	95	4, 30 a. m.	509, 3	.....	90
4, 45 p. m.	505, 5	.....	95	4, 45 a. m.	509, 3	.....	90
5 p. m.	505, 4	+ 1, 08	95	5 a. m.	509, 3	+ 1, 86	91
5, 15 p. m.	505, 4	.....	95	5, 15 a. m.	509, 3	.....	90
5, 30 p. m.	505, 4	.....	95	5, 30 a. m.	509, 7	.....	90
5, 45 p. m.	505, 5	.....	95	5, 45 a. m.	509, 9	.....	90
6 p. m.	505, 5	+ 1, 10	95	6, a. m.	509, 9	+ 1, 98	90
6, 15 p. m.	505, 6	.....	95	6, 15 a. m.	509, 9	.....	90
6, 30 p. m.	505, 7	.....	95	6, 30 a. m.	509, 9	.....	95
6, 45 p. m.	505, 6	.....	95	6, 45 a. m.	509, 9	.....	95
7 p. m.	505, 2	+ 1, 04	95	7 a. m.	510, 1	+ 2, 02	95
7, 15 p. m.	504, 8	.....	96	7, 15 a. m.	509, 3	.....	91
7, 30 p. m.	504, 7	.....	96	7, 30 a. m.	508, 9	.....	91
7, 45 p. m.	504, 6	.....	96	7, 45 a. m.	508, 1	.....	84
8 p. m.	504, 5	+ 0, 90	96	8 a. m.	507, 4	+ 1, 48	83
8, 15 a. m.	504, 2	.....	96	8, 15 a. m.	507, 4	.....	91
8, 30 a. m.	504, 0	.....	91	8, 30 a. m.	505, 9	.....	91
8, 45 a. m.	503, 9	.....	91	8, 45 a. m.	505, 3	.....	91
9 a. m.	502, 9	+ 0, 58	92	9 a. m.	505, 6	+ 1, 12	96
9, 15 a. m.	502, 7	.....	84	9, 15 a. m.	505, 1	.....	96
9, 30 a. m.	500, 9	.....	92	9, 30 a. m.	504, 8	.....	96
9, 45 a. m.	500, 5	.....	84	9, 45 a. m.	504, 5	.....	96
10 a. m.	500, 2	+ 0, 04	84	10 a. m.	503, 7	+ 0, 74	96
10, 15 a. m.	499, 5	.....	84	10, 15 a m.	503, 4	.....	96
10, 30 a. m.	499, 1	.....	84	10, 30 a. m.	502, 9	.....	96
10, 45 a. m.	498, 4	.....	92	10, 45 a. m.	501, 9	.....	96

HOURLY RECORDS OF VARIATIONS IN WEIGHT OF CACAO BEANS AND VARIATIONS OF RELATIVE HUMIDITY—Continued

Fermented Cacao Sample No. 3				Unfermented Cacao Sample No. 5			
Hour	Weight gr.	Per cent variations	Relative humid.	Hour	Weight gr.	Per cent variations	Relative humid.
11 a. m....	497, 4	— 0, 52	92	11 a. m..	501, 0	+ 0, 20	96
11, 15 a. m....	497, 2	.....	92	11, 15 a. m..	500.3	.....	96
11, 30 a. m....	496, 0	.....	92	11, 30 a. m..	500.2	.....	96
11, 45 a. m....	495, 3	.....	92	11, 45 a. m..	499, 3	.....	92
12 a. m....	494, 8	— 1, 04	92	12 a. m..	498, 9	— 0, 22	96
12, 15 p. m....	494, 0	.....	92	12, 15 p. m..	498, 4	.....	93
12, 30 p. m....	493, 9	.....	92	12, 30 p. m..	497, 9	.....	96
12, 45 a. m....	493, 8	.....	92	12, 45 p. m..	497, 3	.....	96
1 p. m....	493, 7	—1 , 26	92	1 p. m...	496, 9	— 0, 62	93
1, 15 p. m....	492, 9	.....	92	1, 15 p. m...	496, 9	.....	96
1, 30 p. m....	492, 4	.....	92	1, 30 p. m...	496, 7	.....	96
1, 45 p. m....	492, 2	.....	92	1, 45 p m...	496, 7	.....	93
2 p. m....	492, 0	— 1, 60	85	2 p m...	496, 5	— 0, 70	93
2, 15 p. m....	491, 7	.....	85	2, 15 p m...	496, 5	.....	96
2, 30 p. m....	491, 3	.....	85	2, 30 p m...	496, 5	.....	96
2, 45 p. m....	491, 2	.....	85	2, 45 p m...	496, 5	.....	96
3 p. m....	491.1	— 1, 78	85	3 p m...	496, 7	— 0, 66	96
3, 15 p. m....	491, 2	.....	85	3, 15 p m...	497, 1	.....	96
3, 30 p. m....	491, 2	.....	85	3, 30 p. m...	497, 1	.....	96
3, 45 p. m....	491, 2	.....	85	3, 45 p. m...	497, 1	.....	96
4 p. m....	491, 3	— 1, 74	85	4 p. m...	497, 2	— 0, 56	96
4, 15 p. m....	491, 8	.....	85	4, 15 p. m...	497, 3	.....	96
4, 30 p. m....	491, 9	.....	85	4, 30 p. m...	497, 0	.....	96
4, 45 p. m....	492, 0	.....	85	4, 45 p. m...	497, 4	.....	96
5 p. m....	492, 0	— 1, 60	88	5 p. m...	497, 4	— 0, 52	96
5, 15 p. m....	492, 2	.....	88	5, 15 p. m...	497, 5	.....	96
5, 30 p. m....	492, 3	.....	88	5, 30 p. m...	497, 6	.....	96
5, 45 p. m....	492, 5	.....	88	5, 45 p. m...	497, 8	.....	91
6 p. m....	492, 8	— 1, 44	88	6, p. m...	498, 0	— 0, 40	91

These results are graphically expressed in the diagram of plate XXXII.

DETERMINATION OF CRITICAL MOISTURE POINT FOR MOULDING

Small random samples of cacao beans, both fermented and unfermented (taken from the samples N. 3 and 5), composed of 20 seeds each, were selected. A mixture of conidia and spores of *Aspergillus niger, A. fumigatus, A. glaucus, A. flavius, Rhizopus nigricans, R. arrhizus* and *Mucor mucedo* from fresh carrot agar cultures was prepared and kept dry in a desiccator. Each sample of cacao was enclosed in a common glass cup, and inoculated by dusting (using a small brush) with the mixture of conidia, then covered with a photographic glass plate. Furthermore, in order to regulate the amount of moisture of the atmosphere of the glass cup and to equilibrate the moisture content of the beans with the moisture of enclosed air, a 20 c.c. beaker containing a definite saline solution was inclosed in the glass cup.* The plate glass cover was sealed to the cup with paraffin.

* For references on the method for maintaining constant humidity, see Spencer, H. M. Laboratory methods or maintaining constant humidity.

The following table shows the saline compounds used and the respective concentration of each. As the experiment was made in the Laboratory, and of course, not at a constant temperature, the relative humidity oscileated between two extreme limits. Maximum, minimum and average relative humidity was approximately calculated from Spencer curves or tables.

CONCENTRATION OF THE SOLUTIONS AND RELATIVE HUMIDITY

Salts	Concentration of solution	32 °C. % humidity (aproxim.)	24 °C. % humidity (aproxim.)	28 °C.% humidity (aproxim.)
H_2O Distilled (test)	.....	100	100	100
H_2SO_4 concentrated (test)	.....	Almost 0..	Almost 0..	Almost 0
$Na_2CO_3.10\ H_2O$	Saturated.	81	87	84
$Na_2SO_4.10\ H_2O$	Saturated.	88	92	90
Na Cl and KNO_3	Saturated.	34	30	32
$(NH_4)_2SO_4$	Saturated.	82	88	81
NaOH	N	80	81	80
NaOH	5N	80	81	81
NaOH	10N	84	85	84
NaOH	15N	84	85	85
NH_4Cl	Saturated.	80	79	79
NaCl and $KC10_3$	Saturated.	34	36	35
$KHSO_4$	Saturated.	83	85	84
NaCl, KNO^3 and $NaNO_3$	Saturated.	28	30	29
$BaCl_2.2\ H_2O$	Saturated.	86	88	87
$ZnSO_4.7\ H_2O$	Saturated.	88	90	89
$Na_2Cr_2O7.2\ H_2O$	Saturated.	48	52	50

The experience begun November 13 and ceased February 15 of the subsequent year. The results were as follows:

CACAO MOULDING AS RELATED TO RELATIVE HUMIDITY

First development of moulds	Days from the beginning	Average relative humidity	Solution of
Fermented cacao beans			
November 21	8	90	Na_2SO_4
November 23	10	84	Na_2CO_3
November 23	10	100	H_2O
November 25	12	87	$BaCl_2$
November 26	13	81	$(NH_4)_2SO_4$
November 26	13	85	NaOH. 15N
November 27	14	84	NaOH. 10N
November 28	15	84	$KHSO_4$
November 28	15	89	$ZnSO_4$
November 29	16	81	NaOH. 5N
November 29	16	80	NaOH N
November 29	16	79	NH_4Cl.
Unfermented cacao beans			
November 19	6	100	H^2O
November 20	7	90	Na_2SO_4
November 20	7	89	$ZnSO_4$
November 21	8	85	NaOH. 15N
November 21	8	84	NaOH. 10N
November 21	8	84	$KHSO_4$
November 22	9	87	$BaCl_2$
November 22	9	81	$(NH_4)_2SO_4$
November 23	10	81	NaOH. 5N
November 23	10	80	NaOH. N
November 23	10	79	NH_4Cl
November 24	11	84	Na_2CO_3

DISCUSSION OF THE RESULTS AND CONCLUSIONS

The results of the experiments may be summarized as follow:

(1) The normal moisture content of fermented and unfermented cacao beans varies from 14 to 21 per cent, based on 9 determinations of 9 samples, with a general average of 19 per cent. Moisture content of unfermented cacao beans is higher than the fermented cacao beans, being respectively, 19 and 15 per cent. Also, the moisture content is very variable in different samples.

(2) Also the specific weight varies from a minimum of 0.883 to a maximum of 0.991; this difference is partially related, in our opinion, to the different moisture content. The general average based on 40 determinations on 4 samples is 0.934, but the average of unfermented cacao is slightly higher than in fermented cacao: respectively 0.890 and 0.887.

(3) The size of fermented and unfermented cacao beans is almost the same, the general average being 20 mm. in length, 12 mm. broad and 7 mm. thick.

(4) The capacity of imbibition of cacao beans when immersed in distilled water at 40° C. is about 18 per cent of the initial weight, but varies from 15 per cent (fermented cacao beans) to 20 per cent (unfermented cacao beans). The imbibition is complete during the first 24 hours of immersion.

(5) The capacity of imbibition of cacao beans when exposed to saturated water vapor at room temperature is about 1.10 per cent of the initial weight; these results were obtained in a closed room during five days, at room temperature. Daily absorption is extremely irregular, varying from 2.38 per cent to 0 per cent. The total absorption during the five days is as irregular as the preceding, oscillating from a minimum of 0.56 per cent to a maximum of 2.77 per cent. The average total absorption of unfermented beans (1.57 per cent) is greater than the average of fermented beans (0.56 per cent).

(6) The effect of drying at different temperatures ranging from 40° C. to 100° C. to the absorption of water vapor, when dried cacao beans are exposed to saturated water vapor during ten days, is very clear on fermented cacao beans. The absorption of water vapor is inversely proportional to the temperature of drying, being maximum at the lowest temperature (40° C. and 1.10 per cent of increase in weight) and minimum to the highest temperature (of 80° C., 90°C.,

and 100° C. respectively 0.42, 0.43 and 0.44 per cent). The best temperature for desiccation of fermented cacao beans, as related to the water vapor absorption, is 80° C. On the contrary, the desiccation of unfermented cacao at different temperatures is apparently without influence on the water absorption, the course of increase in weight being very irregular.

(7) The loss of moisture of cacao beans exposed to direct sunshine during 11 hours (7 hours during the first day, and 4 hours during the second) is as irregular as the imbibition of the seeds when exposed to saturated water vapors. In a general way, it is strongest during the first hour, and gradually but irregularly decreased during the following four hours, falling during the 6th and 7th hours. From the 8th to the 11th hour the loss in weight is almost uniform. The general average of loss in weight is 2.03 per cent, but varying from 3.66 per cent to 1.31 per cent. The desiccation is highest in unfermented cacao beans (average 2.27 per cent) than in fermented cacao beans (average 1.78 per cent), according the greater amount of moisture of unfermented seeds as related to fermented seeds.

(8) The average daily weight of samples of fermented and unfermented cacao, recorded during 100 days, is apparently without direct relation to the average daily relative humidity. It is probable that the relation is not simple and exclusive, and other factors influence the variation in weight of cacao beans, as atmospheric pressure, solar irradiation, and so on. These results are confirmed by the record of hourly variation in weight of fermented and unfermented cacao and relative humidity of the atmosphere.

(9) A 24 hours record of samples of cacao beans, both fermented and unfermented, exposed to the free air, taken at the interval of 15 minutes showed a marked variation of increase and loss in weight of fermented or unfermented beans. Moisture absorption of fermented cacao oscillates between 1.10 per cent and 1.78 per cent, with a total range of 2.88 per cent. Moisture absorption of unfermented cacao varies between 2.02 per cent to 0.70 per cent, with a total range of 2.72 per cent. In spite of the fact that the total range of the weight is almost the same in both samples, fermented cacao lost 0.68 per cent in weight, and unfermented cacao increased 1.38 per cent in weight.

The abundant literature on cacao problems, shows that the most important determinations of moisture content of beans and its variations were made in West Africa on unfermented cacao of Accra

type, by Schwarz (**26**). This author found a moisture content varying from 7.16 per cent to 15.32 per cent. Selecting the beans on the base of dryness, he subdivides them into: (1) beans quite pliable in the shell, (2) beans in which the nib was of a cheesy consistency and presented a wet looking surface when cut, and (3) beans which appeared to be fairly dry. Their respective average for three determinations each were: (1) 14.50, (2) 11.33, (3) 8.15. These results agree well with the results obtained on Santo Domingo cacao. The same author performed three experiments on the relationship between humidity and moisture content of cut and shelled cacao beans, weighing a few seeds as well as bags of cocoa. He demonstrated that cut cacao readily absorbed moisture, and that the amount absorbed varied with the humidity. Percentage of moisture varies from 6 per cent to 14.98 per cent. Since the percentage of absorbed moisture agrees with our results, we cannot demonstrate the relationship between moisture of the air and moisture of the cacao. Shelled beans absorbed from 1.32 per cent to 3.96 per cent of moisture. According Schwarz's results, the maximum increase in weight of cacao in burlap bag was only 0.47 per cent. Finally, according the same author, some data obtained from the Gold Coast Department of Agriculture indicated that beans containing in excess of 10 per cent of moisture are prone to mould. From a general point of view, Accra cacao offers a noteworthy analogy with Sánchez cacao, but a most complete picture of moisture in Gold Coast cocoa is desirable for the purpose of comparison.

One of the most important determination is the critical moisture point. The writer ascertained that a relative humidity of 79 per cent permits the development of moulds after 16 days of incubation on fermented cacao, the minimum time required being 8 days at a relative humidity of 90 per cent. On unfermented cacao, the minimum of moisture required is 79 per cent, the incubation during 10 days, and the minimum time 6 days at 100 per cent of relative humidity. The writer does not express the critical moisture point as moisture content of the beans, but surely the moisture is greater than 10 per cent, as indicated in Cold Coast.

These results may be favorably compared with the experiments performed in the Philippines on moulding of copra by Lava (**17**). He found that the critical moisture point at room temperature is 81 per cent, and the period of incubation from 7 to 21 days.

No continuous series of records of relative humidity of Santo

Domingo are available to date, but records for scattered years for Santo Domingo City, Haina, Moca, Santiago, Puerto Plata and Sánchez. The general average is comprised between 65 and 70 per cent, with two minima 60–65 per cent (December to March, and June to August) and two maxima of 70–75 per cent (April–May, and September–November). From 12 to 4 o'clock, during the night, the percentage of relative humidity very rarely drops to 80 per cent, at sea level, and it is surely higher at 600 meter elevation, which is limit of cacao cultivation in Santo Domingo.

The writer concludes that both climatic and metereologic conditions in Santo Domingo, are very frequently favorable to the development of moulds on cacao beans, taking in consideration the commonly very bad preparation of cacao by farmers, as explained in the latter part of this paper.

PREVENTION OF MOULDING OF CACAO

The problem of the prevention of moulding of cacao must be considered under two aspect: (1) the most general and most important, concerning a better preparation of cacao, chiefly from the standpoint of drying, (2) the more specialized, concerning an appropriate storage on farms, villages and depots at the shipping ports, and in steamship holds.

The first aspect of the problem, or the defects in the preparation of cacao, is, more or less, the same in all the larger cacao-countries of the world. In a previous Report (7), the writer examined the defects common in Santo Domingo, of which the most important are:

(1) The almost general tendency to produce unfermented cacao which is more susceptible to moulding than the fermented cacao.

(2) The very common practice of mixing cacao beans from healthy and mature pods with beans from diseased, over ripe and immature pods which have the tendency to become moldy.

(3) Buying and selling cacao beans on wet or semidry basis, and in a general way, incomplete or imperfect or inadequate drying of cacao. Many causes co-operate to this tendency, as, e. g., indigence of small farmers, imperfect equipment of farmers, keen competition among brokers, lack of attention in drying, inadequate protection and equipment of brokers, storekeepers, exporters, and others.

(4) Inadequate legislation, inspection and grading before and after storage.

The second aspect of the problem, or the conservation of cacao in stores or depots or steamship holds at a satisfactory percentage of humidity could be examined, if necessary, under the basis of Lava's proposition for prevention of copra moulding, using solid NaCl or solid $NaNO_3$ which maintained, at the temperature of 26–33°C., a relative humidity of about 73 per cent. $CaCl_2$ or CaO must be used if a greater reduction of humidity is desired. The use of these substances is economical, since after the complete or partial solution, they can be easily recovered or, in the case of CaO, used as calcium hydroxide. The problem of high humidity in the holds of the vessels and increase of moulding of cacao beans during the shipment must be studied.

In our opinion, the gradual elimination of the unsatisfactory conditions listed will be the best method to improve the Dominican cacao.

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SUMMARY

Experiments on the environmental conditions influencing the moulding of fermented and unfermented Dominican cacao beans were carried.

IV. TESTS FOR ENZYMES OF CACAO

The knowledge of the enzymes of cacao beans, both in fresh and dry seeds (fermented and unfermented), is the basis for the understanding of the mechanism and effect of the fermentation. Scattered notices on this subject are found in the literature of cacao technology, but the first complete study was performed in the Philippine Island by Brill (1). This investigation has been undertaken for a comparison with Brill's results, a few of which are different from the results obtained by previous investigators, as well as for testing enzymes not previously considered.

METHOD OF STUDY

Four series of experiments were carried out, using unselected

Dominican cacao (Sánchez type), harvested in Moca and prepared in the National Agronomic Station, namely: (1) fresh cacao beans with surrounding pulp, (2) fresh and clean cacao beans without the pulp, (3) clean fermented cacao beans, prepared by fermenting the seeds during four days at the temperature of the laboratory (20–30°.C) and then drying, (4) clean unfermented cacao beans, prepared by drying the seeds after carefully washing.

The method used was almost the same as the method employed by Brill: a 10 per cent aqueous solution of ground seeds, standing six hours and then filtered through cloth, was mixed with 2 per cent (by volume) of toluene and 2 per cent of chloroform, both as antiseptic and as facilitating the exosmose of the enzymes.

Each experiment was repeated at least three times, and more, if necessary, until a confirmation of positive or negative results was obtained.

Saccharase. 10 c.c. of water, 10 c.c. of cacao bean extract, 10 c.c. of 40 per cent cane-sugar solution, 3 drops of very diluted chloridric acid (pH of the mixture 4.6), standing 48 hours at 40° C., and testing with Fehling solution.

(1) Fresh beans and slime: strongly positive.
(2) Fresh clean beans: positive.
(3) Fermented beans: negative.
(4) Unfermented beans: slightly positive.
(5) Test: negative.

Maltase. As in the preceding, but using 10 c.c. of 20 per cent maltose solution (pH of the mixture acidized: 4.2).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

As in the preceding, but using bean extract obtained by 10 days of maceration in thermostate at 40°C. (pH of the mixture, acidized: 4.0).

(1) Fresh beans and slime: slightly positive.
(2) Fresh clean beans: positive.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Lactase. As in the preceding, using a 20 per cent lactose solution (pH of the mixture, acidized: 4.4).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

As in the receding, but after 10 days of maceration (see above); (pH of the mixture, acidized: 5.0).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Trehalase. As in the preceding (pH of the mixture, acidized: 4.4).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Melibiase. As in the preceding (pH of the mixture acidized: 4.8).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Amylase. 40 c.c. of soluble starch solution (1 per cent), 10 c.c. of bean extract, standing 48 hours at 40°C., testing the presence of reducing sugars with Fehling solution.

(1) Fresh beans and slime: strongly positive.
(2) Fresh clean beans: slightly positive.
(3) Fermented beans: negative.
(4) Unfermented beans: slightly positive.
(5) Test: negative.

Dextrinase. As in the preceding, but testing the presence of dextrines with Lugol solution.

(1) Fresh beans and slime: yellowish.
(2) Fresh clean beans: reddish.
(3) Fermented beans: violet-bluish.
(4) Unfermented beans: reddish.
(5) Test: violet-bluish.

Pectinase. 25 c.c. of bean extract, 5 gm. of pectine, freshly prepared from carrots (Bertrand and Mallevre method, as referred by Calmette, Negre and Bouquet: Man. techn. de Microbiol. et Sérol., II édit., p. 168. Paris, 1926), testing the presence of reducing sugars with Fehling solution, after 48 hours at 40°C.

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Cellulase. 25 c.c. of bean extract, 5 gm. of stripes of ashless filter paper. After 48 hours of incubation at 40° C., the paper is washed, dried and weighed.

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Emulsin. 10 c.c. of bean extract, 10 c.c. of amygdalin (2 per cent solution), standing 48 hours at 40°C., and testing the presence of cyanhidric gas with picro-sodic paper in a corked Erlenmeyer.

(1) Fresh beans and slime: pinkish.
(2) Fresh clean beans: pinkish.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

As above, but testing with 10 drops of freshly prepared Fe SO_4 solution (3 per cent), one drop of Fe Cl_3 (1 per cent), mixing thoroughly, adding NaOH solution (10 per cent) and dissolving the precipitate with H_2SO_4 solution (10 per cent).

(1) Fresh beans and slime: light Prussian-blue color.
(2) Fresh clean beans: slight Prussian-blue color.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Protease. 5 gm. of coagulated egg albumine, 25 c.c. of bean extract, for 10 hours at 40°C., testing the loss in weight of albumine cylinders (Mett method, Arch. Anat. und Physiol., Physiol. Abth., *68:* 94). Liquid media acidified with a few drops of very diluted chloridric acid (pH 3.8).

(1) Fresh beans and slime: negative (loss gm. 0,003).
(2) Fresh clean beans: negative (loss gm. 0,008).
(3) Fermented beans: negative (loss gm. 0,004).
(4) Unfermented beans: negative (loss gm. 0,008).
(5) Test: negative (loss gm. 0,005).

Tryptase. One cubic centimeter of 30 per cent solution of gelatine in thymol solution (0,1 per cent) is distributed in each test tube of 5 mm. in diameter, being previously alcalinized with 2 per cent of NaOh N/10, adding 1 c.c. of bean extract to each test tube. After 48 hours at 30°C., the height of the cylinder of gelatine is measured (Fermi method, as referred to De Rossi, Microbic. agr. e tecn. 204.1927).

(1) Fresh beans and slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Peptase. 10 c.c. of egg albumen solution (one egg albumen in 100 c.c. of water), 10 c.c. of bean extract, for 48 hours at 40°C (pH of the solution:

6.2). Acidity of dissociated amynoacid is titrated with KOH 1/10 N(Sörensen's tritimetric method, as referred in De Rossi, 1. c., p. 205).

(1) Fresh beans with slime: c.c. 1, 0.
(2) Fresh clean beans: c.c. 0,9.
(3) Fermented beans: c.c. 0,6.
(4) Unfermented beans: c.c. 0,8.
(5) Test: c.c. 0,6.

20 c.c. of egg albumen solution, prepared as above, 10 c.c. of seed extract, standing 48 hours at 40°C. Indole test performed by Goré modification of the technic of Ehrlich-Böhme (as referred by the Comm. on Bact. Techn., Soc. Amer. Bacteriol. Manual meth. pure culture study of Bacteria, p. VI/14. 1930).

(1) Fresh beans with slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

20 c.c. of Bactopeptone solution (2 per cent), 10 c.c. of bean extract, for 48 hours at 40°C. Indole tested as above (pH=6, 2).

(1) Free beans with slime: negative.
(2) Fresh clean beans: negative.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Amynoacidase. 20 c.c. of asparagine (2 per cent solution), 10 c.c. of been extract, for 48 hours at 40° C., testing the alcalinity with KOH N/10.

(1) Fresh beans with slime: c.c. 2, 2.
(2) Fresh cleans beans: c.c. 2, 3.
(3) Fermented beans: c.c. 1, 6.
(4) Unfermented beans: c.c. 2, 0.
(5) Test: c.c. 1, 5.

Lipase. 10 c.c. of water, 10 c.c. of bean extract, 1 c.c. on pure olive oil, 1 drop of diluted acetic acid, for 48 hours at 40°C., testing the acidity with NaOH N/10.

(1) Fresh beans with slime: c.c. 2, 2.
(2) Fresh clean beans: c.c. 1, 7.
(3) Fermented beans: c.c. 2, 91.
(4) Unfermented beans: 5, 9.
(5) Test: c.c. 2, 1.

Glycerophosphatase. 10 c.c. of water, 10 c.c of bean extract, 50 c.c. of 2 per cent solution of sodic glycerophosphate, 48 hours at 40° C. (pH = 5, 8), testing the presence of soluble phosphoric acid with ammonium molybdate solution.

(1) Fresh beans with slime: slight yellow coloration.
(2) Fresh clean beans: slight yellow coloration.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Fitase. 20 c.c. of bean extract, 30 c.c. of 2 per cent solution of fitine, 48 hours at 40°C. (pH=6.2), testing the presence of soluble phosphoric acid with ammonium molybdate solution.

(1) Fresh beans with slime: slight yellow coloration.
(2) Fresh clean beans: slight yellow coloration.
(3) Fermented beans: negative.
(4) Unfermented beans: negative.
(5) Test: negative.

Oxidase and peroxidase 20 c.c. of seed extract, 1 c.c. of alcoholic tincture of guaiacum (10 per cent), the color being observed after ½ hour, 2 hours, 12 hours and 24 hours of thermostation at 40°C.

(1) Fresh beans with slime: light red; red; dull read; dull red.
(2) Fresh clean beans: pinkish; red-pinkish; red; red.
(3) Fermented beans: no change; pinkish; red; pinkish.
(5) Unfermented beans: pinkish; red-pinkish; red; red.
(5) Test: no change.

5 c.c. of bean extract, 1 c.c. of benzidine solution (0.5 per cent) and 5 drops of H_2O_2, 2 hours at 40°C.

(1) Fresh beans with slime: violet-bluish.
(2) Fresh clean beans: light violet-blue.
(3) Fermented beans: very light violet-blue.
(4) Unfermented beans: violet-blue.
(5) Test: unchanged.

5 c.c. of bean extract, 1 c.c. of solution of acid pyrogallic (2 per cent), 5 drops of H_2O_2, after 24 hours at 40°C.

(1) Fresh beans with slime: duty yellow with precipitate.
(2) Fresh clean beans: dirty yellow with precipitate.
(3) Fermented beans: very light yellow.
(4) Unfermented beans: dirty yellow.
(5) Test: unchanged.

Catalase. 5 c.c. of bean extract 50 c.c. of neutralized H_2O_2 (pH=6.8.) 1 per cent solution, measuring the O developed, using a common calcimeter, after 24 hours at the temperature of Laboratory (24–30°C.).

(1) Fresh beans with slime: 31, 1 c.c. of oxigen.
(2) Fresh clean beans: 29, 2 c.c. of oxigen.
(3) Fermented beans: 2, 7 c.c. of oxigen.
(4) Unfermented beans: 11, 8 c.c. of oxigen.
(5) Test: 0, 8 c.c. of oxigen.

Philotion. 10 c.c. of bean extract, 2 gm. of precipitated sulphur contained in closed Erlenmeyer flask in presence of stripes of lead acetate paper, after 24 hours at 40°C.

(1) Fresh beans with slime: very light brown color.
(2) Fresh clean beans: very light brown color.
(3) Fermented beans: no change.
(4) Unfermented beans: very light brown color.
(5) Test: no change.

Reductase. 10 c.c. of bean extract, 10 c.c. of water, 1 c.c. of methylene blue solution at 1 per cent, after 48 hours at 40°C.

(1) Fresh beans with slime: slightly decolorized.
(2) Fresh clean beans: no change.
(3) Fermented beans: no change.
(4) Unfermented beans: no change.
(5) Test: no change.

DISCUSSION OF THE RESULTS

The results of our experiences, as compared with the tests performed by Brill, may be summarized as follows:

Enzyme (1)	Brill's experiments					Author experiments				
	Pulp	Heated pulp	Fresh clean seeds	Heated fresh clean seeds	Fermented seeds	Heated fermented seeds	Fresh seeds with slime	Fresh clean seeds	Fermented seeds	Unfermented seeds
A-HYDROLYZING ENZYMES										
a-DECOMPOSING CARBOHYDRATES										
1-Saccharase (invertase)	P	P	N	N	P	P	P	P	N	P
2-Maltase	N	N	N	N	N	N	P	P	N	N
3-Lactase	.....	.....	.....	.....	.....	.....	N	N	N	N
4-Trehase	.....	.....	.....	.....	.....	.....	N	N	N	N
5-Melibiase	.....	.....	.....	.....	.....	.....	N	N	N	N
6-Raffinase	P	P	P	P	P	P	.....	.....	.....	.....
7-Amylase (diastase) (2)	N	N	N	N	P	P	P	P	N	P
8-Dextrinase (3)	N	N	N	N	?	N	P	N	N	N
9-Inulase	N	N	N	N	N	N	.....	.....	.....	.....
10-Pectinase	.....	.....	.....	.....	.....	.....	N	N	N	N
11-Cellulase	.....	.....	.....	.....	.....	.....	N	N	N	N
b-DECOMPOSING GLUCOSIDES										
1-Emulsin	N	N	N	N	N	N	P	P	N	?
c-DECOMPOSING PROTEIC SUBST.										
1-Protease	.....	.....	.....	.....	.....	.....	N	N	N	N
2-Tryptase	.....	.....	.....	.....	.....	.....	N	N	N	N
3-Peptase (indole test)	.....	.....	.....	.....	.....	.....	N	N	N	N
4-Albuminase (tryptophane test)	?	N	N	N	N	N	.....	.....	.....	.....
5-Protease (tryptophane test)	P	N	N	N	N	N	.....	.....	.....	.....
6-Amynoacidase	.....	.....	.....	.....	.....	.....	?	?	N	?
d-DECOMPOSING FAT SUBSTANCES										
1-Lipase	N	N	N	N	N	N	N	N	N	P
e-DECOMPOSING ETHER. COMP. PHOSPHORIC ACID										
1-Glycerophosphatase	.....	.....	.....	.....	.....	.....	P	P	N	N
2-Fitase	.....	.....	.....	.....	.....	.....	P	P	N	N
B-OXIDING AND REDUCING ENZYMES										
1-Oxidases	P	N	P	N	P	N	P	P	P	P
2-Peroxidases	.....	.....	.....	.....	.....	.....	P	P	P/?	P
3-Catalase	.....	.....	.....	.....	.....	.....	P	P	?	P
4-Philothion	.....	.....	.....	.....	.....	.....	P	P	N	P
5-Reductase	P	P	P	P	P	P	P	N	N	N

(1)—N-Negative results; P-Positive results; ?-Doubtful results.
(2)—The name of enzymes enclosed in the brackets, are used in the Brill's paper.
(3)—In the study of Brill, the dextrinase is comprised in the test for diastase.

The hydrolyzation of the saccharose is a controversial point. According Brill (1), fresh clean cacao beans do not hydrolyze this sugar, while, in our experience, the enzyme is found on seeds with slime, clean seeds and dried cacao beans, but not in the fermented cacao. Different results can be explained by the different environmental conditions of the tests, such as concentration of the sugar solution, hydrogen-ions concentration, etc.

The tests for maltase in the Philippine cacao were negative, and Brill affirmed that "this appears to be one of the rarer cases of diastases unaccompanied by maltase". Our first test, repeating, more or less, the conditions of the tests performed by Brill showed negative results. A second series of tests, varying the duration of

the incubation (ten days instead of two days), showed positive results only in fermented and unfermented cacao beans. It is well known that the maltase is an endoenzyme not easily extracted from the plant cell, and only from dried material, after a long maceration.

Amylase (an enzyme hydrolyzing starch to dextrines) is not present in fermented beans only, but dextrinase (an enzyme hydrolyzing dextrines to maltose) was in beans without slime, fermented and unfermented cacao. These results are in contrast with Brill's results and are confusing.

In our experiment, the presence of amygdaline was demonstrated, at least in fresh seeds (with and without slime), in accordance with the results of Sack (cfr. H. Smith. Ferment of cacao, p. 148. London 1913), but in disagreement with Brill's results.

Of the enzymes hydrolyzing proteic substances, or its derivates, the presence of the amynoacidase is doubtful; hydrolizing enzymes, both in acid and alcaline media, are absent. These results are in partial disagreement with Brill's tests.

Lipase is present only in dry unfermented seeds. Glicerophosphatase and fitase are secreted by fresh seeds.

Oxiding and peroxiding enzymes are contained in fresh and dry cacao beans, as well as, in part, the enzyme transforming sulphur in sulfuretted hydrogen. Only fresh seeds with slime contain reductase. Brill found reductase in pulp, fresh, and fermented seed, also heated.

CONCLUSIONS

From the results of these investigations, definite conclusions cannot be drawn without a deep knowledge of organic compounds contained in slime, fresh fermented and unfermented cacao beans, as well as without the study of enzymatic activities of cacao-fermenting organisms. Unfortunately, these observations are fragmentary and rather incomplete.

Of twenty-two tested enzymes, only oxiding and peroxiding enzymes are universally diffused on different kinds of cacao beans. Fresh seeds with slime are of the amplest range of enzymatic activity (13 enzymes); fresh clean seeds (without slime) contain 11 enzymes, tests for dextrinase and reductase being negative. Fermented dry seeds contain 9 enzymes, the range of the enzymatic activities being limited to the invertase, diastase, lipase, oxiding and peroxiding enzymes, including the doubtful presence of the amynoacidase and emulsin. Positive test for the presence of a

lipolytic enzyme, (splitting fatty substances into glycerol and fatty acids) ascertained only in unfermented seeds should be particularly mentioned, as related with the rank odor of some Dominican unfermented samples of cacao. The presence of the lipase must be confirmed testing fatty substances composed of higher fatty acids as sterinolipoids, recently obtained on cacao beans. In spite of the fact that the presence of a relatively large amount of fats in cacao beans indirectly confirms the necessary presence of a lipolytic enzyme, this test is not very sure for the presence of the glicerophosphatase and fitase. If glicerophosphatids and fitine are present in cacao seeds, the acidity of the test on fatty substances should be derivated, at least in part, from the hydrolyse of esters of phosphoric acid.

Fermented cacao lacks almost totally of enzymatical activities; only three enzymes were obtained, of which two are of doubtful presence. The only enzyme surely present is the oxidase, probably associated with the frequent blackening of old Dominican fermented and unfermented cacao. The poor enzymatic activity (from qualitative standpoint) of fermented cacao, as probably related with a greater stability of the seeds, could be favorably compared with the more active dried unfermented seeds. The fermented cacao bean is a truly dead seed, also from biochemical point of view, while an unfermented cacao bean is apt, under suitable conditions, to many biochemical changes resulting from its latent enzymatic activity.

SUMMARY

Twenty-two enzymes are tested on Dominican cacao. Fresh cacao beans with surrounding slime are the most active; fermented dry cacao beans are almost inactive. A lipase is probably present only in fermented cacao.

V. AN IMPROVED METHOD FOR THE DESICCATION OF CACAO BEANS

This method is based on the application of a distillation box for saline water, when exposed to the sunshine, for desiccation of fermented or unfermented cacao beans.

The principle of this apparatus, attributed to Charles Wilson, is well known: the saline water is contained in a metallic dish, enclosed in a flat wooden box; the top if formed by an inclined glass plate, and exposed to the direct sunshine. To each descent of temperature, the saturated water vapor enclosed in the box is condensed on the glass plate, and is collected in a bottle as distilled water.

This distilling box was used for the first time during the year 1872 for securing the supply of drinking water for the workers employed in the silver mines of Salinas, in the desert of Atacama (Chile), from a saline water containing 140 gr. of salt per liter. Using a series of Wilson's apparatus, with a glass surface of about 5,000 square meters, the production of distilled water was 23 metric tons per day.

The same apparatus was employed for many other uses, by Maurain and Brazier in Paris, Ginestous in Tunisy, Richard in Monac, Lozano in Spain, and others for the industrial drying of fish, fruit, distillation of alcohol, etc. This method and its application was described in detail by Richard (**24**) and by Lozano (**21**).

During the summer of 1929 I constructed an apparatus of about one square meter of glass surface, and during the months of August and September I experimented on the possibility of the using it in cacao bean drying. As these preliminary observations, under certain conditions, gave good results, I delayed the study of the practical application using a larger series of boxes to the summer of 1930, when the National Agronomic Station of Moca was closed.* For this reason the observation here referred to must be considered only as preliminary results, and the publication is made for the purpose of stimulating experiments on an industrial scale.

The structure of Wilson apparatus is explained by the sectional sketch of pl. XXXIII: ** *di* is the metallic dish containing the cacao beans; *wa* are the wooden walls; *pl* the glass plate; *mp* is a metal plate bended to Z shape, for the detention of drops; *co* is a gutter functioning as a water collector; *pi* indicate a small pipe line for conduction of the water to the bottle *bo* and four supports *fo*. One small door, *do* opening at the right side makes it possible to remove the metallis dish. The box is black painted and the joints (chiefly the glass plate joins) are sealed with sealing wax.

During many weeks, the temperature was recorded using a double-recording thermograph (air and soil thermograph) for simultaneous record of inside and outside temperature. In spite of the fact that the registrating pen for the box temperature was set so that the 0°C. line of the chart corresponded to 30°C., with a few

* A few experience on drying of fresh fruit, also made favorable results, using oranges, pawpaw, cashew fruits, *sapote* and *mamey*, but not completely good using star-apple, plantain and banana, also if cutted in small pieces.

** The figure is taken from Lozano paper.

exceptions, the maximum temperature was not included in the graphic. In other words, in a general ways, the maximum temperature is greater than 75°C., while the maximum is, more or less, the same as the free air, or slightly less. As exemplification, the two-hour temperature of the box and the temperature of the air is reported, from Wednesday September 25 to Sunday September 29; the graphic is illustrated in the plate XXXIV.

Day	Hour	Air temperature degrees C.	Averages degrees C.	Box temperature degrees C. (1)	Averages degrees C.
September 25	2 a. m.	24	.....	24	.....
September 25	4 a. m.	23	.....	23	.....
September 25	6 a. m.	22	.....	35	.....
September 25	8 a. m.	21	.....	55	.....
September 25	10 a. m.	28	.....	75	.....
September 25	12 a. m.	31	25, 0	65	46, 0
September 25	2 p. m.	36	.....	45	.....
September 25	4 p. m.	41	.....	29	.....
September 25	6 p. m.	29	.....	26	.....
September 25	8 p. m.	25	.....	25	.....
September 25	10 p. m.	23	.....	24	.....
September 25	12 p. m.	22	29, 0	23	29, 0
September 26	2 a. m.	22	.....	22	.....
September 26	4 a. m.	21	.....	21	.....
September 26	6 a. m.	21	.....	38	.....
September 26	8 a. m.	23	.....	55	.....
September 26	10 a. m.	28	.....	80	.....
September 26	12 a. m.	32	24, 5	74	48, 0
September 26	2 p. m.	40	.....	40	.....
September 26	4 p. m.	39	.....	27	.....
September 26	6 p. m.	28	.....	26	.....
September 26	8 p. m.	24	.....	24	.....
September 26	10 p. m.	24	.....	23	.....
September 26	12 a. m.	23	29, 5	22	27, 0
September 27	2 a. m.	21	.....	23	.....
September 27	4 a. m.	21	.....	24	.....
September 27	6 a. m.	23	.....	27	.....
September 27	8 a. m.	23	.....	37	.....
September 27	10 p. m.	24	.....	22	.....
September 27	12 a. m.	29	23, 5	57	39, 0
September 27	2 p. m.	21	.....	43	.....
September 27	4 p. m.	38	.....	27	.....
September 27	6 p. m.	29	.....	25	.....
September 27	8 p. m.	25	.....	25	.....
September 27	10 p. m.	24	.....	25	.....
September 27	12 p. m.	24	27, 0	25	26, 0
September 28	2 a. m.	24	.....	25	.....
September 28	4 a. m.	24	.....	24	.....
September 28	6 a. m.	23	.....	37	.....
September 28	8 a. m.	24	.....	58	.....
September 28	10 a. m.	28	.....	77	.....
September 28	12 a. m.	31	26, 0	70	48, 0
September 28	2 p. m.	38	.....	43	.....
September 28	4 p. m.	41	.....	28	.....
September 28	6 p.m.	28	.....	27	.....
September 28	8 p. m.	26	.....	25	.....
September 28	10 p. m.	24	.....	24	.....
September 28	12 p. m.	23	30, 0	24	28, 5
September 29	2 a. m.	23	.....	25	.....
September 29	4 a. m.	24	.....	28	.....
September 29	6 a. m.	24	.....	40	.....
September 29	8 a. m.	27	.....	55	.....
September 29	10 a. m.	29	.....	77	.....
September 29	12 a. m.	32	26, 5	70	48, 5
September 29	2 p. m.	40	.....	38	.....
September 29	4 p. m.	41	.....	38	.....
September 29	6 p. m.	29	.....	27	.....
September 29	8 p. m.	26	.....	27	.....
September 29	10 p. m.	26	.....	26	.....
September 29	12 p. m.	26	31	25	28, 5

(1) The figures corresponding to the curves outside of the chart are approximately calculated.

It should be noted that (1) these days were exceptionally warm, (2) the depression of the temperature of the box at about 4 o'clock corresponds to the shading of the same caused by the respective positions of the Wilson apparatus and the walls of the laboratory house, (3) that not only the Wilson's box and the sensitive thermometric cylinder, but also the air temperature registrating element and the body of thermograph were exposed to the full sunshine, on a reflective cement floor, (4) the great thermometric depression from 10 to 12 o'clock of the day September 27 corresponds to a drizzling rain.

A series of records obtained using a maximum and minimum alcohol thermometer showed that the maximum daily temperature exists from 2 to 4 o'clock in the afternoon, and oscillates between 65 and 83°C. when the day is warm and the sky is bright, and from 35 to 50°C. during rainy or dull days. Minimum temperatures correspond to the minimum of free air.

The condensation of water vapor reaches the maximum from 4 P.M. to 6 P.M. and about three fourths of the condensed water is collected during these two hours. Also a depression of the temperature during the maximum elevation is very productive in condensed water. From 4 to 10 A.M. the collection of condensed water is only occasional; no water is collected during the night.

During the first experiment, a layer about 12 cm. in thickness (5 inches) was placed in the metallic dish of the distilling box, the condensation being abundant, but the desiccation not uniform, so that frequent stirring was necessary. The best thickness of the layer of cacao beans is not more than 5 cm. (about 2 inches). Under the best conditions, one to three days are necessary for a complete drying of the seeds, and generally two days, when the cacao is unfermented, and from two to four days (most frequently three days) when unfermented. If the beans are over-dried, they appear slightly wrinkled and friable, but of good flavor and odor.

Two or three consecutive rainy days are very dangerous, if fermented or unfermented cacao beans are freshly stored in the distillation box, as the atmosphere saturated with water vapor favors the continuation of fermentation, and of course, an over-fermentation, or the rotting of cacao beans. In this case, the best prevention is the removal of the cacao from the box, and the storage in a room to protect from the rain. One day of rain followed by one day of full sunshine is not so dangerous, as the beans are par-

tially dried, and the raising of the temperature acts as a partial sterilizer.

The distilled liquor is apparently strongly acid when the beans are fermented; and weakly acid if they are unfermented and have the peculiar odor of cacao fermentation, but they were not examined chemically.

In conclusion, the drying of cacao beans by the utilization of the sunshine in such a box is promising for the cacao industry, as an easy, inexpensive and quick method, but more complete studies, made on industrial bases are necessary before forming a definite judgment.

SUMMARY

The application of Wilson's box of water distillation, utilizing the sunshine, for the desiccation of cacao beans is suggested. This method should be studied more thoroughly.

VI. THE YEASTS OF THE DOMINICAN CACAO

Fragmentary notices on the microorganisms, and particularly the yeasts (including asporigenous forms) found on cacao beans or isolated from fermenting cacao, are easily found in many papers on cacao problems, chiefly on curing of cacao. Our knowledge of yeasts of the cacao beans, during the period before the year 1927, is very incomplete. They are summarized in a comprehensive treatise by Hamel Smith et al. (**14**) on cacao fermentation. The only named and apparently specific yeast of the cacao fermentation is the "*Saccharomyces theobromae*" Preyer, but, according to Hamel Smith (**14**), De Rossi (**10**), Henneberg (**15**), and others, the presence and activity of cosmopolite yeasts (such as the elliptic yeast, the apiculate yeast, the anomalus yeast) was commonly admitted. In 1927 Lilienfeld-Toal (**19**) published a very important and exhaustive paper on yeasts (sporigenous and asporigenous), in the German language, based on material from Ecuador, Venezuela, Trinidad, Brazil, Gold Coast, St. Thomé, Ceylon, Java, etc., which was soon followed by another paper by Busse, Henneberg and Zeller (**1 bis**) referring to the results of experiments on cacao fermentation.

The microorganisms isolated and studied by Lilienfeld-Toal were: (1) *Saccharomyces ellipsoideus* var. *tropicus* Lil.-Toal & Henneb., (2) *Schizosaccharomyces Bussei* Lil.-Toal & Henneb., (3) the "anomalus" yeast, (4) an undetermined "Kamhefe A", probably identic

with *Saccharomyces theobromae*, (5) an undetermined sporogenous yeast from Brazilian cacao, listed as "Weinhefe B", (6) an undetermined "Kahmhefe B"; isolated from Costa Rican and Trinidad beans, (7) an undetermined "Hefe R", asporogenous, isolated from St. Thomé samples and (8) an undetermined "Saccharomyces M", isolated from Trinidad cacao beans.

Samples of the Dominican cacao (so called 'Sánchez' type) was not included in the study of Lilienfeld-Toal. So far as the present writer is able to learn, there exists no record of experiments on Sánchez cacao, with the exception of an anascosporic yeast (*Kloecheria domingensis* Cif.), isolated from rotting cacao pods by the writer (**5**). As independently from the "vexata quaestio" of the industrial importance of the cacao fermentation, the exact nature of the yeasts causing or associated with fermentation, remains one of the major questions, and constitutes the bases for later studies. The most important purpose of this paper is to characterize the yeasts and pseudo-yeasts found on fermenting Dominican cacao beans.

The study of Lilienfeld-Toal was performed on dry cacao beans, and the yeasts isolated were those most likely adapted to survive during curing and drying. A second and minor purpose of this study was the comparison between the yeasts isolated during the fermentation and the yeasts found on dry fermented cacao beans, and their respective numerical distributions.

MATERIAL AND METHOD

During the years 1926, 1927 and 1928, isolations were made from fermenting cacao in fermetation boxes of cacao farmers located at Samaná, Sánchez, Villa Rivas, Pimentel, San Francisco de Macorís, Salcedo, Moca, Santiago, Bajabonico, La Vega, Bonao and San Cristóbal, with a total of 162 isolations. The determination of the yeasts and the distribution during the fermentation, was performed in the Agronomic National Station at Moca. The study of the yeasts remaining on dried cacao beans was made in combination with the experiments on moulds and mouldings of cacao beans.

As soon as the isolated strain was purified, the fermentation was tested, using Lindner's method for small fermentation and the strain stored. According to Redaelli and the writer's procedure (**22**), the optimum temperature for the growth was observed on the "starting medium" pepto-glucose agar, at $pH = 7{,}00 \pm 0{,}4$), and the sporogenicity on Gorodkowa's agar. The macroscopical and microscopical

morphology was deduced from the study of cultures on (1) Raulin neutral solution, (2) carrot agar, (3) malt extract gelatine (10 per cent solution in surrogation of the beer worth), (4) malt extract water (10 per cent solution). Biochemical activities were tested, in advance of the fermentation tests, the assimilation of the carbon from the carbohydrates, alcohols, and organic acids, and assimilation of azote from organic and inorganic nitrogenous compounds. For a more detailed description of methods of study, we refer to Redaelli and Ciferri (**22**).

The classification adopted by Redaelli and the writer (**9**) as well as by the writer alone (**6**) is followed.

Strains N. 151, 168, 169, 174, 183, 189, 203, 207, 211, 221, 230, 249 and 250

CULTURAL CHARACTERISTICS

Optimum temperature of growth: about 40°C.

Starting medium: colony abundant, of rapid growth, yellowish to yellow, creamy, uniform; edges thinner than the center; border irregularly sinuate.

Gorodkowa's agar: scanty growth. Easily forming spores.

Raulin's neutral solution: poor and slow growth; after two weeks, the solution is troubled; no velum; fragments of the ring; deposit not abundant, almost mucilaginous.

Carrot agar: very abundant growth: not possible to distinguish the colony from that on starting medium; a colony one week old is partly collected at the bottom of the tube.

Malt extract gelatine (geant colony): flattened, whitish-yellowish, more or less round, irregularly bordered colony, without characteristic features; center crateriform, with not well marked radial striae; no appreciable liquefaction. Must be referred to the fundamental type I of Will.

Malt extract water: quick formation of one at first pulverulent, then creamy deposit; formation of ring slow and irregular, frequently also incomplete; no superficial pellicle. A good etheric-alcoholic odor.

The color is a somewhat variable characteristic: the colonies of the strains N. 168, 183, 211 and 230 are yellow in color; strains N. 189 and 211 are almost white; other strains are yellowish to white-creamy.

Also the presence of a depressed crater is a rather variable character, well defined in the strains N. 174, 207 and 221, almost absent in the strains N. 168 and 169. Radial striae are, in a similar way, a more or less variable character.

MORPHOLOGICAL CHARACTERISTICS

Spheric, smooth, 2–4 mmm. diameter ascospores, 1 to 4 for each cell (most frequently 3), formed without previous copulation. Sporification of the cells variable from about 75 per cent (strain N. 183) to about 20 per cent (strain N. 168). Vegetative cells normally spheric, rarely sub-spheric to sub-elliptic, 3–4.5 mmm. in diameter; geant cells scarce, 5–7 mmm. in diameter. Very active, normally unipolar budding. Cells normally single, rarely from 2 to

5-chained (in the strains. N. 174 the cells are generally aggregated in little chains of 3 to 4 elements). No special morphological features. Cells of the deposit on liquid cultures are similar to the vegetative cells on solid media, but sub-elliptic in shape, 2.5–4.5 mmm. by 3–5 mmm. Cells of the ring, as rule, forming small chains (of 2 to 4 elements), showing polar or lateral budding.

BIOCHEMICAL CHARACTERISTICS

Gas production in glucose, saccharose, levulose, raffinose and maltose; fails to produce gas in galactose, mannose, rhamnose, lactose, trehalose, arabinose, xylose, sorbitol, dulcitol, melibiose, starch, soluble, destrin and inulin. It inverts saccharose and raffinose but not trehalose. Assimilates readily peptone, but no nitrate and nitrite of potassium, and ammonium sulphate. Glucose and saccharose are readily taken up; glycerin is very scantly assimilated; acetic and tartaric acids are not assimilated, or very slightly. It fails to liquefy gelatine, although it forms the nail.

SYSTEMATIC POSITION

This always present and abundant yeast belongs to the *Saccharomyces ellipsoideus* var. *tropicus* Lil.-Toal & Henneb., an inter-tropical yeast isolated from fermented beans of many tropical countries. This variety, in my opinion, is no other than the common ellipsoideus yeast of the wine of the temperate countries, adapted to the tropical regions. Differences between our strains and the description of Lilienfeld-Toal are slight except for the optimum temperature of growth (in our strains about 40°C., and in the Lilienfeld-Toal strains from 20 to 30°C., the higher limit being 42°C.), and the copulation as a residual sexuality ascertained by the German author. The optimum temperature of the Dominican strains are apparently more closely related to the temperature of the fermentation boxes, normally oscillating from 35 to 45°C.

STRAINS N. 172, 179, 205, 225, 236, 247, 256 AND 268

CULTURAL, MORPHOLOGIC AND BIOCHEMICAL CHARACTERISTICS

Not possible to distinguish from the preceding yeast, except by the absence of the fermenting power of galactose.

SYSTEMATIC POSITION

This yeast, almost as frequent as the *Saccharomyces ellipsoideus* var. *tropicus,* is of the same cycle, and may be distinguished from the type in a new variety: *Saccharomyces ellipsoideus* var. *domingensis* Cif., n. var. As a matter of fact, this variety is, more or less, of the same importance as the Lilienfeld-Toal's "Weinhefe B", found on Brazilian cacao beans, and a separated from the type chiefly

by the fermentation of glucose and gelactose, not of saccharose, maltose and raffinose, as well as by the tuberculated surface of the colonies on agar media. In my opinion, the brazilian strain may be classed in a variety of the same yeast; *Saccharomyces ellipsoideus* var. *brasiliensis* Cif., n. nom.

STRAINS N. 155, 159, 173, 186, 196, 223, 244 AND 258

All these strains were classified on the basis of morphological characteristics as *Endomyces anomalus* (Hans.) Zender, the cosmopolite yeast formerly known as *Saccharomyces anomalus* or *Willia anomala.*

This yeast is exceedingly frequent, and was found by Lilienfeld-Toal on samples of cacao. Judging from the cultural features on starting medium, one may distinguish many forms or varieties, chiefly by the color of the colonies, varying from the whitish to the pinkish, this characteristic probably being related to other distinctive features, but the study of our strains was limited to the micro-morphologic ones.

STRAINS N. 154, 166, 175, 190, 201, 224, 237 AND 255

Of these strains, N. 166, 190 and 237 only were studied in detail; the identification of the remaining was made from micro-morphologic characteristics. All yeasts here listed must be classified as *Schizosaccharomyces Bussei* Lil.-Toal & Henneb., a cosmopolite yeast found by Lilienfeld-Toal as one of the most diffused. The differences between the description and our strains are not appreciable: among the cultural characteristics, the optimum temperature of growth is 40°C. (37°C. according the description); the color of the colonies varies from light yellow and straw-yellow. The concentric rings of the geant colony are more or less marked, as well as the radial striae. It fails to liquefy the gelose. Assimilates peptone easily, glycocol and asparagine not at all or very scantily; nitrate and nitrate of potassium, and ammonium sulphate not at all. It readily inverts saccharose, raffinose and trehalose. It does not seem to assimilate organic acids, (malic, tartaric, acetic and citric) and glycerine (plate XXXV, fig. C).

As rightly expressed by Lilienfeld-Toal, this species is of the cycle of *Schizosaccharomyces Pombe* Lindner, from which it differs as *Schizosaccharomyces mellacei* Jorg. differs from *S. Pombe.*

For the characteristics of the asporogenous race, see *Schizotorulopsis Bussei.*

Strains N. 152, 165, 185, 206, 219, 220, 235 and 240

The complete study of this yeast was made up on the strains N. 152 and 220 only, and the remaining strains classified according the microscopical appearance of the vegetative cells.

CULTURAL CHARACTERISTICS

Optimum temperature of growth: about 35°C.

Starting medium: colony abundant, thick, of very rapid growth, smooth, uniform; border unbroken, or forming very irregular and broad sinuosities, of enameled polish; ivory-white in color and creamy when young, it appears slightly yellowish and pastose when old.

Gorodkowa's agar: poorly developed. Not forming spores.

Raulin's neutral solution: it forms a heavy creamy ring, at first incomplete, later complete; there is no pellicle; the liquid medium remains clear; deposit mucilaginous, very abundant, roping, grayish in color.

Carrot agar: very similar to the starting medium, but the colonies are more developed and of more rapid growth.

Malt extract gelatine (geant colony): flat, smooth, ivory-white, of small size colony, of enameled polish, with uniform irregularly undulate borders, without crateriform central cavity, radial striae or concentric rings. Growth according the fundamental type I of Will.

Malt extract water: very similar to the growth on Raulin neutral solution.

MORPHOLOGICAL CHARACTERISTICS

Asporigenous. On solid media, young cells are typically and more or less regularly apiculate; old cells are irregular in shape, from apiculate to ellipsoid, obovate and spheroid, single. Budding very active, normally bipolar. Geant cells scarce or absent, slightly larger than the normal ones. Vegetative cells from 3 to 6 mmm. in diameter or in length, most frequently from 3.5 to 5 mmm. Cells of the ring on liquid media very similar to the preceding, single to aggregated in short chains composed of 2 to 4 cells. Cells of the deposit more frequently round than apiculated, always single, but mechanically aggregated or tangled, mucous; the protoplasm is filled with oil drops and crystalloids; there may be degenerative forms and absence of budding.

BIOCHEMICAL CHARACTERISTICS

It ferments glucose readily but does not ferment levulose, sorbitol, dulcitol, abinose, xylose, rhamnose, mannose, galactose, maltose, lactose, melibiose, saccharose, trehalose, raffinose, starch, soluble dextrin, glycogen and inulin.

It inverts saccharose, but not trehalose; the inversion of raffinose, if any, takes place with difficulty. Strain N. 185 inverts raffinose; other strains do not invert the same hydrocarbonate. Glucose, levulose and saccharose are easily assimilated; galactose and mannose not readily and lactose not to any extent. Assimilates acetic acid well; tartaric, malic and citric acid not readily; methylic and ethylic alcohol not to any extent. Assimilates glycerin very

slowly. Pepton is preferred, but asparagine is assimilated quite well; nitrate of potassium and sulphate of ammonium are assimilated slowly but not nitrite of potassium nor glycocol. Starch is not hydrolized and gelatinase is not produced. This yeast grows apparently without difficulty in media containing 1 per cent of acetic acid.

SYSTEMATIC POSITION

Many tropical species of the genus *Kloeckeria* Janke (showing apiculate, asporogenous cells) were isolated chiefly from the soils and described by Klöcker, but most of them are not completely characterized and easily distinguished, and a comparison with our species cannot be made satisfactorily. The present writer (**5**) described a species of *Kloeckeria* (*K. domingensis* Cif.), isolated from washing water of rotting cacao pods, very rarely found on fermenting cacao beans, and only at the beginning of the fermentation. *K. domingensis* is very distinct from the strains in study, in having larger cells (6–12 by 4–8 mmm.), very regular in shape when young; ferments levulose and glucose only slightly; assimilates glucose, saccharose, levulose and maltose, but not acetic acid; an hydrogen-ion concentration of pH = 5,0–4,6 interrupts the growth. In expectation of the revision of the species of *Kloeckeria* indicated to date, we describe the form in study as a new species: *Kloeckeria cacaoicola* Cif., n. sp.

STRAINS N. 182, 202 AND 254

The strains in study must be referred to the *Kloeckeria domingensis* Cif., previously described (**5**).

STRAINS N. 158, 163, 167, 184, 197, 199, 210 AND 228

Only four strains (N. 158, 163, 184 and 210) were completely studied, the remaining being determined on the basis of cultural characteristics and morphological aspect of the cells on starting medium, as well as on the basis of the fermentation.

CULTURAL CHARACTERISTICS

Optimum temperature for growth: about 35°C.

Starting medium: thin, flat, white to ivory-white, then white-grayish or white-yellowish, mat, more or less regularly rounded colonies, with smooth surface, and smooth plain or irregularly undulate borders.

Gorodkowa's agar: thin, depressed, almost pellicular, poor colonies; cells without ascospores.

Raulin's neutral solution: quick growth, forming a superficial ring at first incomplete, later complete, but irregularly thickened, with a few very small floating islets, but without a well developed velum; easy formation of an

abundant, more or less pulverulent deposit at the bottom of the test tube; the liquid remains clear.

Carrot agar: very abundant development, similar to the growth on the starting medium.

Malt extract agar (geant colony): flat, white to whitish, finally mat, rounded colony, showing a small slightly depressed center (crateriform), sorrounded by a peripheric ring; colony thinner at the periphery, with a few not well defined radial striae, but without concentric rings; borders undulate or not, finely ragged in the sub-superficial portion of the colony, but smooth at the surface.

Malt extract water: the same growth as in Raulin's neutral solution, but more abundant at the bottom of the tube; the liquid of culture is slightly decolorized; without any peculiar odor (Plate XXXV, fig. B).

MORPHOLOGICAL CHARACTERISTICS

Young cells cultivated on solid media are generally, ovate or elliptic to elliptic-elongate, with unipolar, rarely bipolar budding, 3 to 5 mmm. in diameter or in length; geant cells not very frequent, more or less rounded, 4 to 6.5 mmm. in diameter not clearly distinct form the normal cells; cells single, rarely aggregated in short chains composed of 2 to 4 cells. Cells of the ring developed on liquid cultures similar to the preceding, but frequently aggregated in chains, straight or branched, composed of 2 to 7 elements; also lengthened, ellipsoid to sub-cylindric cells, 6–9, (generally 6–7 mmm.) by 3–5 mmm., single or chained together with the normal cells. Cells of the small floating fragments of the pellicle completely similar to the preceding, but composed of numerous elongate cells, and a few short normal cells. Deposit composed of more or less rounded to ovate or elliptic cells, with scarce single, buds, showing involutive or degenerative forms, and a more or less disorganized protoplasm. All young cells possess one vacuole, not rarely two or more small vacuoles, and at first one or two fat globules, then many small globules.

BIOCHEMICAL CHARACTERISTICS

Ferments glucose easily and abundantly, not arabinose, xylose, rhamnose, mannose, galactose, levulose, sorbitol, dulcitol, maltose, lactose, melibiose, saccharose, trehalose, raffinose, starch, soluble dextrin, and inulin. Fermenting power is very strong in a few strains (as N. 163, 184, 197 and 199), and very reduced in a few other (as N. 184 and 228). Assimilates well glucose, levulose, galactose, maltose and saccharose; also abundantly acetic and citric acid, less tartaric and slightly malic acid; assimilates ethylic alcohol, but not methylic alcohol. It growths very well on peptone solution, and well also in asparagin, nitrate of potassium and sulphate of ammonium solution; the development is poor on nitrite of potassium and on glycocol solutions. Inverts readily saccharose and raffinose, very sparingly trehalose.

SYSTEMATIC POSITION

This form, one of the most frequent asporogenous yeasts, found on fermenting cacao, in spite of the small difference of the cultural and morphologic characteristics, is identical to the "Kahmhefe A"

of Lilienfeld-Toal, found in cacao samples of all countries. In the opinion of the German author, the "Kahmhefe A" is identical, or. at least, very similar to the "*Saccharomyces theobromae*" Preyer, and I agree with him, taking in consideration the necessity of a right interpretation of the so called "spores" of the original description, and the cultural characteristics as referred to the peculiar cultural substratum. For the presence of fat globules in young cells, and the development of the yeast on liquid media according the fundamental form I of Will, this form should be assigned to the genus *Eutorulopsis* Cif. (*Eutorula* Will and not Saccardo). Of the asporigenous yeasts described as species of the genus *Eutorulopsis,* and not producing pigment in culture, two are known: *E. vulgaris* (Will) Cif. & Red and *E. ellipsoidea* (Will) Cif. & Red. The organism studied differs from both strains, in the cultural, morphologic and biochemical standpoint, so that the Preyer's species can be maintained, but transferred to the genus *Eutorulopsis*; *Eutorulopsis theobromae* (Preyer) Cif., n. comb.

Strains N. 157, 171, 187, 198, 222 and 245

Another very common asporogenous yeast, of the same type as the preceding. Only the strains N. 157 and 198 were studied in detail.

CULTURAL CHARACTERISTICS

Optimum temperature for growth, about 40°C.

Starting medium: white-grayish to light-grayish, small, flat, almost pellicular, irregular colonies, very thin, of an uniform thickness or slightly thicker at the borders; borders more or less irregularly sinuate-undulate. Old colonies are gray in color.

Gorodkowa's agar: poor growth, very similar to the preceding, but showing less developed colonies. Not forming spores.

Raulin's neutral solution: quick superficial development, forming a continuous but thin ring and at first cob-webby, then thicker velum; the superficial pellicle is fragmented, but almost entire in old colonies, grayish, finely folded; deposit slimy and abundant, grayish in color; no peculiar odors.

Carrot agar: very similar to the cultures on starting medium.

Malt extract gelatine (geant colony): colony of slow growth, grayish in color; flat, smooth, superficial, forming a great central crateriform depression, without striae, and not well marked concentric rings; borders continous or irregularly and broadly sinuate, in the subsuperficial portion finely striated.

Malt extract water: growth similar to that on Raulin's neutral solution.

One of the most distinctive features is the slow growth of the colonies at the optimum temperatures, in any liquid or solid media (plate XXXV, fig. D and F).

MORPHOLOGICAL CHARACTERISTICS

Cells developed on solid media ovate to elliptic, rarely sub-cylindric or sub-elongate, 2.5–5 by 4–6 mmm., normally 2.5–4 by 3–5 mmm., the longest being 8 mmm. Geant cells not frequent, spheric, 5–7 mmm. in diameter. Cells single or chained (from 2 to 20, rarely more) forming straight or sparingly branched chains; also crown-like aggregation of 10–30 cells are present. Chains and crown are exceptional on solid media cultures, but normal in the superficial velum, and quite frequent in the ring. Budding, unipolar on solid media cultures, and bipolar on the cells of the velum and ring, is very active only on solid media. Cells of the deposit are single or mechanically aggregated by a connective mucosity, or more or less free; they are very scarcely budding, frequently guttulated or vacuolated, showing involutive strange forms. Geant cells are very frequent.

BIOCHEMICAL CHARACTERISTICS

Does not produce gas in arabinose, xylose, rhamnose, glucose, mannose, galactose, fructose, sorbitol, dulcitol, maltose, lactose, melibiose, saccharose, trehalose, raffinose, starch, soluble dextrin and inulin. It inverts saccharose and raffinose, not at all trehalose, Liquid media containing glucose, maltose, levulose and saccharose are preferred. It develops quite well on acetic acid solution, and in presence of ethylic alcohol, less on tartaric, malic and citric acids, glycerin and methylic alcohol. Peptone solution is preferred, but nitrate of potassium and ammonium sulphate solution are admitted. The liquefaction of the gelatine is very small.

SYSTEMATIC POSITION

This yeast, almost as frequent as the preceding yeast, is easily distinguished from the *Saccharomyces theobromae* by the velocity of the growth, which is rapid in the Preyer's fungus, and slow in this species. In our opinion, this yeast is similar or identical to the "Kahmhefe B" of Lilienfeld-Toal, isolated from Costa Rican and Trinidad cacao beans, but this yeast was not as completely studied by the German author as the other yeasts, so that a complete comparison cannot be made.

This asporogenous yeast must be included in the genus *Eutorula* (*Torula* of the zymologists, p. p.), in spite of presence of chains both straight and branched, crown-like figures, etc. These aggregations, if found both on liquid and solid media, are characteristics of the genus *Blastodendrion* (Ota), which geant colonies are intermediary between the typical geant colonies of the Mycotoruleae and the same of the Torulopsideae. In the fungus at hand, cell aggregations are found almost only on liquid media cultures, and the geant colony is, of course, of the type I of Will (Torulopsideae). At this time the systematic position of the colourless species of the genus *Torulopsis* is so chaotic that an efficient compari-

son is impossible, and therefore we should create a new species "ad interim", which we will name *Torulopsis Lilienfeld-Toalii* Cif., n. sp., dedicated to the distinguished German zymologist Dr. O. A. von Lilienfeld-Toal.

This species differs from the formerly studied yeast by the velocity of the growth, the presence of a complete superficial pellicle, the aggregations of the cellular elements on liquid media cultures, as well as by many biochemical characteristics, chiefly by the absence of the fermentative power.

Strains N. 156 and 234

This yeast is probably of accidental presence in the fermenting cacao.

CULTURAL CHARACTERISTICS

Optimum temperature for growth: about 30°C.

Starting medium: growth rapid; colony at first white, later white-yellowish, dense, creamy, opaque, thick; the center of the colony is smooth and uniform, the border is thinner, grossly and irregularly lobate.

Gorodkowa's agar: very poor growth; single colonies small, round, whitish, flat, partially confluent in a larger colony. No ascospores formation.

Raulin's neutral solution: poorly developed; only fragments of the ring are present, and the deposit, pulverulent and whitish, is not abundant; no pellicle; does not produce peculiar odors.

Carrot agar: characters similar to those observed on the starting medium, but more pronounced; most of the colony run to the bottom of the test tube.

Malt extract gelatin (geant colony): white or whitish, flat, circular, small colony, slowly forming an enlarged central depression, but not of the crateriform type; radial striae or concentric rings are not formed; irregularly sinuate, linear, thin, smooth borders. Geant colony of the Will's fundamental type I.

Malt extract water: as in Raulin neutral solution, but more abundant.

MORPHOLOGICAL CHARACTERISTICS

Does not form ascospores. Cells more or less spheric to spheroid, rarely elliptic to avoid, 3.5–5.5 by 4–6.5 mmm., with unipolar, rarely bipolar budding. Cell single, rarely aggregated in short chains composed of 2 to 4 elements, without complicated aggregations. Geant cells scarce on solid media, more abundant on liquid ones, spheric, 6–8 mmm. in diameter, scarcely distinguished from the normal cells. Cells of the ring spheric to elliptic, 3–5 mmm. in diameter, or 3–4 by 4–6 mmm., normally aggregated in short straight chains composed of 2 to 6 elements; the budding is scarcely active. Cells of the deposit in liquid cultures similar to those on solid media, but with a more or less disorganized protoplasm, frequent involutive forms, and without budding.

BIOCHEMICAL CHARACTERISTICS

Does not produce gas in arabinose, xylose, rhamnose, glucose, mannose, galactose, sorbitol, dulcitol, maltose, lactose, saccharose, trehalose, raffinose, starch, soluble dextrin and inulin. Inverts saccharose, raffinose, and trehalose. It is not difficult on the selection of sugars as source of carbon: glucose, levulose, maltose and galactose are of the same importance. Does not develop in methylic and ethylic alcohol solutions, as well as on acetic, citric, malic and tartaric acids, and on glycerine. The growth is very abundant on peptone, and scarce or not at all on glycocol, asparagine, potassium nitrate and nitrite and ammonium sulphate. On gelatine cultures, a slow liquefaction takes place.

SYSTEMATIC POSITION

This species is clearly distinct from the preceding, as well as from the *Eutorulopsis* variety isolated from the Dominican cacao. The problem of the comparison of the species in study among the to date more or less completely described species of the genus Torulopsis, remains the same; thus we should create a new species "ad interim", dedicated to Mr. Hamel Smith, pioneer of the study on cacao fermentation, which we will name, *Torulopsis Hamel-Smithii* Cif., n.sp.

STRAIN N. 161

This strain, isolated together with strains of the *Schizosaccharomyces Bussei,* is indistinguishable by cultural, morphological and biochemical standpoint from this yeast, with the exception of the following characteristics: (1) fails to produce ascospores on Gorodkowa's medium, as well as on chalk cones and on sterilized carrot; (2) the enormous production of arthrospores (or *Oidium*-like cells), chiefly in liquid media; (3) in spite of the absence of the ascospore production, a more or less complete conjugation of the cells may rarely be observed; after the copulation, the cell remains inactive; (4) the fermentative power very reduced, but the same carbohydrates are fermented.

In spite of the fact that the transformation of the *Schizosaccharomyces Bussei* into the asporogenous form or "viceversa" was not experimentally demonstrated, there are no doubts on the relation between ascogenous and anascosporic strains. On the other hand, Lilienfeld-Toal observed the presence of asporogenous strains.

The imperfect form of the genus *Schizosaccharomyces* must be classified under the genus *Schizotorulopsis* Cif., of which only one species is surely known (*S. Alfonsecai* Cif.), not to mention the *S. asporus* Eykman and named: *Schizotorulopsis Bussei* Cif., n. nom.

Strain N. 215–215 bis.

This strain was isolated for the first time in January 1927 from fermenting cacao from a cacao estate located at La Vega. It was generically determined, and the failure to produce fermentation ascertained. Before the transplant of the month of July, the strain died. A new casual reisolation was made during the month of October of the same year from a fermenting cacao of a cacao farm of Moca, and the study was completed without delay. Five months after the reisolation, I also lost the second strain.

CULTURAL CHARACTERISTICS

Optimum temperature for growth: about 40–42°C.

Starting medium: white or whitish, creamy, slimy, not abundant colonies, of slow growth also at the optimum temperature, and very slow at lower temperatures; the colonies are smooth, glassy, without distinctive particularities.

Gorodkowa's agar: very small, lenticular, alabastrine, scattered colonies. Not forming spores.

Raulin's neutral solution: very reduced development, both superficial and at bottom; only small and scattered fragments of rings are formed; no superficial pellicle; very scarce, slimy, white-yellowish deposit.

Carrot agar: of the cultural subtrata tested, this agar appears as the best; colony very similar to those on starting medium, but more abundant.

Malt extract gelatine (geant colony): very small, round, flat, white, smooth colony, without crater, rings or striae; borders plain, smooth, uniform. The type of the colony is the I type of Will.

Malt extract water: development similar to that on Raulin's neutral solution, but slightly abundant; the ring is more developed.

MORPHOLOGICAL CHARACTERISTICS

Cells developed in cultures on solid media clearly biform; most frequently sphaeric to subovate, about 4 to 6 mmm. in diameter, mixed with elliptic-lengthened or cylindroid cells, of 7–12 by 5–8 mmm. The multiplication effected is by a process beginning by budding and ending by fission, as described in the genus *Schizoblastosporion*. Cells of the ring on liquid media cultures similar to the preceding but most abundant cells are the elongate instead of the rounded. Cells of the deposit generally spheric, frequently showing degenerative forms, with a more or less spoiled protoplasm; geant cells present but scarce, not easily distinct from normal spheric cells but for a thicker membrane and a slightly greater (diameter 5–7.5 mmm.).

BIOCHEMICAL CHARACTERISTICS

They do not ferment arabinose, xylose, rhamnose, glucose, mannose, galactose, fructose, sorbitol, dulcitol, maltose, lactose, melibiose, saccharose, trehalose, raffinose, starch, soluble dextrin and inulin. Does not invert saccharose, trehalose and raffinose. Assimilates abundantly only glucose and levulose, not maltose, saccharose, mannose, galactose and lactose, nor organic acids, or alcohols. Growth good only on peptone solution. Fails to liquefy gelatine; does not

produce sulphuretted hydrogen from powdery sulphur; fails to produce indol or trytophane, and does not give the biuret reaction; does not hydrolize starch; methylene blue solution is decolorized very slowly.

SYSTEMATIC POSITION

This strain falls under the genus *Schizoblastosporion,* a genus of asporigenous yeasts characterized by the reproduction by budding-fission. The only species to date described is the type of the genus: *S. Starkeyi-Henricii* Cif. (5), isolated from North American soil. Under the denomination "Hefe R", Lilienfeld-Toal isolated from cacao of St. Thomé another strain of Torulopsidacea, undoubtedly pertaining to the same genus *Schizoblastosporion.* Differential characteristics between *S. Starkeyi-Henricii,* "Hefe R", and our strain are summarized in the following table.

Schizoblastosporion Starkeyi-Henricii	*"Hefe R" of Lilienfeld-Toal.*	*Strains N. 215-215 bis of Ciferri.*
Apparently of strong vitality.	In culture 50% of the cells dead.	of feeble-vitality.
White colony.	Yellowish colony.	White or whitish colony.
No pellicle.	Mucous pellicle.	No pellicle.
Rapid growth.	Slow growth.	Slow growth.
?	Liquefy gelatine.	Not liquefy gelatine.
?	Optimum temp. 20°C.	Optimum temp. 40-42°C.
Cells not very mucous.	Cells very mucous.	Cells not very mucous.
Spheric cells 2-7 mmm. of diameter.	Spheric cells 6, 5-7 mmm. of diameter.	Spheric cells 4-6 mmm. of diameter.
Elongate cells 10-22 by 5.5-7 mmm.	Elongate cells 10 by 20 mmm.	Elongate cells 7-12 by 5-8 mmm.
Without geant cells.	With geant cells.	With geant cells.
Glucose is preferred.	Glucose is preferred.	Glucose and levulose are preferred.

These differences are, in our opinion, distinguishing the "Hefe R" from the strains N. 215–215 bis, and both from *S. Starkeyi-Henricii,* basing our opinion on the cultural, morphological and biochemical differences above summarized. According to these conclusions, we propose the binomial *Schizoblastosporion santhomensis* Cif., n. nom. for the Lilienfeld-Toal's "Hefe H", and *Schizoblastosporion domingensis* Cif., n. sp. for the strains N. 215–215 bis.

In closing, from the study direct or indirect of two other species of the same genus, we are able to perfect the generic description of *Schizoblastosporion* Cif.: asporogenous yeast, in which multiplication begins by budding and ends by fission; frequently of feeble vitality; without fermentative power; assimilation of carbon and azote generally specialized from one or a few determined compounds; cells always bimorphe, the smaller spheric and the longer elliptic to cylindric.

STRAIN N. 216

Isolated only once from dry fermented cacao beans, but here

very abundant. This strain must be considered as the *Torula aurantiaca* Saito (**25**), isolated from the air in Manchury. This species being well known from Saito's description, we are adding only the characteristics not previously observed, or different from those of the Japanese author.

CULTURAL CHARACTERISTICS

Agar cultures are generally abundant, easily developed, smooth, with plain or irregular, simple borders. Gelatine cultures of the same type of all simple Torulopsidaceae (I fundamental type of Will), with a small central depression, and simple, and smooth borders. Cultures in liquid media forming a small, rather incomplete ring, and a more or less abundant deposit, but not pellicle. Optimum of temperature for growth about 30°C. Cells normally not aggregated in chains, and not forming arbuscula, crowns, and so on. Does not ferment arabinose, xylose, rhamnose, glucose, mannose, galactose, fructose, sorbitol, dulcitol, maltose, lactose, melibiose, saccharose trehalose, raffinose, starch, soluble dextrin and inulin. Does not invert raffinose. Develops abundantly on glucose, levulose, maltose and galactose, as well as peptone, less on asparagine, nitrate of potassium and ammonium sulphate.

SYSTEMATIC POSITION

This species is reported for the first time found outside of the type locality, and probably, accidentally present on fermenting cacao, should be transferred to the genus Torulopsis as *Torulopsis aurantiaca* (Saito) Cif. & Red.

Strain N. 204

This strain was isolated only one time, and as the preceding strain, must be considered as an accidental contamination of the fermenting cacao beans. It may be considered as identical with the *Torulopsis mucilaginosa* (Jörgens.) Cif. & Red., a pinkish Torulopsidea, described by Jörgensen (**16**) and revised and reclassified by the present writer and Redaelli (**8**).

Strain N. 153

As the preceding two strains, but exceedingly frequent on the sample examined. In our opinion, this strain is a variety of the *Torula rumosa* Saito (**25**), a Mycotorulea, that, according the description of the Japanese author, as well as the observation on the strain at hand, must be referred to the genus *Mycotorula* Will as *Mycotorula ramosa* (Saito) Cif., n. comb. Only differential characteristics from Saito's description are indicated.

CULTURAL CHARACTERISTICS

On favorable solid media the colonies are abundant, at first smooth, then with plume-like borders, and with an abundant sub-superficial development of root-like mycelium. The borders are finely engraved to dentate, and under the microscope, a dense, net-like work is visible. On gelatine, the geant colony is clearly sulcate by radial, simple or branched, striae, starting from a depressed to crateriform center to the periphery. No or only sketchy concentric rings are visible. Borders dentate and plume-like, with a dense sub-superficial mycelium vegetation. On liquid media, the development is abundant, forming at first a complete and generally dense ring, then a serie of floating islets, finally aggregated into an orange-reddish, finely folded, mat superficial pellicle. The deposit is ropy, brownish in color. The color of the colonies on solid media oscillate form gold-yellow to orange-reddish.

MORPHOLOGICAL CHARACTERISTICS

The morphology of the cell is very similar to that described by Saito, but the cells of the velum reach 30 mmm. in length and 6.5 mmm. of thickness. Geant spheric cells are frequent in the deposit of liquid cultures, being from 6 to 9 mmm. in diameter.

BIOCHEMICAL CHARACTERISTICS

Does not ferment arabinose, xylose, rhamnose, glucose, mannose, galactose, fructose, sorbitol, dulcitol, maltose, lactose, saccharose, trehalose, raffinose, starch, soluble dextrin and inulin. Inverts trehalose, saccharose and raffinose. Liquefies gelatine slowly. Growth good on glucose, saccharose, multose, but not on levulose and galactose. Only the peptone is favorable. The optimum of the temperature for growth is lower than 30°C.

SYSTEMATIC POSITION

This strain differs from the *Mycotorula ramosa* in many particulars of the colonies both on solid and liquid media, as well as in the morphology of the cells and in the biochemical characteristics. For this variety we propose the name: *Mycotorula ramosa* (Saito) Cif. var. *dominicana* Cif., n.var.

STRAINS N. 160, 162, 164, 170, 176, 177, 188, 192, 214 and 227

All these strains are referable to the genus *Geotrichum* (*Mycoderma* of Auct. sensu stricto, or *Oidium* of the zymologists, pro parte). With the exception of the strains N. 164, 177 and 192, all others are not easily distinguished from the morphological and biochemical points of view, while cultural characteristics are but little differentiated. For brevity, the most outstanding characteristics only are summarized.

Strain N. 160

CULTURAL CHARACTERISTICS

On solid favorable media, it forms a poorly developed, folded, white, pellicular colony in which borders are more or less plume-like. In favorable liquid media, the ring is fused to a well developed, white-folded, thin, dusty, at last floating pellicle; the deposit is abundant and slimy. The geant colony on malt extract agar is thin, pellicular, finely but indistinctly folded, chiefly at the borders, but without concentric rings. This yeast does not liquefy the gelatine.

Strain N. 162

CULTURAL CHARACTERISTICS

On solid favorable media, it forms an abundant, white-grayish to white-yellowish mat, thick, at first smooth, then more or less woolly, irregularly but very densely cerebroid or mesentheroid-folded colonies. The borders are thick, almost smooth. In favorable liquid media, the pellicle is abundant, yellowish, cerebriform-sulcate, dense; the ring is fused with the pellicle. The geant colony on malt extract agar is thick, irregularly cerebriform-convolute, with a broad central depression. The gelatine is not liquefied, or very slow.

Strain N. 164

All cultural characteristics are similar to those of the strain N. 160, but the colonies on solid media are more developed.

Strain N. 170

Very similar to the strain N. 160; the deposit is mucous more than slimy, and the geant colony shows more well marked radial striae and almost indistinct concentric rings.

Strain N. 176

Similar to the strain N. 162, but thinner and yellowish in color.

Strain N. 177

CULTURAL CHARACTERISTICS

Very abundant, whitish, dry, plain, abundant and of rapid growth colonies, covered by a dense cottony layer, when developed on solid favorable media. In liquid media, the velum is cottony or densely wool-like, but irregularly developed, almost white in color, at last floating. The geant colony on malt extract agar is very well developed, almost hirsute, under the superficial cotton-like layer, crenulate, dry; the borders are uniform and plain. The center is somewhat prominent, but irregularly, and one or few not well marked concentric rings are visible. The gelatine is not liquefied.

Strain N. 188

Similar to the preceding, but with well marked radial striae, and more or less indistinct concentric rings. The colonies on solid media are more developed, and a slow but diffused liquefaction of the gelatine takes place. The colonies are always smooth, never crenulate.

Strain N. 192

Not distinct from the strain N. 188.

Strain N. 227

Very similar to the strain N. 162.

Strains N. 160, 162, 164, 170, 176 177, 188, 192 214 and 227.

MORPHOLOGICAL CHARACTERISTICS

Very similar in all studied strains. Arthrospore generally from 3.5 to 6 mmm. by 5 to 12 mmm.; mycelic hyphae more or less slender, branched, septate, of the same thickness. Strains N. 160, 164 and 170 have only creeping hyphae; strains N. 162, 176 and 227 abundant creeping hyphae, but also a few, short and poorly developed, sub-erect, hyaline, sparingly branched hyphae; strains N. 177, 188 and 192 possess creeping hyphae as well as erect, densely branched, cottony, septate, apparently true myceliar hyphae. All creeping hyphae easily produces chained arthospores; sub-erected hyphae may produce arthrospores only in contact with the solid substrata; erected, true myceliar hyphae; apparently never produces arthrospores.

BIOCHEMICAL CHARACTERISTICS

The most outstanding difference is the power of liquefying the gelatine. Studied strains do not ferment any tested carbohydrate; growth good on glucose, saccharose, maltose, and levulose; less on galactose; ethylic alcohol, as well as acetic and citric acid (less on tartaric and malic) are accepted; not so well glycerine; not to any extent on methylic alcohol. Peptone, asparagine, nitrate of potassium and sulphate of ammonium are easily accepted; not so well glycocol; not or very little on nitrite of potassium.

SYSTEMATIC POSITION

Up to the present time the species of the genus *Geotrichum* are generally grouped in a few, more or less distinct species, or included under comprehensive determination that is so bewildering that a comparison with our strains cannot be made. For this reason we prefer the temporary grouping into species and variety on the base of the tested differences, according to the following key:

A. Cerebriform-sulcate colonies, more or less woolly, whitish to yellowish, slowly or not liquefying the gelatine (Strains N. 162–type, N. 176 and 227.)—*Geotrichum cerebrinum* Cif. n. sp.

B. Pellicular, smooth, poorly developed colonies, whitish to yellowish, not liquefying the gelatine (Strain N. 160–type, N. 164 and N. 170).—*Geotrichum flexuosum* Cif., n. sp.

C. Colonies showing striae and concentric rings, covered by a cottony layer, white to grayish in color.

a. Crenulate colony; not liquefying gelatine (strain N. 177).—*Geotrichum byssinum* Cif., n. sp.

b. Smooth colonies, liquefying the gelatine (strain N. 188–type and N. 192).—*Geotrichum byssinum* Cif. var. *rigidum* Cif. n. var.

YEASTS FOUND IN SANTO DOMINGO AS COMPARED TO THE YEASTS FOUND IN OTHER COUNTRIES

The yeasts common to Santo Domingo and other tropical countries, as found on fermenting, or fermented cacao beans, are the following:

1. *Saccharomyces ellipsoideus* var. *tropicus* Lil.–Toal & Henneb.—Intertropical.
2. *Endomyces anomalus* (Hans.) Zender.—Intertropical.
3. *Schizosaccharomyces Bussei* Lil.–Toal & Henneb.—Intertropical.
4. *Eutorulopsis theobromae* (Preyer) Cif. ("Kahmhefe A" Lil.–Toal).—Intertropical.
5. *Schizotorulopsis Bussei* Cif.—Intertropical.

The following yeasts are common to Santo Domingo and other countries, but not tropical countries, nor on fermenting or fermented cacao beans:

6. *Torulopsis aurantiaca* (Saito) Cif. & Red.—Manchury.
7. *Torulopsis mucilaginosa* (Jörg.) Cif. & Red.—Europe.

The following yeasts are found only in Santo Domingo, on the same substratum:

8. *Saccharomyces ellipsoideus* var. *domingensis* Cif.
9. *Kloeckeria cacaoicola* Cif.
10. *Kloeckeria domingensis* Cif.
11. *Torulopsis Lilienfeld-Toalii* Cif.
12. *Torulopsis Hamel-Smithii* Cif.
13. *Schizoblastosporion domingensis* Cif.
14. *Mycotorula ramosa* (Saito) Cif. var. *dominicana* Cif.
15. *Geotrichum cerebrinum* Cif.
16. *Geotrichum flexuosum* Cif.
17. *Geotrichum byssinum* Cif.
18. *Geotrichum byssinum* Cif. var. *rigidum* Cif.

The following yeasts, not found in Santo Domingo, are described on fermented cacao of other tropical countries:

19. *Saccharomyces ellipsoideus* Hans. var. *brasiliensis* Cif. (= "Weinhefe B" Lil.–Toal).—Brazil
20. *Schizoblastosporion santhomensis* Cif. (="Hefe R" Lil.–Toal).—San Thomé.
21. *Torulopsis neotropica* (*) Cif., n.nom. (="Kahmhefe B" Lil.–Toal).—Costa Rica and Trinidad.
22. *Saccharomyces theobromae-fermentans* Cif., n.nom. (**) (="Saccharomyces M" Lil.–Toal).—Trinidad.

THE FERMENTATION OF THE CACAO AND THE YEAST DISTRIBUTION

The distribution of the yeasts in relation to the course of fermentation may be deduced from the isolations made during the experiments. Judging from the strains isolated, we may divide the yeasts in: (1) yeasts normally present during the fermentation of cacao, and (2) yeasts of accidental or occasional presence. The yeasts of the first category, and, of course, that may play a role in the fermentation, are: (1) *Saccharomyces ellipsoideus* var. *tropicus*, (2) *S. ellipsoideus* var. *domingensis*, (3) *Endomyces anomalus*, (4) *Schizosaccharomyces Bussei*, (5) *Kloeckeria cacaoidola*, (6) *Eutorulopsis theobromae*. Less frequent, but not of exceptional presence, are: (1) *Torulopsis Lilienfeld–Toalii* and (2) *Kloeckeria domingensis*. Accidentally found on fermenting cacao beans are: (1) *Torulopsis Hamel-Smithii*, (2) *Schizoblastosporion domingensis*, (3) *Torulopsis aurantiacaca*, (4) *Torulopsis mucilaginosa*, (5) *Mycotorula ramosa* var. *dominicana*, (6) *Geotrichum cerebrinum*, (7) *G. flexuosum*, (8) *G. byssinum*, (9) *G. byssinum* var. *rigidum*.

In relation to the frequency of each species or variety of yeast, and the period of fermentation, including the period of post-fermentation, we may consider four phases, namely: (1) beginning of fermentation, (2) fulness of fermentation, (3) end of fermentation, (4) persistance on dry fermented cacao beans, conserved at the normal conditions, six months after the fermentation. In the scheme of the yeasts distribution, the absence of the species is expressed

(*) From the description of Lilienfeld-Toal, it is almost impossible to arrange this yeast in one of the genera of the asporigenous. As species of *Torulopsis* it is not typic of the genus.

(**) According to the German author above quoted, this yeast is similar both to *Willia anomala* and *Saccharomyces lactis* Dombrowski, but it is apparently more like the *Saccharomyces fragilis* Jörg. It differs from the three above quoted species by many cultural, biochemical and morphological characters, and may be a distinct and new species.

by 0; + indicated very rare; + + quite rare; + + + common; + + + + very common.

Yeast	Beginning of fermentation	Fulness of frementation	End of fermentation	Dry beans
Saccharomyces ellipsoideus var. tropicus	+ +	+ + + +	+ + +	+ + +
S. ellipsoideus var. domingensis	+ +	+ + +	+ + +	+ + +
Endomyces anomalus	+ + +	+ +	+ +	+ +
Schizosaccharomyces Bussei	+	+ + +	+ + +	+ +
Kloeckeria cacaoicola	+ + +	+ +	+	0
Eutorulopsis theobromae	+ + +	+ + + +	+ + + +	+ + +
Torulopsis Lilienfeld-Toalii	+ +	+ + +	+ + + +	+
Kloeckeria domingensis	+ + +	+	0	0
Torulopsis Hamel-Smithii	+ +	0	0	0
Schizoblastosporion domingensis	+	0	0	0
Torulopsis aurantiaca	0	0	+	0
Torulopsis mucilaginosa	0	0	0	+
Mycotorula ramosa var. dominicana	0	0	+	0
Geotrichum cerebrinum	0	0	+	+
G. flexuosum	0	0	+	+
G. byssinum	0	0	0	+
G. byssinum var. rigidum	0	0	+	0

KEY TO THE GENERA, SPECIES AND VARIETIES OF CACAO YEASTS

A. Sporogenous yeasts *(Saccharomycales)*.

I. Without true mycelium (*Saccharomycetaceae*).

1. Multiplication by budding (*Saccharomyces* Meyen).

a. The ellipsoid type (*Saccharomyces ellipsoideus* Hansen).

b. Fermenting glucose, raffinose, levulose, saccharose, maltose and galactose. — *S. ellipsoideus* Hans. var. *tropicus* Lil.-Toal.

bb. Fermenting glucose, raffinose, maltose, saccharose, levulose but no galactose.—*S. ellipsoideus* Hans. var. *domingensis* Cif.

bbb. Fermenting glucose and galactose only.—*S. ellipsoideus* Hans. var. *brasiliensis* Cif.

c. Not so.—*S. theobromae-fermentans* Cif.

2. Multiplication by fission (*Schizosaccharomyces* Linder).

Only species.—*S. Bussei* Lil.-Toal & Henn.

II. With a true mycelium (*Endomycetaceae;* genus *Endomyces* Rees).

Only species.—*E. anomalus* (Hans) Zender.

B. Asporogenous yeasts (*Atelosaccharomycetaceae-Torulopsilaceae*).

I. Without a true mycelium (*Torulopsideae*).

1. Multiplication by budding.

a. Cells spheric, ovate to ellipsoid.

b. Young cellules with one or more fatty corpuscles; growing according to Will's fundamental form I (*Eutorulopsis* Cif.)

Only species.—*E. theobromae* (Preyer) Cif.

bb. Young cellules without fatty corpuscules, growing according to Will's fundamental form III (*Torulopsis* Berl. emend).

PLATE XXVIII.

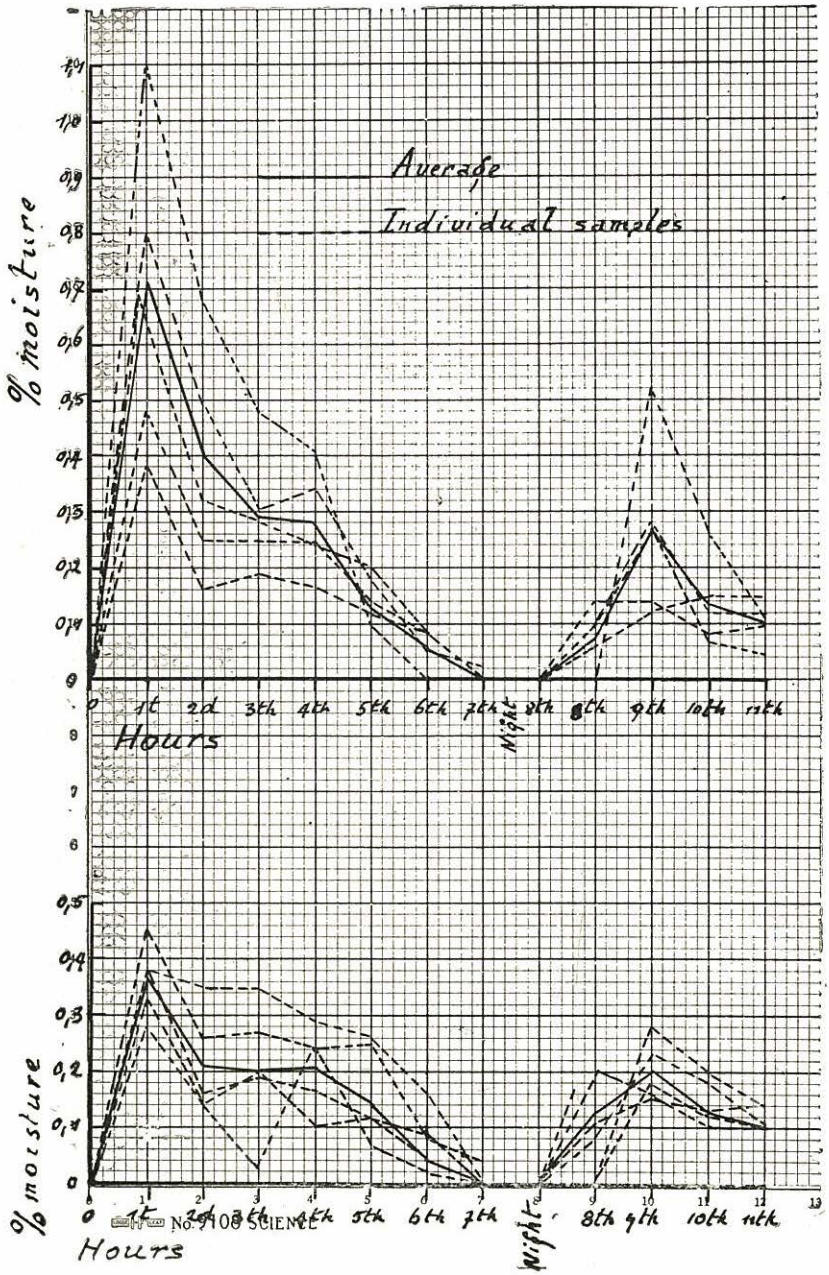


PLATE XXIX.

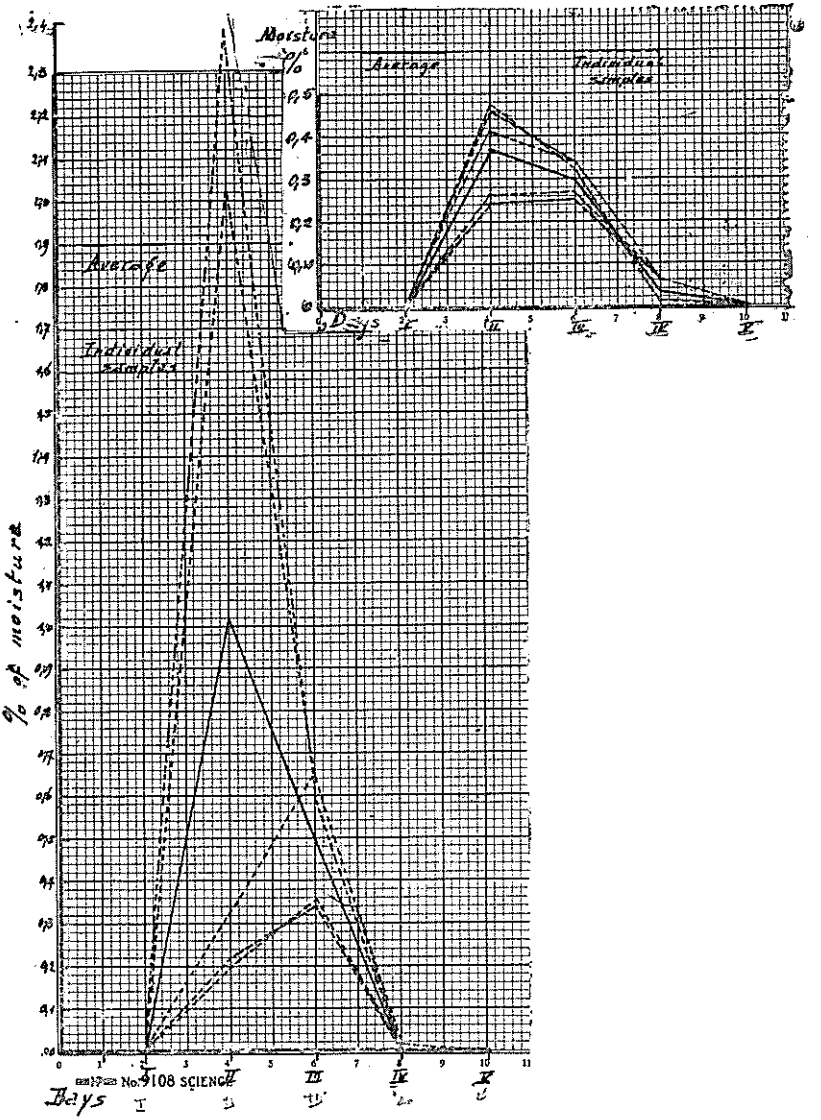
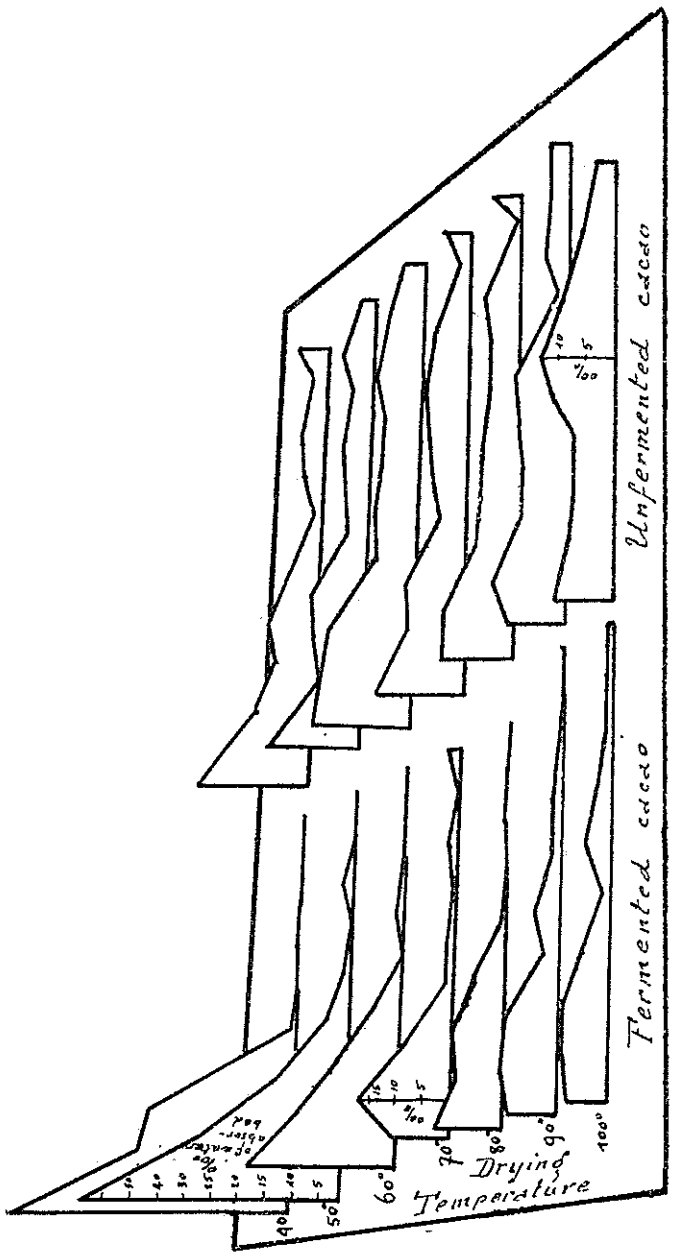

Moisture
%
0.5
0.4
0.3
0.2
0.1
0
Average
Individual samples
Average
Individual samples
% of moisture
No. 9108 SCIENCE
Days

PLATE XXX.


Unfermented cacao
Fermented cacao
Drying Temperature
40°
50°
60°
70°
80°
90°
100°

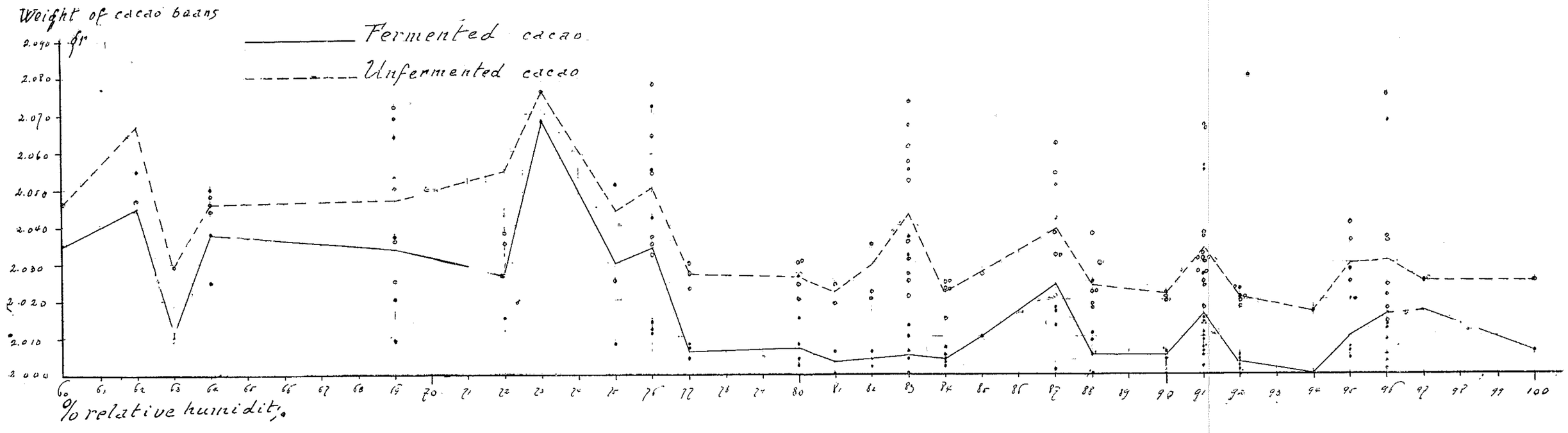

Weight of cacao beans
gr.
Fermented cacao
Unfermented cacao
2.090
2.080
2.070
2.060
2.050
2.040
2.030
2.020
2.010
2.000
60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
% relative humidity

PLATE XXXII.

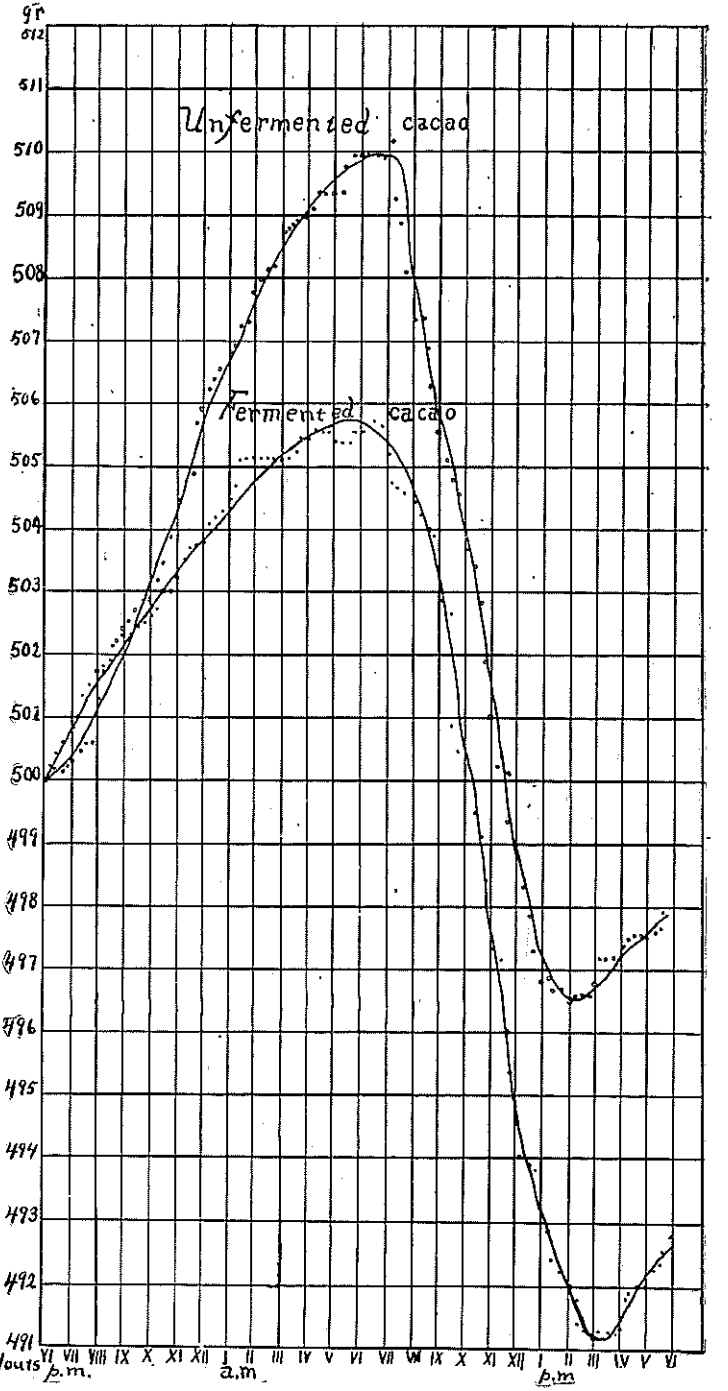
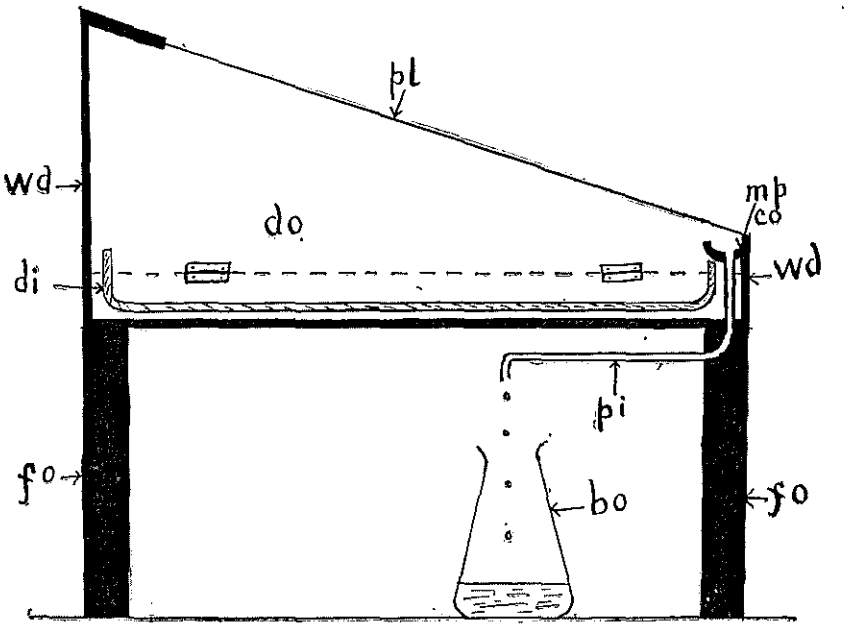

gr
512
511
510
509
508
507
506
505
504
503
502
501
500
499
498
497
496
495
494
493
492
491
Unfermented cacao
Fermented cacao
Hours
VI VII VIII IX X XI XII I II III IV V VI VII VIII IX X XI XII I II III IV V VI
p.m.
a.m.
p.m.

PLATE XXXIII.



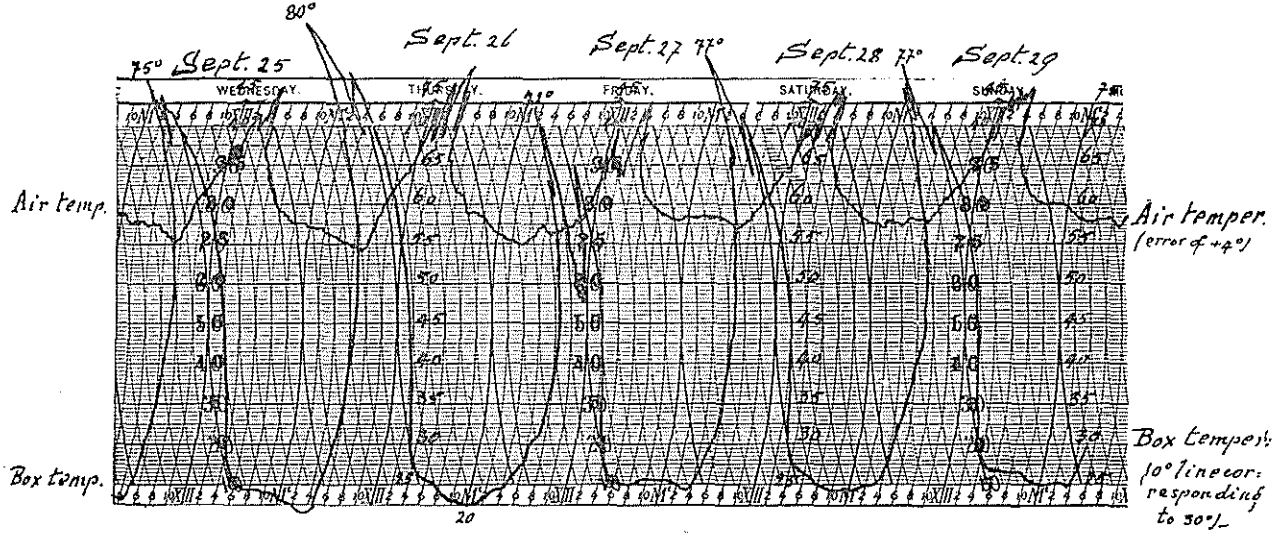
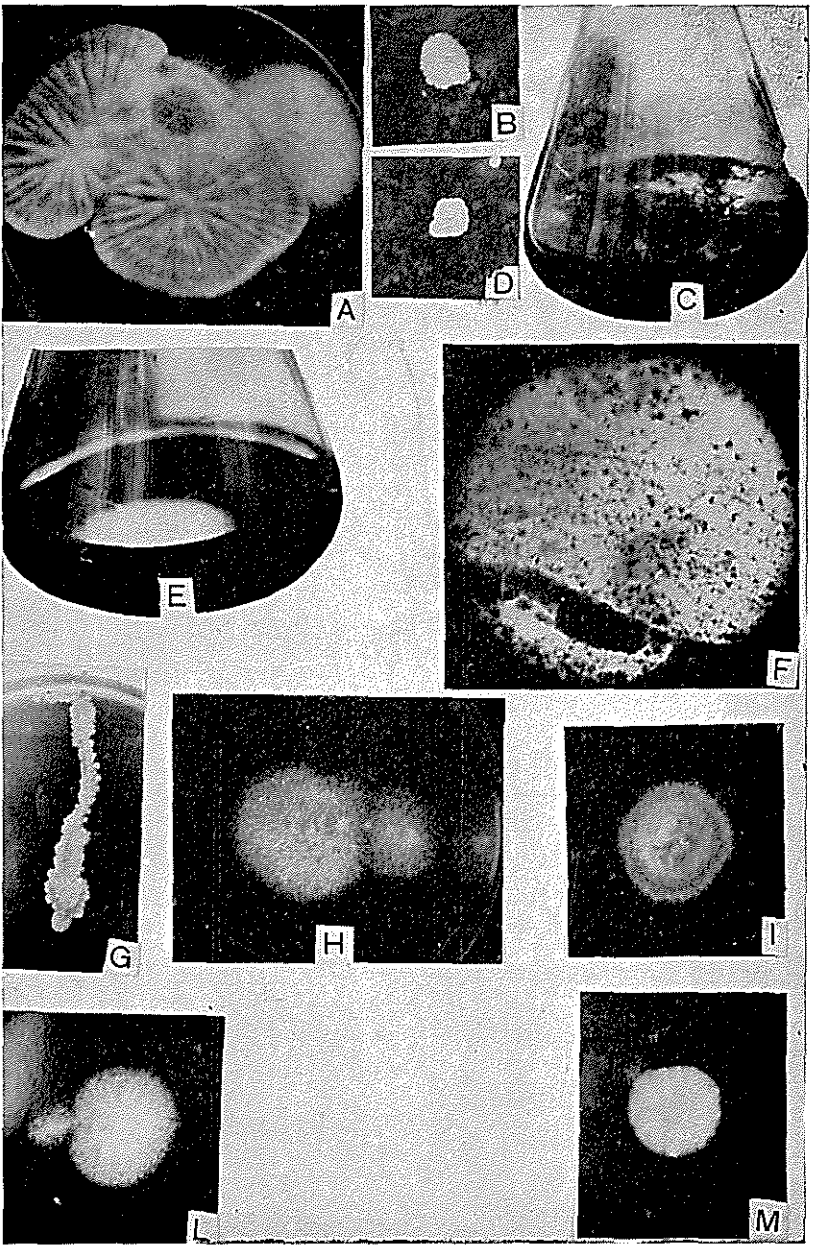

80°
75° Sept. 25
Sept. 26
Sept. 27 77°
Sept. 28 77°
Sept. 29
WEDNESDAY.
THURSDAY.
FRIDAY.
SATURDAY.
SUNDAY.
Air temp.
Box temp.
Air temper.
(error of +4°)
Box temper.
(0° line corresponding to 30°)
20

PLATE XXXIV.

PLATE XXXV.



c. Colonies pink or red to orange in color.

d. Colonies orange in color, developing abundantly in glucose, levulose, maltose and galactose solution.—*T. aurantiaca* (Saito) Cif. & Red.

dd. Colonies pinkish, developing abundantly in saccharose solution only.—*T. mucilaginosa* (Joerg.) Cif. & Red.

cc. Colonies whitish, yellowish or grayish in color.

d. Cellular elements aggregated in liquid media, producing a complete superficial pellicle.—*T. Lilienfeld-Toalii* Cif.

dd. Cellular elements not aggregated or only shortly chained, not producing a complete pellicle or without pellicle.

e. Cells chiefly ovate, colonies grayish, with lacerated borders.—*T. neotropica* Cif.

ee. Cells chiefly spheric, colonies white-yellowish, with linear borders.—*T. Hamel-Smithii* Cif.

aa. Cells generally apiculated or lemon-shaped.—(*Kloeckeria* Jancke.)

b. Cells large, fermenting levulose and glucose, not growing in pronounced acid media.—*K. domingensis* Cif.

bb. Cells smaller, fermenting glucose, growing in pronounced acid media.—*K. cacaoicola* Cif.

2. Multiplication by fission (*Schizotorulopsis* Cif.) Only species.—*S. Bussei* Cif.

3. Multiplication starting by budding and ending by fission.—*Schizoblastosporion* Cif.)

a. White or whitish colonies, not liquefying gelatine, optimum temperature about 40–42°C. Cells small.—*S. domingensis* Cif.

aa. Yellowish colonies; liquefying, optimum temperature about 20°C. Cells larger.—*S. santhomensis* Cif.

II. With a true mycelium (*Mycotoruleae*).

1, With or without occasional arthrospores (*Mycotorula* Will.) One species.—*M. ramosa* (Saito) Cif.

2. Elongated or rectangular long chained arthrospores (*Geotrichum* Link).

a. Cerebriform-sulcate colonies, more or less woolly.—*C. cerebrinum* Cif.

aa. Pellicular, smooth colonies.—*G. flexuosum* Cif.

aaa. Cottony colonies, showing concentric rings and striae.

b. Colonies crenulated, not liquefying gelatine.—*G. byssinum* Cif.

bb. Colonies smooth, liquefying gelatine.—*G. byssinum* Cif. var. *rigidum* Cif.

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Explanation of Plates

Plate XXVIII. Loss in weight of fermented cacao and unfermented cacao exposed to the sunshine.

Plate XXIX. Increase in weight of fermented and unfermented cacao beans exposed to saturated water vapor.

Plate XXX. Increase in weight of fermented and unfermented cacao beans dried at differented temperatures and exposed to saturated water vapor.

Plate XXXI. Daily variations of weight of fermented and unfermented cacao beans in relation to the average of the atmospheric humidity.

Plate XXXII. Hourly variation of weight (increase and loss) of fermented and unfermented cacao beans exposed to the free air.

Plate XXXIII. Sketch of the Wilson's distillation box for the desiccation of the cacao beans.

Plate XXXIV. Five days record of a double recording thermograph, showing the temperature of the Wilson box as compared to the air temperature.

Plate XXXV.

Figure A. *Geotrichum byssinum* Cif. var, *rigidum* Cif. Old geant colony on malt extract gelatine (natural size).

Figure B. *Eutorulopsis theobromae* (Preyer) Cif. Adult geant colony on malt extract agar (natural size).

Figure C. *Schizosaccharomyces Bussei* Lil.—Toal & Henn. Old superficial vegetation on malt extract agar (one-half natural size).

Figure D. *Torulopsis Lilienfeld-Toalii* Cif. Adult geant colony on malt extract agar (natural size).

Figure E. *Geotrichum byssinum* Cif. Young but well developed superficial vegetation on malt extract agar (more than half natural size).

Figure F. *Torulopsis Lilienfeld-Toalii* Cif. Photograph of very young cellular aggregation on starting medium (very much magnified).

Figure G. *Geotrichum cerebrnium* Cif. Young colony on starting medium (about ¾ natural size).

Figure H. *Geotrichum byssinum* Cif. Young geant colony on malt extract agar (natural size).

Figure I. *Geotrichum byssinum* Cif. Old geant colony on malt extract agar (natural size).

Figure L. *Geotrichum byssinum* Cif. Young colony on carrot agar (natural size).

Figure M. *Geotrichum flexuosum* Cif. Very old geant colony on malt extract agar (natural size).