INVESTIGATION OF FROTHING

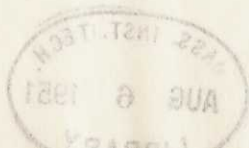## IN MOLASSES

by

Aaron L. Brody

Submitted in partial fulfillment of the

requirements for the degree of

Bachelor of Science

in Food Technology

from the

Massachusetts Institute of Technology

Signature of Faculty Supervisor _____Signature redacted_____

Signature of Head of Department_____Signature redacted_____

Signature of Author _____Signature redacted_____

Date: 18 May 1951

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Date: 10 May 1951

356 Harvard Street
Dorchester 24, Massachusetts,
18 May 1951.

Professor Joseph S. Newell
Secretary of the Faculty
Massachusetts Institute of Technology
Cambridge 39, Massachusetts

Dear Sir:

    The thesis entitled INVESTIGATION OF FROTHING IN
MOLASSES is hereby submitted in partial fulfillment
of the requirements for the degree of Bachelor of
Science in Food Technology.

                         Respectfully submitted,

                 Aaron L. Brody

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SUMMARY

The so-called "froth fermentation" of edible molasses was studied in an attempt to determine its basic cause. The theories of spontaneous chemical action, surface action, enzymatic action, and microbial action were among those investigated. Experiments eliminated microbial action as the basic cause of the froth. It was further shown that the gas, carbon dioxide, was supersaturated in the molasses, and the release of this dissolved gas resulted in the frothing. Surface action might aid in the release of the gas. Other investigations showed that the Maillard reaction could very well be the chemical reaction resulting in the formation of the carbon dioxide. A method of preventing the froth by changing the pH was suggested.

INTRODUCTION

Molasses is that uncrystallizable mother liquor from which has been crystallized out sugar. Due to the extreme difficulty of extracting out the last portions of the sugar present in the molasses, this product is produced in great quantities each year as a by-product of sugar refining. Blackstrap molasses is that molasses from which all the commercially recoverable sugar has been recovered. Edible molasses is obtained when only part of the commercially recoverable sugar has been removed.

Edible molasses is commercially manufactured by adding a quantity of pure sugar to cane blackstrap molasses. (Beet molasses is invariably inedible due to its high mineral content.)

In order to extract the maximum possible sugar from the molasses various processes are used. These include carbonation, sulphitation, and ion exchange. The newest of these, ion exchange, exchanges hydrogen ions for mineral ions in the molasses, thus giving a purer, more dilute liquor from which sugar can crystallize. The first two involve the addition of gaseous products to the blackstrap molasses to obtain this maximum crystallization. Carbonation involves the addition of carbonic acid and calcium oxide to make a direct consumption sugar containing more non-sugar solids than refined cane sugar but fewer of these impurities than raw sugar. Sulfitation involves the addition of sulfur dioxide and lime to give a similar product. However, most mills using the sulfitation process produce edible rather than blackstrap molasses.

There are many types of molasses each used for a different purpose. Blackstrap is used in the fermentation industry and in the animal feed industry as a binder. Invert or high-test molasses is a high concentration, inedible molasses with part of the sucrose inverted to inhibit crystallization. This product is used in the fermentation of molasses to produce ethyl alcohol. Edible molasses, of which Dark, Puerto Rico, Barbadoes, and New Orleans are examples, is used in the home as a spread, in pastries and cakes, etc.; in the New England Style Baked Bean Industry; in the bakery industry; in the soy sauce industry, etc. .

The manufacture of sugar from cane involves pressing the cane to obtain sugar cane juice from the fibers. To this juice is added lime as a precipitating agent to aid in bringing down impurities. The lime and impurities are filtered out, the juice is boiled down, and a portion of the crystallizable sugar is removed from it. This juice is first molasses. Second molasses or second-jet massecuite is first molasses diluted with cane juice and re-extracted. Third molasses is second-jet massecuite diluted with water and re-extracted. Fourth molasses is blackstrap, and no further sugar can be commercially crystallized out from this product.

Basket centrifuges are used to throw the molasses off from the sugar crystals. All of the boiling processes involve high enough temperatures to destroy all but thermophilic micro-organisms and very heat-stable enzymes.

Throughout the history of the sugar refining industry, it has been noted that under certain conditions of storage, these molasses would froth or foam and very often expand with such violence as to

burst their storage container and cause irreperable damage.
Occasionally so much heat develops that a charring of the molasses
occurs, finally turning the molasses into a solid black mass. The
froth is especially noticeable upon heating. When stored in tanks,
molasses often becomes too viscous to flow at any reasonable rate
of speed, and therefore it is necessary to heat the molasses to
lower its viscosity and make it flow rapidly. Such heating often
causes a violent frothing in the molasses.

This frothing has also been noted in massecuites. In this
case the frothing has caused severe economic loss due to 1) over-
flowing the container, and 2) requiring storage in containers which
would hold two to three times the capacity were it not for the large
volume of the froth. When edible molasses is bottled for home con-
sumption, the frothing prevents a legal fill of the container.
Housewives are often skeptical about buying a bottle of the molasses
with the froth on its surface. Further, bottles containing froth-
ing molasses have been known to pop their covers or burst. The
buckled tin of molasses due to internal gas formation is well
known in homes and industry. Many labels on molasses containers
instruct the buyer to uncover the container and store at low tem-
peratures to prevent damage due to the "volatilizing" of the molasses.

It has been observed that once a molasses has commenced foam-
ing, only low temperatures or the addition of an anti-foam agent
will stop the frothing. Generally, molasses froths only during
the summer months or when stored under high temperatures. However,
not all molasses foam under these conditions - some not foaming
under any varying physical conditions. Futher, there seems to be

no particular type of edible molasses which is immune to this phenomenon.

Investigations to date could not have disclosed a satisfactory method of combatting or preventing this phenomenon which is often referred to as the "froth fermentation" since if such a method were found, it would be in use today by the leading molasses producers.

It was with the view of finding a satisfactory preventative that this investigation was begun.

LITERATURE SEARCH

The first reference to the "froth fermentation" in the litera-
ture searched is by Durin in 1883, reported by Zerban in 1947.
Zerban's report indicates Durin believed the phenomenon was caused
by microbial action. [1]

The original classic research on the phenomenon is by Geerligs
in 1894, reported by Tempany in1901. The East Indian sugar expert
beleived the frothing of molasses in storage was caused by the
breakdown of unstable primary products called glucinates (formed
by the action of lime and glucose) in the presence of alkali. [2]

Work by Laxa in 1898, again reported by Zerban in 1947, indi-
cates the isolation of thermophilic bacteria in a fillmass which
had frothed. However, these thermophiles did not produce the
foaming in new samples of molasses. [3]

Geerligs, in his classic volume on the manufacture of sugar
in the East Indies in 1903, made the first reference to the froth-
ing of massecuites upon cooling down, and the frothing of second
and third molasses. Since disinfectants proved useless in inhi-
biting the action, Geerligs deduced that micro-organisms were not
the major causative agent of the froth. The odor of the escaping
gases indicated that the breakdown products of the molasses in-
cluded carbonic, formic, and acetic acids. Other end products
found were humus and caramel. The massecuites frothed only while
cooling down, creating the hypothesis that the unstable bodies
present in molasses break up spontaneously upon concentration of
the solutions in which they are suspended. The cooling down of the
massecuites causes the crystallization of sugar and thus the con-

centration of the impurities. There was still enough heat present
in the cooled massecuite to promote the dissociation of the unstable
constituents was supported by the facts that 1) frothing ceased
upon the completion of the cooling process, and 2) frothing was not
so violent in small tanks which cooled rapidly. Geerligs believed
that the unstable bodies which broke down at high temperatures in-
cluded glucinic acid.[4]

Tempany, in 1907, made some observations on muscovado molasses
and vacuum-pan molasses, and followed up these observations which
tended to prove some of Geerligs' theories. The frothing of vacuum-
pan molasses in storage was always accompanied by a rising to the
surface of a thick, black scum increasing in volume with time.
Samples of a sterile molasses were inoculated with frothing vacuum-
pan molasses and incubated for three weeks to confirm Geerligs'
experimental data that microbes did not cause the foaming. No.
frothing was observed after the three week period, and so again
experimental data showed that microbial activity had no effect on
the "froth fermentation". To further confirm this belief, a sample
of frothing molasses was sterilized for one-half hour at 100 C
after which gas evolution ceased - proving little since the high
sterilization temperature doubtless caused the rapid decomposition
of the gas-evolving compounds. Tempany observed differences in
the rate and manner of gas evolution with the nature of the molasses.
Muscovado molasses was processed at higher temperatures than vacuum-
pan molasses thus causing a greater destruction of breakdown products
and creating a slower gas evolution in the muscovado. The gummy
scum which rose to the surface when isolated frothed violently.

Analyses of the gum showed it to have a five percent higher ash content than molasses itself. This fact went another step toward proving Geerligs' theory that the gas-producing products were compounds of bases with compounds formed by glucose breakdown. Lime salts of glucinic acid are soluble although the basic salts or apoglucinates are not indicating these might be the scums which rose to the surface. [5]

Lafar (1914) believed that micro-organisms caused the bulk of the decomposition of amino acids in molasses to produce nitric oxide and carbon dioxide. Fission or budding yeasts such as Zygosaccharomyces mellis acidi and Saccharomyces thermantitonum produce gas from saccharine media under similar conditions as gas is produced in the "froth fermentation."[6] Zerban reported (1947) that Lafar's earlier studies indicated that he believed a mold fermentation of amino acids to be responsible for the phenomenon.[7] However, Lafar attributed a portion of the gas evolution to the Maillard reaction during which an amino acid such as glycocoll reacts with glucose and water to produce carbon dioxide.

Herzfield, in the same year reported the work of his superior, Kraisy, in which both agreed with Lafar. Kraisy showed that invert sugar plus an amino acid (in this case, glutamic acid) plus heat would yield carbon dioxide. The varying viscosity of different types of molasses appeared to be the reason for the off-again, on-again frothing. Kraisy had a new theory concerning the frothing of massecuites upon cooling down to 65 - 75 C. He believed that heating of themassecuite caused the chemical breakdown to produce carbon dioxide and further resulted in the supersaturation of the

liquid with the gas. The crystals of sugar exert a catalytic action and start the gas evolution which stops only when the mass has cooled down sufficiently that reducing sugars are no longer formed. However, Kraisy believed the bulk of the frothing was caused by microbial action at the expense of amino acids.[8]

In 1915, the International Sugar Journal reviewed the theories presented to date on the phenomenon. This journal concluded that Lafar's theory held for the best molasses with which he worked, and Geerligs' theory was best for cane molasses with which he worked. Reasons advanced for these conclusions were 1)cane molasses has a very low amino acid contant, the total nitrogen content being but 0.4% in cane molasses whereas beet molasses has a nitrogen content on the order of 2%, 2) Under the Lafar-Kraisy theory, frothing should occur in clarifiers, eliminators, etc., in addition to the after product stage, and 3) Amino acid (glycocoll) - reducing sugar reaction yields pleasant smelling products whereas the "frothy fermentation" yields foul aromas similar to those resulting from reducing sugar decomposition by heat in the presence of alkali. Therefore, the journal's editors concluded that cane molasses' spontaneous frothing was due to spontaneous decomposition of products resulting from the heating of reducing sugars in the presence of alkali and/or also of products resulting from superheating sucrose or reducing sugars, this spontaneous decomposition resulting in the formation of humic, acetic, and formic acids, and carbon dioxide and other gases. To support these theories, various experimental data was cited:

> 1) heating a basic reducing sugar solution resulted
>
> in violent frothing,

2) a very concentrated sirup stored at a high temperature for a long period of time frothed to six times its original volume when cooled suddenly, and

3) Second molasses from a raw juice treated with excess lime frothed violently on heating.[9]

Gillet, in 1917, studied the froth fermentation of second jet massecuites in beet sugar manufacture. Examining only one specific sample of frothed product microscopically, he discovered thermophilic micro-organisms, one of which had an optimum temperature for growth of 70 C and evolved carbon dioxide. This organism, upon isolation, was found to thrive on invert sugar in a slightly acid medium. Further, Gillet claims to have observed the frothing in a molasses containing but traces of nitrogen thus seemingly invalidating the Lafar-Kraisy theory. However, Claassen, in the same year, showed that the "frothy fermentation" could occur at 90 C, and Gillet showed by experiment that the micro-organism he isolated could not grow at that temperature.[10]

Claassen believed that the high sugar concentrations in the molasses and the high temperatures to which they were exposed precluded any possibility of microbial action being the cause of the phenomenon. He advanced the theory that the cause was the interaction of invert sugar or other decomposition products by heat and amino acids, oxygen being necessary to start the reaction.[11]

The classic chemical analyses with reference to this phenomenon were made over a fourteen year period and reported in 1929 by the late C. A. Browne of the United States Department of Agriculture. Samples of cane-sugar molasses were allowed to undergo spontaneous

deterioration for fourteen years and were periodically examined
with respect to total sugars, invert sugars, organic non-sugars
and total solids. No samples showed any yeasts, molds, or bacteria,
and, further, toxic formic acid was present in sufficient quantities
(from decomposition) to assume their absence; and therefore it is
safe to assume that organic activity played no role in the decompo-
sition changes noted.

It was found that approximately 10% of the sucrose had been
inverted, and as a result of the dehydroxylation, a partial dilution
had been effected. The percentage of carbon in the organic non-
sugars was found to be 4% higher than those of fresh molasses while
the hydrogen percentage decreased. The progressive dehydroxylation
causing this phenomenon was especially pronounced in molasses made
by the lime clarification method. It was further found that about
10% of the invert sugar had been converted to organic non-sugar.

Of the two purely chemical theories put forward to explain
the deterioration, Browne tended to agree more with the glucic acid
theory. Glucic acid the approximate formula of which is $C_{24} H_{32} O_{18}$
when isolated was shown to be a parent substance of formic acid,
known to be present in deterioration froth. It had been further
shown by both Browne and contemporaries that calcium hydroxide used
for clarification reacts with reducing sugars to give an unsaturated
compound unstable in the presence of atmospheric oxygen or organic
impurities having much the same nature as glucic acid. The poly-
merization and/or reaction products of glucic acid could theoreti-
cally give rise to the organic non-sugars which have been shown to
increase during the storage period. Further, it was suggested that

glucic acid was a parent substance of acrolein which had also been reported present in frothing molasses and also gives rise to some of the acid end products reported.

However, Browne did not exclude the Maillard reaction, but rather believed it played only a minor part. A typical Maillard reaction might involve glucose and glycocoll condensing to give a glucose-glycocoll condensate product, and this product converting to a glucose methyl amino product. The glucose methyl amino product is also highly unstable and dehydroxylates, doubles carbon linkages, and condenses with additional glucose to produce melanoidins, melanoidic acids, and nitrogenous humic substances of the $C_{16}H_{15}NO_6$ type. These substances may be those surface active agents which lower the surface tension to such an extent that foaming may occur. Although not mentioned in any literature covered, it seems likely that a destruction of these surface active agents would go a long way to preventing the formation of the foam.

Browne showed that alanine, glutamic and aspartic acids, and asparagine all of which are present in molasses would act on glucose and fructose to give similar end products and such other substances as $C_{22}H_{25}O_{13}N$, typical of certain organic nitrogenous colloids. However, the reaction of a reducing sugar upon an amino acid produces no volatile acids which have been found as end products of the "froth fermentation." This fact seems to exclude the Maillard reaction as the major cause of the phenomenon under consideration.[12]

Hucker and Brooks of the Geneva Experimental Station of New York State (1942) performed a series of experiments in order to ascertain the optimum conditions of frothing and from these conditions, attempted to explain the reaction. They found that although

an increase in hydrogen ion concentration decreased carbon dioxide production (foaming) in a test reaction of glycine and glucose, the raising of hydrogen ion concentration in the molasses tested did not appreciably decrease the gas production. A study of the relationship between the optimum temperature of carbon dioxide production and volume of carbon dioxide produced showed a direct relationship indicating the foam reaction is primarily a spontaneous chemical reaction. To check this fact, samples of frothing molasses were inoculated into sterile media and incubated at various temperatures. The micro-organisms present were found to produce gas at the optimum temperature range of 45 - 65 C. These micro-organisms did not grow in the undiluted molasses but did grow well in the dilute product. This would indicate that any condensation water on the surface of the molasses would provide and ideal medium for gas production. It would also indicate that once frothing begins, the gas production of the micro-organisms probably plays some small part in aiding in the foaming.

Taking the foaming of the micro-organisms quantitatively into consideration, Hucker and Brooks found that the effect of temperature on carbon dioxide production follows a logarithmic curve with a critical temperature at which gas production is sufficiently pronounced to produce violent molasses foaming. This temperature was found to be dependent upon the type and source of the molasses and varies with the viscosity, specific gravity, and temperature to which the molasses has been subjected during factory processes.

In an attempt to find a practical method of controlling the foam, Hucker and Brooks attempted to vary the viscosity and spe-

cific gravity of molasses by the addition of water. However, the quantity of water necessary to reduce the foaming was excessive and not practical.

In other observations, not closely investigated, the addition of the germicide phenol (25%), was found to reduce the frothing, but not appreciably. The calcium oxide content of the molasses (which some practical men believed had some effect on the reactions) is not a significant factor in the reaction, according to these investigators. [14]

Zerban (1947) reported the work of Sandere in 1946, in which he stated that the "froth fermentation" reaction is catalyzed by metallic iron. [15]

Henry and Clifcorn, in 1949, investigated the hypotheses that the mineral constituents of molasses exert some catalytic effect on the unstable organic constituents causing the gas formation. Cane sirup samples were analyzed and from these analyses, synthetic sirups were created. A sirup of sugar, water, and added minerals produced no gas and were therefore used as controls. The two investigators then added an unstable organic acid naturally occuring in molasses which could conceivably produce carbon dioxide by decarboxylation. The acid used was aconitic and the various minerals were added by ion exchange methods. The minerals used included iron, manganese, alcium, and magnesium.

The authors, integrating their findings with those of other investigators reached some conclusions. A typical Maillard reaction is known to be speeded up by increase of pH, but a decrease in pH was found to increase the rate of reaction in the natural product.

Further, the reduction in amino acid and protein nitrogen was found to be small compared to the tendency to produce gas. Therefore, the Maillard reaction was shown to be a minor factor in the frothing. The authors' investigations showed that the ash constituents (Calcium and magnesium) of the molasses created a catalytic influence upon aconitic acid (or some similar acid) to produce the carbon dioxide which creates the unwanted foam.[16]

A summary of the theories thus far advanced in the literature, none of which have been thoroughly disproved now follows.

The microbial theory believes that micro-organisms cause the decarboxylation of amino acids with the resultant carbon dioxide being evolved to cause the foaming. The Kraisy theory, states that the high processing temperatures during the manufacture of molasses results in chemical decomposition of certain constituents with the resultant formation of carbon dioxide which is dissolved in the hot sirup. This supersaturated sirup releases the gas with foaming at the surface upon cooling down possibly with the aid of sugar crystals formed.

Glucic acid, and end product resulting from the reaction of lime and invert sugar, may decompose with heat to form carbon dioxide. Another theory states that this glucic acid (or possibly other unidentified constituents of molasses) reacts with more invert sugar in the molasses without the presence of heat to produce carbon dioxide. The Maillard theory states that the interaction of amino acids and invert sugar results in the decarboxylation of the amino acids to produce carbon dioxide. A similar theory believes that sucrose decomposition products such as glucic acid reacts with amino acids to produce a decarboxylation reaction. However, this

theory also includes a catalysis by oxygen gas as necessary for the reaction.

The most recently advanced theory in the literature has certain mineral constituents in molasses catalyzing the decarboxylation of amino acids to produce the carbon dioxide of the foam.

The only unanimous fact found in the literature was that carbon dioxide was the gas evolved in the foam, other gases being present in only trace amounts.

In the face of these many varying and conflicting reports it was necessary to attack this problem from many different aspects to prove or disprove each theory advanced in the literature.

PLAN OF RESEARCH

At least one bottler of molasses had certain facts to offer
concerning the "froth fermentation". In ten years of employment,
he had noted the frothing had occured only during the summer months
indicating that a high temperature is necessary to initiate the
reaction. He further stated that if the molasses had not frothed
in the barrel in which it was held, it would not froth in the bottle.
Although most of the barrel gave a constant amount of frothing for
each unit of molasses bottled, the end portion or that containing
crystallized sugar sediment gave a molasses which frothed far more
violently. This bottler handled Dark, New Orleans, Puerto Rico,
and Barbadoes molasses, and of these Dark and New Orleans often
frothed, Puerto Rico not very often, and Barbadoes only rarely.

The statement concerning the relation sugar crystals to foaming
seems to be supporting evidence to the Kraisy theory of supersaturation
of molasses with the gas causing the foam. Since all of the molasses
dealt with in this plant contained sulfur dioxide, it was possible
that the molasses was supersaturated with sulfur dioxide.

Professor L. J. Heidt of the M.I.T. Chemistry Department who
had previously worked with the Sugar Research Foundation offered
the suggestion that the direct cause of the "froth fermantation"
was an enzyme, such as decarboxylase. He believed that although
the boiling processes through which sugar, sirups, and molasses are
put are severe enough to destroy most heat-sensitive enzymes, certain
types of micro-organisms such as the thermophiles or heat stable
microbes, and saccharophiles, or "sugar loving" microbes might pro-
duce an exoanzyme which was the cause of the frothing. The term

exoenzyme was specifically used since a number of experimenters cited
in the literature added germicides to frothing molasses in order to
eliminate microbial action as a source of gas. Such germicides are
effective in doing their jobs but often do not serve to inhibit
enzymes which had been excreted into the medium by the micro-organisms
prior to their death. Such exoenzymes could doubtless cause foaming.

Another former worker in the field believed that the important
factor in the frothing was the action of surface active agents presence
which have concentrateds at the interface of molasses and air. It
was suggested that the protein of molasses (nutritionally insigni-
ficant) made up the bulk of surface active agents, but the presence
of cellulosic and pectinaceous materials which could concentrate
at the interface could not be ignored.

One further factor not mentioned in the literature as a source
of the frothing was the presence of carbonates due to the addition
during carbonation processes. If the molasses turned very acid,
say due to the action of flat sour organisms, this gas could be
released with resultant frothing.

Although the literature searched voted in the majority of cases
against microbial action, various men, both those acquainted with
the problem, indicated a desire to see these results confirmed, i.e.,
they doubted the validity of those experiments stating that micro-
organisms were not the cause of the phenomenon.

The literature, as indicated above, showed various degrees of
enthusiasm for various theories of spontaneous chemical action
causing the evolution of carbon dioxide which in turn resulted in
foaming. Most of the literature, however, agreed that some sort of

spontaneous chemical degradation was, and still is, responsible
for the frothing.

There would therefore seem to be three basic approaches to
the problem. These would include attacking the problem by deter-
mining the cause of the gas formation. This would involve four
basic approaches, chemical, enzymatic, microbiological, or addition
in the form of carbonates. Another approach to the problem would
be the supersaturation theory, and still another the surface active
agent theory.

Because of the wide variety of these approaches, it was decided
to attack each in a more qualitative than quantitative manner in
order to get more fundamental results. Once qualitative results
were obtained, quantitative results would more easily follow. It
was further decided to attempt at least a delving into each aspect
to some extent and to follow the lead which proved most promising
at the start. In practice, this was difficult to do because of
the wide magnitude of results obtained.

The initial phase of the experimental work involved obtaining
a sample of molasses which had been known to foam (as has been pre-
viously stated, a sample which has not been known to foam could never
be made to foam.) and to subject this molasses to varying conditions
of temperature under which optimum foaming would occur. While at
each temperature, the pH and density was also varied to determine
if variations in these properties would influence the foaming. The
importance of density was not considered as much as was viscosity
which decreased with a decreasing density and increased with in-
creasing density though not necessarily in any special relation to

each other.

Once the optimum conditions of temperature, pH, and density for frothing were obtained, these conditions could be, and were, duplicated on samples of molasses on which experiments were conducted, in order to determine if the experimental procedures inhibited or increased the frothing. In all cases, the volume of visible foam was used as the index of extent of the "froth fermentation."

Because most investigators believed that micro-organisms were not the cause of frothing, it was decided to ascertain this fact as the first experimental procedure. Bacteriological counts were taken on samples incubated at various temperatures. Attempts were made to isolate the individual microbes and determine if any one was a gas former. Other experiments along this line involved incubation in various media, especially the more saccharine types, to determine if the molasses contained anaerobic or aerobic gas formers, thermophilic gas formers, or flat-sour formers. The most significant experiments planned concerning the microbial theory involved. The sterilization of the molasses by various methods, and, following this, the determination of the volume of froth forming. Among the methods of sterilization would be cathode ray, ultrasonic, chemical, and heat.

Experiments involving surface-active agents followed these. Various anti-foamers were added to frothing molasses in an attempt to inhibit the foaming. In addition, various agents known to destroy or degrade possible natural surface active agents were those present in the frothing molasses were added to the foaming samples. Further, some of these natural surface active agents were added to non-

foaming samples in an attempt to create the frothing and in such a way, determine the cause.

To inhibit enzymatic action, various experimental procedures were attempted on samples of frothing molasses followed by measurements of foam while the molasses was being held under optimum conditions.

Initial plans called for an investigation of each of the so-called chemical theories. This would involve heat treating non-foaming molasses in and out of the presence of calcium salts as a starting point for determining the validity of the glucic acid theory. The results of this experiment would determine the course of action to follow to prove the various glucic acid theories.

To prove the validity of the Maillard reaction, various amino acids and invert sugar were added to non-foaming molasses to determine the extent of foaming these would create. Plans called for ion exchange and other chemical methods to inhibit these compounds in the foaming sample to determine if such inhibition would inhibit the frothing.

The newest of theories, that of mineral catalysis of a decarboxylation reaction, was to be proven or disproven by the addition of these minerals to a non-foaming sample and the removal or tying-up of these minerals in a foaming sample.

Experimental procedures on the supersaturation theory involved the addition of various inert crystals to molasses, both foaming and non-foaming. A vacuum pulled on molasses samples at sub-optimum conditions would prove the validity of the theory.

As the final phase of the experimental work, a gas analysis was performed on the gas evolved by the foaming molasses to

substantiate any theories formed by results from other experiments
and to clarify any doubts in the minds of any future investigators
in the field.

EXPERIMENTAL WORK

The first step was the construction of a constant temperature device in which molasses could be held to determine optimum frothing temperatures. A water bath was the device used. This water bath consisted of a cylindrical tank roughly one and one half feet in diameter and ten inches deep filled with water. As a thermal regulator, a helical bimetallic thermostat was immersed in the water and connected to two electrical resistance heating knives which were also immersed in the water. A vertical motor with a one-foot long glass shaft on the end of which was a glass propellor served to stir the water and to keep the water temperature constant. The lowest temperature which could be maintained was that of tap water which was supplied to the bath, the temperature of this water ranging up to 25C. Room temperature, being lower than water temperature, tended to cool the water, at which point electrical contact was made in the thermostat, and the heating knives heated up the water. The bath was accurate to within 1 C. of the adjusted temperatures.

As measuring containers in which to hold the molasses in the water bath, six standard 50 cc. laboratory burets were sealed off on their lower end and wired to a horizontal rod above the bath so that the lower nine inches of the burets were immersed in the water. To determine whether the liquid in the burets heated up to water bath temperature in a reasonable time, thermocouple measurements were made using a copper-constantan couple, inserting the measuring junction in the molasses in the immersed burets and inserting the reference junction in an equilibrium mixture of ice and water. These measurements, made at 65 C., showed that it took

less than five minutes for the interior of the molasses to reach
the temperature of the water bath.

The initial sample of frothing molasses used in tests was a
sample of Dark molasses. However, the supply of Dark was eventu-
ally exhausted and was replaced in experimental work by frothing
Barbadoes molasses. There appeared to be no difference in the
action of the two.

Bacteriological tests constituted the first of the experimen-
tal procedures. Samples of dark and dark non-foaming molasses were
diluted, plated out with nutrient agar, and incubated at 30 C. and
37 C. Counts revealed the results tabulated in table (1).

These counts were not very accutately done in as much as no
duplicates were made, but they serve to illustrate that there is
an adequate flora present in molasses, the bulk of which tends to
be thermophilic in character.

From these plates were isolated fourteen different species of
micro-organism, including yeasts and bacteria. These were isolated
by picking out those colonies which, by appearance, were different
from each other, and transfering by means of an inoculating loop
some culture to a Smith gas tube filled with a nutrient broth.
These tubes were incubated at 37 C. for a total of six days and
examined periodically. Only two tubes showed the formation of gas,
these two having been inoculated with micro-organisms isolated from
Dark non-foaming molasses. Again, these tests were iinconclusive
since there may well have been more than fourteen species of mi-
crobes in the samples, and some of those isolated may not have
thrived in the non-sugary nutrient broth or at the comparatively

low temperature for thermophiles of 37 C.

The next series of bacteriological tests were more positive
in nature. Samples of Dark and Dark-non-foaming molasses were in-
oculated into Smith gas tubes containing 1% sugar in a nutrient
broth and incubated at 50 C. (It had been proven by this time that
50 C. was an optimum temperature of frothing.) However, evaporation
of the liquid precluded any accurate results.

At the same time, samples of Dark and Dark non-foaming molasses
were inoculated into tubes containing liver broth, sealed with agar
and vaseline, and incubated at 50 C. for 36 hours as a test for
anaerobic gas formers. Similarly, a 1% sugar nutrient broth was
inoculated with the two samples of molasses, sealed in a like manner,
and incubated thermophilically. In addition, tubes of this nutrient
broth containing gas tubes were inoculated without sealing and in-
cubated as a test for aerobic thermophilic gas formers. Duplicate
tests were made on each sample. In no case was the formation of
gas evident..

Some timelater, the same tests were repeated. However, this
time, the nutrient broth contained 2% sucrose, and in addition to
Dark and Dark non-foaming molasses, a sample of foaming Barbadoes
was inoculated into the media to test for gas formation. Again,
tests were run in duplicate, and again, no gas formation was evi-
dent in any tube.

At the same time, the samples were inoculated in dextrose-
tryptone agar to test for flat sour formers. All samples showed
abundant evidence of flat sour or acid forming microbes.

In two instances, one cc. samples of foaming Dark molasses

were inoculated into 20 cc. of Dark non-foaming molasses and held
at 52 C. for an appropriate length of time to determine if the in-
oculation of a "cold" molasses with "hot" molasses would initiate
the frothing as a confirmation of the above microbial results.
In neither case did the non-foaming sample show any appreciable
frothing at any time after inoculation. This last experiment, in
addition to disproving the microbial theory of frothing also lends
evidence to the case against surface active agents.

The final phase of the bacteriological work consisted of
sterilizing samples of foaming Barbadoes molasses using cathode
ray irradiation. 10 cc portions of molasses were irradiated at
0.5, 1.0, 2.0, and 4.0 million R E P (Roentgen equivalent physical
units-the unit of irradiation.) The irradiated samples were incu-
bated at 50 C. and the following quantities of foam were observed,
respectively, 0.2 cc., 0.2 cc., 0.3 cc., 0.5 cc., and 0.6 cc.
Inoculation of the irradiated molasses into a sugary nutrient agar
and incubation at 50 C. resulted in a zero count after 36 hours -
indicating that the molasses was sterile.

While these microbiological tests were taking place, the water
bath tests were being run to determine optimum frothing conditions.
It was found that holding the molasses at refrigerator temperatures
(10 C.) inhibited the frothing. However, due to tap water tempera-
ture, it was required that water bath temperatures commence at 35 C.
In each case between 15 and 20 cc of molasses was poured into a
buret and held for a minimum of 18 hours. Each buret had a diameter
of about 0.5 inches and therefore presented a surface area of about
0.2 square inches per 20 cc to the molasses which is roughly one-

third of the surface area which is presented by the usual bottled molasses. Results indicate that this difference in surface area was not significant.

The % solids of the molasses was measured by an Abbe refractometer. Again, this % solids was used as an index of viscosity, the higher the % solids, the higher the viscosity. The acidity of the molasses was measured by means of a Beckmann pH meter. The maximum volume of visible foaming was used as the measure of the froth. After some experimentation, it was found that this maximum occurred within one hour after reaching temperature, although periodic measurements were usually taken after one hour. The percentage of foam should probably have been the measure used, but because 20 cc was the usual volume of molasses in the buret, it was decided that a direct measure would be better.

In all cases, the molasses was poured into a graduated cylinder and mixed with the neccessary reagent for 60 seconds. Air was beaten into the molasses in this process, but this was taken into account by controls while taking measurements.

Initially, both Dark and Dark non-foaming molasses had a pH of 5.4 and a solids content of 76.3%. However, both enjoyed a pH drop to 4.8, probably due to the action of flat-sour organisms over a long period of time.

Table (2) indicates the results of the experiments. (D is the symbol for Dark molasses and DNF for Dark non-foaming.)

The tabular values are but typical values indicative of the tendencies of the various molasses. The table shows a sharp break at 45 C. indicating that the critical temperature above which mola-

sses foams vigorously is 45 C. The molasses further showed a non-foaming tendency at pH greater than 6.0.

The foaming molasses showed excellent foaming tendencies at 50 C., and, therefore, this was the temperature chosen as that optimum for frothing. (Higher temperatures gave no real difference in the foaming tendency.)

Surface active agents were then added to Dark and Barbadoes non-foaming molasses held at 50 C., keeping the pH within foaming limits and the density within the range experimental results showed was adequate for foaming in an effort to induce this foaming.

Among the surface active agents which created no foam were silicone oil, Span 20, Span 85, Tween 21, capryl alcohol, emulsol, sodium borate, pectin, and gelatin. The two Spans and Tween are fatty acid esters. Among the surface active agents whose addition resulted in a foam were stearic acid (1.5 cc.), albumin (1.5cc.), casein (0.8cc),and a number of enzymes which will be described later.

In an effort to suppress the frothing in Dark and Barbadoes foaming molasses, various anti-foam agents were added. Among these were Span 20, Span 85, Tween 21, silicone oil, emulsified silicone oil, and Dow-Corning anti-foam A. All gave identical results. None inhibited the foam. Rather, they acted as defoaming agents in making the formation of froth less violent and in causing the suppression of the froth in a much shorter time than normally occurs without their presence.

A protein precipitating agent in the form of trichloro-acetic acid was added to foaming Barbadoes molasses in attempt to precipitate any protein which had collected at the interface of molasses

and air and might be acting as a surface active agent. Substanti-
ally no inhibition of the frothing was obtained.

In other efforts to remove proteinaceous material which might
act as surface active agents, various proteolytic enzymes were added
under different conditions, in varying concentrations, and after
various lengths of time being allowed for reaction time. The en-
zymes included papain, pancreatin, and trypsin. All samples show-
ed a tendency to froth more vigorously after such treatment. A
similar experiment with taka-diastase, a starch digestant also re-
sulted in more violent frothing.

To test the Henry theory of mineral catalysis of decarboxya-
tion, mineral salts were added in varying quantities to non-foaming
Barbadoes molasses. The cations tested were calcium, barium, mag-
nesium, iron, and copper. In no case did a substantial foam result.

A variation of this experiment was to add carbonate precipita-
ting cations to foaming Barbadoes molasses in an effort to tie up
the gas. This experiment worked well when calcium was the cation
but not at all using barium. However, this experiment was not con-
trolled carefully, and so the results may mean little.

In order to determine the validity of the enzymatic theory,
various proteolytic enzymes were added to non-foaming Barbadoes
molasses in varying quantities, under varying conditions. These
included papain and pancreatin, and trypsin, of which papain and
pancreatin created a substantial foam. The amylase, taka-diastase
created a similar foam.

The addition of the enzyme inhibitor potassium cyanide to both
foaming and non-foaming Barbadoes molasses resulted in a violent

eruption of froth indicating that the acid molasses had reacted to form gaseous hydrogen cyanide. However, the use of mercury bichloride as an enzyme inhibitor resulted in no cessation of the frothing in a foaming sample.

The Maillard theory of interaction of amino acid on invert sugar was next attacked. Various combinations of liquid and solid glutamic acid, glycine, and dextrose in various concentrations were added to non-foaming Barbadoes molasses. Further, the amino acids were added alone in another set of runs. All samples showed substantial foaming, although the froth did not have the more or less homogeneous consistency typical of natural molasses foam.

The final phase of the experimental work consisted of ascertaining the validity of the theory of supersaturation of the molasses with gas. The table shows that the addition of sugar crystals and salt crystals at a temperature below the critical resulted in a formation of froth. Other inert crystals, even sand, created similar foaming.

If, as the experiments with crystals seem to indicate, a foaming sample is different from a non-foaming sample only through the presence of dissolved gas, a vacuum pulled on a foaming sample at room temperature will create a froth. Such a vacuum was created above a foaming Barbadoes molasses using a simple laboratory aspirator and pressure flask. Within five minutes the sample was frothing violently. This frothing continued for at least one hour. A similar vacuum pulled on non-foaming Barbadoes molasses at the same room temperature (29 C.) resulted in absolutely no sign of foam even after one hour.

The pH of the molasses prior to frothing under this vacuum was found to be 3.9, and after frothing it had increased to 4.2 indicating an acid gas had been removed.

To settle all doubts concerning the identity of the dissolved gas, an Orsat analysis was performed. 300 cc of foaming Barbadoes molasses was incubated at 55 C. in a specially designed 1000 cc Mason jar for 18 hours. The gas in the jar was run into the measuring buret of an Orsat and then into 30% KOH. Duplicate runs showed 4.5% of an acid gas in the gas in the jar above the molasses. An acid gas would mean carbon dioxide, sulfur dioxide, or hydrogen sulfide. Because of the peculiar odors of the latter two, they are easy to determine organoleptically. Such a test indicated that carbon dioxide was the gas which had left the molasses during incubation, since carbon dioxide is present in air in a concentration of less than 0.15%.

DISCUSSION of RESULTS

As mentioned above, little significance except that there is a substantial microbial flora present in molasses can be attached to the counts made on the fresh molasses. However, experiments run to determine the presence of anaerobic and aerobic gas formers indicate conclusively that although thermophiles are present in molasses (there was definite turbidity in most tubes) no thermophilic gas formers could possibly be responsible for the gas formation in frothing molasses. The presence of thermophilic gas formers could very well cause the formation of acid (and seemingly did as shown by the gradual decrease in pH by molasses over long periods of time) which could release carbon dioxide gas from carbonates which were added during processing. Further evidence countering the microbial theory of foaming include the fact that the molasses which did froth began its foaming immediately upon reaching the critical temperature (microbes require 18 - 24 hours before they begin their work), and the fact that a "hot" molasses could not contaminate a "cold" molasses with gas forming microbes. Further, tabulated data showed that frothing occurred at temperatures as high as 80 C., a temperature at which no micro-organism thrives.

The crowning bacteriological work was the sterilization of foaming Barbadoes molasses by cathode ray irradiation. The sterile molasses frothed although less than normally. Since the maximum irradiation raised the temperature but 3 degrees, it appears that the temperature at which the molasses was held was at some point above the critical, or perhaps some amino acid which might have decarboxylated to evolve carbon dioxide gas was destroyed thus tying up the carboxyl group. Since there was a 24 hour time lapse

between irradiation and examination, it appears that either the
irradiation or the handling of the molasses immediately before or
after irradiation resulted in its foaming during the 24 hour period
and subsequent low foam resulting from incubation. (The molasses
as received from the operator was frothing.)

In determining the optimum conditions of frothing, the factor
of viscosity is probably very important, and it was thought that
% solids was directly proportional to viscosity. However, a lite-
rature search revealed that the relationship is some sort of a lo-
garithmic one, and all that can be said is that if the % solids
was low, so then was the viscosity, etc. The table shows a trend
towards a minimum density (viscosity) below which frothing will
not occur, but this was not followed up. The table also indicates
that there seems to be no maximum % solids (viscoty) above which
molasses will not froth. This tendency is probably arrested at
a % solids well above the normal range of commercial molasses and
need not concern us in this problem.

The critical temperature found below which molasses shows little
tendency to foam agreed well with the findings of Hucker and Brooks.
The maximum pH above which molasses would not froth seemed to be
6.0, but this was not followed up accurately and could be off by
a half a pH unit either way. A change in the pH to the more basic
side of acid might well be the most practical solution to this pro-
blem for molasses producers since sulfur dioxide is usually added
to inhibit microbes and a little less acid would probably not sup-
port the growth of any more micro-organisms.

A possible explanation for the difference between the actions

of the surface active agents may be had by an examination of their individual constituents. Those agents causing frothing included an acid (stearic) and proteins (albumin, casein.) The acid might well have released carbon dioxide from carbonate, or being a solid acid, released dissolved carbon dioxide gas. The protein might well have been broken down to amino acids which in turn decarboxylated. The surface active agents creating no froth were all neutral and non-protein, except gelatin. Gelatin showed a tendency to gel in the saccharine and acid medium and may well have increased the viscosity to above the critical.

Since th pH of molasses is in the range of isoelectric pH of proteins, it is entirely possible (assuming the gelatin viscosity theory accurate), that a surface active agent under optimum conditions is necessary to release chemically formed gas from the medium. The isoelectric point of proteins is that point at which they show their maximum activity. Stearic acid might well be an ideal agent whereas the others used might not be.

The precipitation of the protein might indicate that protein is not the surface active-agent in molasses. The addition of enzymes to remove the surface-active agents resulted in the addition of proteins to the medium (all known enzymes are proteins), and this procedure, regardless of the concentration of enzyme used may well have added surface activity to the molasses. Further, it is possible that the enzymes initiated a chemical reaction resulting in gas formation. It would seem that the surface-activity theory bears much investigation because of the strong contradictory evidence for it. Due to the inconsistencies, this theory deserves much

more careful consideration before it is discarded.

In neither set of experiments concerning cation catalysis or cation tie-up of carbon dioxide were runs carefully made. This aspect deserves a great deal of work since it offers so many possibilities of variation.

The addition of enzymes to a non-foaming molasses, as indicated above, adds a surface agent to a medium which may need such an agent to effect the release of gas from its body. Three of the enzymes created a foam, and one of them was a starch digestant, resulting in the hypothesis that since starch breakdown products are not gaseous and are not chemically converted to gaseous products, the action of these enzymes was surface action. This argument was supported by the action of an enzyme inhibitor in a foaming molasses. Such an agent caused no effect on the frothing indicating that the action of an enzyme, if any, is in the surface rather than chemically. Again, this surface activity theory must be carefully investigated before discarding it.

The Maillard theory of frothing seems well substantiated on the basis of the experimental work conducted. The fact that the foam was not typical may well have been due to the absence of necessary surface active agents to act in combination with chemical reactions.

By far, the most significant experiments conducted were those that proved conclusively that a frothing molasses contains carbon dioxide in a supersaturated form. This fact explains why molasses will froth at below critical summer temperatures after being subjected to violent agitation in barrel-racking processes. It also

explains why molasses containing a sugar crystal sediment froths more violently than does a molasses without sugar crystals. It explains why once a barrel starts foaming, the entire barrel froths. The fact that non-frothing molasses will not foam regardless of how one changes the physical conditions is also explained by supersaturation.

Among those agents which will release gas from a liquid supersaturated with it are the sharp edges of crystals, agitation, another gas, heat which expands the gas and forces it out of solution, and a lowering of the pressure above the surface by a vacuum (so that the vapor pressure of the dissolved gas is greater than that of the gas above the liquid). It is possible that a lowering of the surface tension of the molasses (by addition of a surface active agent) opens the way for the escape of the dissolved gas.

CONCLUSIONS

The most significant finding of this investigation appears to be that carbon dioxide gas is supersaturated in foaming molasses. All findings indicate this fact. However, conclusive evidence is not present. It is possible that when the dissolved gas was pulled out of solution (and this dissolved gas need not have been saturated or supersaturated), a chemical equilibrium was shifted in such a manner as to effect the release of more gas.

However, assuming the supersaturation theory correct, we can further theorize. Carbon dioxide gas, under this assumption has entered the molasses at some earlier stage in the process. It is doubtful that it entered during storage by any chemical or bacteriological means since 1) a non-foaming molasses was shown to contain no gas-formers, and 2) the same and other non-foaming samples were held for a period of eight months (more than an average time for holding molasses) without showing any tendency to froth. Again, this fact is hypothetical and remains to be proved conclusively, perhaps by holding many different non-foaming samples (taken fresh from the plant) for long periods of time and examining them for foaming power.

The supersaturation, if proven conclusively, would indicate that many researchers were wrong in their basic premise, that molasses foams by a chemical reaction upon reaching specific storage conditions. However, any one of a number of chemical reactions may be the correct one accounting for the froth. Even microbes may have produced the froth at some very early stage in processing prior to being destroyed. It is also possible that some catalyst inherent in molasses from the sugar cane stage either accelerated the re-

lease of gas from the molasses thus preventing its supersaturation (to create a non-foaming molasses) or accelerated its supersaturation (to create a foaming molasses.) Such a catalyst may well have been a surface active agent. The addition of carbon dioxide gas to a non-foaming molasses and then to a foaming molasses whose gas has been removed might go a long way to proving or disproving this theory.

Experimental work to prove or disprove the chemical theories of gas formation would involve either a careful step by step analysis of the manufacturing process to determine where the production of foaming and non-foaming molasses differs or a careful study of each of the chemical reactions thus far mentioned in the literature. Of those studied in this investigation, the Maillard reaction offers the most promise as a source of gas. Although this reaction accelerates at higher pH, it could easily account for a portion of the foam. The fact that foaming fell off with increasing pH is due either to a neutralization of carbon dioxide by base or a neutralization of amino acid necessary for the reaction by base. Further evidence for the Maillard theory is had by an examination of the action of various enzymes on non-foaming molasses. It has now been shown that they could not have acted as surface active agents (as indicated above) on non-foaming molasses, and therefore, their most likely action is the formation of amino acids to react with reducing sugar and produce gas. It is very possible that a non-foaming molasses is one extracted in such a manner as to minimize amino acid formation or one from which sufficient gas has evolved to use up all the amino acid present.

All the hypotheses presented above are vague and require further investigation before reaching any definite conclusions. The magnitude of the sugar and molasses industry warrants further experiments along lines outlined in this investigation so as to determine the basic cause of the froth and thus eventually eliminate it.

LITERATURE CITED

1.   F. W. Zerban, "The Color Problem in Sucrose Manufacture," Technological Report Series No. 2, Sugar Research Foundation, Inc. (1947), 15.

2.   H. A. Tempany, International Sugar Journal, 11 (1901), 588-594.

3.   Zerban, Sugar, 15.

4.   H. C. Prinsen-Geerligs, On Cane Sugar and the Process of its Manufacture in Java (London, 1903), 83-84.

5.   H. A. Tempany, " 'Froth Fermentation' of Molasses," West Indian Bulletin, 10 (1907), 130.

6.   Franz Lafar, " 'Froth Fermentation' of Massecuites, Syrups, and Molasses, etc.," International Sugar Journal, 16 (1914),184.

7.   Zerban, Sugar, 15.

8.   A. Herzfield, "Cause of 'Froth Fermentation' of After-Product Massecuites," International Sugar Journal, 16 (1914), 230.

9.   "Cause of the 'Frothy Fermentation' of the After-Products of the Cane Sugar Industry," International Sugar Journal, 17 (1915), 284-285.

10. Rene Gillet, " 'Frothy Fermentation' of Second Jet Massecuites in Beet Sugar Factories," International Sugar Journal 19 (1917), 264-269.

11. Zerban, Sugar, 15.

12. H. Claassen, " 'Froth Fermentation' and Formation of Carbon Dioxide," Chemical Abstracts, 13 (1919), 2775.

13. C. A. Browne, "Spontaneous Decomposition of Sugar Cane Molasses," Industrial and Engineering Chemistry, 21 (1929), 600.

14. G. J. Hucker and R. F. Brooks, "Gas Production in Storage Molasses," Food Research, 7 (1942), 481-494.

15. Zerban, Sugar, 15.

16. R. E. Henry and L. E. Clifcorn, "Chemical Decomposition of Cane Sirup and Molasses during Storage," Industrial and Engineering Chemistry, 41 (1949), 1427.

BIBLIOGRAPHY

Ashby, S. F. "The 'foaming' of Molasses."
    International Sugar Journal, 11 (1909), 302-304.

Browne, C. A. Handbook of Sugar Analysis
    New York: Wiley, 1913.

Browne, C. A. "Spontaneous Decomposition
    of Sugar-Cane Molasses," Industrial
    and Engineering Chemistry 21 (1929), 600.

Browne, C. A. "Spontaneous Combustion of
    Egyptian Sugar Cane Molasses,"
    Industrial and Engineering Chemistry,
    17 (1925), 734.

"Cause of the 'Frothy Fermentation' of the
    After-Products of the Cane Sugar
    Industry," International Sugar
    Journal, 17 (1915), 284-285.

Claassen, H. " 'Froth Fermentation and
    Formation of Carbon Dioxide,"
    Chemical Abstracts, 13 (1919), 2775.

Crawley, J. T. "Spontaneous Combustion of
    Molasses," Journal of the American
    Chemical Society, 19 (1897), 538.

Fort, Charles A. "Variable Mineral Composition
    of Blackstrap Molasses," Sugar,
    November, 1946.

Geerligs, H. C. P. On Cane Sugar and the
    Process of its Manufacture in Java
    London: Altringham, 1903.

Gillet, Rene. " 'Frothy Fermentation' of
    Second-Jet Massecuites in Beet
    Sugar Factories," International
    Sugar Journal, 19 (1917), 264-269.

Hall, H. H., Lawrence H. James, and E. K.
    Nelson. "Micro-organisms causing
    Fermentation Flavors in Cane Sirups,
    especially Barbadoes Molasses,"
    Journal of Bacteriology, 33 (1937),
    577-585.

Henry, R. E. and L. E. Clifcorn. "Chemical
    Decomposition of Cane Sirup and
    Molasses during Storage," Industrial
    and Engineering Chemistry, 41
    (1949), 1427.

Herzfield, A., "Cause of the 'Froth
        Fermentation' of After-Product
        Massecuites," International Sugar
        Journal, 16 (1914), 230.

Hucker, G. J., and R. F. Brooks. "Gas
        Production in Storage Molasses,"
        Food Research 7 (1942), 481-494.

Kauppi, T. A. "Silicones Speed, Sanitize
        Processes," Food Industries
        23 (1951), 44-6.

Lafar, Franz. " 'Froth Fermentation' of
        Massecuites, Syrups,
        and Molasses, etc.," International
        Sugar Journal, 16 (1914), 184.

Lewis, V. M., W. B. Esselen, Jr., and C. R. Fellers.
        "Non-enzymatic Browning of Foodstuffs -
        Nitrogen-free Carboxylic Acids in the
        Browning Reaction," Industrial and
        Engineering Chemistry, 41 (1949), 2591-2594.

Lewis, V. M., W. B. Esselen, Jr., and C. R. Fellers.
        "Non-enzymatic Browning of Foodstuffs:
        Production of Carbon Dioxide," Industrial
        and Engineering Chemistry, 41 (1949), 2587.

Martinez, Raymond J. The Story of
        Molasses. New Orleans: Industries
        Publishing Agency, 1949.

Owen, W. L. "The Deterioration of Cane
        Sugars in Storage; Its causes and
        Suggested Measures for its Control."
        Louisiana Bulletin No. 162, 1918.

Ross, Sydney. "The Inhibition of Foaming,"
        Renssaleer Polytechnic Institute Bulletin,
        Engineering and Science Series, No. 63,
        1950

Saint, S. J. " 'Froth Fermentation in table
        Syrup Manufacture," International
        Sugar Journal, 34 (1932), 40-41.

Spencer, Giulford L. and George P. Meade
        A Handbook for Cane-Sugar Manufactures
        and Their Chemists. N. Y. John Wiley
        and Sons, 1945.

Tempany, H. A. International Sugar
        Journal 11 (1901), 588-594.

Tempany, H. A. "Cane Syrups or Fancy
    Molasses," West Indian Bulletin
    13 (1913), 324.

Tempany, H. A. " 'Froth Fermentation' of
    Molasses," West Indian Bulletin
    10 (1907), 130-137.

Urban, Karl. "Ammoniacal Frothing of
    After-Product Massecuites,"
    International Sugar Journal,
    22 (1920), 467.

Zerban, F. W. "The Color Problem in Sucrose
    Manufacture," Technological Report
    Series No. 2, Sugar Research Foundation,
    Inc. (1947)

Zerban, F. W. and Carl Erb. "Fermentable
    Sugars from Unfermented Reducing
    Substances in Cane Molasses,"
    Industrial and Engineering
    Chemistry, 39 (1947), 1597.

Zerban, F. W. and Louis Sattler. "Unfermentable
    Reducing Substances in Molasses,"
    Industrial and Engineering Chemistry,
    34 (1942), 1180.

APPENDIX

Table No. 1 - <u>Bacteriological counts of Dark and Dark non-foaming molasses.</u>

<u>30 Centigrade</u>

<u>Dark molasses</u>			<u>Dark non-foaming molasses</u>		
Dilution	Count	Actual Count	Dilution	Count	Actual count
$1:10$	235	235	$1:1$	100	100
$1:10_2$	50	500	$1:10_2$	39	390
$1:10^2$	50	5000	$1:10^2$	-	-
$1:10^3$	-	-	$1:10^3$	-	-

Average count: 1900/cc.   Average count: 245/cc.

<u>37 Centigrade</u>

Dilution	Count	Actual Count	Dilution	Count	Actual count
$1:1$	-	-	$1:1$	-	-
$1:10_2$	-	-	$1:10_2$	60	600
$1:10^2$	166	16600	$1:10^2$	41	4100
$1:10^3$	150	150000	$1:10^3$	-	-
$1:10^4$	63	630000	$1:10^4$	-	-
$1:10^5$	30	3000000	$1:10^5$	200	20000000

Average count:1300000/cc.   Average count:7000000/cc.

Table 2. Quantity of foaming under varying physical and chemical conditions.

	Foam (cc)	pH	% solids
35 C.			
D - tartaric acid	1.0	4.2	76
DNF - tartaric acid	0	2.6	75
D - boric acid	0	3.9	63
DNF - boric acid	0	3.6	72
D - NaOH	0	7.9	65
DNF - NaOH	0	8.1	63
D - HCl	0	3.0	57
DNF - HCl	0	3.0	54
40 C.			
D	0	4.9	77
DNF	0	4.9	78
D - HCl	0	4.3	72
DNF - HCl	0	4.1	66
D - acetic acid	0	4.9	66
DNF - acetic acid	0	4.3	64
D - water	0	5.1	73
DNF, - water	0	5.1	75
D - tartaric acid	1.5	4.1	77
DNF - tartaric acid	0	3.8	77
D - Boric acid	0.5	4.6	75
DNF - Boric acid	0	4.2	70
D - sugar crystals	1.0	5.1	78
DNF - sugar crystals	0	5.1	76
D - NaOH	0	4.3	72
DNF - NaOH	0	4.1	66
D - salt crystals	0.4	4.9	77
DNF - salt crystals	0	4.9	77
45 C.			
D	0.8	5.0	79
DNF	0	5.0	80
50 C.			
D	5.4	4.9	79
DNF	0	4.9	80
D - tartaric acid	1.0	3.3	76
DNF - tartaric acid	0	3.8	76
D - HCl	2.5	4.2	70
DNF - HCl	0	4.5	70
D - NaOH	0	6.7	71
DNF - NaOH	0	7.0	70
55 c.			
D - tartaric acid	9.0	4.0	75
DNF - tartaric acid	0	4.0	77
D	10.0	4.8	77
DNF	0	4.8	77

Table 2. <u>Quantity of foaming under varying physical and</u>
          <u>chemical conditions.</u>

	Foam (cc.)	pH	% solids
60 C.			
D	3.0	4.8	78
DNF	o	4.8	79
D - tartaric acid	1.5	3.5	75
DNF - tartaric acid	0	3.0	72
65 C.			
D	5.0	4.9	80
DNF	0	4.9	80
D - HCl	2.5	4.3	70
DNF - HCl	0	4.3	68
D - NaOH	0	6.2	67
DNF - NaOH	0	6.1	69
70 C.			
D	3.0	4.8	80
DNF	0	4.8	73
80 C.			
D	3.0	4.7	80
DNF	0	4.7	80