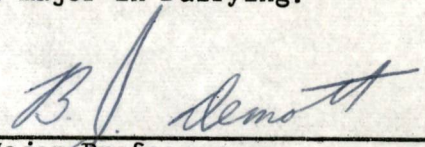


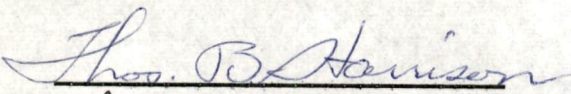
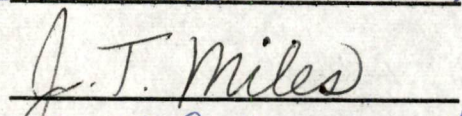
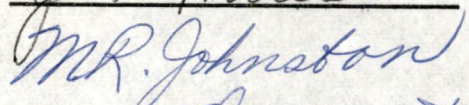
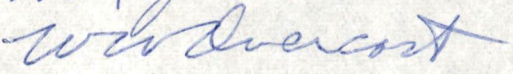
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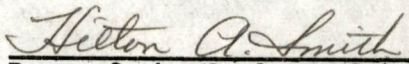
I am submitting a thesis written by Brind Bahadur Singh entitled "The Influence of Vacuum Level and Heat Treatment Upon Volatile Sulphydryls and Cooked Flavor in Milk." I recommend that it be accepted for nine quarter hours credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.


Major Professor

We have read this thesis and
recommend its acceptance:

Accepted for the Council:


Dean of the Graduate School

THE INFLUENCE OF VACUUM LEVEL AND HEAT TREATMENT UPON VOLATILE
SULFHYDRYLS AND COOKED FLAVOR IN MILK

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Brind Bahadur Singh
March 1963

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CHAPTER I

INTRODUCTION

The development of off-flavors in dairy products is sometimes intricate. It is very important to the dairy industry that a knowledge of the causes and prevention of these off-flavors be gained since flavor is perhaps the most important single factor in determining the consumer acceptance and saleability of dairy products.

There are some flavors, disagreeable in themselves, which directly affect the saleability of the product, since they are quite evident to the consumer. There are other flavors not particularly disagreeable nor even noticeable to the average consumer which are believed by the buyer to indicate poor keeping quality. The cooked flavor belongs in the former category.

Relatively recent reviews of off-flavors in milk have been made by Strobel, Bryan and Babcock (48). The heat treatment of milk produces chemical changes in milk resulting in flavors not pleasing to the taste and smell, for example, cooked flavor. The lack of complete knowledge about the chemical nature of these flavor producing substances complicates the problem of evaluating the means of removing them from milk and dairy products.

Taints in milk and dairy products have been removed partially or wholly by:

- (a) Dilution or washing with water or a light weight mineral oil.

(b) Aeration with and without reduced pressure.

(c) Application of vacuum.

(d) Distillation with steam, usually under controlled pressure.

The primary function of vacuum is to accelerate the removal of flavor laden vapors and to assist in temperature control.

Lowering the temperature and pressure, i.e., increasing the vacuum applied:

(a) would facilitate the removal of low boiling substances

(b) may facilitate or may retard the removal of substances with boiling points close to that of water; and

(c) would definitely retard the removal of high boiling substances (43).

Questions which remain yet to be answered include:

(a) what is the chemical nature of tainting substances?

(b) what is the particular combination of time, temperature, vacuum treatment that is most effective and efficient for removal of off-flavors?

(c) can chemical tests be developed for the tainting substances, so that more objective means will be available for evaluating the treatment combinations?

(d) can the treatments be adapted to enough of the dairy products to insure greater economy in the use of the equipment?

(e) can the equipment be adapted to remove chemical substances that are not affected by present treatments?

Studies on the heat volatile sulfides of milk have assumed special importance since work has been reported indicating that the liberation of

these sulfides and the formation of sulfhydryl groups may be closely allied with the production of the cooked flavor and with the prevention of the oxidized flavor in milk (19,30). The results secured in these various sulfides studies have been based largely on qualitative determination. Quantitative determination and the influence of time and temperature have been reported (52).

Influence of time, temperature and vacuum on the sulfhydryl production and subsequent development of cooked flavor has not been studied so far, therefore, the present work was undertaken to study the influence of various combinations of time, temperature and vacuum levels on the production of sulfhydryls in milk during heat treatment. The influence of various levels of vacuum treatment on the development of cooked flavor in milk is reported.

CHAPTER II

REVIEW OF LITERATURE

Flavor Identification Research

Flavor research aims at prevention of off flavors, preservation of normal or desirable flavors and to cut short or eliminate ripening or aging processes required for flavor development in certain products.

Flavor identification research mainly is carried out to investigate the chemistry of compound or compounds responsible for a flavor. Such information is lacking with respect to a large number of flavors of interest in the dairy field including cooked flavor.

Four main steps are involved in the chemistry of food flavor investigation, namely, isolation, concentration, purification, and identification. Flavor compounds are effectively manifested at levels of parts per million, or even per billion. Therefore, it becomes necessary to find methods that are operative on a microscale. Some recently developed research tools like chromatographic methods, spectrophotometers and colorimeters have done much to alleviate the need in this area (29).

Patton, and Josephson (40) have removed heat induced flavor compounds from skim milk, heated to 260° F for 60 minutes, by direct extraction with redistilled ethyl ether. These substances may be concentrated by evaporation of the ether. The judgement of many impartial observers indicate that this concentrate has a strong "Caramelized" odor, typical

of heated milk. Fractionation of the flavor concentrate has been accomplished by selective solvent extraction and vacuum distillation. Solvent extraction has yielded three major fractions, soluble in water, petroleum ether, and ether respectively. The ether soluble fraction contains the "caramelized" principle, whereas the water soluble fraction has more of a sulfide type of "cooked odor". Both of these fractions contain reduced sulfur in significant quantities. This evidence may explain why the sulfhydryl groups in heated milk disappear as the development of caramelized flavor progresses.

Patton and Josephson (42) studied the heat induced flavor changes occurring in heated skim milk using a group of five taste observers. The most important and pronounced changes resulting from the heat treatment were found to be the development of "cooked" (olfactory) and "rough" (tactual) flavors. These same flavors also were manifest to approximately the same degree in heated rennet whey.

In order to determine the importance of serum proteins in these flavor changes, heated (205° F - flash) rennet whey was supercentrifuged at 35,000 r.p.m. (42). Such treatment produced significant reductions in "cooked" as well as "rough" tactual flavor responses. Parallel diminution (in certain cases complete) in the nitroprusside test of the whey occurred as the result of the removal of denatured serum proteins. The primary importance of serum proteins in heat induced flavor changes was demonstrated by these studies. Attempts to remove serum proteins from heated skim milk by supercentrifugation were unsuccessful, as indicated by the nitroprusside test and flavor responses to observers to the skim milk.

following centrifuging treatment. This phenomenon may be attributed to the physical and/or chemical adsorption of serum proteins on the casein micells in heated milk. The serum proteins (sulfhydryl positive) in heated milk are found in the rennet curd of the milk rather than in the whey. Such a phenomenon may explain in part, the soft curd characteristics of highly heated milk.

When samples of skim milk were heated for prolonged periods at 205° F, the quantity of sulfhydryl groups, as determined by the nitroprusside test, was found to diminish substantially. However, in the absence of casein (rennet whey) and lactose (dialyzed skim milk), such a diminution did not occur (42).

Dill et al (8) in an effort to isolate and identify sulfur compounds resulting from various heat treatments, heated skim milk by steam injection to temperatures ranging from 190° F to 300° F with holding times of either 2 or 150 seconds. The milk was then cooled to 130° F by flash evaporation and finally cooled to 40° F by a plate cooler. Measurement of heat activated sulfhydryls was accomplished by amperometric titration using silver nitrate. The volatile sulfur compounds, liberated as a result of the above heat treatments, were collected as precipitates in mercuric chloride solution. Gas chromatography was used to resolve and identify the volatile materials. Volatile components were formed at heat treatments greater than a critical treatment concomitant with a loss in titrable sulfhydryl groups in the milk. The volatile fraction consisted primarily of hydrogen sulfide. The appearance of methyl sulfide in the volatile fraction appeared to correlate more closely to the

presence of a cowy odor in the raw skim milk than to heat treatment.

Flake, et al (12) developed a method for isolating and concentrating a highly volatile flavor and odor material believed responsible for the typical activated flavor produced in milk exposed to solar or artificial source of radiation. Analysis of the material so isolated was not possible because of the small yield of product obtainable. Distillate from the milk containing the flavor yielded a positive test for sulfhydryls, whereas the concentrate yielded only a strongly positive disulfide test. It is believed this is due to oxidation of sulfhydryls during isolation of the flavor concentrate material.

The chemical similarity of the sulfhydryls and disulfide indicates the similarity of the flavor bearing products and the role of sulfur bearing compounds in the source of the flavor.

Stewart, (47) states that the introduction of gas chromatography has provided the research scientist with a powerful tool for determining volatile components. Adoption of this technique has resulted in a flood of research papers on the volatile compounds in food. Most of these studies are being reported in the name of "flavor chemistry". Despite the importance of these studies they show only what is causing the flavor, collectively, and not where it is coming from specifically. Some important components contributing to the aroma of foods have been isolated and identified - but what is usually not being done is to establish which of the compounds isolated are responsible for what sensory properties.

Thus the challenge is to establish the importance of the volatiles in our foods.

Appropriate tests with isolated fractions and compounds will surely help answer such questions as:

- (a) does the compound have sensory properties?
- (b) if so, what is its nature?
- (c) what combinations of components result in the sensory properties typical of the original product from which they were derived?

Bidmead (2) described a simple but useful means of concentrating flavors by slow freezing of a stirred solution. The ice forming peripherally containing no flavor materials and the solution in the center becoming gradually more concentrated.

Roberts and Dill (44) state that the mechanics of control over the steam system have been established.

A number of potential advantages to be realized, particularly from high temperature treatments, are better keeping quality in the finished product, removal of volatile flavor compounds when vacuum treatment is incorporated, and better process control in terms of quality characteristics of the finished product. The purpose of mixing live steam with fluid milk products is for rapid and efficient heat transfer to prevent development of off flavor and result into a nutritious wholesome acceptable product. He has described a steam injection system for heating fluid milk products in which temperatures are automatically and accurately maintained as high as 300° F. The vacuum system is being used to provide quantities of volatile materials

from milk for identification purposes. The system is very adaptable for research and commercial use because of the wide range of treatments and volumes possible with the equipment.

The vacuum treatment is not sufficient to remove all of the objectionable flavoring substances that might be present in milk. The failure to remove all of these substances may be readily understood when one realizes that the usual vacuum applied for flavor removal is seldom above 26 inches (approximately 100 mm pressure) and that there are many flavoring substances with a vapor pressure lower than this at processing temperatures.

Some investigators favor steam because (a) some substances are very steam volatile (b) the temperature of the milk or dairy product can be controlled by steam during processing (c) steam is readily available and (d) the condensate can be disposed of easily and quickly (43).

In discussing the practical application of steam distillation, Roberts (43) stated that the substances to be steam distilled must be insoluble or only sparingly soluble in water, prolonged contact with boiling water or steam must not decompose it, and the vapor pressure must be at least 4 mm in the neighborhood of 100° C.

Heated Flavors

Heating is most fundamental process to which milk and its products are subjected in dairy industry.

Cooked flavor is one of the heated flavors which commences to develop at or about 74° C in milk when heat treatment is given to it momentarily (19,30).

Pasteurization, forewarming, preheating, superheating and heat sterilization are the processes indispensable in dairy products which bring on cooked flavor in lesser or greater degree depending upon the extent and other details of heat treatment.

In modern times pasteurization practice has been accepted to be necessity rather than a preference. It is spreading rapidly all over the world. As such the flavor of good quality pasteurized milk generally is the flavor standard for milk so far as consuming public is concerned.

Cooked flavor arises from the sulfhydryl groups activated by heat denaturation of beta lactoglobulin and proteins of the fat globule membrane (27). The flavor is specifically due to volatile sulfides and hydrogen sulfide in particular. The precise mechanism regarding formation of hydrogen sulfide from sulfhydryl groups in these proteins is not known. Townley and Gould (52) has made an extensive study to find out factors affecting the quantity of sulfides produced in heated milk. In general they have been successful in showing that oxidizing conditions inhibit and reducing conditions favor sulfide formation. Lowering of oxidation and reduction potential have also been observed in the region in which the cooked flavor is formed (30).

The whole field of the development of cooked flavor is obscured in the complexities of protein denaturation, and loss of vitamin B₁. This invites one to accept the challenge of unfolding the secrets in the research for removal of cooked flavor from heated milk.

The Effect of Heat on Milk Proteins

Serum proteins are the only heat-denaturable proteins in milk. This group of proteins amounts to approximately 0.6 to 0.7% of the milk and since they reside in the whey following removal of the casein from skim milk by acid or rennet coagulation, they are designated the "whey proteins".

The valuable tool of electrophoresis available to the protein chemists have enabled them to show the range of protein composition of milk. The research worker of cooked flavor is primarily interested in the fraction of beta-lactoglobulin, which represents about 50% of the total serum proteins, for the simple reason that it figures most prominently in heat induced changes of milk.

The heat induced changes of concern are: evolution of hydrogen sulfide, formation of cooked flavor, and lowering of oxidation reduction potential. The common cause of these phenomena appears to be heat denaturation of the milk serum proteins. Apparently milk serum proteins in their native state have a definite coiled configuration which is maintained by hydrogen bonding, hydration and secondary valence forces. When these proteins are exposed to heat above a certain critical level, the native configuration is disrupted and the characteristic properties of the proteins are altered. One important manifestation

is a change in solubility. They become much less soluble in acid at pH 4.6 and in saturated sodium chloride. Thus they precipitate from heated milk with the casein upon acidification or saturation with salt (29).

According to the method of Harland and Ashworth (23) the heat denaturation of these proteins can be measured by precipitability with saturated sodium chloride. The rate increases regularly as the temperature is raised from 68° to 80° C.

Paper electrophoresis has clearly shown differing degrees of susceptibility to heat denaturation by individual fractions of serum proteins. Immune globulins are rendered acid precipitable by least heat treatment, followed in order by the blood serum albumin, the beta lactoglobulin and finally the alpha lactalbumin, which is very resistant to heat denaturation (29). Complicated interesting phenomenon is also exhibited by interactions among the fractions themselves (54). When a protein particle is subjected to sufficient heat it uncoils and the groups become more accessible and reactive (29).

The activation of sulfhydryl groups in milk by heat treatment first becomes evident at about 167° F (75° C) as measured by the nitroprusside test (30). A reagent that shows similar sensitivity to such change is thiamine disulfide. These groups are also susceptible to oxidation. The lability to origin of these groups is the basis of explanation for the antioxygenic properties of milk so heated.

Josephson and Doan (30) also indicate that oxidized flavor is not produced in the presence of sulfhydryl group and cooked flavor.

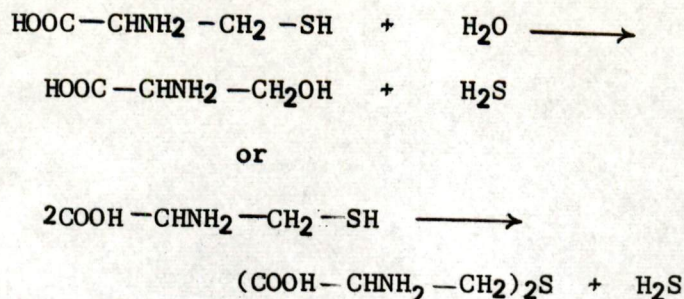
Ascorbic acid is also an antioxidant but its nutritional value is destroyed in the process when oxidized. Some evidence indicate that ascorbic acid is protected or stabilized to a considerable degree when milk is heated to a temperature sufficiently high to produce cooked flavor and positive nitroprusside test even when small amounts of copper are present. The protection is especially noted when a sample is heated to 190° F. By the same token sulfhydryl groups are powerful reducing agents and probably account for the lowering of the oxidation reduction potential that simultaneously accompanies their activation in milk.

Josephson and Doan (30) noted the first lowering of the potential at the temperature which was critical for the production of cooked flavor and production of sulfhydryls:

Temp. of 170° F (76.6°C)-sharp drop-slight cooked flavor, faint
N.P. test.

Temp. of 190° F (87.7°C)-Potential still lower, stronger cooked
flavor, deeper N.P. test.

Hydrogen sulfide seems partly responsible for the cooked flavor in heated milk. The evolution of volatile sulfides and the appearance of cooked flavor in heated milk are closely related to the activation of the sulfhydryl groups. The amino acid cysteine, the principal site of sulfhydryl groups in the milk serum proteins, liberates hydrogen sulfide when heated in aqueous solution. The exact chemistry of this decomposition is not known although it may be effected in the following alternative ways (29).



The protein associated with milk fat globule membrane is activated by heat treatment. The temperature of activation is approximately 7° F lower than that for skim milk protein (29).

Timasheff and Townend (49) identified A and B forms of beta-lactoglobulin differing precisely on the basis of the very few amino acid residues.

Zweig and Block (55) point out that all or almost all the -SH groups in milk are present in the whey proteins.

Kiermeier and Hamed (32) found that through heating milk free -SH groups will be formed from beta-lactoglobulin. SH groups are active antioxidants which protect oxidation of ascorbic acid and further prevent lipid bound taste.

Larson and Roleri (38) found that the immune globulins are the least and alpha-lactalbumin the most heat resistant of the milk serum proteins. The heat treatment of 70° C, for example, denatured 89% of the immune globulins, 52% of the serum albumin, 32% of the beta-lactoglobulin, and 6% of the alpha-lactalbumin.

Gough and Jenness (15) found that beta-lactoglobulin B was heat denatured faster than A in 1% solutions or in milk.

Hetrick and Tracy (24) found that the first noticeable cooked flavor resulted in the denaturation of about 58% of the albumin and globulin.

Larsen and Roleri (38), Josephson and Doan (30), and Townley and Gould (51) found that beta-lactoglobulin is the principal reducing component of the milk proteins. Materials adsorbed on the fat globule are a source of heat labile sulfides in milk.

Gordon et al (14) made a study of hydrolysates of beta lactoglobulin A and B and mixed A plus B and suggested that A contains two times more aspartic acid, 1.6 times more valine, 1.4 less glycine and 1.3 less alanine residues per 35,000 mol. wt. than B. The difference in aspartic acid and glycine contents seem to be significant.

Patton and Josephson (42) point out that use of an argentometric amperometric titration procedure has revealed that the only source of SH groups in skim milk is the serum proteins. It is also shown that beta-lactoglobulin can account for practically all the -SH groups present. Study of the contributions of various major serum protein fractions to heat induced cooked flavor in skim milk demonstrated beta-lactoglobulin to be responsible for the flavor, conversion of -SH groups to H_2S as a result of heat treatment. This, in a general way, explains the mechanism whereby beta-lactoglobulin gives rise to cooked flavor.

Kiermeier and Hamed (32) point out that -SH groups in heated milk increases with the rise of the presence of certain fat globule membrane constituents and beta-lactoglobulin.

Gough and Jenness (16) found that beta-lactoglobulin B was more rapidly denatured than beta-lactoglobulin A by heat treatments.

Zittle et al (54) observed that heating a mixture (0.5% of each) of beta-lactoglobulin and K casein at pH 7 at 90° C for 15 minutes lead to interaction between the two proteins.

Fitzpatrick and Sullivan (10) investigated electrophoretic patterns of milk proteins and found evidence to indicate that protein interactions varied with the type of processing. Interactions occurred between alpha- and beta-casein; in addition, there was complexing between the beta-lactoglobulin and the complexed alpha-casein.

Jenness and Patton (29) indicated that beta-lactoglobulin and blood serum albumin contain cysteine which is important because of its free sulfhydryl (-SH) group. Most of these groups in milk are in beta-lactoglobulin since the blood serum albumin is present in such low concentration.

Josephson and Doan (30) further elaborate on this point to observe that protein free whey is negative for sulfhydryls and cooked flavor during heating to critical temperature and storage. Buttermilk gave a strong nitroprusside test as the oxygen of the milk was taken up by the microbes leaving all -SH groups unoxidized to N.P. test and cooked flavor. Washed cream and buttermilk was weakly positive. Twice washed buttermilk, and cream, had questionable nitroprusside values.

Albumin of milk serum plays a major role in the production of nitroprusside test as compared to globulin constituents of the husk of the fat globule (30).

Fat Globule Membrane Study

Oxidation or oxidized flavor is due to oxidation of phospholipids of milk catalyzed by the presence of copper or iron. The process is further enhanced by the presence of protein. Temperature also influences the process (30). Observations of heat treatment was made at 180° F for 10 minutes with or without 1 PPM of copper added. The oxidized flavor was detected by taste (53).

Gould and Sommer (19) observed that the complexity of the milk system increases with application of heat. When milk is heated, the first major change is the denaturation and subsequent coagulation of whey proteins. At 143° F for 30 minutes, or 160° F for 15 seconds, from 5 to 10% of these proteins are denatured. As the temperature and heating time are increased, rapid denaturation occurs and at 176° F for a few minutes it is essentially complete. Temperatures near boiling produce other changes such as hydrolysis of whey proteins, increases in proteose and non-protein nitrogen and the production of ammonia.

When proteins are heated, exposed to ultraviolet irradiation, ionizing radiations, very high and low pH, sound waves, surface forces, pressure or treated with alcohol, they suffer denaturation indicated by profound changes produced in solubility. Denaturation involves the breaking of hydrogen bonds and salt linkages through which they are maintained in their specific configuration. Denaturation is an irreversible change. Unfolding of the molecule often exposes groups which may undergo chemical reaction. Proteins are stable at room temperature over a pH range from 4 to 8 and some stable over a wider range.

Casein usually does not precipitate upon heat treatments. Drastic heat treatments, such as autoclaving at 120° C, do cause a decrease in solubility of whole casein, but this is accompanied by a splitting out of the esterified phosphate groups and degradation as manifested by the appearance of non-protein nitrogen. These changes do not come within the definition of denaturation (29).

Belec and Jenness (1) found that casein is completely dephosphorised when heated at 120° C for five hours and that approximately 15% of the nitrogenous material was simultaneously released as small molecules.

Maximum quantity of CO₂, nearly 10% by volume or 20 mg. of CO₂ per 100 ml. is present in the raw milk at the time of secretion which is lost to the atmosphere slowly and steadily. The loss is accelerated by heat and agitation of the milk. Due to loss of CO₂ acidity of the milk is reduced and pH is increased (29).

Calcium phosphate is less soluble at high temperatures, therefore, heat treatment of the milk causes the formation of a calcium phosphate colloid. The calcium and phosphate ions do not revert to the soluble state upon cooling of the milk. The effect is as though the following reaction occurred:



In some cases these two opposing factors (CO₂ expulsion and phosphate precipitation) so nearly counter balance each other that there is no net change in pH.

The heat treatment has little effect, if any, on the amount of dissolved citrate in milk (29).

Gould and Sommer (19) observed that salts of milk constitute one of the most complex systems of the milk components. Calcium appears to be the balance wheel in the salt system of milk as it relates to the effect of heat.

Gould and Sommer (19) observed that temperatures near boiling produce changes in lactose which undergoes decomposition to form such acids as formic and lactic and to produce organic compounds such as furfuryl alcohol and maltol. The changes in protein and lactose affect both the color and flavor in the milk.

Influence of Temperature, Time and Other Factors On Sulfhydryl Production and Development of Cooked Flavor

The dairy industry is adopting methods of processing dairy products at higher temperatures and shorter heating times. The benefits claimed are continuous processing, meeting requirements regarding destruction of micro-organisms and enzymes, and minimizing detrimental effects on flavor and other properties. The time required to reach this temperature and to cool the milk is also important. Some research has been done in connection with the time temperature relationship and other factors connected with it in respect of sulfhydryl production which is supposed to be the cause of cooked flavor (29).

Townley and Gould (52) define critical temperature for sulfhydryl production in heat processing of milk to be that temperature at which

sulfhydryl production commences in activated free form and is accompanied by cooked flavor in milk, i.e. 76° C. The sulfhydryl production begins at this temperature.

Above or below 76° C a change in the rate of sulfhydryl production is marked. A prolongation of the heating time also decreases the critical temperature in the liberation of sulfides. The volatile sulfides do not vary much from the normal value if the milk is being heated at temperatures somewhat below the critical temperature. However, there appears to be a maximum critical temperature above which the production of sulfhydryls is decreased, i.e. 97° C. Burton (4) found that once the evolution of -SH groups commenced it was evolved rapidly in its critical temperature range of 70° to 80° C and subsequently fell.

Zweig and Block (55) found the minimum critical temperature to be between 58° and 69° C. At the point of critical temperature they observed cooking taste in the milk. Dill et al (7) found out that volatile components were formed at heat treatments slightly greater than the minimum critical treatment, concomitant with a loss in titrable sulfhydryl groups in the milk. The volatile fraction consisted primarily of hydrogen sulfides.

Harland and Ashworth (22) observed the minimum critical temperature to be 70 - 76.5° C for 30 minutes and one minute respectively. Cooked flavor was observed by heating milk at 76.5° C for one minute, 71° C with 15 minute and 68.5° C with a 30 minute pasteurizing time.

Josephson and Doan (30) obtained positive nitroprusside tests under similar conditions and verified the findings of Harland and

Ashworth (22). According to Kleyn and Shipe (36) hydrogen sulfide was produced rather rapidly, and the production corresponded to a loss in thiamine disulfide values at any given temperature and time within the critical range of reactive sulfhydryl production.

Kiermeier and Hamed (34) citing Goded states the minimum critical temperature to be between 74° and 76° C if held only momentarily, or 66° to 68° C if the milk was held for 30 minutes.

Gould (17) found cooked flavor in milk heated between 75° - 100° C. Gould and Sommer (19) produced cooked flavor by heating milk to 75° - 78° C momentarily, 70-76° C for 3 minutes, or 70-72° C for 30 minutes. Townley and Gould (52) observed cooked flavor in samples exposed to 95° C for periods ranging from 0 to 30 minutes.

Gould (17) and Gould and Sommer (19) state that cooked flavor in the milk is due to formation of free sulfhydryl groups during heat treatment.

Townley and Gould (52) found that cream has lower minimum critical temperature than milk. Critical temperature for 3 minute holding period for milk was 74-76° C, for skim milk, 76-78° C, for 35% cream, 66-68° C, and for 20% cream, 70-72° C.

Kiermeier and Hamed (34) heated whole milk to temperatures between 75° and 95° C and observed a formation of sulfhydryl groups. With a five second period of maintaining temperature a simple linear relationship was obtained. However with periods of 10, 17, and 30 seconds of maintaining temperatures up to 90° C an especially strong formation of -SH groups resulted, while after 55 to 100 seconds at temperatures above

90° C the rate was decreased. Thus not only the heating temperature influences the liberation of sulfhydryl groups but also the duration of maintaining temperature. Fast production of sulfhydryl groups occurred up to 85° C. At temperatures above 85° C this development became slower, at temperatures of 110-150° C a decrease of the liberated sulfhydryl groups was observed. This decrease is not due to the oxidation of -SH groups to -SS groups, since they heated the milk up to a 150° C in vacuum and observed, in spite of it, a decrease of -SH groups.

Dill et al (9) report the effect of direct steam injection heating on the development of heat activated sulfur compounds in skim milk over a temperature and time range of 190 to 300° F for 2 to 150 seconds. The heat activation of sulfhydryl groups as measured by silver nitrate titration, followed a direct relationship to heating temperatures in those samples held for 2 seconds. At twenty and one hundred fifty seconds, maximum responses to temperature were observed at 260° F and 220° F, respectively. A decrease, then occurred as the temperature of heating increased. It was determined that much of the decrease in titration values above the critical treatment resulted from volatilization of sulfur compounds.

Townley and Gould (52) observed that a vacuum distillation method at temperatures of 74-75° C and 77-79° C liberated 0.0033 and 0.0108 mg sulfur respectively from one liter of milk. An increase of temperature increased the quantity of volatile sulfides produced. The volatile sulfide produced at 76° C was 0.018 mg/l, at 80° C, 0.053 mg/l, at 86° C, 0.130 mg/l and at 90° C, 0.240 mg/l. By prolongation of the heating period

at temperatures below 95° C the amount of liberated sulfides increased.

Kiermeier and Hamed (34) concluded from their experiments that with the prolonged heating of whole milk a continuous liberation of sulfhydryl groups took place. The largest effectiveness of the maintaining temperature seems to be in the area from 5-70 seconds.

The decreases of sulfhydryl groups by a very intensive heat treatment may explain why in spite of high sterilizing temperature, sterilized milk, cream or condensed milk, do not have the intense cooked taste one would expect of such heat treatments.

Humbert et al (25), Gibson (13), and Roberts and Dill (44) also found similar results.

The cooked taste did not appear below a heat treatment of 77° C but was always observed at high temperatures, and the higher the temperature, the stronger the cooked taste (34).

Smith and Macleod (46) and Josephson and Doan (30) point out that pasteurization temperatures of 178° F and above, 168° F and above respectively resulted in greater production of sulfhydryls and marked cooked flavor which disappeared completely with storage, acted as an anti-oxidant and tended to prevent oxidized flavor development.

Townley and Gould (52) found that, in general, increasing the temperature between the range of 76 to 90° C increased liberation of sulfhydryls. A 30 minute period of exposure to these temperatures was found to give increased sulfide liberation in vapor. Prolonging the heating period at all temperatures below 95° C increased the sulfide liberation. In general the nitroprusside tests become progressively more

positive the longer the holding time or the higher the heating temperature.

Increasing amounts of sulfides are made available by exposing milk to 95° C for periods up to 60 minutes. When milk is exposed for a period of 90 minutes or longer at 95° C the quantity of sulfides liberated is progressively decreased and after 210 minutes of exposure, sulfide liberation was no longer detectable (52).

Heating milk for a short period at 90 to 95° C in a sealed can or heating milk for a short period to temperatures above boiling decrease the sulfide liberation or prevent it entirely (52).

Vitamin B₁, contains about 12% sulfur. Ten to 50 per cent of this vitamin is lost from pasteurization to sterilization which indicates this vitamin may be a source of the free sulfhydryls in milk.

In addition to vitamin B₁, other sources of non-protein sulfur may exist in milk. About 15.1% of the sulfur of the milk is in the form of non-protein organic sulfur (52).

Boyd and Gould (3) found that the thiamin disulfide values decreased 20 to 22% as a result of liberation of the hydrogen sulfide which, for milk, amounted to a decrease of 1.0 mg. of thiamin disulfide value per 0.1 mg. of hydrogen sulfide removed. Hydrogen sulfide reveals only the volatile portion of the heat labile -SH groups, whereas, the thiamine disulfide values are affected by all -SH compounds which are created or revealed by the heat treatment, including hydrogen sulfide. These workers found that addition of copper, calcium chloride, or disodium phosphate or low temperature fore-warming and condensing,

affected the concentration of heat produced -SH groups. Ninety minutes of aspiration after heating permitted complete recovery of the hydrogen sulfide which had been produced. The volatile sulfhydryl results also reveal the importance of temperature and time of heating in as much as the major portion of the hydrogen sulfide was liberated during the holding and/or aspiration period, especially noticeable in the series involving a 30 minute heating period. The thiamine disulfide values, as cystine hydrochloride were highest at the time the 90° C temperature was achieved, ranging from 19 to 22 mg/l, and become progressively less as the heating and/or aspiration period progressed. This indicated that the compounds responsible for the thiaminedisulfide values are produced before hydrogen sulfide is liberated. The liberation of hydrogen sulfide was accompanied by a decrease in the thiamine disulfide values, with the major changes occurring during the first 30 minutes, following the reaching of the temperature of 90° C. The milk was brought to the highest desired temperature, 90° C, within approximately 8 minutes. Hydrogen sulfide is removed by aspiration only. Vacuum aspiration helps in recovery of hydrogen sulfide produced by heat treatment under vacuum.

Kiermeier and Hamed (34) found free -SH groups, as measured by cysteine content, first becomes evident in milk when heated to about 75° C, increases rapidly with increase in heating temperature to about 95° C, and thereafter rises, but at a steadily diminishing rate, until at a temperature of about 110° C beyond which it declines. Development of a cooked flavor runs more or less parallel to liberated -SH groups.

Dill et al (8) found out that -SH content increased with the severity of the heat treatment. The -SH content decreased during storage in all samples.

According to Nelson (39) the -SH groups play a part in the heat coagulation of milk. The amount of the formed -SH groups depends on the duration and the temperature of the heating process.

Townley and Gould (52) found sulfide liberation from milk is decreased either by low pH values or pH values over 9, by sugars, formaldehyde, cystine, sodium chloride, hydrogen peroxide, iodine, and metals, e.g. copper, silver, mercury, iron. An increase in the heat labile sulfides results by increasing pH values between 6.5 and 9 and by the addition of ethyl alcohol, sodium sulfite and cysteine HCl. Sulfide liberation from milk may be decreased by (a) treating the milk so that denaturation of serum proteins is retarded or prevented or (b) oxidizing or blocking out the sulfhydryl groups as they are formed.

Greenbank and Wright (21) found that heating deaerated milk for the same time and temperature as normal milk developed a higher concentration of sulfhydryl groups which are considered to be antioxidants, and improved the keeping quality of the dried products.

Kiermeier and Hamed (35) found that evacuation of milk is superior to replacement of air by inert gases for the purpose of protecting heat liberated -SH groups against atmospheric oxidation, especially at temperatures below 95° C. Formation of -SH groups is greater where milk is heated in contact with stainless steels than in contact with glass, especially at lower temperatures such as 72° C, but is by no means

identical for different types of steel. Gould (18) was able to prevent the cooked flavor and the oxidized flavor by adding 2 PPM. of copper sulfate to milk before heating.

Other workers have reported also on the cooked flavor that appears in milk (26,45,33).

Strobel, Bryan, and Babcock, (48) have made review on literature on flavors of milk and have cited the work of twenty authors on cooked flavor development in heated milk.

Effect of Ultraviolet Light on Milk

The sulfur of the milk exposed to ultraviolet radiation is more heat labile than that of milk not so exposed to radiation (50).

The increased lability of the sulfur of the milk exposed to radiation also appears to be good evidence of the possible role of sulfur compounds in formation of the activated flavor. Milk exposed to ultraviolet radiation has a lower minimum critical temperature for sulfhydryl production than milk not receiving such radiation.

Milk exposed to radiation may be differentiated from milk not exposed to radiation by use of a colorimetric test for sulfhydryls.

Flake et al (11) found that exposure to radiation increases the lability of sulfur of milk and causes development of off flavor which is activated, sunshine or burnt flavor.

Influence of Vacuum on Sulfhydryl Productions

Graves et al, (20) point out that cooked flavor was obtained by heating milk to 175° F for 19 seconds in a regular high temperature

short time pasteurizer. Because of the high initial temperature at the flow diversion value, boiling occurred at a level between 7.5 and 10 inches of vacuum. An expert panel found a highly significant improvement in cooked flavor as vacuum was increased up to 10 inches. The consumer panel, however, found maximum improvement to be at 7.5 inches level. At 5" vacuum the boiling point of the milk is 203° F., at 10", 192° F., and at 15", 179° F, which is below the temperature of the milk and steam mixture. In these experiments the temperature did not fall to the theoretical level of 179° F but dropped only 5° to 187° F giving, in effect, a super heating of the milk and steam mixture in the first chamber. The vacuum treatment equipment used reduced the intensity of garlic, silage, feed and cooked flavors in milk.

Kleyn and Shipe (36) indicate that combined steam heating and vacuum treatment produced a significant denaturation of whey proteins, as measured by the increase in the activity of sulfhydryl groups and the decrease in whey protein nitrogen. However, the extent of activation of sulfhydryl groups was lower than had been expected. The initial sulfhydryl values for treated milk range from 0.021 to 0.031 meq/liter expressed as cysteine hydrochloride. These values indicate that the heat treatment received by the milk in the ultra high temperature heater is activating the sulfhydryl compounds. However, these values are much lower than the 19-22 mg/liter range (i.e. 0.121-0.139 meq/liter) reported by Boyd and Gould (3) for milk samples at the time they reached 90° C. The reason for the lower values in the case of U.H.T. treated milk probably is because the milk is held at the high temperature for

only a few seconds in the steam vacuum unit, whereas it took approximately 8 minutes for Boyd and Gould (3) to heat milk to 90° C and cool it to 75° C.

Harland and Ashworth (22,23) found a temperature increase of 13.5° F yielded a ten fold increase in the rate of denaturation between 155 and 175° F. The result of experiment demonstrated that the homogenized, treated milks had higher active sulfhydryl contents than the non-homogenized samples.

Boyd and Gould (3) made an observation that the production of volatile sulfhydryl compounds is a two step process, first, the production of materials which reduce thiamine disulfide, (excluding hydrogen sulfide); and secondly, the decomposition of a portion of these materials to permit evolution of hydrogen sulfide. They showed that thiamine disulfide values computed as cysteine hydrochloride, were highest at the time the 90° C temperature was achieved, ranging from 19-22 mg/liter (0.121-0.139 meq/liter) and became progressively less as heating and/or aspiration progressed.

Since the milk was held at a high temperature for only a few seconds in the steam vacuum unit, little time was available for the production of either volatile sulfhydryls or hydrogen sulfide. Of course, some of the volatile sulfhydryls or hydrogen sulfide may have been removed in the vacuum chambers of the steam vacuum unit. The milk treated in the steam vacuum unit had a cooked flavor but a very weak nitroprusside test, contrary to other literature. One might question therefore, the contribution of volatile sulfhydryls to the cooked flavor in milk.

These results indicate that the amount of volatile sulfhydryls necessary for a cooked flavor in milk may be less than that which is actually necessary to yield a positive nitroprusside test. Perhaps all that is necessary for cooked flavor development is mere sulfhydryl activation (36).

According to Kleyn and Shipe (36) the combined steam heating and vacuum treatment was found to produce a significant denaturation of whey proteins as measured by the increase in the activity of the sulfhydryl groups and the decrease in whey proteins. Removal of oxygen by vacuum treatment is considered to be a contributing factor in increasing the activity of -SH groups. Homogenization, steam heating and vacuum treatment play a important role in high retention of ascorbic acid.

Burton (4) found that SH groups in heated milk are not stable, but disappear on storage even in a sealed container. Individual samples were heated at 80° C for 30 minutes in small screw topped containers and were stored after treatment without opening in a refrigerator at about 30° C. The steady fall in SH content was noted in samples titrated by two procedures, i.e. in alcohol or aqueous mediums. The titrations with ethanol give lower end points than those in water. The fall in SH content on storage is slow, about 50% of the original content disappearing after 10 days. The action is irreversible.

The measured SH content falls as the ascorbic acid in the unheated milk falls. The riboflavin acting as an oxidizing agent, depresses the number of free SH groups. When the riboflavin is destroyed and its oxidizing effect is removed, more free SH group can exist.

Demott and Conder (6) collected data regarding the persistence of cooked flavor in milk pasteurized at 194-195° C for a calculated holding time of 5 seconds and concluded that the cooked flavor of milk on the fifth day after pasteurization was less pronounced and on the ninth day after pasteurization was more pronounced than on the same day of pasteurization.

Coulter, et al (5) investigated the reducing capacity of fluid milk and dry whole milk by the thiamine disulfide method and the acid ferricyanide method. The thiamine disulfide method measures essentially only those reducing groups produced as a result of heat treatment of the serum protein fraction of milk. The acid ferricyanide method includes these groups as well as ascorbic acid and reducing material resulting from heat treatment of lactose in the presence of phosphate buffer or protein or both. The thiamine disulfide reducing substances reached a maximum during heat treatment of fluid milk and then decreased. The maximum reached is somewhat higher and the rate of decrease is slower in the absence of oxygen. A higher maximum is reached by high temperature short time heating (95° C for 2 to 5 minutes) than by heating to a lower temperature for longer time. The acid ferricyanide reducing groups, although showing some effect of oxidation during heating, continue to increase over long periods of heating. The acid ferricyanide reducing capacity of dry whole milk may be increased as a result of heat treatment during drying. The thiamine disulfide reducing groups can not be increased by the drying process. As a matter of fact, there is no change in the thiamine disulfide reducing capacity on heating

systems which have a higher solids content than about 70%. The maximum rate of production of acid ferricyanide reducing substances occurs on the heating of milk systems of about 90% solids.

Sodium nitroprusside, $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}_3$, reacts with compounds containing sulfhydryl groups to produce an intensely red but somewhat unstable color. Sulfhydryl containing proteins such as beta-lactoglobulin give this test. In general, the -SH group must be free and accessible and most -SH containing proteins require denaturation before they will give the test. It has been used a great deal in studies of denaturation, both for qualitative tests and as an end point indicator. Its use for detecting changes in heated milk has been discussed by Patton and Josephson (41,42).

In recent years a turbidimetric method devised by Harland and Ashworth (23) has been used to a considerable extent for determining the concentration of undenatured serum proteins in heated and dry skim milks.

Burton (4) measured sulfhydryl groups in milk by an amperometric titration. Hutton and Patton (27) titrated in an aqueous medium (about pH 9.4) and found that the heat activated SH groups were measured. A zero titration was obtained for heat treatments of less than about 70° C for 30 minutes or its equivalent, after which the content rose to a maximum at about 80° C for 30 minutes and subsequently fell. With alcohol added to the medium, an end point is obtained with raw and slightly heated milk, which falls slightly with increasing intensity of heating to approach the curve in aqueous medium when this is at

its maximum. They interpret these results as showing that the alcoholic titration gives the total number of -SH groups in the milk, while the aqueous titration gives the heat activated groups; those which contribute to the heated flavor. The authors concluded that SH groups in raw milk are in an inaccessible state so they can only be titrated with difficulty. When the soluble proteins have been denatured by heat then the SH groups are accessible to titration. Increasing severity of heat treatment causes a fall in the number of SH groups, even when the heating occurs in a sealed container (4).

Although the importance of free -SH groups in the development of heated flavor is well established, the relationship between -SH groups and heat stability is uncertain. It is not even clear whether sulfhydryl groups assist or detract from stability. One might expect them to have an adverse effect, since for example, milk of high beta-lactoglobulin content is known to be unstable (4).

Nelson (39) observed that a saturated H_2S solution has a marked destabilizing action on milk protein.

Acceptable commercial fore-warming processes for the manufacturers of milk solids not fat seem to be such as to give maximum activated -SH content. Before heat stability becomes a problem, e.g. during sterilization of evaporated milk, further heat treatment has been given which will reduce the final SH content. High fore-warming temperatures will cause a lower final -SH content at the time when coagulation might occur. High fore-warming treatments also improve stability. Conversely, a less intense treatment in which the maximum heat activation is not

obtained during fore-warming may cause it to occur later in the process, so causing a stability lower than the normal (5).

The formation of methylene blue from p-aminodimethylaniline by distillation of the sulfhydryls into a hydrochloric acid solution of the reagent in the presence of nitric acid and ferric chloride has been used (12,52). The color formed is stable in light. In the reaction, each sulfur atom distilled as sulfhydryl enables the formation of one molecule of methylene blue. A concentration curve was established from readings promptly obtained using the photoelectric colorimeter. The concentration of the methylene blue formed in the distillates from various samples of milk was determined from the colorimeter concentration curve of the standard methylene blue solutions.

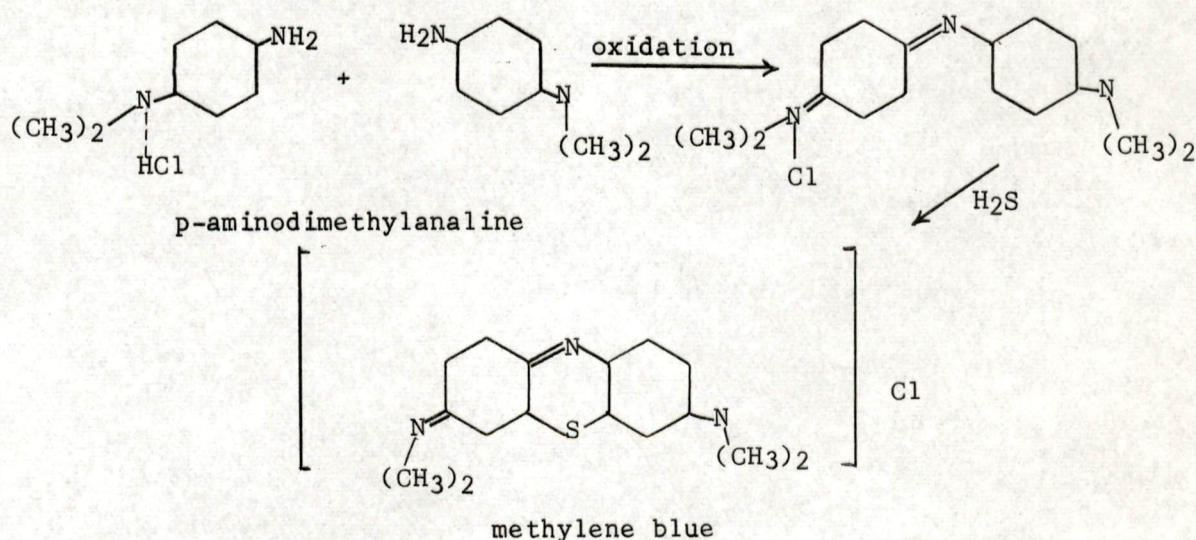
Townley and Gould (52) heated milk in a water bath and took approximately 15 minutes to bring the samples to the desired temperature. The method involves the collection of the liberated sulfides in alkaline zinc acetate and then the production of methylene blue color by the addition of p-aminodimethylaniline and ferric chloride. The intensity of the blue color varies directly with the amount with the amount of sulfides.

Thus, the production of sulfhydryls during heat treatment of milk has been assessed at various temperatures and times. The field of influence of vacuum on sulfhydryl production and subsequent effect on development of cooked flavor seemed to be virgin. The objective of the present investigation was to devise procedures for and collect data in the new field.

CHAPTER III

MATERIALS AND METHODS

In principle the method of distillation of sulfhydryls was followed as used by Townley and Gould (52) with the additional use of mercury vacuum control for controlling the fluctuations at different vacuum levels used in the experiments. All tubing used was sulfur-free neoprene. A water aspiration vacuum source was used to create a vacuum effect in the milk under process. The amount of volatile sulfur produced was measured by the amount of methylene blue color formed which is believed to form according to the following scheme (31).



Two quarts of milk from the University creamery were heated as a single sample. The samples were heated at different temperatures, times and vacuum levels as indicated in Table 1 with the milk in a three-liter flask in water bath. Nitrogen was passed at a rate of approximately

five bubbles per second through distilled water and alkaline pyrogallol solution and into the milk to push the vapors into the receivers containing 20% alkaline zinc acetate. Nitrogen gas was purified by passing through distilled water which dissolves and arrests any impurity in the gas. Pyrogallol solution arrests any oxygen present. The purified nitrogen agitates the milk and causes uniform heating. In every case, the flow of nitrogen was started when the sample was put in the water bath and the aspiration was continued not only during the heating period but for 30 minutes thereafter. Thus the sulfide values reported represents the sulfide liberated during the heating period and for a 30 minute period after heating. The alkaline pyrogallol was prepared as recommended by Townley and Gould (52) whereby thirty grams of pyrogallic acid was dissolved in 100 ml of distilled water and 2.5 volumes of 50% sodium hydroxide added. Blank determination with distilled water showed the apparatus to be free of sulfur. Each volatile sulfide receiver was nearly half filled with 4 mm glass beads and contained 25 ml of the dilute alkaline zinc acetate prepared in stock by mixing 25 ml of 20% zinc acetate and 40 ml of 10% NaOH diluted to one liter by adding distilled water. The reagent arrested nearly all the hydrogen sulfide in the first receiver.

Approximately 15 minutes were required to reach the desired temperature. After attaining this temperature the milk was brought under the vacuum influence and heating continued for the desired time. At the end of the heating period the heat source was removed, the vacuum

released and the nitrogen flow continued for 30 minutes. At the end of the aspiration period the solution and glass beads were removed from the receivers and the receivers rinsed with distilled water, then with 10 ml of 1:4 hydrochloric acid. The acid rinse was withheld from the alkaline receiving solution to avoid the possibility of an error due to the liberation of hydrogen sulfide before the indicators were added. Twenty-five milliliters of diamine reagent, which was prepared in stock by adding 500 ml of concentrated hydrochloric acid, 500 ml of distilled water and 1 gram of the methylene blue precursor dye, was added to the alkaline zinc acetate mixture. Five milliliters of 0.02 molar solution of ferric chloride in 4% hydrochloric acid was added and the acid rinsings from the receiver. The flask was immediately stoppered and a few minutes allowed for color development.

The solution was then placed into a 300 ml volumetric flask, the beads and flasks rinsed several times with distilled water, the rinsings added to the volumetric and made up to volume with distilled water.

The solution was permitted to stand for several hours (usually overnight) to permit the maximum intensity of color to develop. The intensity of the color was then determined with Bausch and Lomb Colorimeter using a 620 wave length and the concentration of methylene blue read from a previously constructed graph.

Six hundred and twenty millimicron wave length was selected after preparing a curve of transmissions at different wave lengths from 350 to 650 with a 20 μ wave length interval.

A colorimeter concentration curve of methylene blue was prepared at wave length 620 which was then used to determine the concentration of methylene blue developed from the hydrogen sulfide collected in alkaline zinc acetate solution evolved in the vapours of different samples of heated milk. The data for the concentration-colorimeter curve was:

Milligram per liter	Colorimeter readings
3.8	34
3.4	42
3.0	43
2.6	42
2.2	53
1.8	57
1.4	71
1.0	75
0.6	85
0.2	94

The quantity of methylene blue so detected was used to calculate the quantity of sulfur evolved from various samples of milk; one molecule of hydrogen sulfide caused the formation of one molecule of methylene blue. The molecular weight of methylene blue is 319.85, hydrogen sulfide 34.082, sulfur 32.066. Therefore, if one knows the mg of methylene blue formed, then

$$\frac{32.06}{319.85} \times \text{mg. methylene blue} = \text{mg. sulfur.}$$

Due to production of sulfhydryls, milk develops cooked flavor which can be appreciated by taste and the sodium nitroprusside test as described by Josephson and Doan (30).

The nitroprusside test as described by Josephson and Doan (30) was used. This consists of measuring five mls of milk into a test tube,

saturating with ammonium sulfate by adding an excess of the solid and shaking the tube and contents. Five drops of 4.5% solution of sodium nitroprusside ($\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$), are added and the mixture agitated gently. Five drops of concentrated (28%) ammonium hydroxide are added and the mixture agitated and the developed color compared to standards. The milk should be cold (20°C) and the tests should be read within 30 minutes after addition of the reagents.

The presence of a free sulfhydryl group will cause the sample to become pink in color. Contaminants such as acetone, creatinine, and indol also give positive tests but these are not likely to be found in milk.

Measurement of Flavor

The two principal means of measuring flavor are organoleptic and chemical analysis. The former appears to be more important because the latter has two limitations regarding its utility. First, flavor compounds frequently are encountered at levels that defy detection by chemical means, but which are readily evident to the taste. Second, chemical methods can not evaluate the like-dislike aspect of flavor, nor can they readily reveal small qualitative differences. However, the chemical methods eliminate human biases that are ever present.

The sodium nitroprusside test is not a reliable test for cooked flavor as noted by Kleyn and Shipe (36). Therefore, the necessity of organoleptic evaluation was apparent.

Raw milk was secured from the U. T. creamery and heated in the range of 75 to 77° C for 30 minutes at vacuum levels of 310, 410, 510 and 610 mm mercury absolute pressure according to procedure already described. One set of four samples was heated within two days and the judging took place on the third day.

Following heat treatment the milk was placed in one pint bottles and stored in a household refrigerator until used for flavor score judging.

The milk sample was identified by putting a code number label on the containers. Each main sample had two sub-samples thus making eight samples of heated milk and two sub-samples of ultra high-temperature pasteurized homogenized milk which was obtained on the day of judging from the creamery. Five trials of ten samples each were judged for cooked flavor score using a scoring system zero to four; zero score signifying not cooked, one, questionable cooked; two, slightly cooked; three, distinctly cooked; and four, objectionable cooked.

CHAPTER IV

RESULTS AND DISCUSSION

The influence of temperature, vacuum treatment and duration of heat treatment upon volatile sulfhydryl production expressed in micrograms of volatile sulfur per liter of milk for four trials are summarized in Table 1.

An analysis of variance of sulfur evolution from milk subjected to different temperatures, pressures and heat durations is shown in Appendix B.

No significant difference between trials has been observed. Treatments taken as a whole are significantly different. At a temperature of 65° C there is no significant difference between milk heated for 20 minutes and that heated for 40 minutes. At 65° C, there is no significant difference between milk heated at an absolute pressure of 510 mm Hg and that at 310 mm Hg. At 65° C, there is no interaction between time and pressure. At 75° C, there is a highly significant difference between milk heated for 20 minutes and that heated for 40 minutes. At 75° C a highly significant difference was observed between milk heated at 510 mm Hg and that heated at 310 mm Hg. At 75° C there is an interaction between time and pressure. The overall temperature effect is significant.

The data in Tables 1 and 2 show that reduced pressures increase the level of volatile and non-volatile sulfhydryl production appreciably

TABLE 1

Influence of temperature, vacuum treatment and duration of heat treatment upon volatile -SH production from milk

Temp.	Pressure	Time	Trial				Avg.
			1	2	3	4	
(°C)	(mm. Hg)	(min.)	(Micrograms volatile sulfur per liter of milk)				
65	510	20	10	7	8	8	8.25
75	510	20	41	38	41	41	40.25
65	310	20	10	6	8	8	8.00
75	310	20	85	84	84	85	84.50
65	510	40	5	6	9	11	7.75
75	510	40	71	72	71	72	71.50
65	310	40	8	11	9	11	9.75
75	310	40	172	171	167	172	170.50
70	410	30	6	7	9	10	8.00
60	410	30	6	7	7	8	7.00
80	410	30	168	169	172	172	170.25
70	610	30	8	9	8	9	8.50
70	210	30	9	12	13	8	10.50
70	410	10	5	6	8	10	7.25
70	410	50	57	56	53	49	53.75

TABLE 2

Influence of temperature, vacuum treatment and duration of heat treatment upon nitroprusside value in milk

Temp.	Pressure	Time	Trial			
			1	2	3	4
(°C)	(mm. Hg)	(min.)	(nitroprusside value)			
65	510	20	0	0	0	0
75	510	20	2	2	2	2
65	310	20	0	0	0	0
75	310	20	2	2	3	2
65	510	40	0	0	0	0
75	510	40	1	1	1	1
65	310	40	0	0	0	0
75	310	40	3	3	2	3
70	410	30	1	1	0	1
60	410	30	0	0	0	0
80	410	30	2	2	4	3
70	610	30	1	1	1	1
70	210	30	1	2	2	1
70	410	10	0	0	0	1
70	410	50	1	1	2	2

at or above the critical temperature of 75° C. It is not appreciable at temperature levels of 60, 65 and 70° C. This was also observed by Kiermeier and Hamed (34). The increase is more apparent in respect to the volatile sulfhydryl production under vacuum effect (34). Water loss due to evaporation from the milk and its effect on the serum proteins which are thought to be the major source of sulfhydryls, seems to be more important when milk is heated under a reduced pressure.

The production of sulfhydryls increases with the rise of temperature from the minimum critical temperature of 75° C. This was the finding of other workers also (52,34).

Previous workers (3) have shown that for each 0.1 mg of volatile sulfhydryls evolved, there is a decrease of 1.0 mg of sulfhydryls present in the milk. This indicates therefore that under some suitable combination of temperature, time and vacuum relationship cooked flavor might be removed.

The time effect was significant (29 ug sulfur at 10 minutes and 215 ug at 50 minutes), a finding which is in conformity with those of others (52).

The data in Table 3 indicate a slightly greater degree of cooked flavor in those samples having been heat treated under the greatest pressure. These differences, however, are not statistically significant (Appendix C).

The data in Table 3 indicate that, however, the scores of the commercially pasteurized samples were different. The average scores of the 25 samples at an absolute pressure of 610, 510, 410 and 310 mm

TABLE 3

Influence of vacuum level upon cooked flavor in milk heated at 75° C
for 30 minutes

	Judge					
Trial	1	2	3	4	5	Avg.
Absolute Pressure 610 mm. Hg						
1	3.5	3.0	4.0	3.5	2.5	2.56
2	2.5	3.0	2.5	3.5	2.5	
3	3.0	2.0	3.0	2.0	1.5	
4	1.5	2.5	2.5	2.5	2.5	
5	1.5	1.5	3.5	1.5	2.5	
Absolute Pressure 510 mm. Hg						
1	2.0	2.5	3.0	2.0	1.5	2.18
2	1.0	2.5	3.5	1.5	2.0	
3	2.0	2.5	2.0	2.0	2.0	
4	2.0	2.0	2.5	2.0	3.5	
5	2.0	3.0	1.5	2.5	1.5	
Absolute Pressure 410 mm. Hg						
1	3.0	2.5	3.0	2.5	1.0	2.14
2	2.0	1.5	2.0	1.5	2.5	
3	2.0	2.5	2.5	3.0	2.0	
4	1.5	2.0	2.0	1.0	1.5	
5	3.0	2.0	2.0	2.5	2.5	
Absolute Pressure 310 mm. Hg						
1	2.5	2.0	2.0	1.5	2.0	2.02
2	3.0	1.5	2.5	3.0	1.0	
3	2.0	3.5	1.0	2.0	3.5	
4	1.0	2.5	1.5	1.5	0.5	
5	2.5	1.5	2.5	1.5	2.5	

Hg were 2.56, 2.18, 2.14, 2.02 respectively. The average score on the commercially pasteurized samples was 1.16.

Though the influence of different levels of pressures is not statistically significant, reduced pressures may have an effect on the reduction of cooked flavor, and when reduced pressures are combined with certain times and temperatures of heating, this might produce a statistically significant effect in reduction of cooked flavor from milk. The milk in these trials was heated for 30 minutes which allows sufficient time for the development of cooked flavor. If the time is reduced to only a few seconds, cooked flavor is not as likely to take place (52,20). Graves (20) states that vacuum treatment has aided in the reduction of cooked flavor.

The data in Table 2 indicate that with increase of temperature, time and vacuum, an increase in nitroprusside tests are also observed. The nitroprusside tests at 60° C to 65° C were negative, indicating that sulfhydryl groups in the milk are not activated. They are not available in free form to be acted upon by the sodium nitroprusside reagents to give the pink color. At temperature of 70° C the milk showed positive nitroprusside tests but insignificant production of sulfhydryls proving that first sulfhydryl groups in milk are activated and then volatile sulfhydryl production occurs as has also been observed by previous workers (3). However the nitroprusside test alone is not the sole indication of cooked flavor in milk but is a good aid along with other tests to determine chemically the development of cooked flavor. The nitroprusside test increases with the rise of temperature of heating of milk

and represents the proportionate denaturation and development of cooked flavor in milk.

Overall, the effect of temperature is important in nitroprusside value.

CHAPTER V

SUMMARY AND CONCLUSIONS

Milk has been heated at various levels of time, temperature and vacuum to study the influence of these factors on sulfhydryl production and subsequent development of cooked flavor. Organoleptic tests of milk at a constant temperature of 75° C and 30 minutes time with varied level of absolute pressure were also performed. The influence of reduced pressure and time is significant at or above the minimum critical temperature of 75° C and insignificant at temperatures of 60, 65, and 70° C. Overall the effect of temperature is significant in the production of sulfhydryl groups.

The influence of reduced pressure on the development of cooked flavor is slight; not enough to be statistically significant under the conditions of these experiments.

Prolonged heating of milk at 70° C for 50 minutes has appreciable effect on the production of volatile sulfide.

Temperatures of 75 and 80° C and prolonged heating of milk increases the production of volatile sulfhydryl groups, the development of cooked flavor and nitroprusside test.

The influence of reduced pressure has also the similar effect though not significant statistically below 75° C.

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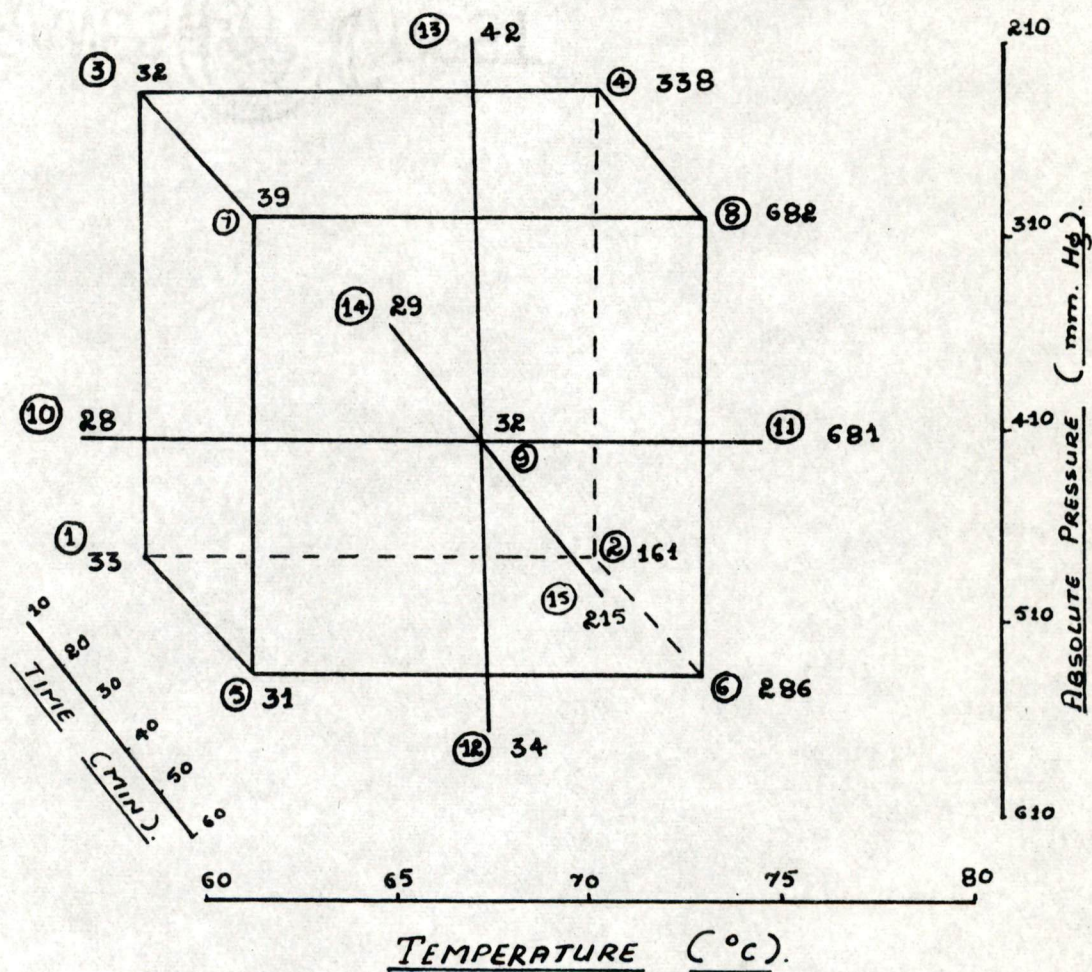
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APPENDIXES



APPENDIX A

DIAGRAMMATIC SKETCH SHOWING THE MICROGRAMS OF VOLATILE SULFUR EVOLVED FROM FOUR ONE-LITER SAMPLES OF MILK SUBJECTED TO DIFFERENT TEMPERATURES, PRESSURES AND HEAT DURATIONS (Numbers within circles are sample numbers)

APPENDIX B

ANALYSIS OF VARIANCE OF SULFUR EVOLUTION FROM MILK SUBJECTED TO DIFFERENT TEMPERATURES, PRESSURES AND HEAT DURATIONS

Source	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	59	183,780		
Trials	3	7	2.3	
Treatments	14	183,616	13,115.4	3,506**
65° C, 20 min vs. 40 min	1		1.6	0.43
65° C, 510 mm vs. 310 mm	1		3.1	0.83
65° C, interaction	1		5.1	1.36
75° C, 20 min vs. 40 min	1		13,747.6	3,675.83**
75° C, 510 mm vs. 310 mm	1		20,520.6	5,486.79**
75° C, interaction	1		2,997.6	801.49**
65° C vs. 75° C	1		55,444.5	14,824.73**
60° C vs. 80° C	1		53,301.1	14,251.63**
610 mm vs. 210 mm	1		8.0	2.14
10 min vs. 50 min	1		4,324.5	1,156.28**
	1		2,211.6	591.34**
Other Effects	1		4,170.0	1,114.97**
	1		13,514.1	3,613.39**
	1		13,366.7	3,573.98**
Trials x Treatments	42	157	3.74	

**Highly significant.

APPENDIX C

ANALYSIS OF VARIANCE ON FLAVOR SCORES

	Degrees of Freedom	Total Sum of Squares	Mean Square	F
Total	99	48.69	0.492	
Pressure	3	3.09	1.030	2.05
Judge	4	1.90	0.470	0.94
Trial	4	3.22	0.805	1.61
Pressure x Judge	12	4.20	0.350	0.70
Pressure x Trials	12	8.58	0.715	1.42
Judge x Trials	16	5.13	0.320	0.64
Error	45	22.57	0.502	