AN ABSTRACT OF THE THESIS OF

MATTHEW V. ANDREWS for the degree of MASTER OF SCIENCE

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Title: FLAVORS ASSOCIATED WITH THE USE OF CHEDDAR CHEESE WHEY POWDER IN ICE CREAM MIX

Abstract approved _____
(Dr. M. E. Morgan)

Flavor problems associated with the use of cheddar cheese whey in the formulation of ice cream and other food products were investigated. During spray drying of whey, which transforms bulky liquid whey into an easily transportable and storable powder, various degrees of off-flavor development may occur depending on the care with which the processing is carried out. Flavor problems present in the dry whey, which usually take the form of heated, stale, or sometimes burned notes, may then manifest themselves in mildly flavored frozen dessert products in which the powder is incorporated. Additional heating given such a finished frozen dessert during pasteurization may also contribute to the whey related off-flavors present by continuing heat induced flavor reactions (mainly Malliard non-enzymic browning and Strecker

degradation reactions) initiated in the original processing of the whey powder.

An ice cream mix model system was used to study the effects of varying whey quality, whey quantity, and heat processing load on ice cream flavor profile. The mix consisted of 27 samples, three parameters (whey quality, whey quantity, and heat processing load) using three levels of intensity within each parameter. There were three control samples containing no whey.

Whey "fingerprint" compounds were identified by headspace GLC/MS analysis of a poor quality whey powder which was later used with two other better quality wheys in the formulation of the model system. Comparison of the identified and some unidentified compound peaks in the "fingerprint" portion of the whey chromatogram to peaks present in the chromatograms obtained from model system analysis provided qualitative correlation. Subsequent quantitation of "fingerprint" compounds in the model system revealed data trends indicating that whey quality and product processing conditions may adversely effect the flavor profile of a frozen dessert product containing whey powder. The quantity of whey added (at least up to the level of substitution used in this work: 25%) did not set trends indicating adverse product effect with increased concentration. These numerical trends were established by using relative quantitation of the whey "fingerprint" or "indicator" compounds to

establish a peak area total for each model system member. When grouped by model parameter, trends in the peak area data were elucidated.

The conclusions reached from the data presented in this work are that whey flavors do carry through from powder to ice cream mix, are detectable, and may effect flavor profile of the ice cream. Also it became obvious that the flavor quality of the ingredient whey and careful selection of heat load for pasteurization of the ice cream mix are of primary importance in maintaining excellent flavor quality in frozen desserts employing whey to reduce manufacturing costs.

Sensory evaluation of the model system members was unable to establish direct off-flavor correlation with increased volatile compound concentration.

Flavors Associated with the Use of Cheddar Cheese Whey Powder in Ice Cream Mix

by

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To my fellow students and the remainder of the faculty of the Department of Food Science and Technology I wish to extend my thanks for your patience and understanding during my studies.

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INTRODUCTION

Spray dried cheddar cheese whey powder[1] has, during recent years, become an important source of non-fat dairy solids in a number of food products such as bread, crackers, cake mixes, ice cream, imitation cheese spread, snack items, and other foods which normally employ non-fat dry milk in their formulations.

Motivation behind the popularity of dried whey solids is mainly economic. The cost of whey powder is about one-tenth that of non-fat milk powder. In 1976 approximately 30 billion pounds of liquid whey were processed, creating about 1.9 billion pounds of powdered or condensed whey, a portion of which (about 20 million pounds) found its way into the manufacture of numerous frozen dessert products.

The many food uses of whey are of enormous economic and, because of the large quantities involved, environmental importance to the dairy industry. However, research and practical experience has led to the elucidation of numerous off-flavors, notably those in ice cream, sometimes described as stale, old ingredient, graham

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[1] Hereafter referred to as whey powder or simply as whey.

cracker or whey taint, which are directly attributable to the use of whey solids in product formulation.

The purpose of this work was to demonstrate that Maillard non-enzymic browning/Strecker degradation products produced in whey powder by high processing temperatures or staling, and the other volatile organic compounds associated with dried whey, become a significant part of the flavor profile of vanilla ice cream or any other mildly flavored food in which these products are incorporated for reasons of economics or flavor enhancement. This flavor effect has been termed whey flavor "carry through".

An ice cream mix model system containing whey powder was employed during the investigation to simulate whey's flavor response in a frozen dessert product. The use of such a system permitted control of the quality and quantity of whey powder added, control of heat processing load, and control of sample and ingredient irregularities which minimized off-flavor effects from mix ingredients other than whey powder.

Recent advances in volatile compound collection techniques using headspace gas entrainment on porous polymer precolumns and subsequent cold trap collection of precolumn eluent were employed with GLC and coupled GLC/MS systems to analyze and compare the compounds which were detected (and thus possibly were flavorful) in whey powder to those detected in the ice cream

mix model with similar whey powder in its non-fat solids component. The results of this analysis were studied to determine the nature and extent of the whey flavor carry through from the powder to the ice cream model.

The research confirms the apparently not so obvious concept that the flavor intensity and quality of a given whey powder are of maximum importance in determining the extent to which that whey will effect off-flavor development in the final (frozen dessert) product. Also confirmed was the relationship of heat processing load to the development of whey related off-flavors in foods where such flavors may have been insignificant prior to processing.

Differences in selectivity and sensitivity of the headspace collection technique employed in this work compared to the solvent extraction methods used in previous investigations limited GLC/MS identification to a small, but possibly more flavorfully significant, portion of the compounds described in that literature. The compounds detected served as whey indicator or fingerprint compounds which demonstrated flavor "carry through" effects in the model system.

The flavor abnormalities in frozen desserts attributed to whey powder addition are less prevelant today than in the recent past mainly because improved whey processing technology and a partial reversal of the poor personal attitude found in whey processing

establishments have improved the overall quality of whey powders available to food manufactures. Of necessity, such changes have occurred in much of the dairy industry concerned with processing large quantities of whey, never-the-less if a food manufacturer is not discriminating in whey product selection, this source of flavor trouble can easily become a major problem.

REVIEW OF LITERATURE

Composition of Whey Powder

In order to fully comprehend the volatile flavor compounds which may develop in spray dried whey powder during processing or storage and subsequently evolve upon its use in food, it is helpful to know the gross composition of the whey product involved. It is also necessary to have some understanding of how the components, fat, protein, carbohydrate, mineral, vitamin, flavor compound, or other constituent, interact to affect the overall flavor; and to observe the effect of heat processing on the flavor of the whey product itself or any food in which whey is incorporated.

The following are examples of the approximate gross analysis of liquid whey, skim milk, powdered dry whey, and non-fat dry milk (NFDM) (19, 6).

Table 1. Composition of some dairy products.

Component	Liquid Whey	Skim Milk	Whey Powder	NFDM
Water	93.2	90.5	4.5	4.0
Fat	0.25	0.10	1.1	0.7
Protein	0.90	3.60	12.9	35.8
Lactose	5.10	5.10	73.5	51.5
Ash	0.55	0.73	8.0	7.9

The presence of certain amino acid residues in the whey protein fraction of liquid whey is of importance in determining functional groups which might be available for participation in chemical browning reactions with other components of the whey upon heat processing. Participation of these amino acids in other reactions besides browning is not contra-indicated. The following is a partial listing of amino acids present which contain the more reactive side chain moieties (6).

Table 2. Amino acid levels in whey proteins.

Amino Acid	β-Lactoglobulin[a]	α-Lactalbumin[a]	Serum Alb.[a]	I_g[a]
Aspartic acid	10.2	17.1	9.4	8.1
Glutamic acid	17.9	11.9	14.4	10.7
Cysteine[b]	0.6	0.0	5.5	2.7
1/2 Cystine[c]	2.3	5.8	included above	
Lysine	10.7	10.9	11.2	6.0
Phenylalanine	3.3	4.2	5.9	3.5
Methionine	2.9	0.9	0.7	0.8
Alanine	5.5	1.5	5.0	3.8

[a] Weight percentage of residues per mole of protein

[b] Not involved in S-S bridges

[c] Bridged Cys

The major functional amino acids, lysine (epsilon NH_2), cysteine, methionine, and phenylalanine are present in the liquid whey in sufficient quantity to be presumed significant in heat induced chemical pathways. The epsilon NH_2 of lysine is suspected to be a participant in the Maillard/Strecker degradation reactions which result in the synthesis of alkylpyrazines (14). These reactions occur in both powdered milk and whey products as heat is applied or as the product stales during storage (12, 13). The amino-carboxylic acid portion of the amino acid moiety and the side chains of Phe, Met, Ala, and Cys, are also believed to participate in Strecker degradation reactions producing alkylpyrazines and other compounds found in whey (38, 40). The free sulfhydral group of Cys is believed to participate in the formation of cooked flavors which develop in milk during pasteurization (8).

Ferritti and Flanagan note that whey is more subject to such browning reactions than is non-fat dry milk, presumably due to the presence of more lactose and the shift in protein profile (11).

Volatile Compounds Found in Whey and Other Products

Ferritti and Flanagan (12) found that Maillard non-enzymic browning/Strecker degradation products such as alkyl substituted pyrazines, furans, and aldehydes; other N-heterocyclics, and several aliphatic and cyclic aldehydes, ketones, and acids (probable

remnants of lipid oxidation) were a principal part of the flavor profile of dried whey solids. Dimethysulfone, while not particularly flavorful, was the single most abundant compound detected. A suggestion from a later article (13) that lipid deterioration is a contributing factor in stale flavor development in dairy products was also of interest. Table 3 lists the major flavor compounds Ferritti and Flanagan found in edible spray dried whey powder.

Table 3. Compounds identified in the steam distillate of spray-dried whey (12).

2-Methylpyrazine	2-Formylpyrrole
2,5-or 2,6-Dimethylpyrazine	n-Methyl-2-formylpyrrole
2,3-Dimethylpyrazine	α-Methyl-γ-butyrolactone
2,3,5-Trimethylpyrazine	Isobutyramide
2-Methyl-5-ethyl- or 2-Methyl-6-ethylpyrazine	n-Methyl-2-pyrrolidione
C_4-Alkylpyrazine	3-Hydroxy-2-butanone
2-Methyl-5-vinyl- or 2-Methyl-6-vinylpyrazine	Benzaldehyde
2-Acetylfuran	Phenol
Furfuryl Alcohol	Benzyl Alcohol
2-Propionylfuran	Maltol
	Dimethylsulfone
	Propionic acid
	Butyric acid
	Benzoic acid

In addition to this work, these authors analyzed freeze dried whey subjected to accelerated browning (11), stale non-fat milk powder (13), and model systems containing lactose-casein (10) and lysine-lactose (14). These last two systems simulated foods containing sugar and amine compounds.

Ferritti and Flanagan's work with edible spray dried whey (12) is descriptive of the type of compounds used in the present research as indicator or fingerprint compounts to demonstrate the whey flavor "carry through" effect.

Comparison of articles by Ferritti and Flanagan pertaining to browning reactions in two different whey powder systems (11, 12) lends support to the notion that either browning or staling may effect the overall flavor of the whey product. Furan, ether, ketone, and pyrrole compound groups appeared in much greater profusion in freeze dried whey subjected to the accelerated browning conditions, 75 C, 75% RH, for four days, than in normal spray dried whey. The absence of pyrazines, as a group, from the rapidly browned whey was an unexpected result (11).

The difference in compound groups isolated from the two whey powders, i.e. abundance of N-heterocyclics in normal spray dried whey powder, and abundance of 0-heterocyclics and other oxy-hydrocarbons in the rapidly browned powder, may suggest a difference in synthetic pathway present when generating heat-formed

Maillard/Strecker products and other products from long time/low temperature staling (pyrazines); and the pathways active during short time/medium temperature staling (furans). The rapidly browned product may have received insufficient heat load to cause pyrazine synthesis yet this load did provide severe enough conditions to degrade its carbohydrate fraction allowing furan and oxy-hydrocarbon synthesis to predominate (14). The following table further examines the effects of time and temperature on compound groups present in whey powder and other systems.

Table 4. Time/temperature relationships in various browning systems.[c]

Compounds				
Pyr	Fur + O_2 Cpds	Product	Heat Processing Load	Storage Load
+	+	Spray-dried whey	150 C/10 min	3 yrs/4 C
-	+	Freeze-dried whey[a]	None	4 days/70 C
+	+	Stale non-fat dry milk	150 C/10 min	2.34 yrs/15 C[b]
+	+	Lactose-casein model	None	11 days/75 C
-	+	Lysine-lactose model	None	22 hrs/75 C

[a] Subjected to accelerated browning

[b] approximate temperature

[c] (10, 11, 12, 13, 14) cited.

Non fat dry milk and spray dried whey powder both received high heat treatments and are found to contain pyrazines; freeze dried whey (rapidly browned) and the two model systems received very little heat processing and two of the three contain no pyrazines, but

do contain furans and oxy-hydrocarbons. The storage or staling conditions of these three non-heat-processed products contains a clue to the low temperature, long time, storage conditions necessary for pyrazine synthesis. The most severly handled non-processed product contains furans, oxy-hydrocarbons, and pyrazines (lactose-casein model system 11 days/75 C), the other two less severly handled products contain only furans and oxy-hydrocarbons.

The Use of Whey in Ice Cream

Dried whey, modified (dialized) dried wheys, and the various whey concentrates, have been used as replacements for milk solids-non-fat in ice cream and sherbet since the middle 1940's (27). The original reasons for the use of whey are much the same today as then: cost, availability (or the lack of it) of milk solids, and flavor enhancement of a few specific products (18). Nielson, Frazeur, Arnold, and Igoe, (2, 17, 18, 23, 35) in separate works, cite statistics concerning cost and consumer or professional panel preference testing which confirm whey's usefulness. Fraseur (18) cites much literature which indicates no preference for ice cream samples containing electrodialized whey over a control sample containing no whey. Nielson(35) points out the possible shortcomings encountered in the use of whey; i.e. flavor, textural, and nutritional (low protein to lactose ratio) problems. The consumer

preference testing done by Frazeur (18, 17) in 1967 showed that NFDM and different quantities of electrodialized whey, as used in ice cream, produced no statistically significant (0.05) variation in flavor quality, up to a 25% whey substitution level. However, excellent quality and average quality non-dialized whey products were statistically inferior to NFDM in consumer acceptance. Arnold's work in 1967 (2) was less critical of the non-modified wheys and states that substitution levels up to 35% in ice cream may be acceptable.

There was no evidence produced by Frazeur or Arnold (2, 17, 18) which demonstrated a textural difference between ice cream containing whey and ice cream containing NFDM.

Whey Flavor in Ice Cream

Bills (4) described a situation in which an experienced flavor panel was able to detect a definite whey solids flavor in seven of 25 samples; four additional samples exhibited off-flavors which were described as probably whey related. In the same article Bills examined the possible connection between decline in overall flavor scores for vanilla ice cream in a state wide dairy product judging contest, during the period 1969-1973, and increased utilization of whey powder for ice cream manufacture in the same period. He concluded that increased usage of whey powder in ice cream

formulation has resulted in flavor and textural problems.

In view of the experience of Bills and the other authors who described consumer testing of whey fortified ice cream, we formed an hypothesis to account for the whey flavor carry through effect and have attempted its confirmation.

The relationship of heat and time and their effect on whey quality have been explored. Flavor changes which occur in the whey before its incorporation into an ice cream product fall into two catagories, those brought about by processing of the liquid whey and those brought about by storage of the dried whey.

It is our contention that some of these flavors found in whey manifest themselves in the flavor profile of the finished ice cream product, to its detriment.

Production Conditions Which Effect Whey Quality

Several sources (20, 1) describe the cyclone spray drier and ancillary evaporation equipment most often used to convert liquid whey (six percent solids) into a non-hygroscopic, edible, spray-dried powder containing four percent moisture. The maximum temperature encountered in the drying process (150 C) and the processing times, even at lower temperatures, provide sufficient heat load to synthesize the volatile flavor compounds found in whey (30).

Gillies (20) describes the DeLaval spray-drier in some detail. From his description, it is evident that the semi-moist whey powder which coats the warm walls of the drier can, as moisture is removed, be badly over-processed if it adheres for a sufficient length of time. Such a system is very susceptable to local over-processing phenomina especially adjacent to isolated hot spots along the drier wall, and places where caking of the powder is particularly pronounced.

Other situations which often result in poor or improper processing of whey are: bacterial contamination, improper plant sanitation, lengthy storage of liquid whey (resulting in oxidative deterioration of the small lipid fraction present), gross over-heating (caramelization), improper seed crystalization of lactose resulting in clumping and water retention in the crystal matrix, and poor human attitude.

Figure 1 is a flow chart describing the whey drying operation (42).

Maillard Non-enzymic Browning/Strecker Degradation Pathways

Many of the N and O aliphatic and heterocyclic compounds present in whey powder, the model system mix, and various other products encountered in this research are believed to be products

Liquid Whey

Heat Shock----- Microbial Protection 64.2 C (147.5 F)

Storage Tanks

Evaporator

Hot well-- 64.2 C up to 87.8 C (190 F) 15-18% solids

Triple Effect Evaporator

Preheat---- 87.8 C (Maintained)

Cool ------- to 48.9 C (120 F)

Finish Pan --- 82.2 C (180 F)

Cool -------- 43.3 C (110 F) 35-40% solids

Seed and Storage Vats (towers)

Cool ------- 15.6 C (60 F)

Seed ------- 15.6 C 52% solids

Spray Dryer

Heat ------ 148.9-154.5 C (300-310 F)

Cool ------ 46.1 C (115 F)

Redryer

Heat ------ 82.2 C (180 F)

Cool ------ 43.3 C (110 F)

Dry, non-hygroscopic Whey Powder (4% moisture)

Figure 1. Flow chart for drying of liquid whey to a non-hygroscopic powder.

of Maillard non-enzymic browning and Strecker degradation-condensation reactions (10, 11, 12, 13, 14). Aldol condensations and polymerization reactions can also be expected to occur (38).

The main synthetic pathways relevant to this research are the pathways which lead to pyrazine, furan, or oxy-hydrocarbon formation. Rizzi (38) recounts work which indicates that pyrazines normally arise from complex pathways involving α-amino acids and carbohydrate fragments. He cites literature which indicates that carbohydrate-ammonia solutions and oxy-α-amino acids have yielded pyrazines upon heating. The reactions of α-dicarbonyl compounds with α-amino acids were also studied; α-dicarbonyls are ubiquitous in food products and are believed to participate in Strecker degradation of α-amino acids.

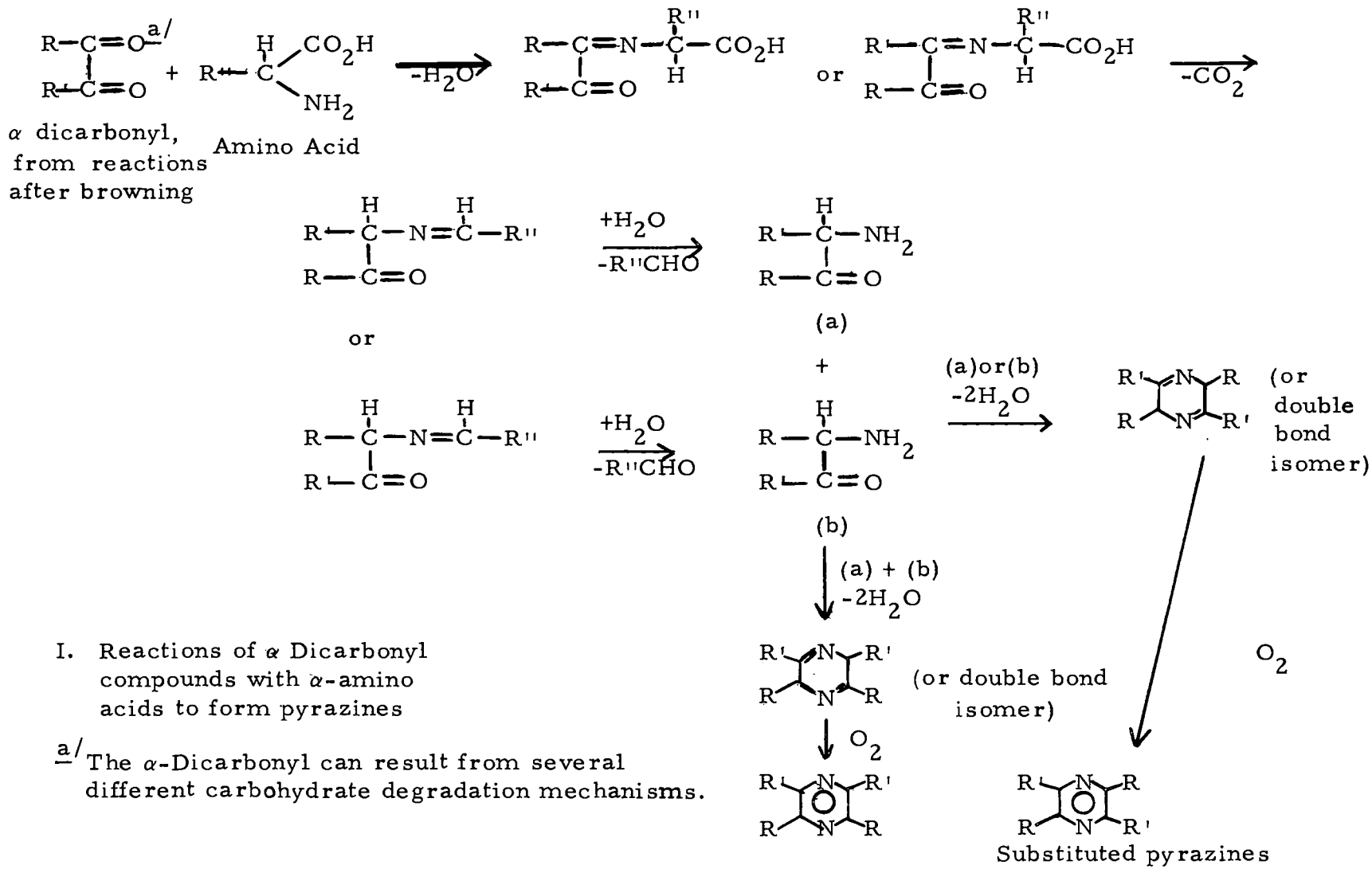
Shutte (40) also described pathways involving α-dicarbonyl induced Strecker degradation of an α-amino acid resulting in amino-aldehyde (ketone) synthesis; two moles of this amino-aldehyde then condense to an alkylpyrazine. Shutte lists several other potential flavor compounds which could arise via Strecker degradation-condensation reactions and presents a pathway by which substituted furans are produced from browning reactions involving hexose (pentose)-amine precursors, (see Figure 3).

Rizzi (38) describes tetraalkylpyrazine synthesis involving the Strecker degradation of DL-phenylalanine with mixtures of

diacetyl and dipropionyl resulting in production of tetramethyl-, diethyldimethyl-, and tetraethylpyrazines. The pathways below, Figures 2, 3, 4, 5, have been selected as representative of the literature; some may be speculative in places and possibly over simplified because they were derived from model system rather than food research, however, they seem reasonable from a synthetic chemistry point of view.

Other Pyrazine Synthesis Conditions

Pyrazine synthesis is influenced by temperature (of heating or storage), moisture content, time, pH, and the molar ratio of sugar to available amine compounds (14). Maga (30) in his review of pyrazine literature was specific concerning synthetic conditions, factors effecting yield, and pathways involving condensation reactions. He cited literature which indicates that pyrazine synthesis during short time periods rarely occurs below 100 C (212 F) and becomes variable above 150 C (302 F) due to decomposition. Pyrazines were rapidly synthesized at 120 C during a 24-hr period and only gradually increased in concentration thereafter. Temperatures as low as 70 C for 30 min were found, in a few cases, to be sufficient for pyrazine synthesis (usually in products such as cacoa). Reaction rate of pyrazine synthesis was influenced by acid-base conditions, and amino acid or carbohydrate source.

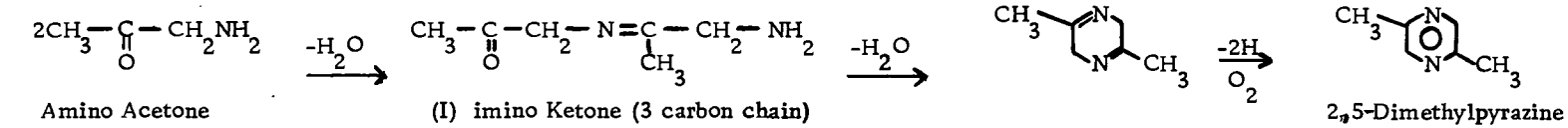


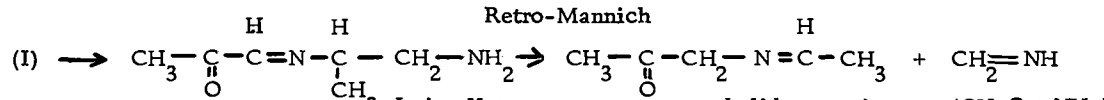
I. Reactions of α Dicarbonyl compounds with α-amino acids to form pyrazines

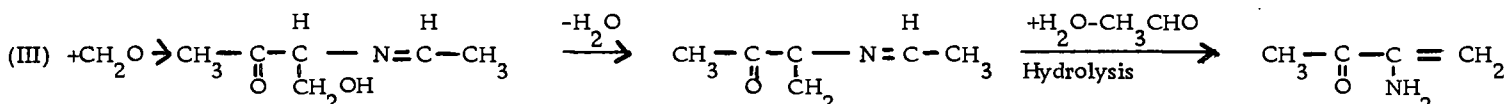
a/ The α-Dicarbonyl can result from several different carbohydrate degradation mechanisms.

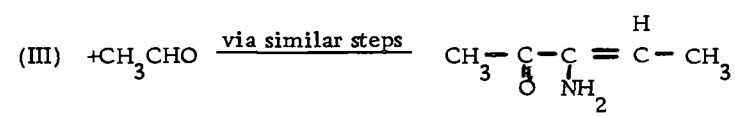
Figure 2. Pathways involved in pyrazine synthesis (38).

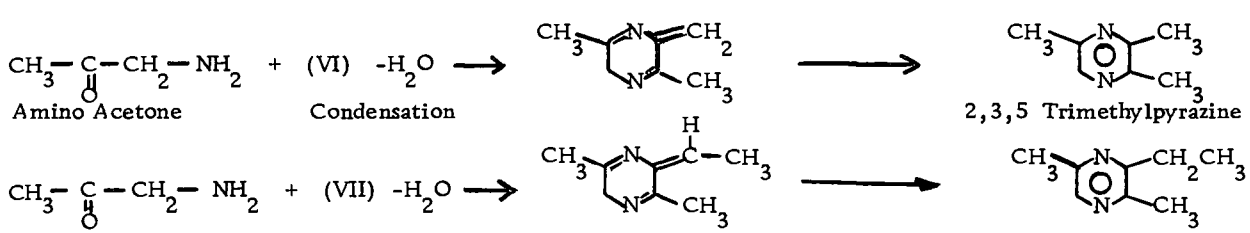
Figure 2. Continued



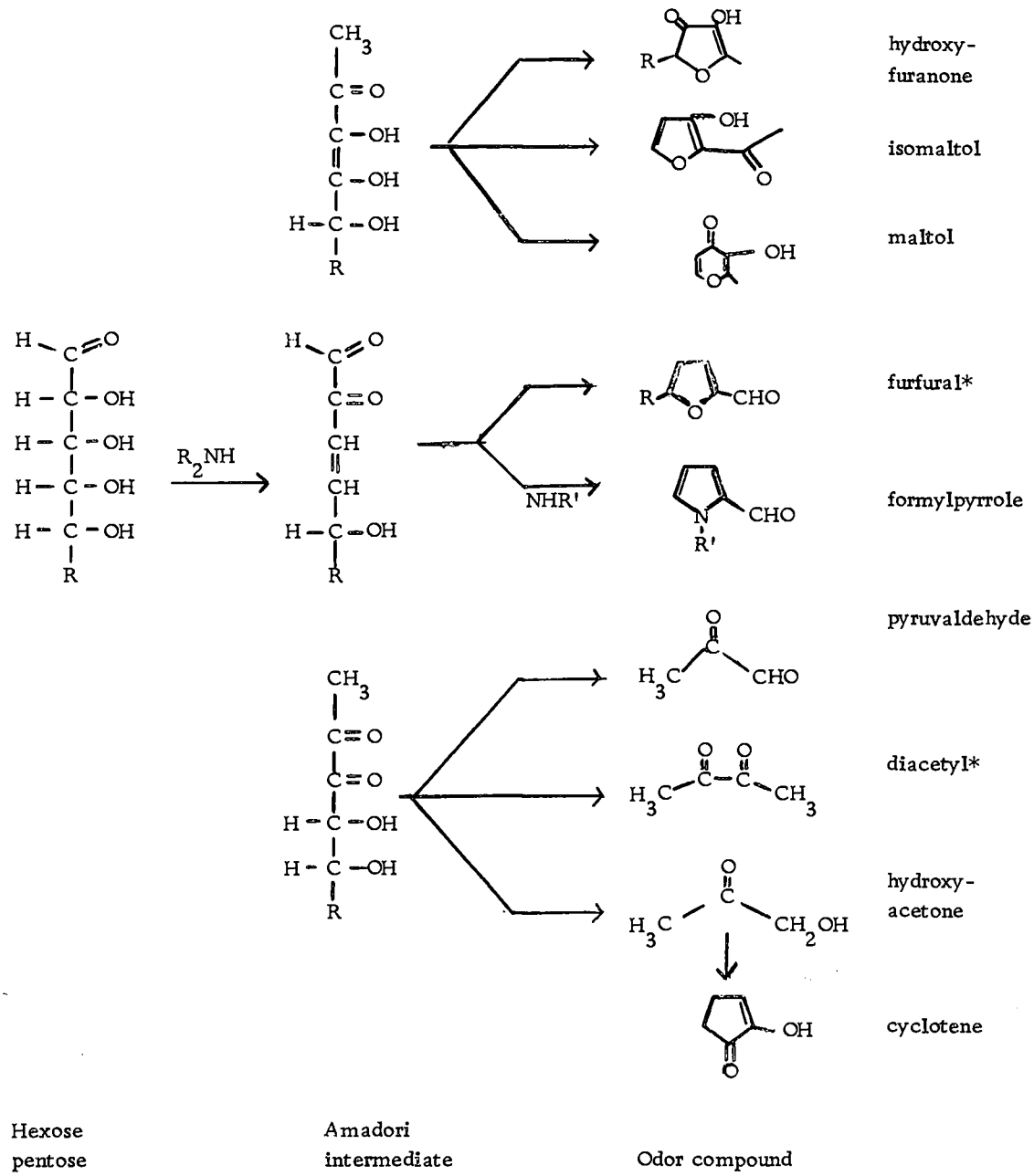








II. Condensation of Amino Acetone to form Pyrazines (38).

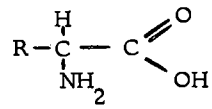


[a] The above pathways account for furan presence in sugar-amine mixtures and indicate the source of many of the dicarbonyl compounds required for subsequent Strecker degradation/condensation reactions resulting in pyrazine synthesis.

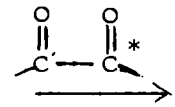
* Isolated in whey or model system.

Figure 3. Furan synthesis pathway involving Amadori intermediates of carbohydrate reactions (including browning) (40).

Reaction Pathway



Amino Acid



Product

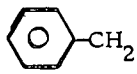
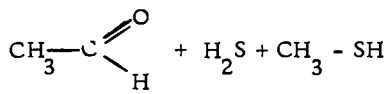
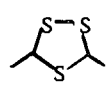
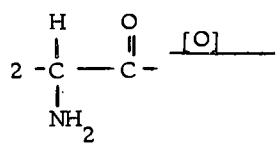
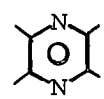
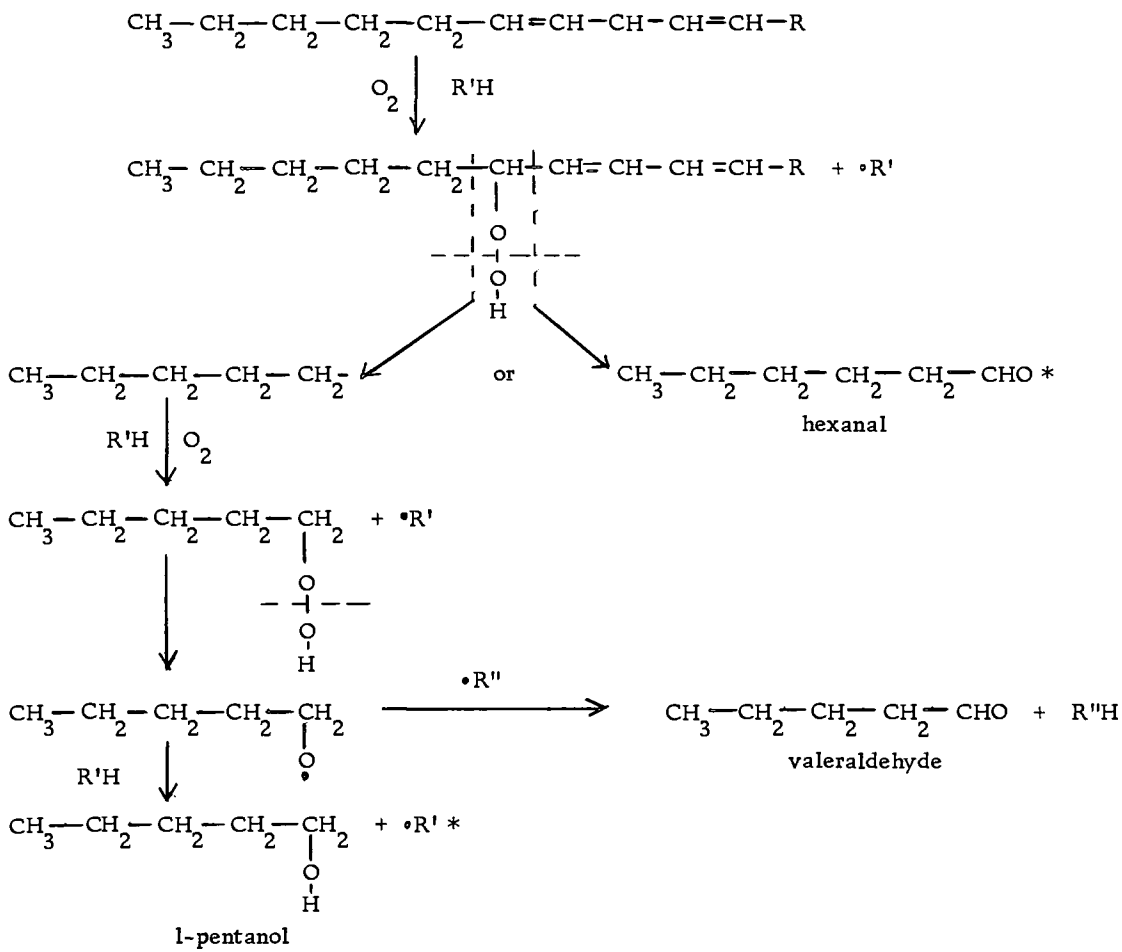
R	PRODUCT
CH_3-	acetaldehyde
$CH_3-S-CH_2-CH_2-$	methional
	phenylacetaldehyde
	benzaldehyde *
CH_3-	
$HS-CH_2-$	$CH_3-CH(SH)-S-CH_3$ 1-methylthioethanethiol
$CH_3-S-CH_2-CH_2$	$CH_3-S-S-CH_3$ di-Me-disulfide
	CH_3-S-CH_3 di-Me-sulfide
	$CH_3-S-S-S-CH_3$ di-Me-trisulfide *
	 di-Me-trithiolane
Any Amino Acid	  pyrazines

Figure 4 provides a useful summary of those compounds in whey which could possibly have arisen from Strecker degradation-condensation reactions. The compounds in Figure 4 marked with an asterisk (*) have been detected in whey or the model system, the remaining compounds are often present in other heated systems.

Figure 4. Other compounds resulting from Strecker degradation of amino acids (40).



[a] The small amount of lipid occurring in whey powder may account for the presence of short chain alcohols, aldehydes, ketones, lactones, and acids.

* Found in whey and model system.

Figure 5. Possible lipid oxidation reactions in whey (15).[a]

Glycerol, acetaldehyde, glyoxal, 2, 3-butanedione (diacetyl), hydroxyacetone, and glucosamine in addition to sugars, were known to react with an amine source and condense to form specific pyrazines.

Maga cited work by Koehler (25) which employed radiolabels to confirm the source of the pyrazine carbon skeleton as carbohydrate and source of the nitrogen heteromolecules as the amine function of an amino acid.

Literature concerning the effects of time/temperature relationships on pyrazine synthesis was discussed by Maga (30). Under low temperature, long-time conditions, a postulated pathway involves reaction of sugars and amino acids to form a ditetrahydroxybutylpyrazine intermediate produced by condensation reactions. This undergoes rearrangement and cleavage to form alkylpyrazines.

Under higher temperature conditions, immediate rearrangement and cleavage of sugars resulted in the formation of small hydroxycarbonyl and dicarbonyl fragments which, as cited earlier, react along various pathways with amino acids (Strecker degradation and condensation reactions) to form alkylpyrazine compounds.

Maga cites work by Wang et al. (46) which proposes pathways by which pyrazine synthesis occurs by condensation of the browning reaction product cis-methyl reductone with glyoxal or pyruvaldehyde and amino acids to produce acetyl- and methyl-acetylpyrazines.

Comparable Analysis of Other Heated Foods

Previously cited literature has established that alkylpyrazines, furans, oxy-hydrocarbons and other volatile organic compounds are produced by synthetic pathways active during the processing of whey powder and other heat processed ice cream ingredients. It seems reasonable that flavor compounds could also arise during processing conditions encountered in production of a whey fortified ice cream, in addition to, and possibly aided by those, induced by the inclusion of whey in the ice cream mix. The following citations describe heated flavors produced in a variety of foods under many different processing conditions which lends support to this belief.

Meat Flavors

Mussinan et al. (33, 34) found pyrazine, methylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 5-ethyl-2,3-dimethylpyrazine, and other more complexly substituted pyrazines, 33 in all, in the steam distilled ether extract of pressure cooked beef. They found 35 similar pyrazines in the steam distillate extract of cooked pork liver. Many substituted furans were also isolated from the pork liver. The authors relate the presence of the pyrazines in these systems to Maillard non-enzymic browning reactions involving carbohydrate and protein precursors present in the meats.

The temperature of cooking in both cases was 162.7 C (325 F) for 15 min.

In addition to pyrazines and furans, the article concerning pork liver compounds presented evidence indicating the presence of a great number of aliphatic hydrocarbons, ketones, esters, thiazoles, other sulfur compounds, and pyrroles. Compounds in the pork study which are related to the present work on whey were: Hexanal, benzaldehyde, 2-furaldehyde (2-furfural), furfuryl alcohol, and 2-pentanone.

An earlier paper by Liebich et al. (28) mentions only a single pyrazine present in the ether extract of vacuum distilled roast beef and roast beef drippings. n-Hexanal, 1-pentanol, benzaldehyde, dimethylsulfone, and dimethylpyrazine were found. In addition, numerous other ketones, aldehydes, alcohols, furans, lactones, various saturated, mono, and di-unsaturated aliphatic hydrocarbons and aldehydes were detected.

A boiled beef system mentioned in the same article had a small fraction of the compounds present in the roast system but did contain two additional pyrazines: trimethyl- and dimethylethyl-pyrazine.

Peterson (37) concludes that differences in flavor profile between canned and fresh beef stew are due to "thermal oxidative fat decomposition, thermal decomposition of amino acids and

carbohydrates, and the browning reactions characteristic of roasting or baking processes."

Wasserman (48) describes processes by which meat flavors are developed (aging, method and temperature of cooking, and so forth). He relates these flavors to compounds and pathways believed to exist in heated meat systems. Amino acid degradation (not Strecker degradation of amino acids) is a high temperature process which would occur rarely in most cooked systems; however, carbohydrate decomposition and amino acid-carbohydrate reactions were described as potential meat flavor pathways. No characteristic meat aroma compounds were cited by the authors. The role of sulfur containing compounds and lipids in meat flavor was also discussed.

Cocoa and Coffee

The CO_2 distillate of the basic fraction of roasted cocoa was found by Vitzthum et al. (44) to contain some 34 alkyl and aryl substituted pyrazines, and nine cyclo-alkylpyrazines; many of which were similar to those found in pressure cooked beef, pork and boiled beef. The 34 pyrazines mentioned were newly identified compounds, some 310 volatile compounds had been previously identified in cocoa prior to Vitzthum's work. Also described in the same article were four oxazoles, seven pyridines, three

quinoxalines and quinoline. No substituted furans were found, except in fused ring systems with pyrazine groups. O-aminobenzoate was also found. No typical cocoa "characteristic compound" was isolated.

Mechanisms believed responsible for synthesis of the pyrazine fraction of the cocoa isolate are believed similar to those responsible for such synthesis in other foods.

Compound groups similar to those reported in cocoa, above, have been detected in coffee. Vitzthum (43) describes previous work indicating the presence of pyrazines, oxazoles, thiazoles, pyrroles, acetylpyridines, acetyl- and furylpyrazines, quinoxalines, indoles, and quinolines, 86 total. He presents new work describing 17 cycloalkylpyrazines found in the basic fraction of coffee isolate.

Vegetable Product Flavors

Roasted filberts and roasted peanuts were investigated by Kinlin et al. (24) and Walradt et al. (45). They found a large quantity of substituted pyrazines, pyrroles, pyridines, furans, terpenes, esters, aldehydes, ketones, lactones, alcohols, phenols, and sulfur compounds. Two organic acids and several aromatic hydrocarbons were detected in filberts. No acids or benzene derivatives were found in the peanuts. Thiazoles as a group were present in the peanuts and absent from the filberts. The mechanisms proposed to

account for the synthesis of pyrazines in both articles are similar to those proposed by most authors, namely, carbohydrate degradation to produce the carbon skeleton of the pyrazine moiety and amino acid or ammonia donation of the nitrogen molecules.

Baked potatoes (36) and sesame seeds (31) both contained pyrazines, furans, and aromatic aldehydes as a majority of the compounds detected, all of which appeared to be heat induced products involving pathways which use sugars, amino acids, or lipids as precursors. Potato chips (7) displayed aliphatic aldehydes, ketones, alcohols, a few sulfur compounds, two aromatic aldehydes, eight substituted furans, and one nitrogen heterocyclic: 2-acetylpyrrole. Alpha-terpineol was also isolated.

Work by Hashiba (21) on soy sauce described many sugar-amino acid Amadori compounds in browned soy sauce. The author suggests that these Amadori compounds play a major role, with oxygen, in soy browning but does not indicate the flavor compounds resulting from these reactions. Carbonyl compounds, normally very reactive, were believed to be of minor importance in soy browning because they are present only in very small quantities.

Lactose Flavor Affinity

Studies by Yabumoto et al. and Lee et al. (49, 26) indicate that anhydrous α-lactose has a high affinity for low molecular weight

compounds similar to those usually found in the flavor profile of food substances. The affinity is mainly for oxygenated compounds such as alcohols, aldehydes, ketones, esters, and so forth but may possibly extend to nitrogen containing molecules. This ability of lactose to bind compounds to itself may partially account for whey's unusual flavor problems in ice cream. Whey is usually a more flavorful product than NFDM and may impart a distinct flavor to ice cream products it is used in. It may be possible to attribute these problems to the higher proportion of lactose in whey (ca 73% lactose in whey; ca 56% lactose in NFDM) and conclude that lactose may influence the flavor profile of a frozen dessert product by transporting flavor compounds present in whey into the ice cream matrix where they manifest themselves as whey taint or other off-flavor.

EXPERIMENTAL

Whey Products

Samples of spray-dried cheddar cheese whey powder were obtained from Tillamook County Creamry Association, Tillamook, Oregon and Kraft Food Co., Chicago, Illinois. The Kraft product is marketed under the trade name Krafen. These products were edible whey powder with low visible browning.

One sealed sample of Tillamook whey, dated 5-75, was stored for one year in a dry, room temperature, storage area which was a reasonable approximation of industrial storage conditions. The other wheys obtained later, Tillamook 5-76, and Krafen 6-76, were stored at room temperature in closed containers prior to use in either model system or whey product analysis.

Ice Cream Model System

The model system used to simulate a commercial ice cream mix consisted of 30, 50-ml samples containing 12.0 percent butterfat and 11.0 percent solids not fat. It was designed to reflect the following variables (system parameters): whey quality, whey quantity, and processing temperature. A supply of all mix ingredients other than whey (i.e. NFDM, sugar, skim milk, 38%

cream) was obtained commercially and subjected to sensory evaluation for flavors such as stale, cooked, and old ingredient which might affect sensory or organic volatile compound analysis of the system. The model was constructed in duplicate, one sample for sensory and one for GLC analysis.

The non-whey mix ingredients were cleanly flavored and demonstrated only minor quantities of whey indicator compounds in control samples (which contained no whey powder) subjected to chromatographic analysis. Care was taken during preparation of model system samples to minimize product exposure to non-refrigerated conditions or oxidizing atmosphere.

Samples in this system were designed to contain 0, 15, 17.5, and 25% whey substituted, by weight, for non-fat milk solids in the serum portion of the mix. Subjectively judged, good (Tillamook 5-76), fair (Krafen 6-76), and poor (Tillamook 5-75), wheys were used at each substitution level. After preparation and mixing, samples were placed in 2.5 x 15 cm screw top, culture tubes and processed in a temperature controlled water bath with the following heat loads: 150 F (65 C) for 50 minutes; 150 F for 68 minutes; and 155 F (68.3 C) for 78 minutes. After processing the samples were cooled to -40 F (-40 C) and stored at this temperature prior to analysis. There were a total of 27 samples and 3 controls.

Selection of Model System Parameters: Whey Powder Percentage

Choice of the 15, 17.5 and 25 percent substitution levels was based on the existing legal maximum substitution percentage, 25%; the hypothesized flavor threshold level of whey flavors, 17.5%; and a percentage believed to be well below the hypothesized threshold level, 15%.

Whey Powder Flavor Quality

Three flavor qualities of whey powder were selected, based on subjective sensory analysis, to reflect the quality of powders commercially available. The "good" powder, Tillamook 5-76, while possessing a distinct flavor, had no stale or old characteristics and was not cooked or over heated. The fair quality whey, Krafen 6-76, was marginally inferior to the good quality whey in overall flavor intensity but had no pronounced off flavor characteristics. The poor quality whey, Tillamook 5-75, was markedly stale and exhibited a much stronger overall flavor and odor than the other two. None were of extremely poor quality.

Model System Processing Loads

There were several methods available to achieve pasteurization

of this dairy product system; batch, high-temperature-short-time, and ultra high temperature; all of which have individual advantages depending on product viscosity, resistance to heat conduction, quantity of product to be processed, and length of storage required. The batch (low-temperature-long-time) method was selected because small sample sizes were involved (50 ml).

Authors differ on the proper combination of time and temperature for batch processing of an ice cream mix. Frandsen and Arbuckle (16) suggested 155 F (68.3 C) for 30 minutes, Igoe (23) advocated 165 F (73.9 C) for 30 minutes. As a compromise between these two heat loads, to bracket the "normal" pasteurization line expressed in Figure 6, and to include processing loads which would be good approximations of the industrial heat processing stress range, the following time-temperature combinations were chosen: 150 F for 50 and 68 minutes, and 155 F for 78 minutes. Figure 6 illustrates the relative position of these heat loads compared to the "normal" ice cream time-temperature processing line. The heat loads used fall below (pt. 1), almost on (pt. 2), and well above (pt. 3), this "normal" line.

Figure 6 is constructed using a semi-logarithmic plot of the two most common time-temperature processing conditions used for ice cream manufacture: 155 F for 30 minutes and 175 F for 25 seconds (.42 minute). The heat loads used for the model system

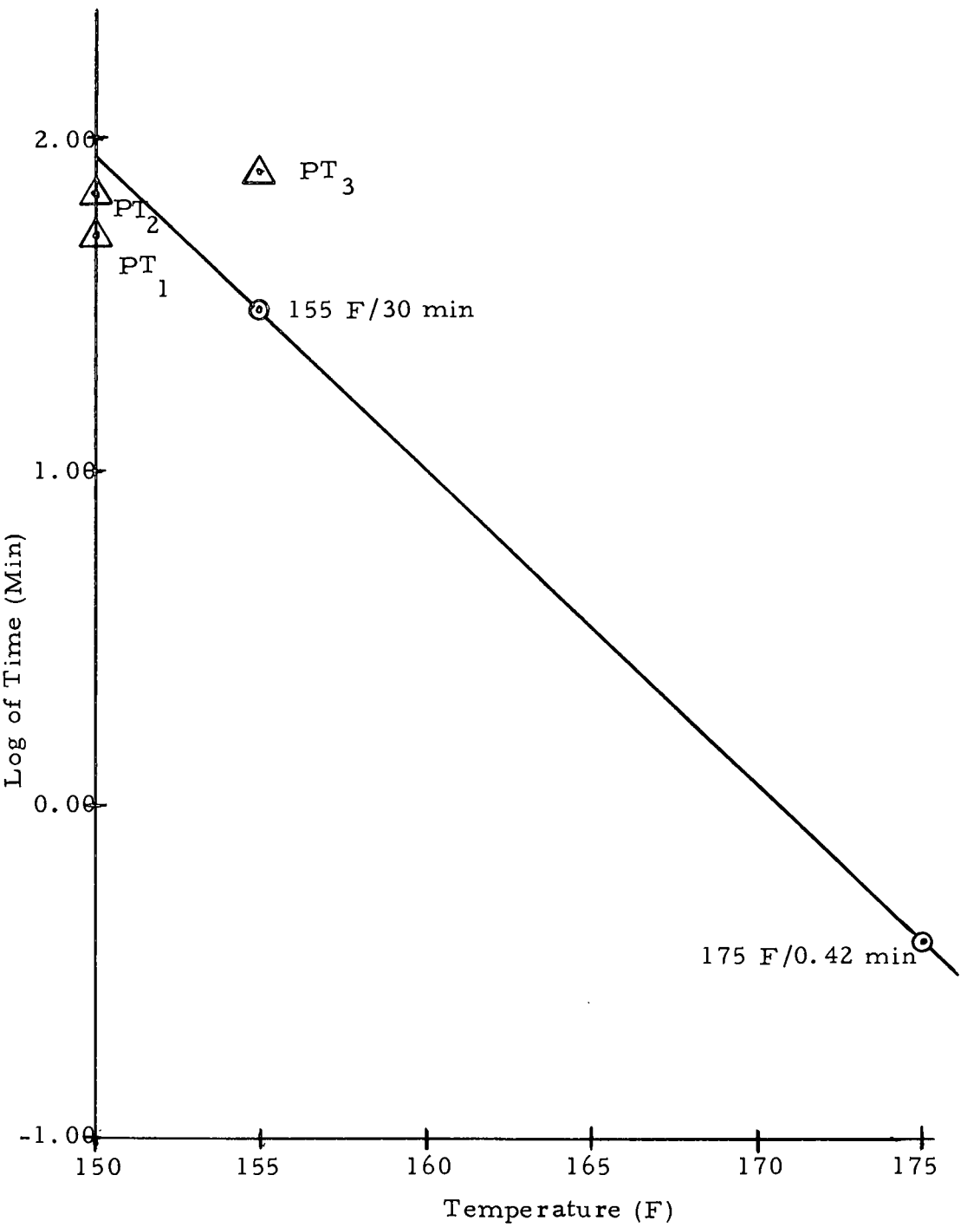


Figure 6. Semi-logarithmic plot of "normal" ice cream pasteurization line and model system pasteurization loads.

are 58.8, 80, and 280 percent, respectively, of the "normal" processing conditions at a given temperature.

Collection of Volitile Compounds from Whey Powder and Model Samples

The collection of moisture free volatile organic compounds from dry whey and model system samples and their subsequent GLC analysis was accomplished using techniques described by Miller et al. (32), Boyko (5), and many others.

Dry Sample Analysis

About 30 grams of dry whey powder was placed in a 2.5 X 15 cm screw-top culture tube fitted with a two-holed plastic lid containing a silicone rubber septum. This assembly was attached to the apparatus in Figure 7. Prepurified N_2 was passed, at 30 ml/min, through a 20 ft X 1/4 in gas cleanup trap packed with 60-80 mesh firebrick in a bath of 2-methoxyethanol cooled with Dry Ice and subsequently through the sample container. The volatile compounds thus entrained from the sample were collected on a 10 cm precolumn of 100/120 mesh Porapak Q maintained at 55 C with an electrically controlled heat gun to prevent condensation of water vapor. Channeling in the dry sample produced by gas flow, was prevented by agitation with a Sonicator. After a 60 min collection

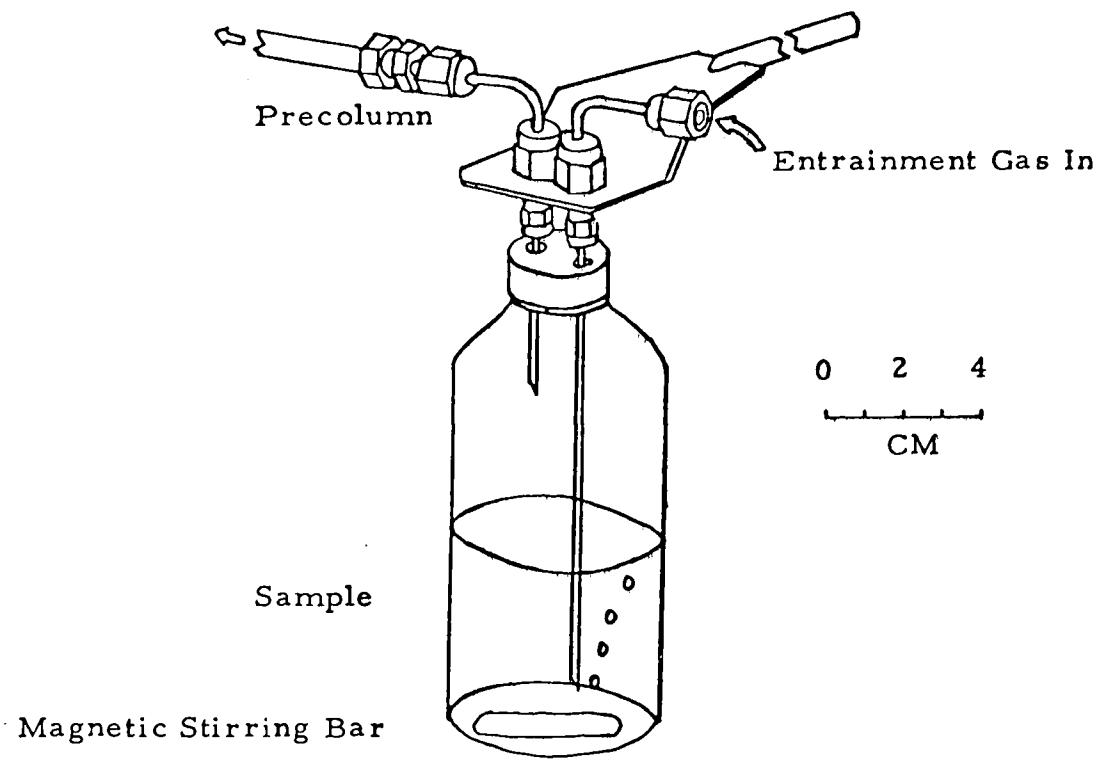


Figure 7. Entrainment (collection) assembly (42).

period, the headspace sampling device was disconnected. The precolumn was connected directly to the N_2 line and gas flow was continued for an additional 20-30 min. (at 55 C) to effect removal of any residual water collected on the polymer.

The precolumn was then heated to 135 C and back-flushed with N_2 at 12 ml/min. The volatile compounds eluted were condensed in a capillary loop trap, 75.4 X 0.076 cm ID, cooled in Dry Ice snow. Dry Ice powder was packed and replenished often to maintain constant temperature conditions.

Wet Sample Analysis

The collection of volatile compounds from the ice cream mix samples necessitated the following modifications of the above technique. Fifty grams of sample were placed in a 250 ml screw-top bottle fitted with a holed cap and silicone septum as above. To this, 50 ml tap water, and 20 gm of anhydrous sodium sulfate (Na_2SO_4) and a magnetic stirring bar were added. (The presence of the sodium sulfate increased the vapor pressure of the volatile compounds present in aqueous solution.) This mixture was maintained in a stirred, temperature controlled water bath at 60 C ($\pm$ 2 C) during the collection procedure. All other collection parameters and techniques used to elute and trap compounds from the precolumn were similar to those used for dry sample analysis.

Analytical Procedure

Using the apparatus diagrammed in Figure 8, the frozen trapped volatile compounds were flashed (using the heat gun) onto a 500 ft X .03 in capillary analytical GLC column, wall-coated with a mixture of eight percent Carbowax 20M and one percent Versamid 900 in $CHCl_3$, for development of the chromatogram.

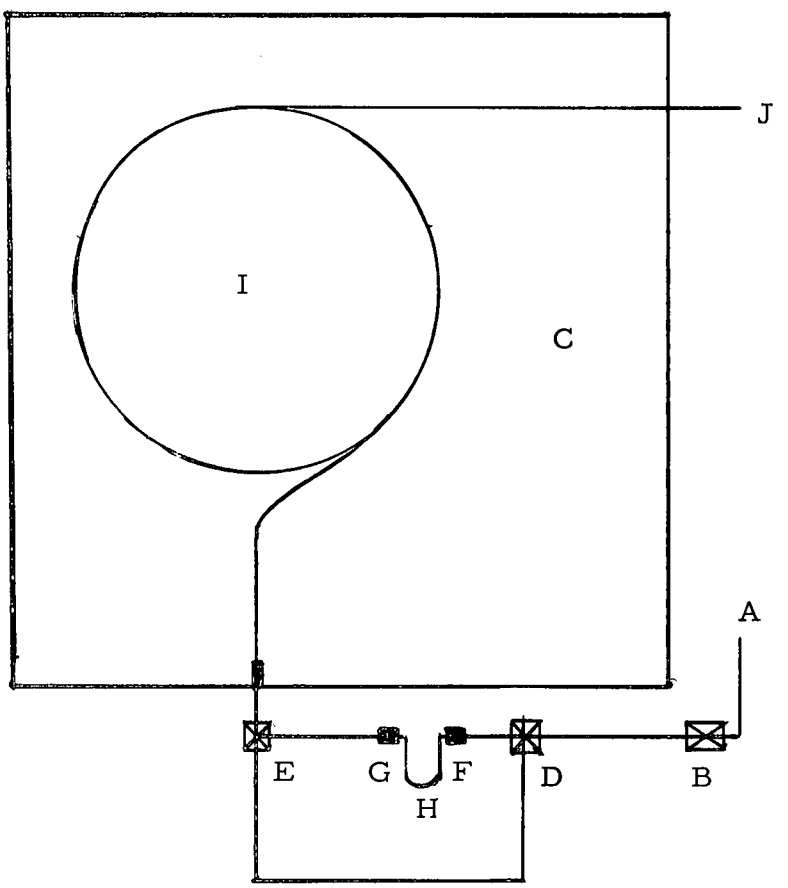
Analyses of the collected volatile fractions by GLC were accomplished using a Varian model 1200 gas chromatograph with external modifications (39) to facilitate attachment and heating of the capillary loop traps containing the volatile flavor compounds, and to direct the carrier gas through the loop trap/analytical column system.

The GLC parameters were as follows:

Detector: Flame ionization; H_2 and air flame. Detector temperature held at 265 C.

Carrier gas: N_2 @ 12 ml/min; stabilized for temperature programming by use of a heated (170 C), capillary restriction and a high pressure carrier gas source (280 psig).

Column: 500' x .03" Carbowax 20M-Versamid 900 wall-coated capillary; eight percent Carbowax 20M and one percent Versamide 900 in $CHCl_3$.



A	External High pressure Gas Source
B	Capillary Isothermal Restriction
C	Column Oven
D & E	Toggle Valve
F & G	1/16 in Swagelok Union
H	Capillary Trap
I	Capillary GLC Column
J	To Mass Spectrometer or FID.

Figure 8. External modifications of Varian 1200 GC.

External Loop Trap Flash Temperature: 180 C peak after ten seconds.

The model 1200 Varian gas chromatograph was used for all chromatographic work which was not associated with MS identification of organic compounds.

Identification of volatile organic compounds detected in the dry whey sample examined was accomplished using a Finnigan Quadrupole Mass Spectrometer, model 1015C, coupled to a Varian model 1400 gas chromatograph with an all glass, jet carrier gas separator. The GLC/MS system used helium as a carrier gas; gas flow and temperature programming parameters were the same as those of the Varian model 1200 used for the basic GLC work.

MS conditions were as follows:

Filament current:	400 microamperes
Electron voltage:	70 eV
Analyzer pressure:	5×10^{-6} Torr
Multiplier voltage:	2.8 Kilovolts
Scanned spectra:	m/e 10 to m/e 250.

MS data were collected and analyzed using a System Industries Model 150 dedicated data system.

Chromatogram Correlation

Correlation of the MS chromatogram to the numerous GLC chromatograms was accomplished using peak retention ratios which compared the retention times and spectrum numbers of all desired peaks to an internal standard (artifact)[1] 2-methoxyethanol, as if it were the solvent front, and the peaks representing 2-furfuryl alcohol or benzaldehyde at the end of the chromatogram. Peak retention ratios were constructed using the spectrum numbers of the MS chromatogram. An artificial retention time (in mm) was calculated from these ratios which, when compared to the measured GLC chromatogram retention time (mm) gave close approximation if peak correlation existed.

Quantitation of Compounds Identified

The areas (representing concentration) of GLC peaks identified as whey flavor compounds in model system members were totaled with other selected peak areas to give a numerical indication of the extent to which whey flavors had either carried through or been synthesized in that model.

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[1] 2-methoxyenthanol with Dry Ice is used as a coolent in the trapping apparatus, during trap hookup and removal, vapors from the cooling bath are also trapped.

Quantitation of individual peaks was accomplished using the peak area estimation method: peak height times width at one-half height. The peak area total, representing each model member, was calculated using selected peaks which were most consistant and most representative (in our opinion) of characteristic compounds present in whey; other criteria were also used in this selection, they are mentioned in the discussion portion of this work. Several subtotals of compound groups such as pyrazines, furans, oxy-hydrocarbons and so forth were also computed.

Grouping of model members by parameter and subsequent statistical analysis (group mean and difference testing) led to the elucidation of trends in the data which were of significance to the research.

Quantitation in Headspace System

Quantitation in any system which employs headspace analysis to ascertain volatile compound composition is strictly relative in nature. That is, analysis of headspace volatile organic compounds is only indicative of those compounds which have a relatively high vapor pressure and is subject to the various liquid-vapor equilbria established in that medium. If analysis conditions are kept constant from sample to sample, relative quantitation for purposes such as flavor analysis is feasible and reproducible. In this system

only the several model members were quantified with any thought to inter-sample comparison. Absolute quantitation could never be accomplished by this technique.

Standard Quantification

Under conditions similar to the collection parameters described previously for a wet sample, 100 microliters of a dilute ethyl ester mixture (series C_5-C_{11}) in ethanol containing 1.0 ng/μl of each ethyl ester produced a peak area of 2346.5 mm^2 for the ethyl hexanoate ester.

Peak Odor Data

During chromatography of selected model system or whey sample, the column effluent was sniffed as peaks emerged to ascertain the presence of any characteristic "whey" odors. An effluent splitter was employed on all samples to facilitate peak sniffing. A split to detector ratio of 2.63:1 was used. Relative quantitation of volatile compounds required no correction factor because the splitter remained in the analysis system for all model members.

Colorimetric Procedure for Determination of Hexose-Amine Compounds

The Elson-Morgan technique (9) for determination of

hexose-amine compounds was employed to confirm the presence of Amadori intermediates in heated whey powder. The technique used required two grams of whey powder dissolved in 50 ml of water. The whey mixture was centrifuged to remove any undissolved suspended particles. Following the procedure outlined in (9); add one ml of acetyl acetone solution (one ml acetyl acetone in 50 ml 0.5 N sodium carbonate) to whey solution; rinse sides of test tube with water. (The heating step next described by Elson and Morgan was omitted in this procedure to prevent formation of Amadori compounds in the whey mixture during the heat application.) Let stand at room temperature for a period of one hour. Add four ml of ethanol followed by one ml p-dimethylaminobenzoate solution (0.8 gram in 30 ml ethanol and 30 ml concentrated HCl). Add one additional ml of ethanol and allow the system to stand for 20-30 min for color development. A red color indicated presence of hexose-amine compounds.

Sensory Evaluation of Ice Cream Mix Model System Members

A panel experienced in sensory analysis of dairy products was employed to correlate sensory response of flavor change to change in model system parameter. The three member panel used a straight forward desirability test, scaled: pronounced, moderate,

slight in order to assign weight to the specific flavors under analysis: stale, whey taint, and old ingredients.

RESULTS AND DISCUSSION

Compounds Detected in Whey Powder by Headspace GC Analysis

Figure 9 depicts a chromatogram of the volatile organic compounds isolated from the headspace above a poor quality whey powder (Tillamook, 5-75) which had been stored for approximately one year at room temperature in stable atmospheric conditions. The whey powder manifested very stale, old ingredient, graham cracker off flavor notes typical of an aged or poorly processed product. A marked yellow-brown tint (over and above that which might be expected to arise from residual Annatto cheese color) was present in the powder. This discoloration is often indicative of non-enzymic browning reactions which have proceeded to a considerable extent.

The volatile organic components of this poor quality whey were analyzed by combined GLC-MS; those compounds present in sufficient concentration to permit spectral identification are listed in Table 5. Those not identified are listed alphabetically.

Confirmation of Relationship between Flavor Isolate and Product

To insure that the headspace volatile compound collection had

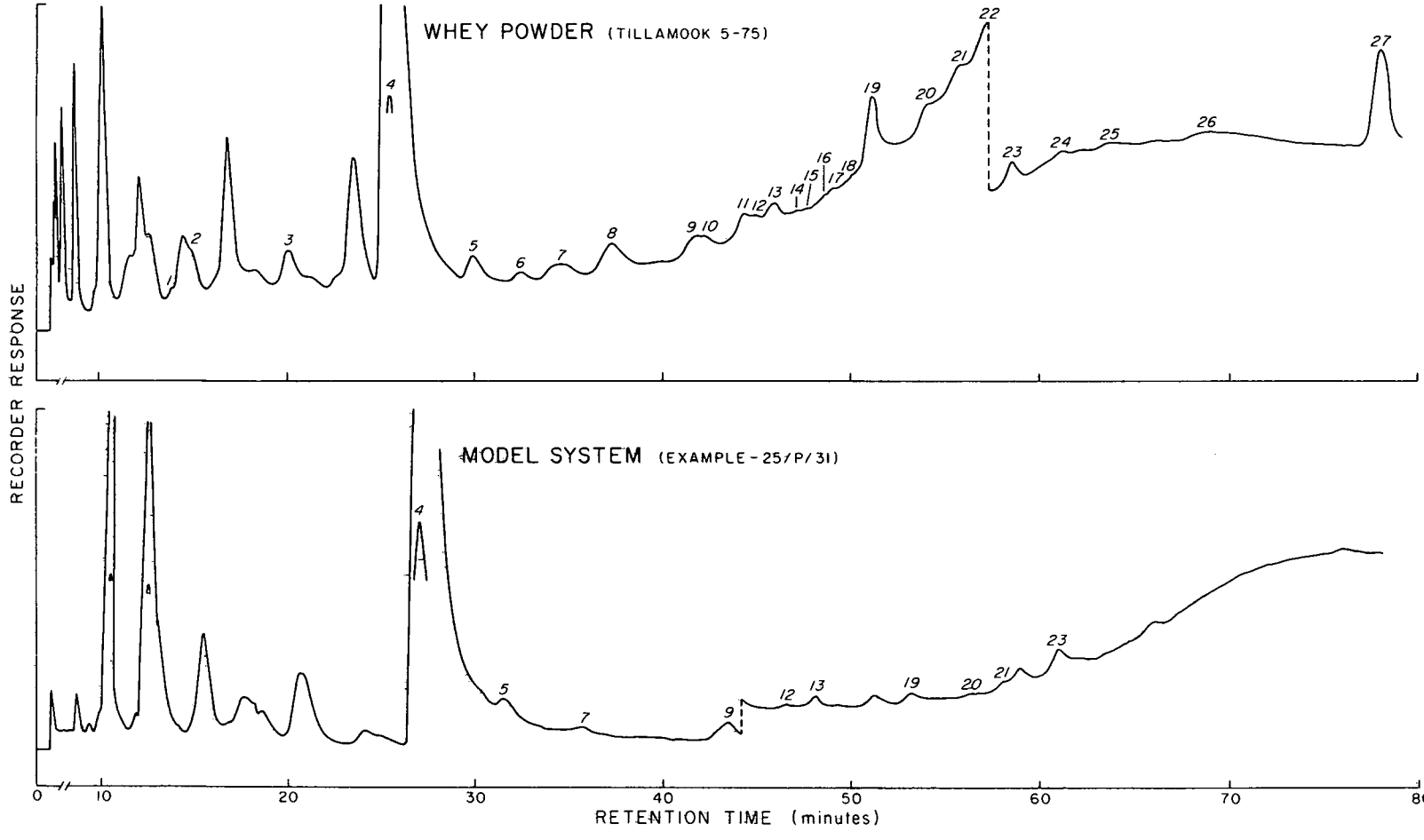

Figure 9. Chromatograms of whey powder and 25/P/3 model system.

isolated a compound mixture representing the actual product under analysis (in our case whey or model system ice cream mix), the total effluent collected in a trap loop was sniffed to observe its relationship to the smell (or taste) of the product.

The poor quality whey powder (Tillamook 5-75) eluted had a very familiar stale, whey-like odor reminiscent of the powder itself.

Correlation between Model System and Ingredient Whey Chromatograms

The chromatogram (FID) from poor quality whey shown in Figure 9 was compared to a chromatogram of identified compounds constructed by multiple limited mass search of data obtained from GlC/MS analysis of the same poor quality whey powder. Identification of comparable compound peaks present in both chromatograms subsequently provided qualitative correlation between the identified whey compounds (MS chromatogram) and the compounds described by the 30 chromatograms (FID) obtained from the whey fortified ice cream model system analysis.

Mathematical correlation between FID and MS chromatograms was necessitated by dissimilarities in chromatogram starting point and in recorder and print out speeds. These differences prevented direct visual or mechanical comparison of compound peaks, and required the use of an internal chromatographic reference point

Table 5. Compounds identified in spray dried whey powder, Tillamook 5-75.

Number	Compound
1.	Diacetyl[a]
2.	2-Pentanone[a]
3.	n-Hexanal[a]
4.	2-Methoxyethanol[b]
*5.	n-Pentanol
*6.	2-Methylpyrazine
*7.	Unidentified Peak C.
*8.	2, 5-Dimethylpyrazine
*9.	Methylethylpyrazine (exact substitution unknown)
*10.	Dimethyltrisulfide
*11.	Unidentified Peak F.
*12.	2-Ethyl-3, 6-dimethylpyrazine
*13.	2-Furfural
14-18.	Unidentified Peaks G, H, I, J, K
*19.	Benzaldehyde
*20-21.	Unidentified Peaks M and N
22.	Unidentified Peak O.
*23.	2-Furfuryl alcohol
24-26.	Unidentified Peaks Q, R, S
*27.	Dimethylsulfone**

* Included in Peak Area Total

** Present in whey powder, not model system.

[a] These compounds identified by GLC/MS but not included in Peak Area Total.

[b] Analytical artifact.

to calculate relative retention times for the compounds present. Since a majority of the peaks considered as whey indicator compounds eluted after the peak representing 2-methoxyethanol, this omnipresent analytical artifact was chosen as the reference point. Actual calculation of relative retention data will be discussed later.

Qualitative correlation between volatile organic compounds present in the dry wheys and those present in the ice cream model system samples, coupled with quantitative comparison of selected compound concentrations between individual model system samples, were meaningful analytical procedures. Quantitative comparison of compounds in dry whey to those in the ice cream model was unnecessary.

Procedure for Interpreting Model System Data

To obtain quantitative assessment of the flavor differences produced by various model system parameter changes, it was necessary to devise a mathematical interpretation of each model system chromatogram which represented the effect of whey powder addition, processing load, or other non-whey variable on the system; and was amenable to statistical analysis. Wide variation in the appearance of whey indicator compounds during model system analysis prevented interpretation of model results based on the

presence or absence of any single compound. This variation may have been due to random differences in synthetic pathways for compounds created during processing, non-uniformity of mix ingredients (especially the whey powders and NFDM), or variations in compound volatility, caused by micro-environment anomilies, which affected analytical results. Never-the-less the overall effect necessitated the use of a broad analytical approach which consolidated the complexity of the system and minimized the effect of these seemingly random factors on interpretation of the change in volatile flavor profile produced by whey powder addition. Using a simple, easily analyzed, mathematical representation termed "Peak Area Total" to quantify the actions of heat, decreasing whey quality, and increasing whey quantity on the system, accomplished this end.

Determination of the peak area total involved summation of the peak areas (in mm^2, representing concentration) of selected compounds representative of whey powder flavors present in the poorest quality ingredient whey and also present in varying quantities in the other two wheys and the model system members. This sum expressed,in numerical terms, the total volatile flavor intensity related to the addition of whey powder or the change in heat processing load for each model member. Sub-totals of peak areas involving groups or classes of compounds such as pyrazines,

oxy-heterocyclics, all oxy-hydrocarbons, and so forth, were also computed.

Selection of Compounds for Inclusion in Peak Area Total

Those compounds listed in Table 5 marked with an asterisk (*) were those most representative of the typically "whey" compounds described in the literature(10, 11, 12, 13, 14) and were also representative of the heated stale flavor characteristics which often appear in foods containing whey. These compounds served as whey indicator compounds for qualitative comparisons and as peak area total compounds for quantitative comparisons. Selection was made using the following criteria: Those compounds in the poor quality (Tillamook 5-75) whey powder which had previously been reported; those whose synthetic pathways, as described in the literature, were known or suspected to operate in heated or stale aldose-amine systems; those with a high flavor potential; and those with a sufficiently high vapor pressure to remain volatile in the ice cream mix matrix; were included in the total. Those compounds known to be analytical artifacts or normally unrelated to whey or ice cream systems, were excluded.

Origins of Compounds Used as Whey Indicator Compounds

Origins of compounds selected for inclusion as whey indicator and peak area total compounds are listed below. Reason for inclusion is also listed.

Pentanol -- carbohydrate or lipid breakdown product; found in other non-enzymic browning systems (24, 28, 34).

2-Methylpyrazine -- product of non-enzymic browning/ Strecker degradation reactions; found in whey (12, 13).

Peak "C" -- unidentified peak characteristic to the chromatograms of whey powders used in the ice cream model.

2,5-Dimethylpyrazine -- browning reaction product; found in whey (12, 13).

Methylethylpyrazine -- browning reaction product; exact position of substituents not definable from spectrum; possibly found in whey (10, 12).

Dimethyltrisulfide -- Strecker degradation product from sulfur amino acids; reaction pathway

possible in system from amino acids present.

2-Ethyl-3,6-dimethylpyrazine -- browning reaction product; similar compounds in whey.

2-Furfural -- browning reaction product; carmelization product; carbohydrate breakdown product; found in rapidly browned whey, stale powdered milk and model systems related to dairy products (11, 13).

Benzaldehyde -- heat induced compound, Strecker degradation; found in whey (12, 13).

2-Furfuryl Alcohol -- carbohydrate breakdown product; browning reaction product from Amadori rearrangement of sugars; from reduction of 2-furfural; found in whey, rapidly browned whey, and other related model systems (11, 12, 14).

Dimethylsulfone -- oxidation of dimethylsulfoxide-a component of fresh milk arising from feed or plant sources, considered an artifact; found in whey

(12), but not detected in model system.

The compounds listed below were also detected in poor quality whey or the model system but were not included as whey indicator or peak area total compounds for the following reasons.

Diacetyl (2, 3-Butanedione) -- possible cheese culture artifact; possible browning reaction product but considered an intermediate, precursor, or catalyst in reactions of alpha-dicarbonyl compounds, Strecker degradation/transamination/condensation reactions; not found in model system.

2-Pentanone -- lipid or carbohydrate breakdown product; found in other browning systems (24, 34), see below.

Hexanal -- probably lipid oxidation product, mainly from lipid portion of ice cream (lipid portion of added whey powder small compared to overall ice cream lipid content).

2-Methoxyethanol -- artifact from analytical technique;

used as internal reference for calculation of retention data.

Note: The portion of the chromatogram eluting after the 2-methoxyethanol peak, from which the whey indicator compounds were picked, might be termed the whey fingerprint portion of these chromatograms. The compounds listed above which were not included in the whey indicator or peak area totals were excluded partially because they were not present in this fingerprint portion of the whey chromatogram. The unidentified peaks labeled C, F, M, and N were present in the fingerprint portion of the poor quality whey powder chromatogram and were also present in many of the model system members, these compounds were included in the list of whey indicator compounds despite the lack of identification.

Whey Powder's Use in Ice Cream

There is ample evidence that whey, from an economic viewpoint, has a definite place among the ingredients used in the manufacture of frozen desserts and other foods requiring low cost milk solids. However, if one considers the possible flavor impact whey might have on the products in which it is present, there is reason to question the use of whey as a milk powder substitute. Ice cream, especially vanilla ice cream, is a very delicately flavored product. The addition of a poor or marginal quality ingredient, in the form of

poor quality whey powder, could mean the difference between an acceptable product and a greatly inferior, unacceptable one.

Table 6 presents flavor threshold data for a few of the compounds detected in the poor quality Tillamook 5-75 whey powder. In most cases the threshold levels in water are a few parts-per-million and, in the case of the pyrazines, even less in oil. Ice cream is a complicated oil-water emulsion which, with a mild flavoring such as vanilla present, would be extremely susceptable to flavor changes induced by minor increases in concentration of compounds such as those listed in the table.

Table 6. Flavor threshold values for selected compounds (21, 41).

Compound	Water Value	Oil Value
n-Pentanol	0.225 ppm	--
2-Methylpyrazine	105	27
2,5-dimethylpyrazine	35	17
2-Ethyl-3,6-dimethylpyrazine	43	24
2-Furfural	80	
Benzaldehyde	3×10^{-3}	
2-Furfuryl alcohol	30	

Results of Ice Cream Model System Analysis

Table 7 lists the retention data for comparison of the FID chromatogram to the MS chromatogram of poor quality whey powder.

Table 8 compares the retention data for the 30 chromatograms obtained from model system analysis to the FID chromatogram of poor quality whey. Correlation between retention data of identified compounds and those of unknown structure was not always exact; slight variations in analytical conditions such as temperature programming rate, gas flow rate, or initial column temperature effect the elution characteristics of the analytical column and thus effect the retention time of individual chromatographic peaks.

GLC analysis of the whey fortified ice cream mix demonstrated, by chemical analysis and relative quantitation, that some of the flavor compounds associated with dry whey powder could be detected in a system simulating commercial ice cream, and flavor trends produced by whey addition could be partially traced. The presence of whey flavor compounds in the model system suggests that there is a "carry through" of flavors associated with whey into the ice cream and suggests their possible importance in the development of the final flavor of the ice cream product. Also of importance

Table 7. Retention data comparing[a] FID whey chromatograms to the MS whey chromatogram.

Compounds of Interest	Measured Retention Time: GLC (FID)	Calculated Retention Time: MS
2-Methoxyethanol	0.00	0.00
n-Pentanol	5.7	6.3
2-Methylpyrazine	8.85	9.34
Peak "C"	11.6	11.9
2,5-Dimethylpyrazine	14.95	15.03
Methylethylpyrazine	21.0	20.72
Dimethyltrisulfide	---	---
2-Ethyl-3,6-dimethylpyrazine	24.85	24.99
2-Furfural	26.05	26.00
Benzaldehyde	32.5	32.3
2-Furfuryl alcohol	42.05	42.05

[a] These data were constructed from a comparison of identified compound peaks to the 2-methoxyethanol and the 2-furfuryl alcohol peaks on the MS chromatogram. Retention ratios calculated from this comparison (similar to R_f calculation in paper chromatography) were used to construct artificial or calculated retention times (in mm rather than spectrum number) for all identified compounds on the MS chromatogram. Comparison of these calculated retention times (mm) to actual measured retention times on the GLC (FID) chromatograms produced peak correlation. Known-compound analysis elucidated the 2-furfuryl alcohol peak on the GLC (FID) chromatogram.

Table 8a. Retention data for comparison of FID whey chromatogram to model system chromatograms.

Compounds of Interest	Model System Members R_{mb}[a] 15[b] g 1	15 f 1	15 p 1	17.5 g 1	17.5 f 1	17.5 p 1	25 g 1	25 f 1	25 p 1	$\bar{x}$	Whey Powder GLC R_{mb}
2-Methoxyethanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
n-Pentanol	.15	.16	.15	.17	.17	.15	.19	.17	.18	0.17	.18
2-Methylpyrazine	--	--	--	-	--	.26	--	--	--	0.26	.27
Peak "C"	--	--	.32	.35	.33	.32	--	.34	--	0.33	.36
2,5-Dimethylpyrazine	--	.46	.45	--	.47	.45	.45	.48	--	0.46	.46
a Methylethylpyrazine	.59	.69	.60	--	.61	--	.69	.62	.66	0.64	.65
Dimethyltrisulfide	--	--	--	--	--	--	--	--	--	--	.66
2-Ethyl-3,6,-dimethylpyrazine	--	--	.75	--	--	--	--	--	--	.75	.76
2-Furfural	--	--	.79	--	.79	.79	--	.77	--	.79	.80
Benzaldehyde	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.00
2-Furfuryl alcohol	--	--	--	--	--	--	--	--	--		1.29

a Correlation based on 2-methoxyethanol and benzaldehyde. Benzaldehyde was used inplace of 2-furfuryl alcohol as the end peak because 2-furfuryl alcohol appears only once in the model system analysis.

b Model system designations: 15 = 15% substitution; 17.5 = 17.5%; 25 = 25%; g = good whey (subjective decision); f = fair whey; p = poor whey; 1 = 150 F (65 C) for 50 min.; 2 = 150 F (65 C) for 68 min.; 3 = 155 F (68.3 C) for 78 min.

Table 8b. Retention data for comparison of FID whey chromatogram to model system chromatograms.

Compounds of Interest	Model System Members R_{mb}[a] 15[b] g 2	15 f 2	15 p 2	17.5 g 2	17.5 f 2	17.5 p 2	25 g 2	25 f 2	25 p 2	$\bar{x}$	Whey Powder GLC R_{mb}
2-Methoxyethanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
n-Pentanol	.17	.14	.17	.17	.18	.17	.18	.17	.16	.17	.18
2-Methylpyrazine	--	.24	.24	--	.26	.29	--	.25	--	.26	.27
Peak "C"	.33	--	.29	.34	.33	--	.35	.33	.31	.33	.36
2,5-Dimethylpyrazine	.47	.47	.46	.48	.47	.46	--	.46	.46	.47	.46
a Methylethylpyrazine	.65	.60	.61	.61	.62	--	.66	.62	.60	.62	.65
Dimethyltrisulfide	--	--	--	--	--	--	--	--	--	--	.66
2-Ethyl-3,6,-dimethylpyrazine	.77	--	--	--	--	.78	--	--	--	.78	.76
2-Furfural	.80	--	.79	.79	.80	--	.80	--	.79	.80	.80
Benzaldehyde	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.00	1.0
2-Furfuryl alcohol	--	--	--	--	--	--	--	--	--	--	1.29

Table 8c. Retention data for comparison of FID whey chromatogram to model system chromatograms.

Compounds of Interest	Model System Members R_{mb}[a] 15[b] g 3	15 f 3	15 p 3	17.5 g 3	17.5 f 3	17.5 p 3	25 g 3	25 f 3	25 p 3	$\bar{x}$	Whey Powder GLC R_{mb}
2-Methoxyethanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
n-Pentanol	.16	.18	.16	.16	.16	--	.15	.15	.18	.16	.18
2-Methylpyrazine	--	--	.28	--	--	.26	--	--	--	.27	.27
Peak "C"	.33	.34	.33	--	--	--	.29	.31	.33	.32	.36
2,5-Dimethylpyrazine	.46	--	.42	--	.41	.46	--	--	--	.44	.46
a Methylethylpyrazine	.61	.61	--	.64	--	--	.60	.58	.63	.61	.65
Dimethyltrisulfide	--	--	--	--	--	.68	--	--	--	.68	.66
2-Ethyl-3,6,-dimethylpyrazine	--	--	--	.76	--	--	.76	--	.75	.76	.76
2-Furfural	.80	.80	--	.79	.80	--	--	--	.80	.80	.80
Benzaldehyde	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2-Furfuryl Alcohol	--	--	--	--	--	--	--	--	1.29	1.29	1.29

is evidence that there is an increase in these whey indicator compounds during heat processing of the ice cream mix. Table 9 lists the peak area totals and subtotals for the model system analysis.

The data generated by this analysis was not, by any means, linear in nature. Many ancillary variables in the model system, which were not accounted for in the interpretation of the analytical data, affected the overall results. However, combination of the technique of peak area total, with a simple statistical grouping of system members by parameter type (whey quality, whey quantity, or heat processing load) established trends in volatile compound concentration change which indicated the parameters from which the most product flavor effect could be expected.

Each of the parameters had three load levels: 15%, 17.5%, and 25% whey substitution level; good, fair, and poor whey quality levelp and 65 C/50 min, 65 C/68 min, and 68.3 C/78 min heat processing load level; which produced a total of 27 samples. Grouping these by load level within a parameter, produced three sample groups of nine members; nine for level one, nine for two, and nine for three of each parameter. The mean peak area total for each nine member group was computed and compared to the means for the other two groups in the same parameter. Numerical trends in these means correlating with parameter load level change became apparent. Trend interference produced by conditions present in the

Table 9a. Peak area totals for model system analysis[a].

Totals	Model System Members									
	15 g l	15 f l	15 p l	17.5 g l	17.5 f l	17.5 p l	25 g l	25 f l	25 p l	0 - l
Total of selected peaks	185.2	737	912.7	1007.2	421	524.7	693	2124	339	342.67
Total oxy-hydrocarbon peaks (including furan)	171.9	279.7	527.3	708	416	384.7	632	24	297	172
Total oxygen heterocyclic peaks (furan)	---	---	146.7	5	---	258	5	---	---	5
Total nitrogen heterocyclic peaks (pyrazine)	13.3	457.3	372	159.2	---	13.3	61	2100	42	80

[a] Areas in mm^2.

Table 9b. Peak area totals for model system analysis.

Totals	Model System Members									
	15 g 2	15 f 2	15 p 2	17.5 g 2	17.5 f 2	17.5 p 2	25 g 2	25 f 2	25 p 2	0 - 2
Total of selected peaks	2144.5	1317	2073	1423.5	1850	2272	778.5	1110	1088.6	0
Total oxy-hydrocarbon peaks (including furan)	32.5	950	1098	900.5	1252	957	570	951.6	674.3	0
Total oxygen hererocyclic peaks (furan)	----	70	5	5	32	----	----	89.8	39.2	0
Total nitrogen hererocyclic peaks (pyrazine)	2083	227	327	253	224.5	1315	150	50	334.7	0

[a] Areas in mm^2.

Table 9c. Peak area totals for model system analysis.[a]

Totals	Model System Members									
	15 g 3	15 f 3	15 p 3	17.5 g 3	17.5 f 3	17.5 p 3	25 g 3	25 f 3	25 p 3	0 - 40
Total of selected peaks	1611.5	2238	3289	706	2670.5	2126	244	262.7	1925	180
Total oxy-hydrocarbon peaks (including furan)	1359	1824	2081	526	2521.5	---	121.3	109	1620	173.3
Total oxygen heterocyclic peaks (furan)	5	52	---	70	364	---	---	---	840.5	27
Total nitrogen heterocyclic peaks (pyrazine)	44	194	828	180	149	2126	74.7	146.7	174.5	---

[a] Areas in mm^2.

other two parameters not under consideration were nullified by the grouping technique. Table 10, 11, and 12 depict the trends found in the mean peak area totals, and indicate their relative statistical significance.

Trends in the Model System Data

The three levels of whey substitution (15%, 17.5%, and 25%) produced mean peak area totals which demonstrated no trend increase in whey related volatile compounds for a corresponding increase in whey powder substitution in the ice cream model. This lack of trend implies that whey substitution quantity may not effect off-flavor development in the final product.

Trends existing in the other two parameters were demonstrated by rearranging the 27 sample peak area totals into groups reflecting change in whey quality and then heat processing load. Results of group mean comparison in these cases revealed trends which indicated that the addition of poor quality whey to the mix or use of an overly large heat processing load, increased the level of whey related volatile compounds present in the ice cream model, as compared to better quality wheys or lower heating loads. These trends indicate the potential adverse flavor effect of poor quality whey or high pasteurization temperatures (times) on commercial frozen desserts.

Table 10a. Table of peak area total means for the model system parameter: Processing load.

Grouping	Mean Peak Area ($\bar{x}$)	Standard Deviation	Statistical Difference @ 0.05 level, 9+9-2 = 16 d.f., t_c = 2.12.
Processing Load Increase			
1. 65 C/50 min.	771.53 mm^2	573.00	
2. 65 C/58 min.	1561.90	537.23	Statistically different from 1 @ 0.05 level t = 3.02.
3. 68.3 C/78 min.	1674.74	1070.29	Statistically different from 1 @ 0.05 level t = 2.23; not statistically different from 2 t = 0.28.

Note: Trend of means gradually increases, i.e. the volatile compound concentration is increasing as product processing load increases. Conclusion: processing load may be important to product flavor (at least flavors induced or indicated by the presence of the selected compounds).

Table 10b. Table of peak area total means for the model system parameter: Whey quality decrease.

Grouping	Mean Peak Area ($\bar{x}$)	Standard Deviation	Statistical Difference@ 0.05 level, 9+9-2 = 16 d.f., t_c = 2.12.
Whey Quality Decrease			
1. Good quality whey[a]	865.93 mm^2	644.17	
2. Fair quality whey	1414.47	853.20	Not statistically different from 1,@ 0.05 level, t = 1.54.
3. Poor quality whey	1616.67	961.00	Not statistically different from 1 @ 0.05 level, t = 0.43 or 2 @ 0.05 level, t = 0.47.

[a] Subjective quality assignments.

Note: Trend of means gradually increases, i.e. the volatile compound concentration is increasing as whey quality decreases. Even though the means are not statistically different at the 0.05 level, trends do exist. From this it appears that whey quality may be of importance to product flavor (at least flavors induced or indicated by the presence of the selected compounds).

Table 10c. Table of peak area total means for the model system parameter: Whey Quantity Increase.

Grouping	Mean Peak Area ($\bar{x}$)	Standard Deviation	Statistical Difference @0.05 level, 9+9-2 = 16 d.f., t_c = 2.12.
Whey Quantity Increase			
1. 15% substitution	1611.99	940.99	
2. 17.5% substitution	1444.54	825.18	Not statistically different from 1 @ 0.05 level, t = 0.40
3. 25% substitution	951.58	690.39	Not statistically different from 1 @ 0.05 level, t = 1.70 or 2 @ 0.05 level, t = 1.37.

Note: Trend reversed from expected, means gradually decreasing, i.e. the volatile compound concentration is decreasing as whey powder concentration increases. Conclusion: whey quantity may not be of importance to product flavor (at least flavors induced or indicated by the presence of the selected compounds).

The results obtained were not unexpected. Several authors (2, 3, 17, 18, 23, 35) have done consumer testing of various whey products as used in frozen desserts. Two, Igoe and Arnold (2, 23), believe that added sweet whey (up to 35% of the non-fat solids in Arnold's case) is not deleterious to product flavor or consumer acceptability. Frazeur (17), however, in earlier work, concluded that only excellent flavored, electrodialized whey was suitable for 25% or greater substitution. Better-than-average and average flavored, non-dialized wheys were believed to be suitable only in the more highly flavored or stabilized products such as sherbet, or ice milk, not in ice cream. The differences in findings related by these authors may, in part, be due to advances in whey processing technology which have come about during the years since Frazeur's work (1967). In fact, very recent work by Bhusri (3) indicates that blended wheys, i.e. combinations of various whey types, may be used as the sole source of milk solids non-fat in an ice cream formulation without loss of acceptable flavor.

The findings of Igoe, Arnold, and to a certain extent, Bhusri, reinforce the findings of this research: i.e. whey substitution quantity may not be critical to product off-flavor, as previously believed. Whey quality and processing environment appear to be more important factors influencing off-flavor development. It should be noted, though, that any lack of trend in our data (pertaining

to whey substitution quantity) might be due to minuscule differences in actual whey content of each system member, rather than the lack of effect of whey quantity on mix flavor. The 15% substitution system contained approximately 0.87 g of whey in 50 g of mix; the 17.5% system, 0.99 g; the 25% system, 1.42 g.

The trends that do exist in our data seem to be of major importance. The trend established indicating an increase in whey-like volatile compounds produced upon increase in heat processing load is possibly the least expected. It appears, from this data, that there is an increase in whey-like compounds (which are present in whey and also may be present in other components of the mix) to a measurable extent when the ice cream is pasteurized. The similarity of these compounds to the whey related compounds is not unusual, it is well known that heating of a food product changes its flavor properties, and many such heated systems display similar compound synthesis. The interesting item present in this data is the increased change in volatile compounds produced by heating an ice cream system containing whey compared to similar heating of control samples containing no whey. See Table 9 a, b, c. We believe this data indicates that the presence of whey in the ice cream system induced further formation of whey-related compounds, over and above those generated in the same system in the presence of non-fat dry milk alone. Precursors present in the ingredient

whey, as reminants of previous heat processing, may account for this increased synthesis.

The trend established indicating an increase in volatile whey compounds upon decrease in added whey quality is an expected result. This author has long believed that the flavor quality of a given whey is the most critical consideration when selecting a usable, non-flavor inducing whey powder for inclusion in an ice cream product.

Tables 11 and 12 a, b, c, deal with trends present in sub-totals of peak areas involving pyrazine, furan, and oxy-hydrocarbon compounds. Model members were grouped in the same manner as total peak areas; and means were computed. The model system trends which became apparent were increased pyrazine concentration with decreased whey quality, and increased oxy-hydrocarbon/furan concentration with increased heat processing load. These trends indicate a staling or high-temperature heat processing pathway for pyrazine synthesis, and a low-temperature (i.e. pasteurization) pathway for oxy-hydrocarbon generation. These findings are consistant with literature cited in this work. The totals and sub-totals of selected peak areas produced no other trends, statistically significant or otherwise.

Table 11a. Peak area total means (sub-total pyrazines) for model system parameter: Processing load.

Grouping	Mean ($\bar{x}$)	Standard Deviation	Statistical Difference @ 0.05 level, 9+9-2 = 16 d.f. t_c = 2.12.
Processing Load			
1. 65 C/50 min	357.5	674.4	
2. 65 C/68 min	551.6	683.73	Not statistically different from 1 @ 0.05 level t = .72.
3. 68.3 C/78 min	435.21	675.69	Not statistically different from 1 or 2 @ 0.05 level, t_{13} = .19 t_{23} = .13.

No trend observed; conclusion: increase in processing load may not effect product flavor quality produced by pyrazine presence.

Table 11b. Peak area total means (sub-total pyrazines) for model system parameter: Whey quality.

Grouping	Mean ($\bar{x}$)	Standard Deviation	Statistical Difference @ 0.05 level, 9+9-2 = 16 d.f. t_c = 2.12.
Whey Quality			
1. good[a]	335.3	659.3	
2. fair	394.28	652.48	Not statistically different from 1 @ 0.05 level, t = .19.
3. poor	614.69	700.34	Not statistically different from 1 or 2 @ 0.05 level, t_{13} = .69 t_{23} = .87.

Numerical trend observed; conclusion: whey quality may effect product flavor quality produced by pyrazine presence.

[a] Subjective flavor comparisons.

Table 11c. Peak area total means (sub-total pyrazines) for model system parameter: whey quantity.

Grouping	Mean ($\bar{x}$)	Standard Deviation	Statistical Difference @ 0.05 level, 9+9-2 = 16 d.f. t_c = 2.12.
Whey Quantity			
1. 15% substitution	505.03	639.8	
2. 17.5% substitution	489.08	731.61	Not statistically different from 1 @ 0.05 level, t = .05
3. 25% substitution	348.18	663 23	Not statistically different from 1 or 2 @ 0.05 level, t_{13} = .46 t_{23} = .51

Reverse trend observed; conclusion: increase in whey quantity at this level may not effect product flavor quality produced by pyrazine presence.

Table 12a. Peak area total means (sub-total furans and oxyhydrocarbon compounds) for model system parameter: Processing load.

Grouping	Mean ($\bar{x}$)	Standard Deviation	Statistical Difference @ 0.05 level, 9+9-2 = 16 d.f. t_c = 2.12.
Processing Load			
1. 65 C/50 min	382.29	218.24	
2. 65 C/68 min	820.22	358.35	Statistically different from 1 @ 0.05 level t = 3.13.
3. 68.5 C/78 min	1129.09	956.33	Not statistically different from 2 @ 0.05 level t = 0.91 but statistically different from 1 @ 0.05 level t = 2.28.

Numerical trend observed; conclusion: increase in processing load may effect product flavor quality produced by furan or oxygen containing compound presence.

Table 12b. Peak area total means (sub-total furans and oxy-hydrocarbon compounds for model system parameter: Whey quality.

Grouping	Mean ($\bar{x}$)	Standard Deviation	Statistical Difference @ 0.05 level, 9+9-2 = 16 d.f. t_c = 2.12.
Whey Quality			
1 good[a]	557.91	418.92	
2. fair	925.31	838.04	Not statistically different from 1 @ 0.05 level, t = 1.18.
3. poor	849.59	666.07	Not statistically different from 1 or 2 @ 0.05 level, t_{13} = 1.11 t_{23} = 0.21.

No trend observed; conclusion: decrease in whey quality may not effect product flavor quality produced by furan or oxygen containing compound presence.

[a] Subjective flavor comparisons.

Table 12c. Peak area total means (sub-total furan and oxy-hydrocarbon compounds) for model system parameter: Whey quantity.

Grouping	Mean ($\bar{x}$)	Standard Deviation	Statistical Difference @ 0.05 level, 9+9-2 = 16 d.f. t_c = 2.12.
Whey Quantity			
1. 15% substitution	924.82	733.29	
2. 17.5% substitution	851.74	726.46	Not statistically different from 1 @ 0.05 level, t = .21.
3. 25% substitution	555.47	505.89	Not statistically different from 1 or 2 @ 0.05 level, t_{13} = 1.24 t_{23} = 1.00.

Reverse trend observed; conclusion: increase in whey concentration (at this level) may not effect product flavor quality produced by furan or oxygen containing compound presence.

Actual Flavor Effects of Volatile Flavor Compounds

The flavor effects of compounds detected in a system and the flavor effect of the system itself are often very different. To substantiate the findings above which demonstrated that the volatile compounds present in whey could be detected in an ice cream model system and quantitative trends established, a flavor panel was employed to attempt detection and quantification of flavor changes in duplicates of the 27 samples previously analyzed by GLC. Table 13 indicates these results. In all cases there were no apparent numerical trends which would indicate flavor correlation withGLC data.

This result was not altogether unexpected. All mixes possessed a cooked note which tended to mask other flavor properties. There were whey taints and other anomalies noted in the samples, but no trending increase or decrease in flavor intensity could be established for any individual parameter change. Flavor analysis in this application is not extremely sensitive without a highly trained panel. It is also possible that all parameter load levels may have been too small or too similar in nature to produce detectable trends in flavor effect. None-the-less the flavor potential of the isolated compounds remains, and the results

of this work indicate that there is reason to believe that compounds characteristic of whey flavor are present, in detectable levels, in ice cream with added whey in its formulation. This knowledge, combined with known threshold levels of the detected compounds, indicates the many serious off-flavor potentialities which may exist in whey powder or any product not employing top quality, fine flavored whey in its manufacture.

Flavor Panel Results

The following table displays the results of the flavor panel testing done in this research. Samples were analyzed for stale, old ingredient, whey taint, cooked, oxidized, and storage flavors. Only the stale, old ingredient, and whey taint flavors were of interest; they were given a numerical weighting of four. The sample flavor intensity was graded on a sliding scale of pronounced, moderate, slight and none, numerically weighted four, three, two, one respectively. No flavor response was weighted as one to facilitate computer analysis. The product of the flavor weighting and the intensity weighting was the flavor score of that particular model member. Similar sample grouping techniques were employed with this data as for peak area total computation. Control scores averaged 33.33. The sample means were as follows.

Table 13. Flavor panel results.

Parameter	Total Flavor Score
Whey Quality	
Good	70.69
Fair	71.56
Poor	68.44
Whey Substitution Quantity	
15%	69.78
17.5%	70.22
25%	70.69
Mix Processing Load	
65 C/50 min	64.44
65 C/68 min	73.78
68 3 C/78 min	72.44

Without statistical analysis one can see that there is very little, if any, difference in the flavor scores of these samples. The model members (those containing whey) are more flavorful than the control samples (those without whey) but are not distinguishable from one another either by the formation of clear numerical trends or by statistical analysis.

Model System Controls

To isolate the volatile compound character of all compounds present in the model system from sources other than the several

whey powders, control samples containing no whey were used. Processing and analysis conditions were the same as for other model members. The results of this work appear at the end of Tables 9 a, b, c. Comparison of the individual whey powders to a non-fat dry milk sample (also used in the model) served as control for the ingredient wheys.

For interest's sake and to partially justify the subjective selection of good, fair, and poor whey powders from the available manufactured products, the following peak area totals for the wheys are provided in Table 14.

Table 14. Peak area totals for whey and NFDM powders.

Tillamook (5-76	2500 mm^2	= Good quality powder
Krafen (5-76)	2816 mm^2	= Fair quality powder
Tillamook (5-75)	3588.5 mm^2	= Poor quality powder
Non-fat Dry Milk	551 mm^2	= Good quality NFDM

Assuming that the selected peaks quantitized above and throughout this work are indicative of off-flavors which are undesirable in a whey product (or NFDM), the above totals indicate "quality" of the whey product.

Heated Whey Samples

To demonstrate that the increase in volatile compounds noted in members of the ice cream model system as processing load increased was a normal phenomenon, identical 30-g samples of whey powder in 50 ml of water with 20 g of sodium sulfate were analyzed; one sample was heated to 150 F (65.6 C) for one hour prior to analysis, the other remained at room temperature. Table 15 below outlines the differences found between heated and non-heated whey powders.

Table 15. Peak area totals for heated and unheated whey powders.

Compound Types	Heated Whey	Unheated Whey	Increase
	----------mm^2----------		%
Total	9952	8169	21.8
Pyrazines	1720	1228	40.1
All 0-Containing	3058	2383	28.3
Furans	2008	1606	25.0

The only compound which showed no quantitative increase upon heating was 2-furfuryl alcohol.

The increase noted for heat induced compounds was not unexpected; however, the low temperature of heating (65.6 C) might not, according to Maga (30) have been sufficient to produce

pyrazine synthesis. This minor experiment demonstrates that whey is very sensitive to heat induced compound synthesis and demonstrates the sensitivity of our analytical technique.

Analytical and quantitation techniques for the above analysis were similar to those used for the whey powders and the model system.

Heated Product Substantiation

To further substantiate literature and analytical findings encountered in this research involving the presence of Maillard reaction products in the ingredient wheys used for the model system analysis, a secondary analytical technique employing wet chemistry (as opposed to MS) was used to analyze for hexose-amine compounds in whey powder. The presence of such compounds could be considered a confirmation of the presence of Maillard browning products, potential precursors for pyrazine and other flavor compound synthesis in whey powder.

The Elson-Morgan test for hexose-amines (glucose-amine) as described in the Experimental section, was used for this analysis. All whey samples gave a positive (red) reaction to this test indicating the presence of Amadori rearrangement products and further indicating that non-enzymic browning had proceded to a measurable extent.

CONCLUSION

The evidence presented herein substantiates the belief that flavors present in whey powder, as it comes from the container, can and do manifest themselves in a finished food product such as ice cream if that product is mildly flavored.

Whey powder quantity, that is, the amount of whey powder substituted for non-fat dry milk in the formulation of an ice cream product, appears not to be of major importance in predicting off flavor induction in that product. Whey powder quality (taste and odor) and the processing conditions to which the product mix is exposed after preparation, appear to have a greater impact on off-flavor development.

Flavors, which in other foods might be desirable (pyrazines, imparting a heated note), are potentially undesirable in ice cream and may not be masked by the other ingredients in the ice cream mix, on the contrary, they may remain volatile and potentially influence off-flavor development in the final product.

Further research is necessary to demonstrate the exact cause of "whey flavor", however this is a very arduous undertaking since no single "whey compound" was detected by this author during months of sniffing peaks eluting from a GLC column. To a certain extent this failure negates the purpose of this work, which

was to demonstrate the carry through effect and, originally, to attempt to suggest ways that whey off-flavors could be eliminated. Unfortunately since no whey compound(s) could be found and no simple processing alteration would rid whey of its off-flavor, it is not possible to offer a "miracle" cure for whey flavors. However, we experienced wheys of reasonable quality in this research. The best suggestion for a food manufacturer wishing to use whey, is to be discriminating in choice of product; the best suggestion for a whey processor is to be as careful and as quality oriented in the handling of whey as one would be with milk. Good sanitation, good temperature and time control of heating processes, proper crystalization techniques and above all good personnel attitude will go a long way toward accomplishing the "miracle" of good flavored whey.

Further research to find a quick analytical assay for added whey in ice cream would be presently useful, as a quality control method--however, as this author and others point out--whey quantity may not (especially after future improvements in whey processing technology) require regulation as a mix ingredient in ice cream.

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