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Title: Some Factors Involved in the Clarification of Whey Wine

Abstract approved _____
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The development of whey beverages including whey wine has been an area of whey utilization research. One study to produce a commercially salable wine from sweet whey, particularly cheddar whey, was undertaken. It included developing a process to clarify the naturally cloudy wine, monitoring the physical and chemical changes which occurred during the wine making process, comparing the sensory differences in the unclarified and clarified wine, and incorporating the wine sediment in a food product.

The criteria for the clarification process were simplicity, legality, reasonable cost, minimal usage of energy and equipment, and maintenance of the wine character and quality. Existing procedures for clarifying grape wine such as filtration, centrifugation, and the addition of fining agents, casein, Cold Mix Sparkolloid, gelatin, tannin, and bentonite were investigated. Also investigated were techniques used to separate the proteins from whey; specifically pH adjustment (with potassium carbonate) and precipitation (with sodium

hexametaphosphate). It was found that the most feasible clarification procedure was the addition of 0.20-0.50 percent bentonite on a dry weight basis followed by a polishing filtration.

Determinations for lactose, protein, fat, ash, and total solids and tests for Brix, pH, titratable acidity, and alcohol content were performed to monitor the effects of fermentation, aging, and clarification. The changes noted during fermentation and aging were primarily due to alcoholic and lactic acid fermentations. Bentonite fining had a dilution effect but did increase the ash content of the wine.

Triangular difference tests with blindfolded tasters indicated that there was no significant taste difference between cloudy wine and bentonite-fined and filtered wine. A cursory gas-liquid chromatographic analysis revealed only a slight difference in the gross volatile components of unclarified and clarified wines.

The wine sediment was dried to a paste and substituted at the five and ten percent levels for nonfat dry milk in a commercial sugar cookie recipe. A preference test showed that the sediment decreased the acceptability because of its "acid" taste. It is likely that the sediment could successfully be utilized in a fermented or cultured dairy product.

Some Factors Involved in the Clarification of Whey Wine

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

SOME FACTORS INVOLVED IN THE CLARIFICATION OF WHEY WINE

I. INTRODUCTION

Historically whey has been used to a limited extent in human and animal nutrition, but generally this by-product of cheesemaking has universally been considered a waste product. Research to develop uses for whey as food and animal feed has recently accelerated. The growing concern about environmental pollution has resulted in stringent water quality standards which have changed the economics of whey disposal (13; 27, p. 153). The practice of discharging whey into natural water systems is now illegal or severely restricted. As a result of these developments, whey has a negative value for many cheese plants.

Another aspect of the emphasis on whey utilization is the concern over present and future food shortages, and that the lactose, vitamins, minerals, and high-quality protein in whey should not be wasted. Even though these constituents are present at relatively low levels in whey, their quantities are significant because of the large volumes of whey involved. The annual increase in whey production, presently about 26 billion pounds in the United States, is an important aspect of the need for whey utilization research.

The development of whey-based alcoholic beverages has been an area of research (67; 19). This particular study was a facet of a research effort by Yang and co-workers (66) to develop a commercially

salable wine from sweet whey, notably cheddar whey, that could be produced with the existing facilities of cheese plants. This wine-making procedure, as developed, allows for the utilization of the whole whey and requires minimal energy inputs and processing equipment. The whey wine has a potentially good economic value and yields nutritious sediments as by-products.

Wine appearance is a critical product attribute, and preliminary tasting of the wine produced by Yang et al. (66) showed clarified (clear) wine to be preferable to unclarified (cloudy) wine. Experimentation to find an acceptable clarification method was based on the assumption that the wine cloud was comprised largely of proteins and yeast materials. The experimental criteria were simplicity, legality, reasonable cost, minimal usage of energy and equipment, and maintenance of the wine character and quality. Specifically, the research focused on the following:

Clarification methods: Various techniques and substances were used to attempt to remove the wine cloud. The experimental approaches were based on commercial wine clarification practices and methods of whey protein separation.

Physical and chemical changes: Analyses and tests were performed to monitor and better understand the nature of changes due to fermentation, aging, and clarification. The whey, cloudy wine, clear wine, and sediments were analyzed for gross composition and tested for such properties as Brix, pH, titratable acidity, and alcohol content.

Cloudy wine versus clear wine: Taste panels were held to determine the acceptability of cloudy and clear wines relative to each other and in absolute terms. A cursory comparison was made of their volatile components using gas-liquid chromatography.

Use of the wine sediment: The wine lees represented a nutritious by-product and were incorporated into a food product as a means of utilizing it. The addition of whey protein concentrates and dried or condensed whey to commercial food products provided direction.

II. REVIEW OF LITERATURE

Separation of Proteins from Whey

Whey from whole milk natural cheeses (such as cheddar) resulting from rennet coagulation is termed sweet whey. That derived from the manufacture of cottage cheese is termed acid whey.[1] Both are basically five percent lactose solutions with lesser amounts of protein, ash, and fat. Sweet whey has a range of pH 5-6 and 0.15-0.25 percent titratable acidity expressed as lactic acid while acid whey has a range of pH 4-5 and 0.26-0.55 percent titratable acidity.

The whey or serum proteins, the noncasein proteins, comprise approximately 20 percent of the milk proteins. The chemistry and nature of these proteins are discussed in the literature (38; 58; 64; 26, p. 101). β-Lactoglobulin and α-lactalbumin are the major serum proteins. Both have isoelectric points near pH 5.1, and both are heat-denaturable. The minor serum proteins present in the largest amounts are blood serum albumin and the immunoglobulins. Those present at trace levels include lactollin, lactoferrin, transferrin and glyco-protein-a. There is also the poorly understood "proteose-peptone" fraction composed of low molecular weight glycoproteins.

Denaturation of the whey proteins is one method of separation. It involves a breakage of covalent bonds which causes a random,

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[1]For purposes of this paper, the term "whey" will mean sweet whey. Acid whey will be designated as such.

unfolded conformation of the protein. This results in a decrease in protein stability or solubility which may lead to aggregation and coagulation. The unfolding causes sulfhydryl groups to become more accessible and reactive. Sulfhydryl groups and disulfide bonds are intimately involved with the aggregation process as explained by Lyster (35).

Denaturation of serum proteins is commonly accomplished with heat. Larson and Rolleri (33) found the immunoglobulins and α-lactalbumin to be the most and least heat sensitive respectively, while β-lactoglobulin and serum albumin exhibited intermediate sensitivity. This contrasts somewhat with results obtained by Park and Kim (49) who employed 15-minute heat treatments at various temperatures. They reported denaturation temperatures as being 75°C for serum albumin and the immunoglobulins, 85°C for β-lactoglobulin, and 95°C for α-lactalbumin. In his review Lyster (35) states that the denaturation of α-lactalbumin follows first-order kinetics while that of β-lactoglobulin follows second-order kinetics under certain conditions of temperature and pH. Gough and Jenness (12), in studying the heat denaturation of β-lactoglobulin, discovered that genetic variant B was more rapidly denatured than genetic variant A. It should be noted that no appreciable denaturation of the whey proteins occurs at the legal minimum pasteurization times and temperatures.[2]

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[2]Sixteen seconds at 161°F for the high-temperature short-time (HTST) method, and 30 minutes at 145°F for the holding method.

Ionic strengths and pH affect the stability of proteins. At their isoelectric point, proteins have a minimum net charge and hence minimum hydration and solubility due to their amphoteric nature. Generally, ionic strengths of 0.01-0.50 percent increase the solubility of proteins; at higher ionic strengths proteins tend to be less soluble because they compete with salt ions for water molecules.

The relationship of whey protein denaturation by heat to pH and ionic concentration has been researched. Jenness (25) reported that heat-denatured whey proteins generally exhibited less solubility in acid solutions and in salt solutions. Nielsen et al. (45) evaluated the effect of temperature, time, pH, and total solids concentration on protein denaturation in Colby cheese whey. The factor ranges were 60-90°C for temperature, 1-30 minutes for time, 4.5-7.8 for pH, and 6-60 percent for total solids. Temperature, followed by total solids, pH, and time were found to have respectively less effect upon protein denaturation. Kenkare et al. (31) found that removing calcium by pH adjustment and dialysis decreased the heat stability of proteins in skimmilk sera. These findings are in agreement with Morr and Josephson (42) who concluded both calcium and casein stabilized whey proteins against heat denaturation.

Serum proteins are precipitated with polyphosphates. Hartman and Swanson (17) achieved best results by adjusting whey to pH 2.5 and adding 0.5 percent sodium hexametaphosphate. Melachouris (40) examined the interaction of β-lactoglobulin and polyphosphates. With one percent solutions of each, one-third of the β-lactoglobulin was precipitated by adding 15 percent polyphosphate by volume.

Precipitation increased with increasing polyphosphate chain length, whereas temperature variation between 5.0°C and 73.5°C had no effect.

Jones and associates (28) precipitated 98-99 percent of the proteins in acid whey with "ferripolyphosphate," a combination of ferric chloride and sodium polyphosphate glass. The precipitate contained 22 percent protein, 12 percent iron, 39 percent phosphorous pentoxide, and 5 percent calcium. Over 75 percent of the iron and 90 percent of the phosphate remained in the liquid fraction.

Morr and Lin (43) researched the feasibility of precipitating serum proteins with alcohols as an initial step in preparing whey protein concentrate. The addition of ethanol at the 75 percent level on a volume basis to calcium-free whey precipitated 40 percent to 60 percent of the proteins. They proposed an aggregation mechanism of protein dehydration followed by adsorption of the alcohol which disorganized the hydrophobic interior of the protein molecule.

Hildalgo and Hansen (18) adjusted whey to pH 3.2 and recovered 90 percent of the protein as a precipitate by adding an equal volume of 0.25 percent carboxymethylcellulose solution. In a subsequent study, Hansen and co-workers (15) obtained maximum protein precipitation from whey at pH 4.0 with an equal volume of 0.13 percent carboxymethylcellulose solution.

Townend and Gyuricsek (59) added sufficient cysteine to acid whey to give a 0.006 M concentration. Subsequent heating at 80°C for 15 minutes precipitated approximately 50 percent of the proteins, more than was precipitated by boiling the whey for 30 minutes. They submitted that the cysteine caused aggregation by reducing the disulfide

bonds in the whey.

Holsinger (19) in her review of whey beverages mentions that proteolytic enzymes and tannins both are used to deproteinize whey. Tannin combines reversibly with the peptide or amide linkage of the protein to form a hydrogen-bonded complex. As part of a patented process for a whey beverage, Murray (44) deproteinized whey by heating it for 10 minutes at 200°F, cooling it to 60°F, and adding 25 ml/l of 50 percent citric acid and 9 ml/l of 10 percent tannic acid.

Reverse osmosis, ultrafiltration, and electrodialysis are membrane processes which are used to concentrate or separate solids in a liquid. Only water is removed by reverse osmosis so it is a concentration process, whereas solutes can be separated by ultrafiltration on the basis of their molecular weight and by electrodialysis on the basis of their electrical charge. Although all three processes operate on simple principles, they are sophisticated techniques and require specialized equipment. Ultrafiltration presently has the widest commercial application of the three to whey processing (8; 20). The retention of from 80 to 98 percent of the whey proteins is attainable depending on the selectivity of the membrane and other parameters. In some instances reverse osmosis is used to concentrate the whey prior to ultrafiltration.

Clarification of Wine

The clarification of wine can involve fining, filtration, centrifuging, and heating. Amerine et al. (1, p. 299) and Amerine and Joslyn (2, p. 528) discuss the subject of wine clarification extensively.

Fining is the addition of a substance or substances which combine chemically with wine colloids or neutralize their charge to produce a coagulum which settles out. Common fining agents include casein, gelatin, tannin, isinglass, and bentonite.

The alkaline nature of casein and sodium or calcium caseinate is the property that determines their fining ability in wine. The acidity of the wine neutralizes the casein which then precipitates and adsorbs suspended material. O'Neal and associates (48) concluded the 0.04-0.06 percent added casein gave good results for most wine types. Best results were obtained at room temperature when the casein was allowed to settle before filtration. In relation to bentonite, casein resulted in less sediment but also gave less clarification.

Joslyn (29) has reviewed the mechanism and chemistry of gelatin fining. Gelatin added to wine possesses a positive charge and forms a precipitate with the negatively charged tannin in wine. The volume of lees in gelatin-fined wine is usually less than two percent. Red wines exhibit a reduction in color and tannin content when fined with gelatin. White wines, which are low in tannin, are fined by first adding tannin and then gelatin in approximately equal quantities. The development of a persistent cloud can occur in white wine overfined with gelatin. The level of gelatin commonly used in wine fining is 0.03-0.05 percent.

Isinglass, a product derived from sturgeon, when added at levels of 0.005-0.015 percent can result in a brilliant wine. However, it is expensive and tends to produce a loose sediment which requires careful filtration procedures.

Bentonite, a montmorillonite clay, is the fining agent of choice for bulk wine production in California and elsewhere. It produces bulky lees but possesses an excellent capacity to remove proteins, a topic discussed by Rankine and Emerson (52). One theory relates the fining ability of bentonite to the mutual flocculation of the negatively charged bentonite and the positively charged proteins. A second explanation is that bentonite has the ability to adsorb organic cations and anions. Sodium or natural bentonite has a swelling capacity two to three times as great as calcium bentonite and is able to remove more protein.

Kean and Marsh (30) report bentonite as being superior to organic fining agents with regard to protein removal and wine stability. Gelencser (11) tested several bentonites and concluded that heat-labile proteins were almost completely removed by bentonite fining. Sommers and Ziemelis (57) discovered that bentonite fining after fermentation efficiently removed both protein and non-protein nitrogen. The amounts of KWK bentonite, a sodium bentonite, used in commercial fining are 0.05-0.20 percent for table wines and 0.40-0.70 percent for dessert wines.

Other fining agents include egg albumin, pectic enzymes, polyvinylpyrrolidone, and Sparkolloid, a proprietary product. Sparkolloid is a complex of refined polysaccharides in a dispersion carrier of diatomaceous earth which goes into wine as a negatively charged macromolecule and is recommended for hard-to-clarify wines. One advantage of Sparkolloid is that it yields compact lees.

The practice of employing heat stabilization and centrifugation to clarify wine is uncommon. Heat stabilization may involve holding the wine at 60°C for 72 hours, but this is too severe a treatment for most wines. Centrifugation unduly aerates wine in many instances and is more usually employed for the clarification of wine lees. One advantage of centrifugation as pointed out by Fessler and Nasledov (9) is the small loss of wine.

Filtration is an important clarification technique in commercial wine production (1, p. 299). Filtration may be used to clarify cloudy wines but is usually employed subsequent to fining or racking. This is because the rate of filtration varies with the clarity of the wine, and a rapid rate is an economic necessity.

A series of asbestos or fiber pads between hollow metal plates mounted on a frame is utilized for a polishing or finishing filtration. Filter aids are often added to wines and held in suspension by agitation prior to filtering. This minimizes the clogging effect of wine particles and allows more wine to be filtered before replacement of the pad is necessary.

III. MATERIALS AND METHODS

Materials

Whey

Unsalted, separated cheddar cheese whey was obtained from several Oregon cheese plants. The whey was collected by plant personnel in ten-gallon stainless steel milk cans, placed in a freezer room for four hours, and transferred to a cooler until pick-up the following day. The cans of whey were transported by car to the research facility in Corvallis. The transport, which took a maximum of three hours depending on the location of the cheese plant, included packing the cans in crushed ice on warm days.

Whey to be used within seven days was held at 34°F and allowed to warm to room temperature prior to inoculation. Whey to be used after a longer storage period was frozen and then thawed at room temperature prior to inoculation.

Yeast

The wine yeast *Saccharomyces cerevisiae* variety *ellipsoideus*, Montrachet strain was used for inoculation. It was obtained from Oregon Specialty Company of Portland, Oregon, in five-gram packets which were stored at refrigerator temperature.

Clarification Agents

Granular KWK bentonite, the sodium bentonite commonly employed

in wine clarification, was obtained from Oregon Specialty Company of Portland, Oregon. C-102 Cold Mix Sparkolloid was supplied by Scott Laboratories of Richmond, California. The other clarification agents tested, casein, gelatin, tannic acid, sodium hexametaphosphate and sodium carbonate, were obtained from the laboratory stock of chemicals.

Analytical Methods

Brix

The Brix is the percent sugar by weight at 20°C. A plastic cylinder was filled with sample at room temperature, and the Brix was measured with a saccharometer, a specific type of hydrometer. For fermenting whey samples, the saccharometer reading was taken five minutes after sampling in order to reduce the carbon dioxide content.

Titratable Acidity

Ten ml of sample were titrated with a standard solution of 0.1 N sodium hydroxide to an endpoint of pH 8.3, and the acidity expressed as percent lactic acid. In the instance of fermenting whey, a 15 ml portion was first removed and after five minutes the 10 ml sample was pipetted.

Alcohol Content

An ebullioscope was used to determine the alcohol content following a procedure similar to that detailed by Amerine et al. (1, p. 696). Fifteen ml of distilled water and 50 ml wine were separately heated to constant boiling temperatures. The percentage alcohol by volume based on the two boiling temperatures was read from the slide rule.

Lactose Determination

The lactose determination used was the Association of Official Analytical Chemists official gravimetric method (4, p. 224). Twenty-five grams of sample were diluted with 400 ml distilled water in a 500 ml volumetric flask. Ten ml of copper sulfate solution[3] and 8.8 ml of 0.5 N sodium hydroxide were added which yielded an acid solution with the copper as a solute. Distilled water was added to the 500 ml mark, and the liquid was mixed and filtered through a Whatman number one filter paper.

Twenty-five ml each of copper sulfate solution and alkaline tartrate solution[4] and 50 ml of filtrate were combined in a 400 ml beaker. The beaker was covered with a watch glass and heated on an electric heater so that the mixture began boiling in exactly four minutes. After boiling two minutes the mixture was filtered through a medium-grade sintered glass filter using suction. The beaker and the cuprous oxide precipitate were washed completely with 60°C distilled water. The precipitate was then washed with ten ml of alcohol and finally with ten ml of ethyl ether. Following this, the precipitate was oven dried for 30 minutes at 100°C, cooled in a desiccator, and weighed. The weight of lactose equivalent to the weight of cuprous oxide was obtained from the appropriate table and expressed as a percentage of the sample.

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[3]34.639 grams of copper sulfate were dissolved in distilled water, diluted to 500 ml, and filtered through a Whatman number one filter paper.

[4]173 grams of Rochelle salt and 50 grams of sodium hydroxide were dissolved in water, diluted to 500 ml, and filtered through prepared asbestos.

Protein Determination

The basis for the protein analysis was a modification of the official Association of Official Analytical Chemists Kjeldahl determination for nitrogen (4, p. 223).

Approximately three grams of sample were weighed into a tared plastic weigh dish, and then rinsed into an 800 ml Kjeldahl flask with a minimum of distilled water. A packet containing eight to ten grams of potassium sulfate and one gram of copper sulfate pehtahydrate, three Hengar granules, and 25 ml of concentrated sulfuric acid were added to the flask. The flask was placed on a digestion rack in a hood and heated over low heat for a period of time ending one half hour after the solution became blue-green. The flask contents were allowed to cool to room temperature, and 400 ml of distilled water were added with the flask in an inclined position. Next, two pieces of mossy zinc were added, and 75 ml saturated sodium hydroxide were layered under the flask solution with minimal mixing.

The flask was connected to a condenser, and the solution swirled until a uniform color was achieved. Two hundred ml of condensate were distilled into 50 ml of four percent boric acid containing indicator.[5] The boric acid-condensate mixture was titrated to a gray endpoint with a 0.02 N hydrochloric acid. The percentage of protein in the sample was calculated according to the formula:

$$\% \text{ protein} = \frac{\text{ml of HCl} \times 1.4 \times 6.25 \times \text{Normality of HCl}}{\text{weight of sample}}$$

[5]The indicator for ten gallons of four percent boric acid consisted of 0.25 grams methyl red, 0.25 grams brome cresol green, and 250 ml ethyl alcohol.

Fat Determination

A modification of the Association of Official Analytical Chemists method for the fat content of raw milk with the TeSa reagent (4, p. 225) proposed by Anderson Laboratories, Inc. of Fort Worth, Texas, (3) constituted the fat analysis procedure. Eighteen grams of sample and nine grams of TeSa reagent were weighed into a Babcock skimmilk bottle calibrated from 0.00-0.25 percent. Two ml of 20 percent sodium hydroxide were added, and the bottle contents were mixed by swirling for five seconds. The bottle was then placed in a boiling water bath for 15 minutes and swirled for five seconds every three minutes. Next, 40 percent ethanol was added to raise the meniscus to near the top of the graduations in the bottle neck. The bottle was then centrifuged two minutes at 140°F, placed in a 140°F water bath, and the amount of fat in the bottle neck read with calipers. The fat was expressed as a percentage of the sample.

Ash Determination

The ash determination procedure was based on the official Association of Official Analytical Chemists method for milk (4, p. 223). Approximately five grams of sample were weighed into a porcelain crucible previously dried to a constant weight. The sample was evaporated to dryness on a steam bath and then ignited in a muffle furnace at 500-540°C. The sample was maintained at that temperature until a gray-white ash with constant weight was obtained. This commonly required ten hours, after which time the crucible was cooled in a desiccator. The ash was then weighed and expressed as a percentage of

the sample.

Total Solids Determination

The Association of Official Analytical Chemists official method for milk was the basis of the total solids determination. Approximately three grams of sample were weighed into an aluminum dish containing approximately 25 grams of washed, ignited sand. The dish was heated on a steam bath for 15 minutes and placed in an air oven at 100°C for three hours. Afterwards, it was cooled in a desiccator, weighed quickly, and the percentage total solids calculated. The sand was used to prevent error due to crust formation by the lactose (51, p. 487).

Gas-Liquid Chromatography

The chromatographic analyses were performed on a Varian Aerograph 1200 with a hydrogen flame ionization detector. The injection port temperature was 200°C, and detector temperature was 260°C. The range was one with an attenuation of 128.

The column was a 12-foot by one-eighth inch stainless steel column packed with five percent butanedial succinate (BDS) and 0.05 percent igepal on Chromosorb G. It was conditioned overnight at 175°C and maintained at 135°C during the analysis. The samples of ten μl were injected directly with a 30 ml per minute flow rate of nitrogen carrier gas. The chart speed was 30 inches per hour (55).

Wine Making Procedure

A ten-gallon can of fresh cheddar cheese whey served as the starting material. Sufficient dextrose, 22 percent of the whey by weight, was mixed into the whey to give a Brix reading of approximately 22.0. Ten numbered one-gallon glass jugs were each filled with 3600 ml of sweetened whey and inoculated. One gram of yeast was dissolved in ten ml of water at 110°F and added to each container without mixing. The jugs were fitted with fermentation locks, and the fermentation was allowed to proceed at room temperature. The fermentation was judged as finished when the Brix reading remained unchanged for three consecutive days. At this point the wine was racked into new glass jugs, and the sediment from each jug was frozen separately for subsequent analysis. The new jugs were purged with carbon dioxide and sealed with screw caps. The wine was racked a second time after 17 additional days and a third time after another 33 additional days following the same procedures. At this point the wine was considered cloudy or unclarified wine. In some instances, small amounts of material of a lipid nature were observed floating on the surface of the wine and were skimmed off prior to racking.

Clarification Procedures

Filtration

The filtration system consisted of an Ertel ESP Model self-priming stainless steel pump and an Ertel E-1 Model stainless steel filter fitted with a number nine grade Ertel asbestos filter pad. The

pad was placed in the filter and washed with one gallon of one percent lactic acid solution. Sufficient water was then pumped through the system until no "paper taste" could be detected. The wine was then filtered at 15-20 pounds of pressure.

Centrifugation

A Model UV centrifuge manufactured by International Equipment Company was used to centrifuge 250 ml portions of cloudy wine at 3000 revolutions per minute for various times.

pH Adjustment

Amounts of 20 percent potassium carbonate solution were added to 50 ml aliquots of unclarified wine using a burette. The addition was monitored with a pH meter, and the amounts were designed to give a continuum from pH 4.5 to pH 5.9. The mixtures were then transferred to test tubes, shaken vigorously for ten seconds, and sealed with corks. After 72 hours the results were noted.

Precipitating Agent

Various amounts of a five percent solution of sodium hexametaphosphate were added to 50 ml aliquots of cloudy wine in test tubes with ten seconds' vigorous shaking. The tubes were sealed with corks, and the results were noted after 72 hours.

Fining Agents

The fining agents were prepared as slurries or solutions and added to 50 ml aliquots of unclarified wine in test tubes with ten seconds of vigorous shaking. The degree of resulting clarification

was judged visually after 72 hours.

The bentonite was pre-swelled before addition to the wine in accordance with present industry practice. Sixty-four grams of bentonite and 800 ml water were combined in a 2000 ml Erlenmeyer flask and injected with steam for one hour with magnetic stirring. The mixture was covered and kept at room temperature until the following day when it again was subjected to one hour of steam injection. The amount of water introduced by the steam resulted in a final slurry of six percent bentonite.

Following the manufacturer's directions, 100 ml of cold distilled water and 2.4 grams of Cold Mix Sparkolloid were mixed at high speed to yield a smooth slurry ready for use.

Solutions of casein, gelatin, and tannin were prepared per guidelines given by Amerine et al. (1, p. 310). A two percent casein solution was prepared by dissolving 0.5 gram sodium carbonate in 100 ml of water and adding two grams of casein. A one percent solution of gelatin in water was made up by heating one gram of gelatin in 100 ml of water and adding 0.5 gram of citric acid and 0.1 gram of sodium benzoate. One gram of tannic acid was dissolved in 100 ml of water to give a one percent tannin solution.

Sensory Evaluation Methodology

Triangular Difference Test, Blindfolded

A section of counter space in a quiet area of the Oregon State University sensory evaluation facility (flavorium) was utilized to conduct triangular difference tests. Twenty tasters were enlisted and

were seated one at a time at the counter and blindfolded. One-ounce samples at room temperature in six-ounce drinking glasses were presented to the taster in a randomized order. A facilitator aided the taster with the initial tasting and with any retasting requested. The taster was asked verbally to identify the different sample.

Preference Test

Professor Lois McGill and her staff conducted the preference test in the Oregon State University flavorium. Twenty tasters were presented three samples in randomized order in paper cups marked with randomly chosen three-digit numbers. Tasters judged each sample on a ballot using a nine-point hedonic scale with the value one representing "dislike extremely," five representing "neither like nor dislike," and nine representing "like extremely."

Use of Wine Sediment

Preparation of Sediment

The frozen sediment from the first racking was thawed at room temperature and filtered through a number one Whatman filter paper until a paste-like consistency resulted. The total solids in the wine sediment paste was determined to be 33.8 percent.

Preparation of Food Product

A commercial sugar cookie recipe (36, p. 128) with a base of 100 pounds of flour was scaled down to a base of one-half pound of flour. Cocoa and vanilla were added as flavorings, and the wine sediment paste was substituted for five percent and ten percent of the

nonfat dry milk (see Appendix I). The finished dough was rolled out into a cylinder approximately two inches in diameter and sliced into one-fourth inch thicknesses. The cookies were then baked at 350°F for 12 minutes.

IV. RESULTS AND DISCUSSION

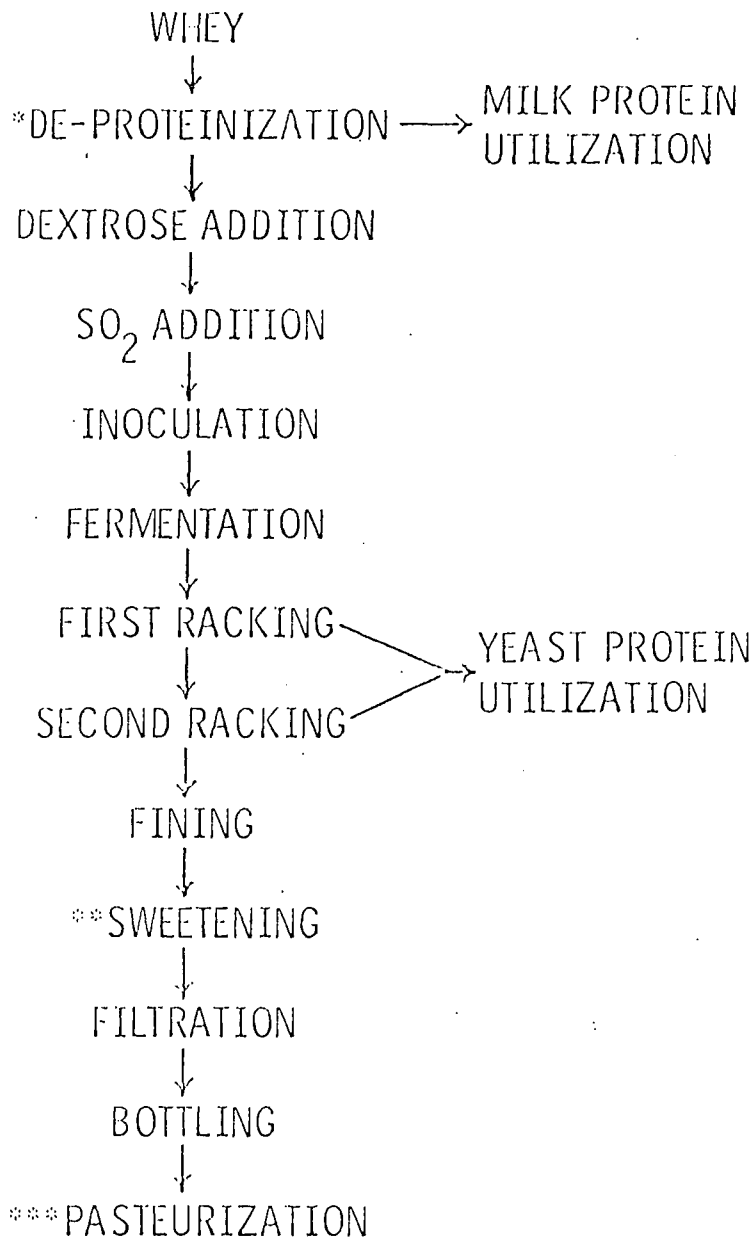
Wine Making Procedure

The methodology developed by Yang and associates (66), schematically diagrammed in Figure 1, was the basis for the wine making procedure. Differences exist in the two approaches because Yang's procedure reflects the results of additional research. For instance, partially deproteinizing the whey by heating it for five minutes at 180°F was found to decrease the wine cloud without significantly affecting wine taste. Research into yeast strain, fermentation temperature, and the need for nutrients and preservatives was also undertaken.

The Montrachet strain of yeast was compared against four other wine yeast strains, namely Burgundy, Port, Champagne, and Sherry. All of the yeasts produced wines with similar sensory qualities and with alcohol levels over ten percent, but Montrachet gave the most rapid fermentation. At room temperature this yeast required an average of seven days for fermentation as opposed to eight to 14 days for the other strains.

Fermentation temperatures of 55°F, 72°F, and 90°F were tested. The fermentation rate was faster with increasing temperature, but at 90°F the wine possessed an unacceptable flavor and slightly less alcohol. Besides requiring little or no energy input, fermentation at 72°F was preferred in terms of wine taste and alcohol content.

To determine if the whey-dextrose mixture lacked nutrients for optimum yeast growth, vitamins B_1 and B_2 and two levels of nitrogen,



*Omitted if a cloudy wine is produced
**Omitted when a dry wine is produced
***Omitted if an aseptic method is used

Figure 1. Production of sweetened clear whey wine.

added as ammonium phosphate and ammonium chloride, were tested separately for their effect on fermentation rate and alcohol production. Considering these criteria, none of the added nutrients produced a wine significantly different from the control.

The need for a preservative was also investigated. The two preservatives legally approved for wine making in the United States, sulfur dioxide and sorbic acid, were individually added to whey prior to inoculation at several concentrations from 50 ppm to 400 ppm. Standard plate counts of the wine after 19 weeks revealed that all wines, including the control, were nearly sterile. The conclusion was drawn that the inherent preservative effect of the alcohol and lactic acid precluded the need for added preservatives. Mention was made that 100 ppm of sulfur dioxide may be desirable since it produced a cleaner tasting wine.

The Effects of Clarification Techniques

Sensory evaluation tests by Yang et al. (66) indicated filtered whey wine was preferable to unclarified whey wine. Tasters gave the former a rating of 6.3 and the latter a rating of 5.2 on the same nine-point hedonic scale used in the present research. The color of the clear wine is a light amber similar to a white grape wine while that of cloudy wine is a dull, opaque yellow-gray.

The wine cloud was assumed to be composed mostly of protein colloids with some yeast materials. Only a small amount of the colloidal content of new grape wines is due to yeasts as they tend to settle rapidly after fermentation.

Heat denaturation is a common method of precipitating whey proteins. However, only cursory experimentation was undertaken in this direction because it was presumed the times and temperatures involved would cause substantial alcohol loss and undesirable flavor and odor changes. Pofahl and Vakaleris (5) noted a 25 percent increase in the concentration of sulfhydryl groups with heat denaturation of the serum proteins. Hutton and Patton (22) reported that heating whey proteins lowered the oxidation-reduction potential and "liberated" sulfhydryl groups and volatile sulfides, particularly hydrogen sulfide, to impart a cooked flavor. The cysteine portion of the β-lactoglobulin was the major source of these sulfhydryl groups. In actuality, whey wine heated for 90 seconds at 100°C possessed a cooked flavor and odor, exhibited only moderate clarity, and had a reduced alcohol content of 8.7 percent.

The obvious clarification technique was filtration, but experimentation revealed it to be impractical when used alone. A filter pad designed for close filtration was needed to remove the wine cloud, but the operation was characterized by a slow flow rate and frequent replacement of the pad. The filtration efficiency was not significantly increased by employing a filter aid. It was apparent that filtration would have to be preceded by removal of all or most of the wine cloud. The research approach to effect this cloud removal was to test techniques and substances utilized in clarifying grape wines and in separating the proteins from whey.

Centrifugation as a means of clarification represented energy input and the need for expensive equipment at the commercial production

level. These reasons, together with the fact that promising results were being obtained with bentonite, limited experimentation in this area. In one instance, whey wine centrifuged for 30 minutes at 3000 revolutions per minute exhibited only a slight decrease in cloudiness.

The adjustment of the wine pH to the isoelectric point of the major whey proteins was investigated. The results of mixing 0.65 ml to 1.30 ml of 20 percent potassium carbonate solution into 50 ml of whey wine to produce a continuum from pH 4.5 to pH 5.9 are given in Table I. A range of pH 4.9-5.9 resulted in wines with good clarity and a white coagulum. Maximum clarity was observed at pH 5.3-5.5. This pH is slightly higher than the isoelectric points for β-lactoglobulin and α-lactalbumin, but ionic concentration can shift the isoelectric point of proteins. The precipitation may also have been due to a combination of the hydrogen ion concentration and the alcohol level.

Since the wine tasted flat from acid neutralization, each sample was racked and its titratable acidity increased to 0.7 percent with lactic acid. No change in clarity was seen, but an unacceptable salt taste, presumably carbonate, was detectable.

Sodium hexametaphosphate was added to the cloudy wine at concentrations of 0.0025-0.15 percent. Good clarity was observed at levels of 0.10 percent and higher, but the wine possessed an off-flavor. The ability of the trivalent phosphate anion to form aggregates of the positively charged protein molecules is the probable cause of precipitation.

TABLE I. EFFECT OF pH ADJUSTMENT ON WHEY WINE CLARIFICATION.

Sample number	20% Potassium carbonate added	Percentage potassium carbonate added	pH	Appearance
	Milliliters	Dry weight basis		
1	0.65	0.26	4.5	Slightly cloudy
2	0.80	0.32	4.7	Slightly cloudy
3	0.95	0.38	4.9	Clear
4	1.10	0.44	5.1	Clear
5	1.20	0.48	5.3	Very clear
6	1.25	0.50	5.5	Very clear
7	1.30	0.52	5.7	Clear
8	1.35	0.54	5.9	Clear

The ability of wine fining agents to remove a colloidal suspension of animal origin was untested, but their negligible or minimal effect on grape wine character was a desirable attribute. Casein, gelatin, tannin, Cold Mix Sparkolloid, and bentonite were the fining agents tested. Their ability or inability to fine whey wine when added in various concentrations on a percent dry weight basis is shown in Table II. Most of these data are reported by Yang et al. (66).

Casein exhibited no fining action when incorporated at levels of 0.01-0.20 percent. Since the acidities of grape and whey wine are somewhat similar, it is reasonable to expect casein to precipitate with a clarifying action in whey wine as it does in grape wine. Furthermore, the ethanol present would tend to decrease the charge on the casein particles and their solubility by reducing the dielectric constant.

Casein exists in milk as part of a complex system (26, p. 305) so its behavior upon addition to whey wine is speculative. Above its isoelectric point of pH 4.7, casein tends to bind divalent cations, particularly calcium and magnesium, which favors its aggregation and precipitation. The pH of whey wine is approximately 4.0, however, so casein should favor binding with water molecules. Also, only one-third of the calcium and three-fourths of the magnesium in milk remain in the whey, as pointed out by Verma and Sommer (62). Lastly, the casein, as in the fining of grape wines, was dissolved in a dilute sodium carbonate solution to increase its solubility, and sodium causes a higher degree of hydration and dispersion of the casein particles. To increase the likelihood of precipitation, casein dissolved in a dilute calcium

TABLE II. EFFECT OF FINING AGENTS ON WHEY WINE CLARIFICATION.

Fining agent and percentage added	Clarifying action[1]			
Dry weight basis	Poor	Fair	Good	Excellent
Casein				
0.01-0.20	x			
Cold Mix Sparkolloid				
0.01-0.20	x			
Gelatin				
0.01-0.16	x			
Tannin, gelatin				
0.04, 0.01-0.16	x			
0.08, 0.01-0.16		x		
0.16, 0.01-0.16		x		
Tannin				
0.04	x			
0.08		x		
0.16			x	
Bentonite				
0.10	x			
0.20		x		
0.30			x	
0.50				x

[1]Based on visual observation 72 hours after addition of fining agent.

carbonate solution was added to the wine at levels from 0.01-0.80 percent, but only slight fining action was observed.

Cold Mix Sparkolloid was introduced into the wine at concentrations of 0.01-0.20 percent with no observable change in the wine cloud. It solubilizes as a negatively charged macromolecule in grape wine and would be expected to flocculate with the protein colloids of whey wine. This failure to flocculate may involve an inability of the polysaccharide to disperse properly.

The degree of cloudiness was not reduced when gelatin was mixed with the wine at concentrations of 0.01-0.16 percent. This was somewhat expected since gelatin would be positively charged at the pH of whey wine. In the fining of grape wines, tannin, whether added or present naturally in the wine, combines with gelatin to form a precipitate. Several combinations of tannin and gelatin in the respective ranges of 0.04-0.16 percent and 0.01-0.16 percent were added to whey wine with the tannin being introduced four hours before the gelatin. Fining action was observed to be correlated to higher amounts of tannin and resulted in a granular sediment of light violet color. The fined wine had a distinct astringent taste.

Tannin by itself produced acceptable fining at a concentration of 0.16 percent. The coagulum was dark violet, and the wine again possessed an unacceptable astringent note. The oxidation of phenolic compounds in the tannin to ultimately form colored polymers is probably the explanation for the violet sediment.

Bentonite was introduced at levels of 0.01-0.50 percent and was found to be the most effective fining agent tested. It is noted

for its ability to clarify recalcitrant wines as well as to remove proteins. Amerine and Joslyn (2, p. 533) offer an extensive review of bentonite fining studies.

At concentrations of 0.30 percent and 0.50 percent respectively, bentonite produced an acceptably clear and a brilliantly clear wine after 72 hours, each with a loose, white sediment. Fining continued with time, and after ten days wine containing 0.20 percent bentonite achieved good clarity.[6] Figure 2 is a graphical representation of the relationship between the amount of bentonite added to the wine and its protein content as determined ten days after bentonite addition. The amount of protein and the degree of cloudiness decreased with increasing bentonite. Only a slight cloud was visible at 0.15 percent bentonite, and, as stated, concentrations of 0.20 percent or more produced clear wines. From these results and observations it can be theorized that the cloud is comprised largely of proteins, that there is a limit to the amount of proteins that bentonite will remove, that over-fining with bentonite will still result in a clear wine, and that a significant quantity of proteins soluble under those conditions still exist in the clear wine.

There are two problems apparent with bentonite fining. One problem is the bulkiness of the lees which would result in product loss or necessitate additional steps to recover the wine from the lees. Approximately eight percent sediment by volume resulted from the wine from

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[6]Observation after 20 additional days showed 0.20 percent bentonite to be the lowest amount which gave a clear wine.

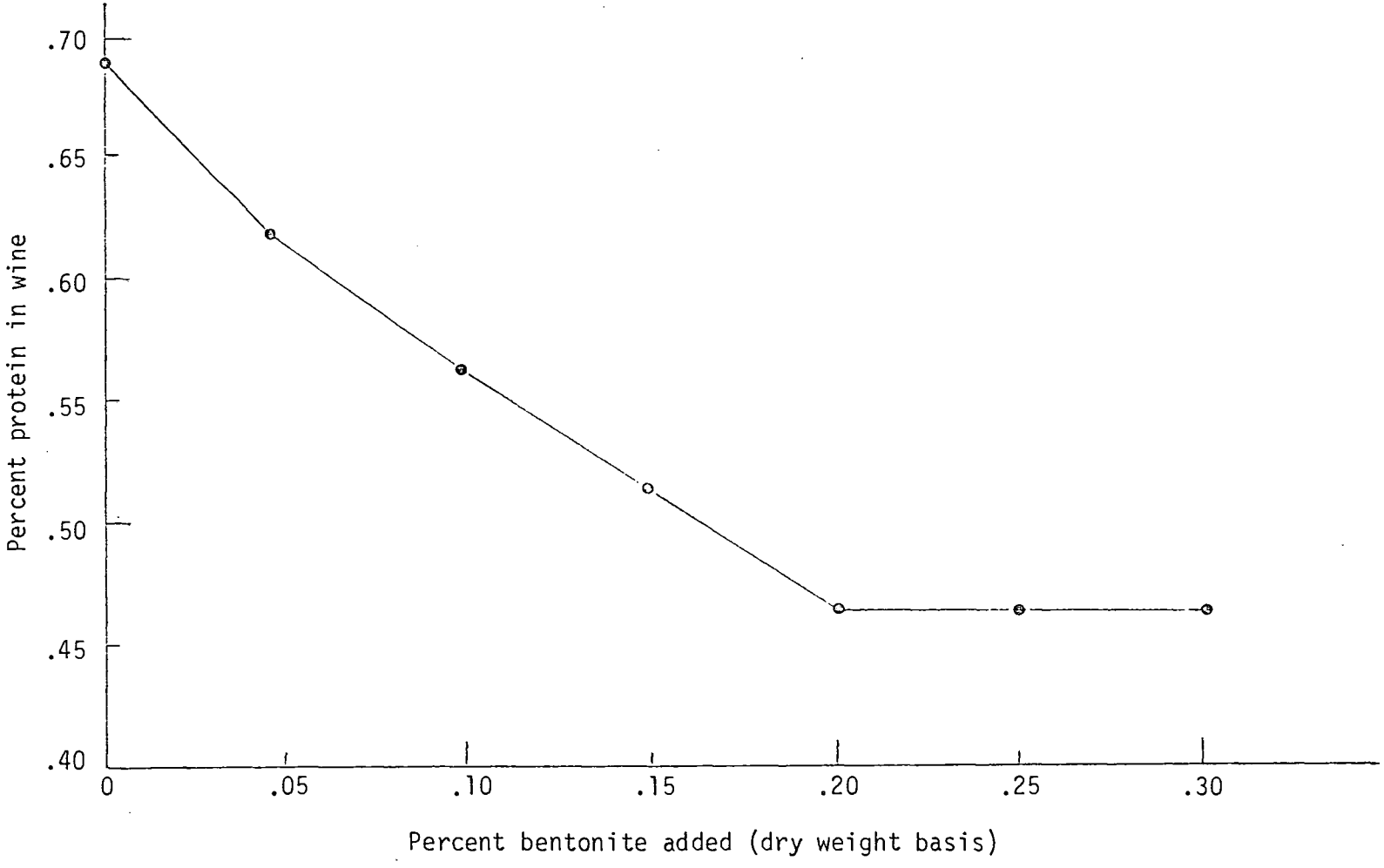

Figure 2. Removal of protein from whey wine by bentonite.

the addition of 0.20 percent bentonite. The other problem is that the concentration of the bentonite suspension and the amount of suspension necessary for fining may dilute the wine too much. Federal regulations (60) limit the use of bentonite in the fining of grape wines. They state that not more than two gallons of water shall be added to each pound of bentonite, and that the total quantity of water shall not exceed one percent of the volume of the wine treated. The six percent suspension as prepared in the present study complies with the first provision; but, for example, 0.20 percent bentonite on a dry weight basis means adding water equivalent to 3.35 percent of the wine volume. Whether bentonite usage in the fining of whey wine would be subject to such limitations is unknown.

Further research with bentonite is advisable. Several different bentonites with different chemical and physical properties are employed in wine making world-wide. A comparative study of a number of bentonites was undertaken by Vasari (61), and a similar study by Wegner is cited by Amerine and Joslyn (2, p. 313). Even calcium bentonites which remove less protein and produce less sediment than sodium bentonites in the fining of grape wines may perform differently with the protein colloids of whey wine. Amerine and Joslyn (2, p. 535) report that a powdered bentonite is now available which requires no pre-swelling and gives a heavy, compact lees. This is probably the new F-1 bentonite tested by McLeod and associates (39) against the usual KWK bentonite. Twice as much of the F-1 bentonite was necessary to achieve equivalent protein stabilization, but the lees were reduced by 40 percent. Some researchers have reported best results were obtained using bentonite

in conjunction with other fining substances.

The use of polyacrylamide with bentonite offers potential. Drboglav and co-workers (6) found that polyacrylamide added to bentonite reduced the clarification time for grape wine and the volume of lees. Balanca (5) introduced bentonite and polyacrylamide into grape wine at respective levels of 0.75 g/l and 2.5 g/l and reduced the clarification time from ten to 20 days to one to two days. Dzhanpoladyan et al. (7) concluded that a very stable wine resulted from fining with bentonite in the range of one to two g/l and polyacrylamide in the range of one to five mg/l.

The improved procedure by Yang et al. (66) for producing clear whey wine may be relevant. It included heating the whey for five minutes at 180°F to cause partial protein precipitation followed by pouring the liquid off the precipitate before inoculation. The cloud in the resulting wine would no doubt contain less protein, so fining it would require less bentonite.

Effects of Fermentation, Aging, and Clarification

A chronology of the wine making procedure based on determinations of pH, titratable acidity, Brix, and alcohol content is given in Table III. Determinations on the sampled contents of wine jugs number one, two, and three, one determination per jug, were averaged to provide the data. "Bentonite-filtered wine" denotes the addition of 0.30 percent bentonite to cloudy wine followed seven days later by racking and filtration.

TABLE III. CHANGES IN pH, TITRATABLE ACIDITY, BRIX, AND ALCOHOL CONTENT DURING WINE MAKING.

Day	Material	pH	Titratable acidity[1]	Brix	Percent alcohol
1	Whey	4.7	0.26	6.8	---
1	Whey plus dextrose	---	---	21.9	---
13	First racking	4.3	0.63	3.1	9.7
68	Third racking	4.2	0.53	3.0	10.6
85	Cloudy wine	3.9	0.59	2.9	10.6
89	Filtered wine	3.9	0.50	2.9	10.5
92	Bentonite-filtered wine	4.0	0.48	2.6	9.2

[1]Expressed as lactic acid.

Lactic acid and alcoholic fermentations are the two major processes in the conversion of the whey to wine. The former decreases the Brix by utilizing lactose and increases the titratable acidity by the production of lactic acid. This accounts for the titratable acidity increasing from 0.26 percent in the whey to 0.59 percent in the cloudy wine. Titrations of whey wine aged several months indicated that the acidity stabilized at approximately 0.6 percent. The relationship between the pH and titratable acidity is influenced by the high buffering capacity of the system due to the presence of lactate, phosphate, citrate, and proteins.

Alcoholic fermentation affects all four physical and chemical determinations in Table III. The decrease in Brix and increase in the alcohol content coincides with the production of ethanol and trace amounts of higher alcohols from dextrose. An alcohol content of 10.6 percent from 22.0 percent dextrose represents a yield of 48.2 percent. This is 94.3 percent of the theoretical yield of 51.1 percent which is usual for grape wines. It can be seen that the final ten percent of the fermentation occurred after the first racking. The production of carbon dioxide which remains in the wine as carbonic acid for a time no doubt inflated the titratable acidity reading at the time of the first racking. The acidity would also be affected by the small amounts of lactic, acetic, and succinic acids which are normal by-products of alcoholic fermentation. The final Brix reflects the lactose not fermented to lactic acid. No lactose is utilized in the alcoholic fermentation because the Montrachet yeast does not produce lactase.

The decrease in the acidity by filtration could involve both the loss of volatile acids and the removal of proteins. Also, some alcohol could be expected to volatilize. Bentonite fining would have the additional effects of diluting the system and causing the loss of alcohol due to mixing and racking.

The gross compositions of the whey and the wine at various points in the wine making process are presented in Table IV. The figures given are the averages of three analyses, either from the can of whey or one each from wine jugs number one, two, and three. The data in Tables III and IV lend themselves to joint consideration.

Besides experimental error, some comments on the validity of the five analyses are in order. The accuracy of the ash and total solids determinations may be assumed due to their simplistic methodology and their basis on Association of Official Analytical Chemists official methods. Results on the same sample were, in fact, very reproducible varying less than plus or minus 1.5 percent for total solids and less than plus or minus 4.0 percent for the ash. To test the validity of the lactose determination procedure, three samples of a 5.00 percent lactose solution were analyzed with the results being 4.92, 4.94, and 4.98 percent. In analogous fashion a 1.00 percent solution of purified bovine serum albumin gave results of 0.87, 0.87, and 0.86 percent using the protein analysis procedure with a nitrogen conversion factor of 6.25. The analysis was assumed to be valid despite the seemingly low percentages obtained. The bovine serum albumin, although purified, contained about three percent salts and other impurities. Also, the moisture content was probably four to five percent owing to its

TABLE IV. CHANGES IN GROSS COMPOSITION DURING WINE MAKING.

Day	Material	Percent				
		Lactose	Protein	Fat	Ash	Total Solids
1	Whey	4.58	0.88	0.0	0.52	6.33
85	Cloudy wine	4.14	0.69	0.0	0.34	6.33
89	Filtered wine	3.72	0.56	0.0	0.31	5.68
92	Bentonite-filtered wine	3.59	0.47	0.0	0.40	5.40

hygroscopicity and the presence of bound water.

The TeSa fat test for raw milk, an Association of Official Analytical Chemists official method, has been compared to other official methods in several studies. Washburn (63) found it to correlate well with the Roese-Bottlieb and Babcock tests. Ibrahim and Shipe (23) reported TeSa and Babcock results to be in close proximity, while O'Dell (47) found an average error of 0.82 percent between the two tests. The ability of the TeSa test to determine the low levels of fat found in whey was verified when an average reading of 0.13 percent was obtained for a mixture of one part whole milk containing 3.9 percent fat and 19 parts whey.

The composition of the whey in Table IV is in agreement with various literature sources (32, p. 201; 46, p. 19) with the exception of fat which is commonly reported as being 0.1-0.3 percent in whey. However, the cheese plant which supplied the whey achieved very efficient separation and routinely obtained fat readings of less than 0.005 percent using the Babcock test. There is the likelihood, though, that to insure a sample with representative fat the whey must be tempered to allow even distribution of the lipids. Using the TeSa test, whey from another cheese plant found to contain no fat based on samples taken after mixing subsequently gave an average reading of 0.22 percent fat when tempered to 105°F.

The decrease in the lactose during fermentation is due to the lactic acid fermentation. The reduction of the protein level involves the utilization of nitrogenous materials by the wine yeasts and their removal after settling by racking. Racking also removes the proteins

which precipitate during fermentation and aging. Bentonite fining flocculates the proteins and diminishes both the lactose and soluble protein concentrations by dilution.

Bentonite fining increased the concentration of ash, however. This phenomenon has been reported in the literature as occurring with grape wines. Amerine and Joslyn (2, pp. 534 and 537) cite one study by Hennig and another by Jakob in which the use of bentonite increased the ash content as much as 300 mg/l. An increase of 900 mg/l seems possible with whey wine, considering that more bentonite is added. The metabolism of minerals by the wine yeast during fermentation would account for the lessening of the ash level. They require relatively large amounts of potassium, magnesium, and calcium salts plus inorganic phosphate and sulfate for growth.

The percentages of total solids in the wine analyses are significantly more than the totals for lactose, protein, and ash. The probable explanation is that yeast and bacterial metabolism produced by-products such as cellular materials and fixed acids which would contribute to total solids but not to the other categories.

Table V presents the gross compositions of two sediments, the lees from the first racking and the coagulum from bentonite fining. Both sediments show a concentration of the non-soluble solids, protein and ash, and a dilution of the soluble lactose. The large increase in the ash of the bentonite coagulum is no doubt due to the fining agent itself.

On a dry weight basis the lees contain roughly 35 percent lactose, 31 percent protein, and 5 percent ash. All three are significant for their nutritional potential, but the proteins are of singular importance

TABLE V. GROSS COMPOSITION OF WINE SEDIMENTS.

Sediment	Percent				
	Lactose	Protein	Fat	Ash	Total Solids
Lees from first racking	3.87	3.43	0.0	0.54	10.95
Coagulum from bentonite fining	3.37	1.25	0.0	1.25	6.84

because of their high nutritional quality. Forsum (10) documented the high nutritive value of whey proteins, particularly α-lactalbumin, in feeding trials with rats. Wingerd (65) found that lactalbumin, the whey protein fraction composed of β-lactoglobulin, α-lactalbumin, and blood serum albumin, was nutritionally better than whole egg protein and much better than whole milk protein.

The functional properties of whey proteins in the lees would be very important in food applications. Morr comments on the solubility and whippability of whey protein concentrates in one study (41). Jaynes and Asan (24) succeeded in preparing fibrous proteins from cheese whey, an accomplishment with very real significance. The process consisted of freeze drying a whey protein concentrate of 70 percent protein prepared by precipitation with sodium hexametaphosphate at pH 3.0. The material was then extruded into a setting solution of 12 percent acetic acid and 12 percent sodium cloride. The tensile strength of the fibers was improved by adding detergents to the protein suspensions.

Protein is also present in the yeast portion of the lees. Reed and Peppler (53, p. 328) review the content of dry yeasts, and Harris (16, p. 192) discusses the biology and chemistry of nitrogen metabolism by yeasts. Various studies indicate that Saccharomyces cerevisiae contain approximately 20 percent nitrogen, of which half is protein. Although media and growth conditions are influencing factors, most yeasts, Saccharomyces cerevisiae included, have an amino acid composition closely resembling that of soy protein. Yeast is also an excellent source of the B vitamins.

The bentonite coagulum probably contains a higher ratio of whey to yeast proteins than the lees. The presence of bentonite in this sediment would prohibit its use as human food. Bentonite in feed rations, however, can be desirable. Rundsig et al. (54) studied the effects of incorporating bentonite into high-grain dairy rations. Whereas a grain-containing diet normally depresses the fat level of the milk, the addition of five to ten percent bentonite increased both the fat content and milk yield significantly. Lotif (34) concluded that five percent bentonite added to the diets of commercial laying hens increased their body weight, egg production, and feed efficiency.

Both sediments could be processed into foods and feeds with present whey processing technology. Drying, condensing, ultrafiltration, and various fermentations are all possibilities.

Sensory Differences in Cloudy and Clear Wines

The basis for the relative preference of the clarified wine by taste panelists was investigated. One question was whether or not clarification, in this case filtration, changed the gross volatile composition of cloudy wine. Both wines were analyzed using gas chromatography, and reproductions of typical chromatograms are shown in Figure 3. With the exception of peak number two, the volatile components and their amounts in the two wines are nearly identical. The relatively smaller size of peak number two in the clear wine could be due to loss from volatilization during filtration.

Research on the volatile composition of wines is reviewed in the literature (1, p. 201; 2, p. 433). The major by-products of alcoholic

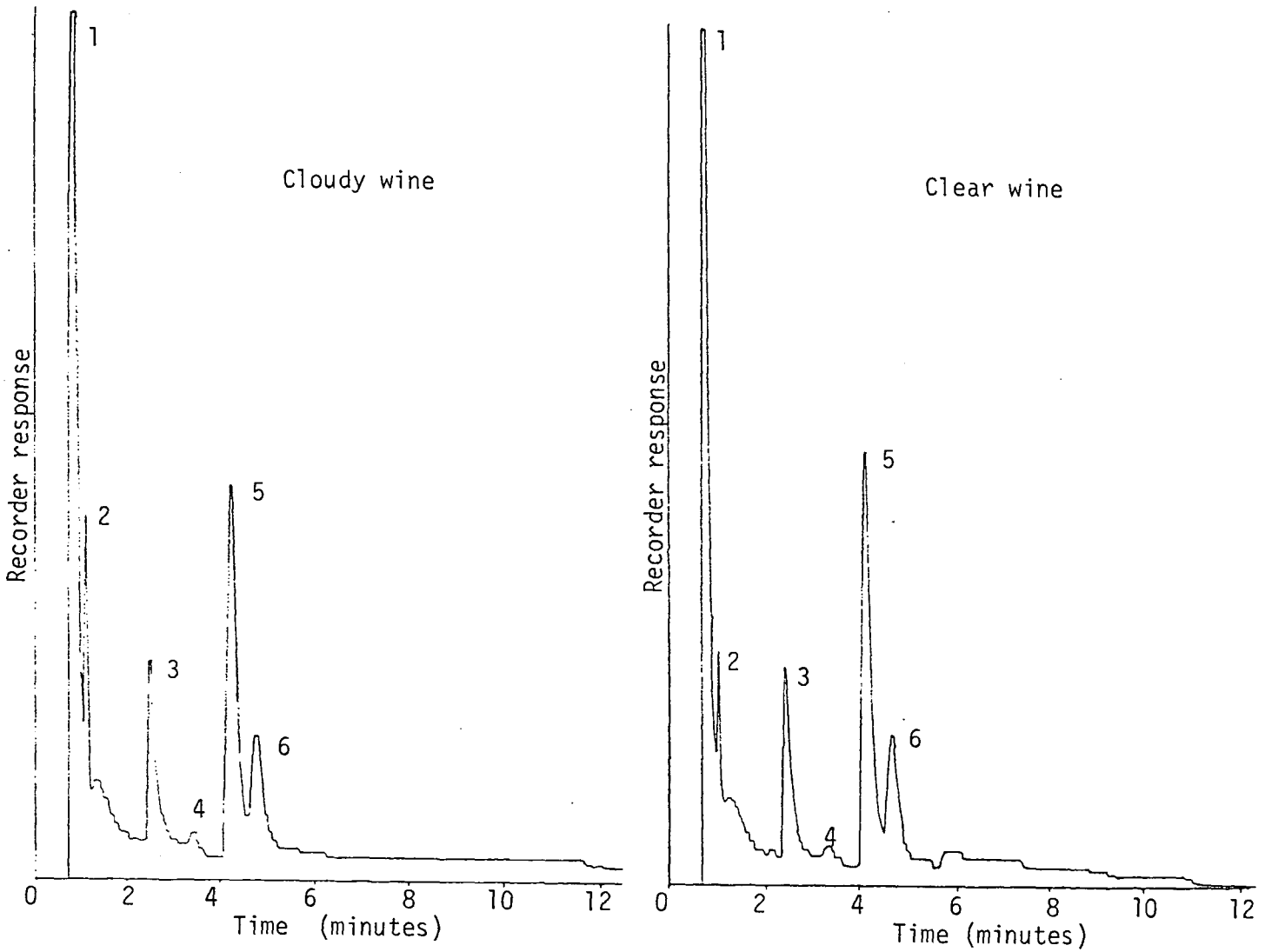

Figure 3. Gas chromatograms of whey wine.

fermentation are considered to be glycerol, acetaldehyde, isobutyl and isoamyl alcohols, 2,3 butylene glycol, acetoin, lactic and acetic acids, and ethyl acetate. Methanol is believed to result from pectin hydrolysis so would not be present. Speculating on a quantitative basis, ethanol could be peak number one, while likely candidates for peaks number two, three, and five could be acetaldehyde, lactic acid, and glycerol.

Admittedly, a great amount of study could be directed towards profiling the flavor components of whey wine. The fact that few conclusions can be drawn from this limited amount of research is fully realized. It appears, though, that the major volatiles of whey wine are not appreciably changed by filtration.

Another question was whether the appearance of clarified wine or a taste difference was the more significant factor in its preference relative to cloudy wine. Unclarified and filtered wines were compared in a triangle difference test involving 20 blindfolded tasters. Both wines had six percent added sugar, and their acidities were adjusted to 1.0 percent with lactic acid. Eight of the twenty tasters, a statistically insignificant number, chose the different sample which indicated wine appearance and not taste to be the major reason for clear wine preference.

The same 20 tasters took part in a similar test of filtered wine and wine fined with 0.30 percent bentonite and filtered seven days later. There was no detectable difference between the two wines since only seven people selected the odd sample. This supports the view that

bentonite fining does not apparently alter the taste and odor of the wine.

Use of the Sediment in a Food Product

Processed whey is added to a growing number of commercial food products including ice cream, confections, infant formula, and sausages (21). Whey is also utilized extensively in such bakery products as bread, cakes, biscuits, and doughnuts (56). Guy (14) mentions that cookies made with whey solids have excellent flavor and color.

The lees of whey wine were dried to a paste which had 33.8 percent total solids, a moderately strong "acid and wine" taste, and a smooth texture. Because of the concentration, it contained roughly 11.5 percent lactose, 9.5 percent protein, and 1.5 percent ash. The paste was substituted for five and ten percent of the nonfat dry milk in a commercial sugar cookie recipe (see Appendix I). Twenty-nine tasters evaluated the cookies in a preference test using the previously mentioned nine-point hedonic scale. The average scores were 4.98 for the ten percent substitution, 5.34 for the five percent substitution, and 5.96 for the control. All three scores were significantly different at the five percent level. Comments indicated that tasters experienced an "acid" aftertaste in the two treated samples.

Time limitations prevented the incorporation of this paste into another food product. It would seem prudent to use its acid flavor to better advantage, an obvious fault with the sugar cookie. A better choice may be to use it as a source of additional solids in a fermented or cultured dairy product such as yogurt or buttermilk.

V. SUMMARY AND CONCLUSIONS

Wine was made from sweet whey following a procedure developed by Yang and associates (66). The effects of fermentation, aging, and clarification were monitored by various tests and analyses. The major thrust of the research involved the investigation of several techniques and substances as to their ability to clarify the naturally cloudy wine. Clarification of the wine was desirable because preliminary tastings revealed a definite preference for clear whey wine. The techniques included filtration, centrifugation, heating, and pH adjustment of the wine. Fining agents tested were casein, Cold Mix Sparkolloid, gelatin, tannin, and bentonite.

The best clarification procedure proved to be the addition of KWK bentonite, a montmorillonite clay widely used in the fining of commercial grape wines, followed by a polishing filtration. Depending upon the time and degree of clarity desired, 0.20 percent to 0.50 percent of bentonite added on a dry weight basis produced acceptably clear wine. Bentonite with subsequent filtration generally met the experimental criteria of simplicity, legality, reasonable cost, minimal usage of energy and equipment, and maintenance of the wine character and quality.

Results with blindfolded tasters indicated that neither bentonite fining nor filtration significantly changed the taste of the wine. Gas chromatographic analysis of cloudy wine and bentonite fined and filtered wine revealed no appreciable differences in their volatile

components.

The wine lees were dried to a paste and substituted at the five and ten percent levels for nonfat dry milk in a commercial sugar cookie recipe. Taste panelists judged the paste to be both detectable and to impart an undesirable "acid" taste.

This study points towards several possibilities for additional research. The bulky lees and dilution effect of fining with KWK bentonite suggests investigation into the clarification parameters of different types of bentonite, the addition of polyacrylamide to bentonite, or the use of other fining substances in conjunction with bentonite. Also, identification of the flavor components in the wine, the incorporation of the wine lees into other food products, and the utilization of the bentonite sediment in animal feed rations are potential areas of study.

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APPENDIX

APPENDIX I

SUGAR COOKIE RECIPE WITH ADDED WINE SEDIMENT PASTE[1]

Ingredient	COMMERCIAL RECIPE[2]		LABORATORY RECIPES[3]		
			no paste	5% paste	10% paste
	Pounds	Ounces	Grams		
Flour	100		277.0	277.0	277.0
Granulated sugar	60		136.2	136.2	136.2
Invert syrup	6		13.6	13.6	13.6
Nonfat dry milk	3		6.80	6.45	6.12
Shortening	25		106.8	106.8	106.8
Monocalcium phosphate		10	1.4	1.4	1.4
Salt	1		2.3	2.3	2.3
Whole eggs	4		9.1	9.1	9.1
Sodium bicarbonate		12	1.7	1.7	1.7
Water[4]	22		52.0	51.3	50.7
Wine sediment paste			---	1.02	2.04
Cocoa			20.0	20.0	20.0
Vanilla[5]					

[1]Wine sediment paste substituted for nonfat dry milk.

[2]Based on 100 pounds flour per Matz (36, p. 128).

[3]Based on one-half pound of flour.

[4]Amounts reflect the fact that wine sediment paste contained roughly 67 percent water.

[5]One-fourth teaspoon added to laboratory recipes.