USE OF SOY PROTEIN ISOLATE AND YEAST PROTEIN AND GLYCAN IN SYNTHETIC ICE CREAM AND ICE MILK

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KIRK F. OTTO

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ABSTRACT

USE OF SOY PROTEIN ISOLATE AND YEAST PROTEIN AND GLYCAN IN SYNTHETIC ICE CREAM AND ICE MILK

by Kirk F. Otto

Utilization of soy protein isolate, yeast protein and yeast cell wall polysaccharide (glycan) in synthetic ice creams and ice milks was studied. All ice cream and ice milk containing these ingredients was processed and frozen by standard, accepted procedures. Soy or yeast protein had no effect on freezing time, draw temperature or overrun at the maximum level incorporated (80% replacement of milk protein). Viscosity of the mix, in general, increased directly with the amount of protein incorporated; at 40% replacement of milk protein, yeast protein caused a decrease in viscosity. Viscosity of mixes containing yeast protein was higher than mixes with corresponding concentrations of soy protein. Both proteins increased meltdown time, particularly at high concentrations. Sensory evaluation indicated that acceptability varied inversely with the amount of yeast or soy protein incorporated. Products flavored with cocoa had better acceptability than vanilla ice creams or ice milks containing these proteins. Neither pasteurization or homogenization of the

mixes caused significant changes in properties of the mixes or frozen products.

The color of the mixes darkened with increasing levels of yeast or soy protein, and to a lesser extent, with yeast glycan. The glycan increased mixed viscosity, lowered overrun (in a batch freezer) and had no effect on freezing time or draw temperature. The flavor of the glycan was bland and the optimum level for improved boxy and texture properties varied with the fat content of the system studied. A completely non-dairy synthetic ice cream containing whey, soy and yeast protein was formulated and processed.

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By

Kirk F. Otto

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INTRODUCTION

The quality of ice cream is markedly determined by ingredients, composition, and processing conditions. There are many possible ingredients which may be used to supply the protein, fat, carbohydrate, minerals, vitamins, stabilizer and emulsifier in the mix. Likewise there are variations possible in the processing of the mix with respect to time and temperature of pasteurization and pressure of homogenization. Control of these variables is important in production of a high quality mix.

The effects of substitution of different ice cream constituents on properties of the finished ice cream have been widely studied and for some products, are well known. However isolates of protein and glycan from *S. cerevisiae* are new products and thus their effects have not been evaluated in dairy products.

The main purpose of this investigation was to study the effects of protein and fat substitution upon various properties of ice cream. Parameters such as mix viscosity, overrun, homogenization pressure, meltdown, pasteurization temperature and freezing time were used to evaluate the ice cream produced by the incorporation of soya or yeast derivatives in the mix prior to processing.

LITERATURE REVIEW

Filled and Imitation Dairy Products

A great variety of filled and imitation dairy products have been made to date. These include products made in semblance of ice cream, milk, sherbet, whipped topping, coffee cream and cheese (Moses 1969, Lambert 1970). A number of these, such as coffee whitener, margerine, filled ice cream (Mellorine) and whipped toppings have gained wide acceptance with the consumer. Longer shelf life, lower cost and possible dietetic advantage are the major reasons, as claimed by Hetrick (1969), for consumer use of these products.

Filled products are a combination of nonfat milk or nonfat milk solids with a fat other than milkfat. The fat is usually a partially or fully hydrogenated vegetable fat and coconut oil is often used because of its stability and mild flavor. The Federal Filled Milk Act of 1923 banned interstate commerce in filled milk products, thereby restricting sale to the state in which it is manufactured (Hedrick 1969). Imitation dairy products may not contain any dairy product ingredients. They usually include fat, protein, carbohydrate, emulsifier, various flavoring compounds and stabilizer. Sodium caseinate, lactose, and whey solids are not considered milk products but rather by products with the definition of chemicals approved for food use and thus may be utilized in imitation dairy products. Imitation milk may enter interstate commerce although the manufacture of this product in some states has been declared illegal (Anon. 1968).

The nutritive value of many synthetic dairy products has been a major obstacle to their acceptance by consumers and nutrition oriented groups. Filled milks usually use coconut fat which is highly saturated, contains no essential fatty acids and has no cholesterol (Brink 1968). Problems with excessive viscosity has also limited the protein and calcium contents of such products to the detriment of nutritive value. Great difficulty has thus far been encountered in producing an imitation product of the high nutritive value and flavor quality of bovine milk. To date, if a product of acceptable flavor has been produced it has also been low in protein content while products with protein content equivalent to cows milk have exhibited an objectionable flavor (Kosikowski 1968). Even a product containing high quality soy protein isolate may have a characteristically "beany" flavor which many Americans find objectionable. The fat used in many imitation dairy products is coconut although soybean, corn or any other edible oil certainly may be used. Problems inherent in the choice of any oil include susceptibility to oxidation, rancidity and other off-flavors due to degradation of the fat (Norris 1964). Many proteins derived from plants are available as sources of protein for imitation products as well as milk derivatives such as caseinates and whey proteins

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(Nielsen 1963, Anon. 1959). Many conventional carbohydrates in the form of sucrose, glucose, lactose and low and high DE corn sweeteners are available (Trempel 1964). Carboxymethylcellulose and the exudate gums are among the widely used stabilizers available today and mono and diglycerides and other polyoxyethylenic surface active agents comprise the emulsifiers useful in formulation of imitation dairy products (Moss 1955). From this limited resume of ingredients it can be seen that the composition and thus the quality of imitation dairy products may, and does, vary widely. Spilman (1963) and others, have shown that the processing of either filled or imitation dairy products can be accomplished with regular dairy processing equipment with only minor adjustments in processing.

Ice Cream Constituents and Properties

Ice cream is normally composed of water, milkfat, serum solids, sugar, stabilizer, emulsifier and flavor. Sources of each of these components can be quite varied. The milkfat may be derived from any combination of sweet cream, unsalted butter, anhydrous milkfat, sweetened condensed milk, and concentrated or dehydrated whole milk. Sommer (1946) believed that ice cream made from fresh cream was superior to that formulated from other concentrated fat products. Similar conclusions have been reached by other researchers but these observations are somewhat dated today because of the technological advances which have been made in food processing.

Serum solids are usually added in the form of non fat dry milk or concentrated skim in addition to that derived from fresh fluid products (Arbuckle 1948). The sweetness of ice cream can be provided by sucrose, dextrose, invert sugar, corn syrup (many types), honey, molasses, lactose, fructose, or any other approved sweetener. Numerous stabilizers have been suggested for improving the body and texture of the ice cream. Among these are agar-agar, alginates, the exudate gums, carageenan, carboxymethylcellulose, gelatin and others (Ludwig and Gakenheimer 1967). The choice of primary emulsifiers is more limited and usually mono- and diglycerides, microcrystallinecellulose, and synthetic esters of fatty acids and polyoxyethylenesorbitan are used. Normally a single stabilizer or emulsifier is not used in modern day ice cream technology. Rather, a proprietary blend of stabilizer, emulsifier and solubilizing agents are used to achieve maximal effects (Moss 1955).

Effect of Constituents on Ice Cream Quality and Properties

Milkfat

Milkfat level not only determines whether an ice cream is legal but the fat also imparts good tactual qualities, adds a subtle flavor quality and acts as a flavor carrier for other flavor components (Doan and Keeney 1965). The milkfat gives the ice cream a smooth and creamy mouthfeel (Arbuckle 1954). The fat globules cluster on the surface of the air cells and tend to retard the rate of whipping (Keeney 1958).

The fat content has no effect upon the freezing point of the mix.

Serum Solids

The serum solids, usually added as nonfat dry milk, have subtle effects upon the flavor and also enhance palatability by imparting desirable body characteristics to the product. The serum solids increase viscosity and melting resistance while lowering the freezing point and slightly reducing whippability (Arbuckle 1969). The improved body and increased viscosity is due mainly to the protein of the nonfat dry milk. The protein rehydrates and acts as a stabilizer. Too high a serum solids content will cause a cooked flavor, a salty flavor due to the mineral salts, or a sandy flavor defect due to the elevated lactose level (Dahle, 1931).

Carbohydrate

Carbohydrates add sweetness to the mix and enhance the creamy flavor of the ice cream. Carbohydrates also improve the body and texture of the ice cream and are important contributors to the total solids and viscosity of the mix (Dusendahl 1963). The freezing point of the mix is lowered by carbohydrates and this effect is of course directly influenced by the molecular weight of the sweeteners used (Wolfmeyer 1963, Reid *et al* 1942). Sugar, at one time was the easiest and cheapest way to increase the total solids of the mix but the inflated price of sucrose, sugar syrups and corn sweeteners has reduced the cost spread between sweetener and other solids in the mix.

Stabilizer

Stabilizer is used to prevent the formation of large, objectionable ice crystals caused by cabinet temperature fluctuations during storage. Stabilizers have a high water binding capacity which imparts a smoother texture and better body to the finished product (Bassett 1969). They tend to increase mix viscosity and limit mix whippability but have virtually no effect upon the freezing point because of their high molecular weights. Stabilizers contribute to a uniform product and improve handling but excessive amounts may give undesirable metldown characteristics (Arbuckle 1972).

Emulsifier

Emulsifiers are used to improve and provide uniform whipping quality. They tend to reduce whipping time and give smaller, more uniform air cell distribution (Rothwell 1965). Emulsifiers help to give a drier ice cream with smoother body and texture (Redfern 1949). Excessive amounts result in defects in texture and meltdown (Josephson 1943).

Water and Air

Two major components of ice cream which are often overlooked are water and air. Water comprises between 50 and 60 percent of ice cream mix and provides the continuous phase of ice cream. Arbuckle (1972) states that the water in ice cream is "a partially frozen emulsion with ice crystals and solidified fat globules embedded in a layer of unfrozen material." This supports the findings of Sommer (1946) who made the observation that the "fat-serum interface is covered by a layer of fat-emulsifying agent and the solidified fat may be dispersed in the unfrozen serum." According to Berger (1972), it is desirable to have uniform air distribution as this affects the quality of the ice cream. The amount of air incorporated influences product weight (the minimum weight being specified by law) and profit. Ice cream must weigh at least 4.5 pounds per gallon to be a legal, saleable product in most states. Overrun is the volume of ice cream obtained in excess of the volume of mix expressed as a percent. The increased volume is composed mainly of air incorporated during the freezing process. The higher the overrun the higher the profit. To a point, higher overrun increases quality but beyond that point quality begins to decrease due to formation of an excessively "fluffy" or light body.

Properties of the Ice Cream Mix

Viscosity

The viscosity of the ice cream mix has been the focus of research for a number of decades. To date no conclusive, definitive work has been published relating mix viscosity to quality. There are, however, some basic points to be considered relative to the viscosity of a mix. Viscosity is inversely related to temperature up to the point of temperature induced denaturation and aggregation. The composition of the mix naturally influences the viscosity. Chocolate ice cream mix usually has a higher viscosity due to the cocoa solids and higher levels of sweetener used in such mixes. Aging the mix may increase the viscosity due to more extensive hydration of the protein and stabilizer and the adsorption of material onto the surface of the fat globule. Aging of the mix for 2 to 4 hours was at one time recommended although modern stabilizer blends and continuous freezers have made the relationship of aging to freezing properties of relatively little consequence.

Whipping Ability

The whipping ability of an ice cream is the ease and speed at which a desired final overrun can be reached. It was originally thought to be controlled by viscosity but the later theory postulated by Leighton *et al* (1927) suggested that tensile strength and the strength of the lamellae around the air cells accounted for whippability. Turnbow (1928) demonstrated that high processing temperatures, proper homogenization and aging increase the whipping ability. According to Leighton (1934), mix with good whipping ability may have high viscosity, contrary to previous beliefs. If the ice cream mix is homogenized with a two stage homogenizer the formation of clusters of fat globules is minimized and good air incorporation and retention is achieved.

Effects of Processing Variables

Pasteurization

The main purpose of pasteurization is to destroy pathogenic organisms. Most states require pasteurization at time-temperature combinations recommended by the United States Public Health Service. In addition to being a legal requirement, pasteurization destroys much of the bacterial load thus greatly extending the storage stability of the mix. Pasteurization also aids in the solubilization and blending of mix ingredients, and improvements in flavor may also result from heat treatment (Arbuckle 1951).

Homogenization

Homogenization results in the reduction of fat globule size to an average of two microns or less in diameter. According to Trout (1950) this is accomplished by any or all of the following as the mix flows from the high pressure region to low pressure: shearing, impingement and cavitation. The net result of homogenization, according to information in Jenness and Patton's text (1959) is a four to six fold increase in surface area of the fat globule surface and a reduction in size to an average diameter of 1-2 microns. Homogenization causes an increase in viscosity which may be due to an altering of the proteins and the adsorption of casein onto the surface of the fat globules. Homogenization causes milk to become whiter in color which is caused by the increased reflection of white light from the surface of more globules.

Pasteurization and homogenization should be accomplished as a continuous operation or an inferior product may result due to action of lipase on unpasteurized, homogenized mix. Pasteurization is most often performed just prior to homogenization because the latter process is best accomplished at pasteurization temperature and because this heat treatment also inactivates lipase enzymes. Immediately following homogenization and pasteurization the mix should be quickly cooled to below 40°F to inhibit bacterial growth and facilitate subsequent freezing.

Freezing

Freezing is one of the most important operations in ice cream manufacture because initial and subsequent texture and smoothness depend on the amount of water frozen under continuous agitation and the size and number of ice crystals formed during freezing. The mix is pumped into the freezer barrel and is agitated by a rapidly revolving beater bar to incorporate air. The barrel is refrigerated, causing the mix to freeze on the inside of the barrel. Attached to the beater bar is a blade which scrapes the semi frozen mix from the barrel wall surface. When the contents of the barrel reach a desired temperature and overrun the frozen mix is drawn off into cartons and placed in a hardening cabinet. The ice cream is then rapidly cooled to -15° or colder. The more rapid the temperature drop the smaller will be the ice crystals formed. It is then desirable to hold the product at this temperature as fluctuations in the temperature cause a coarsening of texture due to the formation of larger ice crystals during melting and refreezing of ice and water.

Other Food Additives of Microbial Origin

Protein from Single Cells (Yeast)

Yeast has been used by man for many centuries and is considered one of his oldest foods according to McCormick (1973). Dried yeast has been used for years as a source of the vitamin B complex. Yeast has also been used to leaven bread, brew beer and ferment fruit juices (Baird 1963). More recently yeast has been produced by a number of companies (Peppler 1967) and various fractions taken from it. According to Rose and Harrison (1970) the annual production of dried yeast exceeds 180,000 metric tons of which only one percent of this is currently used as food for man. Recently techniques have been published for the fractionation and production of protein and cell wall polysaccharides from edible yeast cells (Sucher 1973). Protein is extracted from yeast cells following mechanical rupture of the whole cell. The protein is then extracted, washed, heat treated and recovered by centrifugation. The extracted protein may then be spray dried to a free-flowing, cream colored powder (Sidoti 1973a). The proximate composition of yeast protein prepared in this fashion is 75% protein, 12% carbohydrate, 10% lipid, 2% ash, <1% nucleic acids, <1% crude fiber, and 4% moisture (Sidoti 1973a).

Yeast protein has a PER of 2.2 as compared to 2.5 for casein (Robbins 1973). With the addition of .1% and .2% dl Methionine the PER may be increased to 2.9 and 3.2, respectively. Robbins (1973) has shown the essential amino acid distribution of yeast proteins to be very similar to that of the proteins in milk. Because all of the protein thus isolated is metabolizable, there is no caloric reduction if used in substitution of other proteins in food formulations. According to Sidoti (1973) the level of usage of yeast protein in certain foods is not limited due to flavor but its effect upon texture. This of course is dependent on flavor blandness, just as is the case with any protein isolate. The dried yeast protein may be readily dispersed (Sidoti 1973a) but due to the method of isolation it is very insoluble and not a suitable replacement protein if high solubility is a requirement as in some beverage applications. Yeast protein has been used in the preparation of some food prototypes, according to Sidoti, 1973a.

Proteins for incorporation into imitation dairy products may be derived from a number of sources. Enebo (1970) and others regard microorganisms as a logical protein source, due to their ability to reproduce rapidly on simple media and their high protein content. Molds, yeast, and bacteria are the source of the proteins described as "single cell proteins" (SCP). Tannenbaum (1971) concluded that single cell protein is the only major source which can fill the protein shortage in the world. According to a report by Anderson (1958b), microbial proteins vary in amino acid content dependent upon the propagation medium although yeast protein appears to be fairly consistent in regard to the proportions of 10 essential amino acids. Recently, both yeast protein and polysaccharide glycan have been approved by the Federal Food and Drug Administration for use as food additives in certain foods.

<u>Glycan from Yeast</u>

Another product which has been isolated from the yeast cell is called a glycan. The glycan is separated from the ruptured cells by centrifugation and is then washed to reduce flavors, pasteurized and spray dried to a light colored powder (Sucher 1973). Cook (1958) has shown that the yeast cell wall consists mainly of straight chain polymers made up of the simple sugars β- glucan and α- mannan in a 3:2 ratio. The actual structure of the glycan has yet to be elucidated. The glycan contains approximately 57% of the carbohydrate present in the intact cell. The proximate composition of the food grade glycan is 87% carbohydrate, 11% crude protein, <1% ash, <1% fat, <1% nucleic acids, and 6.2% moisture (Sidoti 1973b). The glycan is claimed to have a unique thickening and fat sparing effect upon food systems. The suggestion has been made (Sidoti 1973b) that yeast glycan may be able to replace all or part of the fat in certain foods. The caloric content of a food containing glycan as a replacement for fat, protein, or carbohydrate would be reduced, since only about 40% of the glycan is bioavailable. Because the glycan has a definite thickening effect upon liquid food systems it may be capable of replacing stabilizers, although there are no data available to substantiate such a possibility. Yeast glycan has been found to be compatible with both the natural hydrocolloids and synthetic gums. The glycan will disperse readily in a cool liquid system and at levels over 4.5% will hydrate extensively, causing high increases in viscosity or even gelling. Glycan absorbs about six times its own weight in water (Sidoti 1973b).

Prototypes of foods containing glycan such as salad dressing, have a smooth texture and a creamy mouthfeel. These properties are, of course, highly desirable in ice cream. According to Jeanes (1973) the new food grade hydrocolloids, of which yeast glycan is an example, will become more prevalent in food applications in the future.

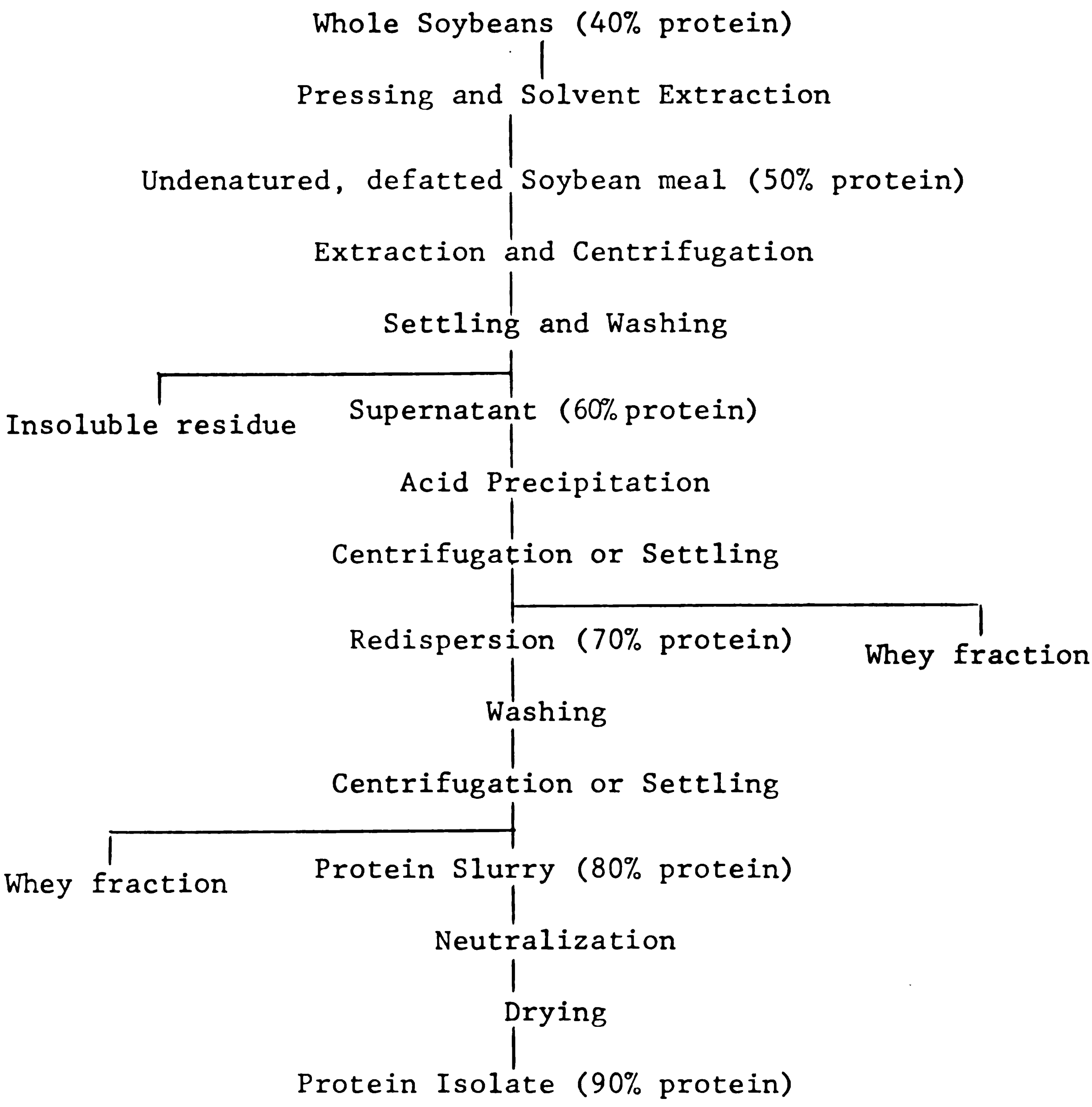
Soybean Derivatives

Soybean Composition and Soy Isolate Production

Soybeans normally contain 40% protein, 18% fat, 24% carbohydrate, 8% moisture, 5% crude fiber and 5% ash (Peng 1970). Conditioned, flaked soybeans are defatted by solvent extraction. A schematic for the production of soy protein isolate is presented in Table 1.

The finished dehydrated soy protein isolate has a composition of 92% protein, 4% moisture, 4% ash and a trace of crude fiber (Anon 3, Anon 4 1973). Wolf (1972) has found that

TABLE 1. The Production of Soy Protein Isolate from Whole Soybeans.



the protein of soy protein is only partially soluble, with solubility varying from one commercial product to another. Johnson and Circle (1959) observed that soy isolates have functional properties such as water binding, thickening, gelling and stabilizing ability. Similar conclusions have been reached by Catsimpoolas and Meyer (1970) in regards to the viscosity and gelation properties of soy isolate. They have shown that protein-protein interaction is responsible for the increase in gelation and viscosity. In addition, soya isolates are described by Smith and Wolf (1960) as being bland in taste and nearly devoid of carbohydrates, fats and fiber. Nutritional analysis of soy isolates has shown the PER to be 1.75. Supplementation with methionine may raise the PER to 2.5 (Robbins 1973). The cost of soy protein is much lower than other similar quality protein sources such as dairy, beef, cheese or egg protein (Williams 1972, Johnson 1959).

The uses of soy protein isolate are limited only by the ingenuity of the processor. It has been used to prepare prototypes of dairy products, bakery goods, beverages, meat products and confections. Much research has been directed to the study of the functional properties of soy protein. Considerable effort has been expended to show how soy protein can be used in foods (Maga 1970, Wolf and Cowan 1971). Very recently great attention has been focused on soy protein because of the low cost of this protein compared to protein from animal sources.

Taste Panel Usage and Statistical Analysis

Taste Panel Uses and Limitations

Man has many and varied feelings toward food. The factors which affect man's food preferences are also extremely diverse. Food likes and dislikes are affected by cultural background, race, economic or social status or the fad of the day. Some are deeply rooted, others only superficial. New food products resulting from food product research and development efforts are ultimately evaluated by taste panels. Panels can be used to determine like or dislike, intensity of a flavor characteristic or degree of difference from a standard. Foods are submitted to taste panel evaluation to provide information which can lead to further product improvement, quality maintenance, new product development, or to determine consumer reaction. The accuracy of the results of these panels can be very good if the tests and their results are handled correctly.

Scoring tests are best used in comparisons of a control sample with several experimental samples. Hedonic scales are used when the judge is asked to express his degree of like or dislike by marking a point on a scale ranging from extreme like to extreme dislike. These scales are usually a 5 to 9 point balanced scale. The two evaluation methods are applicable to both trained and untrained panels. There are many other panel evaluation methods which are applicable to food evaluation. These include the triangle, duo-trio, and single sample tests. Amerine *et al* (1965) lists numerous considerations which should influence the selection of a taste panel. These include the two major requirements of a panelist which are sensitivity to flavor and consistancy of response. Other considerations in relation to panel size, environment, training and number of samples are used to determine the type of tests to be used. Although it is usually desirable to optimize all the panel conditions in order to get the most accurate, reliable results, this is unfortunately not always possible.

Once a taste panel has been run it is highly desirable to be able to obtain some kind of qualitative or quantative results from it. The Analysis of Variance Method and Duncan Multiple Range Method are two ways of obtaining useable data and results.

Analysis of Variance

The analysis of variance is a least squares method for detecting significant differences between samples and can be used to assign sources of variance. The analysis of variance is applicable to the solution of problems of varying complexity, including those with multiple variables and sample interactions. The procedure involved in computation of analysis of variance is explained in Kramer and Twigg (1962) and Amerine et al (1965). When a value has been determined it is compared to that of statistical F-distribution tables to see if they exceed the book values. If they do, it is assumed that there is a significant difference between samples.

Duncan Multiple Range Test

At this point it is still not possible to tell which individual treatment means are statistically significantly different. To do this we use a procedure presented by Duncan (1955). In this method the treatment means are listed in increasing order, the standard deviation is determined, and then multiplied by the appropriate value from a 5% confidence, Multiple Range Test factor table. The value found is the gap which must be exceeded between samples for a significant difference at the 5% level. From these data, interactions may be determined in relation to treatments, replicates and sample types. Further explanation of the Duncan Multiple Range Method can be found in the original paper by Duncan (1955).

Rank Scoring

Another useful method of analyzing taste panel data is the Rank Total method. In this procedure the judges rank the samples according to specific attributes. The samples may be ranked on as many as three or four different criteria, as long as they are ranked individually (Amerine *et al* 1965). All judges must use the same criteria in judging the samples. This is often accomplished through the use of expert panelists such as expert dairy judges (Bliss 1943). Results are summed for each treatment and compared to Rank Total Tables to determine if there is a significant difference among samples (Anderson 1958a).

EXPERIMENTAL PROCEDURES

Manufacture of Ice Cream

Ice cream was made according to the method and procedure as outlined by Arbuckle (1972) using pilot plant equipment. The vanilla ice cream mix for the Yeast Protein (YP) substitution was made to the specifications shown in Table 2. The composition of the chocolate mix is shown in Table 3. The protein of the mix was replaced by YP at levels of 20 and 40 percent in the vanilla mixes and at 20, 40, and 80 percent in the chocolate mixes. Because the amount of YP powder necessary to replace the protein of the mix is less than that of non fat dry milk, lactose was used to maintain an equivalent solids level. Lactose normally makes up about 52 percent of non fat dry milk so the addition of lactose along with YP was not likely to change the resultant ice cream. The composition of the vanilla mix used in experiments involving the glycan from yeast (YG) is shown in Table 4. The composition of the chocolate mix used in YG formulations is shown in Table 5. For the YG runs the level of milkfat used were 0, 1, 3, 5, and 10 percent. For each fat content four levels of YG were evaluated: 0, 1, 3, and 5, percent. The last set of runs involved the production of a synthetic or nondairy ice cream. The formula for this product is shown in Table 6. Three runs of the synthetic ice cream were made.

TABLE 2. Control Vanilla Ice Cream Mix Formulation for Yeast Protein Substitution

10.0%	Milkfat
12.0%	Serum Solids
15.0%	Sucrose
0.1%	Stabilizer-Emulsifier
37.1%	Total Solids

TABLE 3. Control Chocolate Ice Cream Mix Formulation for Yeast Protein Substitution

8.0%	Milkfat
12.0%	Serum Solids
16.0%	Sucrose
2.0%	Cocoa
0.1%	Stabilizer-Emulsifier
38.1%	Total Solids

TABLE 4. Control Vanilla Ice Cream Mix Formulation for Yeast Glycan Substitution

0, 1, 3, 5, or 10%	Milkfat
10.0%	Serum Solids
12.0%	Sucrose
4.0%	Corn Syrup Solids
0, 1, 3, 5.0%	Yeast Glycan
0.1%	Stabilizer-Emulsifier
26.1 -41.1%	Total Solids

TABLE 5. Control Chocolate Ice Cream Mix Formulation for Yeast Glycan Substitution

3.0%	Milkfat
11.0%	Serum Solids
13.0%	Sucrose
6.0%	Corn Syrup Solids
2.0%	Cocoa
0, 1, 2, 3.0%	Yeast Glycan
0.1%	Stabilizer-Emulsifier
35.1- 38.1%	Total Solids

One run was made with whey as the only source of protein; a second employed whey plus two percent YP; the third utilized whey plus two percent soy protein. In all of the above ice cream mixes the dry ingredients were sifted together to aid in their solubulization and then the appropriate amount of water and cream was added. The mix was then thoroughly stirred with a Lightnin Mixer (Model 10X) and pasteurized.

Pasteurization and Homogenization

Pasteurization of the YP and soy protein ice cream mixes was accomplished at either 155°F for 30 min or at 175°F for 10 min. Pasteurization of the YG and nondairy mixes involved only 155°F for 30 min. All pasteurization was done in a steam heated water bath. Immediately after pasteurization the mix was cooled to 150°F and homogenized in a Manton-Gaulin (Model M-3) homogenizer. The YP and soy mixes were homogenized at 2000 psi on the first stage and 500 psi on the second or at 4000 psi on the first stage and 500 psi on the second stage. The YG and nondairy ice cream mixes were homogenized only at the lower pressure. Immediately thereafter the mix was cooled to 33°F and held in a cooler overnight prior to freezing and hardening.

Vanilla Addition and Mix Viscosity

Just prior to freezing and/or measurement of viscosity, vanilla was added according to recommendations of the manufacturer. In all cases the vanilla was 100 percent pure vanilla with no vanillin fortification. The vanilla was thoroughly stirred into the mix. Viscosity measurements

TABLE 6. Synthetic Vanilla Ice Cream Mix Formulation

10.0%	Vegetable Fat (Coconut Oil)
8.0%	Electro-Dialized Whey
12.0%	Sucrose
6.0%	Corn Syrup Solids
0.2%	Stabilizer-Emulsifier
0 or 2.0%	Soy Protein (Promine F)
0 or 2.0%	Yeast Protein
36.2 - 38.2%	Total Solids

were made immediately with the mix at 33°F using a Brookfield Synchro-Lectric Viscometer (Model RVT-7).

Freezing and Hardening

Freezing of the ice cream mix was accomplished on a Glacier (Model F) ice cream freezer. In all cases 800 ml of mix was added to the machine and the control knob set to position 4. Calculation of the freezing time was made from the time the compressor was activated until it shut off at the end of the freezing cycle. Draw temperature measurements were made with a mercury thermometer (0 to 300°F). Overrun was measured on a Pelouze (Model Z-32) overrun scale. Ice cream was drawn out of the freezer (at an average temperature of 21°F) into cyclindrical pint Sealright containers and placed in a freezer set at -15°F for hardening.

Taste Panel Evaluation

Yeast Protein and Soy Protein Substitution of Milk Protein

Hedonic scoring was used to obtain the expressed degree of like or dislike of the panel members. The scale used has a 7 point range with 7 being the most desirable, 4 being neither like nor dislike, and 1 being least desirable (Table 7). The evaluation covered both flavor and body and texture. The Hedonic ratings were converted to the numerical scores and then treated by Analysis of Variance and the Duncan Multiple Range Test. The panelists were untrained in ice cream evaluation but had been given the necessary information to do the proper taste testing. Panels were run in a taste panel room under normal fluorescent lights. The same panel members were used throughout each flavor variation or ice cream type. The panels were all held at the same time of day and panel size was kept constant. Sample sizes were kept as uniform as possible as was their appearance. Samples were placed and numbered at random.

Yeast Glycan Substitution and Nondairy Ice Cream

The taste panel form used to evaluate these products is shown in Table 8. The panelists involved in this taste evaluation were all expert dairy judges. Results of their evaluation were analyzed by Rank Total analysis. In this evaluation only four experts were available which accounts for the small panel size.

TABLE 7. Taste Panel Evaluation Form for Ice Cream Containing Yeast Protein and Soya Protein

Name ____________________ Date ____________________

Evaluate these samples on their flavor and body and texture. Please mark the space which best corresponds to your evaluation.

SAMPLE NO.

		1	2	3	4	5	6	7
FLAVOR	Like very much 7							
	Like moderately 6							
	Like slightly 5							
	Neither like nor dislike 4							
	Dislike slightly 3							
	Dislike moderately 2							
	Dislike very much 1							

SAMPLE NO.

		1	2	3	4	5	6	7
BODY AND TEXTURE	Like very much 7							
	Like moderately 6							
	Like slightly 5							
	Neither like nor dislike 4							
	Dislike slightly 3							
	Dislike moderately 2							
	Dislike very much 1							

TABLE

These
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below

FLAVOR	A
	C
BODY AND TEXTURE	

TABLE 7 (Continued . . . .)

These terms are used by judges to describe defects in flavor or body and texture of ice cream samples. Please pick the term(s) which best describe the sample if you chose to rate it below 4.

		1	2	3	4	5	6	7
FLAVOR	After taste							
	Old ingredient							
	Soy taste							
	Unnatural							
BODY AND TEXTURE	Coarse							
	Crumbly							
	Gritty							
	Gummy							

ANALYTICAL PROCEDURES

Kjeldahl

The protein content of the YP powder and various ice cream mixes was determined to check mix calculations and formulation. Total nitrogen was determined using the official Kjeldahl method (AOAC 1970).

Babcock

The Official Babcock Cream Test was used to determine the fat content of the cream used to produce the ice cream (AOAC 1970).

TeSa Fat Test

The TeSa fat test (Anderson 1959) was used to determine the fat content of the homogenized mixes. A 9 g sample was weighed into the test bottle and 9 ml of water added. Next, 20 ml of fat test reagent (Appendix) was added to the bottle, mixed, and placed in a boiling water bath for 15 min, swirling often. Hot water was added to collect the fat at the base of the neck of the bottle. The sample was centrifuged for 4-5 min and 50 percent methanol (Appendix) added to float the fat column into the graduated portion of the neck. The bottle was centrifuged 30 sec and placed in a tempering bath for 5 min. A pair of calipers were used to measure the fat column.

Vacuum Oven

A 1 g sample was weighed accurately into a total

TABLE 8. Taste Panel Evaluation Form for Ice Cream prepared with Glycan and for Synthetic Ice Cream

Name ______________________ Date ______________________

Independently rank each sample on each of the four characteristics.

Sample		Richness	Smoothness	Body	Flavor
	A				
	B				
	C				
	D				
	E				
	F				

Comments:

solids dish and placed in a vacuum oven at 100^{o}C, 5 mm Hg for 5 hours.

Solubility Index

The Solubility Index of the non fat dry milk was determined according to the procedure of the American Dry Milk Institute (1965). A 10 g sample of NFDM was added to a special mixing jar along with 100 ml of distilled water at 75^{o}F and 3 drops of Diglycol Laurate S. The jar was placed in the mixer, mixed for exactly 90 sec and allowed to stand for 15 min. The solution was mixed and poured into a conical centrifuge tube to the 50 ml mark. The tube was centrifuged for 5 min at 848 rpm (on a 16 inch diameter head). The supernatant was then siphoned off to within 5 ml of the sediment and 50 ml of 75°F water added. The sample was mixed and centrifuged 5 min. The sediment was read from the tube and compared to USDA standards (Solubility Index 1958). The readings showed that the NFDM met the solubility index requirements for U.S. Extra Grade NFDM.

Results and Discussion

The Yeast Protein (YP) portion of this study was undertaken for two major reasons. It was hoped to determine whether YP had functional properties which were desirable and compatible to allow its use in ice cream as a replacement for nonfat dry milk. A second reason for the study hinged on the rising prices of nonfat dry milk in relation to the estimated price of YP. If the cost of nonfat dry milk rose to the point of parity with YP then its substitution in ice cream might be economically feasible. An understanding of the properties of YP will facilitate its usage not only in ice cream but in all food products.

In the initial taste panel studies involving YP, the results obtained for body and texture evaluation were abandoned due to a lack of reliability. It was found that panelists were not sufficiently trained to separate evaluations of flavor from body and texture. Evaluation scores for body and texture ran parallel to the scores for flavor when there was no significant differences in actual body and texture. This conclusion is based upon evaluation of the ice cream samples by trained judges. Flavor scores were presumed to be reliable because they were found to closely parallel replacement levels even though the taste panel members did not know sample identity at the time of evaluation. Further substantiation for the flavor scores come from the method of rating samples: Panelists were asked to rate the samples on how well they liked or disliked the ice cream and not on an absolute scale.

Effect of Protein Replacement Upon Mix Parameters and Taste Panel Scores

Evaluation of the effect of replacement of milk proteins with yeast or soy protein was both objective and subjective. The objective methods involved viscosity measurements, duration of freezing, draw temperature and percent overrun. Subjective analysis included taste panels and meltdown quality.

It was found that the duration of freezing, that is the time required for the mix to reach drawing condition was nearly constant. Most variations can be attributed to machine function. Draw temperature remained constant at 21 to 22^{o}F throughout and thus was a function of machine operation (Figure 1). Similar consistancy of results were obtained with percent overrun even though this is not necessarily a function of the machine as shown in later sample runs. The viscosity of the mix was the only parameter to vary directly with replacement level (Figure 2). It can be seen that as the percent replacement increased to about 40 percent the viscosity also rose. After the 40 percent level the viscosity dropped despite increased YP replacement. This can be explained to be a function of protein solubility. Milk proteins are quite soluble and thus significantly affect

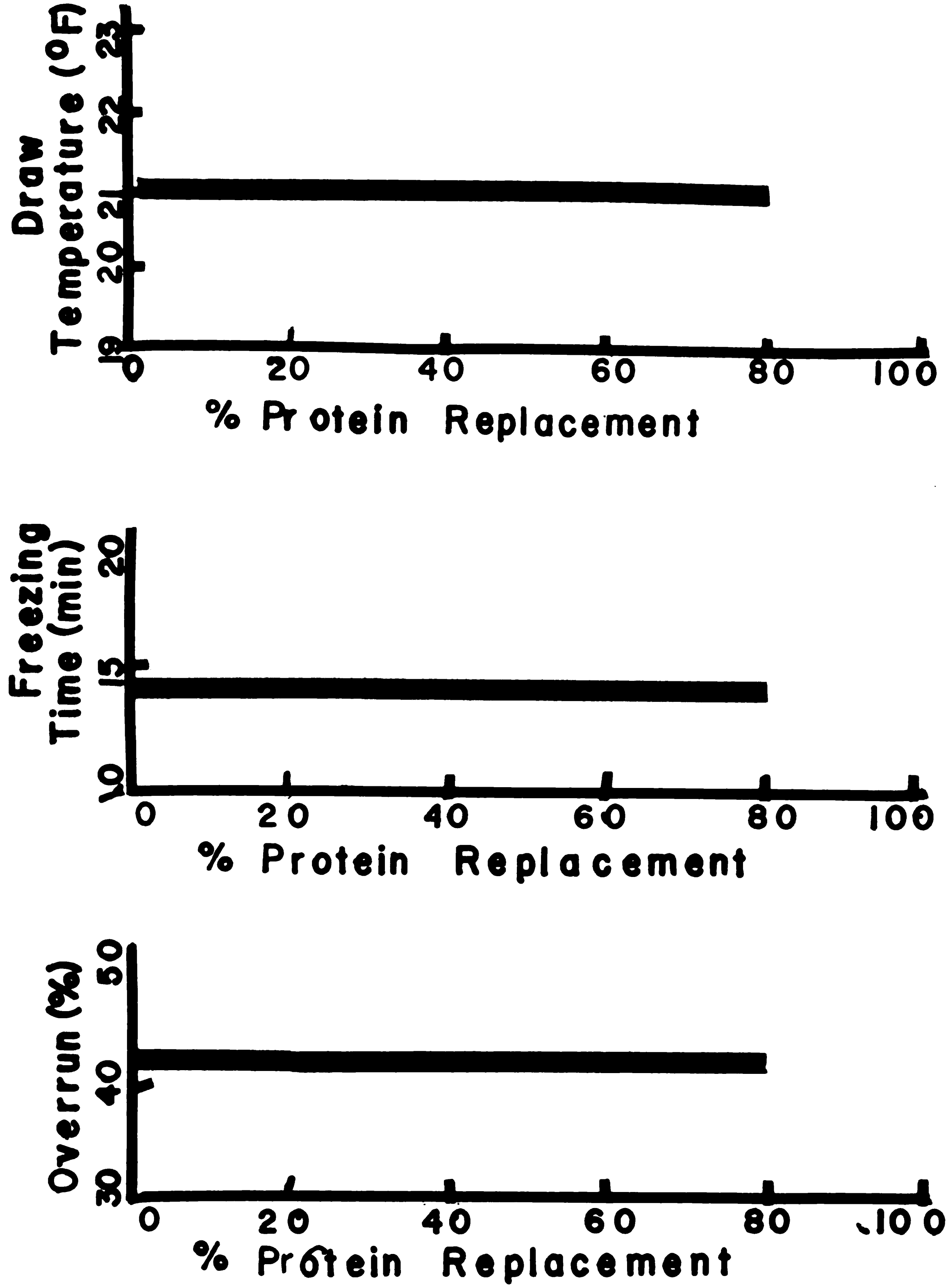

FIG. 1. The effect of protein replacement on draw temperature, freezing time and overrun.

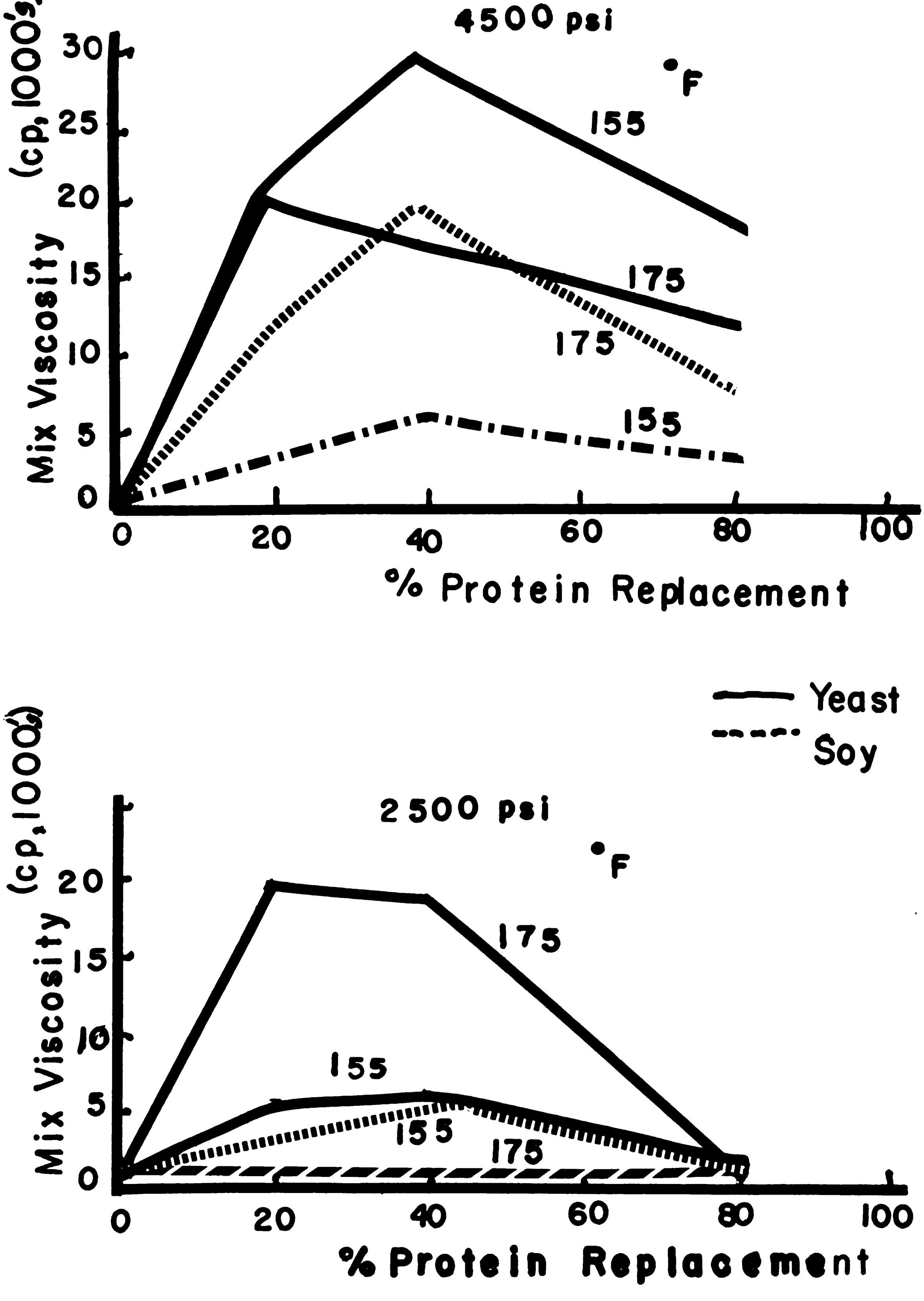

FIG. 2. The effect of protein replacement on mix viscosity.

serum viscosity. The YP is quite insoluble. At initial levels of YP addition, due to increased absorption or hydration of the protein, the mix viscosity increased. This offset the viscosity drop caused by the removal of the milk proteins. However, when a certain point was reached (around 40%) the low solubility of the YP caused the mix viscosity to rise only a fraction. Though more YP was added it did not make up for the milk proteins which were removed (Figure 2). This might be an advantage if a high viscosity were not desired in the final mix. Leighton and Williams (1927) postulated that casien micelles, fat globules, and stabilizer hydrocolloids form a loose network and by individually or collectively aggregating, form a structure. This would account for the apparent viscosity of the mix. It is possible that the YP is not able to function in such a manner which might account for the viscosity drop. The initial viscosity rise can be explained by the high water binding capacity of the YP as it rehydrates. To a point this counteracted the lowering of total milk protein.

It was also found in this study that for the same level of replacement the viscosity of the YP mixes were higher than that for soy protein. This is probably due to the type of protein involved and the different water binding capacities of the yeast and soy protein. Proteins, due to their water binding abilities, restrict the amount of free water and thus act as stabilizers in ice cream mix. They tend to thicken the mix and suspend other solids (Doan and Keeney 1965). In doing this they tend to restrict the migration of solids and give the melting ice cream a creamier consistancy. The effect of the level of yeast and soy protein substitution is shown in Figure 3. It can be seen that the effect of protein substitution is to produce an ice cream which increasingly resists melting as the percent substitution is increased. The effect is more dramatic in the YP samples where the 80 percent substitution level will remain almost unchanged even after two hours at ambient temperature. Though the photograph does not show this clearly the liquid runoff from the 20 percent sample is much more viscous and homogenous than the control which is thin and partially separated. The progression shown in the photograph occurred for all four homogenization-pasteurization combinations as well as vanilla and chocolate. It appears in the 80 percent substitution that even though the viscosity of the mix was dramatically decreased, the resultant hardened ice cream showed the characteristic of overstabilization.

Taste panel evaluation showed that pasteurization and homogenization had no effect upon the flavor of the ice cream. This shown by the proximity of the points in Figure 4 and Table 9. A major conclusion from taste panel evaluation of the vanilla samples indicated that the degree of acceptability of the ice cream product varied inversely with the percent protein replacement. The bland flavor of the vanilla

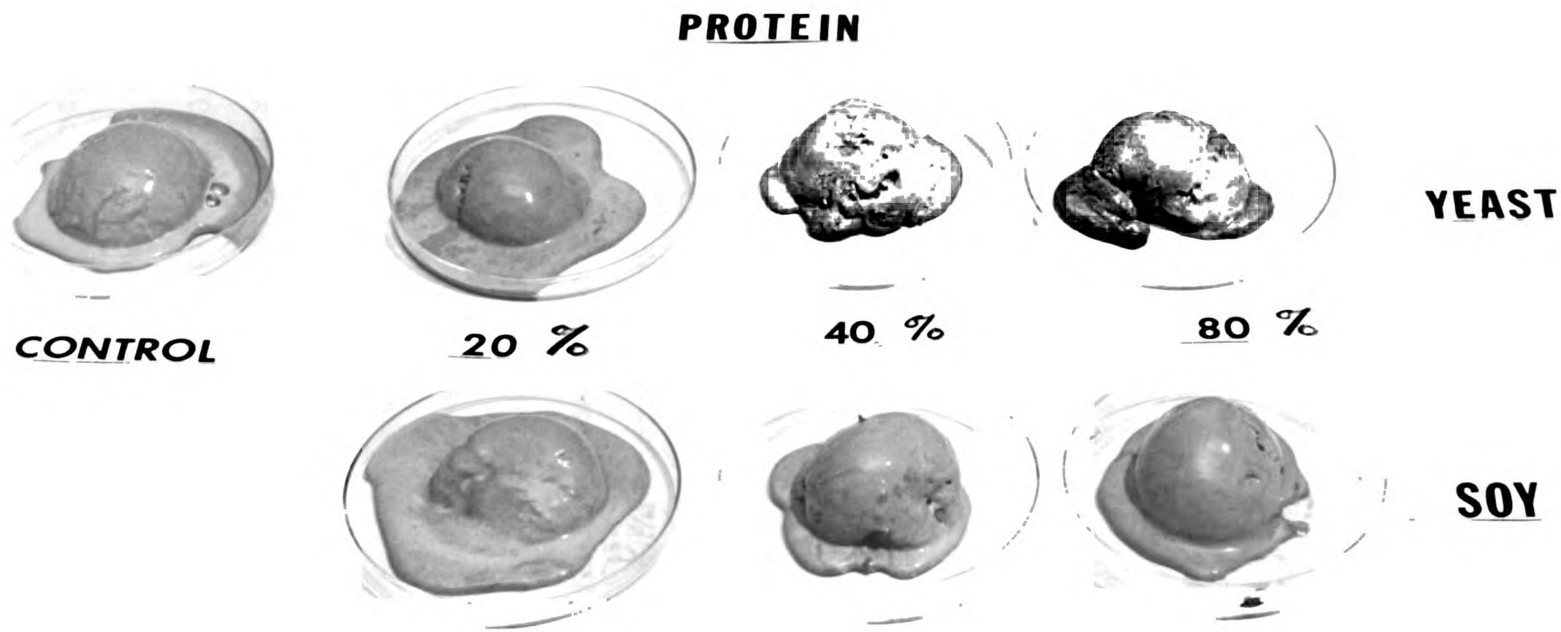

FIG. 3. Effect of Replacement of Milk Proteins by Various Percentages of Yeast or Soy Proteins on Meltdown of Ice Cream (percentage of Total protein).

ice cream was not sufficiently intense to mask the flavor inherent in the yeast and soy powders. The YP powder flavor was classified as "nutty" or "yeasty" while that of the soy was termed "beany". Both were considered atypical and somewhat undesirable in a vanilla ice cream product. With the vanilla, the mean scores of both the yeast and soy substituted samples were deemed equally undesirable (Figure 4). This is in contrast to the chocolate samples (Figure 4) where the soy was rated superior to samples containing equivalent concentrations of yeast products. This may be due in part to the "newness" of the flavor of yeast protein as compared to that of soy which has been used in foods quite extensively. It may also be due to some type of interaction or synergistic effect between the flavoring compounds and the yeast protein. Once again, the data indicate that homogenization and pasteurization have little or no effect upon flavor of the chocolate ice cream. The mean flavor score for the chocolate samples was significantly higher than the vanilla especially in the case of the chocolate soy products. This is to be expected because of the flavor intensity and masking effects of cocoa.

Subjective analysis of the body and texture indicated that samples with 40% replacement of milk proteins had excessive body. This was intensified by 80% replacement. In these samples the ice cream when consumed clung to the mouth, giving a gummy or sticky sensation. These observations

TABLE 9. Mean Flavor Score for Vanilla and Chocolate Ice Cream as Related to Percent Protein Replacement

	VANILLA						CHOCOLATE							
	YEAST			SOY			YEAST				SOY			
% Protein Replacement	0	20	40	0	20	40	0	20	40	80	0	20	40	80
Past.Temp Homo PSL														
155 2500	6.5	3.9	1.6	6.5	4.0	1.8	6.5	4.0	3.0	1.5	6.5	5.5	4.5	3.0
155 4500	6.6	3.8	1.8	6.4	4.0	1.9	6.3	4.1	2.9	1.4	6.3	5.4	4.4	3.0
175 2500	6.4	3.9	1.4	6.6	4.1	1.9	6.5	4.1	3.0	1.3	6.4	5.6	4.6	2.8
175 4500	6.6	4.0	2.0	6.4	3.8	1.6	6.6	4.0	3.0	1.6	6.6	5.5	4.4	3.2

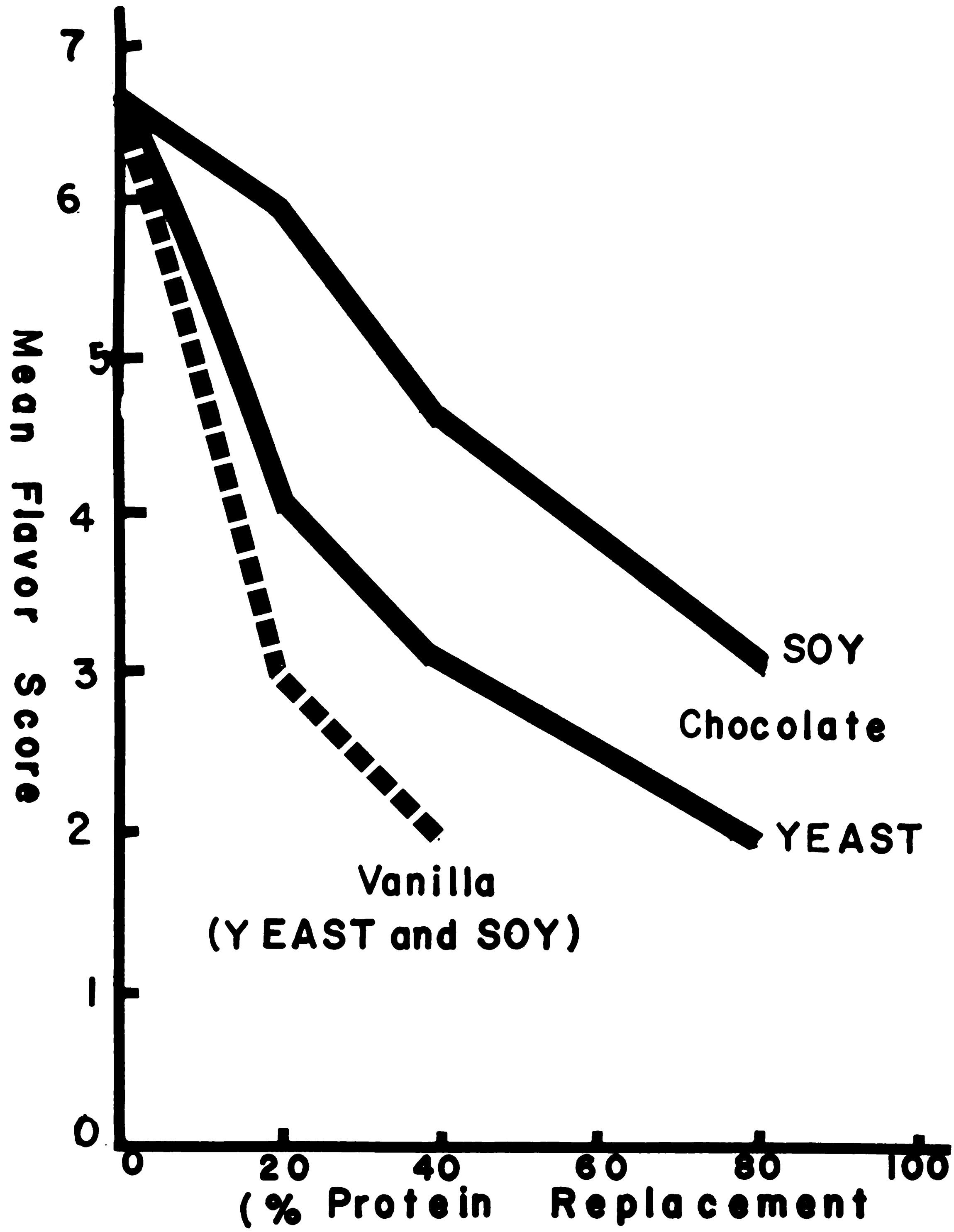

FIG. 4. Mean Flavor Score for Chocolate Ice Cream as a Function of Protein Replacement

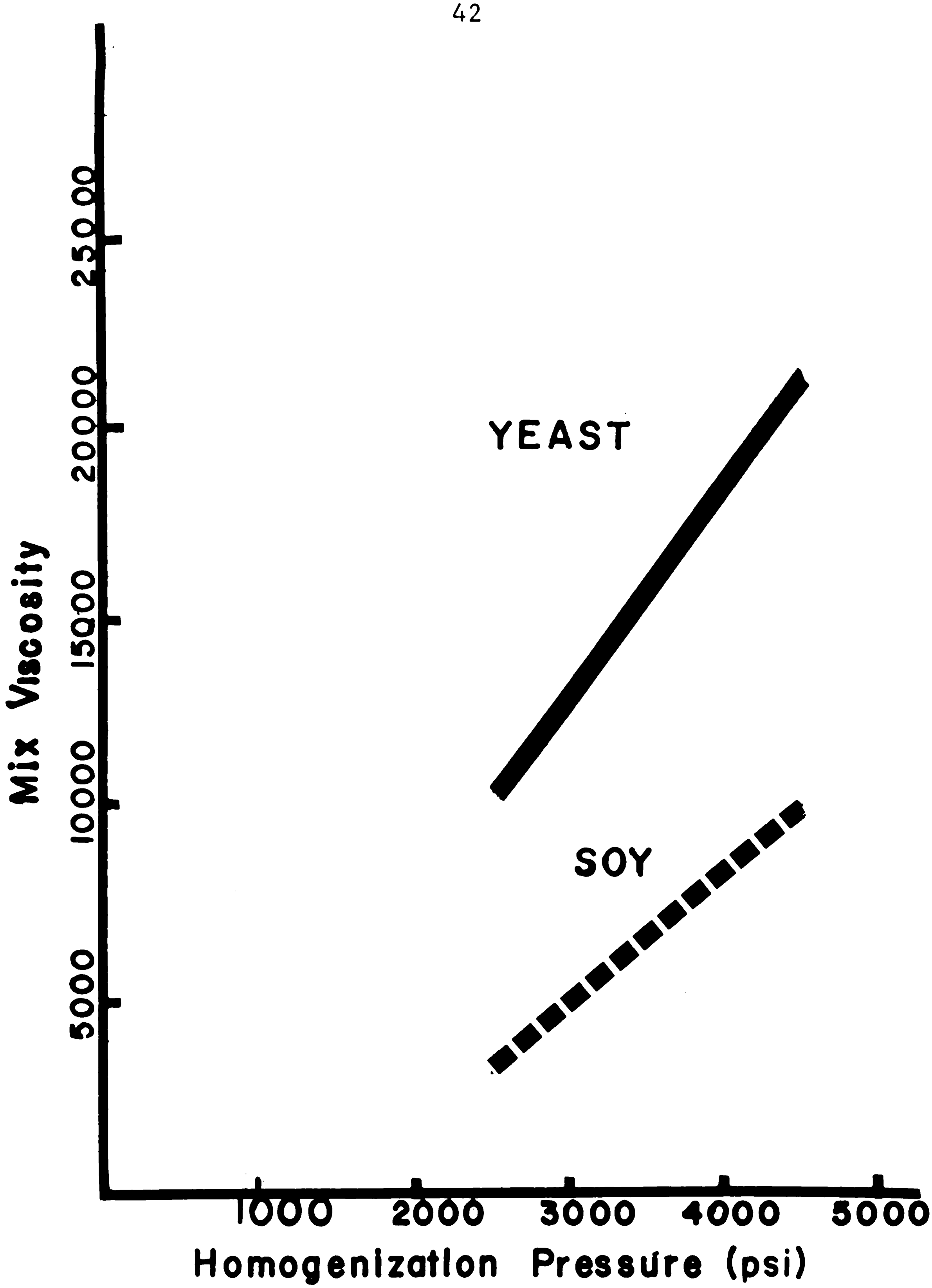

FIG. 5. Effect of Homogenization Pressure on Mix Viscosity

correlate quite well with the melting resistance shown by the samples.

Effect of Homogenization and Pasteurization on Mix Parameters and Taste

Pasteurization appeared to have no effect upon mix parameters, meltdown properties or taste panel evaluation. Homogenization did not show any influence over meltdown properties or taste panel evaluation but it did appear to affect mix viscosity. Higher homogenization pressure resulted in a higher mix viscosity (Figure 5). Similar results were obtained by Whitnah (1956) and others regarding the near linear increase in viscosity as homogenization pressure was increased.

Color

The color of the yeast and soy powders is slightly gray as compared to nonfat dry milk which is yellowish-white. This produced a serious color defect in the samples of vanilla ice cream. The samples were sufficiently different in color to necessitate serving only one sample at a time in the taste panels in the hope that with a single sample presentation, the color differences could be bypassed. Masking lights could have been used but it was felt that this might bias panel evaluation. It would be necessary, if yeast or soy protein were to be used in ice cream, to either reduce or change the color of the protein or to modify the color of the mix through the use of a color additive such as egg yolks or artificial color.

It appears that the flavor intensity of the yeast and soy powders was too great to be masked by even the chocolate flavor. It would thus be necessary to reduce the flavor level of the yeast and soy powder in order to get a product which could be used in a vanilla or even a chocolate ice cream mix, if consumer preference demanded a bland ice cream.

Yeast Glycan

The investigation of the possible use of Yeast Glycan (YG) in ice cream had three major objectives: (1) to study the properties of the glycan in relation to the ice cream system to learn its effects upon the finished product; (2) to determine if cost reduction were possible through its use; and (3) to see if a reduction in caloric content could be achieved. The cost of milkfat is approximately one dollar per pound. If the milkfat content can be lowered through the addition of glycan with no change in body and texture then the cost can be lowered at a rate of ten cents per percent fat removed. Concomitant with reduced fat content is a lowered caloric load. The nature of the linkages of the glycan polysaccharide structure is such that apparently very little of the pure glycan is bioavailable. However the carry through of whole yeast cells and adsorbed lipid and protein gives a level of about 0.5 to 1.8 Kcal per gram of food grade glycan. The exact caloric content will vary with the YG powder composition. Thus removing fat, at a caloric level of 9 Kcal/g, and replacing it with glycan, at a caloric level of

1.8 Kcal/g, the caloric content of an ice cream may be substantially reduced. The color of the glycan is slightly gray which gives a slight off-color to the prepared mix which would need to be masked. The color did appear slightly different between samples, which were presented simultaneously, but the panelists were asked to ignore this in their evaluation. Because the panelists used in this part of the investigation were all expert dairy judges it is hoped that the obvious color differences did not bias their evaluation. Since most ice cream mixes prepared commercially contain added yellow color to convey richness this color effect noted with glycan might be masked. Additional research is needed to verify or refute this. The flavor is very bland and fits well into the vanilla ice cream system. The milkfat of ice cream normally provides a rich flavor characteristic and a smooth texture (Arbuckle 1954). It also tends to lower the rate of whipping and increases the mix viscosity (Leighton 1927). It was found in making ice cream and ice milk that the YG had much the same effect upon the mixes. As the level of glycan was increased the mix viscosity increased (Figure 6) and the level of overrun obtainable decreased (Figure 7). It appears that in both cases the effect of the YG on viscosity was much stronger than that for fat. It appears that in the cases of the five and ten percent fat mixes (Figure 7) the fat content had a moderating effect upon the glycan induced inhibition of overrun. In regard to the viscosity

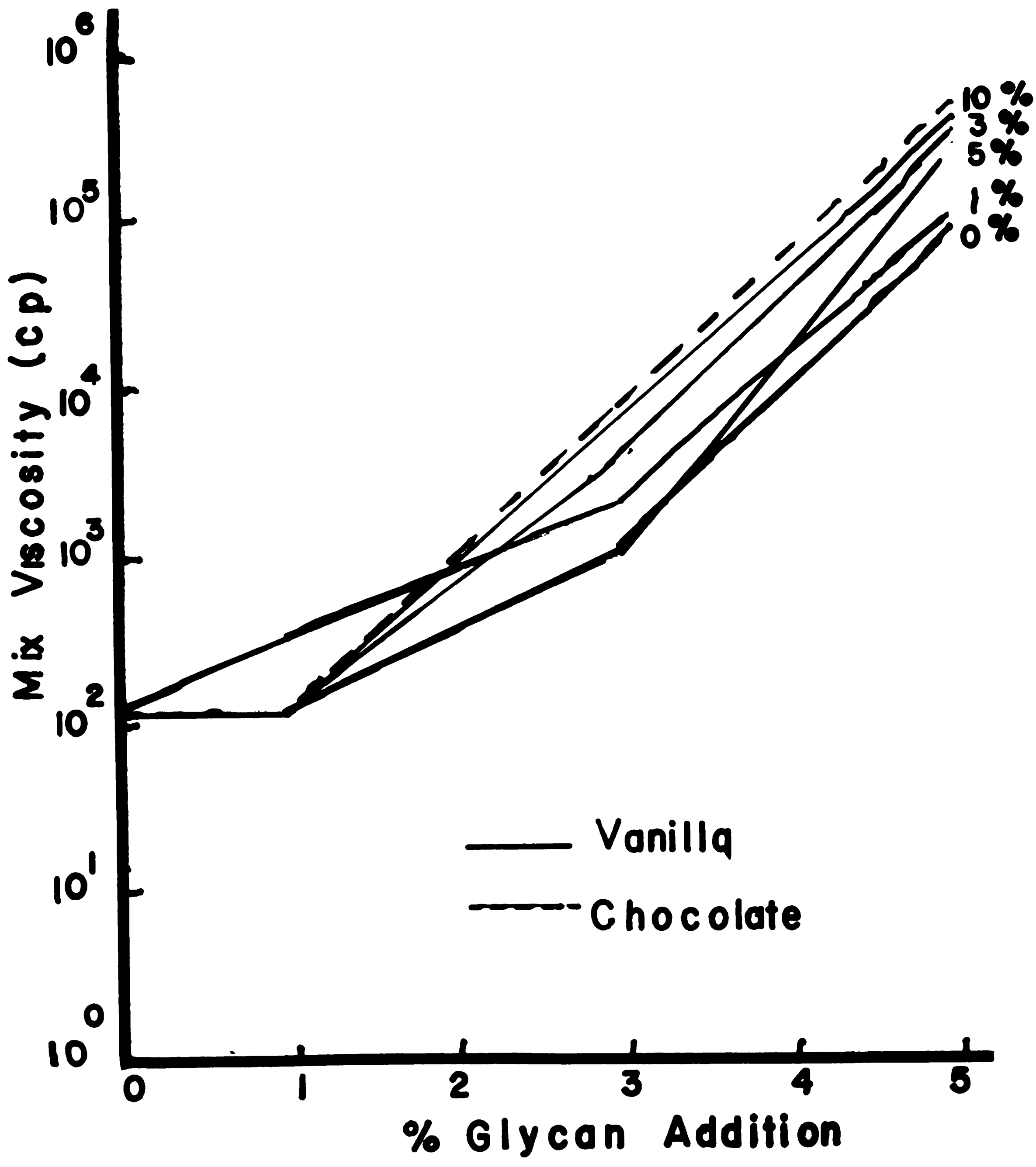

FIG. 6. Effect of Glycan Addition on Mix Viscosity.

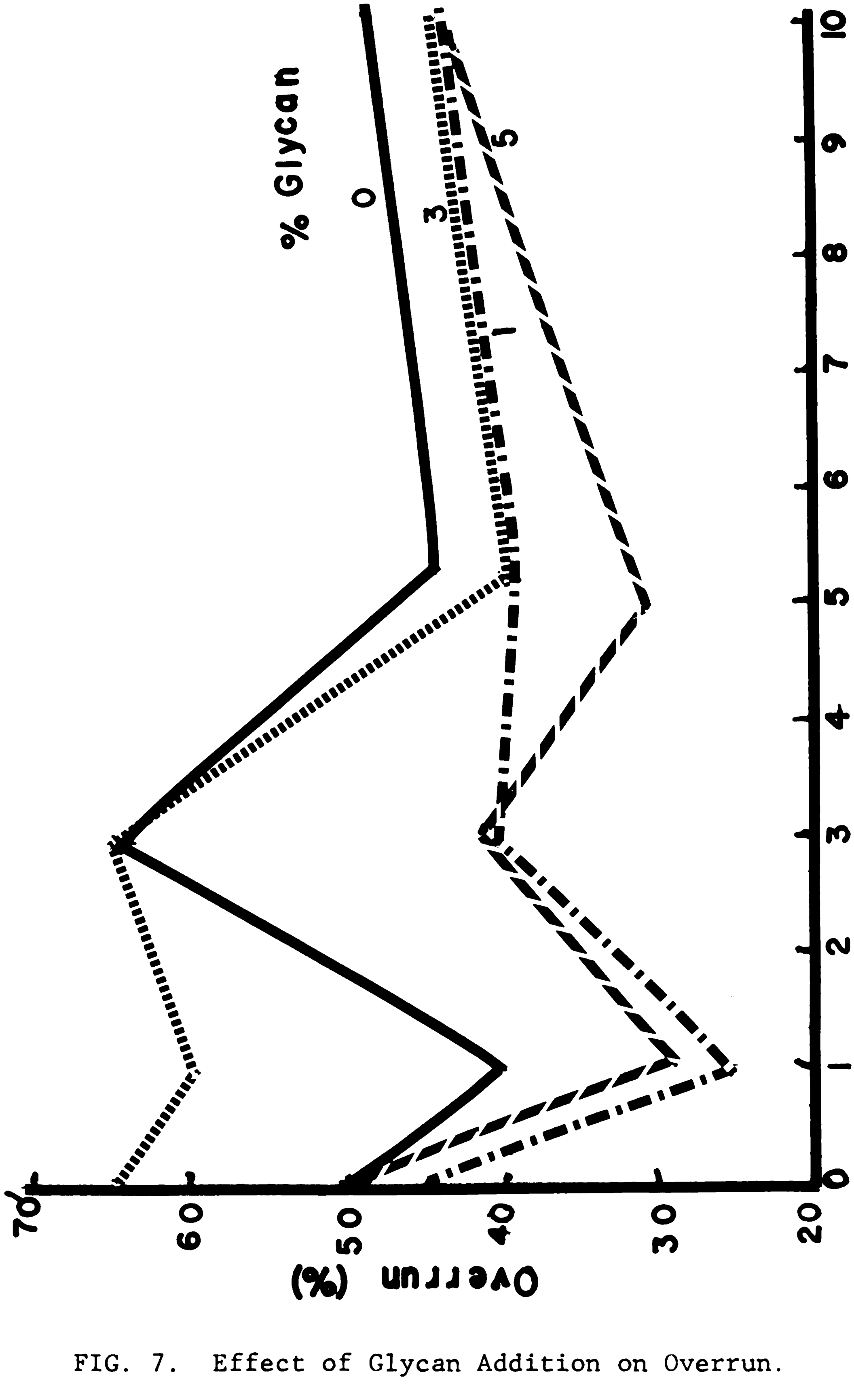

FIG. 7. Effect of Glycan Addition on Overrun.

increase due to the glycan, it would appear that interaction of the fat and glycan was fairly strong as shown in Figure 6, specifically at the five and ten percent fat levels. The viscosity rise in the 0, 1, and 3 percent fat mixes was much lower at the 3 percent glycan level but rose to nearly the same point as the 5, 10, and chocolate glycan mixes at the 5 percent glycan level.

Glycan did not affect the time required to freeze the mix or the temperature of draw. The effect of the glycan added was apparent in the rigidity of the freshly frozen product as it was discharged from the freezer barrel and in the ease with which it discharged. At the highest glycan level the ice cream was removed only with great difficulty, often requiring manual extraction. This was especially true with samples of higher fat content. When the mix hardened the effect of the glycan was again apparent. The ice cream in the cartons varied in resistance in scooping in direct relation to the amount of glycan. The lower glycan levels were easily scooped while the samples containing 5 percent glycan were nearly impregnable. The cause of this phenomenon may be due in part to the total solids resulting from addition of glycan. This seems remote because ice cream of the same solids level had been prepared previously, without glycan, and no difficulty was encountered in drawing the semi-frozen mix from the freezer. The greatly increased viscosity as a result of high levels of glycan might well have accentuated these characteristics.

The meltdown studies showed (Figure 8) that as the percent glycan increased so did the resistance to melting. It has been found (Turnbow 1928) that resistance to meltdown increases directly with viscosity. A rise in viscosity and overstabilization with resultant poor meltdown characteristics was observed in the high level glycan products. This is probably due to the water binding capacity of the glycan (6:1). It appeared that as glycan level increased the amount of separation of the melting ice cream was decreased. The melted ice cream had a thicker, richer look. Sidoti (1973a) also noted that heating (pasteurization) and shear (homogenization) tended to increase viscosity significantly. This would probably account for part of the dramatic viscosity rise in certain mixes.

Taste panel evaluation indicated that for each fat level there was also an optimum level of glycan addition (Figure 9). This optimum appeared to shift with the fat level. At lower fat levels the optimum appeared to be between 3 and 5 percent glycan while at 10 percent fat the optimum appeared to be at or below 1 percent glycan. At glycan levels below the optimum, panelists criticized the ice cream or ice milk for weak body while at higher than optimum levels the product was criticized as gummy or sticky. All panelists used in the glycan evaluations were expert dairy judges with adequate knowledge and training to rate samples purely

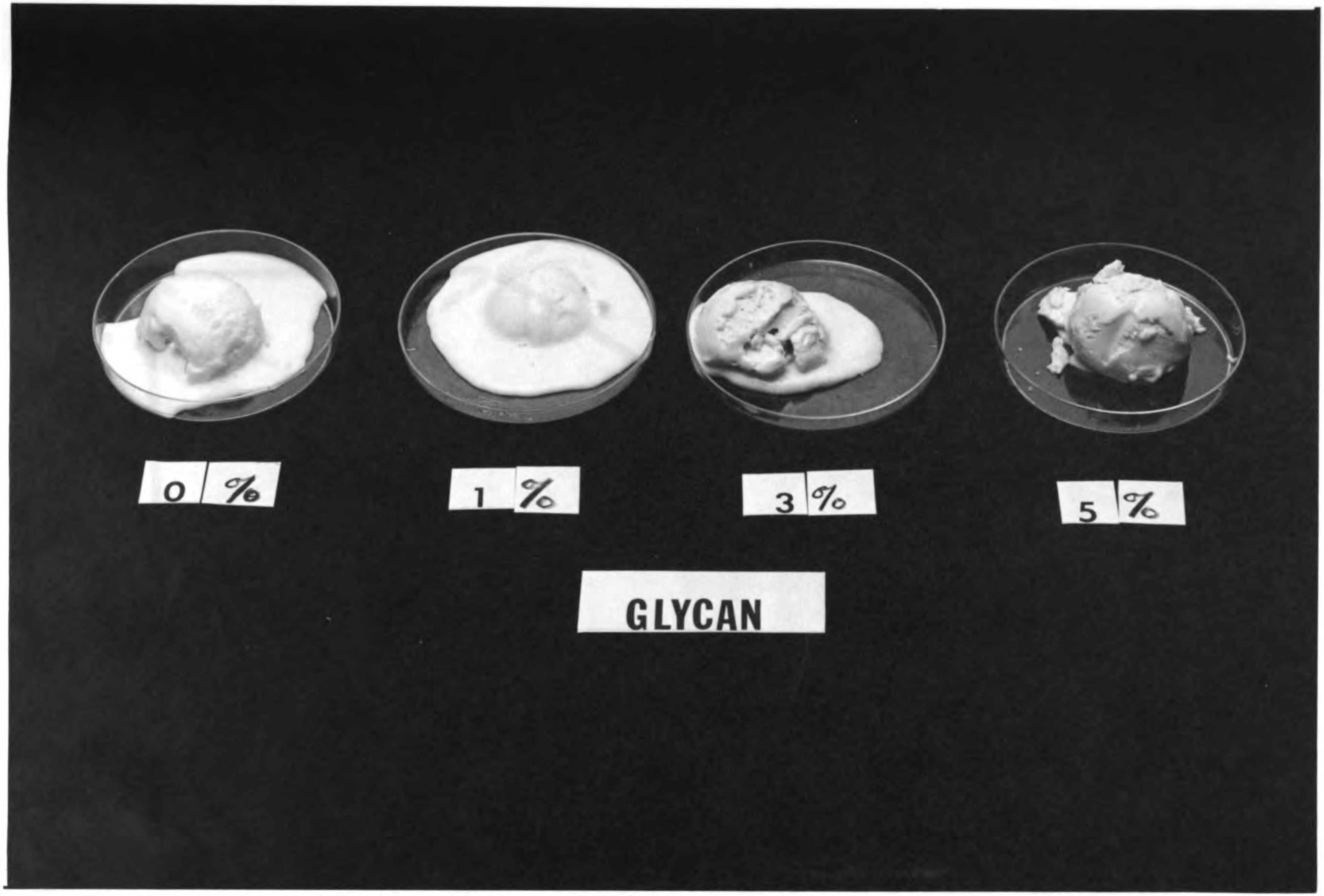

FIG. 8. Effect of Added Yeast Glycan on Meltdown of Ice Cream (percentage of original mix).

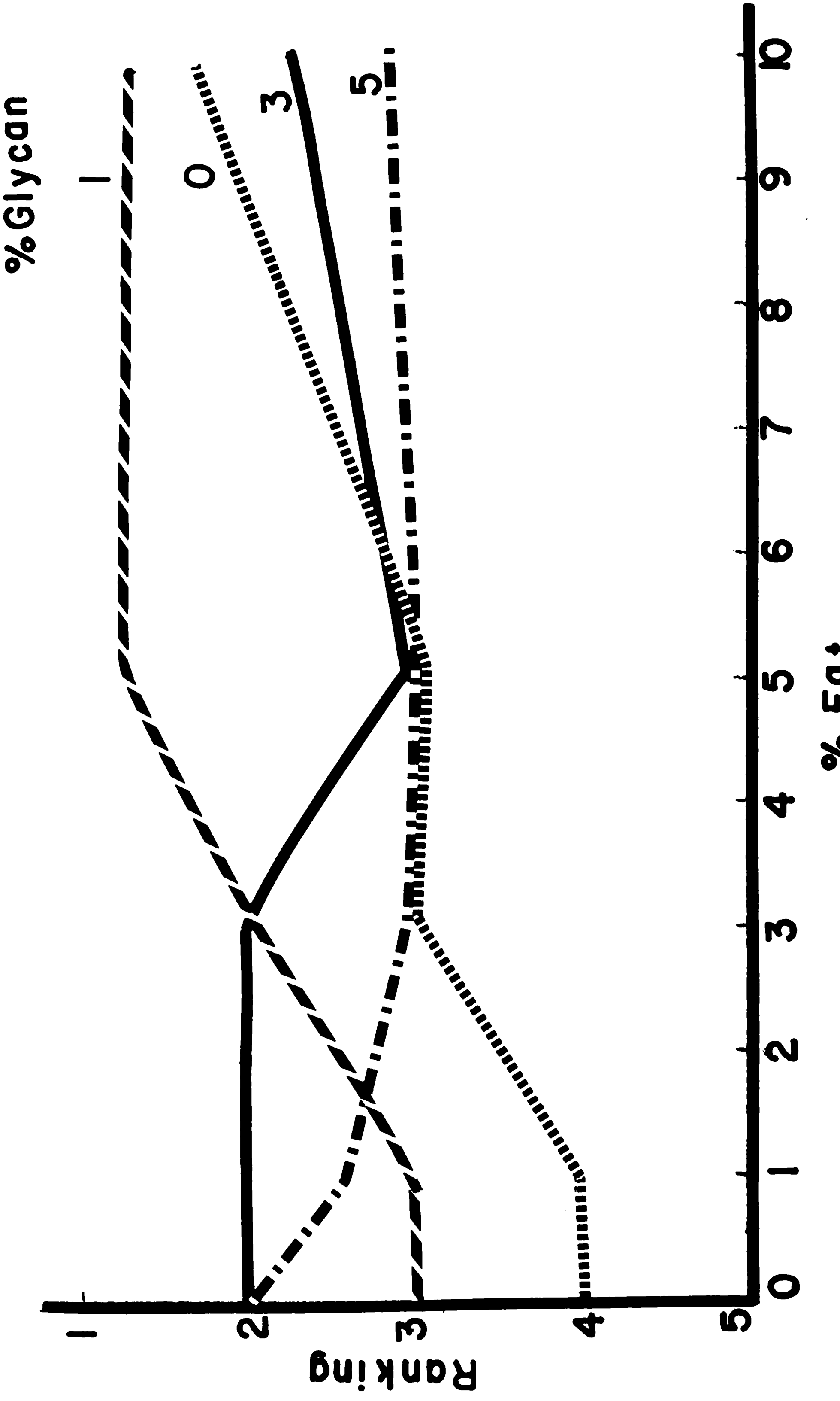

FIG. 9. Effect of Added Yeast Glycan on Ranking of Ice Cream Samples.

on body and textural characteristics. YG might be used to enhance the mouthfeel and texture of an ice cream or ice milk without the necessity of a higher fat content, caloric level or cost. The glycan might be used in place of other mix components to simplify processing and/or reduce costs. It might also be used for its functional properties to replace additives which might not be considered natural by some consumers.

A chocolate glycan mix was made with 3 percent milkfat. As a result of glycan addition, viscosity increased and approximated the viscosity of either 5 or 10 percent fat mixes (Figure 6). This increase may be due also to the additional cocoa solids and slightly higher concentrations of sucrose. The overrun followed the pattern set by the lower level fat mixes. Taste panel evaluation showed an optimum level of glycan addition which was higher than that for a 3 percent fat vanilla mix. The reasons for this are unknown.

NonDairy Ice Cream

Three nondairy ice cream products were made using whey, soy protein, and yeast protein, which provided the protein to the mix along with part of the total solids. As with previous mixes the freezing time and draw temperature were unchanged (Table 10). In this set of mixes the overrun also remained unchanged. The viscosity of the plain whey mix was one-half of the soy-whey and one-third of the YP-whey mixes. This is no doubt due to the water binding capacities of the soy and yeast proteins.

Taste panel evaluation showed the plain whey to be more acceptable than the mixes containing soya or yeast protein. This was especially true in regard to flavor (Table 10). The soy mix was labeled as "beany" while the YP mix was termed as having a severe off-flavor. The color of the mixes were quite similar to a normal vanilla mix although slightly more white. The meltdown showed the whey sample to be very thin. The soy sample was much thicker and looked quite creamy in texture. The YP separated upon melting giving an undesirable appearance. The synthetic product containing YP retained its structure longer than the other two samples but not to the point of not melting down.

TABLE 10. The Effect of a Nondairy Ice Cream Formulation on Mix Parameters and Taste Panel Evaluation

	Whey	Whey + 2% Soy	Whey + 2% YP
Viscosity (cp)	56	112	152
Overrun (%)	50	50	50
Average Ranking (B&T)	1.25	1.75	2.12
(Flavor)	1.00	2.00	3.00
Freeze Time (min)	12	13	12
Draw Temp. (^{o}F)	22	22	22

SUMMARY AND CONCLUSIONS

When milk proteins were replaced by soy protein isolate or yeast protein in ice cream or ice milk the following observations were made:

1. Yeast protein (YP) and soy protein (SP) stabilized the structure of the products and increased resistance to meltdown.
2. Viscosity increased, in general, directly with replacement levels with both proteins; however the increases were greater with YP to a replacement of 40%, after which viscosity decreased.
3. Neither pasteurization nor homogenization caused serious changes in the samples containing YP or SP.
4. YP had no effect on freezing time, draw temperature or overrun.
5. Excessive body and gumminess were reported in samples containing 40% or more yeast or soy protein.
6. Pasteurization had no affect upon mix parameters, meltdown properties or taste panel evaluation.
7. Homogenization did not influence meltdown properties or taste panel evaluation but did affect mix viscosity in that with a higher homogenization pressure a higher viscosity resulted.

8. In general, flavor acceptability by consumer taste panel varied inversely with replacement of milk protein. Chocolate flavored samples had greater acceptability than vanilla and samples containing SP were more acceptable than those with YP.
9. YP, SP and yeast glycan (YG) contributed color (varying degrees of brownness) to the mixes.
10. YG caused mix viscosity to increase, increased meltdown time, lowered overrun in a small batch freezer and had no effect on freezing time or draw temperature.
11. Samples containing YG showed a "fat sparing" effect up to a maximal level which varied with fat content of the sample. Samples with optimal levels of YG were significantly smoother and had good mouthfeel properties. Beyond the optimal level, YG contributed to excessive hardness and crumbliness.

APPENDIX

TeSa

Fat Test Reagent

Reagent was prepared by grinding together 3 parts (by weight) Urea, 3 parts Na_2CO_3, 2 parts EDTA, and 1 part Na_2HPO_4 until finely divided. Add 4 parts polyoxyethylene esters of mixed fatty and resin acids and mix thoroughly. (Prepared reagent is available from Technical Industries, 2711 S.W. Second Ave., Fort Lauderdale, Fla. as TeSa Reagent Concentrate.)

Working reagent was prepared by dissolving 156 g solid reagent in distilled water and diluting to one liter. The reagent was allowed to stand at least 6 hours prior to use and fresh reagent was prepared every 2 weeks.

50% Methanol

500 ml of absolute methanol was diluted in a 1 liter volumetric flask to volume.

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