SOME PHYSICAL PROPERTIES OF MILK PROTEINS AND SOYBEAN PROTEIN ISOLATES IN A SIMULATED MILK SYSTEM

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Elizabeth A. Hoffman

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ABSTRACT

SOME PHYSICAL PROPERTIES OF MILK PROTEINS AND SOYBEAN PROTEIN ISOLATES IN A SIMULATED MILK SYSTEM

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A study of the interactions of various milk proteins and soybean protein isolates was undertaken to see if such mixtures were compatible in a fluid system. Sols of 2.5% casein and 0.8% whey protein concentrate were replaced with increasing increments of soybean protein isolates. After their preparation the sols were either evaluated or heat treated for 145°F/30 minutes, 165°F/flash, or 175°F/ 15 minutes, cooled and subsequently evaluated. Sensory evaluation included subjective observation of color and odor. Viscosity was measured with a Brookfield viscometer. The concentration of soluble protein was determined by Lowry protein assay. Whipping ability was measured by the volume of foam produced and foam stability by the amount of liquid collected from the foam. The amount of oil emulsified signified the emulsification ability.

Sensory evaluation revealed a significant change in color and flavor as casein sols were replaced with increasing amounts of soybean protein isolate. The color remained unchanged but flavor and odor changed markedly with increasing

concentration of the soy protein in the casein-soy replacements, but increased slightly with the whey-soy sols. Whipping ability appeared to be little affected by replacing milk proteins with soy protein. Foam stability decreased greatly as casein was substituted by soy protein but improved as soy protein replaced whey proteins. Emulsification ability decreased for both the casein-soy and whey-soy replacements. There was no indication in any of the evaluations that any interactions of casein, whey protein concentrate, and soybean isolate had occured.

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INTRODUCTION

The need to increase world protein supply has been widely accepted and studied. According to Altschul (1966), about fifty percent of the world's children receive adequate protein nutrition. In 1970, there was an estimated world protein deficit of twelve percent. Even if the amount of protein is increased, this will not necessarily solve the problem, since it is the "amino acid profile" of the protein which determines its nutritional value. Futhermore, it is necessary to maintain production with world population growth, which is increasing by seventy million each year, (Kosikowski, 1969). Also, those areas with the peak population growth are currently both overcrowded and underfed. The staple foods in Asia and Latin America are grain, beans, fish and rice. In the Near East and Africa there is some milk consumption, but still an insufficient amount for good nutrition. This obvious need for increased protein supply has stimulated definite interest in the development of vegetable protein foods. While a typical acre of land may produce forty-three pounds of animal protein, it could produce four hundred fifty pounds of soybean protein. Such production would also increase caloric output four times. Increased consumer acceptability, desirable sensory qualities and dietary reduction of cholesterol and fat are desirable attributes of vegetable protein use (Childers, 1972).

A way of extending animal protein has been devised (Chandrasekhara, 1968; Chandrasekhara et al., 1971). This process, developed at the Central Food Technological Research Institute, produces Miltone, which is buffalo milk "toned" (extended), through the use of peanut isolate. The object was to make rich buffalo milk available to the vast population of India. Miltone, which can be stored up to six months at room temperature, and has the accepted color, flavor, mouthfeel, and nutritional value of milk, has been widely accepted in India. In 1971 (Anonymous), it received the Food Technology Industrial Achievement Award from the Institute of Food Technologists. In 1973 (Anonymous), Miltone was being produced in two cities in India, with two more plants being planned. Considering the wide growth of soybean production of the United States, as described by Ioanes (1973), it would be feasible to consider a similar process using soybean protein isolate. For that reason the project of studying the interactions of various milk proteins and soybean isolates was undertaken to see if such mixtures were compatable in a fluid system.

REVIEW OF LITERATURE

World Usage of Soybean Protein

The composition and general properties of soybeans have been described by many authors (Williams, 1971; Wolf, 1972; Holland, 1968). The most important consideration is that soybeans are approximately forty percent protein. This protein is made available through a variety of forms: whole beans, full fat flours, defatted flakes and flours, soy protein concentrates and soy protein isolates. Each form has an increasing amount of protein. All of these forms contain the essential amino acids necessary for growth, except for a deficiency of methionine, which may be added as a supplement (Anonymous, 1972; Badenhop and Hackler, 1973). Whole soybeans, in various forms, have been used in the Orient for many centuries, but the taste and odor, often described as painty, beany, or bitter (Nelson et al., 1971), is often objectionable to Western taste. However, the destruction of the enzyme lipoxidase by the heat generated in grinding (Holland, 1968), or blanching (Nelson et al., 1971), during the extraction process eliminates most of this problem.

One of the earliest attempts to increase world protein intake was through the development of what is known as CSM, a mixture of corn, soya protein and milk, which produces a nutritionally balanced food (Inglett et al., 1968). This product was found to be stable up to one hundred degrees Fahrenheit for one hundred eighty days, and by adding water, could produce a dough for an unleavened bread. So far, it has been distributed in ninety countries, and has been well accepted in all. According to Bockwalter et al. (1971), one hundred grams of CSM must contain three hundred fifty kilocalories, eighteen to twenty-two grams of protein, two grams of fat and one gram of linoleic acid. Studies are now being carried out to determine which formula provides maximum nutrition at a minimum cost.

Mustakas et al. (1971) have developed another way of making soy protein available. First produced at the Northern Agricultural Research Service (Anonymous, 1970), their protein concentrate, as it is called, makes available a product similar to milk at a lower cost. It is produced by adding soybean oil, emulsifier, sucrose, salt, flavoring, vitamins, and minerals to a full fat soy flour. It is made available in a form which can be reconstituted in water. The product has a good shelflife, acceptable flavor, and nutritional quality similar to cow's milk. At the time, the cost was estimated to be only ten cents per gallon, which would make it extremely competitive with other foods for feeding people in developing nations. Kosikowski (1969) believes that this type of beverage is nutritionally superior to any kind of imitation milk, especially when a native protein base is used. However, the acceptance of a new food by the uneducated and the role of their governments in allowing new, competitive industries to develop are limiting factors in the processing and distribution of a protein beverage concentrate.

Synthetic milk includes both filled and imitation milks. The distinction between the two has been described by many (Holland, 1969; Brink et al., 1969; Council of Foods and Nutrition, 1969; Moses, 1969; and Anonymous, 1969). As defined by Congress, filled milk is a milk product to which a fat or oil has been added to form an imitation milk. On the other hand, there is no regulation for imitation milk, other than a regulation prohibiting the use of any milk products.

Soybean milk, the main type of imitation milk, has been used in the Orient for centuries, (Standal and Kian, 1969), but it was first introduced to this country to be used by infants who were hyperallergic to cow's milk (Anonymous, 1963). Shih (1970) also prescribes soybean milk for cystinosis, homocystinuria, and lowering tendency for athersclerosis. The most widely accepted consideration for most American consumers, for both imitation and filled milks, is its lower cost. Standal and Kian (1969) feel the lower cost of imitation milk could make it available in developing countries to assist in the prevention of mild and even severe kwashiorkor. In such countries, cow's milk is often expensive, diluted, and may be unhygenic. As first described by Collins-Willims (1956), Powdered Sobee, a soy based infant formula, contains 3.4% protein, 2.7% fat, 8.1% carbohydrate, 0.2% fiber, 0.5% ash, and 85.1% moisture. Forman et al.(1964), Holt (1965), Howard et al. (1956), Kay et al. (1960), and Tiling et al. (1961), all believe that such infant formulae are nutritionally adequate for infants. However, Theurer and Sarett (1970) caution that when soy isolate is used instead of soy flour, it is necessary to add those nutrients absent from the isolate. When soy isolate formulae are supplemented with methionine, they have ninety percent or more of the efficiency of casein.

With the success of these infant formulae came the development of imitation and filled milks for the consumer. Holland (1968; 1969),USDA (1969), and Anonymous (1968), list typical formulations of synthetic milks. Imitation milks generally contain soybean isolate, sodium caseinate, corn syrup solids, vegetable fat, stalilizer, water, vitamins and minerals. Filled milks may contain nonfat dry milk solids, coconut fat, emulsifier, vitamins, and water. Noyes (1969) gives the step-by-step processes of three companies in their preparation of synthetic milks.

The major factor which prevents the synthetic milks from being widely accepted is their taste. Shih (1970) believes that soybean milk could be consumed just as easily by American adults as well as infants, but that the taste is the major deterrent. According to Holland (1968), imitation milks do not taste like fresh milks, but they are palatable. Chocolate, black cherry, and black and red raspberry flavors all improve the sensory qualities of the product. Holland (1969) states that sodium caseinate, not soy protein isolate, should be used in an imitation milk to obtain milk flavor.

Other factors than taste affect consumer response to these products (Jacobsen, 1969; Kosikowski, 1969). The willingness to accept imitation products and their cost are also important factors. Milk substitutes range from five to twenty cents per half gallon less than homogenized whole milk (Anonymous, 1968). However, Koskowski (1968), Brink (1969), and Council on Foods and Nutrition (1969) state that these products actually are more expensive than milk nutritionally, because of insufficient protein being used for a good amino acid profile. They have urged that the nutritional value of these products be increased so that they can be used to provide the needed nutrients to both American children and those in less fortunate countries.

The Properties of Soybean Isolates

According to Central Soya Chemurgy Division (a and b), soybean protein isolate is a vegetable protein derived from the major storage globulins of the soybean. It is at least ninety-six percent protein on a moisture free basis, and contains all the amino acids essential for human nutrition. Johnson and Circle (1959) found it comparable in nutritional value with casein on a weight basis. When supplemented with methionine or used with methionine-rich foods, it has outstanding nutritional value (Central Soya Chemurgy Division, a and b). The product, which is obtained by solvent extraction, acid precipitation, and spray drying, is recommended by Central Soya Chemurgy Division (a and b) and Johnson and Circle (1959) for a wide variety of uses, particularly in bakery goods, cereal and dairy products, meat type products, speciality foods, and synthetic milks. Fujmaki et al. (1968) found that it is difficult to remove characteristic soybean flavor, even from soybean isolate, and this hinders its use in certain food products. Yasumatsu et al. (1972 a) found that raw soybean flavor is absent from soybean isolate, but that it still contains a major soybean flavor characteristic. Nonetheless, when this flavor is masked, the isolate may contribute a variety of desired characteristics to a product. According to Belshaw (1971), these properties include thickening, emulsion forming capabilities, film forming abilities and gelling capacity and water binding properties similar to milk or egg proteins. Yasumatsu et al. (1972 b) agreed that soybean protein isolates have good whipping and emulsifying capabilities. Williams (1972) stated that isolates have a pH-solubility relationship similar to casein and may therefore be substituted for casein and caseinates in food products. Belshaw (1971) found the isolate he studied to be ninety percent soluble in aqueous solutions with moderate agitation, but Wolf (1972) cautioned that commercial isolates vary appreciably in solubility and therefore should be evaluated individually. Chakraborty and Hansen (1969) did find that several caseins stabilized the solubility of soy isolates in milk salt systems. Sugimoto and Van Buren (1971) attributed stabilizing effects in soy solutions to monosaccharides, disaccharides and corn syrups. At concentrations above seven percent, viscosity will increase and gelation will occur upon heating (Wolf, 1972; Williams, 1972).

Finally, cost will determine the availability of the isolate for use in milk extension. Belshaw (1971) found that soy isolate made protein available at half the cost of skim milk, and one quarter that of whole egg solids. Ultimately, it will be the cost and availability of soybeans on the world markets that will affect its being used as a milk toning agent.

The Proteins of Milk

Although milk does not contain an especially high amount of any one particular nutrient, it is a uniquely balanced source of man's dietary requirements of protein, fat, carbohydrates, vitamins, and minerals (Jenness and Patton, 1959; Hartman and Dryden, 1965; Lampert, 1970; and Kon, 1972). Also, it contains a significant amount of protein, which is the most essential nutrient which cannot be produced synthetically (Kon, 1972). More importantly, milk contains the daily requirements of almost all the essential amino acids (Lampert, 1970). Milk proteins themselves are classified as caseins, which precipitate out of milk at a pH of 4.6, or whey proteins, which remain in solution at a pH of 4.6. Thompson et al. (1965) gave a thorough review of the many forms of both caseins and whey proteins. In a more recent review, Lyster (1972) reviewed the nomenclature, amino acid sequences, and primary, secondary, and tertiary structures of caseins and whey proteins.

The Properties of Casein

Caseins exist in large colloidal particles containing the protein and considerable quantities of calcium and phosphate, and some magnesium and citrate (Jenness and Patton, 1959). Many models have been offered as to how these are arranged in the casein micelle (Rose, 1969). These micelles can be removed from milk by several processes (Jenness and Patton, 1959; Gordon and Whittier, 1965). High speed centrifugation will remove casein and leave whey proteins and other dissolved constituents in solution. Isoelectric precipotation can be achieved by lowering the pH of milk to 4.6. Casein may also be coagulated and removed from the whey by rennet, as is done in the cheese making process. Casein will also precipitate from milk saturated with sodium chloride.

The physical and chemical properties of casein have been studied by many authors. Jenness and Patton (1959) discussed the solubility, rate of browning, and combining capacity of casein. Another important aspect of milk, flavor evaluation, was discussed by Cayen and Baker (1963).

The food uses of commercial casein are rather limited. In 1967, two thirds of the casein in the United States was used for industrial uses, mainly as a binder in paper, paint, and adhesive products (Webb and Whittier, 1970). New products now being developed and available for consumer use include filled and imitation milks, coffee whiteners, instant breakfasts, cereals, sausages and loaves, toppings, ice cream mixes, sour cream, yoghurt, doughnut mix, and other foods where it is used to supplement the proteins already present (Lampert, 1970; Webb and Whittier, 1970). The many properties of casein are advantageous in these various products. Casein hydrolysate, with a fundamental flavor characteristic which suggests meat, is used to accentuate or suggest the flavor of meat in precooked, cured, stored, comminuted and dehydrated meats and in dry soup preparations. In candies, casein forms a firm, resilient, chewy type of body (Webb and Whittier, 1970). It also improves the body of sour cream and yoghurt, minimizes shrinkage and improves overrun in ice cream, and prevents excessive absorption of fat in doughnuts during deep-fat frying (Lampert, 1970). In coffee whiteners casein serves in emulsification, whitening ability, and body and flavor improvement by contributing its own flavor and reducing the acridity of tannic acids (Knightly, 1969).

The Properties of Whey Proteins

The nutritive value of whey proteins has been known for many years (Osborne and Mendel, 1924). Their physical properties were also studied (Peter and Bell, 1930; Black et al., 1953), but no practical need for the recovery of whey from the cheese making process was demonstrated. However, with the present need for an economical waste disposal system, more cheese plants are now turning to a profitable whey disposal system. Swanson and Ziemba (1967) described a process which turns the economic burden of whey waste disposal into a profitable operation which produces a product of high nutritional value. Wingerd (1971) and Wingerd et al. (1970) found that rats gained more weight on a whey diet than on a casein diet. Another similar study found whey and casein had an almost equivalent protein efficiency ratio (PER) (Anonymous, 1970 b). However, this nutritious product was only available in an insoluble, gritty, denatured form which could be used to supplement cereals (Wingerd et al., 1970; Wingerd, 1971). It is now available in a bland, soluble form of whey protein concentrate which can coagulate with other proteins. The functional properties of this product - solubility, whipping capacity, emulsification, pH value, buffer capacity, viscosity - have now been studied by many (Hansen and Black, 1972; Morr et al., 1973; Kuehler and Stine, 1974; Richert et al., 1974). These properties could hinder their use in specific products, but they also can enhance the value of a food if used correctly (Whey Utilization Conference Proceedings, 1970). It is believed that reverse osmosis may be used to produce high-protein whey preparations (HPW) at a lower cost than nonfat dried milk. Another product which would be cheaper than nonfat dried milk is vegetable protein whey (VPW), which can be obtained by fortifying whey with a vegetable protein such as soy. This mixture yielded a sweet product with a flavor of cereal and milk when reconstituted in water, and had a good storage life and oxidative resistance.

EXPERIMENTAL PROCEDURES

Exploratory Tests

Exploratory tests were run to determine the best possible model system for experimentation. The soybean isolates used were Promine-F and Promine-D, from Central Soya, and Edi-Pro A and Supro 610, from Ralston Purina Company. The solvents used were distilled water and a solution designed to have the salt composition of milk ultrafiltrate (Jenness and Koops, 1962). Each soybean isolate was taken in 0.50 to 2.00 g samples and diluted to one hundred ml with either water or the milk ultrafiltrate solution (Koops' buffer). These samples were agitated for one hour at highest speed on a Burell Wrist-Action Shaker, filtered through Schleicher and Schuell handfolded S and S American filter paper #605, and suitable aliquots of the filtrate were taken for micro-Kjeldahl analysis (McKenzie, 1970), to determine the amount of soluble protein in the filtrate. Hammarsten casein (Modler, 1973) was prepared and the same solubility tests were run.

The Model System

Samples of soybean isolate and casein were combined to a total of 2.5 g/100 ml of Koops' buffer (5.0 g/200 ml) in a four hundred ml beaker. Samples of whey protein concentrate (Enrpro 50, Stauffer Chemical Company), and soybean isolate were combined to a total of 0.8 g/100 ml of Koops' buffer (2.4 g/ 300 ml) in a four hundred ml beaker. These samples were then stirred for one hour on a Cole-Parmer Instrument Company "magne-4", model number 4820-4, magnetic stirrer at setting number nine, and then either subsequently evaluated, or submitted to either one of three heat treatments (1) 145^{o}F/30 minutes, 2) 165^{o}F/flash, 3) 175^{o}F/15 minutes), then cooled to room temperature in cold water and evaluated. The first and third heat treatments were achieved by holding in a water bath for the appropriate time. The second heat treatment was attained by agitating the sample in a boiling water bath.

Sensory Evaluation

Color and odor were evaluated subjectively and each sample was compared with the others being tested at the same time. Any other apparent physical characteristics (clarity, aggregated materials, etc.) were noted.

Viscosity

Viscosity was measured at room temperature (24°C) with a Brookfield syncho-lectric viscometer, model RVT, at one hundred rpm using spindle number two. All samples were used in their entirety in the four hundred ml beakers in which they were prepared.

Soluble Protein

The concentration of soluble protein present in each sample was determined by filtering several ml of sample through Schleicher and Schuell handfolded S and S American filter paper #605, and removing a suitable aliquot of filtrate for a Lowry protein assay (Lowry et al., 1951). Bovine serum albumin dissolved in distilled water was used to prepare the standard curve. Absorbance was read at 550 nanometers on a Bausch and Lomb Spectronic 20 spectrophotometer.

Whipping Properties

Twenty-five ml of casein-soy sample or fifty ml of whey-soy sample was placed in a six hundred ml beaker and whipped until still peaks of foam formed in the beaker with a Hamilton Beach Model 97 Mixette on high setting. The height of the foam was measured to determine the volume of foam produced (Tamsa et al., 1969). The stability of the foam was measured by filling a fifty ml beaker with foam and inverting it on a wire screen placed over a funnel. The amount of drip was measured in a graduated cylinder after thirty minutes. When the foam was too unstable to fill a fifty ml beaker, the amount of time it took for the foam in the six hundred ml beaker to decompose was noted.

Emulsification

Emulsifying ability was measured as suggested by Webb et al. (1970). Five ml of casein-soy sample and twenty-five ml of distilled water or ten ml of whey-soy sample and twenty-five ml of distilled water were placed in a four hundred ml beaker and weighed. The beaker was covered with a rubber stopper with holes for a stirrer, two electrodes, and tubing. The beaker was then attached to a Hamilton Beach malt mixer, number 17 Arnold Model, and the electrodes attached to a Triplet multimeter, model number 630. Mazola corn oil was added through the tube to the mixture which was stirred until the ohmeter read infinite resistance. The beaker was detached from the apparatus and reweighed to determine the weight of oil emulsified.

RESULTS AND DISCUSSION

Exploratory Tests

The solubility of Hammarsten casein was the main consideration of the exploratory tests. As reported in Table 1, the casein was almost completely insoluble in water. Consequently, the model system had Koops' buffer as the solvent. Although four soyprotein isolates exhibited comparable solubilities in Koops' buffer, Edi-Pro A and Supro 610 were rejected because their dispersibility made them difficult to handle with any accuracy. The method of mixing samples also proved inefficient; soyprotein isolates tended to float to the top of the volumetric flask and stick in the neck. This prevented complete mixing of the soyprotein isolate and solvent, giving imprecise results for solubilities. The use of magnetic stirrers in beakers improved the precision and accuracy of measurement.

Sensory Evaluation

The replacement of casein with soyprotein isolate was quite apparent visually and by the aroma of the respective sols. Pure casein mixtures were an opaque white color, with only a few distinct undissolved particles which sank to the

Table 1. Preliminary tests for solubility of soyprotein isolates in distilled water and Koops' buffer.

Protein	Protein (%) (w/v)	Solubility Water (%) (w/v)	Solubility Koops' buffer (%) (w/v)
Promine-F	0.50	12.3	4.2
	1.00	19.5	3.5
	1.50	16.4	4.0
	2.00	18.1	6.0
Promine-D	0.50	34.8	11.3
	1.00	14.5	13.5
	1.50	43.1	15.4
	2.00	33.2	11.1
Edi-Pro A	0.50	1.6	23.4
	1.00	0.8	14.5
	1.50	0.8	9.4
	2.00	0.6	9.2
Supro 610	0.50	10.4	13.2
	1.00	10.3	7.7
	1.50	13.6	6.6
	2.00	12.4	7.2
Hammarsten Casein	0.50	1.0	90.5
	1.00	0.0	65.0
	1.50	0.0	69.1
	2.00	0.3	61.4

bottom. They exhibited a mild to strong "gluey" odor depending on the heat treatment. Soyprotein isolate mixtures appeared cream colored as described in commercial literature. The undissolved soyprotein isolate formed a layer of the top of the mixture, and would aggregate and sink to the bottom during heat treatment. A beany odor was very apparent, especially after heat treatments. The various mixtures exhibited the characteristics of both casein and soyprotein isolate, and as the increments of soyprotein isolate increased and the casein decreased, the properties of the soyprotein isolate predominated.

The whey protein concentrate (Wpc) mixtures were similar visually to the soyprotein isolate mixtures. Both exhibited approximately the same color. However, all the Wpc appeared to dissolve while the soyprotein isolates again floated to the top. Wpc mixtures had a cooked milk odor and the soyprotein isolates had a less intense beany odor than in higher concentrations. Increasing replacement of Wpc by soyprotein isolate did not noticeably change the color, but the floating layer increased and the odor changed from cooked milk to beany. These mixtures also had a pronounced odor.

To date, the objectional flavor of soyprotein isolates has prevented their use and acceptance in milk substitutes. Normal milk flavor can be described as very slightly sweet and salty, not bitter or sour. It has little flavor and is not easily characterized. However, off flavors and odors are easily identified in milk and have been attributed to oxidation, rancidity, sunlight, microbiological deterioration and absorption of foreign flavors. The distinct cooked flavor exhibited by the Wpc develops at approximately 74°C due to the activation of -SH groups from β-lactoglobulin and the milk fat membrane proteins to form H_2S and other sulfides (Jenness and Patton, 1959). Wingerd (1971) characterizes this as a "bland" flavor. Ramshaw and Dunstone (1969) attributed the gluey off flavor of the casein to non-enzymatic browning which develops during storage. The low level of lactose in the Hammarsten casein reduced browning to a minimum, thus preventing a very strong flavor and odor development.

According to Kirimura et al. (1969), the amino acids and peptides present in a food contribute to its flavor. Individual amino acids can be characterized as being sweet, salty, sour, bitter, or MSG-like. Cowan et al. (1973) have listed the flavor components which they feel are responsible for the beany and bitter flavors in soyprotein isolates. Maga (1973) reviewed current work with the flavor of soybean products, and concluded that while the flavor problem of soybeans is complex, technological advances and new instrumentation will stimulate research in this area . Badenhop and Hackler (1970) suggested soaking in NaOH as a pretreatment to improve flavor and mouthfeel. This product must then be supplemented with methionine to replace nutrients lost during soaking (Badenhop and Hackler, 1973). Central Soya Chemurgy Division (a and b) reports a mild alkaline pretreatment of the soybeans before being processed into isolates. It is this off flavor that will have to be either removed or masked before the flavor and odor of soyprotein isolates is acceptable in a bland milk system.

Viscosity

The data in Figure 1 are a plot of the viscosity of casein and soya sols in Koops' buffer. The protein content of the solutions was maintained at 2.5% and varied from all casein to all soya protein. Temperature of heating varied from none to 175°F/ 15 minutes. In general, the viscosity of the suspensions decreased with increasing replacement of the casein by soya protein. The data in Figure 2 show that the initial viscosity of Wpc and soya protein sols is lower than observed with casein. A slight increase is noted as both soyprotein isolate replacements increase. Table 2 presents a statistical analysis of variance of the individual data points and indicates that the changes are all significant. However, several factors must be considered when evaluating these results. Viscosity is defined as the resistance of liquids to flow or pour. Viscosity is important in milk because of its relation to milk's "richness". While the viscosity of milk is a complex property, its normal range is between 1.5 and 2.0 centipoise at 20°C. Lactose, whey proteins, and milk salts contribute very little to this, with the main contribution coming from casein (Jenness and

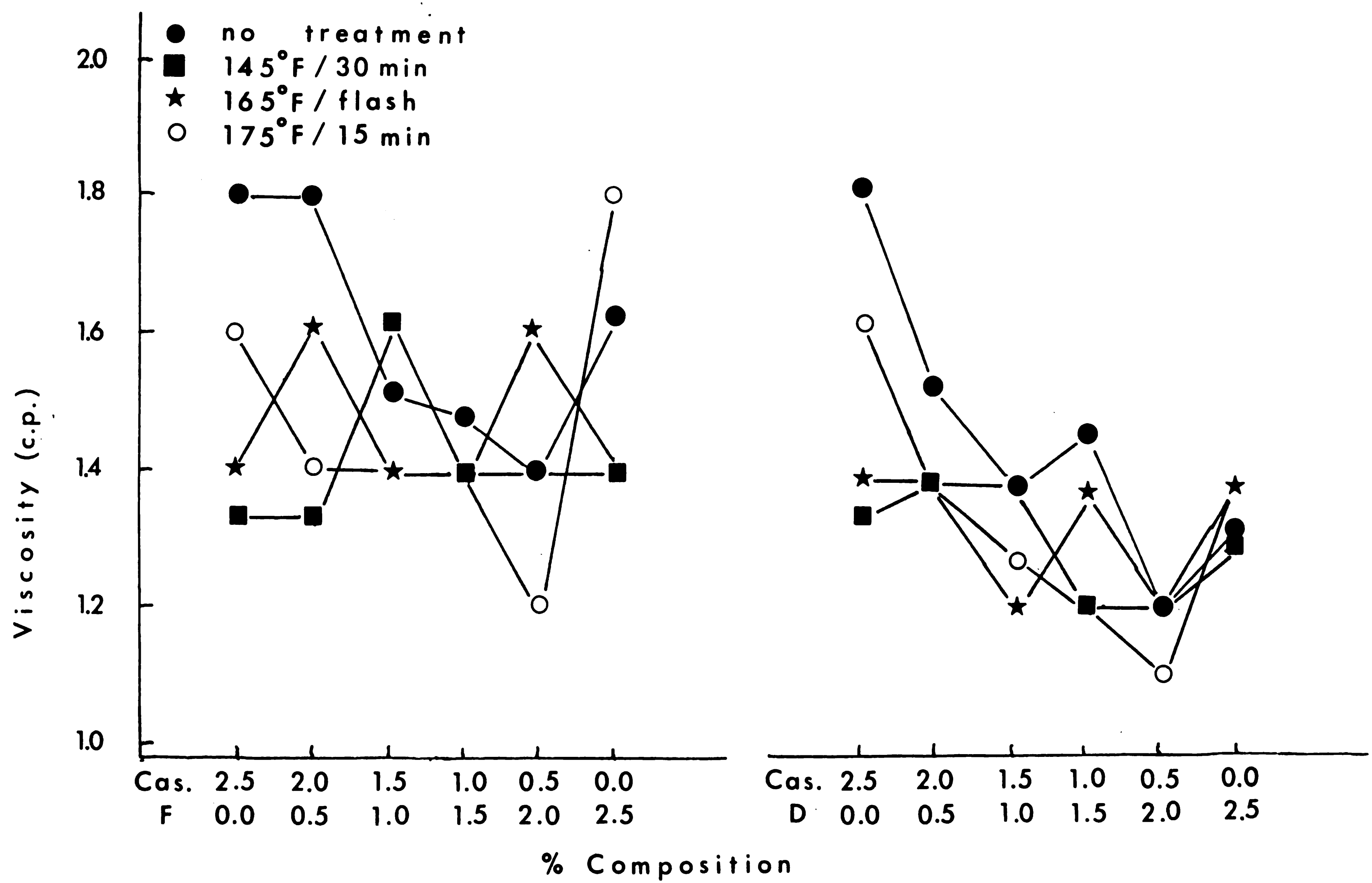

Figure 1. The viscosity of Promine-F and Promine-D replacements of casein in Koops' buffer.

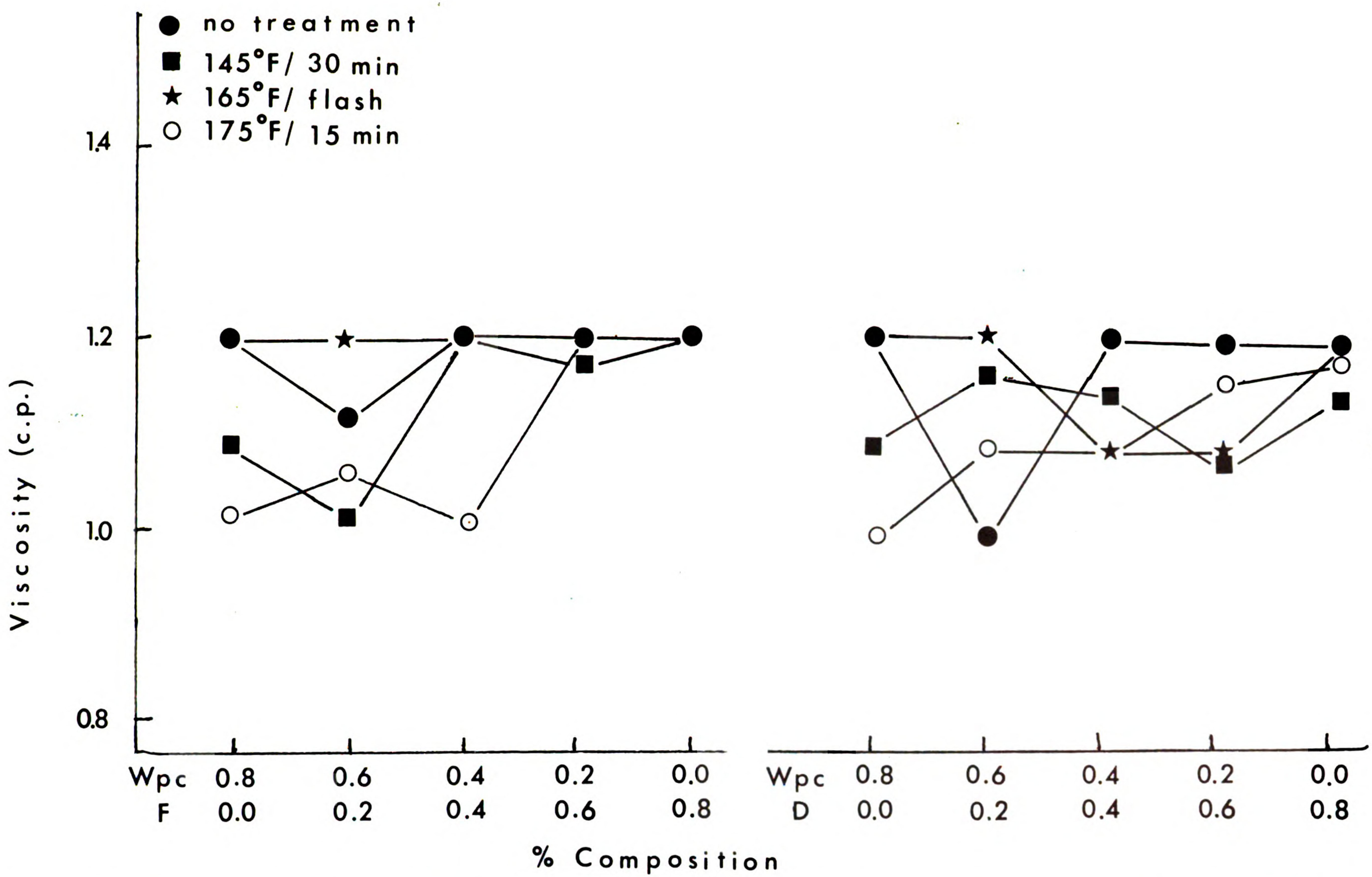

Figure 2. The viscosity of Promine-F and Promine-D replacements of Wpc in Koops' buffer.

Table 2. An analysis of variance for individual data points.

Source of Variance	Approximate Significant Probability			
	Viscosity	Whipping ability	Whipping stability	Emulsification
Casein[1]	<0.0005	<0.0005	<0.0005	<0.0005
Soy[2]	<0.0005	<0.0005	0.012	<0.0005
Temperature[3]	<0.0005	0.007	0.002	0.015
Casein-Soy	<0.0005	0.001	<0.0005	0.006
Casein-Temp.	<0.0005	0.001	<0.0005	0.676
Soy-Temp.	<0.0005	0.072	0.012	0.376
Cas.-Soy-Temp.	<0.0005	0.017	0.093	0.299
Whey[1]	<0.0005	<0.0005	*	<0.0005
Soy[2]	<0.0005	<0.0005		0.130
Temperature[3]	<0.0005	<0.0005		<0.0005
Whey-Soy	<0.0005	<0.0005		0.001
Whey-Temp.	<0.0005	<0.0005		<0.0005
Soy-Temp.	<0.0005	<0.0005		0.033
Whey-Soy-Temp.	<0.0005	<0.0005		0.001

* Data obtained could not be analyzed.

1 Change in percent

2 Promine-F or Promine-D

3 Change in heat treatment

Patton, 1959). As in milk, a very small proportion of the viscosity was due to the solvent. The Koops' buffer alone measured a viscosity of 1.04 centipoise. Jenness and Koops (1962) made no mention of viscosity when describing it as a milk ultrafiltrate. Nonetheless, the data show that the viscosities were significantly higher for casein than for whey protein. Jenness et al. (1965) state that viscosity depends on temperature, and amount and state of dispersion of solid constituents. This was evident when measuring the viscosity of the sols because casein mixtures, with more dissolved particles in solution had a greater viscosity than soyprotein isolate mixtures. The soyprotein isolate aggregates made the viscosity reading higher for whey mixtures.

Soluble Protein

The concentration of soyprotein isolate in the buffer system had a substantial effect on the soluble protein present in the mixture. Figure 3 shows that casein was almost completely soluble in Koops' buffer while both soyprotein isolates were much more dispersible than soluble. An almost linear relationship exists in the decline of the amount of casein and decrease in the amount of soluble protein present. In the case of the Wpc-soya mixtures in which total protein content was lower, there was very little decrease in the amount of soluble protein present with increasing soyprotein

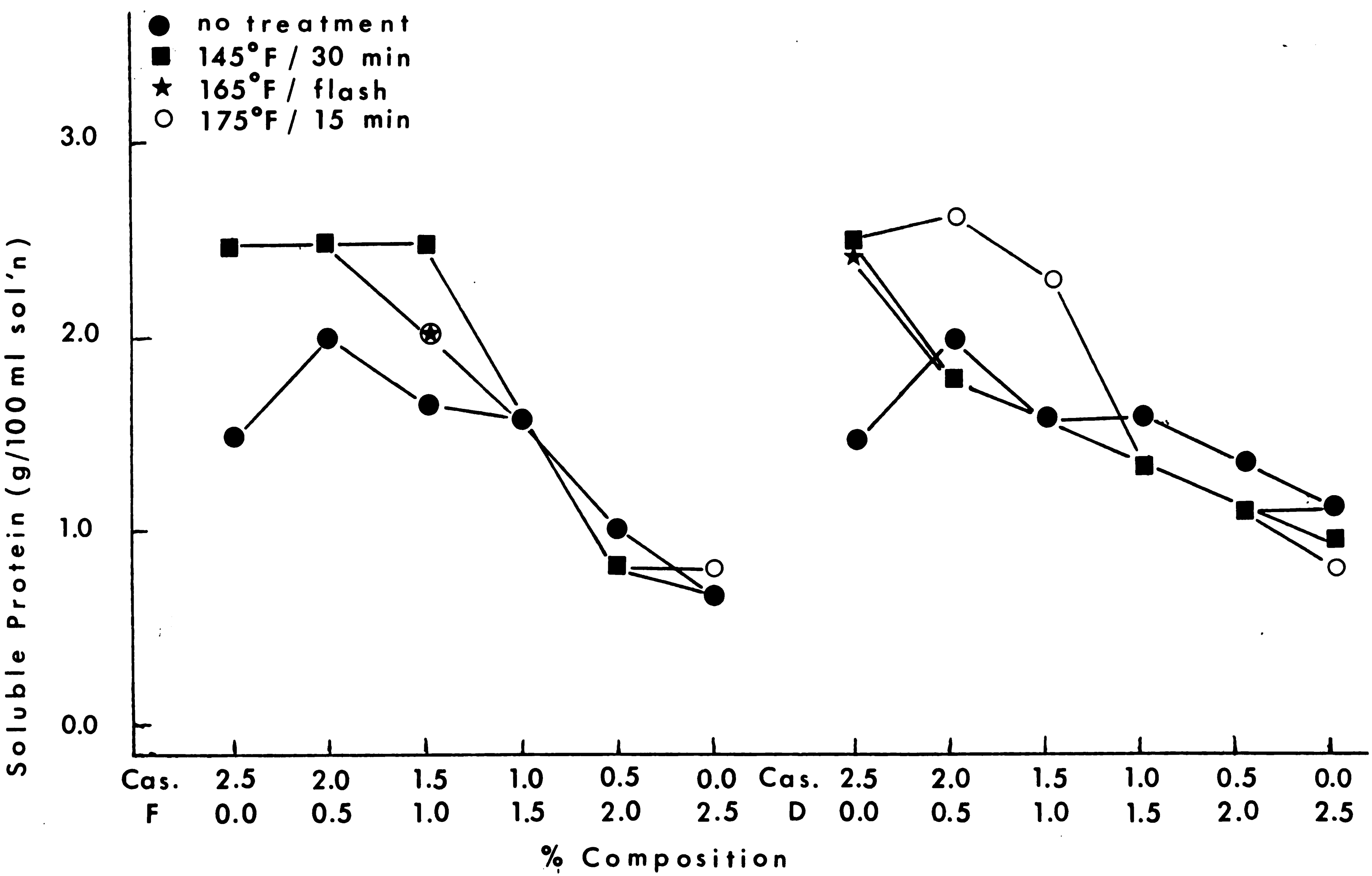

Figure 3. The soluble protein of Promine-F and Promine-D replacements of casein in Koops' buffer.

isolate replacement of Wpc, as seen in Figure 4. No statistical analysis was available for these data because there was no variance among observations for individual data points.

A protein is considered soluble when the attraction of individual molecules for the solvent is greater than that between the protein molecules. Since solubility depends on both pH and salt concentration, minimum solubility is at the isoelectric point of the protein with no salts present. Here there is a maximum attraction of the positive and negative forces of adjacent protein molecules. As the pH increases or decreased from the isoelectric point, a net positive or negative charge develops on the protein molecules and they are attracted to the water molecules, causing the protein to dissolve. At low concentrations of neutral salts there is an increased net charge and increased protein solubility. However, at very high salt concentrations the ions compete with the protein for attraction with the water molecules, and protein solubility decreases. Casein binds Ca^{++} and other bivalent cations very tenaciously at pH values alkaline to the isoelectric point which causes aggregation and decreased solubility. Whey proteins are solublized by a small concentration of neutral salts or small changes in pH (Jenness and Patton, 1959). Gordon and Whittier (1965) point out that the amount of casein dissolved by many solvents varies with the quantity of casein added to a definite quantity of solvent, so that many available data on casein

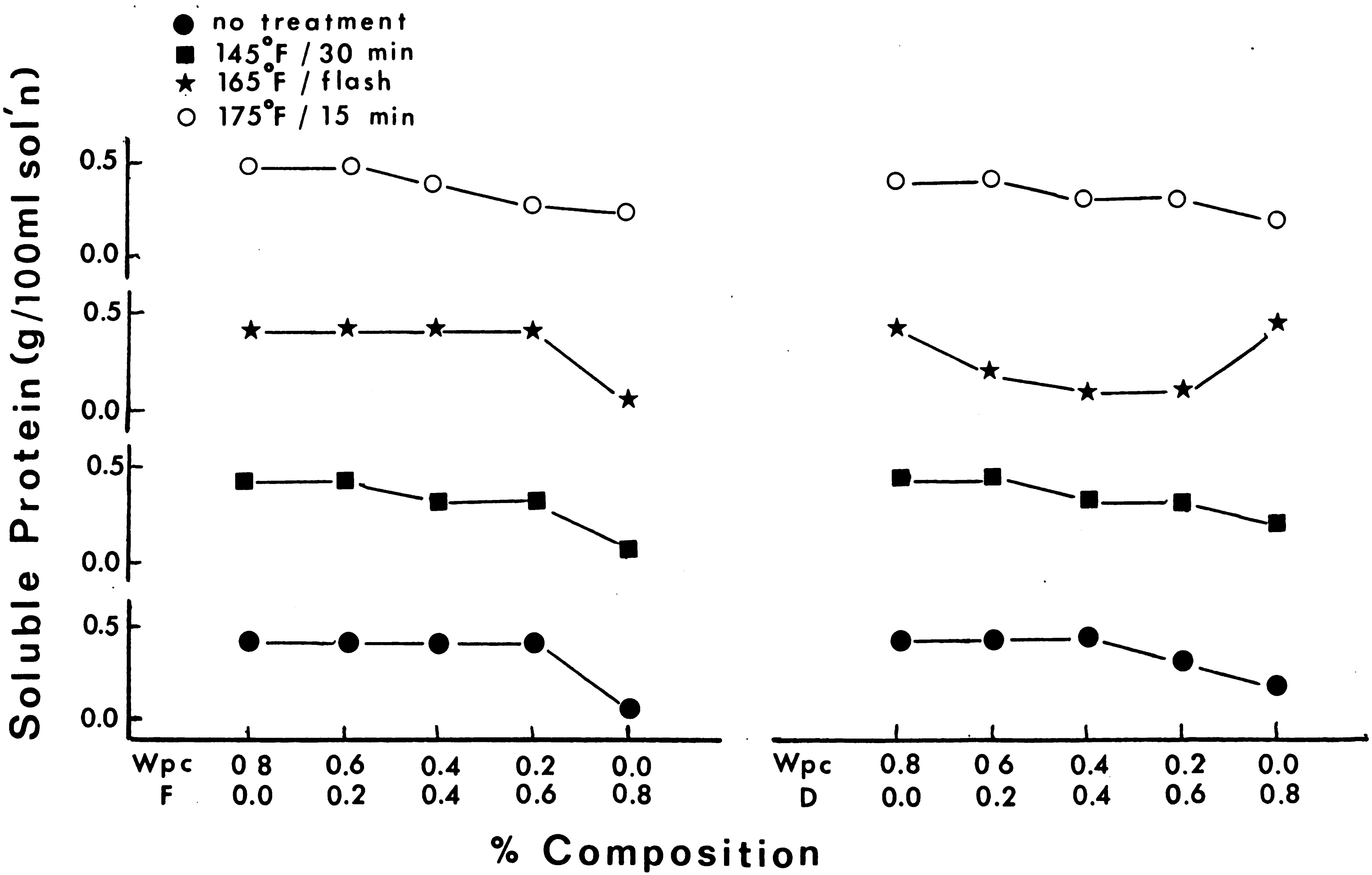

Figure 4. The soluble protein of Promine-F and Promine-D replacements of Wpc in Koops' buffer.

solubility have limited uses. This results from the fact that most casein preparations consist of more than one molecular species. However, casein can be dissolved in aqueous solutions of acids, alkalies, and alkaline salts such as the potassium, calcium, and magnesium chlorides which are present in Koops' buffer. Since this solvent has the same pH, salt content and ionic strength as milk ultrafiltrate, the casein is almost completely soluble in it.

Both soyprotein isolates used are considered water dispersible (Central Soya Chemurgy Division, a and b) with 65% of Promine-F and 75% of Promine-D water soluble. Williams (1972) and Wolf (1972) state that soyprotein isolates are fairly insoluble, but that solubility can be increased by adding 0.01 mercaptoethanol to the solvent. This breaks most of the disulfide cress-links which cause polymerization and thus insolubility during isoelectric precipitation. A portion of the isolate still remains insoluble after adding mercaptoethanol. Wolf (1972) cautions that commercial isolates vary in solubility due to processing variations. Chakraborty and Hansen (1969) and Sugimoto and Van Buren (1971) suggested that carbohydrates be added to milk-soy systems not to increase solubility, but to stabilize the dispersions.

Data from Stauffer Chemical Company and Morr et al. (1973) agree that Wpc is almost totally soluble in the pH and ionic strength range of Koops' buffer.

Whipping Properties

The replacement of casein with soyprotein isolate appeared to have very little effect on whipping ability except for a slight decrease in the case of pure soy mixtures, as seen in Figure 5. Whey mixtures also appeared to maintain their whipping ability, except for 0.2% Wpc, 0.6% soyprotein isolate, which has the least amount of protein present and formed the least amount of foam (Figure 6). All of these values appear to be statistically significant (Table 2). In spite if their fairly stable whipping ability, the whipping stability of the casein-soy mixtures was greatly affected by the introduction of soya protein. Even though the same amount of foam was formed, the foam stability decreased with increasing concentration of soya isolate, as can be seen in Figure 7. Table 2 shows this is also statistically sound. The Wpc and soyprotein isolate mixtures were so unstable that drip could not be measured, and they could not be evaluated statistically. Table 3 shows the amount of time it took for the foam to completely collapse. The pure soyprotein isolate mixtures were stable enough to measure drip, showing increasing foam stability with increasing soyprotein isolate replacement of this low level of protein.

According to Morr et al. (1973) nonfat dried milk, with casein as its main protein, appears superior to whey proteins for formulating a whipped topping mix. Tamsa et al. (1969)

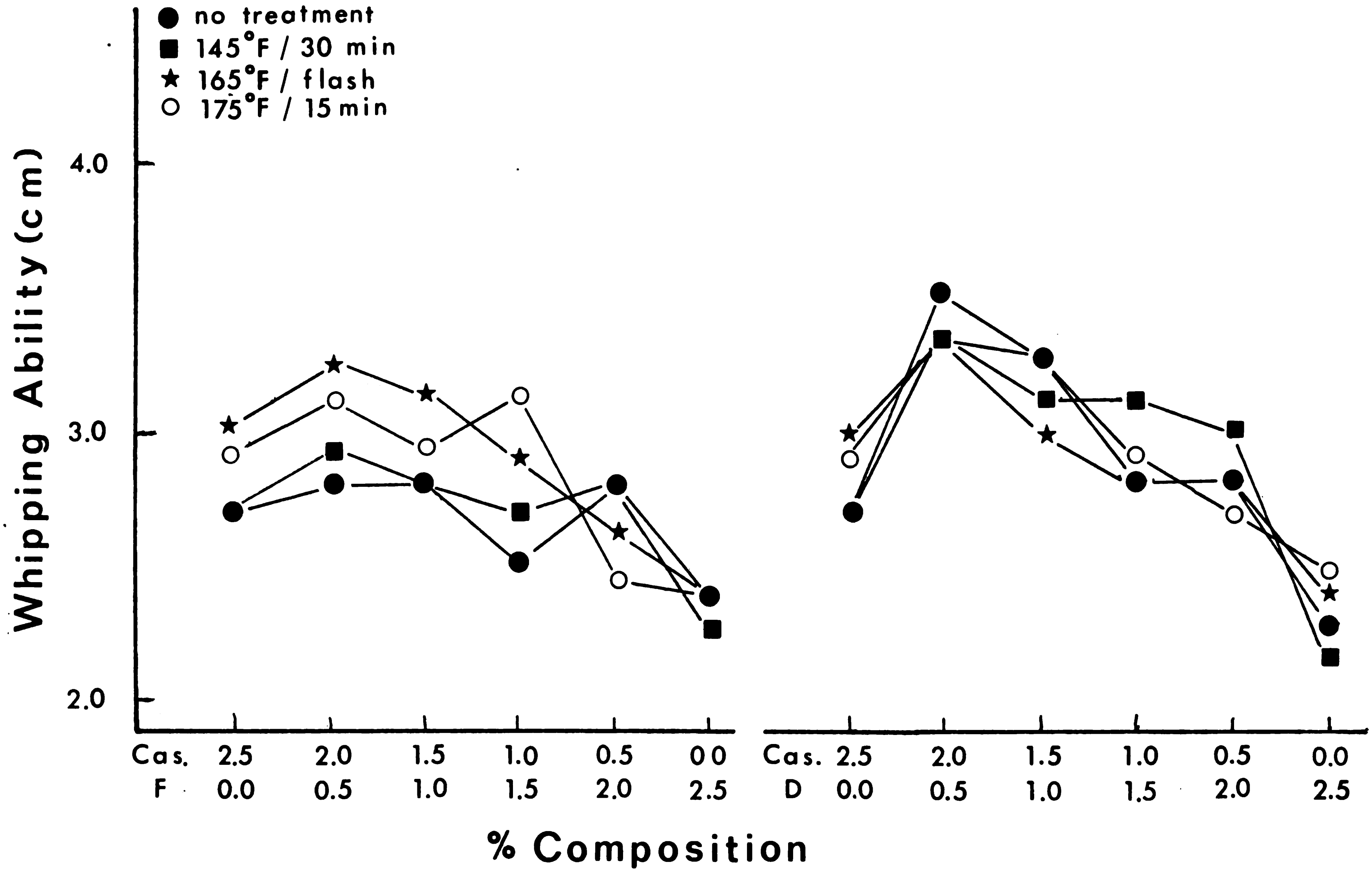

Figure 5. The whipping ability of Promine-F and Promine-D replacements of casein in Koops' buffer.

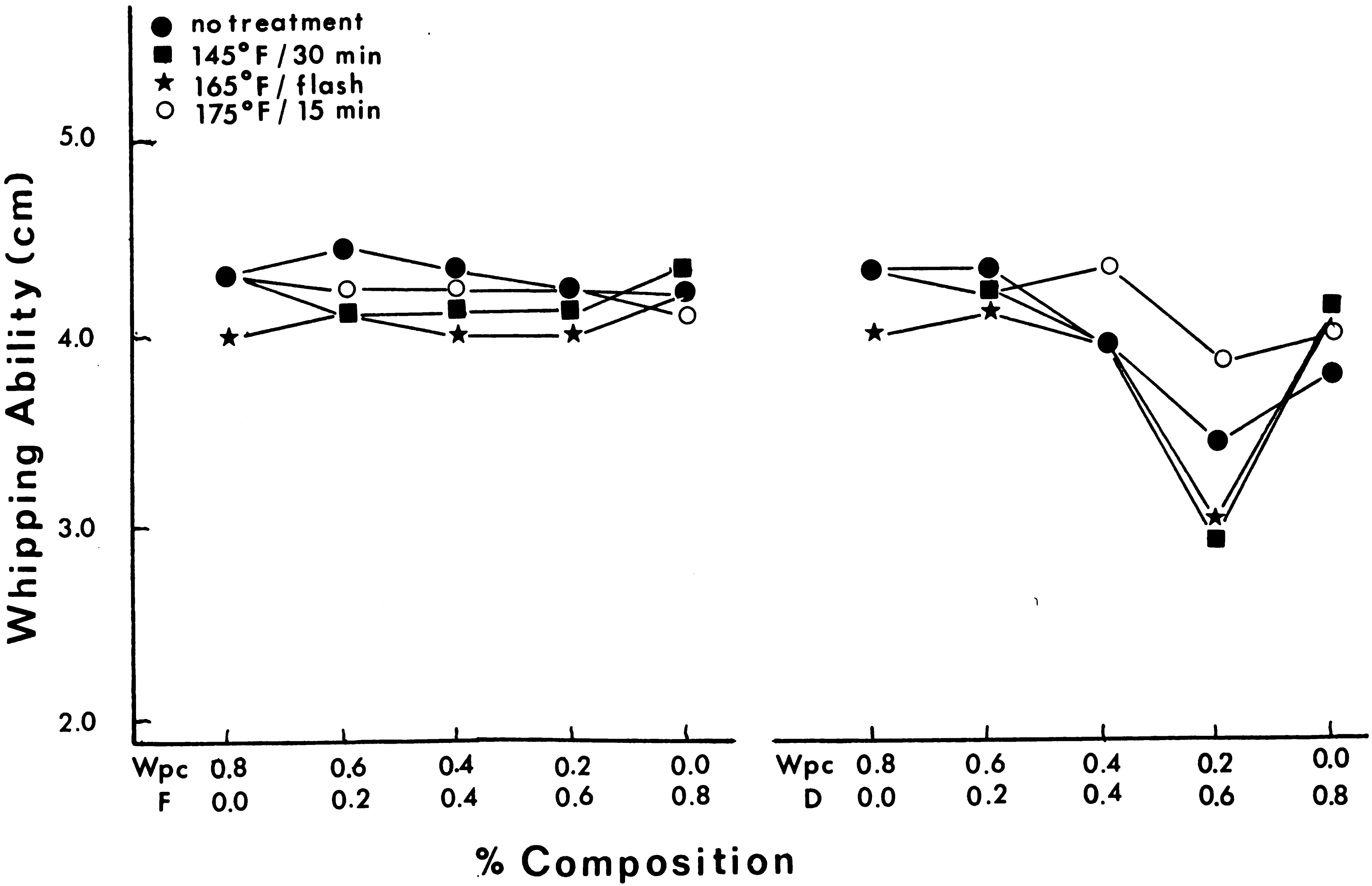

Figure 6. The whipping ability of Promine-F and Promine-D replacements of Wpc in Koops' buffer.

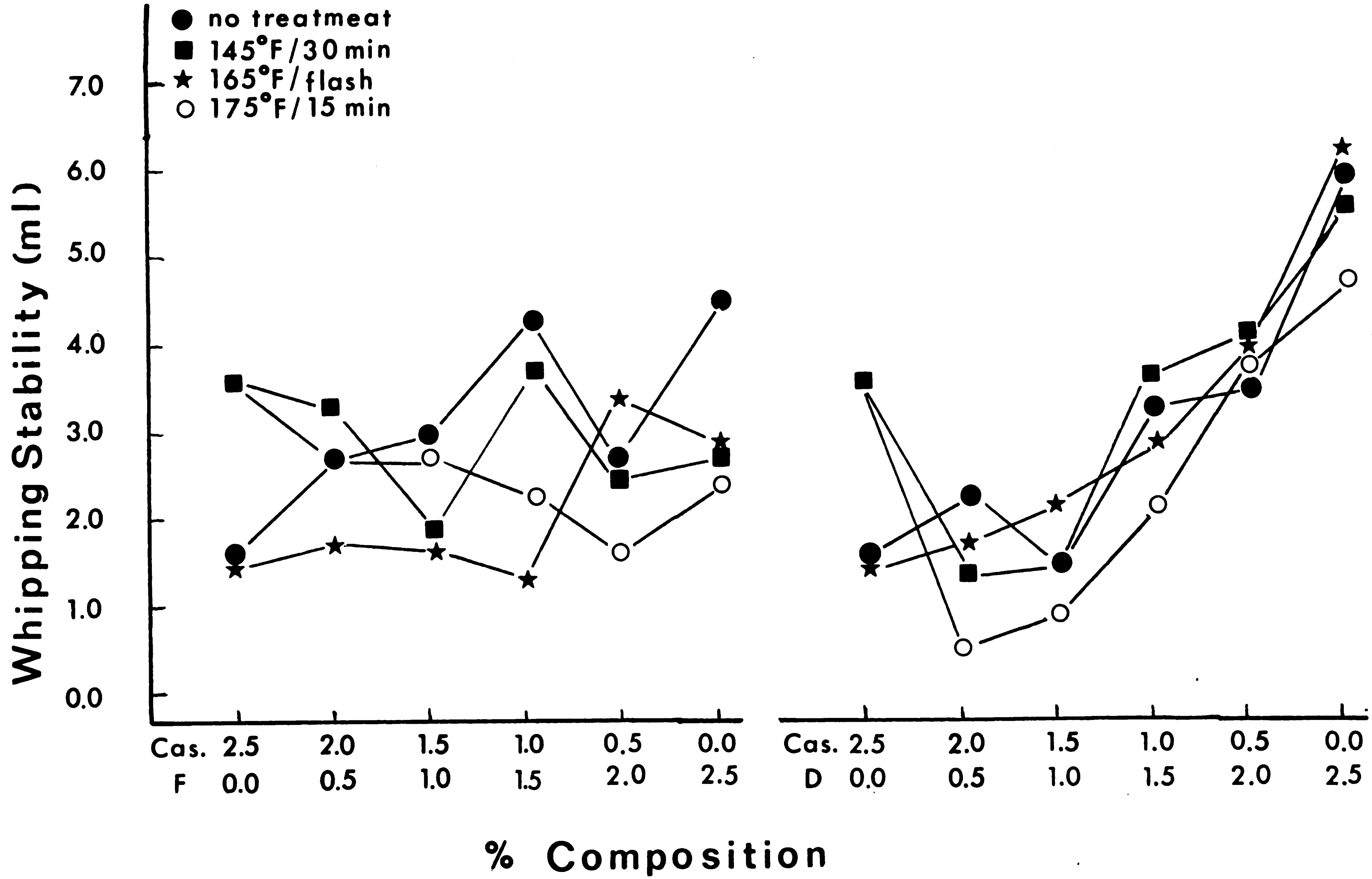

Figure 7. The whipping stability of Promine-F and Promine-D replacements of casein in Koops' buffer.

Table 3. The whipping stability of Promine-F and Promine-D replacements of Wpc in Koops' buffer.

Percent Composition (g/100 ml Koops')		Heat Treatment	Whipping Stability (min. or ml)	(min. or ml)
Wpc	Soybean isolate		Promine-F	Promine-D
0.8	0.0	none	5	3
0.6	0.2		5	3-4
0.4	0.4		5	3-4
0.2	0.6		5	3-4
0.0	0.8		5.2	9.3
0.8	0.0	145°F/30min.	5-10	5-10
0.6	0.2		5-10	10-15
0.4	0.4		15-20	10-15
0.2	0.6		10.7	35-40
0.0	0.8		6.4	6.9
0.8	0.0	165°F/flash	15-20	15-20
0.6	0.2		5-10	5-10
0.4	0.4		10-15	50-55
0.2	0.6		12.9	60-65
0.0	0.8		6.5	10.1
0.8	0.0	175°F/15min.	5-10	5-10
0.6	0.2		10-15	15-20
0.4	0.4		40-45	30-35
0.2	0.6		12.7	65-70
0.0	0.8		5.3	7.0

also agreed that nonfat dried milk can be whipped into a stable foam. Hansen and Black (1972) found that whey protein whipped best when mixed with nonfat dried milk. However, the lower cost of whey proteins and soyprotein isolate has led toward their use in food products, but in mixtures with a much higher percent than tested here. Eldridge et al. (1963) found that soybean protein can form stable whips both above and below the isoelectric point. Both heating and increasing protein concentration enhance foam stability. Yasumatsu et al. (1972 b) found that soy proteins exhibit poor foam stability in spite of their high foam expansion because of their denaturation during processing. Central Soya Chemurgy Division (b) suggests Promine-F has excellent overrun qualities and good stability comparable to sodium caseinate, but this was shown not to be the case at a level of 2.5%.

Apparently the amount of Wpc used was much too low for good whipping properties. Kuehler and Stine (1974) found good whipping ability and stability on 4% Wpc sols. Morr et al. (1973) made toppings with 27% total solids. Richert et al. (1974) also used 4% protein solutions. They found that severe heat treatment required longer whipping times and produced lower overrun foams with highly variable stability. Whey Utilization Conference Proceedings (1970) also recommended 38% whey solids to produce 200 to 300% Overrun.

Emulsification

The emulsifying capacity of casein-soy protein mixtures also decreases as soy protein increases in the mixture. As seen in Figure 8, there is an increase in emulsion ability for Promine-F which maximizes at 1.5% casein, 1.0% soy-protein isolate, and then it decreases sharply. However, from the analysis of variance of the data, Table 2, an extremely wide variance is found among the data, and their validity is in doubt. It was extremely difficult to control the amount of undissolved material that went into the al-iqout being tested, and more of this material would result in a higher amount of oil being used. The emulsions used were also extremely thick, and it was difficult to judge exactly when complete emulsification was achieved. The data for Promine-D and the Wpc mixtures are much more reliable. The ability of Wpc mixtures to emulsify decreased significantly as soyprotein increased. An additional test with coconut oil, which is a saturated fat, was tried with casein and Wpc mixtures with Promine-F with no heat treatment. Figure 10 shows a significant decrease in the amount of oil emulsified, but it is difficult to determine if this is due to the saturation of the fat or to the necessity to use this oil at 92^{o}F to keep it melted, and its consequent hardening upon mixing with the room temperature sample.

Casein is not a widely used emulsifying agent. Both

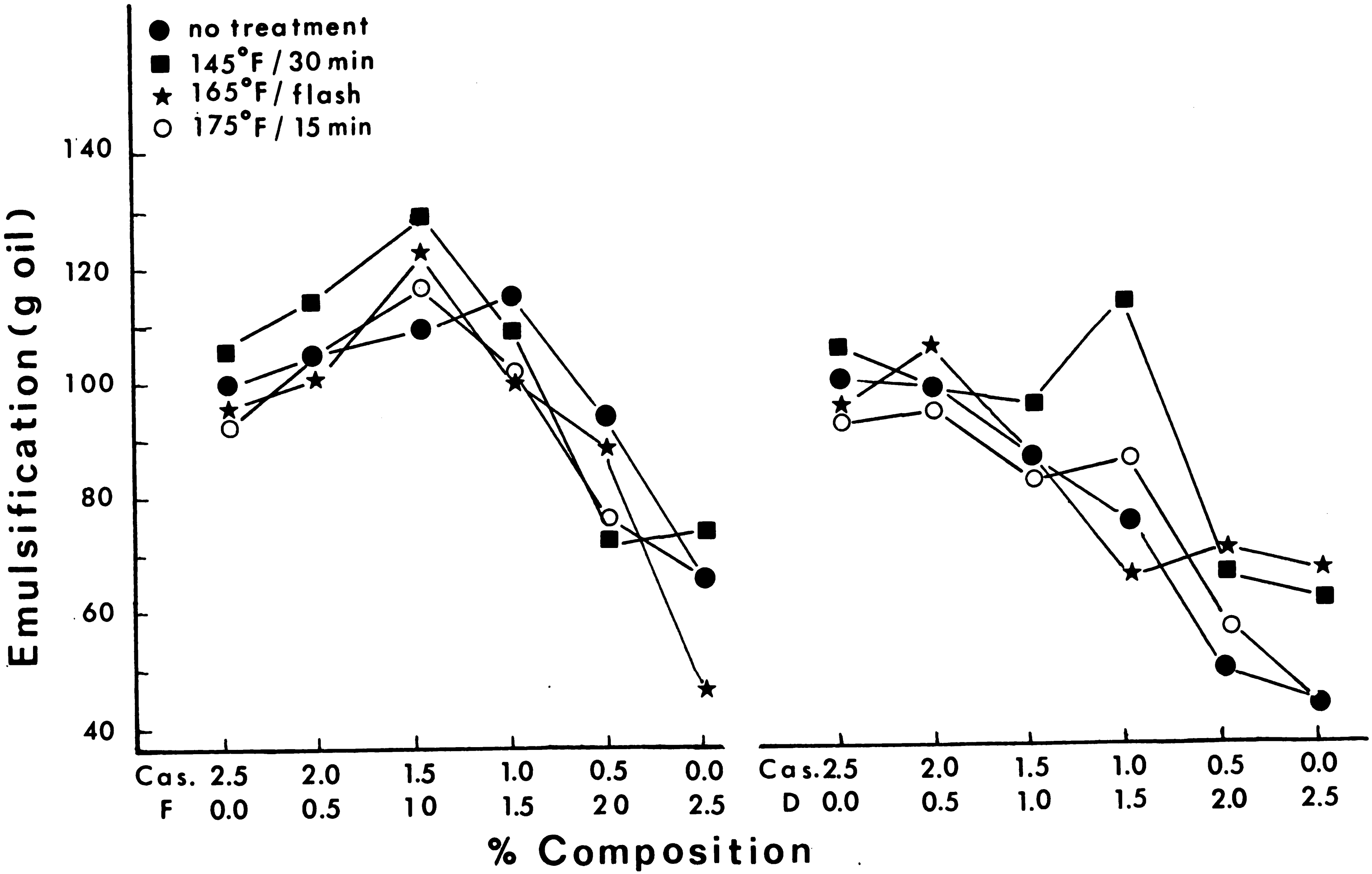

Figure 8. The emulsification of Promine-F and Promine-D replacements of casein in Koops' buffer.

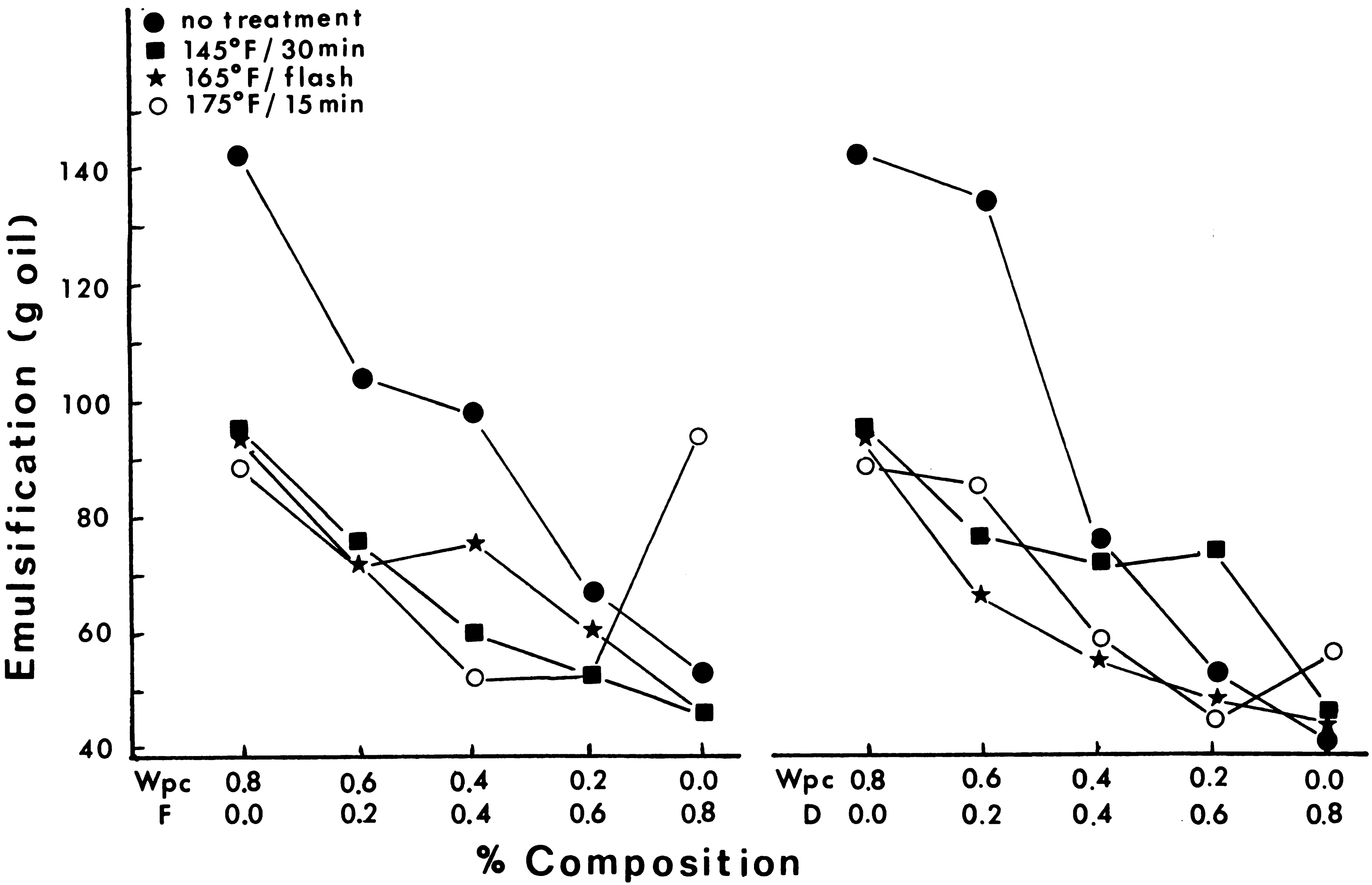

Figure 9. The emulsification of Promine-F and Promine-D replacements of Wpc in Koops' buffer.

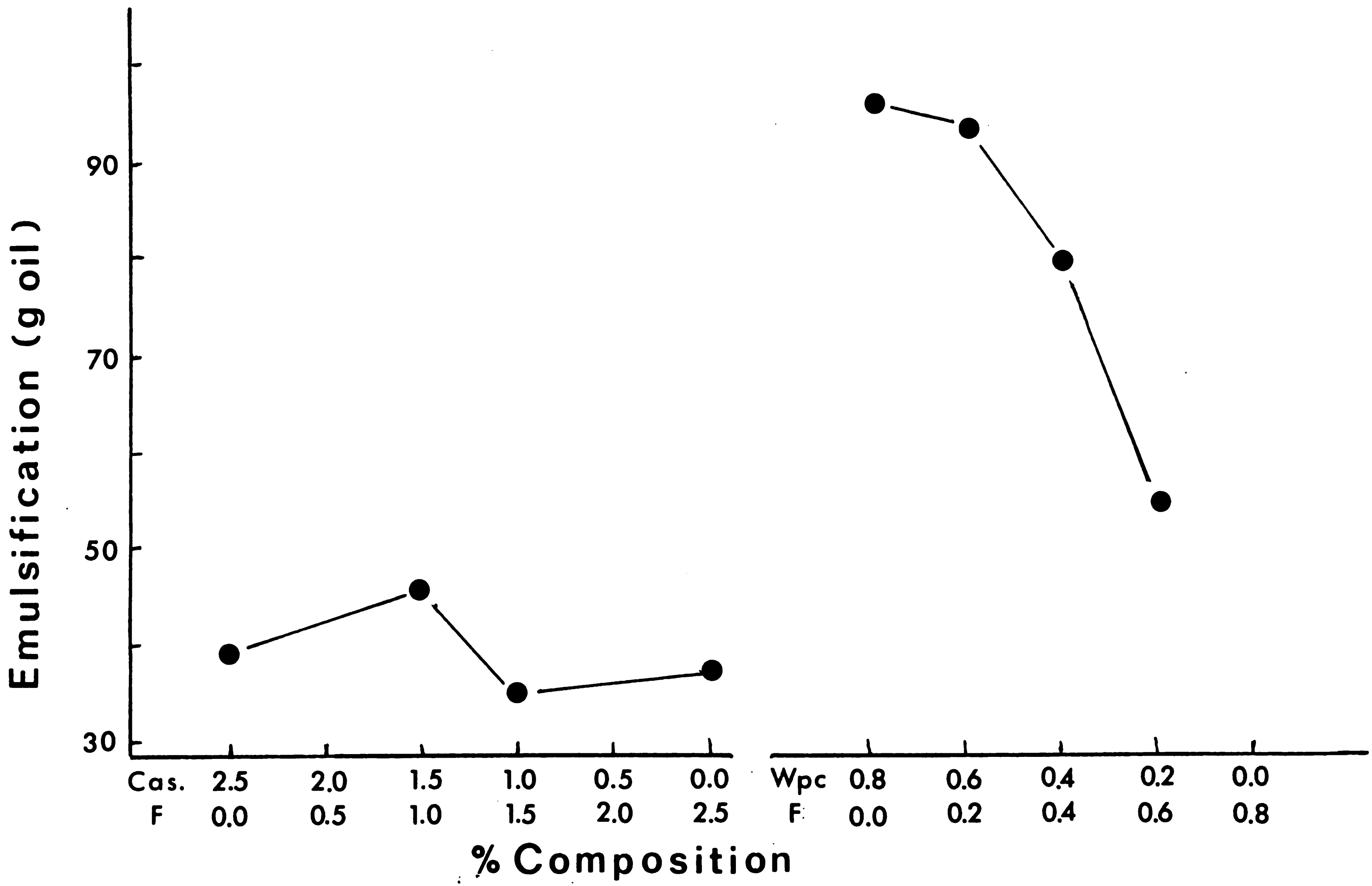

Figure 10. The emulsification of Promine-F replacements of casein and Wpc with coconut oil with no heat treatment.

pure casein and nonfat dried milk show a lower emulsifying capacity than whey protein (Kuehler and Stine, 1974). This would be seen of casein and whey had been teated at the same concentrations. However, the replacement of both with soy-protein isolate resulted in thinner emulsions. Central Soya Chemurgy Division (a and b) recommends both Promine-F and Promine-D as emulsifying agents, but it appears that a mixture of casein and these isolates would form a better product.

SUMMARY AND CONCLUSION

1. Sensory evaluation revealed a significant change in flavor as casein sols were replaced with increasing amounts of soyprotein isolate. The color changed from opaque white to a cream white with noticeable aggregation. The odor changed from gluey to beany. No color change was evident on replacing Wpc with soyprotein isolate, but aggregation was apparent. The odor changed from cooked milk to beany. No new color or odor, signifying protein interactions, appeared.

2. Viscosity decreased with replacement of casein by soyprotein isolate, and increased slightly with replacement of Wpc. No significant deviation appeared to indicate interaction of proteins.

3. The amount of soluble protein present greatly decreased as soyprotein isolate replaced casein, but remained fairly constant as Wpc was replaced. An almost linear relationship indicated no protein interaction.

4. Whipping ability appeared to change very little with either replacement. Whipping stability decreased greatly for soyprotein isolate replacement of casein, but improved for replacement of Wpc. No protein interactions were apparent.

5. Emulsification ability of both casein and Wpc

decreased with increasing replacement by soyprotein isolate. The increase noted in Promine-F and soyprotein isolate is apparently due more to difficulty in procedure than any protein interactions.

The apparent lack of interaction between the casein, Wpc, and soyprotein isolates suggests that these proteins are compatible in a fluid system. However, such problems as color, odor, flavor, viscosity, etc. will have to be overcome before their use as a milk-type product could gain consumer acceptance.

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