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Functional and cake-baking properties of egg white, bovine blood plasma and their protein fractions

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Functional and cake-baking properties of egg white, bovine blood plasma and their protein fractions

by

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This dissertation is lovingly dedicated to my parents, husband, son and children to be.

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1. GENERAL INTRODUCTION

Egg whites are essential ingredients for obtaining desirable volume and texture in many baked products. However, egg whites are also one of the most expensive ingredients that the baking industry uses. Finding low-cost substitutes for egg proteins has been the subject of much interest over the past fifteen years and bovine blood plasma is a likely candidate. Bovine blood is a by-product of the animal slaughtering industry and is produced in vast amounts. For example, bovine blood production in the United States is in excess of approximately 1,000,000 liters daily (Jobling 1986, Johnson 1988) and, thus, is readily available as a raw material. In addition, when collected under sanitary conditions from USDA inspected animals, bovine blood is acceptable for human consumption, and the plasma fraction of blood is currently available from commercial sources in food-grade form. Thus, with such a vast and relatively inexpensive supply of protein, bovine blood plasma is a potential source of proteins to replace egg whites.

Bovine blood plasma has been evaluated as an egg white replacer in cakes, but it was found that full replacement did not produce the same volume, texture and profile as egg white (Brooks and Ratcliff 1959, Johnson et al 1979, Khan et al 1979, Lee et al 1991). However, partial replacement with blood plasma did produce acceptable cakes; thus, there is potential for further improvement. To further extend the use of bovine plasma as an egg replacement, additional research is necessary to investigate and compare the functional properties of both proteins.

The most important functional property of egg whites in the production of cakes is the ability of proteins to solubilize quickly in water and to heat coagulate during baking (Shepherd and Yoel 1976). In addition, foaming and emulsification properties of egg proteins contribute to expansion of cake batters during baking and to stabilizing oil-in-water emulsions (Baldwin 1977). The above properties of egg white and its component proteins are relatively well understood, but little is known about the functional properties of blood plasma and its component proteins.

This dissertation addresses characterizing and comparing the functional and baking properties of blood plasma and egg whites. Since both egg white and plasma have components with unique chemical and physical properties, they were individually examined as well to determine whether certain proteins are superior or inferior to others. Prior to evaluating the baking properties of small (but expensive) quantities of proteins, a micro method for cake-baking was developed using a high-ratio white layer cake formulation.

Dissertation Organization

This dissertation contains three papers. The first paper, which will be submitted to the Journal of Food Science, examines the heat denaturation, foaming, and emulsification properties of bovine blood plasma, egg whites and their protein fractions. The second paper reports on developing a micro method for cake-baking to evaluate baking properties of small quantities of protein fractions. The third paper describes the cake-baking properties of egg whites and blood

plasma proteins in a high-ratio white layer cake formulation and the relationships between the functional properties of the proteins in paper I and cake quality. The second and third papers will be submitted to the Cereal Chemistry journal. The three papers are preceded by a General Introduction and a Literature Review, and followed by a General Conclusion.

2. LITERATURE REVIEW

Blood Composition

Whole blood contains 17-19% protein (Jobling 1986), 0.090-0.012% carbohydrate, and 0.4-0.9% lipid (Oser 1965). The carbohydrate fraction is primarily glucose with small quantities of other reducing sugars. Fatty acids (0.25-0.39%), triglycerides (0.03-0.14%) and cholesterol (0.15-0.25%) are the major whole blood lipid fractions (Oser 1965).

Blood is a suspension of red cells (erythrocytes), white cells (leukocytes) and platelets in plasma. The plasma accounts for 65-67% of total blood weight and contains 8% protein while the cellular element comprises the remaining 33-35% (Gordon 1971, Morrissey 1991). The red cells are the major cellular component and contain 65-75% of the total proteins in the blood. Hemoglobin is the major red cell protein, constitutes 35% of the red cell weight (Oser 1965), and gives blood its characteristic color. Hemoglobin is a conjugated protein containing a colorless protein moiety (globin) and a reddish prosthetic group, the heme pigment (Oser 1965).

Processing Blood Plasma for Food Applications

Blood is collected directly from the stunned animal to the container through a completely closed system which prevents external contamination. On collection, blood is quickly mixed with an anticoagulant (citric acid or sodium citrate), then

chilled and held until the animals pass U. S. Department of Agriculture inspection. To separate red blood cells from plasma, the collected blood is centrifuged at 14,000 rpm on a continuous basis or 58,000 rpm for 10 min on a non-continuous basis (Knipe 1988).

All equipment which comes into contact with blood throughout the collection and separation of fractions are previously wetted with an isotonic solution, as well as, an anticoagulant. An aqueous solution of 0.85% NaCl creates an osmotic pressure outside red cells which is equal to that inside (Howell and Lawrie 1983). If the osmotic pressure is not equalized, the red cells will burst, releasing hemoglobin which will color the plasma red. Hemoglobin forms a true solution in plasma, and it cannot be separated by centrifugation. This is undesirable since red colored plasma will produce much darker colored products after dehydration and restricts the end usage of the product (Halliday 1973). Also, hemoglobin contains iron which promotes oxidation of lipids in plasma and results in an undesirable fishy flavor (Knipe 1988).

The shelf-life of liquid plasma is relatively short; therefore, plasma must be quickly processed into either frozen form and stored at -25°C (Halliday 1973, Akers 1973) or, most commonly, spray-dried into a free-flowing powder (Brooks and Ratcliff 1959, Delaney 1975). Prior to spray drying, the plasma is often concentrated to 21% solids content by evaporating under vacuum at low temperatures to increase the efficiency of the drier (Halliday 1973). Brooks and Ratcliff (1959) reported that an inlet air temperature of 120°C and an outlet air

temperature of 65°C gave powders with moisture contents of 8-9%, and higher drying temperatures caused insolubility and browning. Later, Knipe (1988) stated that a drying temperature lower than 80°C is necessary to maintain functional properties of blood proteins. A work of Matzinos and Hall (1993), showed that bovine plasma dried at an outlet air temperature as high as 90°C was still completely soluble.

Utilization of Blood

Food uses

Blood proteins contain all of the amino acids that are present in both whole milk and eggs. All of the essential amino acids are present at levels greater than those specified by the Food and Agriculture Organization of the United Nations with the exception of isoleucine and methionine, which are the growth-limiting amino acids (Tybor et al 1975).

Blood has been and continues to be a source of protein in many cultures. During World War I, blood was used in Germany for protein fortification of bread (Kobert 1915). Fresh blood from living animals is consumed by some African natives as a regular part of their diet (Pals 1970). Several European and Asian countries also consume blood as puddings and sausage products (Bates et al 1974). Blood is also utilized in sausages, canned meat, and vegetable products in countries of the former USSR (Gorbatov 1988).

Human consumption of the plasma fraction of blood is well accepted in

Canada, the Pacific Rim, and Western European countries (Johnson 1988). Plasma is added to meat products, such as sausages, frankfurters, beefburgers, extended ham, turkey breast and turkey franks, to increase protein levels and to increase fat- and water-holding capacities (Knipe 1988).

Bread-baking properties of blood plasma protein isolate (PPI) were studied by Khan et al (1979). Loaf volume of bread made with 2-6% PPI was significantly higher than that of the control bread. However, increasing levels of PPI darkened crust and crumb colors and made the texture coarse and open. When 2% wheat flour was replaced with PPI, the bread protein and lysine content increased by 15 and 75%, respectively.

Other commercial uses

The major uses for animal blood are as animal feed, pet foods, and fertilizer (Bates et al 1974, Johnson 1988). Blood or blood derivatives are also used in various pharmaceutical applications (Gorbatov 1988) such as tissue and microbiological culture media, RH factor determination, and immunological preparations. Another use of blood is as a binding agent in waterproof glue, in radiator sealing compounds, and in plywood adhesives (Hirchberg, 1957).

Blood Plasma Proteins

Blood plasma contains a variety of proteins having different functions and structures. The major plasma proteins are classified as serum albumin, globulins,

and fibrinogen. Figure 1 displays the distribution of the different plasma proteins in the electrophoretic fractions of serum as obtained by moving-boundary electrophoresis. Some of the properties of blood plasma proteins are also given in Table I.

Serum albumin is the predominant protein in the plasma fraction and accounts for approximately 50-60% by weight of the total plasma proteins. Its concentration in plasma is in the range of 35 to 50 g/L (Putnam 1984). Albumin is acidic and highly soluble in water (30%, w/v) at pH 7, and can resist heating at 60°C for 10 hr in the presence of stabilizers such as caprylic acid (Peters 1985). Some of the physico-chemical properties of human albumin are listed in Table I. Serum albumin consists of a single polypeptide chain containing 17 disulfide bridges. These disulfide bridges organize the molecule into three similar domains, and each domain has a site for certain physiological ligands. For example, long chain fatty acids bind to a site in the third domain (Putnam 1984). These fatty acids are difficult to remove and play an important role in modifying the physical behavior of plasma albumin (Peters 1985).

Globulins are classified as α, β, and γ (immunoglobulins) globulins, and their concentrations in plasma are in the range of 6.8-16.2, 4.6-8.5 and 9.6-25.1 g/L, respectively (Putnam 1984). The physical, chemical, and biological properties of the globulins have been previously reviewed by Putnam (1984). α-Globulins contain about 20 glycoproteins, and the amount of carbohydrate changes from protein to protein and may reach up to 40% or more by weight (Putnam 1984).

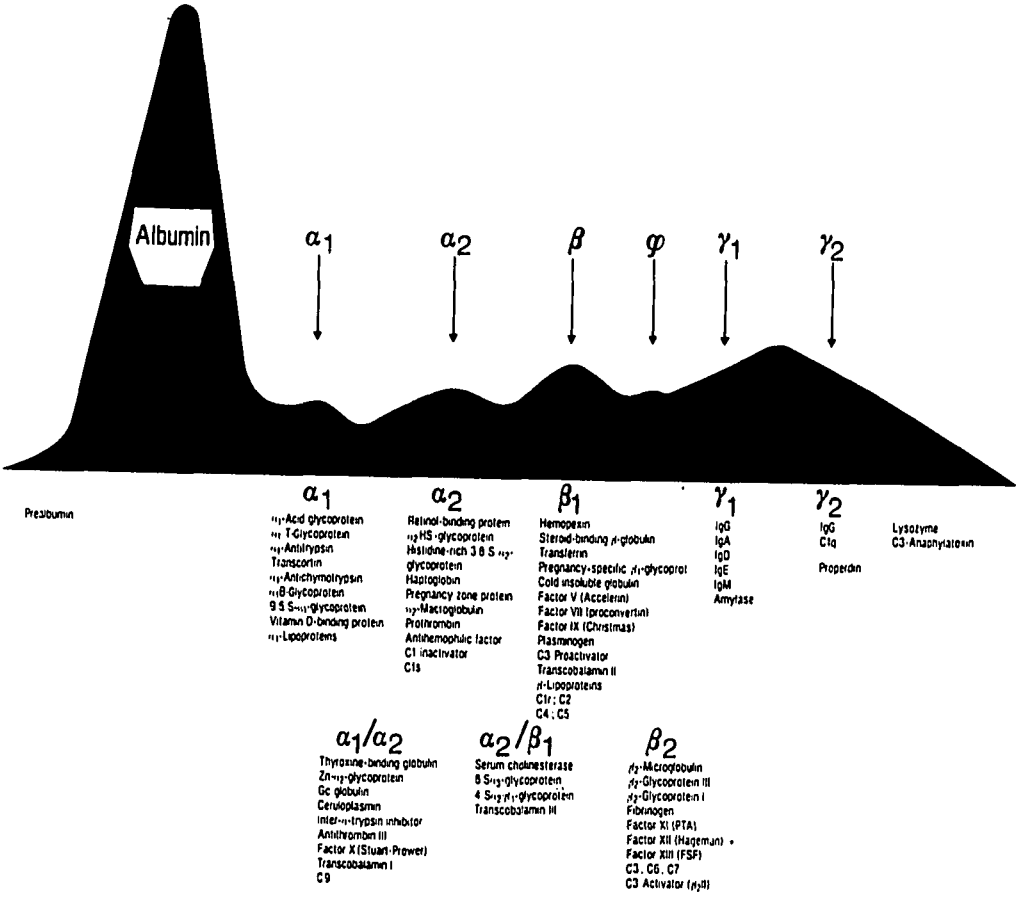

Fig. 1. Plasma proteins in electrophoretic fractions; φ refers to fibrinogen (Heide et al 1977)

TABLE I

Major Proteins in Human Blood Plasma[a]

Protein	Molecular weight	Concentration (mg/100 mL)	pI[b]	Carbohy-- drate content (%)
Albumin	66,500	3500-5000	4.7	0.0
α-Globulins		950-2171		
α_1-Acid glycoprotein	40,000	55-140	2.7	42.0
α_1-Antitrypsin	54,000	200-400	4.8	13.0
Ceruloplasmin	132,000	15-60	4.4	7.0
α_1-Antichymotrypsin	68,000	30-60	-	26.0
α_2-Macroglobulin	725,000	150-420	5.4	9.4
α_2-HS Glycoprotein	49,000	40-85	4.2	14.0
Haptoglobin	86,000	100-220	4.1	16.4
β-Globulins		461-849		
Haemopexin	60,000	50-115	-	22.0
Transferrin	79,500	200-320	5.5	5.9
Complement Component-C3	185,000	55-120	-	2.0
Immunoglobulins		964-2514		
Immunoglobulin G	150,000	800-1800	5.8-7.3	3.0
Immunoglobulin A	~ (160,000)n	90-450	-	8.0
Immunoglobulin M	950,000	60-250	-	10.0
Fibrinogen	340,000	200-450	5.5	4.0
α-chain	66,115	-	-	-
β-chain	52,314	-	-	-
γ-chain	46,468	-	-	-

[a]Taken from Putnam (1975 and 1984).
[b]Isoelectric point.

Transferrin comprises much of the β-globulin in plasma and is responsible for most of the iron bound in plasma (Putnam 1984). Immunoglobulins are heterogeneous in physical properties and chemical structure, and contains antibodies. Immunoglobulins are classified into IgG, IgA, IgM, IgD, and IgE. IgG, IgA, and IgM are the major classes, and IgG accounts for 72-83% (w/w) of the immunoglobulin concentration.

Fibrinogen is a relatively insoluble protein and occurs in the plasma at concentrations in the range of 2-4.5 g/L. It is a hexamer containing two sets of three nonidentical disulfide-bonded chains having a covalent molecular weight of 340,000.

Egg White Proteins

Egg white consists of 88.9% water, 9.7-10.6% protein, 0.03% lipid, 0.5-0.6% ash, and 0.4-0.9% carbohydrate. About 0.4% of the carbohydrate of egg white is in the free form, and 98% of the free form is glucose.

The major proteins of egg white are classified as ovalbumin, conalbumin, ovomucoid, lysozyme, and globulins. The relative amounts of various proteins in egg white and some of their properties are shown in Table II.

Functional Properties of Egg White and Blood Plasma Proteins

Heat denaturation

Denaturation involves any modification in secondary, tertiary, or quaternary conformation of a protein. The strength of the interactions or linkages that stabilize the

TABLE II

Proteins in Egg Albumen[a]

Proteins	Albumen (%,dry basis)	pI[b]	Molecular weight	HΦ[c] (cal/res)	T_d[d] (°C)
Ovalbumin	54	4.5	44, 500	1110	84.0
Conalbumin	12	6.1	76,000	1080	61.0
Ovomucoid	11	4.1	28,000	920	70.0
Ovomucin	3.5	4.5-5.0	$5.5\text{-}8.3 \times 10^6$	-	-
Lysozyme	3.4	10.7	14,300	970	75.0
G_2 Globulin	4.0	5.5	$3.0\text{-}4.5 \times 10^4$	-	92.5
G_3 Globulin	4.0	4.8	-	-	-
Ovoinhibitor	1.5	5.1	49,000	-	-
Ficin inhibitor	0.05	5.1	12,700	-	-
Ovoglycoprotein	1.0	3.9	24,400	-	-
Ovoflavoprotein	0.8	4.0	32,000	-	-
Ovomacroglobulin	0.5	4.5	$7.6\text{-}9.0 \times 10^5$	-	-
Avidin	0.05	10.0	68,300	1060	-

[a]Taken from (Powrie and Nakai 1985) .
[b]Isoelectric point.
[c]Average hydrophobicity.
[d]Denaturation temperature.

protein's structure determines its sensitivity to denaturation. Since structures vary from one protein to another, the effects of denaturing agents vary depending on the protein (Cheftel et al 1985). Besides the nature of the protein itself, the stability of the protein to heat denaturation depends on many other factors such as protein

concentration, water activity, pH, ionic strength, and the kind of ions present.

Differential scanning calorimetry (DSC) is a direct technique for studying thermally-induced transitions. In this technique, a sample holder and a reference holder are maintained at the same temperature while the temperature of both are increased at a programmed linear rate. The continuous and automatic adjustment of heater power (energy per unit time) necessary to keep the sample holder temperature identical to that of the reference holder provides a varying electrical signal depending on the thermal behavior of the sample. Any thermally induced changes occurring in the sample are then recorded as a differential heat flow, normally displayed as a peak on the thermogram.

The denaturation temperature of proteins is most commonly defined as the peak maximum temperature. Unfortunately, this temperature suffers from the disadvantage of being concentration dependent (Wright 1982). It is however, in the case of overlapping transitions, the only accessible temperature. Peak onset temperature (the intersection of the steepest slope and baseline) is the preferred temperature because it is independent of protein concentration (Wright 1982).

Applications of DSC in food research were reviewed by Wright (1982), Biliaderis (1983) and Lund (1983). Of particular relevance to this thesis, is the application of DSC to the study of protein behavior in foods. DSC studies of protein can be listed as: protein denaturation; denaturation kinetics; protein interactions with protein, water, carbohydrates, and other miscellaneous protein interactions; finger

printing and compositional analysis; protein quality and functionality; processes and processing effects.

Egg white proteins. Donovan et al (1975) studied the heat denaturation of egg white and its component proteins by DSC (Table III). In this study, egg white at pH 7 gave major endotherms at 65 and 84.5°C, produced by the denaturation of conalbumin and ovalbumin, respectively. The stability of conalbumin to heat denaturation in egg white increased by 4.5°C when pH was increased from 7 to 9. However, this increase was only 1°C when isolated conalbumin was utilized. Heg et al (1978) found that the denaturation temperature (T_d) of conalbumin was independent of pH in the range of 5 to 10, while T_d decreased below pH 5. In this study, T_d was also found to be independent of NaCl concentrations at all pH values above 4. Addition of aluminium ion increased the heat stability of conalbumin by 12°C (Donovan et al 1975). Lysozyme was more stable to heat denaturation at pH 7 than at pH 9.

Ovalbumin maintained its conformational stability in the pH range of 6 to 9, and below pH 6 the denaturation temperature of ovalbumin steadily decreased (Hegg et al 1979). Later, the work of Arntfield et al (1989) showed that the denaturation temperature of ovalbumin was not significantly different between pH 5 to 9. When the calorimetric experiments were carried out in the presence of NaCl (0.1-0.4 M), only slight increases in the denaturation temperatures of ovalbumin were observed which implies that NaCl has little influence on the maintenance of

TABLE III

Denaturation Temperatures of Egg White Proteins[a]

	Denaturation Temperature[b]			
	In Egg White		Isolated[c]	
Protein	pH 7	pH 9	pH 7	pH 9
Ovalbumin	84.5	84.0	84.0	84.0
Lysozyme	74.0	C[d]	75.0	72.5
Conalbumin	65.0	69.5	61.0[e]	62.0[e]
Ovomucoid	C	C	79.0[f]	77.0[f]
Globulins	C	C	92.5	ND[g]

[a]Adapted from Donovan et al (1975).
[b]Defined as peak in endotherm at 10 °C/min heating rate.
[c]In water, except where noted.
[d]Concealed by denaturation of conalbumin or ovalbumin in egg white.
[e]2 M tris buffer at pH 7.10 and pH 8.68.
[f]0.1 M phosphate buffer, pH 6.8; 0.1 M TAPS buffer (3-{tris (hydroxymethyl)methyl]amino} propanesulfonic acid), pH 9.0.
[g]Not determined.

conformational stability of ovalbumin (Arntfield 1989). However, even at a concentration as low as 17 mM, $CaCl_2$ destabilized protein structure and decreased ovalbumin thermal stability by 2-3°C at all pH values above 5.0 (Hegg et al 1979). Addition of 10% sucrose increased the denaturation temperatures of major egg white proteins by 2°C, presumably a suppressive effect of this solute on water activity (Donovan et al 1975). The irreversible transformation of ovalbumin into its more heat-stable form, *S*-ovalbumin, during storage of eggs at elevated

temperatures was determined by Donovan and Mapes (1976). The denaturation temperature of *S*-ovalbumin was 8°C greater than that of ovalbumin.

Blood plasma proteins. DSC has been used to study thermal stabilities of ligand-serum albumin complexes (Gumpen et al 1979). The fatty acids, lauric acid and stearic acid, stabilized the protein molecules and increased the denaturation temperature of serum albumin. A maximum increase in the denaturation temperature of 30°C was obtained with lauric acid. The denaturation peak temperature of ligand-free serum albumin increased from 68 to 83°C with stearic acid and to 92°C with lauric acid. pH variations in 0.9% NaCl affected the heat stabilities of both ligand-free and ligand-rich albumin; the former being more sensitive in alkaline pH. The fatty-acid-free form of the protein was destabilized when the salt concentration was decreased below 0.9% and was significantly stabilized by the addition of more salt, contrary to fatty acid-stabilized protein which was slightly destabilized by the addition of small amounts of salt.

The effects of pH and ionic strength on defatted and SH-blocked bovine serum albumin were studied by Yamasaki et al (1990). They found that bovine serum albumin (BSA) was stabilized in the neutral-alkaline pH range by the presence of NaCl, but was destabilized in the acidic pH range. The enthalpy of denaturation was maximum in the pH range 5.6-7.0. The stabilizing effect of NaCl on BSA was attributed to its neutralizing effect at low concentration on the electrostatic forces in the pH range 5.6-7.0.

Foaming properties

Foams are defined as colloidal dispersions in which gas bubbles are dispersed in a liquid or semi-solid phase that contains a soluble surfactant (Cheftel et al 1985). A surface-active agent is necessary to maintain the interface against coalescence of gas bubbles by lowering the interfacial tension at the gas/liquid interface and forming an elastic protective barrier between gas bubbles. Many foams are unstable, and the main destabilizing mechanisms are: 1) drainage of lamella liquid due to gravity and pressure difference; 2) gas diffusion from small to large bubbles; and 3) rupture of the liquid lamellae separating gas bubbles (Cheftel et al 1985).

Some proteins can form protective films at gas/liquid interfaces. In protein-based foams, two adjacent bubbles consist of two adsorbed protein films separated by a thin liquid layer called "lamellae." The three main foam formation methods that have been used in studies of proteins are (Halling 1981, Cheftei et al 1985): 1) bubbling gas through a porous sparger (such as sintered glass) into an aqueous solution of a low protein concentration (0.01-2% w/v); 2) whipping/beating an aqueous protein solution (1-40% w/v) in the presence of a bulk gas phase; and 3) shaking an aqueous protein solution (foam formation resembles whipping). The later method has been used only rarely because of difficulty in standardizing of the method.

Foaming properties of proteins depend upon the intrinsic physical properties of protein such as molecular size, shape, conformation, flexibility, rigidity,

compactness, surface polarity, charge, hydrophobicity, etc., (Kinsella 1981). These, in turn, are affected by the processing history, and by the physical and chemical environment in which the protein is used. In a protein foaming system, foam formation involves three sequential stages: 1) diffusion of the soluble proteins to the air-water interface and adsorption at the interface; 2) unfolding and rearranging of polypeptides (polar moieties toward the water) at the interface; and 3) interactions between the polypeptides to form a continuous film (Kinsella 1981, Morrisey et al 1991).

The effect of protein conformation on the surface properties of proteins were determined using three well-defined proteins by Graham and Phillips (1975). Interfacial adsorption and foaming properties of β-casein (non-structured flexible random coil), lysozyme (highly ordered, rigid, inflexible, globular), and BSA (less ordered, more flexible, globular) were compared under standard conditions and at several protein concentrations. β-casein rapidly adsorbed at the surface and attained a stable film pressure. It took much longer for albumin to reach a constant film pressure, and lysozyme adsorbed very slowly. The relative foaming abilities (time to reach half maximum volume) of β-casein, albumin, and lysozyme were 4, 12, and over 30 min, respectively.

Molecular flexibility is an important structural factor governing the foaming properties of proteins (Kato et al 1985). Flexible proteins unfold, adsorb, and spread rapidly at the interface facilitating foam formation. When the molecular flexibility of serum albumin or lysozyme is reduced by the introduction of additional

intramolecular cross-linking, there is corresponding losses of foamability and foam stability (Kato et al 1986a). Prior denaturation of globular proteins, such as ovalbumin and lysozyme, by heat without loss of solubility leads to enhanced foamability due to increased flexibility as determined by susceptibility to protease digestion (Kato et al 1986b), and to an increase in surface hydrophobicity and protein-protein interactions (Kato et al 1989). Mitchell (1986) attributed the beneficial effect of heat denaturation on foaming properties of proteins to two factors, increased surface activity on denaturation and stabilization by solid particles.

It has long been recognized that many properties of proteins can be related to the proportion of amino acids having non-polar side chains. Townsend and Nakai (1983) showed that foaming capacity increased with Bigelow's average hydrophobicity (this parameter was evaluated by adding together the side chain hydrophobicity of the individual non-polar amino acid residues and then dividing by the total number of residues in the protein) and the relationship between surface hydrophobicity and foaming properties were much less significant (Kato et al 1983).

The stabilities of foams reflect the rheological properties of interfacial films of proteins. Foam stability requires formation of a thick, cohesive, elastic, viscous, continuous, and air-impermeable protein film around each gas bubble through hydrophobic and possibly hydrogen and electrostatic interactions (Kinsella 1981). The formation of cohesive films around gas bubbles is facilitated by some degree of surface denaturation which encourages protein-protein interaction and enhances

cohesive forces between proteins in the film. However, complete denaturation causes membrane fragility and foam collapse; therefore, it is not desirable.

Besides the intrinsic physical properties of proteins, several other factors, such as protein concentration, pH, solubility, salt, sugars, and lipids, can affect the foaming properties of proteins (Kinsella 1981, Halling 1981). Foam volume and stability usually increase with increasing protein concentration (Kinsella 1981, Halling 1981). Increased stabilities at higher protein concentrations were attributed to the formation of thicker interfacial films (Kinsella 1981) and to the increase in liquid viscosity brought about by protein (Halling 1981). However, Dickinson and Stainsby (1982) stated that there was no correlation between foam stability and film thickness. Halling (1981) also suggested that in solutions which contain more than one protein an important surface role of a minor component may account for the continued increase of foam stability at higher protein concentrations. The dependence of both foam capacity and stability on viscosity was attributed to three factors by Mitchell (1986): a high viscosity will 1) slow down the rate of drainage of lamellae; 2) decrease the rate at which the bubbles move through the solution, thereby increasing the time for protein adsorption and unfolding; and 3) be indicative of a more unfolded protein in solution.

Since pH of the dispersing medium has a direct effect on the net charge and conformation of the protein, it consequently affects foaming properties, especially foam stability. Generally, completely soluble proteins show good foam stability at their pIs due to electrostatic intermolecular attractions at the pI increase the

thickness, elasticity, viscosity, and rigidity of the protein films adsorbed at the air/water interface (Kinsella 1981). Egg white has maximum foam stability at either its natural pH (8-9) or around the pI of its component proteins (4-5) (Halling 1981). Plasma exhibited better foaming properties at pH 4.8, around the pI of its component proteins, than at its natural pH (8-9) (Tybor et al 1975).

The relationships between protein solubility and foaming properties are rather complex. Although some studies show a correlation between protein solubility and foam formation or stability, others do not. These studies are listed in the review of Halling (1981).

Since ions can affect the solubility, viscosity, unfolding, and aggregation of proteins, they can also affect foaming properties. NaCl weakens interpeptide attractions and reduces surface viscosities and rigidities of protein films (Cumper 1953). NaCl usually increases foaming capacity but may reduce film strength that cause decreased foam stability (Kinsella 1981). However, some proteins give better foams with finer bubbles as ionic strength increases (Cumper 1953). Sucrose and other sugars increase viscosity and, therefore, improve foam stability. Foam stability of egg albumen was related to its glyco-residues (Kinsella 1981). Presumably the glyco-residues of egg albumin are exposed to the lamellar water and increase the viscosity of this water thereby retarding drainage. Foaming properties of proteins can be significantly reduced by the presence of polar lipids (e.g., phospholipids) which compete for the air-water interface and disrupt the

interfacial film by causing reorientation of the hydrophobic surface of protein (Kinsella 1981, Halling 1981).

Egg white proteins. Egg white is composed of different protein components and each of these components play a significant role in its excellent foaming properties. Globulins produce fine bubbles and contribute high foaming capacity. Ovomucin, ovomucoid, and globulins increase viscosity and, therefore, contribute foam stability. Lysozyme complexes with other proteins, especially ovomucin and increases film strength. Ovalbumin and conalbumin denature upon heating and convert foam from a liquid to a solid foam during baking.

MacDonnell et al (1955) fractionated egg white into its major protein components and demonstrated the importance of each of the major proteins in angel food cake preparation. Removal of globulins and ovomucin increased whip time and decreased cake volume. Replacement of globulins alone recovered cake volume but replacement of ovomucin alone did not recover cake volume. Globulins, ovomucin, and ovalbumin together produced angel food cakes equal in volume to the egg white. Ovalbumin alone produced coarse-textured cakes. It was concluded that globulins are extremely good foamers and important for a good textured cake while ovomucin stabilizes foam because of its very rapid insolubilization at the surface of the bubble. However, they indicated that presence of ovalbumin was necessary to set the structure of the baked cake due to its heat denaturation property. Nakamura and Sato (1964) stated that ovomucin increases

the viscosity of egg white and, thus, stabilizes foam. Johnson and Zabik (1981) investigated the foaming ability of egg white proteins. Globulins had good foaming properties and produced angel food cake with excellent texture and volume. As reported previously, ovalbumin produced coarse cake. Ovomucin, lysozyme, ovomucoid, and conalbumin showed little or no foaming capacity. High levels of globulins in egg white reduced cake volume due to its excessive insolubilization at the air-albumen interface. They also found that an egg white protein solution with high ovomucin content, but without lysozyme, gave excellent foaming, but produced smaller cakes; whereas with lysozyme present the foam capacity was greatly reduced but the cake volume was considerably increased. Previously, MacDonnell et al (1955) also showed the detrimental effect of added amounts of ovomucin in egg white on cake volume. They found that excessive insolubilization of ovomucin at the bubble surface decreased film elasticity and prevented cake expansion during baking. However, Johnson and Zabik (1981) have expressed the view that it was the lower heat coagulative properties of the film surrounding the air cells that was primarily responsible for low cake volumes.

In egg albumen, the constituent proteins possess different isoelectric points, and, thus, carry different charges. At the natural pH of egg white (pH 7-8), the basic protein lysozyme (pI 10.7) is positively charged and can interact electrostatically with negatively charged proteins. Attractive electrostatic interactions between proteins may enhance the stabilities of egg white foams .

Blood plasma proteins. There are only a few studies available on the foaming properties of whole blood plasma (Tybor et al 1975, Khan et al 1979, Etheridge et al 1981, Hill and Hall 1987). Foaming capacity of blood plasma proteins was reported to be equivalent to that of egg albumen (Tybor et al 1975, Hill and Hall 1987) and superior to those of casein, whey proteins, and soy isolate proteins (Hill and Hall 1987). However, the stability of blood plasma foam was lower than that of egg albumen (Tybor et al 1975, Khan et al 1979).

Etheridge et al (1981) reported that complexing plasma proteins with hexametaphosphate improved its foaming properties. The phosphated protein gave higher foam volume and greater stability than those observed in either egg albumen or blood plasma proteins.

Among the blood plasma protein components, serum albumin has a sufficiently flexible structure to allow partial unfolding and adsorption at the foam interface, and the residual structure of the adsorbed molecule is sufficient to result in good foam stability.

Effects of pH, NaCl, and sucrose on the foaming properties of BSA were investigated by Poole et al (1984). Foaming capacity and stability of BSA were greatest at pH 5, which is near its pI. At this point, the net charge on BSA is minimum, and electrostatic repulsive forces are at a minimum. At pH 8, BSA carries a net negative charges which prevents association and impairs foaming properties. Reduction of electrostatic repulsion of BSA molecules by the addition of NaCl favored association of BSA at pH 8 which increased foaming capacity and

stability. Foaming capacity of BSA increased slightly in the presence of sucrose at pH 8.0, but foaming stability did not. Poole et al (1984) also reported that foaming capacity and stability of BSA (at pH 8) increased markedly by the addition of low concentrations of lysozyme (at a molar ratio of 1:1). Since BSA and lysozyme carry opposite net charges in the pH range between their isoelectric points, they can interact electrostatically to form complexes. Consequently, the film around the air cells is stronger, and the foam is more stable.

Emulsification properties

An emulsion is a dispersion of two immiscible or sparingly soluble liquids, usually an oil phase and a water phase, separated by a third component, an emulsifier. Many food products are emulsions and protein constituents often play a major role in stabilizing these colloidal systems. Proteins reduce interfacial tension by diffusing and adsorbing at the interface between dispersed oil droplets and the continuous aqueous phase.

Emulsifying properties are important in many food applications of ingredient proteins, and these are commonly determined as emulsifying capacity (EC) and emulsifying activity (EA). EC is determined by stirring an aqueous protein solution while oil is added steadily. After a certain volume has been added, the emulsion in the mixture undergoes a sudden change referred to as either "inversion" or "breaking". This is the end point of titration, and the volume of oil added is taken as a measure of the emulsifying capacity. The EC denotes the maximum amount

of oil that is emulsified under specified conditions by a standard amount of protein. EA requires small quantities of protein and is determined by a turbidimetric technique. In this method, the emulsion is prepared by homogenizing a measured amount of oil and aqueous protein solution together in a blender. The emulsion is diluted and the turbidity of the diluted emulsion is determined at 500 nm. The EA of proteins is expressed as the absorbance at 500 nm of diluted emulsions. The absorbance is proportional to the total interfacial area (m^2/g, defined as emulsifying activity index) and, hence, the inverse square of droplet size (Pearce and Kinsella 1978, Halling 1981).

A number of studies have been done on the relationship between protein structure and emulsification properties (Kato and Nakai 1980, Voutsinas et al 1983, Kato et al 1983, 1985, 1986a, 1986b, 1989, 1990). Proteins with high surface hydrophobicities and high solubilities have good emulsifying properties (Kato and Nakai 1980, Halling 1981, Voutsinas et al 1983, Kato et al 1983). A significant correlation ($P < 0.01$) was obtained between emulsifying activities and the hydrophobicities of proteins (Kato and Nakai 1980). The dependence of emulsifying properties on surface hydrophobicity, compared with the dependence of foaming properties on overall hydrophobicity, has been interpreted by Nakai (1983) as implying that the protein was more unfolded at the air-water interface. This was related to the fact that tension at the air/water interface (73 dyn/cm) was far greater than that at the oil/water interface (13-19 dyn/cm). Kato et al (1986b) observed an increase in surface hydrophobicity and emulsification activity upon

heat denaturation of ovalbumin and lysozyme under conditions that did not reduce solubility. As with foaming properties, flexibility of protein structure is also an important factor governing emulsification properties (Kato et al 1985, Kato et al 1986a, b). Intramolecular cross-linking of serum albumin and lysozyme lowered their molecular flexibilities and, therefore, foaming and emulsification properties decreased (Kato et al 1986a). However, the decrease in molecular flexibility had a more significant effect on the foaming properties. Kato et al (1990) related enthalpy of denaturation (determined by DSC) to foaming and emulsifying properties of heated egg white proteins in the dry state. Good linear correlations were observed between the decrease in enthalpy and increased foaming and emulsification properties of these proteins so long as solubility was not affected.

Emulsification properties of proteins were studied by Hill and Hall (1987). Bovine blood plasma and serum had higher emulsification capacities than egg, whey, and soy isolate proteins. Emulsions prepared with blood plasma proteins were more stable than those prepared with egg. Data from Tybor et al (1973) indicate that the emulsification capacity of plasma protein was greatest at pH 9.4, and a maximum response to protein concentration occurred in the vicinity of 1 g of protein/100 mL. BSA is a good emulsifier because of its molecular size, charge, surface hydrophobicity, disulfide bonds, and ability to unfold to a limited extent, re-orient at the interface and undergo interactions between the protein loops (Morrisey et al 1991).

The effects of ionic strength (0-1.5 NaCl) and pH on the EA of BSA were

investigated by Waniska et al (1981). The EA of BSA was increased at intermediate ionic strengths (0.1-0.6 N) due to neutralization of surface charges, reduced electrostatic repulsion, increased rate of protein adsorption, and greater protein-protein interactions. The EA of BSA increased progressively with increasing pH from 4 to 9, indicating that as net charge increased, the ability to form a film was enhanced. The EA of BSA decreased below pH 4 and above pH 9. Below pH 4 and above pH 9, BSA undergoes conformational transitions in its tertiary structure which impairs formation of a stable interfacial film required for emulsion formation.

The importance of native structure and molecular flexibility to the emulsifying properties of BSA was indicated by Waniska et al (1981). Reduction of the disulfide bonds of BSA resulted in a more expanded conformation and a decrease in EA in the pH range 4-10 compared with that of native BSA, suggesting that native BSA, which has more tertiary structure, forms a stronger, more cohesive interfacial film than reduced protein. Complete disruption of the secondary and tertiary structures of BSA by urea (8M) eliminated emulsifying activity except for slight activity at alkaline pH values. These studies reflect the importance of the native tertiary and secondary structures of proteins to the formation of a stable interfacial film which is required for emulsion formation. Succinylation of BSA markedly improved EA in the pH range 5-10 compared with that of native BSA. This may reflect increased molecular flexibility, which facilitates diffusion to the interface and rearrangement within the interfacial film.

Saito et al (1988) fractionated plasma into albumin-rich, γ-globulin-rich and transferrin-rich fractions. γ-Globulin-rich fraction exhibited the highest emulsification activity among the fractions, followed by transferrin and serum albumin.

Functionality of Egg White in Cake Making

A cake batter can be considered as a fat-in-water emulsion system and the baked cake as a heat set foam, in which the egg white proteins play an important functional role. Cake making can be divided into three main stages (Shepherd and Yoel 1976): 1) batter preparation 2) baking stage and 3) structure development.

Batter preparation

Batter preparation involves mixing ingredients and incorporating air cells and nucleii into the batter. All the air cells and nuclei which ultimately create the cake texture are incorporated in this stage, no new cells can be created by chemical leavening during baking (Carlin 1944). Cake volume is closely correlated with the amount of air in the batter (Dunn and White 1939, Ellinger and Speck 1968).

Cakes can be divided into three types depending on the air incorporation into the batter (Hoseney 1986). The first type utilizes multistage mixing. In multistage mixing, air is incorporated into the fat phase. This type of mixing produces a finer texture (due to the large number of air cells incorporated) and more stable batter because air is in the fat where it has limited mobility. The second type is a single stage mixing that is applied to box mixes. In this type of

mixing, liquids are added to the mix, and the batter is mixed. Since the mix contains surfactants that lower the interfacial tension, air is incorporated directly into the aqueous phase. In the third type of cake, air is directly incorporated into the aqueous phase by mechanical means using a high-speed mixing machine. Single-stage mixing produces very delicate cakes and is not suitable for commercial cakes.

Egg proteins play a role in stabilizating the emulsion system in cake batter. Cake emulsion was related to general theories of emulsions by Shepherd and Yoell (1976), and the possible stabilizing roles of egg in cake batter systems were identified: the lowering of interfacial tension at water/fat surfaces by an emulsifier will ease the formation of an emulsion by aiding the breakdown of larger fat particles to finer-sized particles. The rheological nature of the interfacial film formed around oil globules will affect emulsion stability. The surface charge of the fat droplets may be important. Approach of fat droplets to one another can be resisted by the mutual repulsion of their electrical double layers, thus, preventing coalescence. The viscosity of the continuous aqueous phase to which the egg proteins contribute can also effect emulsion stability.

Baking stage

During the baking stage, cake batter undergoes a number of changes: when batter temperature reaches to 37-40°C, the fat in the batter melts and air bubbles are released from the fat phase to the aqueous phase where they are stabilized by

egg protein molecules at the air bubble-water interface (Shepherd and Yoel 1976). Shepherd and Yoell (1976) reported that when a simple aerated fat and water system was heated air bubbles coalesced within the fat phase. Any water-in-oil emulsion portions of the batter in this stage invert to oil-in-water emulsion. Cake batters undergo bulk flow, up the sides and down the center as temperature rises. Batter viscosity decreases as batter temperature increases from ambient to 60°C (Mizukoshi, 1983; Shelke et al 1990). Egg white significantly increases the batter viscosity both at ambient temperature and at minimum viscosity during heating (Shelke et al 1990). Air bubbles incorporated in the batter preparation stage expands due to movement of water vapor and carbon dioxide (from baking powder) into the air cells. Diffusion of gas from small bubbles to large bubbles begins at 49-56°C and ends at 87-98°C (Bell et al 1975).

Structure development

In the final stage, cake structure develops due to coagulation of egg protein and partial gelatinization of starch. Cake setting starts between 60-70°C and this initial viscosity increase is caused by protein denaturation (Shepherd and Yoel 1976) and a rapid viscosity increase starts between 80-85°C due to starch gelatinization (Shelke et al 1990). Viscosity increase in a heated batter prevent coalescence, migration and loss of air cells before the batter sets. Expansion of air bubbles becomes very rapid at 70-80°C (Bell et al 1975, Shepherd and Yoel 1976). The presence of egg proteins in the membranes around air bubbles

provides elasticity which supports the rapid expansion (Shepherd and Yoel 1976). All of the movements in the batter cease at 99-100°C in high ratio cakes, and the structure becomes fixed.

Donovan (1977) used DSC to investigate the baking process as applied to the manufacture of angel food cake. He found that sucrose used in the standard angel food cake batter increased the denaturation temperatures of egg white proteins and gelatinization temperature of starch by about 13 and 30°C, respectively. Gelatinization of starch and denaturation of the major portion of egg white proteins occured near 95°C, at approximately the maximum temperature attained by the cake when it reaches maximum volume in the oven.

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3. FUNCTIONAL PROPERTIES OF BOVINE BLOOD PLASMA AND EGG WHITE PROTEINS

A paper to be submitted to the *Journal of Food Science*

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ABSTRACT

We investigated the functional properties of egg white, bovine blood plasma, and their component proteins to explain differences in their cake-baking potential. All blood plasma proteins denatured at lower temperatures than the major protein of egg white (ovalbumin). γ-Globulin was the most heat stable and fibrinogen was the most heat-sensitive protein of blood plasma. Blood plasma had similar foaming capacity as egg white, but the foam stability of blood plasma protein was significantly less than for egg white. Among blood plasma fractions, serum albumin, fibrinogen, and Cohn fraction IV-1 (predominantly α-globulin) had good foaming capacities and stabilities. Globulins were the only protein fractions in egg white with good foaming properties. Blood plasma and its component proteins were better emulsifiers than egg white and its component proteins.

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INTRODUCTION

Egg white is an essential ingredient to achieve desirable volume and texture in many baked goods because of its unique solubility, heat coagulation, foaming, and emulsification properties (Shepherd and Yoel, 1976). However, egg products are also one of the most expensive ingredients used by the baking industry; therefore, efforts in searching for low-cost egg substitutes have increased over the past few years. Because of its substantially lower cost, bovine blood plasma, a by-product of the animal slaughtering industry, has received recent attention as an egg substitute in cakes. It has already been shown to have the potential to partially replace egg whites in cakes (Brooks and Ratcliff, 1959; Johnson et al., 1979; Khan et al., 1979; Lee et al., 1991). However, full replacement does not quite reproduce the quality of cakes made with egg white.

The most important functional property of egg white in the production of cakes is believed to be the ability of the soluble proteins to coagulate during baking (Shepherd and Yoel, 1976; Donovan, 1977). The combination of protein denaturation and starch gelatinization increases the viscosity of the batter, which prevents coalescence of air cells and sets the crumb structure (Shepherd and Yoel, 1976; Donovan 1977). Differential scanning calorimetry (DSC) has been used as a technique for studying thermal denaturation of proteins. Heat denaturation properties of egg white, its component proteins, and the baking process have already been studied by using DSC (Donovan et al., 1975; Donovan,

1977). However, thermal properties of plasma proteins have received little attention.

Denaturation temperature of ovalbumin, the major protein of egg white, coincides with the gelatinization of starch in the batter and enables maximum cake volume to be achieved prior to heat setting of the structure (Donovan, 1977). Upon storage of eggs at elevated temperatures, ovalbumin is converted to a more heat stable form, *S*-ovalbumin, which has a denaturation temperature about 8°C higher than that of ovalbumin (Donovan and Mapes, 1976). When eggs containing *S*-ovalbumin are used in the manufacture of angel food cakes, delayed denaturation causes excessive oven spring along with a subsequent collapse, which decreases cake volume (Meehan et al., 1962; Donovan, 1977). These results suggest that heat denaturation properties of egg white proteins play significant roles in cake volume and texture. Therefore, we applied DSC to bovine blood plasma and its constituent proteins in an effort to gain insight into the thermal properties of this complex system.

Besides heat denaturation properties, emulsification and foaming properties of egg white contribute to stabilization of oil-in-water emulsions and expansion of cake batters during baking, respectively (Baldwin, 1977). Foaming capacity of egg white protein components were studied by MacDonnell et al. (1955) and Johnson and Zabik (1981). Globulins had good foaming properties whereas ovomucin, lysozyme, ovomucoid, and conalbumin showed little or no foaming capacity. Foaming capacity of blood plasma was reported to be equivalent to that of the egg

albumen (Tybor et al., 1975; Khan et al., 1979; Hill and Hall, 1987). However, the stability of the blood plasma foam was lower than that of the egg albumen (Tybor et al., 1975; Hill and Hall, 1987).

Blood plasma has three main protein components, albumin, globulins (α,β,γ), and fibrinogen. These protein components have different structures and physical properties. Thus, it is necessary to investigate the aforementioned functional properties of each fraction to determine whether certain components are superior to others to direct process changes that might improve the potential to use blood proteins to replace egg proteins in cakes. The objective of this research was to characterize and compare the heat denaturation, foaming, and emulsification properties of whole plasma, egg white and their component proteins.

MATERIALS AND METHODS

Materials

Fresh eggs were obtained from the Iowa State University Poultry Farm. The whites were separated from the yolk and blended in a Waring Blendor for 40 sec. The speed of mixing was reduced with a Variac transformer and the slowest speed was used for homogenization. Half of the blended fresh egg white was freeze dried, and the other half was spray dried using a Yamato Pulvis Mini-spray model GA-31 with an air inlet temperature of 90°C and an air outlet temperature of 50°C.

Frozen blood plasma was obtained from AMPC, Inc., Ames, IA; and thawed at 4°C; and centrifuged at 613 x g for 15 min at 4°C in a Beckman J2-21

Refrigerated Centrifuge with JA-14 rotor (Beckman Instruments Inc., Palo Alto, CA). Half supernatant was freeze dried and the other half, spray dried. During drying, the spray drier outlet temperature was maintained at 50°C.

Commercially spray-dried bovine blood plasma and egg whites (type P-110) were provided by AMPC, Inc., Ames, IA, and Henningsen Food, Inc., Omaha, NE, respectively. The following purified protein fractions of bovine blood plasma and egg white were purchased from Sigma Chemical Co., St. Louis, MO: bovine serum albumin (product no. A7638, 99% purity); γ-globulins (product no. G5009, 99% purity); Cohn fraction IV-1 (product no. G8512, 60-80% α-, 15-40% β-, 0-2% γ-globulins); Cohn fraction III (product no. G4633, 56% β- and 44% γ-globulins); fibrinogen (product no. F4753, 95% of protein clottable); ovalbumin (product no. A5503, 99% purity); conalbumin (product no. C0755, iron free); ovomucoid (product no. T2011); lysozyme (product no. L6876); and egg white globulins (product no. EG, substantially free of albumin).

Protein Analysis

Protein contents of the blood plasma and egg white samples were determined by using the macro-Kjeldahl method (AOAC, 1984) using a nitrogen conversion factor of 6.25. Protein contents of the fractions were calculated from absorbance measured with a Shimadzu spectrophotometer (model UV-160, Shimadzu Corp., Kyoto, Japan). The following optical factors ($A^{1\%}_{1cm}$) were used: bovine serum albumin, 6.67 at 279 nm (Aoki et al., 1973); γ-globulin, 13.5 at 275

nm (Ruegg et al., 1977); ovalbumin, 7.12 at 280 nm (Glazer et al., 1963); conalbumin, 11.3 at 280 nm (Glazer and McKenzie, 1963); and lysozyme, 26.3 at 281 nm (Sophianopoulos et al., 1962). Protein contents of ovomucoid and fibrinogen were determined by using the Biuret method (Gornall et al., 1949) using bovine serum albumin as a standard. Protein contents of egg white globulins and Cohn fraction IV-1 were also determined by using the Biuret method but using γ-globulin as a standard. Protein content of Cohn fraction III was estimated by using the micro-Kjeldahl method (AOAC, 1984).

Solubilities of egg white and plasma samples in 0.06 M phosphate buffer containing 0.11 M NaCl at pH 7.0 were determined by using the method of Regenstein and Regenstein (1984).

Differential Scanning Calorimetry (DSC)

DSC studies were performed by using a Perkin-Elmer DSC 7 analyzer equipped with a thermal analysis data station (Perkin-Elmer Corp., Norwalk, CT). All proteins except fibrinogen were dissolved in 0.06 M phosphate buffer containing 0.11 M NaCl at pH 7.0 at a concentration of 56 mg/mL. The protein solutions of whole egg white and plasma were centrifuged at 2204 x g for 30 min at 4°C (using the JA-17 rotor) to remove any insoluble substances. The protein content in the supernatant was then determined by using the biuret procedure with bovine serum albumin as a standard.

In all DSC runs, exactly 70 μL of about 5.6% protein solution was

hermetically sealed in a stainless steel pan. Another pan containing 70 μL buffer with no protein was used as the reference. Fibrinogen was directly weighed into the pan and sufficient buffer was added to obtain a protein concentration of 5.6%, and then the sample pan was allowed to equilibrate for 2.5 hr. Samples were heated from 25 to 130°C at a rate of 10°C/min. Enthalpy (ΔH), onset (T_o), and peak (T_p) temperatures were computed automatically. Enthalpies were calculated on the protein content basis.

Foaming Properties

Foaming properties were determined by measuring the volumes of foams produced when air at a constant flow rate of 90 mL/min was introduced through a glass filter for 15 sec into 5 mL of either 0.1 or 1% protein buffer solutions in a glass column (2.6 X 33 cm) with a coarse glass filter in the bottom. The column and glass filter were cleaned with chromic-sulfuric acid between determinations.

Foaming capacity was defined as the volume of the foam at 15 sec after the air was introduced. Foam stability was determined by measuring the remaining volume of the foam V_t at 15 and 30 min after air was introduced. The stability of foam was expressed as the percentage of volume foam remaining and calculated as shown below (Regenstein and Regenstein, 1984):

$$\% \ foam \ remaining = \frac{V_t}{V_o} \ x \ 100$$

where V_o is the initial volume of the foam (foam capacity) at t = 15 sec.

Emulsification Properties

The emulsification activities of protein fractions were determined by using the method of Pearce and Kinsella (1978). Emulsions were prepared by homogenizing 7 mL of peanut oil and 21 mL of 0.2% protein buffer solution with a Janke and Kunkel blender (Model A10, Chemical Rubber Co., Cleveland, OH) at 20,000 rpm for 20 sec at 20°C. Turbidity was measured by diluting (1000X) the emulsion with buffer containing 0.1% sodium dodecyl sulfate (SDS) and the reading absorbance at 500 nm.

Emulsification capacities of whole blood plasma and egg white samples were measured by using a modified method of Swift et al. (1961). About 50 mL of 1% protein buffer solution was emulsified with corn oil at 7000 rpm with a household hand mixer (Model M122, Biospec Products Inc., Bartlesville, OK). Emulsification capacity was defined as the amount of oil (mL) required for 1 g of the protein to reach its capacity.

Statistical Analyses

All determinations were replicated three times, and the data were analyzed with the Statistical Analysis System (SAS, 1990). Least significant differences at $P \leq 0.05$ were used to compare sample means.

RESULTS AND DISCUSSION

Heat Denaturation Properties of Proteins

Egg white. When samples of whole egg white were subject to DSC analysis, thermograms of the type shown in Figure 1 were produced. Two peaks at 67 and 84.5°C were clearly discernible, corresponding to the thermal process taking place in the egg white. A third intermediate peak was noticeable in the thermograms of freeze-dried egg white. The denaturation onset (T_o), peak temperatures (T_p) of the component transitions, and the total enthalpies of denaturation (ΔH) for egg white samples are given in Table 1. T_o and ΔH were similar for freeze- and lab-scale spray-dried egg whites; however, the values obtained for commercially spray-dried egg white were significantly lower. The lower denaturation temperatures and enthalpy values suggest that some molecular conformational changes, such as unfolding of the tertiary structure had already occurred in the commercial sample. These thermal changes are most likely due to higher drying temperatures used during commercial spray-drying.

To resolve the complex thermogram of whole egg white into components associated with its major protein fractions, we individually performed DSC analysis of purified ovalbumin, conalbumin, ovomucoid, lysozyme, and globulins. The DSC results of these proteins are given in Table 2, and Figure 2 shows the corresponding DSC thermograms. The heat sensitivity of the fractions in decreasing order were conalbumin, lysozyme, ovomucoid, ovalbumin, and globulins. Conalbumin exhibited a peak at 64.3°C and was the most heat sensitive

protein component in egg white. T_p of lysozyme was 74.9°C and had the largest ΔH, followed by ovalbumin and conalbumin. The thermogram of ovomucoid exhibited a shoulder around 77°C instead of a peak; therefore, ΔH could not be calculated. The ovalbumin thermogram showed a peak at 87.7°C with a T_o of 80°C, which corresponded to the T_o of the major peak in the whole egg white thermogram. Two overlapping peaks with T_p values of 86.6 and 97.1°C were apparent in the thermograms of globulins. Thus, the two discernible peaks (67 and 84.5°C) in the whole egg white thermogram were attributed to denaturation of conalbumin and ovalbumin, respectively, by considering their T_p values and concentrations in the egg white. However, the positions of the transitions in whole egg white did not correspond exactly to those of its fractions. This can be ascribed to the protein interactions that can take place in egg white. The intermediate peak, which was slightly visible in the freeze-dried egg white thermogram, was attributed to denaturation of lysozyme because of its intermediate T_p value between conalbumin and ovalbumin, large ΔH, and cooperative denaturation profile.

The assignments of the peaks in the whole egg white thermogram to specific components were verified by spiking the whole egg white solution with individual constituent protein standards. For this experiment, freeze-dried egg white was dissolved in the buffer to prepare 5.6% protein solution and centrifuged at 2204 x g for 30 min (using the JA-17 rotor). Then, this stock solution was divided into five samples which were used to prepare 3% protein solutions of component proteins. DSC analyses of these spiked solutions were conducted as

described earlier in the method section. Thermograms of spiked egg white solutions with constituent proteins are shown in Figure 3. Spiking freeze-dried egg white solution with conalbumin increased the height of the first peak (68°C) in the egg white thermogram, lysozyme increased the height of the intermediate peak at 73°C, and ovalbumin increased the height of the third peak at 85°C. Addition of ovomucoid gave a shoulder around 76°C, and addition of globulins did not change the egg white thermogram because they were concealed by the large ovalbumin peak. Thus, we conclude that the main peaks in the whole egg white thermogram arise from the thermal denaturation of conalbumin (T_p at 67°C) and ovalbumin (T_p at 84.5°C), and the small intermediate peak from denaturation of lysozyme (T_p at 73°C). Our conclusion is in agreement with previous reports of Donovan et al. (1975).

Turning to analysis of the component proteins, we noted that conalbumin was more stable to heat denaturation in egg white than when present alone in solution (Tables 1 and 2). In contrast, lysozyme was more sensitive to heat denaturation in egg white than when present alone as was also shown by Donovan et al. (1975). This may be because of protein interactions in egg white. Although the denaturation peak temperature of freeze-dried ovalbumin was about 3°C higher than that in egg white, the onset of denaturation was the same in both cases (80°C).

Blood plasma. Blood plasma samples gave a plateau-shaped denaturation profile with three visible overlapping peaks at about 70, 77, and 83°C (Fig. 1). Table 3 summarizes the denaturation properties of the blood plasma samples. Because the denaturation profile of blood plasma proteins overlap, the onset temperature could not be determined for the individual thermogram peaks. Therefore, the onset temperature in Table 3 was assigned only to the first peak in the thermogram. Processing did not affect denaturation temperatures of blood plasma samples. However, the enthalpy of freeze-dried blood plasma was higher than for spray-dried forms.

To assign the peaks in the whole blood plasma thermogram to its major protein components: albumin, γ-globulin, α-globulin, β-globulin, and fibrinogen, we followed the same steps as we did for egg white. DSC analysis of these fractions were performed, results are summarized in Table 2, and the corresponding thermograms are shown in Figure 4. γ-Globulin was the most heat stable protein in blood plasma, requiring the greatest amount of heat to denature.

Serum albumin consists of a single polypeptide chain containing 17 disulfide bridges. These disulfide bridges organize the molecule into three similar domains, and each domain has a site for certain physiological ligands. Long chain fatty acids bind to a site in the third domain (Putnam, 1984), stabilize the protein molecule, and increase the denaturation temperature of serum albumin (Gumpen et al., 1979; Peters, 1985). Bovine serum albumin used in this study was not essentially free of fatty acids and gave a peak at about 79°C with an onset

temperature of 69°C. In the study by Gumpen et al. (1979), the denaturation peak temperature of ligand-free serum albumin was 68°C.

The DSC thermogram of fibrinogen exhibited one major, low-temperature peak at 57°C and one smaller, high-temperature peak at 101°C. This showed that fibrinogen is a very heat-labile protein and that onset of denaturation can start as low as 47°C. α- and β-Globulins were not pure proteins; therefore, it was not possible to determine their precise denaturation properties. However, Cohn fraction IV-1, which is primarily composed of α-globulin, gave two overlapping peaks at 71 and 79°C. Because the rest of the blood plasma proteins gave denaturation peak temperatures higher than 78°C, we concluded that the lower peak temperature (71°C) was α-globulin. Cohn fraction III contains β- and γ-globulins in almost equal proportions. However, the thermogram of this sample showed only one peak because the β-globulin was concealed by the γ-globulin peak. The presence of β-globulin broadened the peak and tailed it to the lower temperature end more when compared with a thermogram of γ-globulin alone (Fig. 4). This result suggests that β- globulin was more heat sensitive than γ-globulin. According to the data we obtained so far, we assigned the first peak of the whole blood plasma endotherm to denaturation of α-globulin, the second peak to denaturation of albumin, and the third peak to denaturations of both β- and γ-globulins.

To verify this assignment we spiked freeze-dried blood plasma solution (5.6%) with purified constituent protein standards (3%), separately as described for

egg white. Spiking the blood plasma solution with albumin, β- and γ-globulins changed the appearance of the thermogram and transformed it into a single peak (Fig. 5). Albumin-spiked blood plasma produced a single peak at 77.9°C. β- and γ-Globulins spiked plasma solutions gave peaks at 79.5 and 81.8°C, respectively. Spiking with α-globulin increased the size of the first peak (71°C) in the plasma thermogram. Fibrinogen-spiked plasma exhibited two additional peaks outside the typical thermogram of plasma. This showed that either centrifugation of plasma solution prior to DSC analysis precipitated fibrinogen or the concentration of fibrinogen in the solution was too low to produce a visible denaturation peak. Thus, we concluded that the first peak in the blood plasma thermogram arises from denaturation of α-globulin, the second peak arises from denaturation of albumin, and the third peak arises from denaturation of β- and γ- globulins.

Foaming Properties

Egg white and blood plasma. Foaming properties of whole egg white and blood plasma samples were determined by using both 1 and 0.1% protein solutions (Tables 4 and 5). Blood plasma and egg white produced the same amount of foam volumes under our experimental conditions at both concentrations. However, the stability of blood plasma foams were significantly lower than those of egg white foams at 15 min. Whereas blood plasma samples practically lost almost all of their foam after 30 min, egg white samples retained more than 85% of their initial foams.

No processing effects were observed on foam capacities of both egg white and blood plasma samples, but foam stabilities were affected. Freeze-dried samples produced weak and open foam whereas spray-dried samples produced fine bubbles. Therefore, freeze-dried samples produced less stable foams than did spray-dried samples. This effect was more pronounced for blood plasma proteins. These results suggest the occurrence of protein-protein interactions might be facilitated by spray-drying, resulting in the formation of a stronger foam film. In fact, the reduced ΔH values of spray-dried samples indicated that some denaturation occurred during the processing of these samples. This is in agreement with a previous report by Kato et al. (1990) who found linear correlations between decreased enthalpy and improved foaming and emulsification properties of egg white proteins.

Prior denaturation of globular proteins by heat without loss of solubility leads to enhanced foaming and emulsifying properties because of increased molecular flexibility (Kato et al., 1986b; Kitabatake, 1989) and increased surface hydrophobicity and protein-protein interactions (Kato et al., 1989). In addition to these factors, stabilization by solid particles is also possible in heat-denatured samples (Mitchell, 1986). Our solubility data showed that all of the egg white and blood plasma samples were completely soluble in the buffer used in this study except for commercial spray-dried plasma which, was 97.5% soluble.

When the protein contents of the solutions were reduced from 1 to 0.1%, foaming stabilities of the proteins decreased (Tables 4 and 5). The effect of

protein concentration on the stability of blood plasma foam was more significant than on the stability of egg white foam. Freeze-dried and spray-dried blood plasma in the lab lost about 80% of their initial foam volumes after 15 min. Commercially spray-dried blood plasma was able to maintain only 56% of its original foam volume with a relatively large standard deviation at the end of 15 min. The effect of increasing protein concentration on foam stability has been investigated by several groups, and their results were summarized by Halling (1981). Although foam stabilities of a few proteins were nearly constant or slowly increased above 0.1%, most proteins displayed increased foam stability with increased concentration. This effect was attributed to increased liquid viscosity brought about by the protein.

Protein fractions of egg white and blood plasma. Foaming capacities and stabilities of egg white and blood plasma protein fractions were determined by using 0.1% protein solutions (Table 6). Egg globulins were the only protein fraction of egg white that had good overall foaming properties (Table 6). Ovalbumin exhibited good foaming capacity but low stability, lysozyme did not foam, and the remaining of the egg white protein fractions had poor foam capacities and stabilities. The poor foaming properties of lysozyme and ovalbumin were attributed to their rigid, folded molecular structure and very low surface hydrophobicity (Kato et al., 1983, 1985).

Although lysozyme alone does not foam, electrophoretic analysis of egg

white foams and drained liquid showed that lysozyme was preferentially retained in the foam phase (Poole et al., 1984). Proteins carry a net negative charge at pHs above their isoelectric points and a net positive charge at pHs below their isoelectric points. Perhaps at pH 7, positively charged lysozyme electrostatically interacts with other negatively-charged egg white proteins leading to a more structured and viscoelastic film than its component proteins alone. Earlier work of Poole et al. (1984) indicated that addition of low concentrations (0.01-0.10% w/v) of lysozyme (pI 10.7) to solutions (0.50% w/v) of several acidic proteins (pI 4.7-6.0) greatly improved their foaming properties at pH values between the isoelectric points.

Contrary to whole blood plasma, some of its constituent proteins, such as serum albumin, α-globulins, and fibrinogen, exhibited good foaming capacities and stabilities (Table 6). Their foaming properties were similar to those of egg white globulins. Foaming capacity of γ-globulin was significantly lower than the rest of the blood plasma proteins and exhibited very poor foam stability. Cohn fraction III, a mixture of β- and γ-globulins, lost all of its foam in less than 5 min.

Since albumin is a hydrophobic protein with a flexible structure, it has a high affinity for an air-water interface (Kato et al., 1985, 1986a). α-Globulins contain about 20 glycoproteins, and the amount of carbohydrate changes from protein to protein reaching up to 40% or more by weight (Putnam, 1984). Thus, good foam stability of α-globulins can be attributed to its high carbohydrate content. Perhaps the carbohydrate part of this protein increases the viscosity of the lamella and

retards its drainage.

Emulsification Properties

Blood plasma emulsified more oil than egg white (Table 7). The emulsification capacity of commercial spray-dried egg white was significantly higher than those of egg white samples prepared in the lab. This can be attributed to the same reasons associated with foaming properties as explained earlier. Proteins with high surface hydrophobicity and high solubility have good emulsifying properties (Kato and Nakai, 1980; Halling, 1981; Voutsinas et al., 1983; Kato et al., 1983). Commercial spray-dried plasma emulsified slightly less oil than those of freeze- and spray-dried blood plasma samples prepared in the lab. This may be attributed to its slightly lower solubility.

Component proteins of blood plasma also exhibited better emulsification activities than those of egg white protein fractions (Table 8). γ-Globulin had the highest emulsification activity followed by Cohn fraction III (56% β- and 44% γ-globulins) and serum albumin. Among the egg white protein fractions, ovomucoid had the highest emulsification activity. Both lysozyme and ovalbumin had very poor emulsification activities.

CONCLUSIONS

Considerable differences were found in the thermal denaturation temperatures of egg white and blood plasma proteins. Blood plasma proteins

denatured at lower temperatures than ovalbumin (the major protein in egg white) and globulins of egg white. The major protein of blood plasma (albumin) had an onset of denaturation 11°C lower than that of ovalbumin. Although some of the plasma protein fractions had good foam stabilities (albumin, fibrinogen, and Cohn fraction IV,1), whole plasma exhibited significantly lower foam stability than did egg white. These differences probably account for our observations that cakes made with blood plasma have lower volume, coarser crumb structure, and flatter profile than cakes made with egg white.

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Table 1 - Thermal properties of egg white[a]

Samples	Peak 1		Peak 2		$\Sigma\Delta H^d$
	T_o^b (°C)	T_p^c (°C)	T_o(°C)	T_p (°C)	(J/g)
Freeze-dried egg white	64.1 ± 0.4 a	67.5 ± 0.2 a	80.0 ± 0.5 a	84.7 ± 0.2 a	19.3 ± 0.5 a
Spray-dried egg white (laboratory)	64.1 ± 0.2 a	67.4 ± 0.1 a	80.1 ± 0.3 a	84.8 ± 0.3 a	18.0 ± 0.9 a
Spray-dried egg white (commercial)	62.9 ± 0.6 b	66.9 ± 0.3 b	77.8 ± 0.3 b	84.4 ± 0.4 a	13.6 ± 1.2 b

[a]Data are the means of triplicate determinations (means ± standard deviations), and values with the same letter in a column are not significantly different at the 0.05 level.
[b]Denaturation onset temperature.
[c]Denaturation peak temperature.
[d]Total enthalpy of denaturation.

Table - 2 Thermal properties of blood plasma and egg white protein fractions[a]

Protein	T_o[b] (°C)	T_p[c] (°C)	$\Sigma \Delta H$[d] (J/g)
Blood plasma fractions			
Albumin	69.1 ± 0.3	78.8 ± 0.2	18.8 ± 0.7
γ-Globulin	71.8 ± 0.9	83.5 ± 0.5	25.0 ± 2.0
Cohn fraction IV-1 (60-80% α, 15-40% β, 0-2% γ)	66.0 ± 0.3	(1) 71.2 ± 0.5 (2) 78.9 ± 0.2	6.8 ± 1.1
Cohn fraction III (56% β-, 44% γ-globulins)	70.1 ± 0.5	82.9 ± 0.6	16.1 ± 1.4
Fibrinogen	(1) 47.9 ± 1.3 (2) 95.6 ± 0.5	57.3 ± 0.5 100.9 ± 0.3	12.2 ± 0.7
Egg white fractions			
Ovalbumin	80.1 ± 0.8	87.7 ± 0.4	19.8 ± 0.5
Lysozyme	69.0 ± 0.0	74.9 ± 0.1	32.3 ± 0.9
Globulins (53% α, 47% β globulins)	75.1 ± 1.2	(1) 86.6 ± 1.0 (2) 97.1 ± 0.3	14.3 ± 1.5
Conalbumin	60.1 ± 0.3	64.3 ± 0.2	17.7 ± 0.7
Ovomucoid	69.0 ± 0.3	77.2 ± 0.4	ND[e]

[a]Data are the means of triplicate determinations (means ± standard deviations).
[b]Denaturation onset temperature. (1) and (2) indicate the number of endotherms exhibited.
[c]Denaturation peak temperature.
[d]Total enthalpy of denaturation.
[e]Not determined.

Table 3 - Thermal properties of blood plasma[a]

Samples	Onset T_o[b] (°C)	Peak 1 T_p[c] (°C)	Peak 2 T_p(°C)	Peak 3 T_p (°C)	$\Sigma\Delta H$[d] (J/g)
Freeze-dried blood plasma	62.6 ± 0.2 a	69.8 ± 0.4 ab	77.1 ± 0.4 a	83.7 ± 0.1 a	14.2 ± 0.6 a
Spray-dried blood plasma (laboratory)	62.7 ± 0.2 a	71.2 ± 0.6 a	76.5 ± 0.8 a	83.4 ± 0.3 a	12.9 ± 0.6 a
Spray-dried blood plasma (commercial)	62.5 ± 0.4 a	68.9 ± 1.2 b	77.1 ± 0.5 a	83.8 ± 0.4 a	12.3 ± 0.7 b

[a]Data are the means of triplicate determinations (means ± standard deviations), and values with the same letter in a column are not significantly different at the 0.05 level.
[b]Denaturation onset temperature.
[c]Denaturation peak temperature.
[d]Total enthalpy of denaturation.

Table 4 - Foaming capacities and stabilities of egg white and blood plasma[a]

Samples	Initial volume (mL)	Remaining foam (%)	
		15 min	30 min
Freeze-dried egg white	40.3 ± 2.5 a	90.9 ± 1.7 a	84.7 ± 4.8 a
Spray-dried egg white (laboratory)	40.7 ± 1.5 a	91.4 ± 1.8 a	87.7 ± 2.0 a
Spray-dried egg white (commercial)	42.0 ± 1.0 a	91.6 ± 1.8 a	91.6 ± 1.8 a
Freeze-drled blood plasma	41.0 ± 1.0 a	27.6 ± 3.1 c	21.4 ± 5.7 c
Spray-dried blood plasma (laboratory)	41.3 ± 1.5 a	86.2 ± 0.2 b	28.0 ± 7.8 bc
Spray-dried blood plasma (commercial)	41.7 ± 0.6 a	86.6 ± 3.6 b	38.6 ± 14.3 b

[a]Protein concentration in the solution was 1%. Data are the means of triplicate determinations (means ± standard deviations). Values with the same letter in a column are not significantly different at the 0.05 level.

Table 5 - Foaming capacities and stabilities of egg white and blood plasma[a]

Samples	Initial volume (mL)	Remaining foam (%)	
		15 min	30 min
Freeze-dried egg white	38.3 ± 1.5 a	85.2 ± 5.9 a	79.2 ± 4.7 a
Spray-dried egg white (laboratory)	39.3 ± 0.6 a	85.6 ± 5.5 a	84.9 ± 4.6 a
Spray-dried egg white (commercial)	40.0 ± 0.0 a	88.8 ± 4.7 a	83.7 ± 3.8 a
Freeze-dried blood plasma	39.2 ± 0.8 a	22.3 ± 2.0 c	16.4 ± 1.6 b
Spray-dried blood plasma (laboratory)	39.2 ± 0.3 a	28.3 ± 4.3 c	19.7 ± 3.8 b
Spray-dried blood plasma (commercial)	39.5 ± 1.8 a	56.2 ± 17.0 b	22.9 ± 2.4 b

[a]Protein concentration in the solution was 0.1%. Data are the means of triplicate determinations (means ± standard deviations). Values with the same letter in a column are not significantly different at the 0.05 level.

Table 6 - Foaming capacities and stabilities of blood plasma and egg white protein fractions[a]

Samples	Initial volume (mL)	Remaining foam (%)	
		15 min	30 min
Blood plasma protein fractions			
Albumin	38.2 ± 1.6 bc	88.2 ± 0.8 a	84.6 ± 1.0 a
γ-Globulin	35.3 ± 1.5 d	34.1 ± 7.5 c	13.6 ± 6.1 c
Cohn fraction IV, 1 (60-80% α-, 15-40% β-, 0-2% γ-)	40.7 ± 1.2 a	87.1 ± 1.3 a	84.7 ± 1.0 a
Cohn fraction III (56% β-, 44% γ-globulins)	37.0 ± 0.0 c	0.0 ± 0.0 e	0.0 ± 0.0 d
Fibrinogen	38.9 ± 0.4 b	89.7 ± 0.1 a	82.6 ± 0.9 a
Egg white protein fractions			
Ovalbumin	38.8 ± 0.3 b	43.1 ± 7.3 b	30.1 ± 3.6 b
Lysozyme	2.5 ± 0.5 f	0.0 ± 0.0 e	0.0 ± 0.0 d
Globulins (53% α-, 47% β-globulins)	37.5 ± 0.5 bc	88.8 ± 2.1 a	86.3 ± 2.3 a
Conalbumin	35.3 ± 0.6 d	36.1 ± 2.7 c	29.4 ± 8.3 b
Ovomucoid	33.7 ± 0.6 e	21.0 ± 4.8 d	12.9 ± 3.0 c

[a]Protein concentration in the solution was 0.1%. Data are the means of triplicate determinations (means ± standard deviations). Values with the same letter in a column are not significantly different at the 0.05 level.

Table 7 - Emulsification capacities of egg white and blood plasma[a]

Samples	Emulsification capacity (mL oil/g protein)
Freeze-dried egg white	853 ± 25 d
Spray-dried egg white (laboratory)	857 ± 29 d
Spray-dried egg white (commercial)	933 ± 12 c
Freeze-dried blood plasma	1075 ± 14 ab
Spray-dried blood plasma (laboratory)	1079 ± 12 a
Spray-dried blood plasma (commercial)	1035 ± 33 b

[a]Data are the means of triplicate determinations (means ± standard deviations). Values with the same letter are not significantly different at the 0.05 level.

Table 8 - Emulsification activities of blood plasma and egg white protein fractions[a]

Protein	Absorbance (500 nm)
Blood plasma protein fractions	
Albumin	0.433 ± 0.007 bc
γ-Globulin	0.469 ± 0.003 a
Cohn fraction IV, 1 (60-80% α-, 15-40% β-, 0-2% γ-)	0.412 ± 0.015 c
Cohn fraction III (56% β-, 44% γ-globulins)	0.453 ± 0.010 ab
Fibrinogen	0.311 ± 0.024 d
Egg white protein fractions	
Ovalbumin	0.079 ± 0.010 g
Lysozyme	0.038 ± 0.004 h
Globulins (53% α-, 47% β-globulins)	0.272 ± 0.014 e
Conalbumin	0.207 ± 0.020 f
Ovomucoid	0.410 ± 0.034 c

[a]Data are the means of triplicate determinations (means ± standard deviations). Values with the same letter are not significantly different at the 0.05 level.

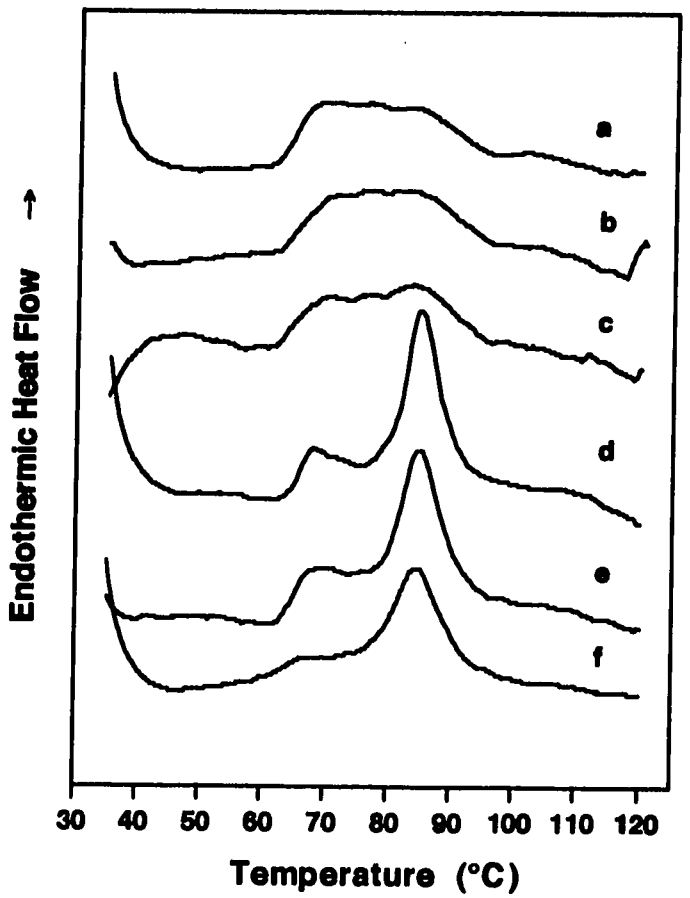

Fig. 1 - Differential scanning calorimetry thermograms of bovine blood plasma and egg white samples (a) freeze-dried blood plasma; (b) spray-dried blood plasma (lab); (c) spray-dried blood plasma (commercial); (d) freeze-dried egg white; (e) spray-dried egg white (lab); and (f) spray-dried egg white (commercial).

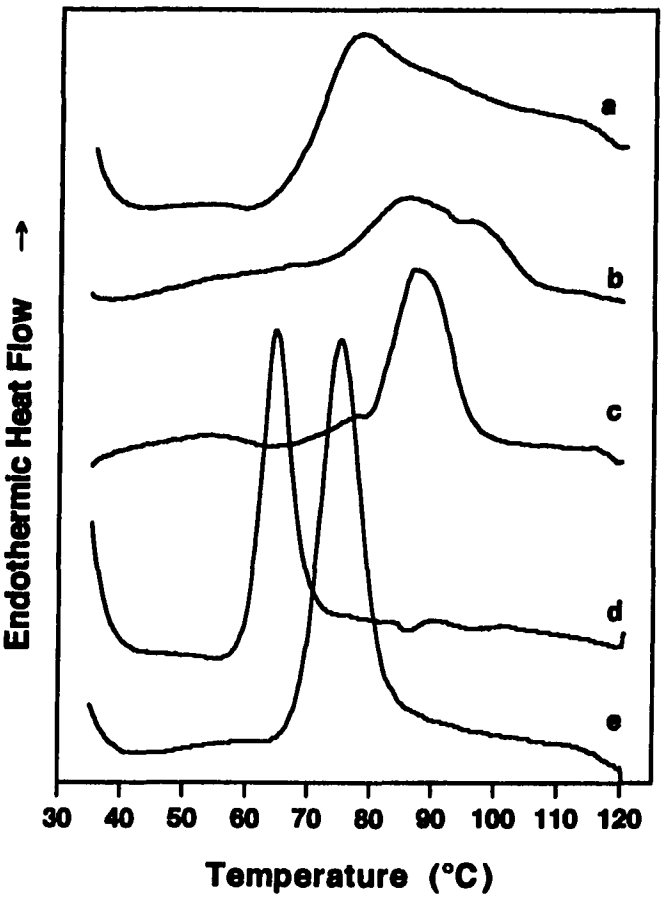

Fig. 2 - Differential scanning calorimetry thermograms of egg white protein fractions (a) ovomucoid, (b) globulins, (c) ovalbumin, (d) conalbumin, and (e) lysozyme.

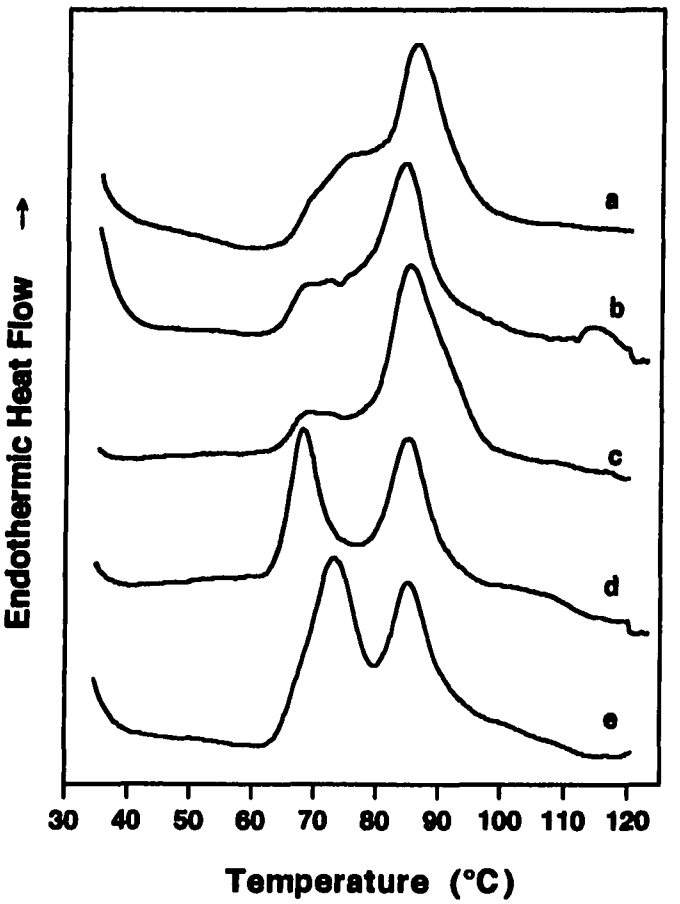

Fig. 3 - Differential scanning calorimetry thermograms of egg white spiked with its protein fractions (a) ovomucoid, (b) globulins, (c) ovalbumin, (d) conalbumin, and (e) lysozyme.

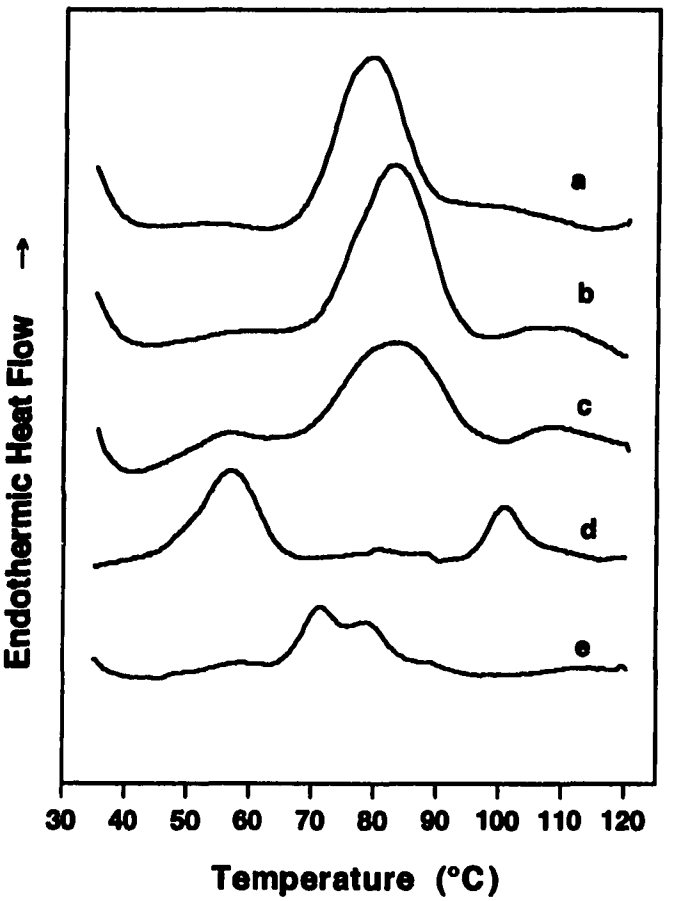

Fig. 4 - Differential scanning thermograms of bovine blood plasma protein fractions (a) albumin, (b) γ-globulin, (c) Cohn fraction III (56% β- and 44% γ-globulins), (d) fibrinogen, and (e) Cohn fraction IV-1 (60-80% α-, 15-40% β-, and 0-2% γ-globulins).

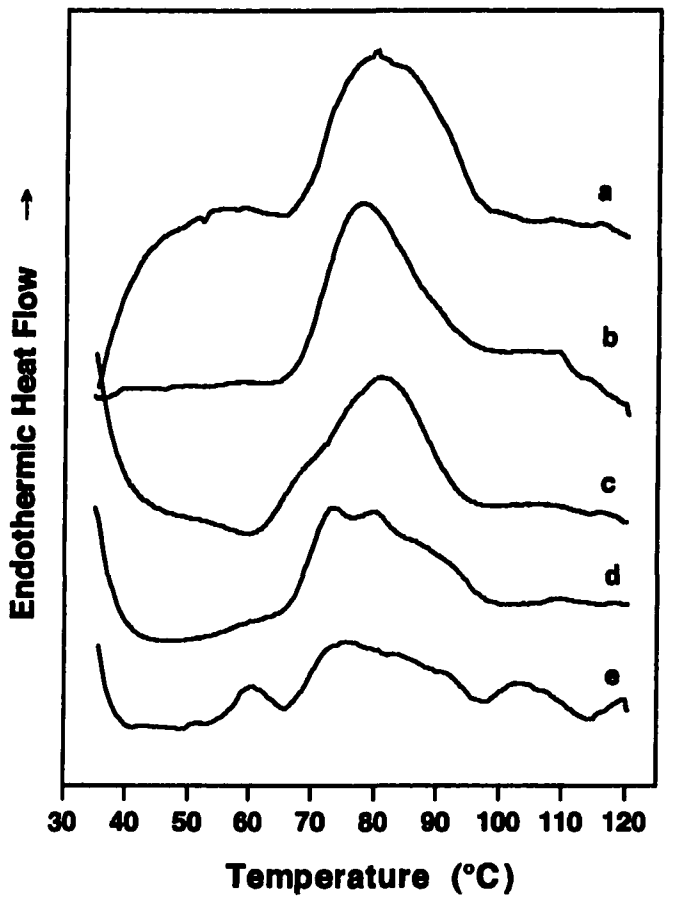

Fig. 5 - Differential scanning calorimetry thermograms of bovine blood plasma spiked with its protein fractions (a) Cohn fraction III (56% β- and 44% γ-globulins), (b) albumin, (c) γ-globulin, (d) Cohn fraction IV-1 (60-80% α-, 15-40% β-, and 0-2% γ-globulins), and (e) fibrinogen.

4. A MICRO METHOD FOR CAKE-BAKING[1]

A paper to be submitted to the *Journal of Cereal Chemistry*

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ABSTRACT

A micro method for cake-baking requiring only 5 g of flour in a high-ratio white layer-cake formulation was developed to test small quantities of experimental ingredients. Optimum mixing time, mixing speed, mixer design, and baking temperature were determined for the micro method. Among all the conditions tested, three mixing stages (4+2+2 min) at 670 rpm speed, using a flat paddle and baking at 191°C gave similar cake properties as AACC method 10-90. These are similar conditions to those used by AACC method 10-90, which uses 200 g of flour instead of 5 g. The 5- and 200-g cake baking methods were compared by using different levels of spray-dried egg whites and bovine blood plasma (0, 25, 50, 75, and 100% of the normal level of egg white protein). Correlation coefficients between the 5- and 200-g cake baking methods were determined for specific

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gravity, cake volume, and symmetry index measurements were 0.80, 0.99, and 0.98, respectively, for egg white and 0.84, 0.99, and 0.99, respectively, for blood plasma.

INTRODUCTION

The standard AACC (American Association of Cereal Chemists) baking methods have limited applications because of the relatively large quantity of ingredients required. Thus, micro-baking procedures have been developed by laboratories in which only limited amount of experimental materials are available. For example, Finney et al (1950) described micro cookie- baking procedure to evaluate the quality of flour obtained from strains developed in wheat breeding programs. A micro method for bread-making, using 10 g of flour, was developed by Shogren et al (1969) and improved by Shogren and Finney (1984) for fractionation and reconstitution studies. However, a micro method for cake-baking has not been described.

we plan to evaluate purified fractions of egg white and bovine blood plasma as substitutes for egg ingredients in cakes. Because these fractions are expensive and/or difficult to prepare in quantities sufficient for the current AACC method, a micro procedure for cake-baking using a high-ratio white layer-cake formulation is needed. This paper describes such a procedure. In the first part of this study, we used different mixing and baking conditions, including those of AACC 10-90, to determine the optimum conditions for a micro test for cake-baking. The second

part compares this micro procedure with AACC method 10-90.

MATERIALS AND METHODS

Materials

Cake ingredients and their sources were: cake flour (Super Cake Flour, moisture 13.6%, protein 7.5%, ash 0.4%; values reported on an as-is basis), Mennel Milling Co., Fostoria, OH; spray-dried egg whites (type p-110), Henningsen Foods, Inc., Omaha, NE; spray-dried beef blood plasma, AMPC, Inc., Ames, IA; and Betrkake emulsified shortening (mono- and diglycerides), Durkee Industrial Foods Corp., Cleveland, OH. The remaining ingredients, powdered sugar 6X, dry milk solids, double-acting baking powder, and salt were all local retail products.

Samples were analyzed by standard procedures for moisture, protein, and ash (AOAC 1984). Protein contents of samples were calculated using the nitrogen-to-protein conversion factor of 5.7 for flour and 6.25 for egg white and plasma.

Equipment

Mixing equipment for micro preparation of cake batter are shown in Figure 1. A 50-mL beaker, to be used as the mixing bowl, was modified so that the bottom was round like a bowl. A bowl holder was designed to prevent heat transfer from the hand to the batter through the glass bowl during mixing and to

provide more uniform mixing of the batter by allowing the bowl to turn during mixing. A miniature flat mixing paddle was fabricated from stainless steel to have the same edge structure and a shape similar to a regular cake paddle. A miniature whisk was purchased from a local department store. A laboratory stirrer (model SL 600, Fisher Scientific Co., Itasca, IL) was used to mix the batter (not shown in the picture). Non-stick surface muffin pans (cup size 7.6X3.2 cm, bottom diameter 5.2 cm) were used as baking pans.

Cake Preparation

To determine the optimum conditions, three mixing times, each consisting of three mixing stages(3+1+1, 4+2+2, and 5+3+3 min); two different speeds (518 and 670 rpm); two types of paddles (flat and whisk); and three baking temperatures (177°, 191°, and 204°C) were tested in a split-plot experimental design. Baking temperatures were assigned at random to the whole plots. Paddle type, mixing speed, and mixing time (12 combinations) were assigned at random to the subplots within each whole plot. The three baking temperatures resulted in 36 (12X3) cakes per replication. The experiment was replicated three times.

Procedure. The batter formula of AACC method 10-90 was used except that the amounts of ingredients were reduced by a factor of forty (Table I). All ingredients, except shortening, were weighed, sifted, and transferred into the mixing bowl and then shortening was added. Mixing and water additions were completed in three stages as in the AACC standard method. Ingredients and 60%

of the water (4 mL) were mixed at the lowest speed for 40 sec, and, thereafter, batter was mixed at a specified speed (518 or 670 rpm) for a specified time (3, 4, or 5 min). After adding the first half (1.4 mL) of the remaining water, the batter was mixed at the lowest speed for 20 sec and then mixed again at either 518 or 670 rpm for a specified time (1, 2, or 3 min). Finally, the remaining water (1.3 mL) was added and the batter was mixed at the lowest speed for 20 sec then mixing continued for 1, 2, or 3 min at either 518 or 670 rpm. The specific gravity of each batter was then determined.

Cake batter (17.5 g) was transferred to a greased and parchment paper-lined pan and baked at either 177° (350°F), or 191° (375°F), or 204°C (400°F) for 16.5, 13.5, or 10.5 min (until done).

Cake preparation procedures for comparison of micro and AACC standard methods. Cakes were prepared by using either the AACC approved method 10-90 (AACC 1983) or the micro method for cake-baking previously described. Because we had previously determined that acceptable cakes were produced by three stages of mixing (4+2+2 min) at 670 rpm speed, using a flat paddle, we used this set of conditions to prepare the micro cakes for the comparison study. Formulations used for both methods are given in Table I. AACC cake batter (425 g) was transferred into each of two 20.3 cm (8 in) diameter cake pans and baked at 191°C for 25 min in an electric oven. AACC baking trials were replicated two times. Micro cakes were baked at 191°C for 13.5 min and replicated three times.

To determine the correlation between the micro and AACC methods, cakes

were prepared with spray-dried egg whites and blood plasma at 0, 25, 50, 75, and 100% of the normal level of egg-white protein. The amount of water added in the first mixing stage was adjusted to compensate for the different moisture contents of blood plasma and egg whites.

Cake Quality - Physical Evaluation

Batter specific gravity was determined as the ratio of the weight of a standard container filled with batter to that of the same container filled with water. Micro cake-volume, by amaranth seed displacement, was measured after cooling for 1.5 hr at room temperature. Then, the micro cakes were stored in plastic bags overnight at 4°C and evaluated the next day for symmetry index (profile), crust color, and texture of the crumb. Volume of the AACC cakes were determined 2 hr after the cakes were removed from the oven by rapeseed displacement. Symmetry index and height were measured by using the layer cake measuring template as described in AACC method 10-91 (AACC 1983). For our micro cakes, the layer-cake measuring template was reduced to one fourth of its original size. Crust colors of cakes were measured with a Hunterlab Labscan Spectro colorimeter (Model LS-5100, Hunter Associates Laboratory, Reston, VA). *L* (lightness), *a* (redness), and *b* (yellowness) values were obtained after calibrating the instrument by using a white tile with standard values of $X = 78.46$, $Y = 83.14$, and $Z = 85.53$. Fluorescent light was used to illuminate the sample.

Statistical Analyses

Statistical analyses were performed with Statistical Analysis System (SAS, 1990). Multiple comparisons were performed after a preliminary *F* test. When the *F* test was significant at the 0.05 or 0.01 level, means were compared by the Least Significant Difference test. Correlation coefficients among cake measurements and between the two methods for cake measurements were calculated by using the PROC CORR procedure.

RESULTS AND DISCUSSION

Effects of Mixing and Baking Conditions on Micro Cakes

Batter specific gravity was significantly affected ($P < 0.01$) by paddle type, mixing time, and mixing speed (Table II). The whisk produced batters with lower specific gravities (0.804 g/cm^3) than the flat paddle (0.825 g/cm^3). The shortest mixing time (3+1+1) gave the highest specific gravity (0.838 g/cm^3). Specific gravities of the other two mixing times (4+2+2 and 5+3+3) were not significantly different (0.803 g/cm^3). Mixing at 518 rpm also resulted in batters with higher specific gravities (0.825 g/cm^3) than mixing at 670 rpm (0.804 g/cm^3). These results indicate that the whisk, higher mixing speed, and longer mixing times incorporated more air into the batter during mixing.

There were also mixing time X paddle type, mixing time X speed, and paddle type X speed interaction effects on batter specific gravity (Table II). The

difference between the mean values of the two paddle types decreased with increasing mixing time, and at 5+3+3 min mixing both the flat paddle and the whisk gave almost the same batter specific gravity. The influence of mixing speed with mixing time also followed the same trend. Speed had greater effect on the ability of the whisk to incorporate air into the batter than that of the flat paddle.

As with batter specific gravity, cake volume was also significantly affected ($P < 0.01$) by paddle type, mixing time, and mixing speed (Table II). The mean differences for volume by the LSD test indicated that volume significantly increased with increasing mixing times. The whisk produced higher volumes (45.6 cm^3) than the flat paddle (45.1 cm^3). Volumes of the cakes (45.8 cm^3) prepared at the higher speed were larger than those of the cakes (44.9 cm^3) prepared at the lower speed. Although the *F* test was not significant for baking temperature, the greatest volume was obtained when cakes were baked at 191°C, and the next greatest volume at 204°C.

Symmetry index (crowning profile) and height of the cakes significantly increased as baking temperature and mixing time increased (Table II). Mixing speed also significantly affected cake height; cakes mixed at 670 rpm were higher than cakes mixed at 518 rpm.

From observations, cakes mixed for 3+1+1 min gave coarse, dense crumb structure. Cakes mixed for 4+2+2 min and 5+3+3 min were similar, having fine, light-crumb cell structures.

Relationships among cake measurements. Correlation coefficients (r) among cake measurements were calculated for each baking temperature and for the composite data over all temperatures (Table III). As expected, based on experience with the AACC cake method, the specific gravity of the micro cake batter was significantly and negatively correlated with cake volume and height. An inverse relationship between specific gravity and volume has also been observed by Dunn and White (1939) and Ellinger and Shappeck (1963) for standard-size cakes. Strong positive correlation coefficients among cake volume, symmetry index, and height (at the center) were observed in our micro cake trials.

Selection of Optimum Mixing and Baking Conditions for Micro Cakes

From the previous study, three sets of mixing and baking combinations (4+2+2 min, whisk, 670 rpm, 350°F; 4+2+2 min, flat, 670 rpm, 375°F; and 5+3+3 min, whisk, 670 rpm 375°F) were chosen as potential optimum micro cake conditions and tested further (Table IV). This time, cakes were prepared with egg white, blood plasma, and without any protein to see which of these micro mixing and baking combinations would give similar results to AACC method 10-90.

In all three mixing and baking combinations, volumes of the cakes prepared without protein (for egg white substitution) were significantly lower than those in which egg white and blood plasma were used. There were no significant differences between egg white and blood plasma cakes. However, the volume of the egg white cakes mixed with the flat paddle trended to be higher (not

significantly) than those made with blood plasma. Because the flat paddle produced results similar to the AACC method 10-90, the flat paddle was chosen for the subsequent comparison study.

Comparison of the Micro Method with AACC Method 10-90

Comparison of the methods were made by preparing cakes with spray-dried egg white and bovine blood plasma at the levels of 0, 25, 50, 75, and 100% of the normal level of egg white protein in the formula. Specific gravities, volumes, and symmetry indices of the cakes prepared by using AACC and micro cake-baking methods are shown in Figures 2-4. Specific gravities of the cake batters slightly trended to lower values as the levels of egg white and blood plasma proteins increased (Fig. 2), but these differences were not significant at the 0.05 level in either the AACC method or in the micro method for both egg white and blood plasma cakes. This may be because air is largely incorporated in the fat phase during the mixing process (Carlin 1944, Bell et al 1975). Wootton et al (1967) report soluble proteins are directly involved in air incorporation only when the batter contains fluid shortenings.

Cake volume decreased as the levels of spray-dried egg white and blood plasma decreased in both methods (Figs. 3, 5, and 6). Reducing the egg white level from the 100% to the 75% level did not significantly reduce cake volumes in either method. Likewise, the 75% egg white level produced cake volumes similar to the 50% egg white level in both methods. The volumes of plasma cakes at the

100 and 75% levels were not significantly different in the AACC method, but were significantly different in the micro method.

The symmetry index (crown or profile) increased as the level of spray-dried egg white and blood plasma increased in both the AACC and micro methods (Figs. 4, 5, and 6). In both methods, the crowning profiles of egg white cakes increased rather gradually with the level of protein through 25-100% (Fig. 4), whereas the crowning profile of the blood plasma cakes increased significantly between 25-50% levels and then remained similar thereafter in either method (Fig. 4).

Correlations with standard method. Fig. 7 shows the relationships between the specific gravities, cake volumes, and symmetry indices (profiles) for egg white and blood plasma cakes prepared by using the AACC standard method and by using the micro method. Highly significant correlation coefficients ($P<0.01$) between cake volumes ($r = 0.99$) and profiles ($r = 0.98$ and 0.99) for the two methods suggest that the micro method provides a simple and reliable alternative to the AACC standard method.

Comparison of Egg White and Plasma Cakes

Egg white and plasma proteins were compared in both methods to see if we could come up with the same conclusions irrespective of the method used. When cakes were prepared by using the AACC standard method, volumes of the plasma cakes at the 100% protein level were about 3% lower than egg white cakes at the same level, but this difference was not significant at the 0.05 level. This is smaller

than the 7% reduction in cake volume previously reported by Lee et al (1991). Volumes of the cakes containing 100% blood plasma were not significantly different from cakes containing 50-75% egg whites (Fig. 8). Likewise, at 75% of the normal egg white level, plasma cakes were not significantly different from cakes made with 25-75% egg white. These results confirm that blood plasma protein is slightly less efficient than egg white protein is used at the same levels.

When cakes were prepared by using the micro method, blood plasma protein at the 100% level produced about the same volume as did egg white protein. As with AACC 10-90, the volume of blood plasma cake at the 100% level was not significantly different from the volumes of egg white cakes at the 50-75% levels (Fig. 8). Cakes made with blood plasma protein at the 75 and 50% levels were equivalent to cakes made with egg white protein at the 50% and 25% levels, respectively. The micro-baking results also show that blood plasma protein was less efficient than egg white protein at the same levels as was shown when using the AACC 10-90 method. In both methods, egg white gave more crowned profiles than blood plasma at the same protein levels. However, blood plasma cakes prepared with the micro method produced more crowned profile than blood plasma cakes prepared with the AACC method.

Crust color of cakes. Crust colors of blood plasma cakes were significantly darker than those of egg white cakes by either methods (Table V). Because crust colors of micro cakes were lighter than standard cakes, another experiment was undertaken to determine a baking time for micro-baking that would give a similar

crust color to standard cakes. Cakes were prepared with 100% egg white and baked at 191°C for 13.5, 14.5, 15.0, 15.5, 16.0, 16.5, and 17.0 min. Cake volume and crust color were determined. In general, cake volume decreased with longer baking times, but differences were not significant at the 0.05 level. Similar crust colors to the standard cake were obtained when micro cakes were baked for 15.5 min.

CONCLUSIONS

A micro method for cake-baking, which used a high-ratio white layer cake formulation, was developed to test expensive experimental ingredients. Acceptable cakes were produced by using three-stage mixing (4+2+2 min) at 670 rpm mixing speed, using a flat paddle, and baking at 191°C. Cake property measurements obtained from this micro method were highly correlated with those prepared by the AACC method 10-90. This micro method reduced the amount of ingredients by forty times compared with the AACC standard method. Therefore, the micro method for cake-baking is applicable for use in situations in which there are only a limited amount of ingredients available and also offers a convenient, simple, and rapid alternative to the AACC standard method.

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TABLE I

High-Ratio White Layer Cake Formulations for AACC and Micro Cake-Baking Methods[a]

Ingredients	AACC Method	Micro Method
Cake flour	200	5.00
Sugar	280	7.00
Shortening	100	2.50
Non-fat dry milk	24	0.60
Dried egg whites	18	0.45
Salt	6	0.15
Baking powder	10	0.25
Water	270	6.70
Stage 1	162	4.00
Stage 2	54	1.40
Stage 3	54	1.30

[a]All values are in grams.

TABLE II

Analysis of Variance for Properties of Micro Cakes Prepared with Different Mixing and Baking Conditions

Source	df[b]	F-Values[a]			
		Specific Gravity	Volume	Symmetry Index	Height
Replicate (REP)	2	17.94**	3.86*	ns	ns
Temperature (TEMP)	2	ns	ns	31.14**	30.25**
REP X TEMP (error A)	4				
Paddle type (PTY)	1	29.54**	17.26**	ns	ns
Mixing time (MTM)	2	36.05**	86.99**	13.34**	26.30**
PTY X MTM	2	4.71*	ns	ns	ns
Speed (SP)	1	28.83**	63.09**	ns	11.97**
PTY X SP	1	4.32*	ns	ns	ns
MTM X SP	2	5.43**	ns	ns	ns
PTY X MTM X SP	2	ns	ns	ns	ns
TEMP X PTY	2	ns	ns	ns	ns
TEMP X MTM	4	ns	ns	ns	ns
TEMP X PTY X MTM	4	ns	2.77*	ns	ns
TEMP X SP	2	ns	ns	ns	ns
TEMP X PTY X SP	2	ns	ns	ns	ns
TEMP X MTM X SP	4	ns	ns	ns	ns
TEMP X PTY X MTM X SP	4	ns	ns	ns	ns
Error B	66				

[a]* and ** significant at $P<0.05$ and $P<0.01$, respectively. ns = Not significant $P>0.05$.
[b]Degrees of freedom.

TABLE III

Correlation Coefficients Among Micro Cake Measurements within Baking Temperature and for Composite Data (all temperatures)

	171°C[b]			191°C[b]			204°C[b]			Composite Data[c]		
Variables[a]	VOL	SYM	HT	VOL	SYM	HT	VOL	SYM	HT	VOL	SYM	HT
SPGR	-0.68**	-0.27	-0.42*	-0.83**	-0.42*	-0.47**	-0.73**	-0.22	-0.34*	-0.71**	-0.15	-0.27**
VOL		0.45**	0.63**		0.49**	0.67**		0.40*	0.50**		0.33**	0.49**
SYM			0.88**			0.82**			0.90**			0.92**

[a]SPGR = specific gravity; VOL = volume; SYM = symmetry index; HT = height at the center.
[b]n = 36.
[c]n = 108.
* and ** significant at $P<0.05$ and $P<0.01$, respectively.

TABLE IV

Effect of Preparation Conditions on Volume of Micro Cakes

Mixing Time (min)	Paddle Type	Mixing Speed (rpm)	Baking Temperature (F)	Protein Used	Volume (cm^3)
4 + 2 + 2	Whisk	670	350	None	42.5 b
4 + 2 + 2	Whisk	670	350	Plasma	46.3 a
4 + 2 + 2	Whisk	670	350	Egg white	46.2 a
4 + 2 + 2	Flat	670	375	None	42.1 b
4 + 2 + 2	Flat	670	375	Plasma	46.4 a
4 + 2 + 2	Flat	670	375	Egg white	47.4 a
5 + 3 + 3	Whisk	670	375	None	42.4 b
5 + 3 + 3	Whisk	670	375	Plasma	48.7 a
5 + 3 + 3	Whisk	670	375	Egg white	48.3 a

[ab]Means with the same letter within the same mixing and baking conditions are not significantly different at the 0.05 level.

TABLE V

Crust Colors of Cakes Baked with Micro and AACC Cake-Baking Methods Using Different Levels of Egg Whites and Blood Plasma[x]

	Micro Cake-Baking Method			AACC Cake-Baking Method		
Protein and Level	**"L"**	**"a"**	**"b"**	**"L"**	**"a"**	**"b"**
None (0%)	81.2 ± 0.1 a	4.6 ± 0.3 d	26.7 ± 0.0 b	67.4 ± 0.2 a	9.5 ± 0.3 b	25.5 ±0.5 ab
Egg white (50%)	74.7 ± 0.8 b	8.4 ± 1.2 c	28.2 ± 0.8 a	66.9 ± 3.3 a	10.5 ± 0.7 b	26.9 ± 1.0 a
Egg white (100%)	70.9 ± 0.1 c	10.6 ± 0.7 b	28.6 ± 0.1 a	65.4 ± 2.0 a	12.0 ± 0.7 a	27.4 ± 0.3 a
Blood plasma (50%)	61.6 ± 0.6 d	12.9 ± 0.1 a	25.4 ± 0.6 c	58.5 ± 1.6 b	12.2 ± 0.2 a	23.4 ± 1.5 bc
Blood plasma (100%)	58.4 ± 1.2 e	13.9 ± 0.0 a	24.4 ± 0.4 c	52.3 ± 0.5 c	13.4 ± 0.6 a	22.1 ± 1.1 c

[x]Mean values of two replicates, ± one standard deviation.
[abcde]Means with the same letter within a column are not significantly different at the 0.05 level.

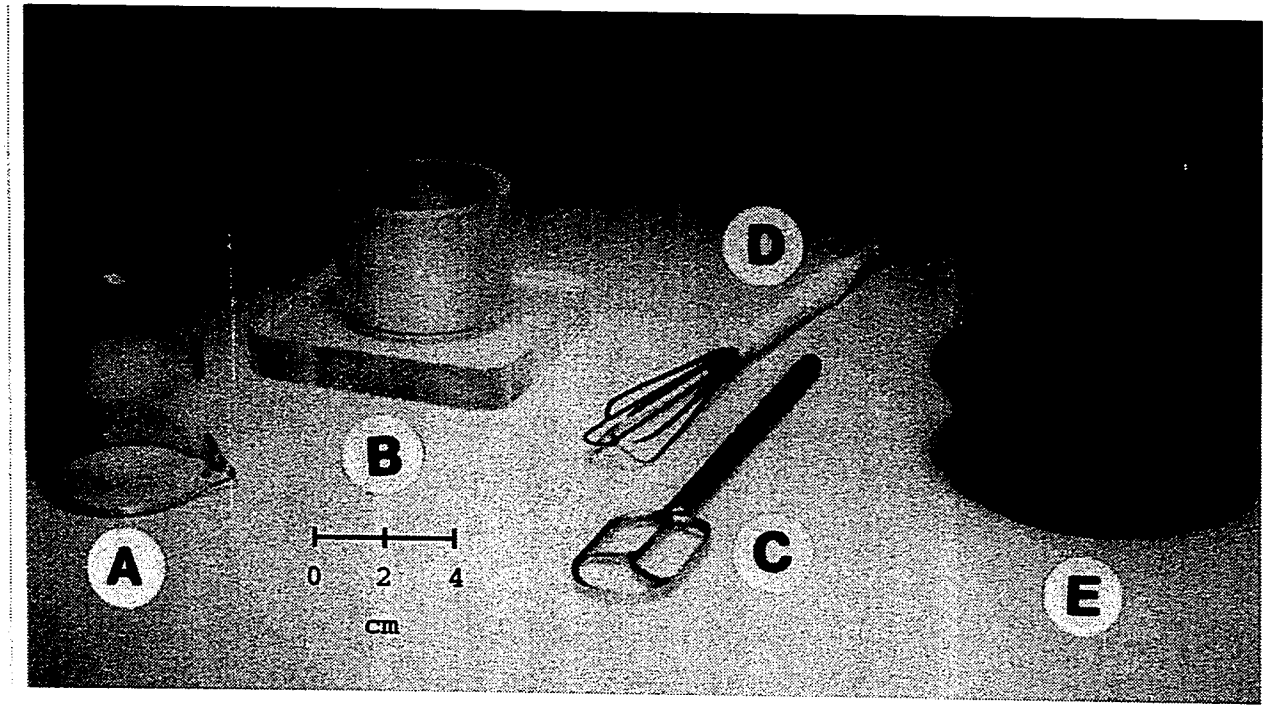

Fig. 1. Mixing equipment for micro cake baking. A, mixing bowl; B, bowl holder; C, flat paddle; D, whisk; and E, baking pan.

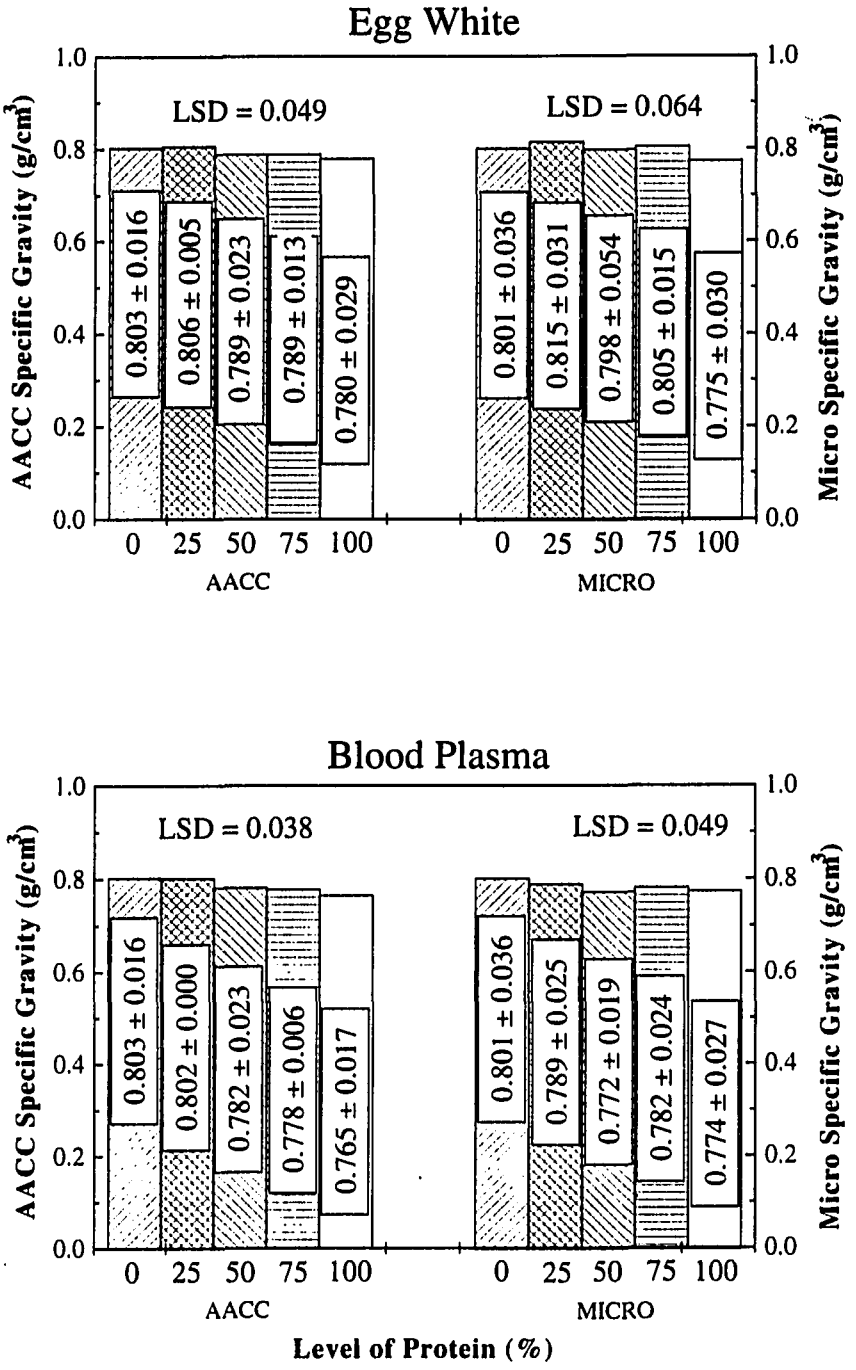

Fig. 2. Specific gravities of cakes prepared with different levels of spray-dried egg white (top) and bovine blood plasma (bottom) using micro and AACC methods.

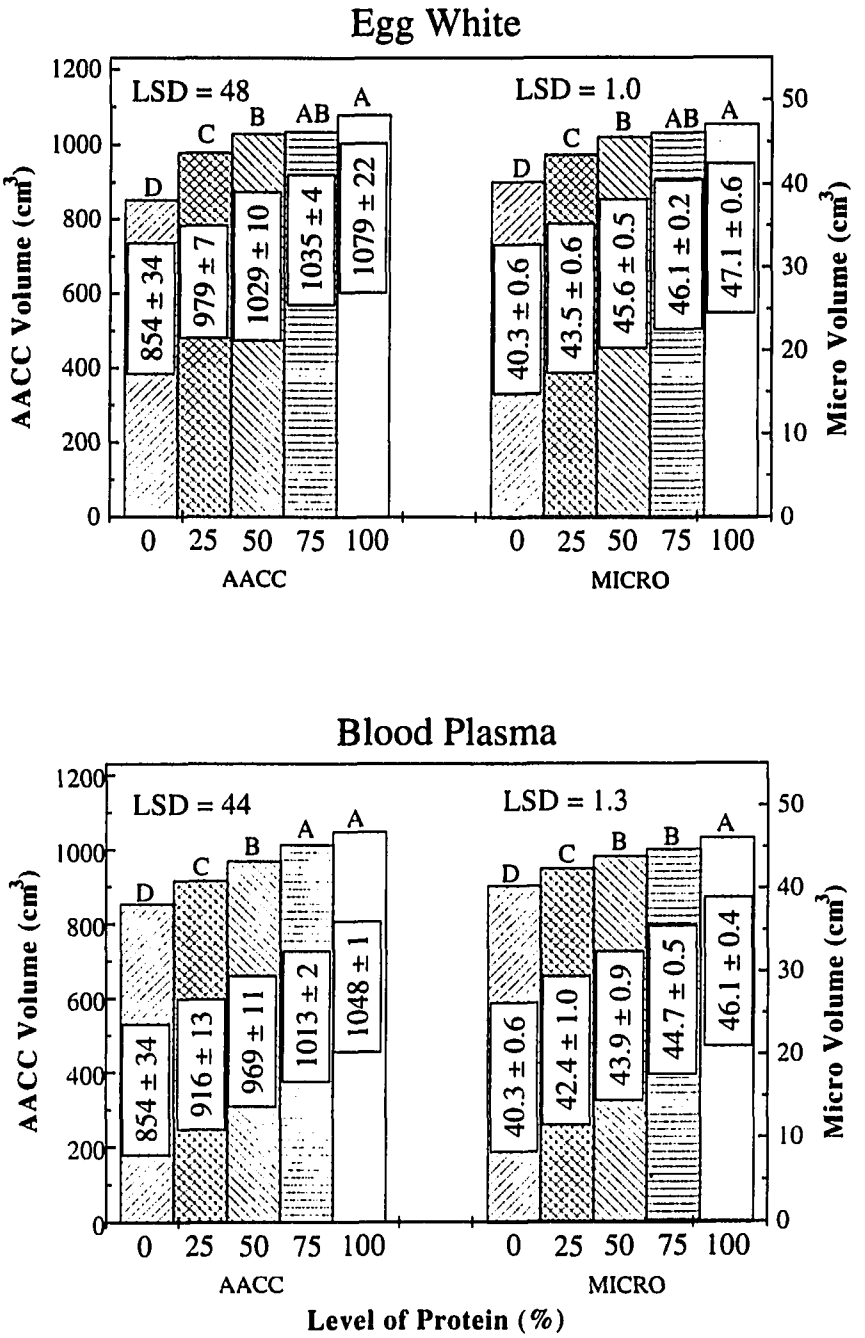

Fig. 3. Volume of cakes prepared with different levels of spray-dried egg white (top) and bovine blood plasma (bottom) using micro and AACC methods.

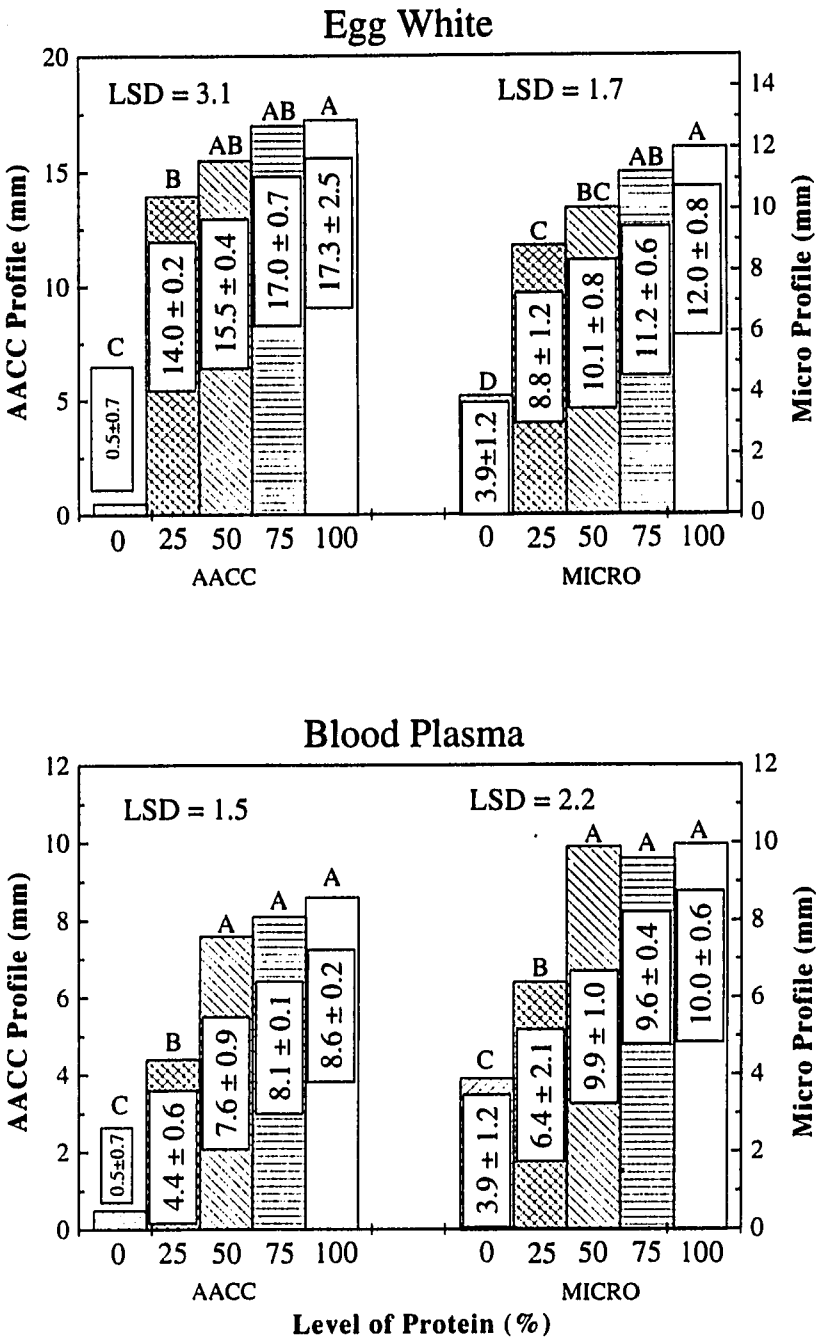

Fig. 4. Profiles of cakes prepared with different levels of spray-dried egg white (top) and bovine blood plasma (bottom) using micro and AACC methods.

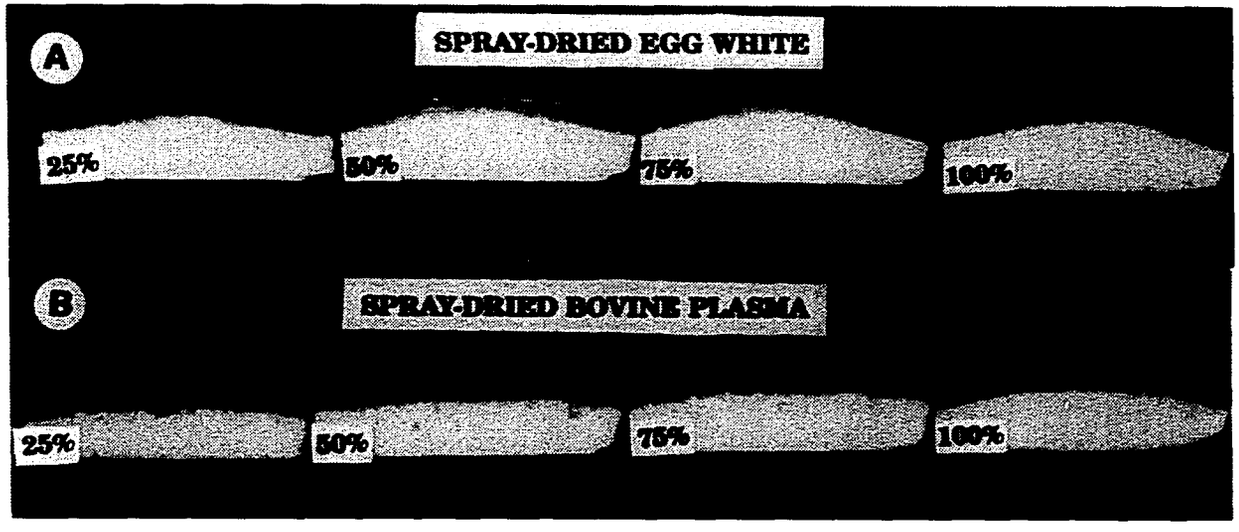

Fig. 5. Cakes baked with AACC method 10-90 using different levels of spray-dried egg white (A) and bovine blood plasma (B) proteins.

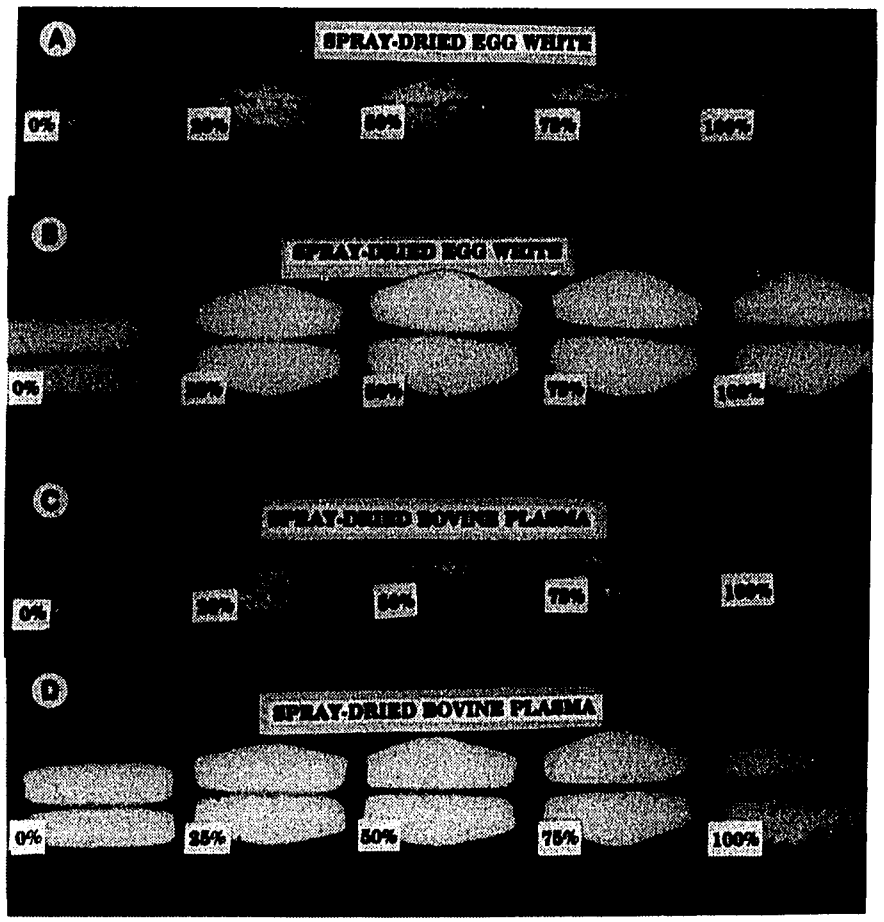

Fig. 6. Cakes baked with micro cake-baking method using different levels of spray-dried egg white (A, B) and bovine blood plasma (C, D) proteins.

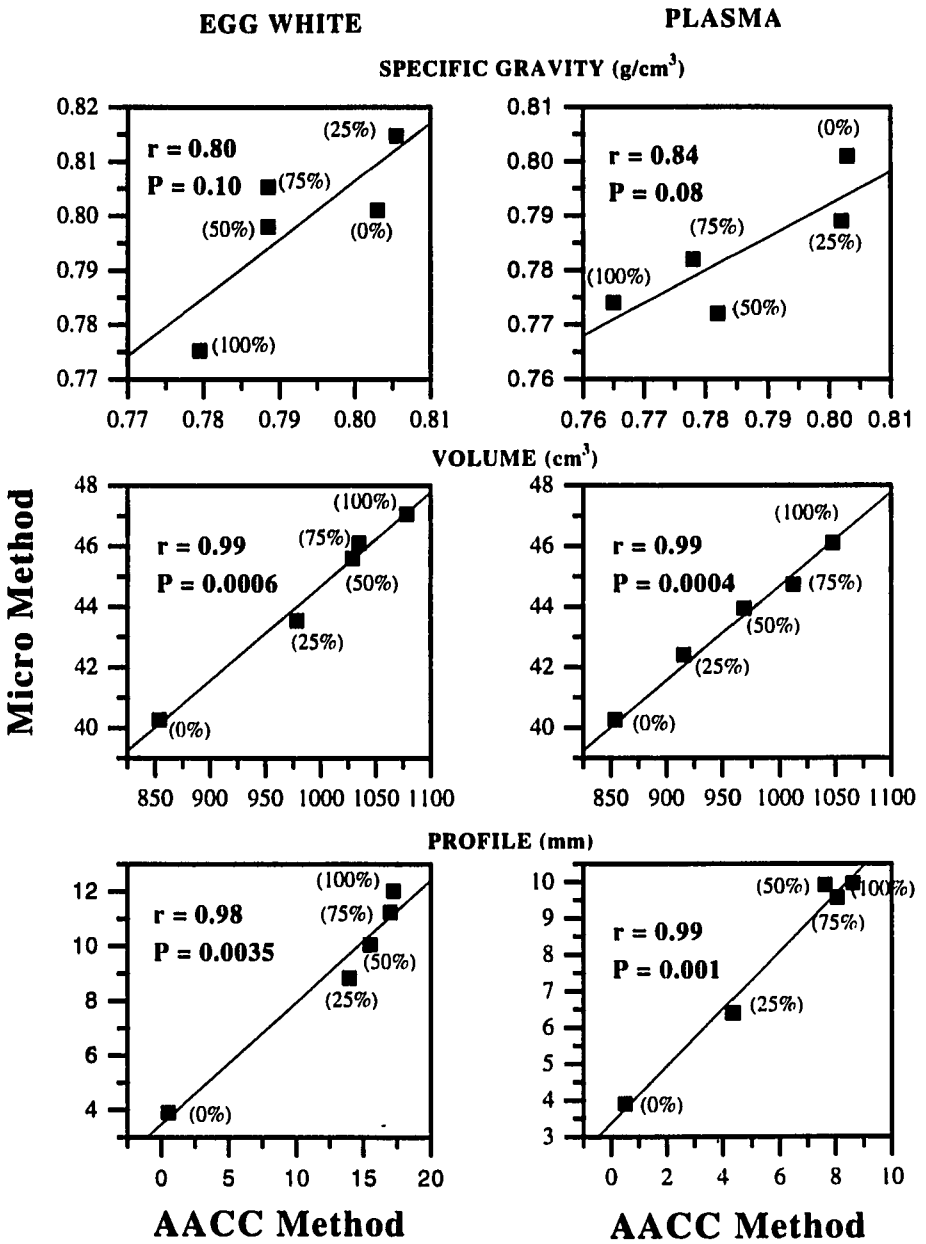

Fig. 7. Relationships between batter specific gravity (top), cake volume (middle), and cake profile (bottom) determined by AACC method 10-90 and by the micro method using egg white (left) and blood plasma (right) at the levels of 0, 25, 50, 75, and 100% of the normal level of egg white protein in the formula.

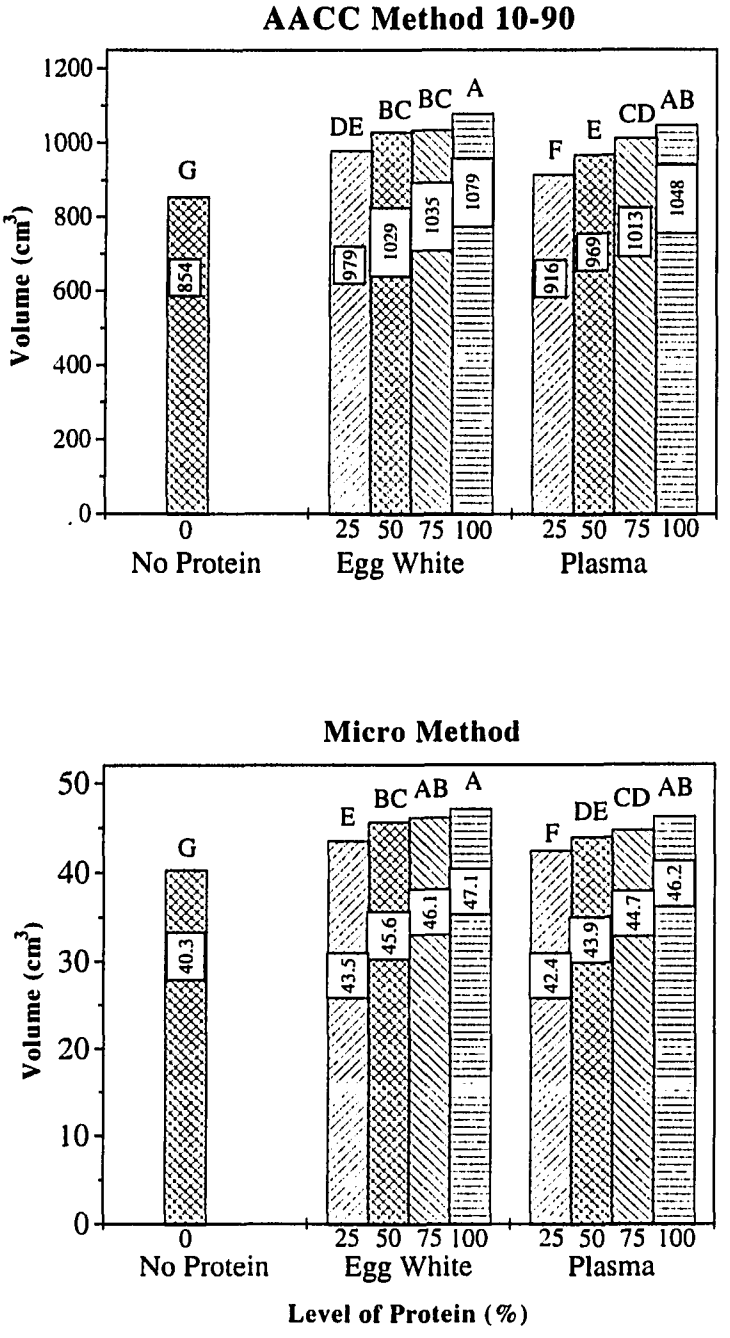

Fig. 8. Effects of different levels of egg white and bovine blood plasma on AACC cake volumes (top) and on micro cake volumes (bottom).

5. CAKE-BAKING PROPERTIES OF EGG WHITE, BOVINE BLOOD PLASMA, AND THEIR PROTEIN FRACTIONS [1]

A paper to be submitted to the *Journal of Cereal Chemistry*

Maide Özbay Raeker[2] and Lawrence A. Johnson[2,3]

ABSTRACT

We have previously shown that egg white and blood plasma proteins have different heat denaturation, foaming, and emulsification properties (Raeker and Johnson 1994a). In the present study, we investigated cake-baking properties of egg white, blood plasma, and their component proteins in a high-ratio white layer cake formulation and described the relationship between the above functional properties of the proteins and cake quality. Egg white produced slightly larger volume, significantly more crowned profile, and finer texture than did blood plasma. Among egg white proteins, cakes made with globulins had the highest volume, finest texture, and most crowned profile compared with cakes made with other fractions. Ovalbumin produced similar volumes and profiles as egg white, but the texture was coarser. Ovomucoid did not coagulate during baking, and cakes made

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with conalbumin and lysozyme had less volume and very dense crumb structures. Fibrinogen produced the smallest cake volume among the blood plasma proteins. Albumin, the major protein in blood plasma, had inferior cake-baking properties to whole blood plasma, whereas γ-globulin had superior properties. α-Globulin produced cakes with high volumes but coarse textures. Separation of fibrinogen from blood plasma increased cake volumes and profiles. The correlation coefficient between cake volume and denaturation peak temperatures of the protein was significant ($r = 0.944$, $P = 0.001$). Foaming and emulsification properties did not significantly affect cake volume.

INTRODUCTION

Bovine blood plasma is a significant source of high quality proteins and exhibits functional properties of utility in food product formulations. Spray-dried beef blood plasma protein concentrate is now commercially available in food-grade form and is a likely low-cost candidate to replace egg products in baked goods.

Brooks and Ratcliff (1959) found that 33% of whole egg could be replaced with plasma in cakes other than sponge cakes. Reportedly, these cakes prepared with blood plasma were equal in volume, texture, crumb, and flavor to those of cakes made with whole egg. Khan et al (1979) reported that blood plasma protein isolate could replace 30% of the egg white solids in angel food cakes. Johnson et al (1979) evaluated bovine blood plasma in high-ratio layer cakes and found that cakes made with fresh lyophilized plasma were substantially the same as those

made with egg products. However, functional properties and replacement efficiency of dried blood plasma solids decreased as storage time increased. In more recent study of Lee et al (1991), nearly equivalent performance was achieved when egg white protein was replaced by 1.1 times as much blood plasma protein in high-ratio white layer cakes.

In the aforementioned studies, full replacement with blood plasma did not quite produce the same volume, texture, and profile as egg white. Cakes made with plasma had slightly less volume, coarser crumb structure, and a flatter profile than those made with egg white. However, partial replacement with blood plasma did produce acceptable cakes, and there is potential for further improvement by producing fractions rich in more functional proteins.

Functional properties of egg white, plasma, and their component proteins were investigated by Raeker and Johnson (1994a), and considerable differences in the heat denaturation temperatures of egg white and blood plasma proteins were noted. Blood plasma proteins denatured at lower temperatures than both ovalbumin (the major protein in the egg white) and globulins. Differences were also observed in foam stabilities between blood plasma and egg white. The stability of blood plasma foam was lower than that of egg white (Tybor et al 1975, Khan et al 1979, Raeker and Johnson 1994a). However, among blood plasma fractions, serum albumin, fibrinogen, and Cohn fraction IV-1 (predominantly α-globulin) were reported to have good foaming capacities and stabilities (Raeker and Johnson 1994a).

Cake-baking properties of egg white component proteins were investigated in angel food cakes (MacDonnel et al 1955, Johnson and Zabik 1981), but to our knowledge, no study has been done on baking properties of blood plasma component proteins. The objectives of this study were to determine cake-baking properties of egg white, blood plasma, and their component proteins in high-ratio white layer cakes; and to determine the relationships between the functional properties of these proteins and the qualities of cakes made with these proteins.

MATERIALS AND METHODS

Materials

Fresh eggs were obtained from the Iowa State University Poultry Farm. The whites were separated from the yolk and blended in a Waring Blendor for 40 sec. The mixing speed was reduced using a Variac transformer and the slowest speed was used for homogenization. The blended fresh egg white was freeze-dried. Frozen blood plasma was obtained from AMPC, Inc., Ames, IA; thawed at 4°C; and divided into two parts. Part was freeze dried; the other part was centrifuged at 613 x *g* for 15 min at 4°C in a Beckman J2-21 Refrigerated Centrifuge with JA-14 rotor (Beckman Instruments Inc., Palo Alto, CA). After that, half of the supernatant was freeze dried and the other half was spray dried using a Yamato Pulvis Mini-spray model GA-31 at an air inlet temperature of 90°C and an air outlet temperature of 50°C. Commercially spray-dried beef blood plasma, albumin-rich,

and IgG-rich spray-dried blood plasma fractions were provided by AMPC, Inc., Ames, IA. Spray-dried egg white (type P-110) was obtained from Henningsen Food, Inc., Omaha, NE. The following protein fractions of bovine blood plasma and egg white were purchased from Sigma Chemical Co., St. Louis, MO: bovine serum albumin (product no. A7638, 99% purity); γ-globulins (product no. G5009, 99% purity); Cohn fraction IV-1 (product no. G8512, 60-80% α-, 15-40% β-, 0-2% γ-globulins); Cohn fraction III (product no. G4633, 56% β- and 44% γ-globulins); fibrinogen (product no. F4753, 95% of protein clottable); ovalbumin (product no. A5503, 99% purity); conalbumin (product no. C0755, iron free); Ovomucoid (product no. T2011); lysozyme (product no. L6876); and egg white globulins (product no. EG, substantially free of albumin).

Cake flour (Super Cake Flour, moisture 13.6%, protein 7.5%, ash 0.4%; values reported on an as-is basis) was obtained from Mennel Milling Co., Fostoria, OH and Betrkake emulsified shortening (mono- and diglycerides) was purchased from Durkee Industrial Foods Corp., Cleveland, OH. All other ingredients, powdered sugar 6X, non-fat dry milk solids, double-acting baking powder, and salt were local retail products.

Protein Determination

Protein contents of blood plasma, egg white, and albumin-rich and IgG-rich spray-dried blood plasma fractions were determined by the macro-Kjeldahl method (AOAC 1984), using a nitrogen conversion factor of 6.25. The protein contents of

fractions were calculated from the absorbance measured with a spectrophotometer. The following optical factors ($A^{1\%}_{1cm}$) were used: bovine serum albumin, 6.67 at 279 nm (Aoki et al 1973); γ-globulin, 13.5 at 275 nm (Rueg et al 1977); ovalbumin, 7.12 at 280 nm (Glazer et al 1963); conalbumin, 11.3 at 280 nm (Glazer and McKenzie 1963); lysozyme, 26.3 at 281 nm (Sophianopoulos et al 1962). Protein contents of ovomucoid and fibrinogen were determined by using the Biuret method (Gornall et al 1949) with bovine serum albumin as a standard. Protein contents of egg white globulins and Cohn fraction IV-1 were determined by using the Biuret method with γ-globulin as a standard. The protein content of Cohn fraction III was estimated by the micro-Kjeldahl method (AOAC 1984).

Cake Preparation

Cakes were prepared by using the micro method for cake-baking (Raeker and Johnson 1994b). The amounts of each cake ingredient used in the formula were as follows: 5 g, cake flour; 7 g, sugar; 2.5 g, shortening; 0.6 g, non-fat dry milk; 0.45 g, dried egg whites; 0.15 g, salt; 0.25 g, baking powder; and 4.0, 1.4, and 1.3 g, water in mixing stages 1, 2, and 3, respectively. The dried egg white in the formula was replaced by equivalent amounts of protein from the various forms of blood plasma, egg white, and protein fractions. Cakes were baked at 191°C (375°F) for 15.5 min in a rotary electric oven. Specific gravity of the batter was determined as the ratio of the weight of a standard container filled with batter to that of the same container filled with water. Cake volume and profile (symmetry

index) were determined as previously described (Raeker and Johnson 1994b). Baking trials were replicated three times.

Functional Properties of Albumin and IgG Rich Plasma Fractions

Heat denaturation properties of the proteins were determined by using the Differential Scanning Calorimetry (DSC) method described in the previous article (Raeker and Johnson 1994a). Three determinations were made on each protein.

Foaming and emulsification properties of proteins were determined as previously described (Raeker and Johnson 1994a) by using a 1% protein solution in 0.06M phosphate buffer containing 0.11M NaCl at pH 7. Determinations were done in duplicate for foaming and in triplicate for emulsification properties.

Statistical Analysis

The data were analyzed with Statistical Analysis System (SAS 1990). When the F test was significant at the 0.05 or 0.01 level, means were compared by the Least Significant Difference test. Multiple linear regression analyses were run by using the stepwise procedure with forward, backward, and maxR options between cake volume and the functional properties of proteins (Raeker and Johnson 1994a).

RESULTS AND DISCUSSION

Egg White and Blood Plasma Cakes

When frozen blood plasma is allowed to thaw at 5°C, fibrinogen stays in a solid phase and can be separated by centrifugation (Ware et al 1947). This fibrinogen-free plasma is called serum. Therefore, in the present work we term the centrifuged plasma as serum and after spray and freeze drying, as spray- and freeze-dried serum to differentiate them from blood plasma samples.

Volume and crowning profile (symmetry index) of the cakes prepared with egg white, blood plasma, serum, and their fraction proteins are given in Table I. Spray- and freeze-dried egg whites gave cakes with similar volumes and crowning profiles. Cakes made with egg whites had slightly higher volumes than cakes made with freeze- and commercial spray-dried blood plasma. However, cakes made with egg whites produced significantly more crowning than those produced with blood plasma samples (Fig. 1). Freeze-dried blood plasma produced cake with properties similar to those of commercial spray-dried blood plasma. Removal of fibrinogen from blood plasma increased cake volume and the crowning profile of cakes; values were similar to those made with egg white (Table I, Fig. 2).

In general, blood plasma cakes had coarser and darker crumb structures than those of egg white cakes. Freeze-dried blood plasma gave the coarsest crumb structure. Commercial spray-dried blood plasma produced denser crumb structure than serum samples, which had loose, fragile, and crumbly texture.

Cakes made with spray-dried egg white had smoother texture than cakes made with freeze-dried egg white.

Cakes Prepared with Egg White Protein Fractions

Significant differences were observed in the volumes of the cakes when different protein fractions were used (Table I, Fig. 3). In agreement with Johnson and Zabik (1981), egg white globulins produced cakes with the largest volumes followed by ovalbumin. In comparison with whole egg white, ovalbumin produced similar cake properties, whereas egg white globulins produced significantly higher volume and more crowned profile. Previously, Johnson and Zabik (1981) reported that angel food cakes made with lysozyme and conalbumin were smaller in volume because of less air inclusion and instabilities of the foams. However, in our study, we attributed the small cake volumes of conalbumin and lysozyme to their lower denaturation temperatures (Raeker and Johnson 1994a). Early denaturation of these proteins at the bubble surface probably decreased film elasticity and prevented cake expansion during baking.

Ovomucoid produced cakes with very small volume, even less than cakes prepared without any protein. Cakes fell in the middle, which resulted in negative values for profile crown. Ovomucoid cakes expanded normally during baking, but then collapsed in the last stage of baking. This shows that ovomucoid did not coagulate during baking in the oven. Lack of heat coagulation of the protein film around the air cells at maximum expansion was likely the reason for small cake

volumes. Ovomucoid has been reported to remain soluble in dilute solutions when its biological activity is completely destroyed by heat (Lineweaver and Murray 1947). A 20% solution of ovomucoid was found to gel between pH 8 to 10 if placed in a boiling water bath for 5 to 15 min. Because the pH of the cake batter is around 7, our results show that ovomucoid remains soluble during baking, and therefore, by itself, could not support cake structure. However, in egg white, ovomucoid, because of its resistance to heat coagulation during baking, may increase flexibility and viscoelasticity of the films around the gas bubbles and contribute to the stability of egg white foam during baking.

Foaming properties of the proteins played significant roles in determining the texture of the finished cakes. From observations, cakes prepared with lysozyme had very dense crumb structures followed by conalbumin. The previous study of Raeker and Johnson (1994a) showed that lysozyme did not produce foam. Conalbumin and ovomucoid had low foaming capacities and stabilities. Ovomucoid cakes had very coarse, rough crumb cell structure. Ovalbumin and globulins produced similar foaming capacities; however, egg white globulin foam exhibited very good stability, whereas ovalbumin foam was unstable. Consequently, ovalbumin cakes had somewhat nonuniform cell structure with bigger gas cells, whereas globulins produced cakes with good texture.

Cakes Prepared with Blood Plasma Protein Fractions

Fibrinogen, the most heat-sensitive protein in plasma (Raeker and Johnson 1984a), produced very small cake volume and flat crowning profile (Table I, Fig. 3). The early denaturation of the protein films surrounding the air cells prevented them from expanding as the temperature increased during baking.

Albumin, the major protein in blood plasma, produced cakes with slightly less volume than did whole blood plasma, whereas globulin fractions of plasma produced larger cake volumes with more crowned profiles than did whole blood plasma. Among the globulins, Cohn fraction IV-1 (primarily α-globulins) produced the largest volume followed by γ-globulins and Cohn fraction III (mixture of β- and γ-globulins). In previous research, γ-globulins were the most heat stable proteins, followed by Cohn fraction III (Raeker and Johnson 1994a). However, α-globulins are a group of 20 glycoproteins, and the amount of carbohydrate varies from protein to protein. For example α_1- acid glycoproteins contain 42% carbohydrate, and such high carbohydrate content may increase resistance of the protein to heat denaturation and, in turn, increase the elasticity of the film around gas bubbles. α-Globulins (Cohn fraction IV-1) also had very good foaming capacity and stability. Batter prepared with Cohn fraction IV-1 had the lowest specific gravity among all proteins showing that more air was entrapped during mixing. Since volume reflects the amount of air incorporated during mixing and CO_2 expansion during baking, it is not very surprising that this protein produced a large volume cake despite having a relatively low denaturation temperature of some of the other proteins in the

fraction.

Albumin produced almost the same crumb structure as did whole blood plasma but the crumb was much less crumbly and denser. In general, cakes prepared with blood plasma globulins were crumbly and had crumb structures with open cells. γ-Globulins produced better crumb structure than did albumen, Cohn fraction III, and Cohn fraction IV-1. Cohn fraction IV-1 cakes had the coarsest the most crumbly, and the most irregular textures. This was an unexpected result because this protein had very good foaming capacity and stability. Fibrinogen cakes had very elastic and smooth texture. No blood plasma protein fractions produced cake texture as fine as did egg white globulins.

Albumin- and IgG-Rich Spray-Dried Plasma Fractions

Albumin- and IgG-rich spray-dried blood plasma fractions were better emulsifiers than spray-dried plasma (Table II). The IgG-rich fraction emulsified more oil than did the albumin-rich fraction. Foaming capacities of the IgG-rich fraction were less than those of the albumin-rich fraction and the whole blood plasma samples (Table II). Albumin- and IgG-rich plasma fractions had very good foam stabilities. They maintained more than 85% of their original foam even after 30 min; whole blood plasma was unable to maintain more than 40% of its original volume.

The DSC thermograms show that these spray-dried fractions are not pure, and there are at least two proteins in each fraction (Fig. 4). IgG-rich plasma

fraction exhibited a shoulder around 70°C and a peak at 78.2°C. As shown in Fig. 4, the albumen-rich fraction exhibited one major peak at 71.1°C and one minor peak at 78.8°C. These denaturation peak temperatures were lower than those obtained by using lyophilized pure albumin and γ-globulins (Raeker and Johnson 1994a).

The albumin-rich spray-dried fraction produced significantly less cake volume than did freeze-dried albumin and blood plasma (Table II, Fig. 2). Textures of the cakes made with albumin-rich protein were denser and crumblier than blood plasma cakes. The IgG-rich spray-dried fraction produced volume and texture similar to the freeze-dried γ-globulin, and cakes made with this protein had finer crumb structure than cakes made with whole blood plasma.

Relationships Between Cake Volume and Protein Functionality

From the previous study (Raeker and Johnson 1994a), the proteins that gave only one denaturation peak were chosen to determine the relationship between cake volume and protein functionality. These proteins were bovine serum albumin, γ-globulin, Cohn fraction III, conalbumin, lysozyme, and ovalbumin. Although fibrinogen exhibited one very low major and one very high minor denaturation peak, we assumed that the lower denaturation temperature would govern the cake properties. Therefore, we also included this protein in this part of the study. Multiple linear regression equations were generated relating cake volumes to the denaturation peak temperature, foaming properties (capacity and

stability), and emulsification activity of proteins (Tables III and IV).

There was a highly significant correlation coefficient between cake volume and denaturation peak temperatures of proteins ($r = 0.944$, $P = 0.001$). The MaxR procedure used in the analysis begins by finding the one-variable model that produces the highest R^2. Then another variable that yields the greatest increase in R^2 is found, and so on. This analysis showed that the protein denaturation peak temperature produced the highest R^2, the square of the correlation between the actual volumes and the predicted volumes by the model, of 0.891. When foaming capacity was entered into the model (Table III), R^2 was improved by only 2% to 0.916 and contribution to the model from foaming capacity was not significant ($P = 0.33$). When emulsification activity was entered into the model, R^2 did not improve, and emulsification activity in the model was not significant ($P = 0.95$). When foam stability instead of capacity was used in the multiple regression analysis (Table IV), the contribution from foam stability to the model was also not significant ($P = 0.64$). These results suggest that the denaturation temperature of the protein is the only important functional property that determines the volume of the finished cake.

CONCLUSIONS

Various proteins used in this study demonstrated that the temperature at which a protein denatures during baking is the determining factor in the final shape and volume of cakes. The higher the denaturation temperature of a protein, the

larger the cake volume and the more crowned profile. This high correlation between protein denaturation temperature and volume indicates that protein denaturation temperature determined by DSC is a reliable predictor of cake volume.

Since γ-globulin had the highest denaturation temperature and produced cakes with larger volumes and better texture than did whole blood plasma, the use of blood plasma as an egg white substitute in cakes can be improved by fractionating it into its γ-globulin- or γ-globulin-rich fractions.

Another solution is to separate fibrinogen from blood plasma because it was responsible for low cake volumes and flat profiles of blood plasma cakes. This would be a more practical solution because separation of this component from blood plasma by centrifugation will be low in cost. However, additional research is necessary to investigate texture and sensory properties of cakes prepared with fibrinogen-free plasma (serum) because crumb texture of cakes prepared with serum were fragile.

In general, spray-dried proteins produced a better crumb texture than did their freeze-dried forms. This was attributed to the better foam stability of spray-dried samples as was shown in the previous study of Raeker and Johnson (1984a).

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TABLE I
Qualities of Cakes Made with Egg White, Plasma, and Their ProteinFractions[a]

Protein	Batter Specific Gravity (g/cm^3)	Cake Volume (cm^3)	Cake Profile (mm)
Spray-dried egg white (commercial)	0.757 ± 0.011	49.0 ± 0.5	9.3 ± 0.4
Freeze-dried egg white	0.780 ± 0.008	48.4 ± 0.6	9.0 ± 0.4
Spray-dried blood plasma (commercial)	0.752 ± 0.012	47.8 ± 0.4	6.8 ± 0.5
Freeze-dried blood plasma (no centrifugation)	0.761 ± 0.006	47.7 ± 0.5	7.2 ± 0.3
Spray-dried serum (laboratory)	0.757 ± 0.009	49.8 ± 0.8	8.9 ± 0.8
Freeze-dried serum (after centrifugation)	0.750 ± 0.012	48.7 ± 1.4	8.9 ± 0.8
Egg white fractions			
Ovalbumin	0.793 ± 0.016	49.4 ± 0.6	9.4 ± 0.6
Globulins	0.774 ± 0.013	51.0 ± 0.7	10.5 ± 1.1
Conalbumin	0.798 ± 0.029	45.6 ± 0.5	8.3 ± 0.5
Lysozyme	0.786 ± 0.021	45.5 ± 0.5	7.7 ± 1.3
Ovomucoid	0.778 ± 0.014	38.6 ± 0.6	-1.8 ± 0.3
Blood plasma fractions			
Albumin	0.781 ± 0.013	46.9 ± 0.5	8.0 ± 0.3
γ-Globulin	0.788 ± 0.018	49.5 ± 0.7	9.0 ± 0.3
Cohn fraction III	0.762 ± 0.007	48.5 ± 1.0	8.7 ± 0.4
Cohn fraction IV-1	0.741 ± 0.002	49.9 ± 0.8	10.4 ± 1.1
Fibrinogen	0.929 ± 0.024	42.2 ± 1.1	3.2 ± 1.1
$LSD_{0.05}$	0.025	1.2	1.2

[a]Values are the means of three replicates (means ± SD).

TABLE II

Emulsification, Foaming, and Cake Properties of Blood Plasma, and Albumin-Rich and IgG-Rich Fractions[a]

Protein	Emulsification Capacity (mL oil/g protein)	Foaming Capacity and Stability			Cake Properties	
		Initial Volume (mL)	Remaining Foam (%)		Volume (cm^3)	Profile (mm)
			15 min	30 min		
Albumin-rich	1173 ± 55 b	41.8 ± 0.4 a	86.7 ± 1.1 a	85.9 ± 1.6 a	45.3 ± 0.6 c	8.1 ± 0.8 ab
IgG-rich	1317 ± 25 a	39.5 ± 0.7 b	88.0 ± 1.0 a	87.1 ± 0.5 a	49.1 ± 0.2 a	8.5 ± 0.8 a
Blood plasma	1035 ± 33 c	41.7 ± 0.6 a	86.6 ± 3.6 a	38.6 ± 14.3 b	47.9 ± 0.4 b	6.8 ± 0.5 b

[a]Means ± SD. Values with the same letter in a column are not significantly different at $P = 0.05$.

TABLE III

Relationships Between Cake Volume and Functional Properties of Proteins[a]

Independent variables[b]	R^2	Model	F value[c]
Step 1	0.891	Volume = 29.9271 + 0.2232 T_p	
T_p			40.94**
Step 2	0.916	Volume = 28.9812 + 0.2223 T_p + 0.0314 FC	
T_p			42.23**
FC			1.20
Step 3	0.916	Volume = 28.995 + 0.2220 T_p + 0.0300 FC + 0.1835 EA	
T_p			31.27**
FC			0.55
EA			0.00

[a]Values and description are listed in Raeker and Johnson (1994a).
[b]T_p denaturation peak temperature; FC, foaming capacity; EA, emulsification activity.
[c]** = $P \leq 0.01$.

TABLE IV

Relationships Between Cake Volume and Functional Properties of Proteins[a]

Independent variables[b]	R^2	Model	F value[c]
Step 1	0.891	Volume = 29.9271 + 0.2232 T_p	
T_p			40.94**
Step 2	0.901	Volume = 29.6974 + 0.2208 T_p + 1.4537 EA	
T_p			34.86**
EA			0.40
Step 3	0.909	Volume = 30.6934 + 0.2100 T_p - 0.0194 FS + 0.1898 EA	
T_p			20.73*
FS			0.28
EA			0.50

[a]Values and description are listed in Raeker and Johnson (1994a).
[b]T_p, denaturation peak temperature; FS, foaming stability (remaining foam volume after 15 min); EA, emulsification activity.
[c]** and * significant at $P < 0.01$ and $P < 0.05$, respectively.

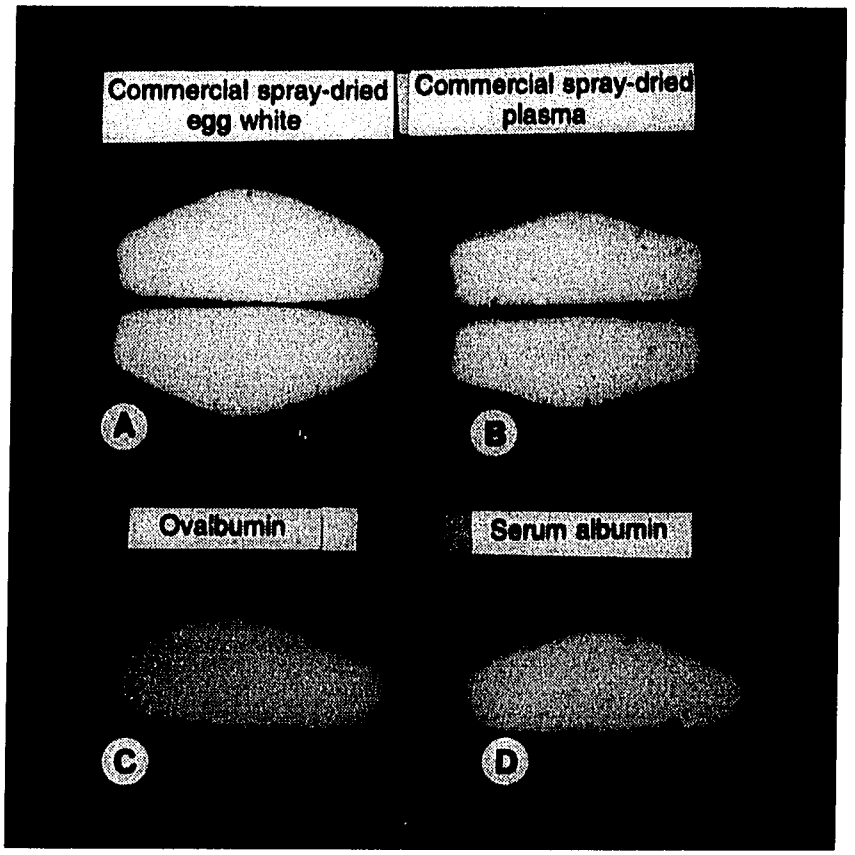

Fig. 1. Cakes prepared with A, egg white; B, blood plasma; C, ovalbumin; and D, serum albumin.

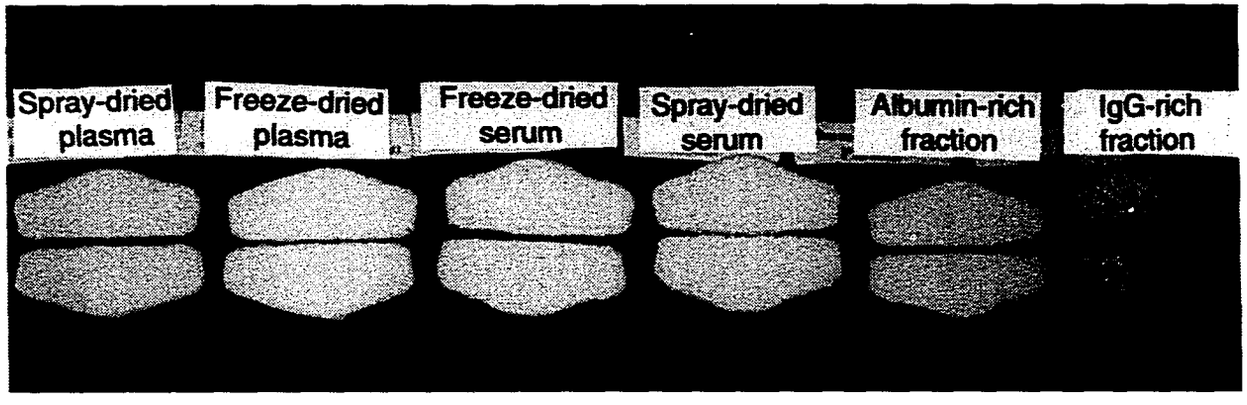

Fig. 2. Cakes prepared with (from left to right) commercial spray-dried blood plasma, freeze-dried blood plasma, freeze-dried serum, spray-dried serum, albumin-rich fraction, and IgG-rich fraction.

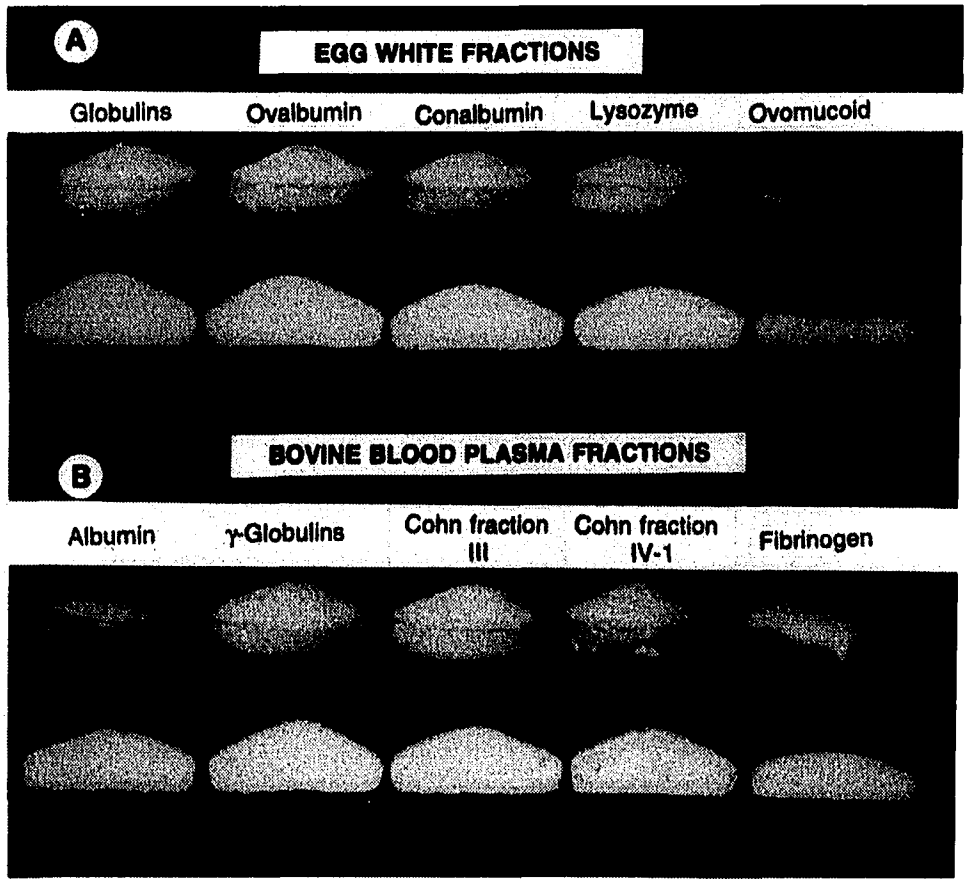

Fig. 3. Cakes prepared with A, egg white protein fractions (from left to right): globulins, ovalbumin, conalbumin, lysozyme, and ovomucoid; and B, bovine blood plasma protein fractions (from left to right): albumin, γ-globulins, Cohn fraction III (56% β- and 44% γ-globulins), Cohn fraction IV-1 (predominantly α-globulins), and fibrinogen.

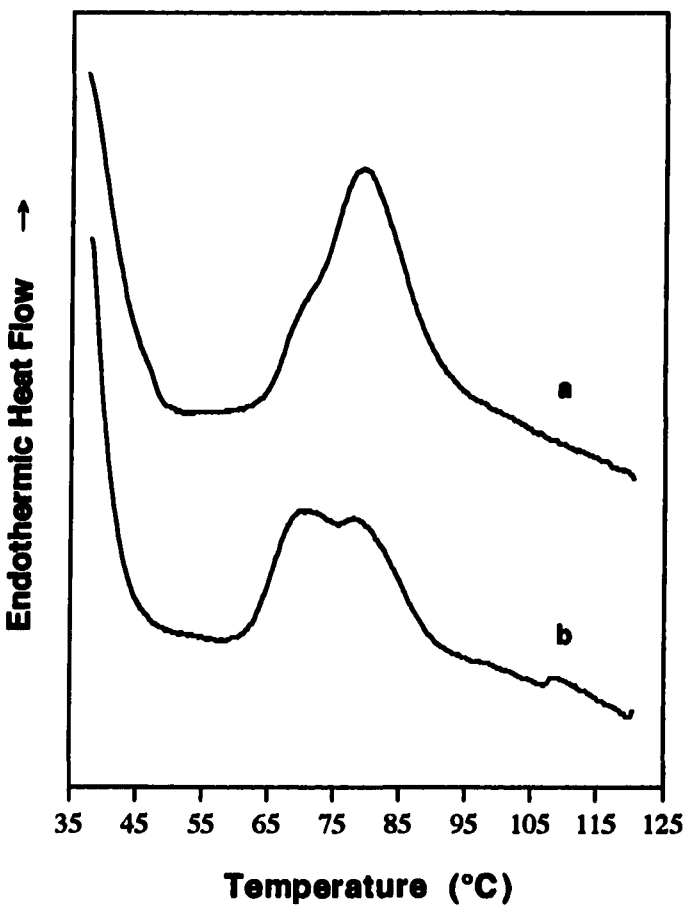

Fig.4. Differential scanning calorimetric thermograms of (a) IgG-rich and (b) albumin-rich spray-dried blood plasma fractions.

6. GENERAL CONCLUSIONS

Functional properties of egg white and blood plasma proteins that are necessary to obtain desirable volume and texture of cakes were characterized and compared to explain differences in their cake-baking potential. Considerable differences in the thermal denaturation temperatures of egg white and blood plasma proteins were found. The most heat sensitive protein of blood plasma (fibrinogen) had a denaturation temperature about 10°C lower than that of the most heat sensitive protein in egg white (conalbumin). The predominant proteins of egg white (ovalbumin) and blood plasma (albumin) denatured about 9°C apart from each other, the former being more stable. γ-Globulins were the only proteins in blood plasma that denatured at a temperature close to ovalbumin. The other difference observed between blood plasma and egg white was in their foam stabilities. Although some of the blood plasma protein fractions had good foam stabilities (albumin, fibrinogen, Cohn fraction IV-1), whole blood plasma exhibited significantly lower foam stability than did whole egg white. Since all of the blood plasma proteins are acidic, addition of a basic protein in blood plasma may increase the electrostatic interactions among proteins and thereby increase the stability of blood plasma foam.

To evaluate purified fractions of egg white and bovine blood plasma as substitutes for egg ingredients in cakes, a micro method for cake-baking was developed requiring only 5 g of flour in a high-ratio white layer cake formulation. The micro cake-baking method was compared with AACC method 10-90 using

different levels of spray-dried egg whites and bovine blood plasma. Correlation coefficients between the micro method and the standard AACC method which utilizes 200 g of flour were determined for specific gravity, cake volume, and symmetry index measurements were 0.80, 0.99, and 0.98, respectively, for egg white, and 0.84, 0.99, and 0.99, respectively, for blood plasma.

Various proteins utilized in this study demonstrated that the temperature at which a protein denaturates during baking is the determining factor in the final shape and volume of a cake. The higher the denaturation temperature of a protein, the larger the cake volume and the more crowned profile. Therefore, protein denaturation temperature obtained from differential scanning calorimetry is a reliable predictor of cake volume. When proteins had different denaturation temperatures, foaming and emulsification properties of proteins were not significant variables to predict cake volume.

Cakes prepared with egg white had a slightly higher volume, significantly more crowned profile and finer texture than did cakes prepared with blood plasma. Since γ-globulin had the highest denaturation temperature and produced cakes with larger volumes and better textures than did whole blood plasma, utilization of blood plasma as an egg white substitute in cakes can be improved by fractionating into γ-globulin- or γ-globulin rich fractions. Another suggestion from this study is to separate fibrinogen from plasma since it was depressing cake volume and profile of blood plasma cakes. This would be a more practical solution since separation of this component from plasma by centrifugation will be low in cost. However,

additional research is necessary to investigate instrumental texture and sensory properties of cakes prepared with fibrinogen free plasma (serum) since crumb texture of cakes prepared with serum were fragile.

It was interesting to note that two of the serum albumin samples (lyophilized pure fraction and albumin-rich spray-dried plasma fraction) utilized in this study had different denaturation profiles, and consequently, different cake properties. This difference probably comes from the variations in their fatty acid contents. It is known that long chain fatty acids bind (Putnam 1984), and stabilize the protein molecule and increase the denaturation temperature of serum albumin (Gumpen et al 1979, Peters 1985). In the study by Gumpen et al (1979) the denaturation peak temperature of fatty acid free serum albumin increased from 68 to 83°C with addition of stearic acid and to 92°C with addition of lauric acid. This shows that there is a potential to improve the cake baking quality of serum albumin. Therefore, future research is recommended to determine the effects of fatty acids on cake-baking quality of serum albumin.

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