RHEOLOGICAL AND FUNCTIONAL PROPERTIES OF PASTEURIZED LIQUID WHOLE EGG DURING FROZEN STORAGE

By

Thomas Joseph Herald

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ABSTRACT

RHEOLOGICAL AND FUNCTIONAL PROPERTIES OF PASTEURIZED LIQUID WHOLE EGG DURING FROZEN STORAGE

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This study investigated the variability in quality of frozen, pasteurized liquid whole egg (LWE) reported by processors of baked goods. Homogenized LWE control and LWE heated at 60°C, 64°C, and 68°C for 3.5 min were evaluated during 4 mos storage at -24°C. An 11% loss in protein solubility was observed between unfrozen LWE control and 68°C LWE. Protein solubility decreased 17% and 11% in LWE control and 68°C LWE, respectively, during frozen storage. Polyacrylamide gel electrophoresis was used to monitor changes in soluble protein composition. The percentage of soluble conalbuminn and globulin proteins decreased while ovalbumin increased during frozen storage. Unfrozen control, 60°C, and 64°C LWE exhibited time independent behavior while unfrozen 68°C and all frozen LWE treatments were thixotropic. Peak viscosity increased approximately tenfold during frozen storage. Correlation between functional and rheological properties of LWE were determined. This study illustrates that combined effects of heat and frozen storage

may be more detrimental to LWE quality than either effect alone.

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INTRODUCTION

Egg products are processed and convenient forms of eggs that are used by commercial establishments, food service, and in the home. Over 3.4×10^8 Kg of egg products are produced annually in the United States. The average egg consumption in the United States has declined from 377 eggs per capita in 1950 to a low of 273 in 1975 (Table 1). There are two reasons for the decrease in egg consumption. First, shelled eggs contain an average of 260 mg of cholesterol. Elevated blood cholesterol levels have been associated with an increased risk of heart disease although the degree of influence of dietary cholesterol on blood cholesterol has not yet been determined. Second, decreases in egg consumption have also been attributed to the changing household. A rise in two income and single parent homes has decreased the time available for preparation of homemade baked goods. Commercially prepared baked goods have partially reversed the decline in egg consumption (Cotterill, 1981).

Egg products are categorized as refrigerated liquid, frozen, dried (Table 2) and speciality products (Cotterill,

Table 1. Average per capita civilian consumption of eggs in the United States for selected years.

Year[a]	Number of eggs	Pounds
1950	377	49.0
1955	360	46.9
1960	324	42.5
1965	306	39.8
1970	304	39.5
1975	273	35.4
1980	281	36.5

[a]Data based on calendar year.

Source: Adapted from U.S. Department of Agriculture (1968, 1972, 1982).

Table 2. Liquid egg production and disposition in the United States, 1985 (in millions of pounds).

Liquid for immediate consumption or processing	Frozen product produced	Dried product produced
578	324	96

Source: Stadelman & Cotterill, 1986.

Table 3. Frozen egg production in the United States, 1985 (in millions of pounds).

Whole liquid egg	Egg white	Egg yolk
214	46	64

Source: Stadelman & Cotterill, 1986.

1986). Liquid whole egg (LWE) is a blend of whites and yolks containing 23-25% egg solids. About, one third of the total liquid egg market is frozen (Table 3). Frozen eggs are mainly marketed as food ingredients. Dried eggs compose 10% of the processed egg market and are used in bakery foods and mixes, mayonnaise, salad dressing, ice cream, pasta and many other convenience foods. Specialty egg products are increasing in popularity and include microwavable entrees such as omelets, scrambled egg, and quiches which are convenient and readily prepared (Cotterill, 1981).

Egg products are pasteurized to destroy Salmonella and extend shelf life ensuring optimum storage stability. Refrigerated liquid egg is stored below 4°C. York and Dawson (1973) demonstrated that pasteurized LWE has a 5 and 12 day shelf life, at 9°C and 2°C, respectively. If liquid eggs are to be frozen for storage, the pasteurized chilled eggs are placed in containers and frozen in a blast freezer at -40°C. LWE are usually defrosted below 4.4°C for less than 24 hr.

Objectives

Processors of baked products have reported variability in quality of frozen pasteurized LWE. Changes in functional properties are apparently caused by alteration of one or more proteins during pasteurization or frozen storage. These alterations have been measured by performance tests, such as volume changes in sponge cakes. The objectives of this

research include: (1) evaluation of the effect of heat treatment and frozen storage on protein composition, rheological properties and functional properties of LWE, and (2) correlation of changes in functional properties to changes in rheological properties and protein composition. Evaluation of LWE by the aforementioned objectives would give processors information to make necessary adjustments prior to product formulation, such as addition of dry egg albumen or starch. This research information could prove economically desirable to the processor by reducing unwarranted ingredient addition.

REVIEW OF LITERATURE

Composition

Eggs consist of water, protein, lipid, carbohydrate, and ash (Table 4). Egg whites are composed of 87.9% water, 10.2% protein (the major solid constituent representing 82.8%, 1.0% carbohydrate, and traces of fat (Watt and Merrill, 1963).

The solid fraction of egg yolk is four fold larger than the egg white. Fats are the major solid constituent comprising 34% of the egg yolk. Proteins comprise 16% of egg yolk mostly in the form of lipoproteins. There is also 2% ash and traces of carbohydrate in the egg yolk (Marion et al., 1964).

Eggs are a good source of high quality protein, containing the essential amino acids, methionine, lysine, cysteine, and tryptophan. Experimental nutritionists often use eggs as a standard for measuring the quality of other food protein. Eggs contain unsaturated fatty acids, iron, phosphorus, and all of the vitamins, except vitamin C. The composition of white and yolk depends on the age, breed and diet of the hens.

Table 4. Nutrient composition of liquid whole egg (per 100 g).

Nutrients and units	Whole[1]	White[1]	Yolk[2]
Proximate composition			
Solids, g	24.5	12.1	51.8
Water, g	75.5	87.9	48.2
Calories, C	152	50	337
Protein (N x 6.25), g	12	10.2	16.1
Total lipid, g	10.9	0	34.1
Ash, g	1	.68	1.69
Lipids			
Free fatty acids, g	0	0	0
Saturated, total	3.67	0	11.42
Monounsaturated, total	4.6	0	14.67
Polyunsaturated, total	1.32	0	4.2
Cholesterol, g	.48	0	1.52
Lecithin, g	2.32	0	7.2
Vitamins			
A, IU	480	0	1527
D, IU	50	0	161
E, mg	1.6	0	5.1
B_{12}, mcg	.88	0	2.83
Biotin, mcg	20.0	6.8	49.1
Choline, mg	430	1.2	1400
Folic acid, mg	.060	.016	.154
Inositol, mg	10.8	4.0	25.8
Niacin, mg	.082	.092	.061
Pantothenic acid, mg	1.52	.24	4.3
Pyridoxine, mg	.119	.021	.334
Riboflavin, mg	.33	.28	.44
Thiamin, mg	.09	.011	.28
Minerals, mg			
Calcium	53	10	148
Chlorine	175	174	176
Copper	.061	.023	.145
Iodine	.047	.003	.141
Iron	1.97	.14	6.0
Magnesium	11.5	10.8	12.9
Manganese	.038	.007	.11
Phosphorus	202	22	599
Potassium	135	150	100
Sodium	129	165	52

Table 4. (cont'd)

Nutrients and units	Whole[1]	White[1]	Yolk[2]
Sulfur	164	163	165
Zinc	1.30	.12	3.89

[1]Based on 24.5% and 12.1% solids, respectively for LWE and egg white liquid.

[2]Pure yolk containing 44% egg solids, diluted with egg white only.

(Cotterill & Glauert, 1979)

Functional Properties

The major functions of eggs in foods are to coagulate, foam, or emulsify. In addition eggs are added to enhance the color and flavor or inhibit crystal formation.

Coagulation Proteins in the native egg are colloidally dispersed in a sol. When heat is applied, there is a change in structure of the egg protein molecules resulting in a loss of solubility and thickening. Changing of the egg protein from a sol to a gel is known as coagulation. (Bennion, 1980; Baldwin, 1986)

The mechanism of coagulation has been investigated by many researchers (Chick and Martin, 1921; Kauzman, 1959; Smith, 1964; Ma and Holme, 1982). Basically, the proteins are denatured, unfolding from their native conformation and reforming new cross-links joining the unfolded molecules together in a three-dimensional matrix.

Time, temperature, salt, sugar, and pH influence coagulation and affect the consistency of cooked eggs and egg containing foods (Palmer, 1972). Coagulation by heat is important in the preparation of custards and other egg dishes. Over-coagulation and toughening result from too high a temperature or too long a heating period (Bennion, 1980).

Egg coagulation is used in food systems to assist in binding. There are several ways to measure the relative degree of coagulation. Recent methods include back extrusion

(Hickson et al., 1982), compression test on the Universal Instron (Egelandsdal, 1980), and Small Amplitude Oscillatory Testing (Beveridge and Timbers, 1985). Others such as Griswold (1962) and Smith (1964) used methods such as amount of sag, viscosity, optical rotation and solubility.

Foams

Foams are colloidal dispersions of gas bubbles in a liquid or semi-solid phase. Gas bubbles are separated from each other by a thin liquid or semi-solid walls that are elastic in a stable foam.

A foam is formed by incorporating air during beating or whipping an egg. Foaming agents such as egg globulins, reduce the surface tension in the continuous phase. The foaming agent will assist in the formation of a surface layer which will resist coalescence of the gas bubbles (Powrie, 1976). As whipping time increases the air bubbles will decrease in size and the color will change from a translucent greenish yellow to an opaque white as the protein denatures at the interface. The protein network gives ridigity and stability to the foam. As whipping continues, stiffness and volume of the foam increases until excessive beating results in rupturing of air cells and syneresis (Palmer, 1972).

The mechanism of foam formation reported by Griswold (1962) is related to the unfolding of the protein molecules.

The change in molecular structure causes coagulation or loss of solubility of a portion of the albumen. The main foaming component are globulins. Globulins increase viscosity, decrease foam drainage, and initially help increase the volume. Globulins are conducive to the formation of small bubbles and give smooth texture to the foam. Ovomucin forms an inflexible and insoluble film that coagulates and rapidly precipitates at the interface. This film is necessary for stability of the foam (MacDonnell et al., 1955). In the presence of globulin, ovomucin is more effective in foam stability. Johnson and Zabik (1981) suggested that ovomucin, ovomucoid, conalbumin and lysozyme have little influence in foam formation when acting singly. Lysozyme and globulin in combination contributed to foam formation. Sauter and Montoure (1972) stated that foam volume decreased with increasing concentration of lysozyme. MacDonnell et al. (1955) reported that ovalbumin coagulated and stabilized foams during cooking. Other factors such as acids, salt, sugar, fat, temperature, dilution and whipping blades all influences foam volume (Bennion, 1980; Baldwin, 1986). Foam is measured either in model or food systems. Foam stability of egg white is determined by measuring the volume of liquid that drains from a given amount of foam in a specified time. Specific volume of the foam measures the relationship between the volume and weight of the foam.

Emulsifiers An emulsion is defined as a system of two immiscible liquids, one of which is dispersed into the other. The dispersed droplet phase of an emulsion is referred to as the discontinuous or internal phase. The phase which forms the matrix is the external or continuous phase. The purpose of emulsifiers is to stabilize the suspension of the discontinuous phase within the continuous phase. Surface active agents, possess a balance of both polar (hydrophilic) and non-polar (hydrophobic) groups. Surface active agents are adsorbed at the interface, forming a film around the oil globules decreasing surface tension and preventing coalescence (Powrie, 1976).

Egg yolks are excellent emulsifiers. Yolk lecithoproteins (lipoproteins containing lecithin) are responsible for emulsifying properties (Palmer, 1972; Stadelman and Cotterill, 1986). Chapin (1951) identified protein and lipoprotein as the most important emulsifying substances in whole egg and found that addition of lecithin reduced emulsifying ability. Excess emulsifying agent can be detrimental to emulsion formation (Cunningham, 1975). Eggs are used as emulsifying agents in baked goods to retard staling by inhibiting starch retrogradation (Becher, 1957). Emulsifying capacity is one way to measure an emulsion. Emulsifying capacity is measured in grams of oil emulsified per milligram of protein. Measurements of electrical resistance or phase inversion are used to determine emulsifying capacity.

Color and Flavor Color is not the primary reason for the use of egg in foods; albeit egg color is desirable in products such as ice cream, custards, and omelets. Deethardt et al. (1965) reported color differences in sponge cake made from eggs laid by hens on different diets.

One of the most important factors influencing the acceptability of eggs is flavor. Flavor differences in eggs are distinguishable in egg products that are not masked by highly seasoned ingredients. Yolk tends to absorb flavors more readily than the white due to a higher fat content (Gaebge, 1940). Koehler and Bearse (1975) and Pearson et al. (1979) reported that diet has a major influence on egg flavor.

Crystal Inhibition Egg white plays a major role in sugar crystal control in candy making (Swanson, 1929). Swanson (1929) and Cotterill et al. (1963) noted a decrease in crystal size of candy when egg whites were added.

Pasteurization

Pasteurization of egg products in the United States became mandatory on June 1, 1966 with the regulation "Pasteurization of LWE" (Federal Register, 1965). The purpose of pasteurization is to destroy pathogenic bacteria such as Salmonella (the target organism in LWE), decrease

the viable bacteria count and improve the shelf life of egg products. The USDA requires that LWE be heated to at least 60°C and held for no less than 3.5 minutes for the average particle.

Eggs are pasteurized using a high temperature short time (HTST) process. Stainless-steel plate heat exchangers (Fig.1) or triple tube heat exchangers (Fig.2) are utilized due to their high efficiency. Batch pasteurization of eggs is not a USDA approved process. Normally less than 1% of the bacteria in raw egg survive pasteurization, which contains less than 1,000 microorganisms per gram. The American Egg Board suggested specifications for LWE which are in Table 5 (Cotterill, 1981).

Quality of egg products is related primarily to denaturation of egg proteins. Denaturation of whole egg proteins, as indicated by a change in viscosity, occurs between 56°C and 66°C. Fractional precipitation and coagulation of proteins occurs rapidly above 66°C (Baldwin, 1986). Torten and Eisenberg (1982) observed that pasteurization increased not only viscosity, but also shear rate dependency. Relative viscosity has been used as an indicator of changes in functional properties of whole eggs (Scalzo et al., 1970). Sugihara et al. (1966) reported that the quality of pasteurized whole eggs, as evaluated by the functional properties of sponge cake, was not significantly damaged until pasteurization temperatures exceeded 63.3°C for 3.5 min. Carbohydrates help to stabilize liquid egg

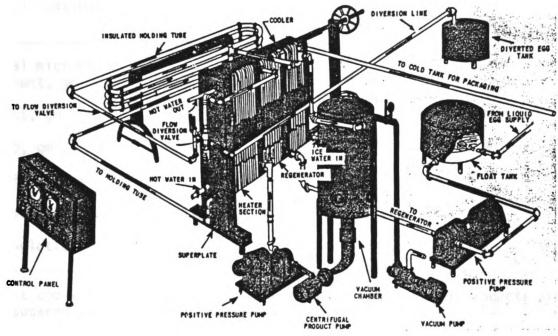

Figure 1. Plate heat exchanger, holding tubes, vacuum chamber, and accessory equipment (Stadelman and Cotterill, 1986).

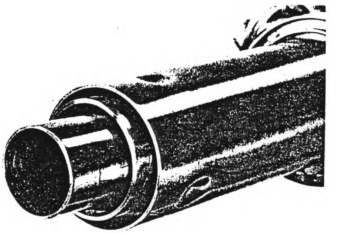

Figure 2. Triple-tube heat exchanger (Stadelman and Cotterill, 1986).

Table

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Table 5. Suggested microbiological specifications of liquid or frozen egg (Cotterill, 1981).

Specifications	Liquid or frozen		
	White	Yolk[1]	Whole
Total microbial count, gm	5,000	<5,000	<5,000
Yeast, gm	10 max	10 max	10 max
Mold, gm	10 max	10 max	10 max
Coliform, gm	10 max	10 max	10 max
Salmonellae, gm	Neg.	Neg.	Neg.
Granulation[2]	-	-	-

[1]Most egg white solids are desugared. Whole egg and yolk products are desugared if specified on purchase (SOP).

[2]U.S. Bureau of Standards Screen No. 80.

against heat denaturation by delaying formation of disulfide bonds between proteins (Cunningham, 1986). Woodward and Cotterill (1983) observed the electrophoretic and chromatographic changes in whole eggs heated from 57-87°C for 3.5 min. Livetins and some globulins were most heat sensitive, while conalbumin and ovalbumin were the most stable. Sucrose and salt increased the heat stability of LWE proteins.

Presently there is not a specific test for determining adequate pasteurization of LWE. Tests have been investigated using endogenous enzymes as indicators of adequate pasteurization. All of the enzymes tested are inactivated either above or below the legal pasteurization temperature and thus, are not accurate indicators. Enzymes that have been investigated include: Phosphatase, alpha amylase, catalase, and beta-N-acetyl glucosaminidase (Donovan and Hansen, 1971; Cunningham, 1966).

Frozen Storage

Freezing and frozen storage at -20°C resulted in at least a doubling in the viscosity of LWE (Torten and Eisenberg, 1982). The increase in viscosity has been attributed to lipoprotein gelation. Powrie et al. (1968) proposed that water plays an important part in gelation. Ice crystal formation dehydrates protein as a result of the increased salt concentration and the breakdown of the water

shell surrounding the molecules. This could promote rearrangement and aggregation of the yolk lipoproteins. Wakamatu et al. (1983) studied the role of NaCl in controlling gelation and observed many of the above phenomena. Cornford et al. (1969) and Torten and Eisenberg (1982) reported changes in the rheological characteristics of unfrozen and frozen LWE. The researchers noted that unfrozen LWE possessed Newtonian behavior, while LWE had non-Newtonian characteristics after freezing and thawing.

The use of frozen yolk as a food ingredient is limited due to lipoprotein gelation. The gelled yolk is difficult to blend with other food ingredients. Many functional properties of whole egg are not affected by freezing (Imai et al., 1985), although decreases in foaming, whipping, and sponge cake volume have been reported (Jordon et al.,1952; Janssen, 1971; Mori, 1971).

Egg White Proteins

Albumen is a protein system consisting of ovomucin fibers in an aqueous solution of numerous globulin proteins (Powrie, 1976). Proteins in the albumen fraction are ovalbumin, ovotransferrin, ovomucoid, ovomucin, lysozyme, globulin, ovoinhibitor, and other smaller protein fractions (Table 6) (Parkinson, 1966). Johnson (1980) evaluated foaming index ($cm^3/g/min$) and angel food cake volume (cm^3) of egg white proteins (Table 7).

Table 6. Protein composition of liquid whole egg (Parkinson, 1966).

Protein	Amount of albumen (%)	pI[a]	Molecular weight
Ovalbumin	54	4.5	45,000
Ovotransferrin	12	1.1	76,000
Ovomucoid	11	4.0	28,000
Ovomucin	3.5	4.5-5.0	$5.5\text{-}8.3\times10^6$
Lysozyme	3.4	10.7	14,300
G_2 Globulin	4.0	5.5	unknown
G_3 Globulin	4.0	4.8	14,000
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	$7.6\text{-}9.0\times10^5$
Avidin	0.05	10	68,300
Phosvitin	3-4		36-40,000
Livetins	4-10		
α			80,000
β			45,000
γ			150,000
Lipovitellin	16-18		
α			40,000
β			40,000
Vitellenins	12-13		
LDL1			10,000,000
LDL2			3,000,000

[a]Isoelectric point.

Table 7. Physicochemical characteristics of albumen protein solutions and angel food cake parameters (Johnson, 1981).

	Con-albumin	Globulins	Ovo-albumin	Ovo-mucin	Ovo-mucoid	Lysozyme
Foaming index $cm^3/g/min$	.34	4.71	.59	No foaming capacity 0.0	0.0	.12
Volume cm^3	157	330	308	52	54	107

Ovalbumins Ovalbumin is the major protein component in egg white. It is one of two proteins which contain all the essential amino acids. The other is lactalbumin in milk. Ovalbumin is a phosphoglycoprotein and exists as three forms, A_1, A_2 or A_3, which differ in phosphorus content. A_1 contains two phosphates per molecule, A_2 has one and A_3 does not contain phosphorus. Ovalbumin contains the most sulfhydryl groups of the albumen proteins. The polypeptide chain of ovalbumin has 4 sulfhydryl and 1 disulfide bond per molecule (Johnson, 1980). Ovalbumin is readily denatured by shaking, but is resistant to thermal denaturation (Powrie, 1976). Woodward and Cotterill (1983) reported ovalbumin was heat stable at 74°C and coagulated at 84°C. Ovalbumin is converted to a more heat stable S-ovalbumin form due to a sulfhydryl-disulfide interchange during storage (MacDonnell et al., 1955). Lineweaver et al. (1967) found that minimal alteration occurred in protein structure when heated to 62.5°C at pH 9 for 3.5 min. Hegg et al. (1979) found that ovalbumin had maximum thermal stability between pH 6-10, and that NaCl did not affect the denaturation temperature. In food preparation, ovalbumin denatures and contributes to the structure of baked products (Baldwin, 1986).

Conalbumin Conalbumin is also known as ovotransferrin because it is an iron binding protein. The iron is obtained from the yolk. Conalbumin is a glycoprotein with most of the carbohydrates in the form of a single oligosaccharide chain

with four residues of mannose and eight residues of N-acetylglucosamine (Williams, 1962a; 1962b).

The conalbumin-iron complex inhibits the growth of certain microorganisms by chelating available iron. The conalbumin-iron complex does occur below pH 5.5, and is stable at 78°C in whole egg (Woodward and Cotterill, 1983). The conalbumin-iron complex is more resistant to denaturation by heat, proteolytic attack, and physical treatments than is the apoprotein (Azari and Feeney, 1958; 1961). At very high and low pH values, Conalbumin has a high net charge causing intermolecular repulsion and an unstable structure. A low net charge, which would occur near the isoelectric point, favors intermolecular interaction and a more stable structure. Gelation can be influenced by pH and additives, such as salt and ionic detergents (Hegg et al., 1978). Other di- and trivalent metal ions bind firmly to conalbumin. Two atoms of Al (III), Cu (II), and Zn (II) per molecule form stable complexes which are colorless, yellow and colorless respectively, whereas the iron complex is red.

Johnson (1980) reported conalbumin complexed with metal ions increased cake volume by decreasing thermal denaturation. The high number of disulfide bonds (15) contribute to thermal stability, but decrease foamability of the proteins. Johnson (1980) reported conalbumin in combination with lysozyme produced fewer air inclusions than conalbumin alone, providing a better foam.

Ovomucoid ovomucoid is a glycoprotein which is heat resistant due to the presence of disulfide bonds (Kilara and Sharkasi, 1985). The use of starch gel electrophoresis by Wise et al. (1964) produced three distinct bands of ovomucoid all of which possessed trypsin inhibiting abilities (Lineweaver and Murray, 1947). Carbohydrate is present as three oligosaccharides, each attached to the protein through an asparaginyl residue. Ovomucoid, with eight disulfide linkages, contains 22% alpha helical structure (Powrie, 1976). About 12% of egg white protein is ovomucoid. In acidic solutions ovomucoid is resistant to heat denaturation. Thermal denaturation of ovomucoid occurs in the alkaline region (pH 9). The helical structure is hypothesized to change during heat treatment (Powrie and Nakai, 1986).

The high resistance of ovomucoid to denaturation is related to the large number of disulfide bonds which stabilize the protein structure. There is little foam formation during mechanical shearing in angel food cakes made with ovomucoid solution.

Ovomucin Ovomucin is a large glycoprotein containing sulfate esters which contributes to the gel-like character of the thick albumen. Ovomucin polymerizes to provide filamentous and fiber like structures (Whitaker and Tannenbaum, 1977). The carbohydrate content of the purified protein is around 30%, which is composed of 10-12% hexosamine, 15% hexose, and

2.6-8% sialic acid (Feeney et al., 1960). Two protein fractions with different carbohydrate content have been isolated from ovomucin. The fractions contain 50% and 15% carbohydrate (Kato and Sato, 1971; Robinson and Monsey, 1971). Ovomucin in solution is resistant to heat denaturation. Cunningham and Lineweaver (1965) found that the solutions with pH values between 7.1 and 9.4 did not change in optical density during heating for 2 hr at about 90°C, indicating no change in conformation.

When eggs are fresh, the thick white is firm. Ovomucin is apparently involved in the thinning of thick egg whites. Several mechanisms have been proposed to explain the role of ovomucin in egg white deterioration which include: (1) the complexing of ovomucin with lysozyme, (2) the dissociation of a complex between ovomucin and lysozyme, (3) a breaking of disulfide bonds, (4) a loss of carbohydrate from ovomucin and (5) the interaction of glucose with the protein (Whitaker and Tannenbaum,1977).

MacDonnell et al. (1955) and Johnson and Zabik (1981) reported that excessive insolubilization of ovomucin at the bubble interface decreased film elasticity and prevented cake expansion during baking, therefore producing low cake volume. This suggested that the reduced heat coagulative properties of the film surrounding the air cells were primarily responsible for the low cake volume. Cunningham and Lineweaver (1965) observed that ovomucin lacked the heat coagulative properties necessary to form the gel network in

cakes. Johnson and Zabik (1981) reported that ovomucin is able to stabilize foams, primarily by increasing the solution viscosity.

Globulins Globulins represent about 8% of egg white proteins. Longsworth et al. (1940) found the globulin fraction consisted of three proteins G_1, G_2, G_3, with isoelectric points of 10.5-11.0, 5.5, and 4.8, respectively. The molecular weight of G_1 is 14,300, G_2 is 30,000 and G_3 is unknown. The globulins are important for foaming properties and because they coagulate during heating (MacDonnell et al., 1955).

Globulins in LWE are heat stable (81°C) due to the interaction of the egg yolk and egg white protein (Woodward and Cotterill,1983). Globulins have the highest foaming capacity of all the egg white proteins. Removal of globulins and ovomucin from angel food cake caused a large increase in whipping time and decreased cake volume. Globulin proteins alone will make excellent meringue (MacDonnell et al., 1955). Globulins contribute to high viscosity and decrease surface tension which promotes small bubble formation and smooth texture in foams (Baldwin, 1986).

Lysozyme Lysozyme is an enzyme that hydrolyzes polysaccharides in the cell wall of certain bacteria, and therefore, contains antibacterial properties that protect the developing chick. Lysozyme contains 129 amino acids

residues, four disulfide linkages, and no free sulfhydral groups (Powrie, 1976). Thermal inactivation of lysozyme is dependent on pH and temperature (Cunningham and Lineweaver, 1965). Lysozyme is much more heat sensitive in egg albumen than when isolated in phosphate buffer between pH 7 and 9 (Powrie and Nakai, 1986). Ovomucin-lysozyme complex formation during heating resulted in loss of foaming properties of egg whites (Garibaldi et al.,1968). The disulfide bonds inhibit the foaming ability of lysozyme. Lysozyme in combination with ovomucin and globulins is able to improve angel food cake volume (Johnson, 1980).

Egg Yolk Protein

Yolk is a complex system containing a variety of particles suspended in a protein (livetin) solution. The types of particles include yolk spheres, free floating drops or granules, profiles or low density lipoproteins and myelin figures (Powrie and Nakai, 1986). The yolk protein is present mainly as lipoproteins (Parkinson, 1966).

Phosvitin Phosvitin is a heat stable (100°C) phosphoprotein with a molecular weight between 36,000 and 40,000 (Cook, 1968). Phosvitin contains 10% phosphorus which represents 80% of the yolk phosphorus (Mok et al., 1961). Little or no cysteine, cystine, methionine, tryptophan, and tyrosine have been found in phosvitin, although 31% of the total amino

acid residues are serine (Mok et al., 1961). Phosphate in the protein is esterified with serine (Powrie, 1976). Mecham and Olcott (1949) calculated that the serine and phosphorus contents of phosvitin were approximately equal. Taborsky (1963) reported that the ferric ions are bound tightly to phosvitin in a soluble complex. Greengard et al. (1964) concluded that phosvitin is the iron carrier in the yolk.

Livetins Livetins are lipid free globular proteins found in the plasma and comprise 10.6% of the total yolk solids (McCully et al., 1962). Shepard and Hottle (1949) reported three isozymes of livetin designated alpha, beta, and gamma by Martin et al. (1957).

Lipovitellins Lipovitellins in the granules of egg yolk contain about 20% lipid and small amounts of phosphorus. The lipid contains approximately 60% phospholipid (75% phosphatidylcholine) (Bennion, 1980). At pH values below 7, lipoprotein exists as a dimer. Alpha and beta monomers are formed at pH values of 10.5 and 7.8, respectively. (Bernardi and Cook, 1960).

Vitellenins Vitellenins are low density lipoproteins (LDL) with a density of 0.98. Vitellenin is an insoluble lipoprotein. Two fractions, LDL_1 and LDL_2, have been isolated from vitellenin. The total lipid for LDL_1 and LDL_2 is 88% and 85%, respectively. The lipid from the LDL

fractions consists of 25-28% phospholipid. The average molecular weights of the two isolated fractions are 10 million and 3 million, respectively (Martin et al., 1957). Yolk lipoproteins are responsible for the gelation that occurs in the yolk and the whole egg during frozen storage (Powrie et al.,1963; Wakamatu, 1983). Vitellenin gel strength increases in the neutral and alkaline pH range, but is weakened to some extent by salt (Wakamatu,1983; Kojima and Nakamura, 1985). Lipoproteins containing lecithin contribute to the emulsifying ability of egg yolk that is essential for producing the characteristic structure of mayonnaise (Forsythe, 1964).

MATERIALS AND METHODS

LWE PREPARATION

Egg Samples LWE was prepared from day old eggs provided by the Michigan State University poultry farm. The eggs were removed from 4°C storage, immediately broken by hand and blended for 20 sec, without foaming, using a Virtis homogenizer (Model 6-109 AF, Virtis Company, Gardiner, NY) at a speed setting of 15 to produce a homogeneous mixture.

Heat Treatment Approximately 50 ml of LWE was poured into each of 15 test tubes (15.24 cm length by 2.54 cm i.d.) which were then placed into a test tube rack. The test tube rack was then immersed in a water bath (Model 4-8600 American Instrument, Silver Springs, MD) and agitated at 75°C for the time needed to reach the desired treatment temperature (60°C, 64°C, and 68°C). Thermocouples were inserted into two test tubes in the rack to monitor the temperature. The rack was then transferred immediately to another bath (Model 8600A American Instrument) set at the treatment temperature (60°C, 64°C or 68°C) and held for 3.5min. A T-line laboratory stirrer (Model 104) with agitating attachment was inserted into a test tube slot to

agitate the rack for quick and even heat transfer through the LWE. A rheostat (Talboy Engineering Corp., Emerson, NJ) controlled the speed of agitation to eliminate foaming. The test tube rack was inverted in an ice bath for 3 min to stop the cooking. LWE was immediately packaged in polyurethane pouches, labeled and vacuum sealed. LWE samples were analyzed at day zero (unfrozen) and after frozen storage in a still freezer for 1, 15, 30, 45, 80 and 120 days at -24°C. LWE samples were thawed at 4°C for 24 hr prior to testing.

Proximate Analysis

Moisture and protein content was determined on the fresh and unpasteurized LWE using AOAC (1984) 17.006-17.007 and AOAC (1984) 17.008-17.009, respectively. The pH of the LWE was determined at room temperature before each testing period using a Corning pH meter (Model 145, Halstead Essex, England).

Protein Solubility

Protein solubility was analyzed as described by Morr et al. (1985) with modifications. One modification involved mechanical breakdown of the LWE with a Haake Viscometer (Model RV12) at 25°C and 110 rpm until the torque equilibrated. This procedure was necessary to ensure reproducible percent soluble protein. This procedure

produced a homogeneous mixture and eliminated any aggregation of protein that may erroneously alter the protein solubility data. Approximately 5g of LWE was accurately weighed and mixed while adding aliquots of 0.1M NaCl to a total volume of 40 ml. The pH of the dispersion was monitored and adjusted to pH 7.0 with 0.1N HCl. The dispersion was mixed for 1 hr and the pH maintained at 7.0. The dispersion was then transferred into a 50 ml volumetric flask and diluted to the mark with additional 0.1M NaCL. An aliquot of the dispersion was then centrifuged at 20,000 x g for 30 min. The supernatant was filtered through Whatman No. 1 filter paper. Microkjeldahl was used to determine protein content in the filtrate. The average percent protein on the fresh LWE was used to calculate the percent soluble protein for all treatments.

Pie Filling

Pie fillings were prepared using the following formula (Chef Pierre, 1986):

Ingredients	Proportions
corn syrup (42 DE)	236g
sucrose	112g
liquid whole egg	153g
salt	1.4g

A syrup was prepared by continuously stirring by hand the corn syrup, sugar and salt while heating to 43°C. The syrup was immediately removed from the heat and the LWE was stirred in. The syrup was poured into custard dishes until the center depth reached 26 mm (approx. 115g). All treatments were baked at 175°C until the center temperature reached 103°C (approx. 25 min). Center temperature was monitored with an Omega Type T Thermocouple (Stamford, CT). Pie fillings were taken out of the oven and cooled at room temperature for 30 min covered with parafilm, and placed in a 0°C cooler until analyzed (no longer than 24 hr). LWE quality was analyzed based on the percent change in depth of the pie filling from before to after baking. Observations on pie filling after baking included surface aberrations such as bubbles, color differences, glossy or dull surfaces, and the ability of the pie filling to adhere to the dish in which it was baked.

Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was performed as described by Woodward and Cotterill (1983). Purified protein standards (ovotransferrin, globulin, and ovalbumin) were purchased from Sigma Chemical Company (St. Louis, MO). LWE, egg white and egg yolk standards were prepared according to the solublilty procedure of Morr et al. (1985) with modification as discussed in the protein solubility

section. Electrophoresis was performed with a SE 600 series vertical slab unit (Hoefer Scientific Instruments, San Francisco, CA) and a constant voltage power supply (Model 1P-17 Heathkit, Benton Harbor, MI) by using the tris-glycine buffer system described by Woodward and Cotterill (1983). This electrophoresis process includes two systems: (1) stacking gel consisting of 0.25M Tris, pH 6.8 and 4% polyacrylamide and (2) resolving gel of 0.75M Tris, pH 8.8 and 7% polyacrylamide. Both systems used tetramethylenediamine (TEMED) as a catalyst, ammonium persulfate solution (freshly made each time) and N,N'-methylene-bisacrylamide used for polymerization. A 250µl sample was prepared containing 100µl of supernatant obtained from the protein solubility procedure, 50µl glycerol and 100µl of buffer (25mm Tris, pH 7.25). A 50µl sample containing 120µg protein was applied to the stacking gel. Bromophenol blue (0.005%) tracking dye was used to observe the protein movement of the protein. A constant current of 25 mA was maintained until the protein migrated into the resolving gel at which time the current was increased to 50 mA. Electrophoresis was stopped when the tracking dye reached the bottom of the gel (5-6 hr). Gels were stained in 0.4% Coomassie Brilliant Blue in 9/45/45 acetic acid-methanol-water (v/v/v) and destained in 7.5/25/67.5 acetic acid-methanol-water (v/v/v). The destain solution was stirred during the destain procedure. Gels were preserved in a 7.5% acetic acid solution. Densitometer tracings were used

to quantify the bands using the Shimadzu Dual-Wave Length Thin-Layer Chromoto Scanner (Model cs-930, Kyoto, Japan). Protein bands were identified using the protein standards and previously published results (Chang et al., 1970; Galyean and Cotterill, 1979; McBee and Cotterill, 1979; Dixon and Cotterill, 1981; Matsuda et al., 1981; Woodward and Cotterill, 1983). The densitometer results were represented as area under the peak for each protein(s). The protein(s) were expressed as a percentage of the total area of the tracing. The amount of protein(s) in each peak was expressed as a percentage of the total soluble LWE protein.

Rheology

A Rheometrics Fluids Spectrometer (RFS) Model 8400 (Rheometrics Inc., Piscataway, NJ) was used to characterize the rheological properties of LWE using oscillatory and steady shear tests under conditions of controlled strain and shear rate at 24°C. A cone (2.5 cm radius, 0.02 radians and a 0.05 mm gap) and plate configuration was used. The single mode (frequency 35 sec^{-1} and strain 35%) was used first to release the tension on the shaft of the cone and plate configuration after the sample was loaded. Next, a steady thixotropic loop was applied to calculate torque, shear stress and viscosity at a shear rate range from 0-60 sec^{-1}. The time to cover the shear rate range was 120 sec. Torten and Eisenberg (1982) evaluated LWE at shear rates between 0

and 800 sec^{-1}, with some shear rates approaching 2000 sec^{-1}. A smaller shear rate was selected to: (1) minimize degradation of the LWE, (2) prevent viscous heating causing denaturation of LWE protein, (3) prevent throwing LWE out of the cone and plate geometry due to centrifugal force, and (4) investigate the fluid behavior at lower ranges of shear. A sample size of 3ml was applied between the cone and plate geometry. A larger sample would cover the top of the cone and plate geometry and obstruct the readings. Linear regression was used to fit the rheological model type for each treatment. Thixotropic behavior was quantitated by cutting out the area between zones 1 and 2 of the rheogram and determining its mass on an Mettler AE 160 analytical balance (Mettler Instrumentation Corp., Heightstown, NJ). The combined mass of the area between zones 1 and 2 was compared to a standard ($4cm^2$ area=0.0307g mass).

Statistical Analysis

MSTAT (Michigan State University, 1985) was used for basic statistics and complete randomized design 2 factor factorial testing on the experimental variables. Variables were shear stress, apparent viscosity (at shear rate of 30 sec^{-1}), treatment, time, peak viscosity, pie filling depth, and percent soluble protein. The complete randomized design using 2 factor factorial (treatment and time) was used to determine the correlation between variables in the

experiments. Duncan's Multiple Range Test was used to evaluate the significant differences between the means at the 0.05 level of probability.

RESULTS AND DISCUSSION

The average moisture and protein content of fresh LWE was 76.8% and 11.4%, respectively. Average pH of the LWE was 7.57 and there was no significant change due to pasteurization or frozen storage. These values are similar to others reported in the literature (Staldeman and Cotterill, 1986).

Protein Solubility

As pasteurization temperature of the LWE increased the protein solubility decreased (Fig.3). The unpasteurized LWE at zero contained 77.2% soluble protein while the solubility of 68°C LWE protein was 66.1%. A significant difference ($p<0.05$) existed between the unpasteurized and 60°C LWE at day zero. Parkinson (1968) reported that soluble protein concentration in the pasteurized fraction was lower than the unpasteurized fraction of frozen eggs. Percent soluble LWE protein decreased from day 1 through day 80 of frozen storage. Protein solubility in the control and 68°C LWE decreased by 16.4% and 10.9%, respectively, during 80 days

PROTEIN SOLUBILITY (%)

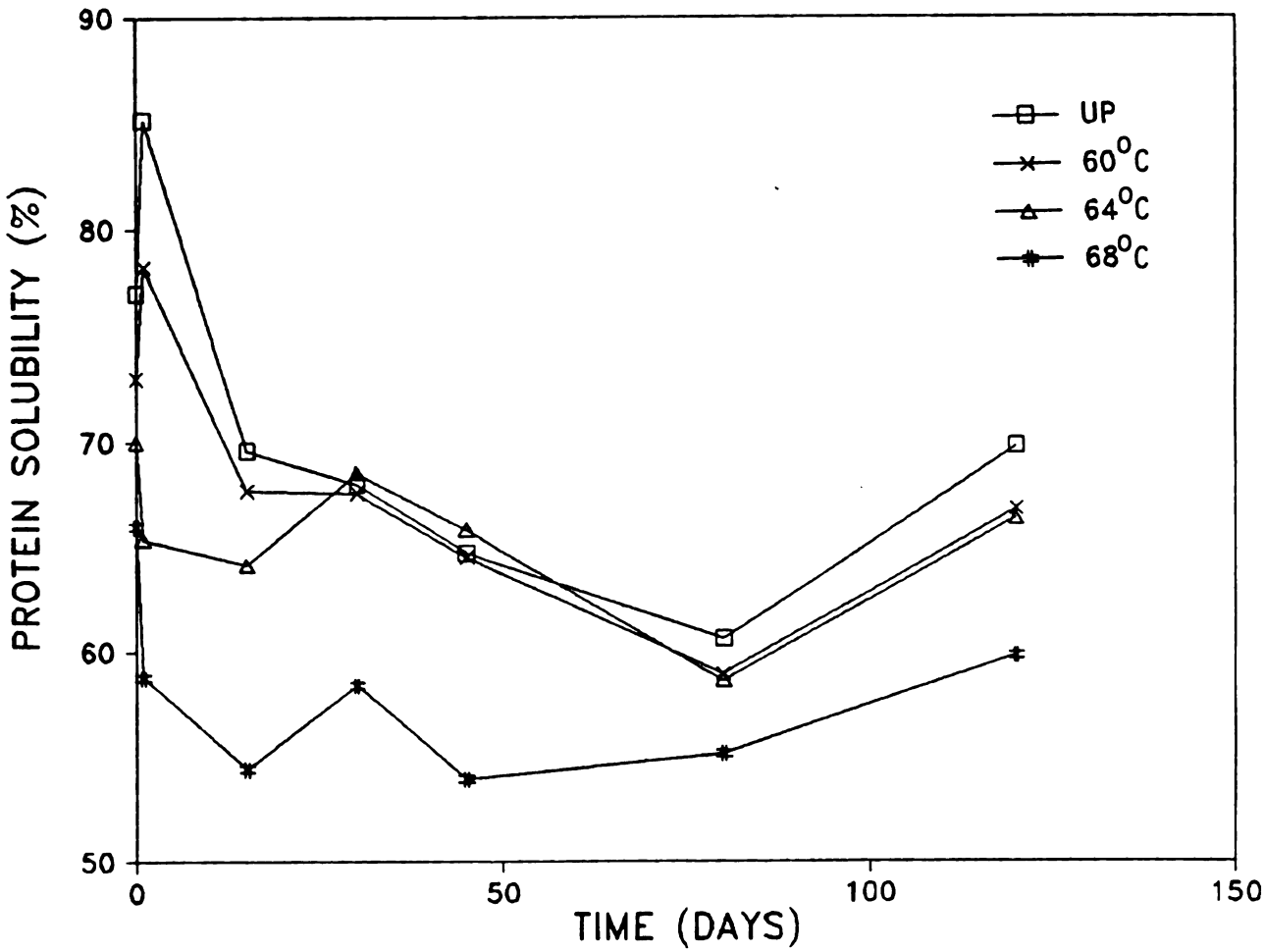


Figure 3. Effect of pasteurization and storage time at -24°C on protein solubility of LWE measured at 23°C. SE=±0.96 (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min.)

of frozen storage. A significant difference ($p<0.05$)in solubility existed between day zero and day one of storage in unpasteurized LWE. The decrease in percent soluble protein with frozen storage could be the result of lipoprotein gelation causing precipitation of the high molecular weight molecules (Wakamatu et al., 1983) or denaturation of LWE proteins, such as conalbumin (Wootton et al., 1981). The percent soluble protein increased in LWE evaluated at day 120. It is possible that the increase in percent soluble protein may have resulted from hydrolysis of peptide bonds releasing amino acids from insoluble protein or from increases in non-protein nitrogen, giving the impression of additional soluble protein.

Electrophoresis

LWE proteins separated by electrophoresis were identified using protein standards as well as published data on relative mobilities (Table 8) (Chang et al., 1970; Galyean and Cotterill, 1979; McBee and Cotterill, 1979; Dixon and Cotterill, 1981; Matsuda and Cotterill et al., 1981; Woodward and Cotterill,1983). Densitometer tracings of protein standards separated by PAGE included ovotransferrin (Fig. 4), egg albumens (Fig. 5), egg white globulins (Fig. 6), fresh egg white (Fig. 7) and fresh egg yolk (Fig. 8). PAGE was used to observe the changes in soluble protein that occurred with pasteurization and frozen storage of LWE.

Table 8. Relative mobilities of liquid whole egg proteins on polyacrylamide gel electrophoresis.

Peak	Protein	Relative Mobility
1	Ovomacroglobulin	0.02
2	Lipovitellin	0.08-0.19
3	Ovotransferrin	0.27-0.32
	Ovoinhibitor	0.29-0.32
	γ Livetin	0.29-0.32
	G_2 Globulin	0.29-0.32
4	Unknown	0.37
5	G_{3A} Globulin	0.45-0.50
6	G_{3S} Globulin	0.52-0.55
	G_{3B} Globulin	0.66-0.68
	β Livetin	0.58-0.64
7	Ovalbumin A_3	0.70-0.72
8	Ovalbumin A_2	0.75-0.80
	α Livetin	0.76-0.80
9	Ovalbumin A_1	0.83-0.91

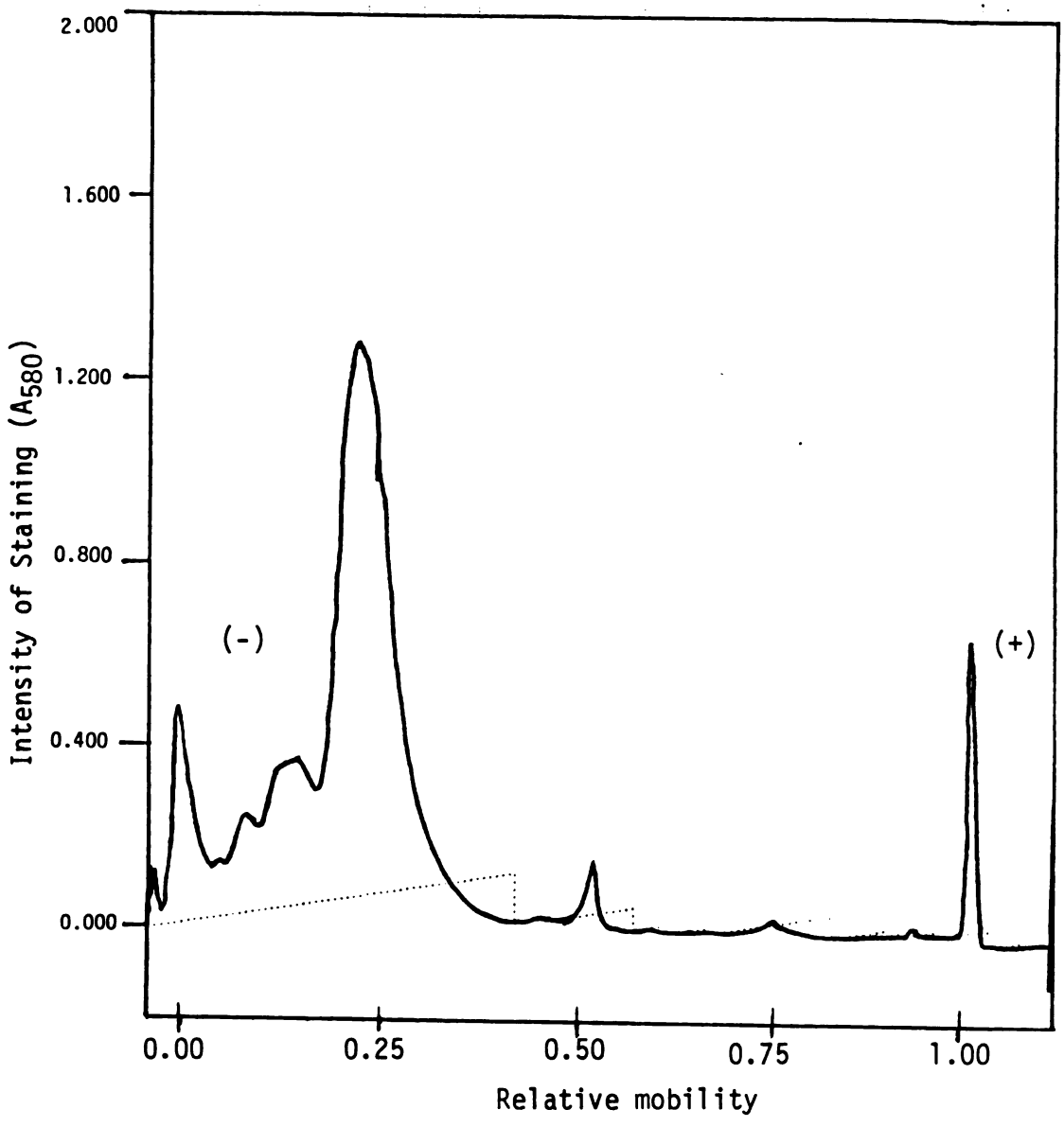


Figure 4. Densitometer tracing of an ovotransferrin standard electrophoregram.

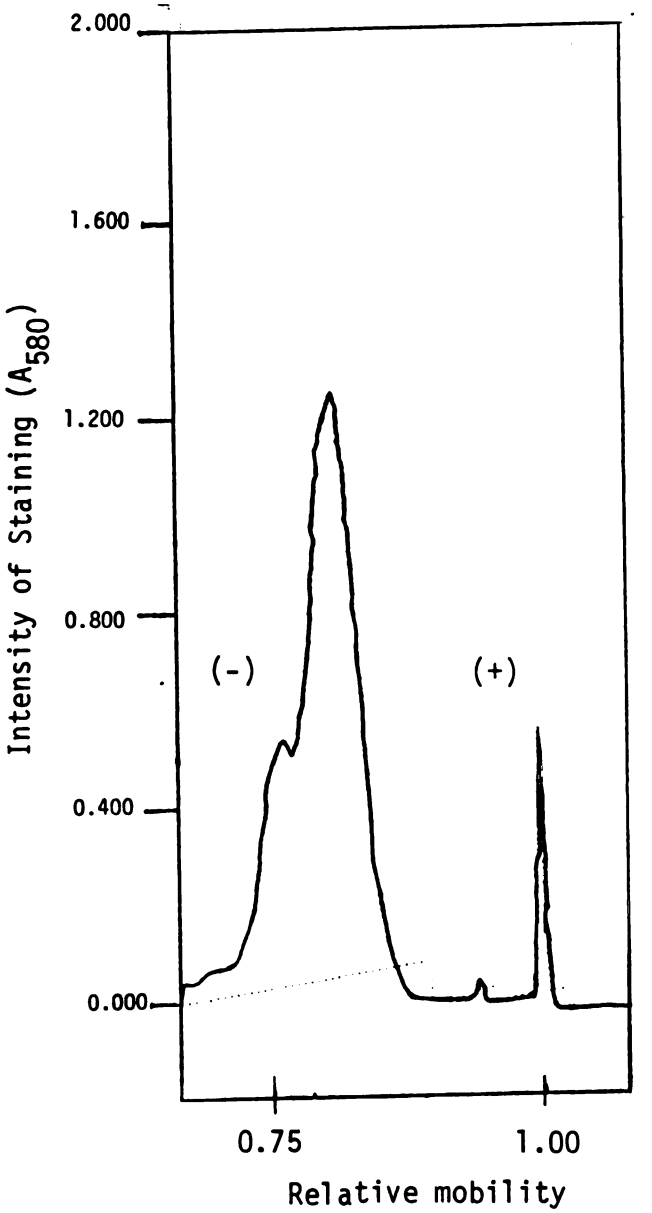


Figure 5. Densitometer tracing of an egg albumen standard electrophoregram.

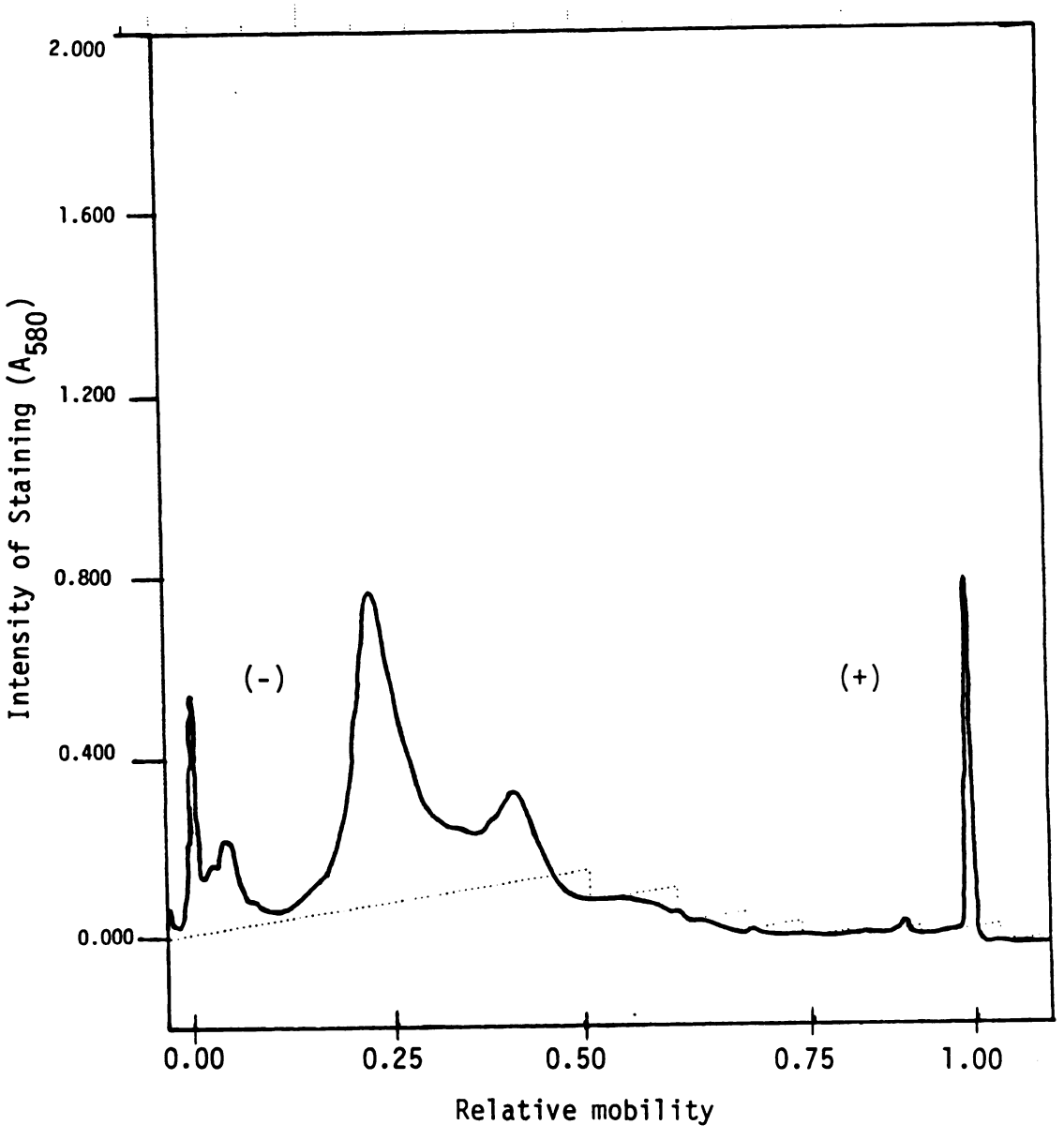


Figure 6. Densitometer tracing of an egg white globulin standard electrophoregram.

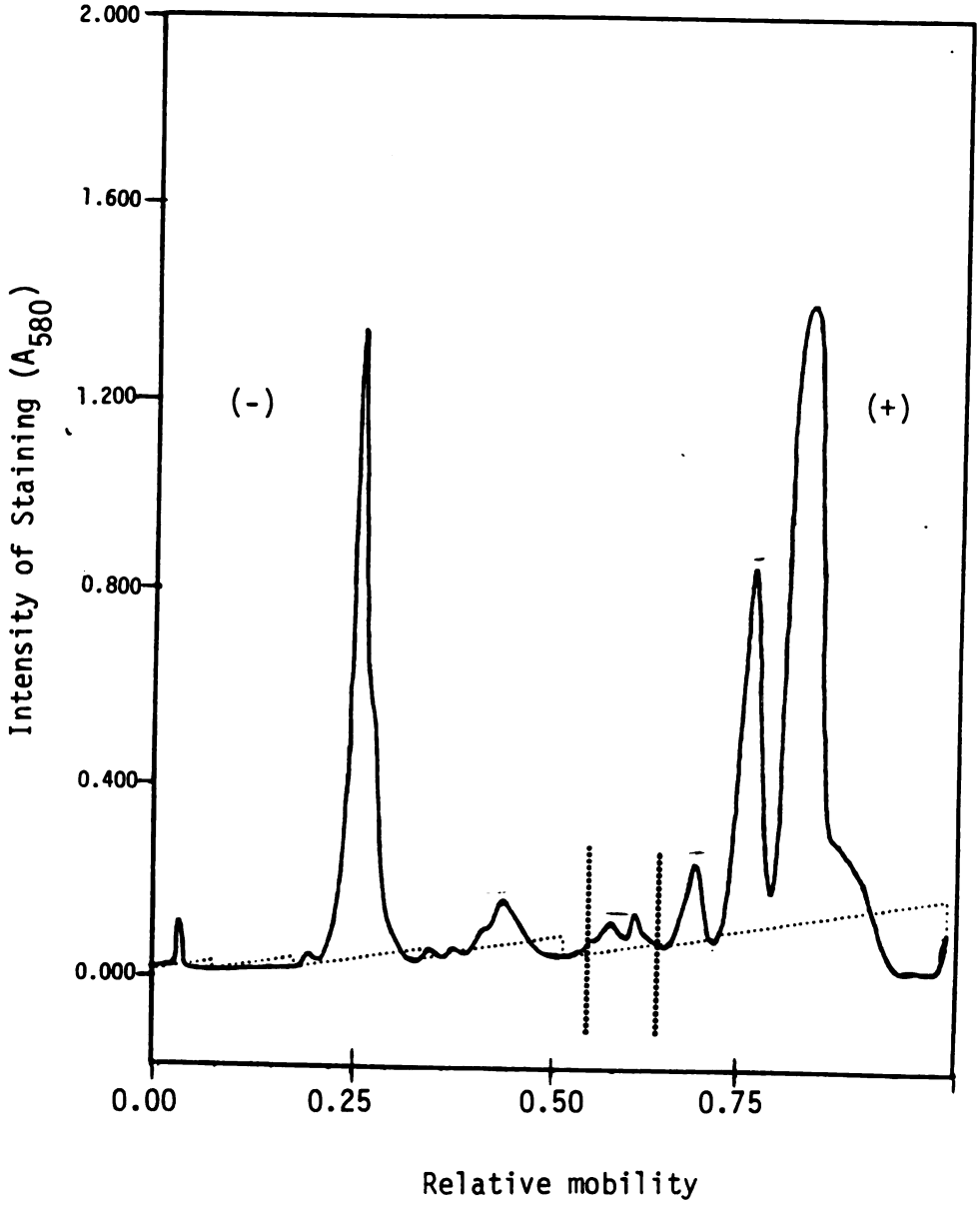


Figure 7. Densitometer tracing of an electrophoregram from soluble fresh egg white proteins.

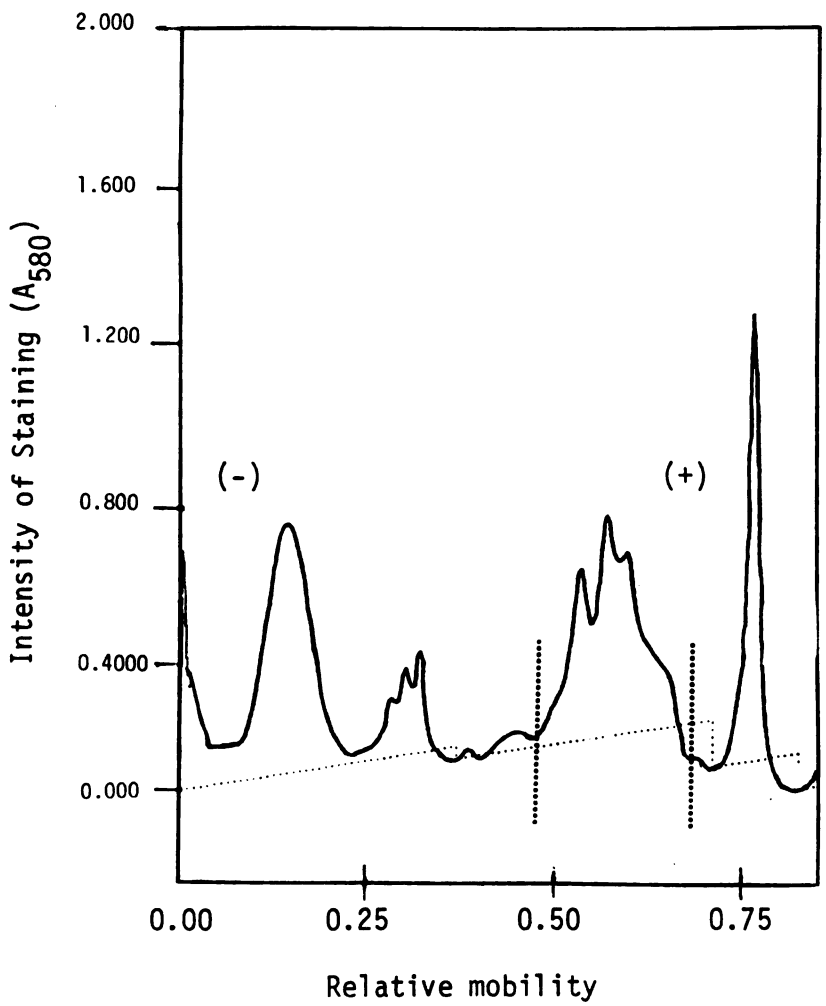


Figure 8. Densitometer tracing of an electrophoregram from soluble fresh egg yolk proteins.

Electrophoretic separation of soluble LWE protein resulted in 9 distinguishable protein bands (Fig.9). Representative electrophoregrams of Day 0 and Day 120 unpasteurized LWE soluble protein are in Fig. 10 and 11, respectively. In general, the percent total soluble protein in the lipovitellin, conalbumin, ovoinhibitor, livetin and G_{3A} protein fractions decreased and the unknown peak, ovalbumin A_1, A_2, A_3 and α livetin fractions increased due to heat treatment. Lipovitellin and conalbumin, ovoinhibitor, G_2, and β livetin decreased, while ovalbumin A_2, A_3, and α livetin increased during frozen storage.

The relative percent change of each soluble LWE protein resolved by PAGE is listed in Table 9.

Peak 1 (Table 9a):Ovomacroglobulin (OMG). OMG did not always appear on the densitometer tracing. For example, it appeared only in the unpasteurized LWE at day 120. Researchers have reported that OMG does not migrate far into the gel due to the high molecular weight (1×10^6). Woodward and Cotterill (1983) observed that OMG, when heated, may break into subunits and migrate into the ovalbumin region.

Peak 2 (Table 9b): Lipovitellin. Lipovitellin is a heat stable (72°C) egg yolk protein (Woodward and Cotterill, 1983). This protein decreased with pasteurization from 11.6% for the unpasteurized LWE to 0% for the 68°C LWE treatment on day zero. Frozen storage decreased lipovitellin from

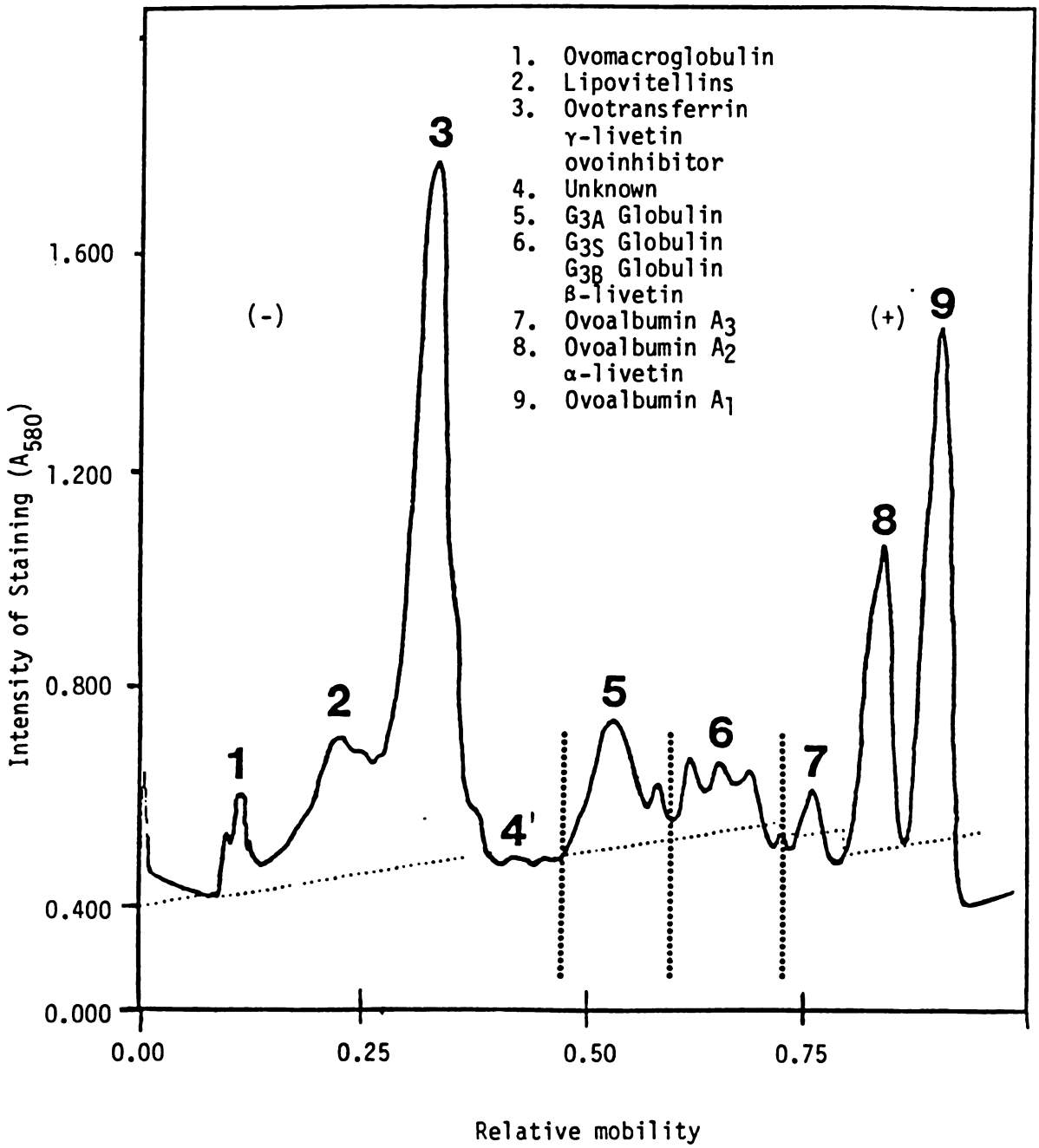


Figure 9. Densitometer tracing of an electrophoregram from unpasteurized and unfrozen soluble liquid whole egg proteins.

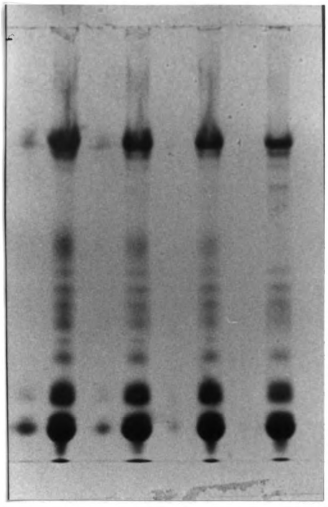


Figure 10. Electrophoregram of heat treated and unfrozen fresh liquid whole egg soluble protein resolved on 7% polyacrylamide gels.

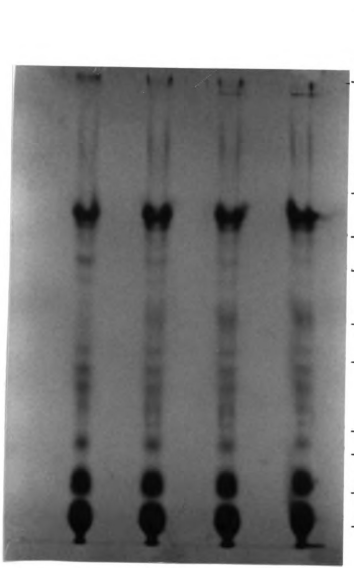


Figure 11. Electrophoregram of unpasteurized liquid whole egg soluble proteins after 120 days of frozen storage resolved on 7% polyacrylamide gels.

Table 9a. Peak 1. Effect of heat treatment and frozen storage on ovomacroglobulin expressed as a percentage of the total soluble LWE protein.

Length of frozen storage (days)	Unpasteurized	60°C	64°C	68°C
	Percent of total soluble protein			
0	2.7	1.0	0.7	2.5
1	0.8	1.1	1.1	1.0
15	0	0	1.6	3.2
30	2.5	1.2	1.4	0
45	3.2	4.3	1.2	4.2
80	3.2	4.3	1.3	4.2
120	0.8	0	0	0

Table 9b. Peak 2. Effect of heat treatment and frozen storage on lipovitellin expressed as a percentage of the total soluble LWE protein.

Length of frozen storage (days)	Unpasteurized	60°C	64°C	68°C
	Percent of total soluble protein			
0	11.6	0	3.3	0
1	2.6	2.7	3.3	2.4
15	0	0	2.8	1.3
30	3.4	0	0.9	1.3
45	1.7	3.2	3.4	0
80	0	0	0	0
120	1.6	0	1.8	2.4

11.6% on day zero to 1.6% on day 120 for the unpasteurized LWE. According to Parkinson (1977) and Dixon and Cunningham (1981) lipoproteins aggregate during frozen storage and may become insoluble with time. Chang et al. (1970) suggested that proteins aggregate during heat treatment to form large molecular weight complexes unable to migrate into the gel. These observations could explain the disappearance of the lipoprotein with pasteurization and frozen storage.

Peak 3 (Table 9c): Ovotransferrin, ovoinhibitor, G_2, and γ livetin. These proteins are considered constituents of the same electrophoresis peak. It was reported that ovotransferrin masked the other proteins (Woodward and Cotterill, 1983). Peak 3 decreased with pasteurization from 38.8% to 16.9%, in unpasteurized and 68°C LWE, respectively, at day zero. The unpasteurized LWE decreased with frozen storage from 38.8% to 14.7% at day zero and day 120, respectively. The decrease in peak 3 could be due to a decrease in γ livetin and ovotransferrin. γ livetin is heat labile and denatures at 63°C (Chang et al.,1970: Dixon and Cotterill, 1979). McBee and Cotterill (1979) reported that γ livetin was more concentrated in the precipitate than the supernatant of the centrifuged LWE protein dispersion. Ovotransferrin is more stable in LWE (78°C) than in egg white (63°C due to the complexing of the iron from the yolk) (Woodward and Cotterill, 1983). Ovotransferrin denatures due to frozen storage (Wootton et al., 1981). Ovoinhibitor and

Table 9c. Peak 3. Effect of heat treatment and frozen storage on ovotransferrin, ovoinhibitor, G_2 and γ livetin expressed as a percentage of the total soluble LWE protein.

Length of frozen storage (days)	Unpasteurized	60°C	64°C	68°C
	Percent of total soluble protein			
0	38.8	27.0	17.8	16.9
1	25.0	18.7	18.3	13.7
15	18.5	15.8	24.7	15.4
30	6.1	21.9	13.1	10.8
45	17.7	19.3	15.3	9.5
80	22.2	15.9	12.7	11.9
120	14.7	27.2	17.5	22.9

Table 9d. Peak 4. Effect of heat treatment and frozen storage on the unknown protein complex expressed as a percentage of the total soluble LWE protein.

Length of frozen storage (days)	Unpasteurized	60°C	64°C	68°C
	Percent of total soluble protein			
0	0	0	0	1.3
1	0	0	0.8	1.3
15	0	0	1.2	1.5
30	0	0	0	0
45	0	0	0	1.6
80	0	0	1.0	1.3
120	0	0	1.0	1.8

G_2 globulin are heat stable proteins denaturing at 78°C and 81°C, respectively (Woodward and Cotterill, 1983).

Peak 4 Unknown (Table 9d): This peak was only present as a small percentage in the densitometer tracings for the 64°C LWE and 68°C LWE treatments. This peak was not identified with any of the protein standards that were examined. Peak 4 may be a complex of certain egg white and egg yolk proteins. Parkinson (1967) reported that electrophoresis of soluble proteins in LWE produced additional peaks that were not present in electrophoregrams of white or egg yolk proteins.

Peak 5 G_{3A} (Table 9e): G_{3A} decreased with pasteurization from 9.04% to 0% at day zero for the unpasteurized and 68°C LWE, respectively. Woodward and Cotterill (1983) reported that G_{3A} denatured at 63°C. Frozen storage did not substantially affect the solubility of G_{3A}.

Peak 6 G_{3S}, G_{3B}, and β livetin (Table 9f): These proteins did not change substantially with heat treatment of frozen storage. G_{3S} and β livetin are heat labile proteins and denature at 63°C (Woodward and Cotterill, 1983). Any changes in this peak may be due to the heat denaturable proteins.

Peak 7,8 and 9 Ovalbumins A_1, A_2, A_3 and α livetin (Table 9g, 9h, and 9i): These proteins were identified as the ovalbumin proteins A_3, A_2, α livetin and ovalbumin A_1,

Table 9e. Peak 5. Effect of heat treatment and frozen storage on G_{3A} expressed as a percentage of the total soluble LWE protein.

Length of frozen storage (days)	Unpasteurized	60°C	64°C	68°C
	Percent of total soluble protein			
0	9.0	6.8	4.7	0
1	5.9	4.1	5.1	1.4
15	4.9	8.8	4.8	0
30	10.3	8.0	7.7	1.4
45	6.5	4.4	4.3	0
80	8.4	7.2	7.2	4.8
120	7.1	8.8	4.9	0

Table 9f. Peak 6. Effect of heat treatment and frozen storage on G_{3S}, G_{3B}, and β livetin expressed as a percentage of the total soluble LWE protein.

Length of frozen storage (days)	Unpasteurized	60°C	64°C	68°C
	Percent of total soluble protein			
0	6.6	7.4	8.7	7.2
1	6.7	6.2	7.8	12.7
15	4.6	12.2	6.5	1.6
30	7.9	10.6	8.2	0
45	6.6	2.4	6.7	0
80	10.6	12.8	9.8	15.2
120				

Table 9g. Peak 7. Effect of heat treatment and frozen storage on ovalbumin A_3 expressed as a percentage of the total soluble LWE protein.

Length of frozen storage (days)	Unpasteurized	60°C	64°C	68°C
	Percent of total soluble protein			
0	1.2	2.8	4.0	3.1
1	2.0	1.8	2.7	4.3
15	1.9	3.1	1.4	3.5
30	3.1	2.1	3.1	3.2
45	2.5	2.4	3.3	4.3
80	2.6	4.5	5.4	6.5
120				

Table 9h. Peak 8. Effect of heat treatment and frozen storage on ovalbumin A_2 and α livetin expressed as a percentage of the total soluble LWE protein.

Length of frozen storage (days)	Unpasteurized	60°C	64°C	68°C
	Percent of total soluble protein			
0	11.5	20.3	23.0	22.1
1	19.7	22.0	21.7	21.1
15	21.0	23.6	16.7	25.2
30	25.3	23.5	22.4	28.6
45	21.4	21.7	23.3	29.7
80	22.6	24.1	25.2	22.2
120				

Table 9i. Peak 9. Effect of heat treatment and frozen storage on ovalbumin A_1 expressed as a percentage of the total soluble LWE protein.

Length of frozen storage (days)	Unpasteurized	60°C	64°C	68°C
	Percent of total soluble protein			
0	19.2	34.0	36.5	46.6
1	37.5	43.0	38.9	40.8
15	48.9	36.2	39.7	49.4
30	41.1	32.4	42.9	54.6
45	40.0	42.0	42.2	50.5
80	33.4	35.1	36.7	37.7
120	43.2	30.8	39.8	41.6

respectively. Ovalbumins are heat stable (78°C) and did not decrease as a percentage of total soluble protein with either pasteurization or frozen storage. The increase in percentage of total soluble LWE proteins in these peaks was due to the decrease in other proteins.

Coomassie blue dye is used to stain proteins present in electrophoresis gels by binding to available sites on the protein molecule. Coagulation decreases the available binding sites on the protein molecule, therefore color intensity of the stained bands decreases with higher pasteurization temperatures. The decrease in color intensity could cause error when evaluating densitometer tracings. Another possible error may result from variation in the amount of sample placed in the gel.

Rheology

Rheology is the study of the flow and deformation of matter. Rheological data is used for: (1) engineering applications, (2) examining structure of materials, (3) quality control and (4) to substitute for or confirm sensory evaluation (Muller, 1973).

Rheological characteristics of LWE were determined in the fourth zone of the thixotropic loop (Fig. 12) unless otherwise noted. This zone was selected because, LWE has reached equilibrium. Before this point, mechanical

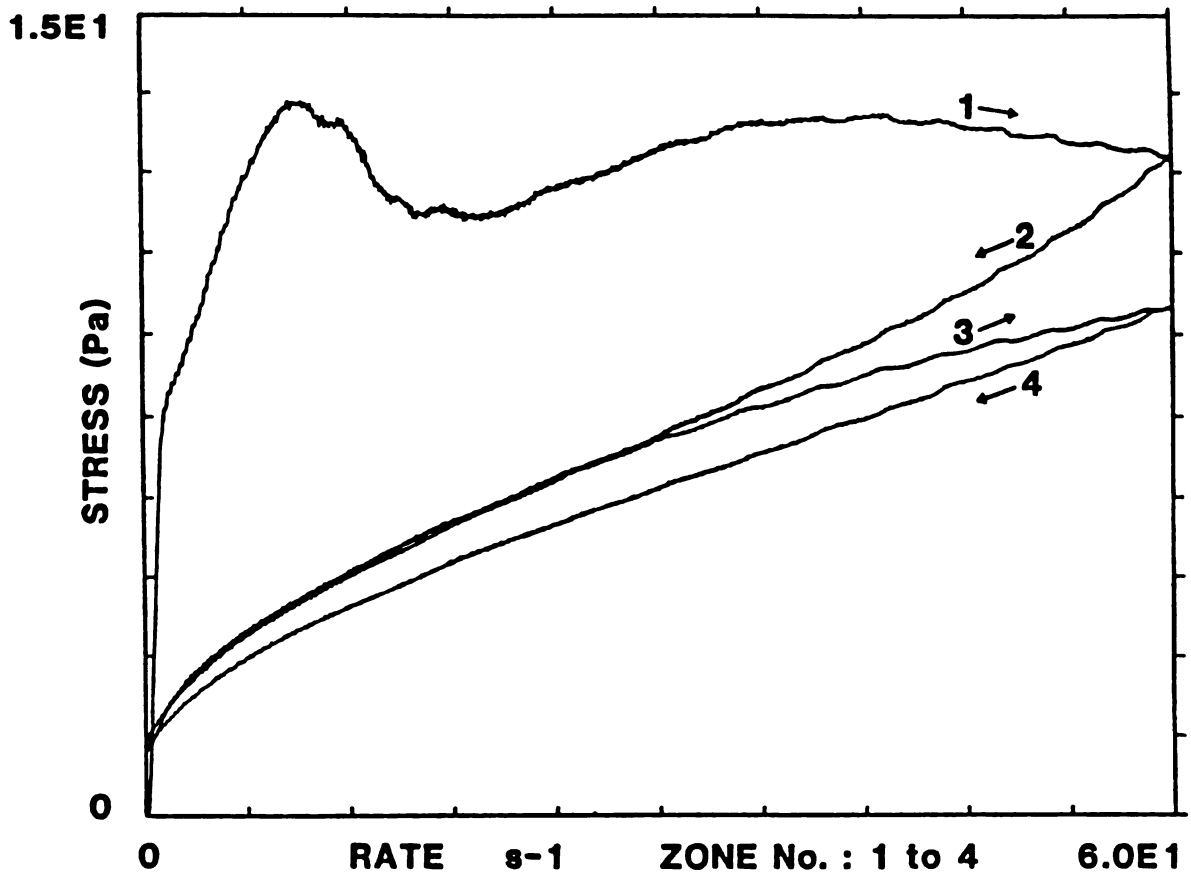


Figure 12. Rheogram of liquid whole egg.

degradation may not be complete. Both heat treatment and length of frozen storage altered the rheological properties of LWE. The following discusses the specific rheological parameters and their changes during the experiment.

Peak Viscosity Peak viscosity was defined as the highest viscosity determined from the rheogram (Fig. 12). The peak viscosity value indicated the presence of egg protein aggregates and was always found in the first zone of the thixotropic loop before mechanical shearing disrupted the aggregated protein structure of LWE. All LWE treatments exhibited increased peak viscosity with frozen storage (Fig.13). Peak viscosity increased from 0.02 Pa-s for the 68°C LWE treatment at day zero. Peak viscosity increased with frozen storage. Unpasteurized LWE day zero was 0.02 Pa-s and increased to 1.1 Pa-s at day 120. Significant differences ($p<0.05$) were found with both pasteurization and frozen storage. A significant difference ($p<0.05$) in peak viscosity with pasteurization was first noted between the unpasteurized LWE day zero and the 60°C LWE day one treatments. With respect to frozen storage, a significant difference ($p<0.05$) in peak viscosity was noted between unpasteurized LWE day zero and unpasteurized LWE day 30. Peak Viscosity of LWE is not of primary interest to the bakery industry because during commercial processing a high shear rate used. Shearing destroys any protein aggregates that may form as a result of frozen storage.

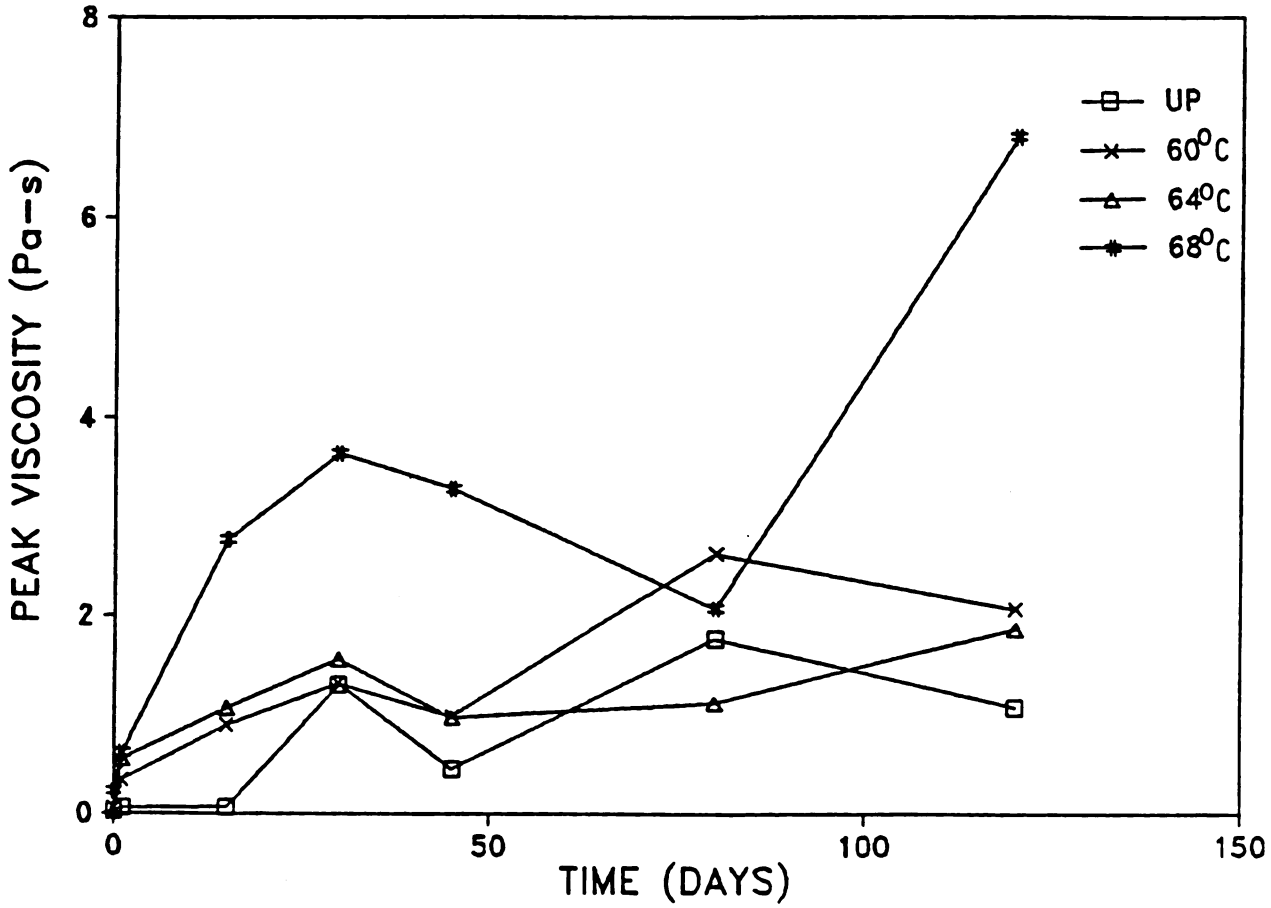


Figure 13. Effect of pasteurization and storage time at -24°C on peak viscosity of LWE measured at 23°C (S.E.=±1.48) UP= unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min.)

Time Dependency Time dependency was evaluated using the first and second zones from the rheogram of shear stress versus shear rate (Fig.12). Time independency was observed for the day 0 unpasteurized LWE, 60°C LWE and 64°C LWE. Day zero 68°C LWE exhibited shear thinning and time dependency which is characteristic of thixotropic behavior. Thixotropic behavior was observed in all of the LWE treatments on freezing. Thus, heating at 68°C or above, and frozen storage can alter rheological properties of LWE. This is the first report of thixotropic behavior which has been quantified (Torten and Eisenberg, 1982) and contradicts previous literature which states that LWE is pseudoplastic with frozen storage (Cornford et al., 1969; Scalzo et al., 1970). Better instrumentation allowed for sensitive testing in a lower shear rate range, and facilitated the thixotropic identification. The area between zones one and two of the thixotropic loop was used to quantify thixotropic behavior. The area of the thixotropic unpasteurized LWE, 60°C LWE, and 64°C LWE all increased with frozen storage due to protein aggregation (Fig. 14). The 68°C LWE thixotropic loop was largest at day zero. This may be due to proteins coagulating during pasteurization with no further aggregation occurring during frozen storage. Decreases in the thixotropic loop area of the LWE with frozen storage may be due to degradation of the aggregated complex. The proteins

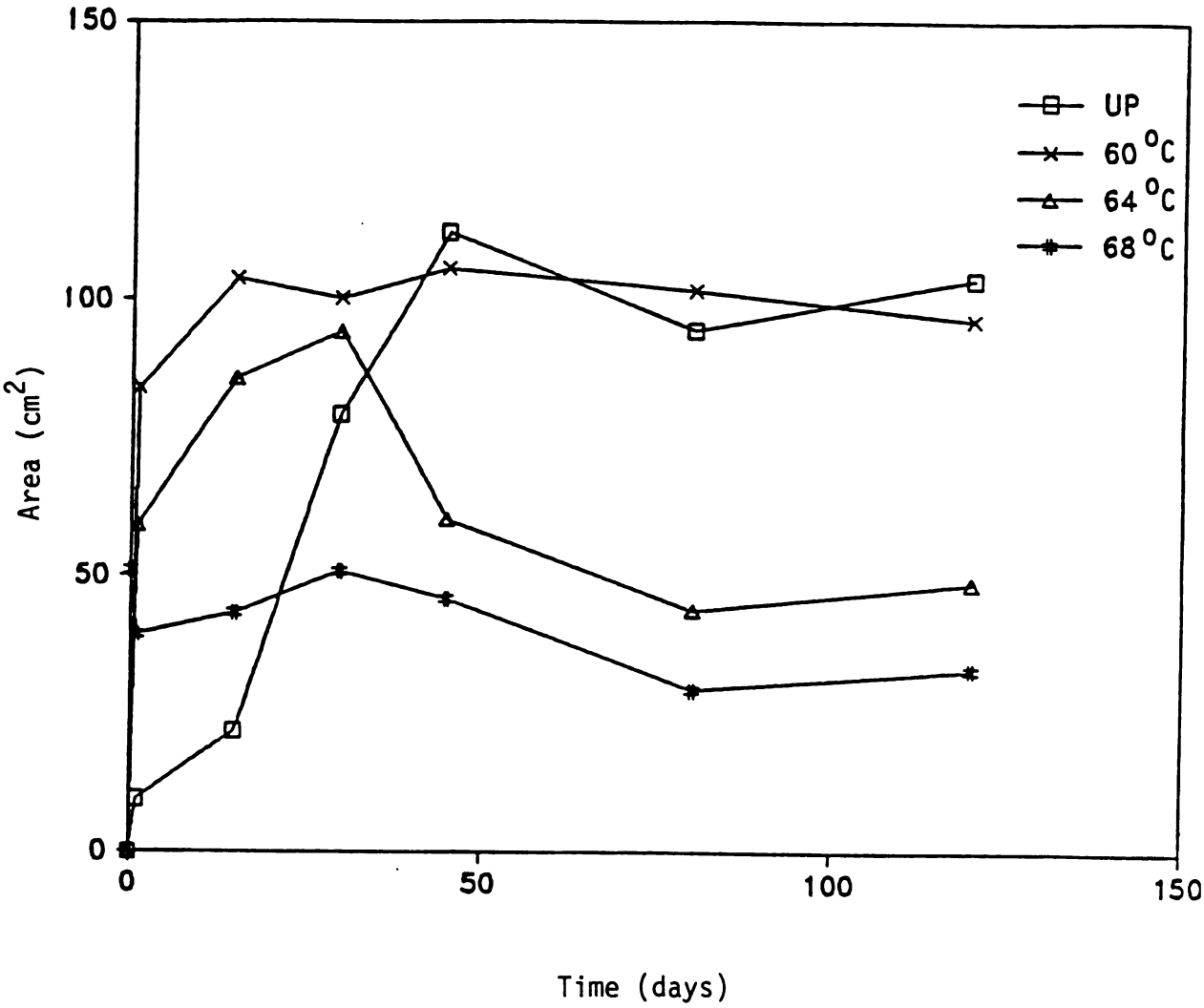


Figure 14. Hystersis curve area of LWE stored at -24°C for 120 days after having undergone pasteurization for 3.5 min. SE=4.79 (unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min.)

containing lipid and carbohydrate may enhance the breaking up of the large protein aggregates during frozen storage.

Elasticity was tested to evaluate the memory of the LWE. After shearing the 64°C day 45 LWE in the thixotropic loop, it was allowed to rest for 1 hr and the thixotropic loop was performed again. The 64°C LWE day treatment returned to 93% of its original peak viscosity. This phenomena also was observed by Tung et al. (1971) for egg albumen.

Apparent Viscosity Viscosity determined at a specific shear rate is defined as "apparent viscosity" (Muller, 1973). Apparent viscosity is useful when dealing with non-Newtonian fluids which do not have constant viscosities over a range of shear rates. The apparent viscosity was calculated in this experiment at shear rate of 30 sec^{-1} in the fourth zone of the thixotropic loop, which was the mid-point of the shear rate range examined. The apparent viscosity increased with treatment temperature and time of frozen storage (Fig. 15). Apparent viscosity increased with heat treatment from 0.01 Pa-s for the unpasteurized LWE treatment day zero to 0.06 Pa-s for the 68°C LWE treatment day zero. Apparent viscosity increased with frozen storage from 0.01 Pa-s for the unpasteurized LWE day zero to 0.08 Pa-s for the unpasteurized LWE treatment day 120. A significant difference ($p<0.05$) due to heat treatment was first noted between the unpasteurized LWE and 68°C LWE day zero.

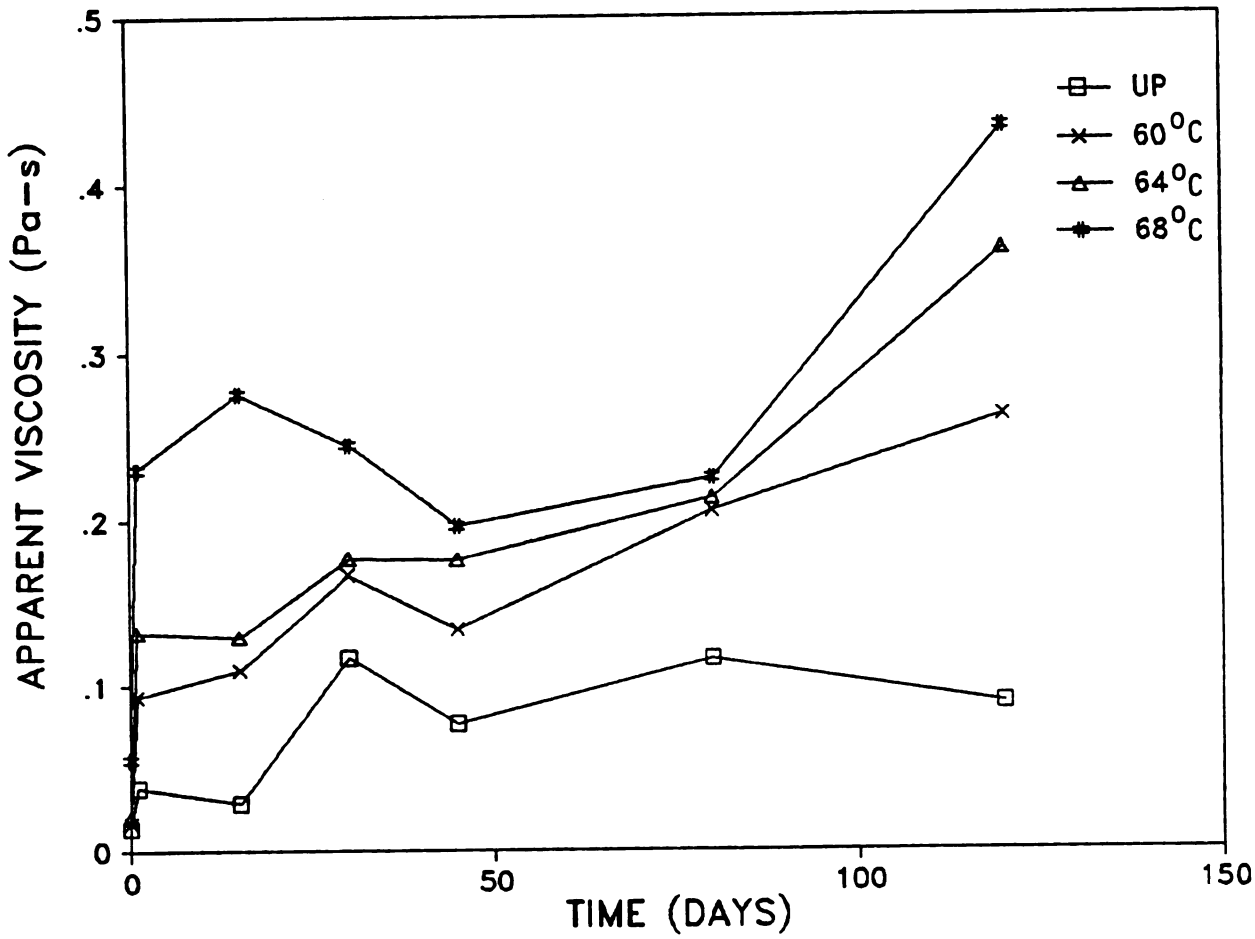


Figure 15. Effect of pasteurization and storage time at -24°C on apparent viscosity of LWE measured at 23°C. SE=±0.01 (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min.)

unpasteurized LWE day zero and unpasteurized LWE day 30 apparent viscosity was significantly different ($P<0.05$) during frozen storage. Torten and Eisenberg (1982) reported lower apparent viscosities for pasteurized and frozen stored LWE than those observed in this experiment, possibly due to the higher shear rate used. Torten and Eisenberg (1982) suggested that increases in viscosity due to heat treatment and frozen storage could result from: (1) asymmetric protein aggregation and changes in intramolecular bonds, or (2) minor changes in intermolecular contacts in the three dimensional system of LWE. Mayo and Baker (1965) noted that the apparent viscosity of thawed LWE can be influenced by the method of freezing, rate of freezing, length of storage, and length of time of thaw.

Steady Shear Shear dependency describes material that still has structure and is being broken down to an equilibrium state. Structure in LWE is due to protein aggregation caused by coagulation and freezing. Shear independency is characteristic of a material that is at an equilibrium state. Shear dependency and shear independency varied with the LWE treatment and shear rate range. Scalzo et al. (1970) also reported that LWE shear dependent range increased with pasteurization temperature and length of frozen storage.

Day Zero (Fig. 16) Unpasteurized LWE, 60°C LWE, and 64°C LWE were shear independent throughout the shear rate range. The

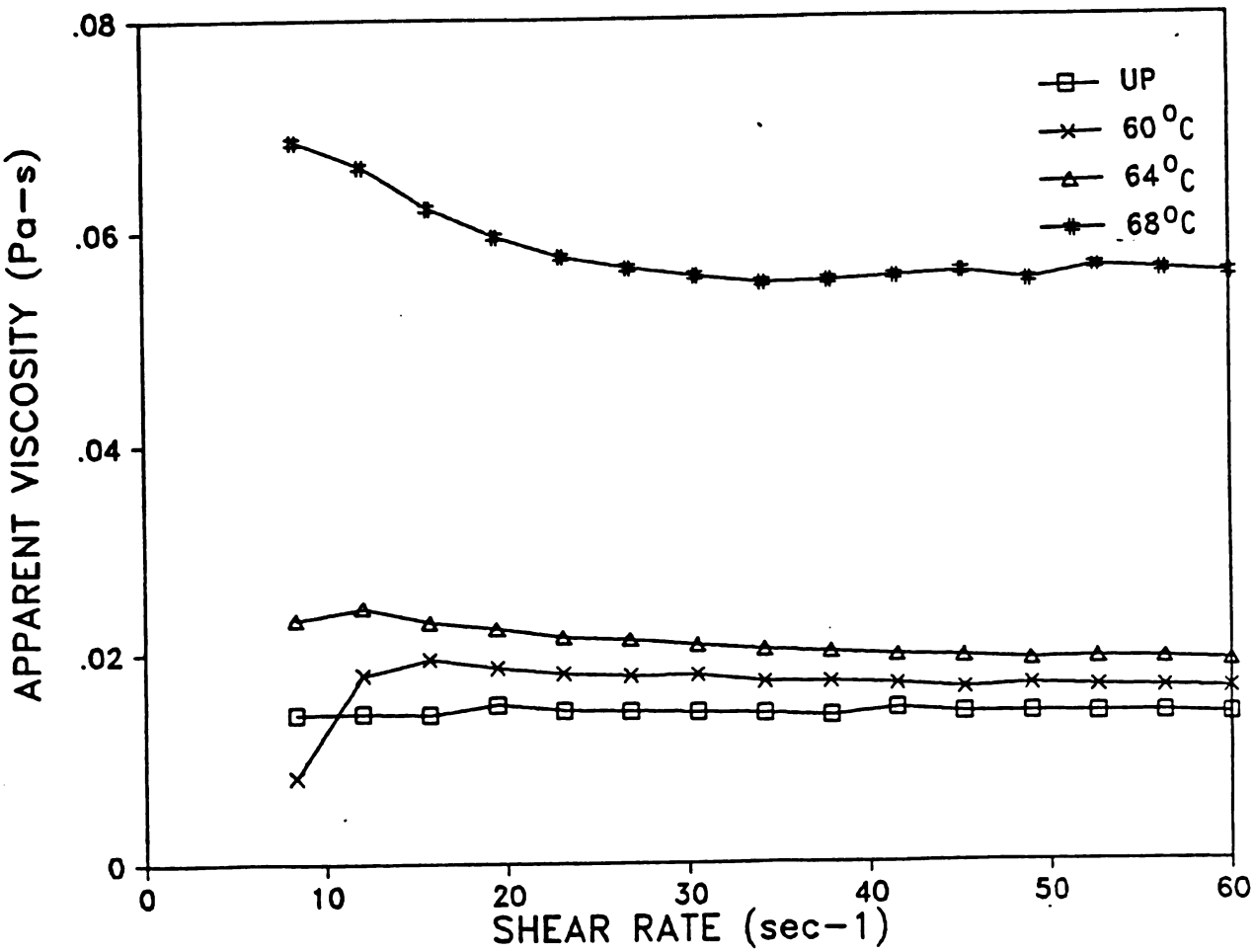


Figure 16. Effect of pasteurization on shear rate dependency of LWE (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

68°C LWE was shear dependent in a shear rate range of 0-30 sec^{-1}, then shear independent for the rest of the range.

Day 1 and Day 15 (Fig. 17 and 18) Unpasteurized LWE remained shear independent throughout the shear rate range evaluated. The 60°C LWE and 64°C LWE exhibited shear dependency over a shear rate range of 0-30 sec^{-1}, then became shear independent for the rest of the shear rate range.

Day 30 (Fig. 19) Unpasteurized LWE had some shear dependency between 0-10 sec^{-1}, then was shear independent throughout the remainder of the shear rate range at days 30, 45, and 80. The 60°C LWE and 64°C LWE were shear dependent from 0-30 sec^{-1}, then were shear independent for the rest of the shear rate range. The 68°C LWE was shear dependent throughout the entire range of shearing.

Day 45 (Fig. 20) Unpasteurized LWE had approximately the same shear history as day 30. The 60°C LWE was more shear dependent than on day 30. The 64°C LWE and 68°C LWE were shear dependent throughout the entire shear rate range.

DAy 80 (Fig. 21) Unpasteurized LWE showed little change from previous days. The other LWE treatments were shear dependent throughout the entire shear rate range.

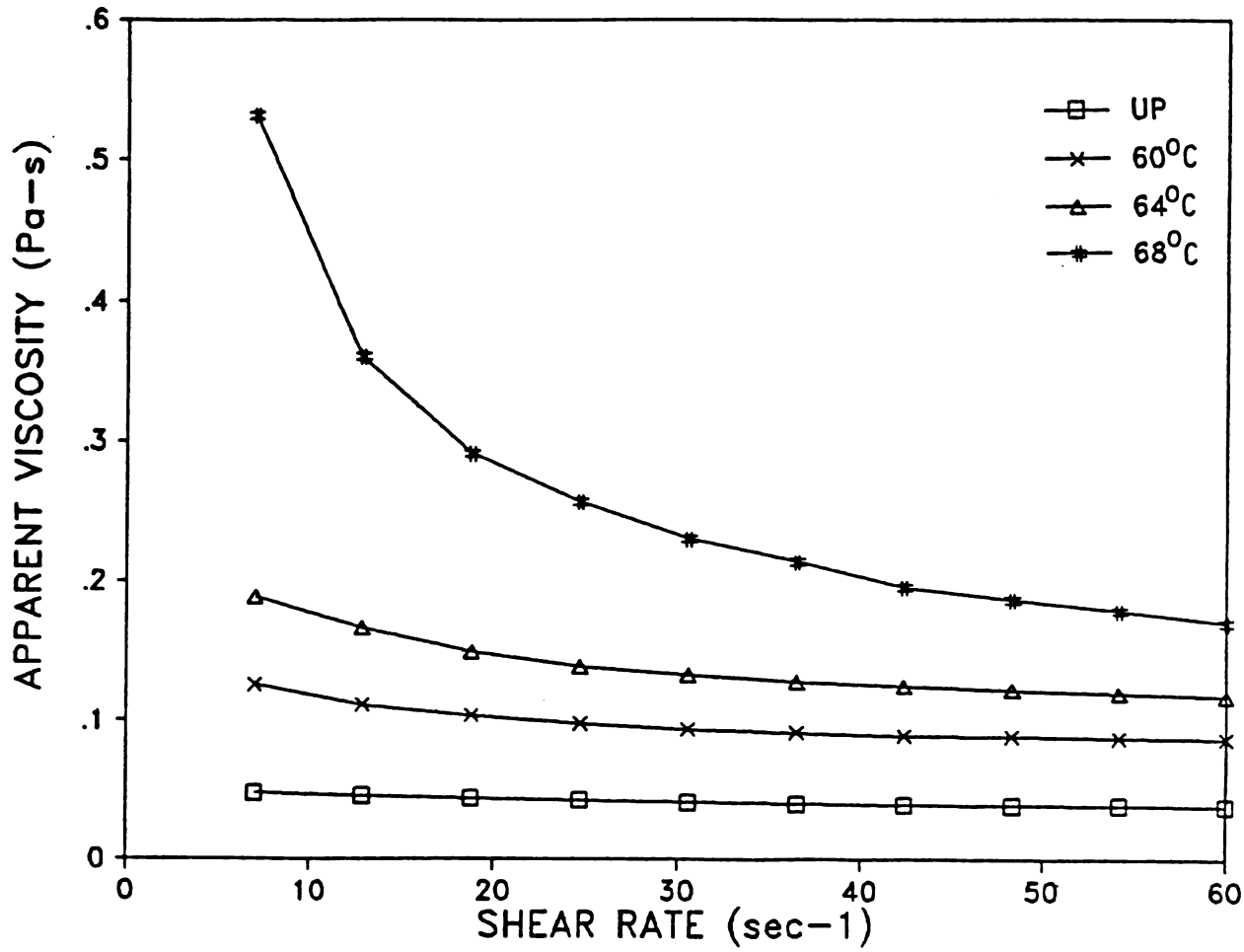


Figure 17. Effect of pasteurization on shear rate dependency of LWE stored at -24°C for one day (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

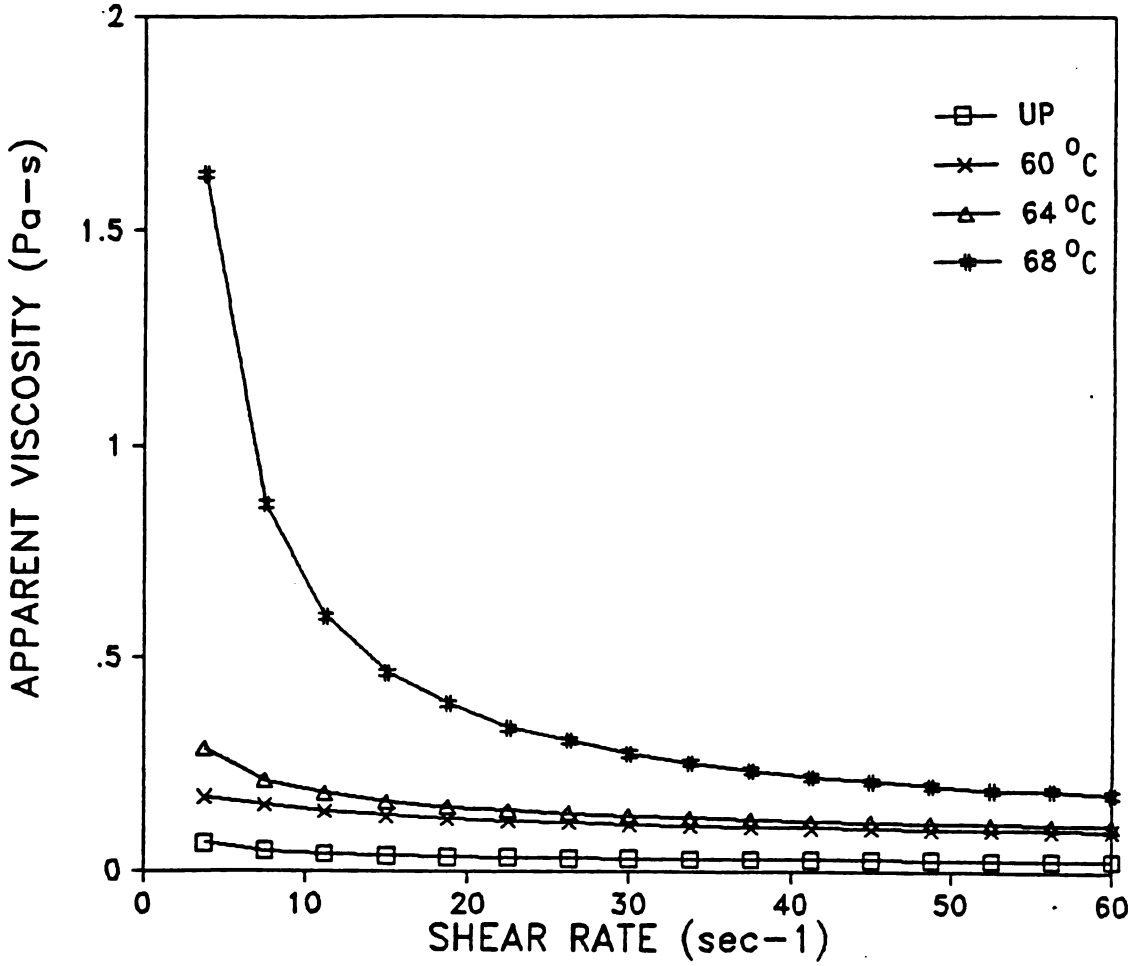


Figure 18. Effect of pasteurization on shear rate dependency of LWE stored at -24°C for 15 days (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

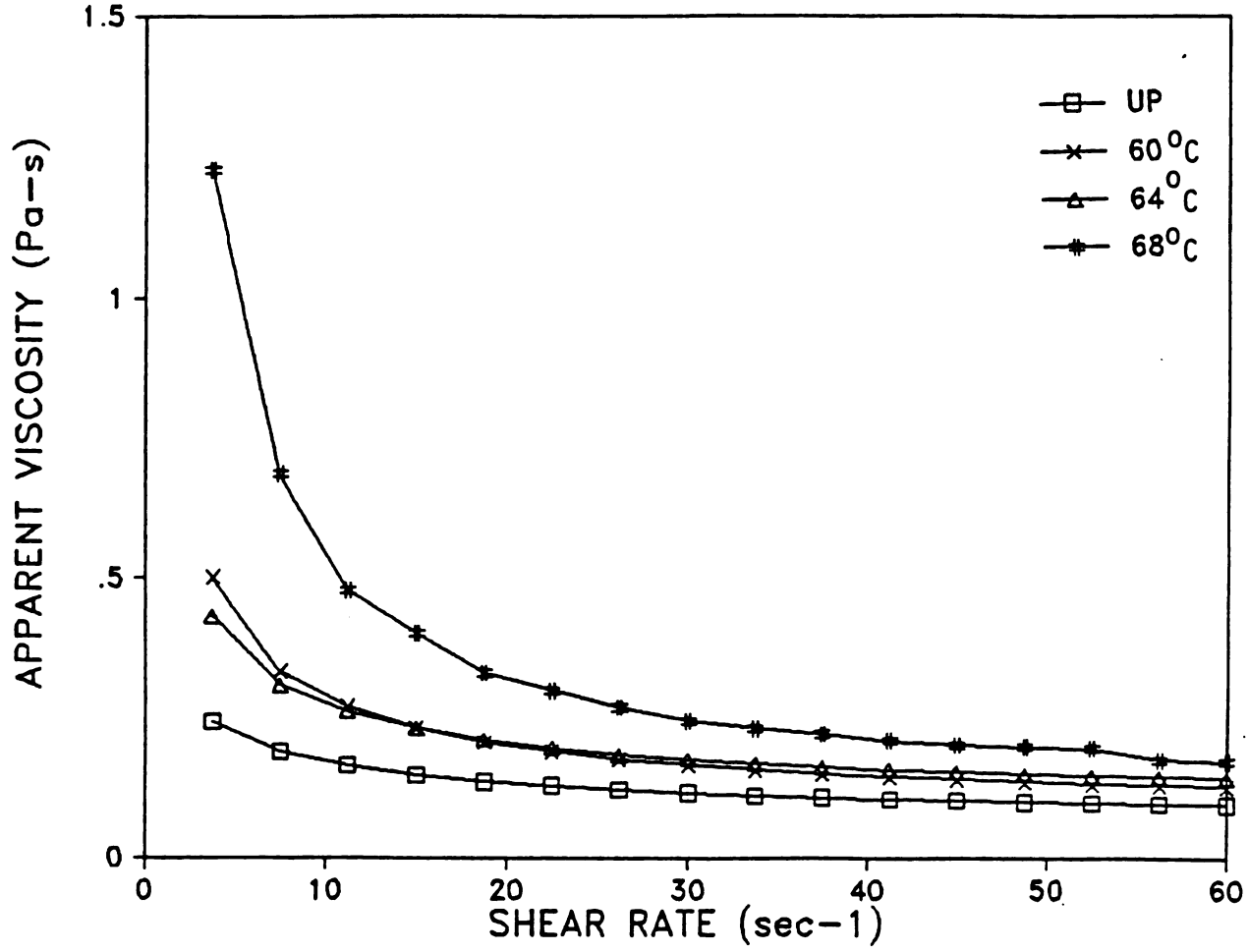


Figure 19. Effect of pasteurization on shear rate dependency of LWE stored at -24°C for 30 days (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

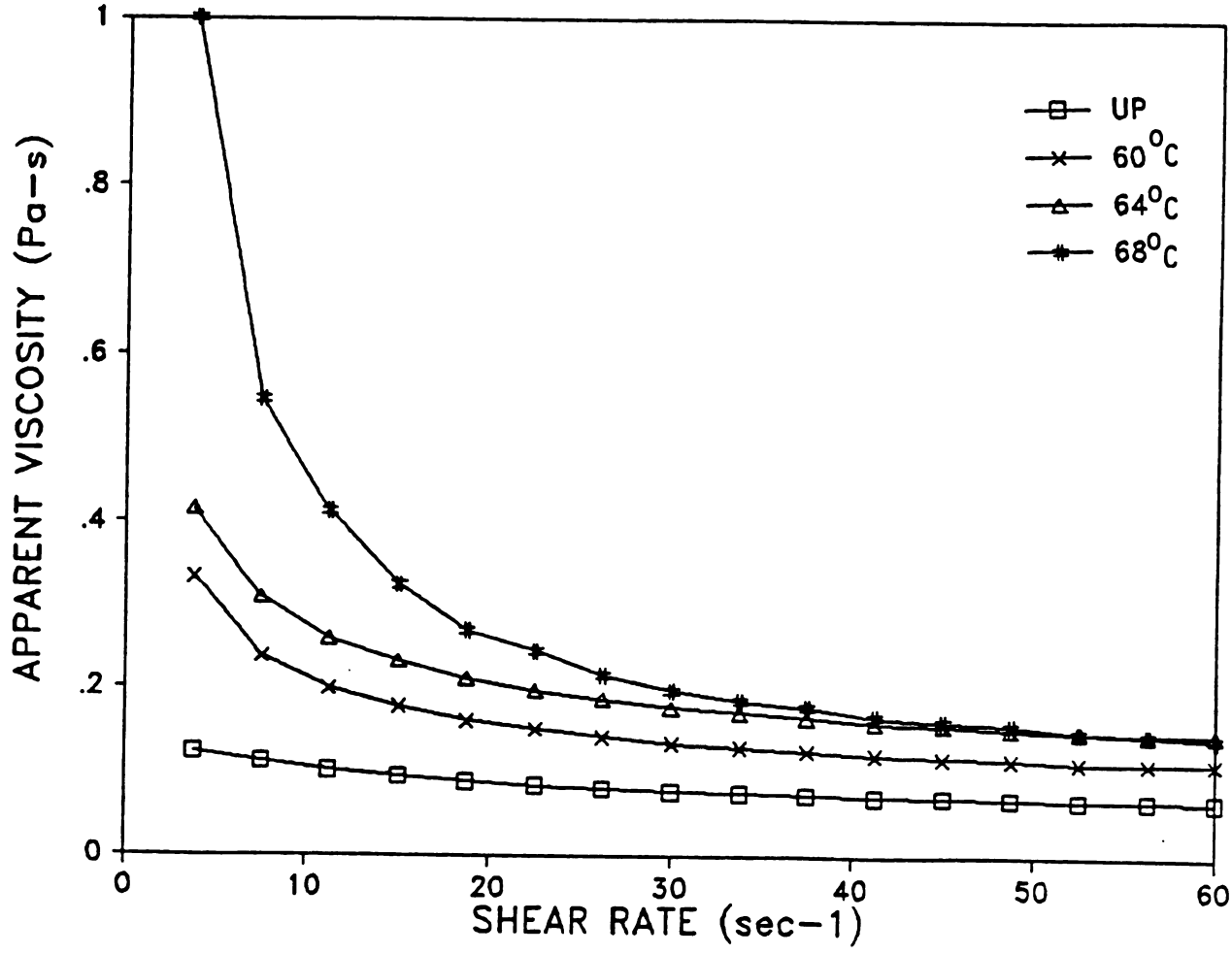


Figure 20. Effect of pasteurization on shear rate dependency of LWE stored at -24°C for 45 days (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

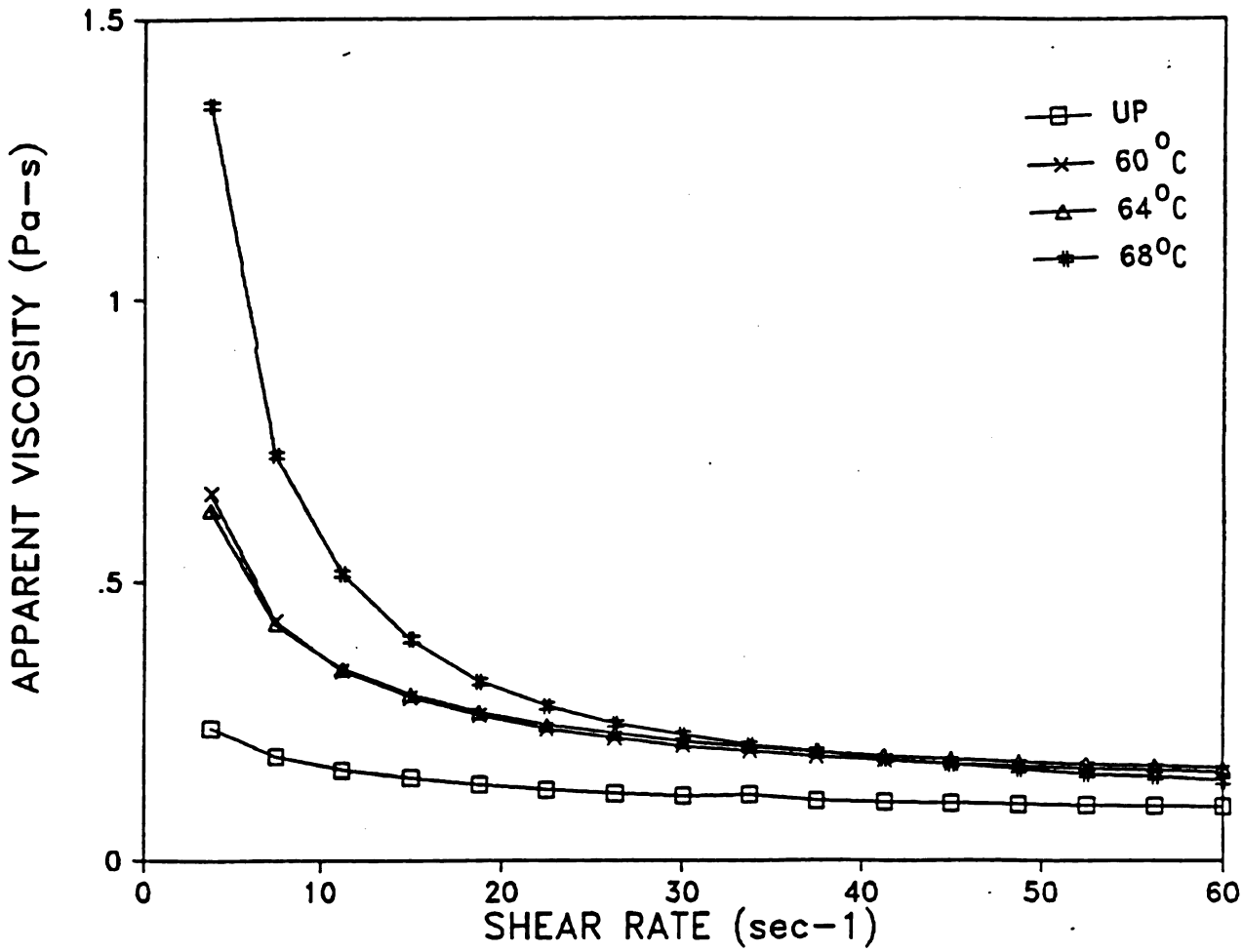


Figure 21. Effect of pasteurization on shear rate dependency of LWE stored at -24°C for 80 days (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

Day 120 (Fig. 22) Unpasteurized LWE was shear independent throughout the entire shear rate range. The 60°C LWE was shear dependent from 0-40 sec^{-1}. The 64°C LWE and 68°C LWE were shear dependent for the entire shear rate range.

Determination of fluid model Curve fitting using linear regression was used to determine the LWE model type from the graphs of shear stress versus shear rate. LWE treatments were either Newtonian or Bingham Plastic in zone four and the correlation coefficients were all greater than .99 (Appendix I). Shear stress and shear rate are proportional giving a constant viscosity in Newtonian and Bingham Plastic fluids (Muller, 1973). Bingham Plastic fluids differ from Newtonian fluids as they exhibit a yield stress. A yield stress is a force that is needed for a fluid to flow other than that provided by gravitational force (Muller, 1973). Yield stress is important in pipe design such as pumping eggs between two points. Frozen stored LWE will need larger pumps to overcome the yield stress for transportation. Yield stresses were observed using linear regression in all LWE treatments except unpasteurized LWE (Fig. 23). Yield stress increased with frozen storage in the Bingham Plastic model.

Day Zero All LWE treatments were Newtonian (Fig. 24). Conford et al. 1969 and Scalzo et al. (1970) reported Newtonian behavior for eggs heated between 5°C and 60°C.

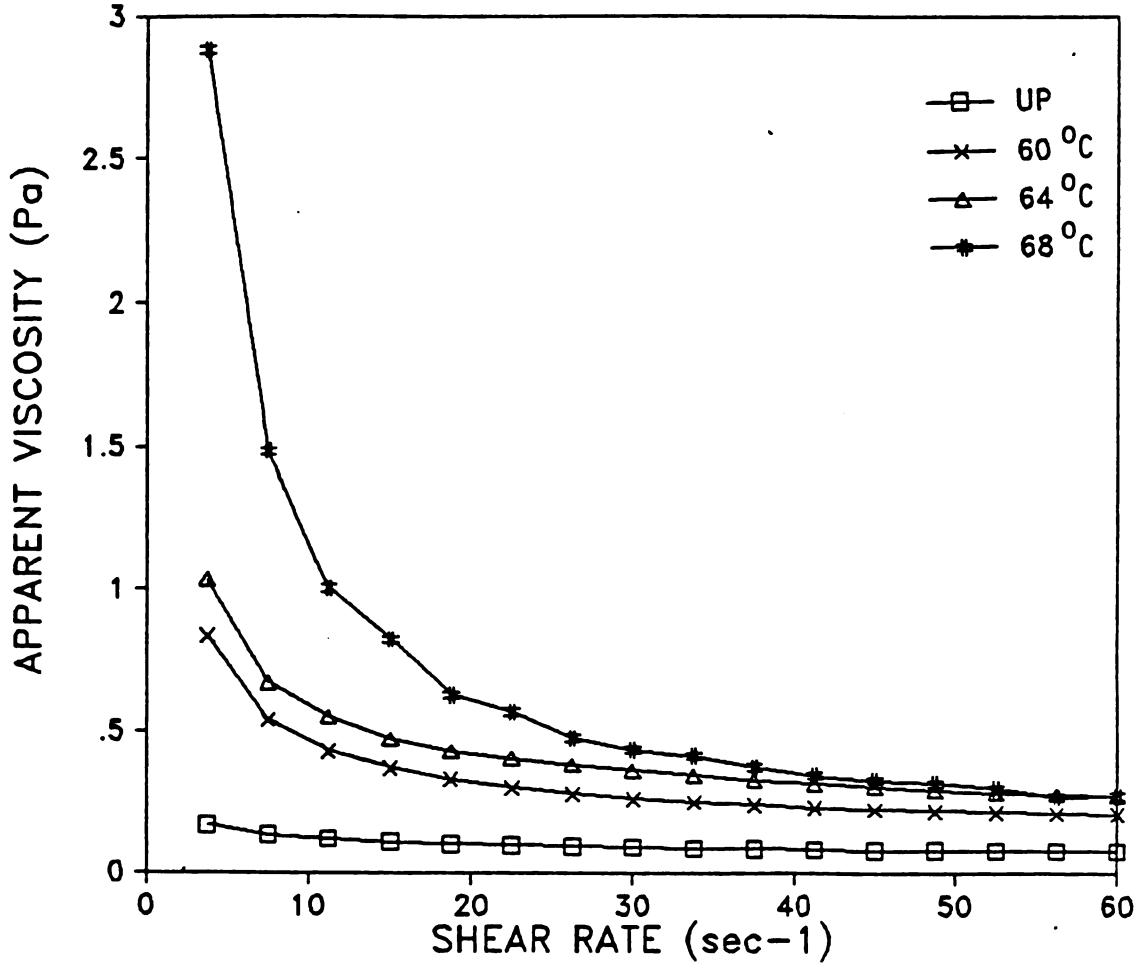


Figure 22. Effect of pasteurization on shear rate dependency of LWE stored at -24°C for 120 days (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

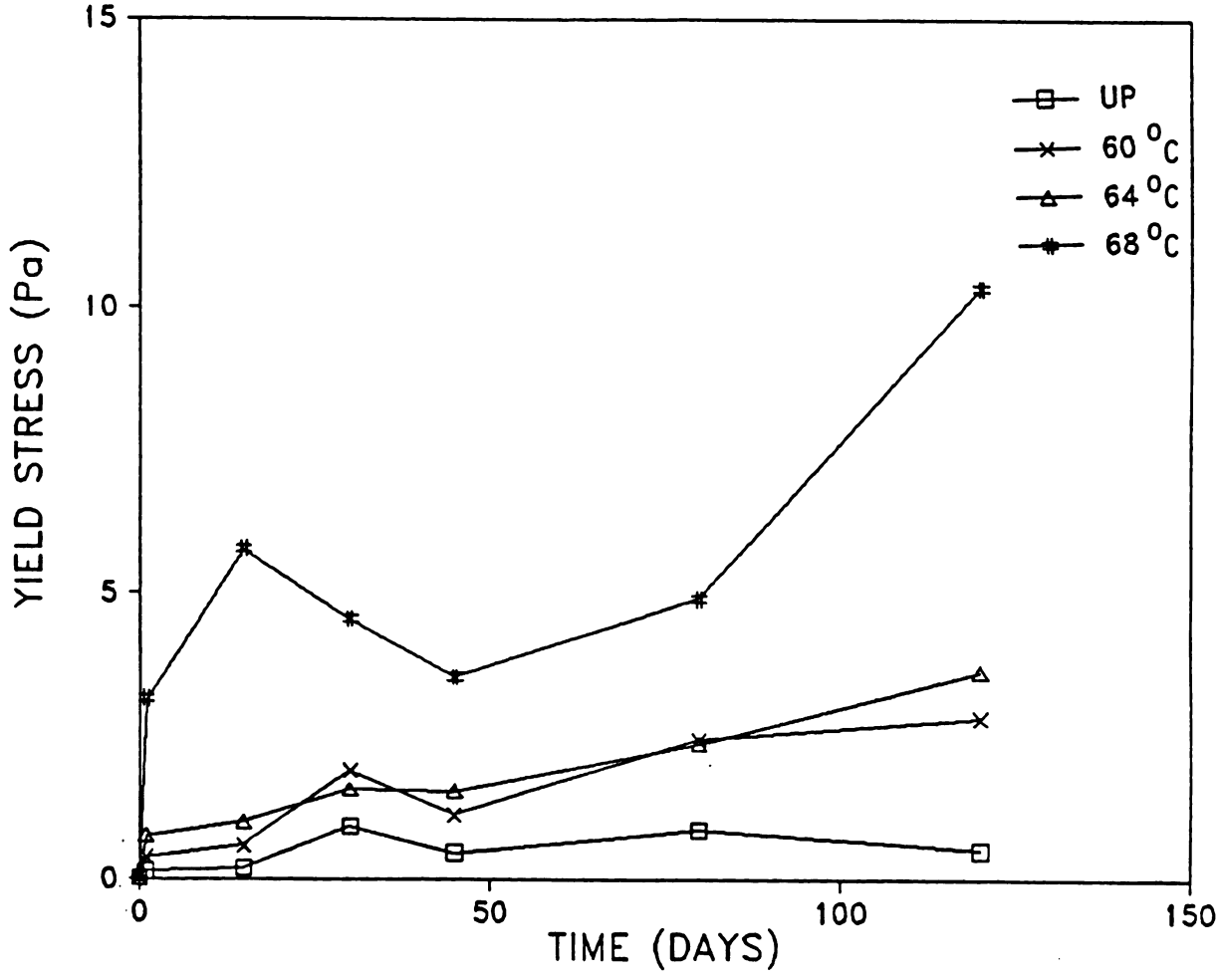


Figure 23. Effect of pasteurization and storage time at -24°C on yield stress of LWE measured at 23°C. SE=±0.30 (up= unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min.)

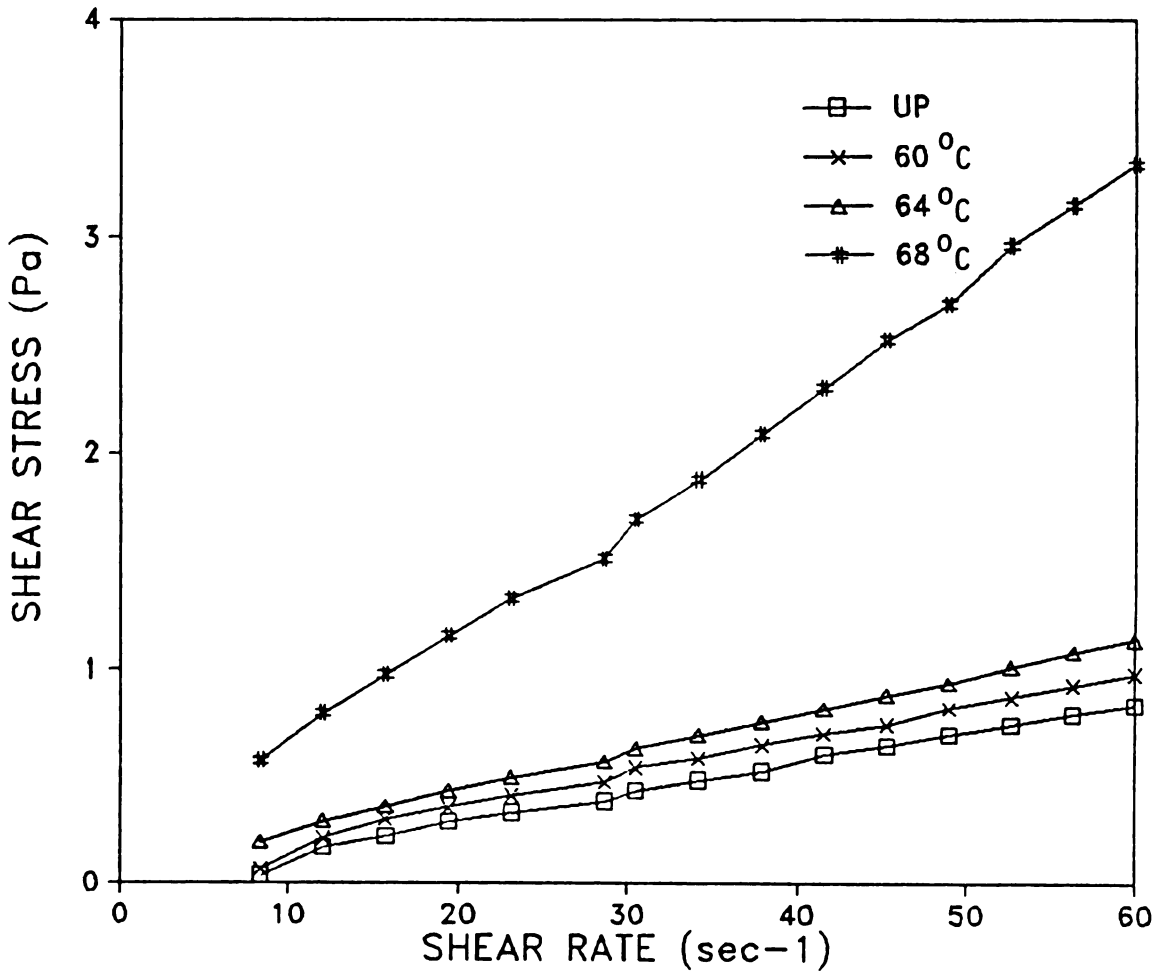


Figure 24. Effect of pasteurization on the rheogram of LWE in the fourth zone of the thixotropic loop (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

Day 1 Unpasteurized LWE, 60°C LWE, and 64°C LWE were Newtonian while the 68°C LWE was Bingham Plastic (Fig. 25).

Day 15 Unpasteurized LWE and 60°C LWE remained Newtonian, while the 64°C LWE and 68°C LWE exhibited yield stress. Therefore, they were characterized as Bingham Plastic (Fig. 26).

Days 30, 45, 80, 120 After Day 30 unpasteurized LWE was the only treatment which did not show a yield stress and, therefore, was Newtonian (Fig. 27, 28, 29, 30). All other LWE samples exhibited Bingham Plastic behavior after 30 days of frozen storage.

Pies

Difference in percent change in depth of pie filings made with the LWE treatments is shown in Fig. 31. Large volume changes were observed in the unpasteurized and 60°C LWE treatments during the first 15 days of frozen storage. The unpasteurized LWE treatments exhibited a larger percent change in depth than the pasteurized LWE treatments during 30-120 days of frozen storage (Fig. 31). No literature was found relating pie filling volume to LWE quality.

Subjective observations were made on the unpasteurized and 68°C LWE treatments during frozen storage. Four categories were used to focus on the most frequently

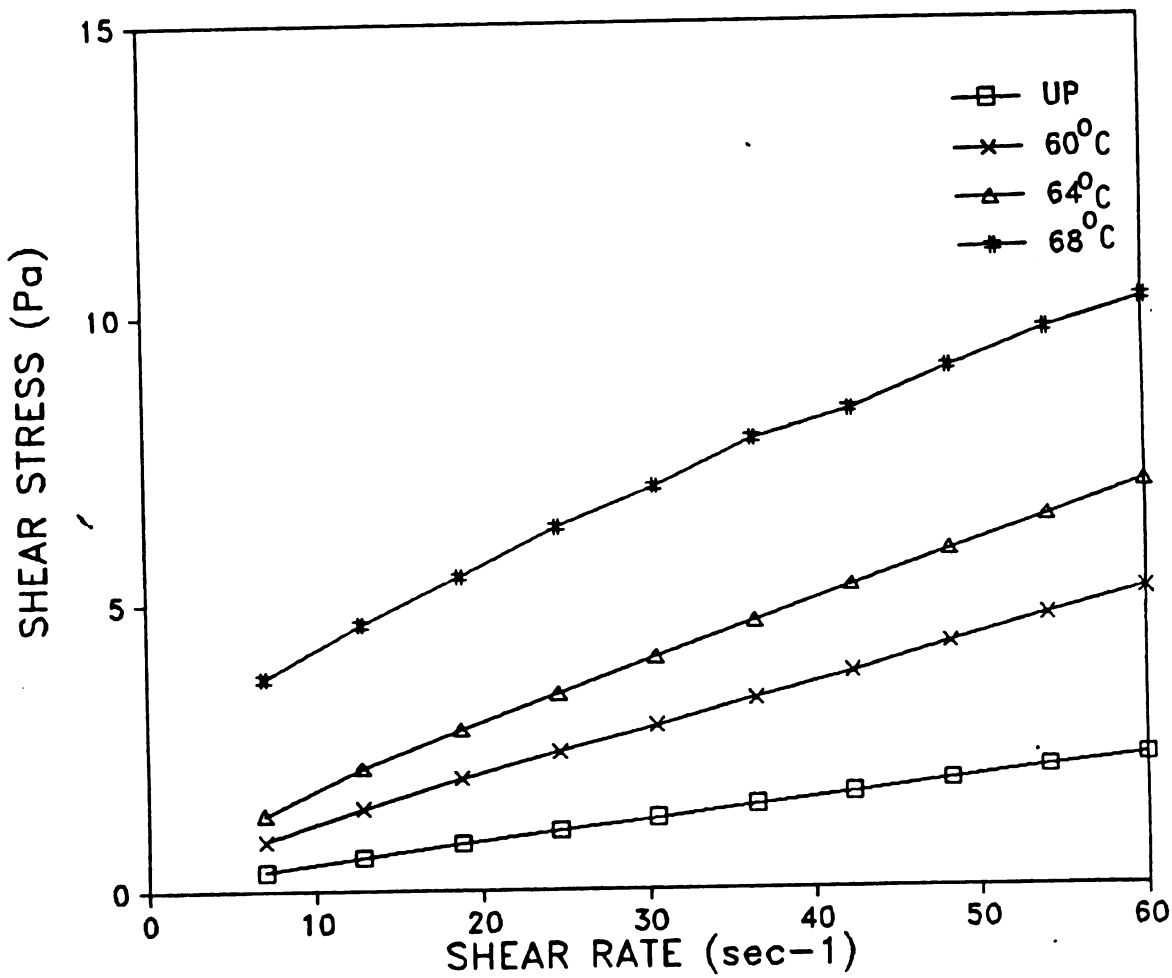


Figure 25. Effect of pasteurization and storage at -24°C for one day on the rheogram of LWE determined from the fourth zone of the thixotropic loop (up=unpasteurized; 60°C, 64°C and 68°C= heat treatment at temperatures indicated for 3.5 min).

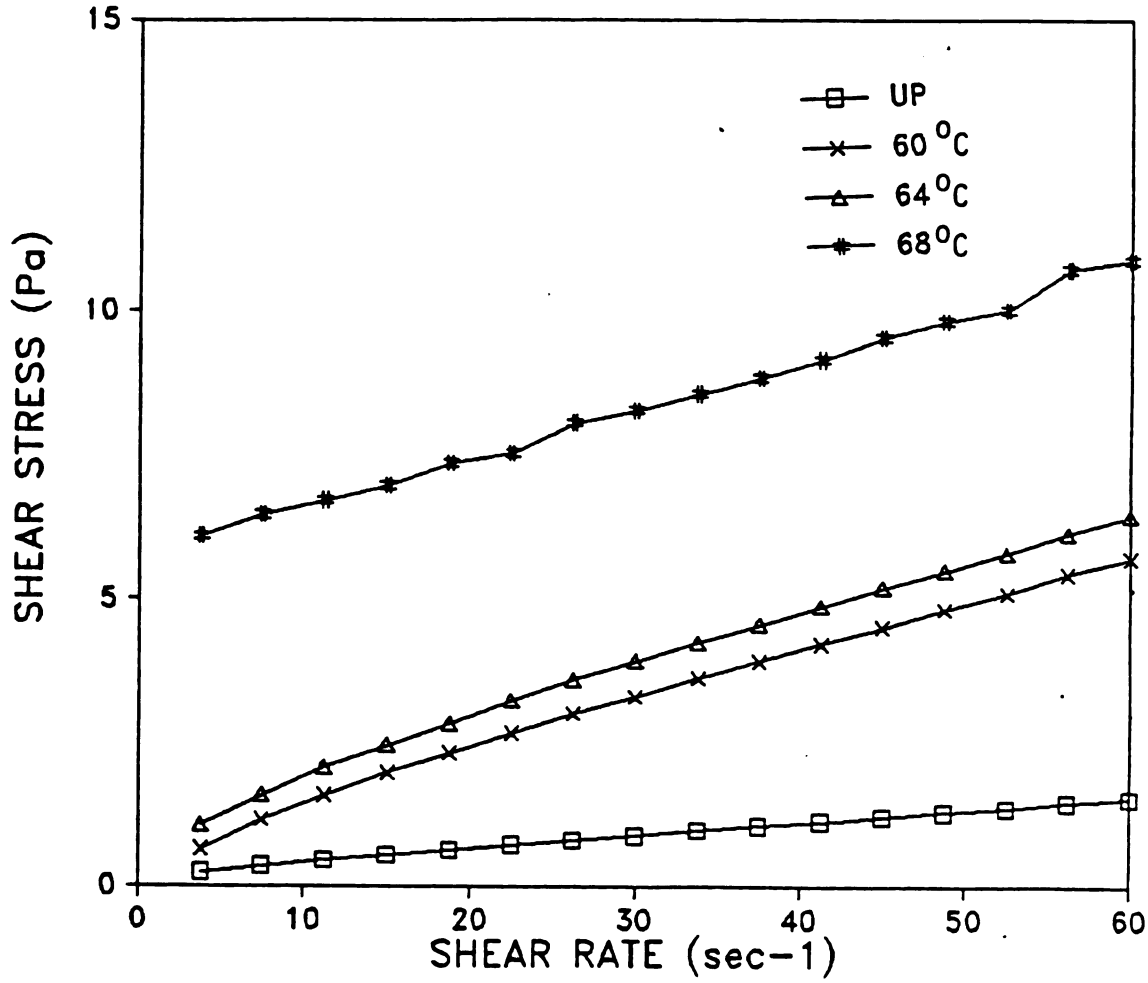


Figure 26. Effect of pasteurization and storage at -24°C for 15 days on the rheogram of LWE determined from the fourth zone of the thixotropic loop (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

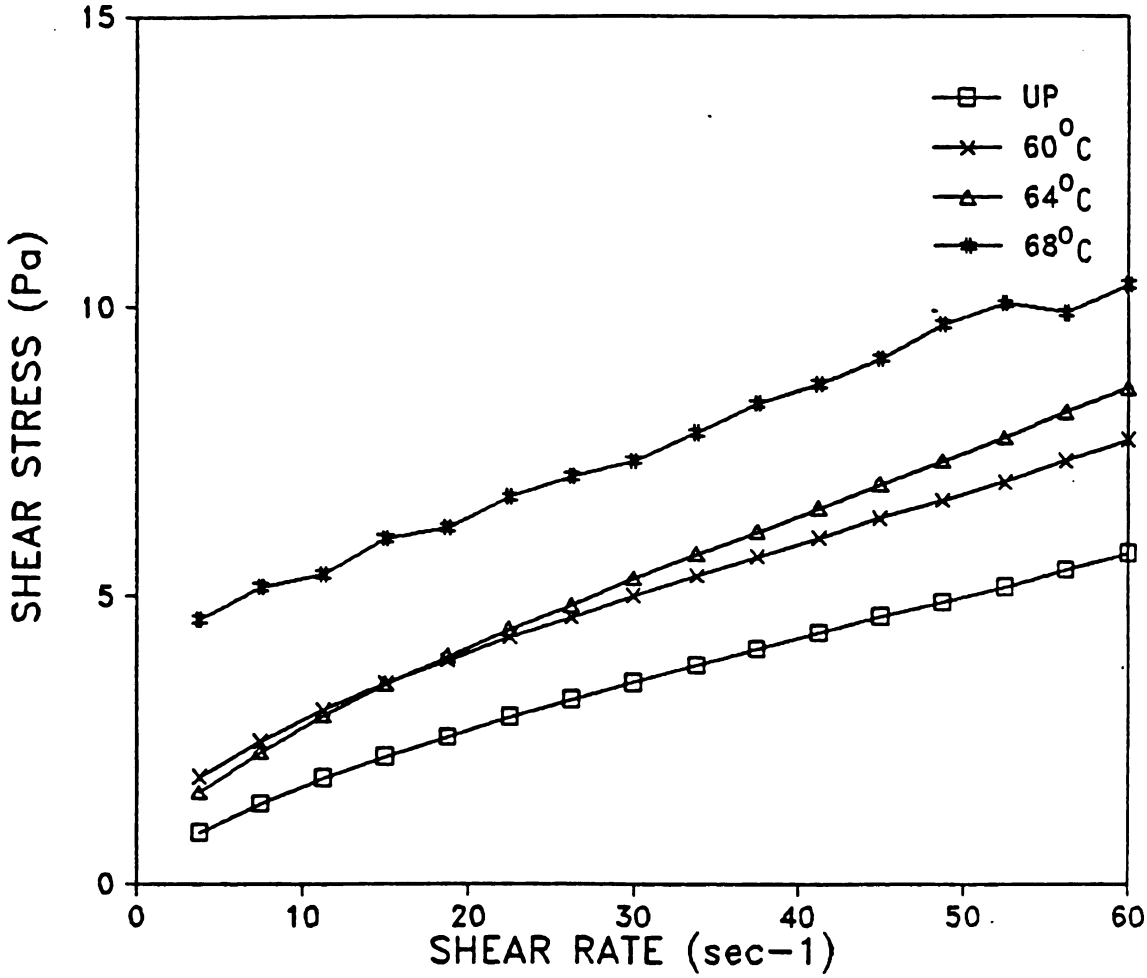


Figure 27. Effect of pasteurization and storage at -24°C for 30 days on the rheogram of LWE determined from the fourth zone of the thixotropic loop (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

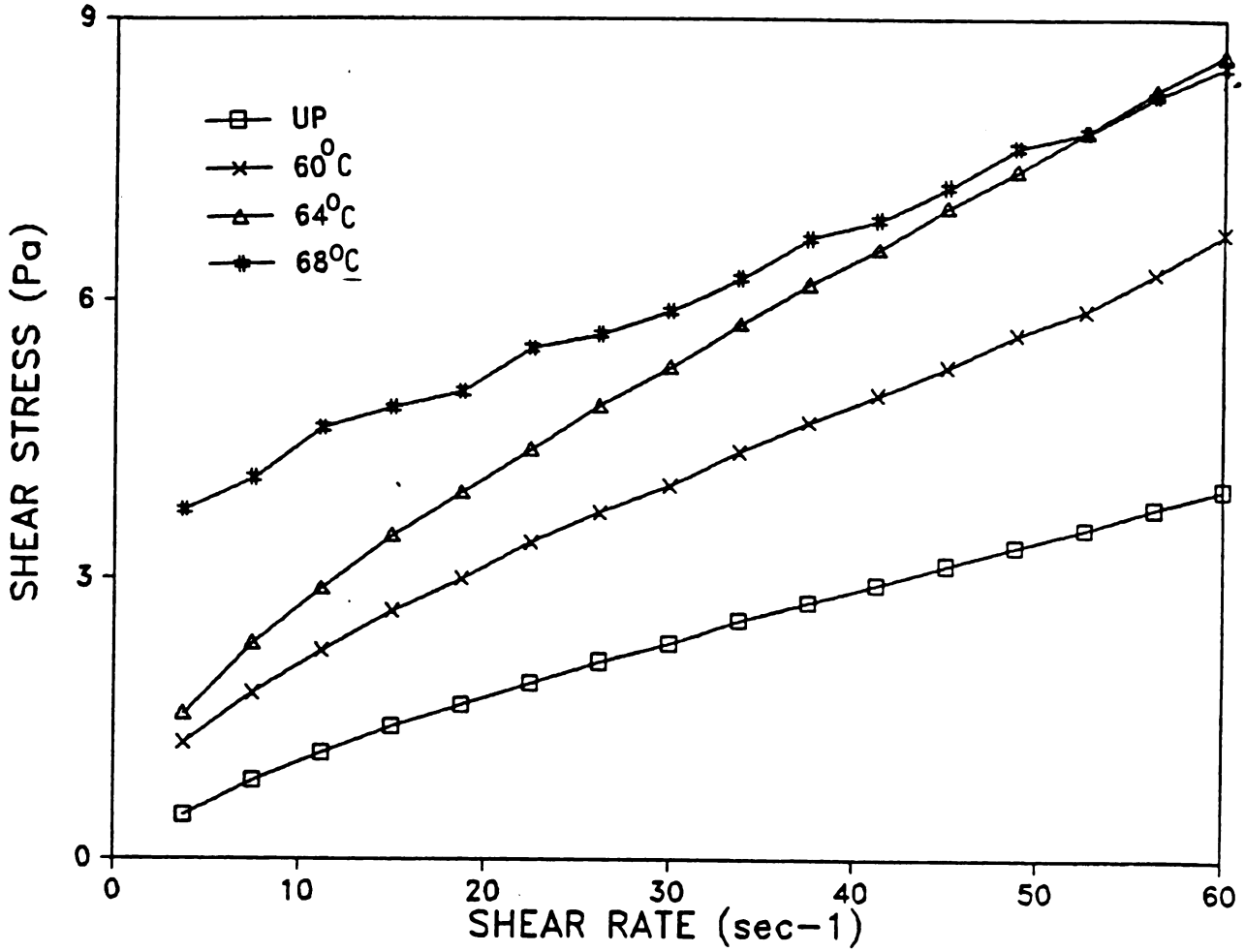


Figure 28. Effect of pasteurization and storage at -24°C for 45 days on the rheogram of LWE determined from the fourth zone of the thixotropic loop (up=unpasteurized; 60°C, 64°C and 68°C= heat treatment at temperatures indicated for 3.5 min).

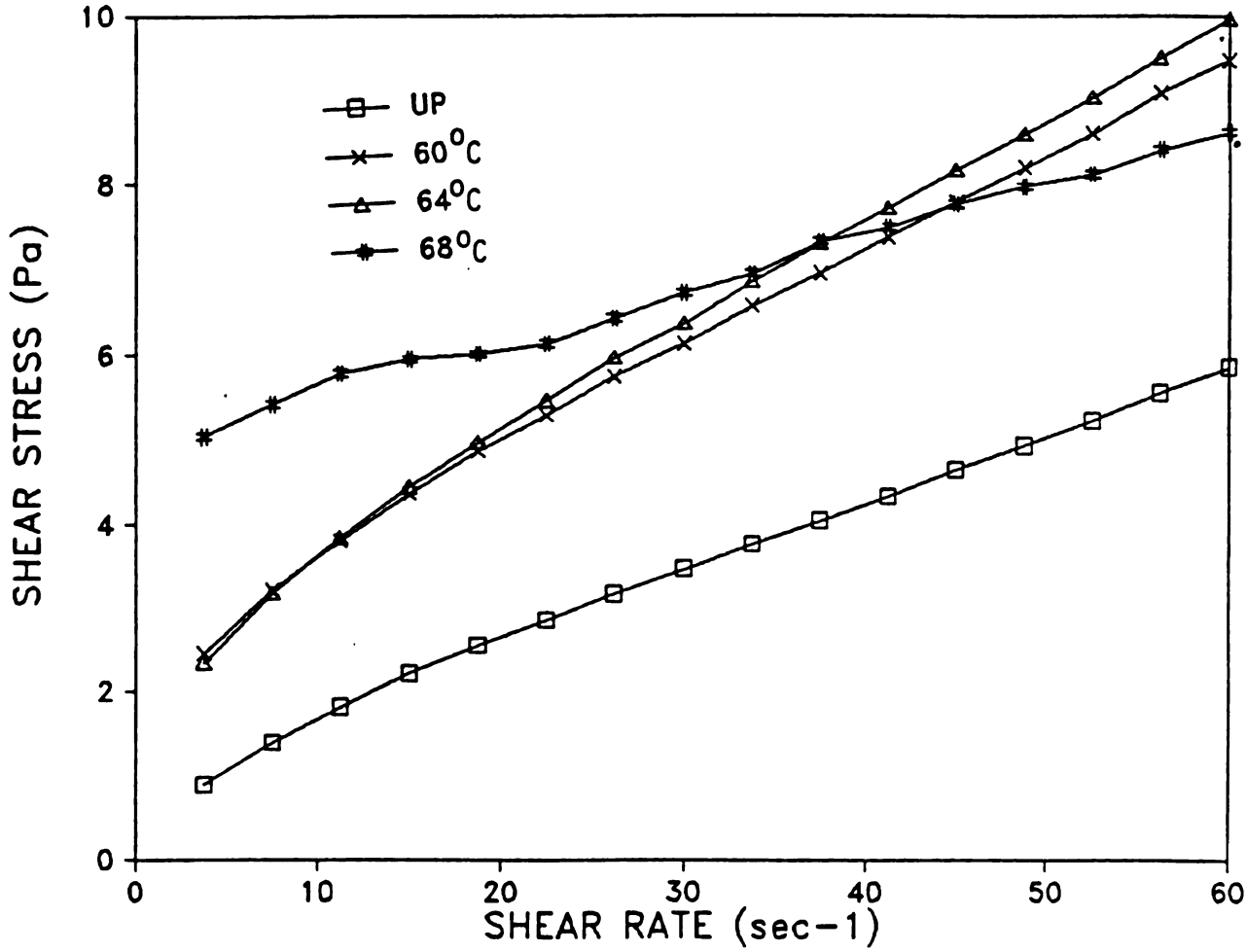


Figure 29. Effect of pasteurization and storage at -24°C for 80 days on the rheogram of LWE determined from the fourth zone of the thixotropic loop (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min).

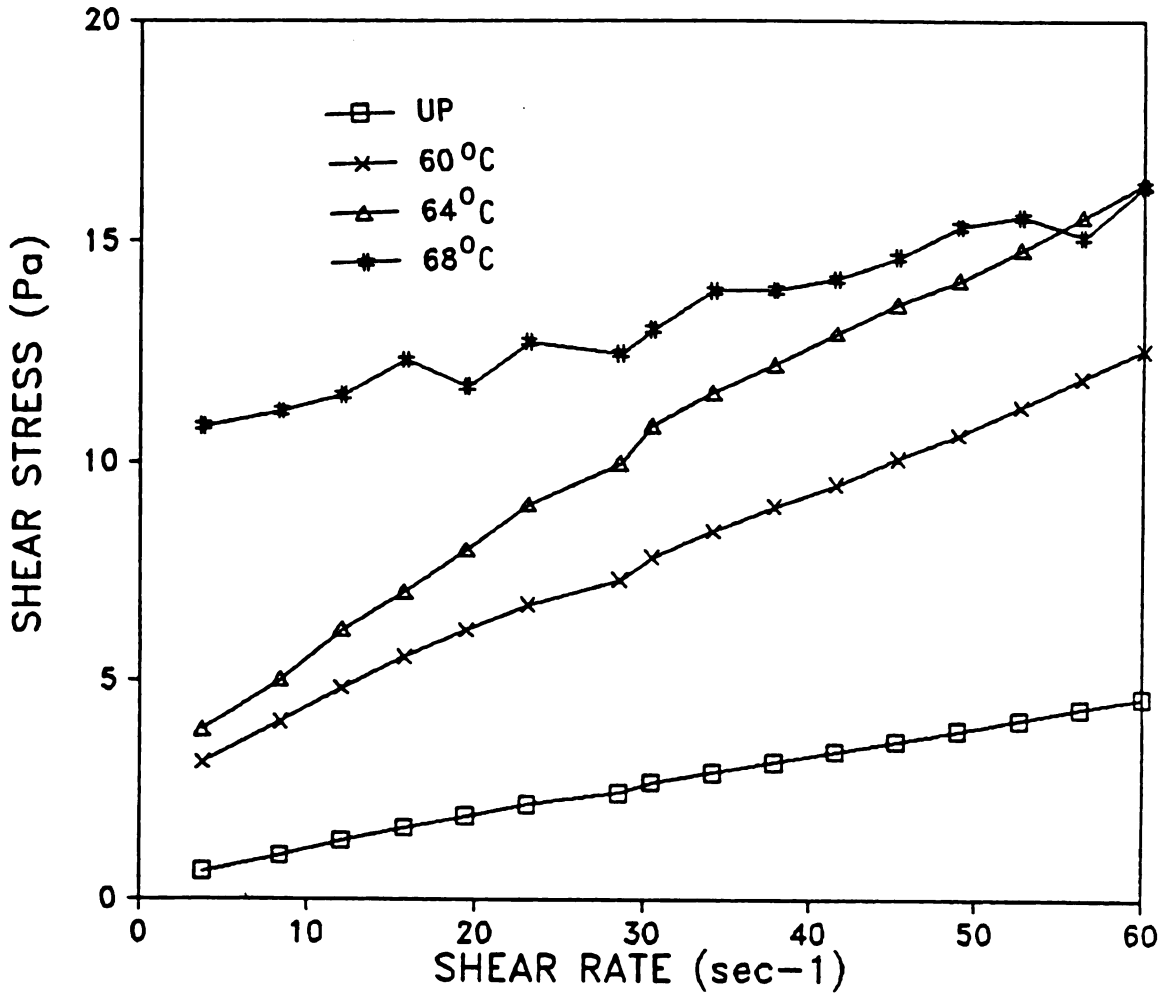
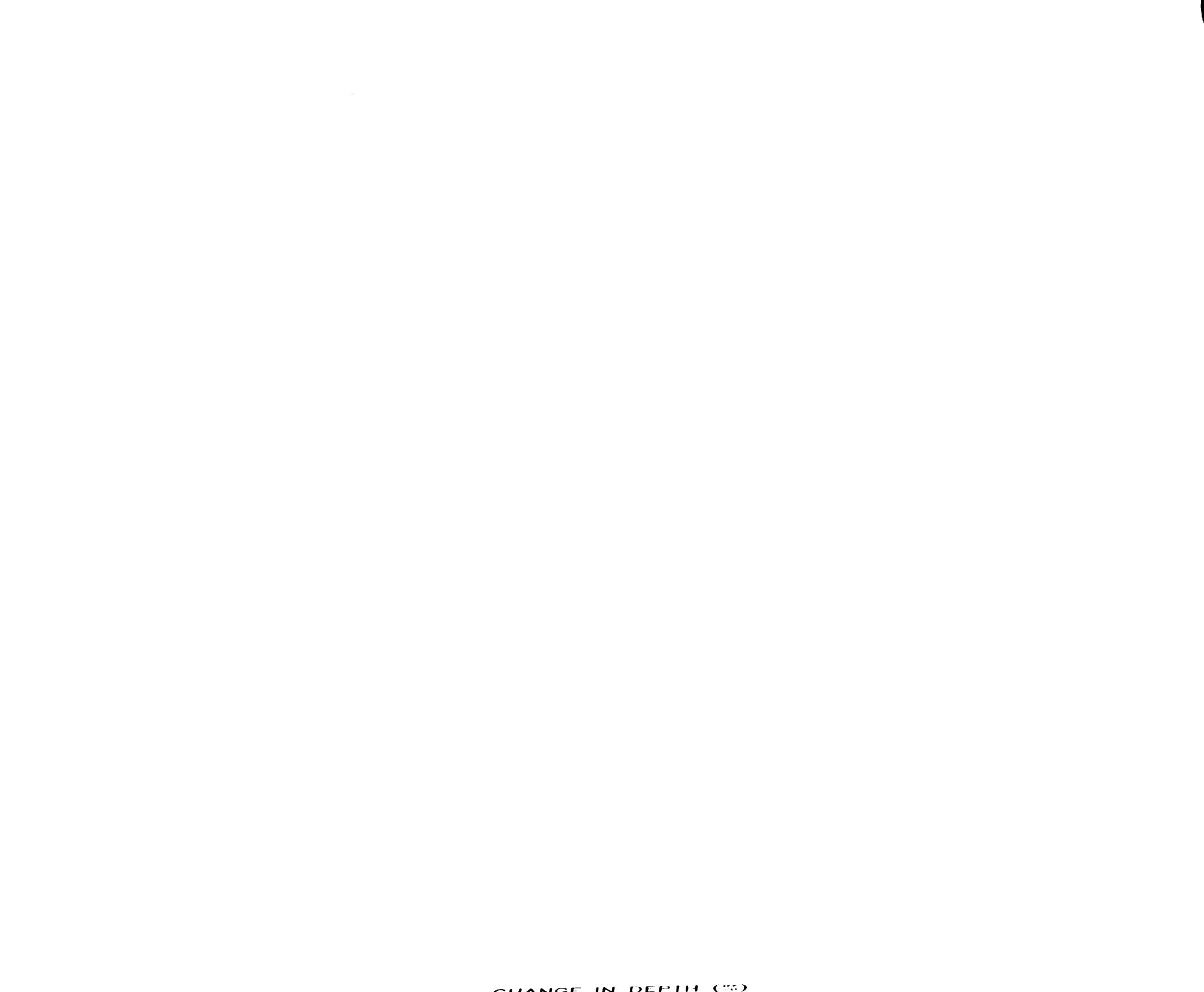


Figure 30. Effect of pasteurization and storage at -24°C for 120 days on the rheogram of LWE determined from the fourth zone of the thixotropic loop (up=unpasteurized; 60°C, 64°C and 68°C= heat treatment at temperatures indicated for 3.5 min).


CHANGE IN DEPTH (%)

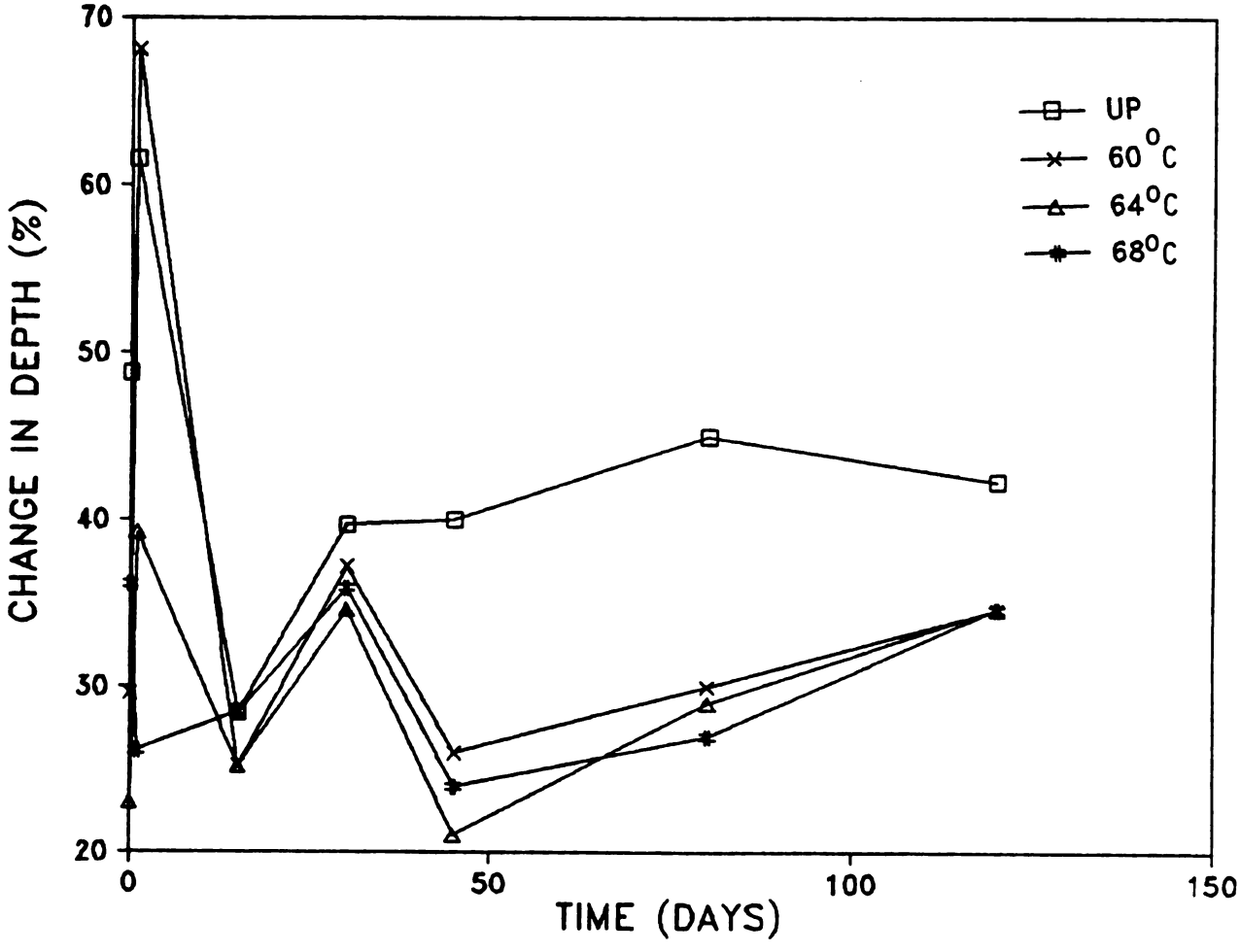


Figure 31. The effect of LWE pasteurized and stored at -24°C on pie filling depth. SE=±1.58 (up=unpasteurized; 60°C, 64°C and 68°C=heat treatment at temperatures indicated for 3.5 min.)

observed pie filling characteristics (Table 10). Surface rupture included any aberrations on the pie filling surface such as bubbles. Extreme aberrations existed on the unfrozen and day one unpasteurized LWE pie filling surface. A V-shaped surface with the apex of the V at the bottom of the custard dish was exhibited. After one day of frozen storage the pie filling made with unpasteurized LWE exhibited aberrations. These bubbles on the surface of the pie filling support the suspension of ingredients, such as pecans, which is a desirable quality factor. The 68°C LWE treatments did not exhibit any surface aberrations during the experiment, although, a rough and crusty surface was observed. Color differences were distinguishable between the unpasteurized and 68°C LWE at all times of frozen storage, except day one. Unpasteurized LWE was light in color and the 68°C LWE dark. Unpasteurized LWE was glossy for all treatments, except day 80 and 120 of frozen storage. A glossy appearance is a desirable characteristics for this particular pie filling. The 68°C LWE pie filling was not glossy during 1-30 days of frozen storage. UNpasteurized LWE adhered to the custard dish, while 68°C LWE did not during 1-30 days of frozen storage. After 30 days of frozen storage, none of the treatments adhered to the custard dish. Poor adhesion appeared to correlate with decreased pie filling volume. Adhesion of the pie filling to the custard dish is a desirable characteristic, as commercial processors want the pie filling to adhere to the pie crust.

Table 10. Subjective observations of pie fillings made with LWE that have been heat treated and frozen.

	Day 0		Day 1		Day 15		Day 30		Day 45		Day 80		Day 120	
	UP	68	UP	68	UP	68	UP	68	UP	68	UP	68	UP	68
Surface rupture	Yes V Shape	Yes	Yes V Shape	No Rough & Crusty	Yes	No Rough & Crusty	Yes	No Rough & Crusty	Yes	No Rough & Crusty	Yes	No Rough & Crusty	Yes	No Rough & Crusty
Color	Light	Light	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Slightly Dark	Dark	Slightly Dark	Very Dark
Glossy	Very Glossy	Glossy	Very Glossy	No	Yes	No	Yes	No	Yes	No	No	No	Yes	No
Adhesion to dish	No	Slight Pulling Away	Yes	Slight Pulling Away	Yes	No	Yes	No	No	No	No	No	No	No

Correlation Coefficient

This is the first time that changes in protein solubility, rheological characteristics and functional properties of LWE have been correlated (Table 11). Correlation between protein solubility and pie filling depth (r^2 = .92) was high. Pie filling volume is related to the functional properties of individual proteins in the LWE. Globulin proteins give the characteristic foaming and leavening properties of baked products. The decrease in soluble globulin proteins with heat treatment may have been responsible for changes in pie filling depth, G_3 globulin proteins are heat labile at 63°C. Percent soluble protein and rheological properties (peak viscosity and apparent viscosity) were not highly correlated (r^2 = .66 and .74, respectively). Soluble proteins, therefore, are not necessarily the ones which are responsible for all the changes in rheological properties. Heat resistant and denatured proteins may be important for changes in the rheological properties. The denatured proteins may have aggregated, increasing the apparent viscosity of the LWE. Apparent viscosity and peak viscosity increased with frozen storage. The changes in protein structure during frozen storage may have caused the rheological changes. Wootton et al. (1981) reported that freezing denatured ovotransferrin, while Kurisaki et al. (1980) reported lipoprotein

Table 11. Correlation coefficients for rheological and functional properties of heat treated and frozen LWE.

	P.Sol[b]	SS[c]	AV[d]	PV[e]	Pies
TRT[a]	0.76	0.81	0.82	0.67	0.63
Time	0.75	0.84	0.85	0.71	0.64
P.Sol[b]		0.66	0.66	0.74	0.92
SS[c]			1.00	0.87	0.55
AV[d]				0.87	0.55
PV[e]					0.40

[a]TRT - Treatment

[b]P.Sol - Protein solubility

[c]SS - Shear stress

[d]AV - Apparent viscosity

[e]PV - Peak viscosity

aggregation during frozen storage. Changes in rheological properties of LWE due to pasteurization and frozen storage have been observed by Conford et al. (1969) and Parkinson (1977). with further research it may be possible to enhance the correlation between these variables.

SUMMARY AND CONCLUSION

The objective of this study was to determine the effects of pasteurization and frozen storage of LWE on rheological properties, changes in protein composition and functional properties. The Rheometrics Fluids Spectrometer was used to characterize rheological behavior (apparent viscosity, peak viscosity and modeling) of LWE. Changes in specific proteins were monitored using PAGE and densitometer tracings. Changes in functional properties of LWE were determined using volume changes of pie fillings.

A control and three pasteurized (60°C, 64°C and 68°C) LWE samples were prepared. Protein solubility was determined for each LWE treatment. Pasteurization caused an 11% decrease in protein solubility in the unfrozen samples. Partial loss of soluble LWE protein during pasteurization was attributed to G_3 globulin. G_3 globulin contributes to the foaming ability of LWE, thus its loss is a major concern with respect to functional properties. Rheological properties all increased with higher pasteurization temperature. Peak viscosity increased from 0.02 Pa-s for unpasteurized LWE day zero to 0.22 Pa-s 68°C LWE treatment day zero. Unpasteurized, 60°C, and 64°C LWE treatments exhibited time independent behavior at day zero while 68°C LWE treatment showed time dependent

behavior at day zero. Apparent viscosity determined at a shear rate of 30 sec^{-1} increased with higher pasteurization temperatures from 0.10 Pa-s to 0.06 Pa-s for the unpasteurized and 68°C LWE treatments, respectively. Unpasteurized, 60°C, and 64°C were shear independent for day zero, while 68°C LWE exhibited shear dependency in the shear rate range 0-30 sec^{-1}, then became independent for the rest of the shear rate range. Curve fitting, using linear regression, was used to determine fluid model type of the LWE treatments. The unpasteurized LWE at all storage times fit the Newtonian model. LWE treatments at extended storage times exhibited a Bingham Plastic fluid model. Major differences in percent change (70%) in depth of the pie fillings during cooking were determined for the unpasteurized LWE at day zero. This decline in volume was attributed to a V-shaped apex formed in the pie filling. The 68°C LWE treatment did exhibit a decline in depth (40%), but this change was less than the unpasteurized LWE treatment. The subjective observations (surface aberration, color, glossiness and adhesion) were similar for the unpasteurized and 68°C LWE unfrozen treatments.

Heat treated LWE was stored at -24°C for 0, 1, 15, 30, 45, 80, and 120 days. LWE protein solubility decreased from 77.2% to 60.6% after 80 days of frozen storage. PAGE revealed decreases in the solubility of lipovitellin, ovotransferrin, ovoinhibitor, livetin, and G_{3A} protein fractions. Rheological properties changed with frozen

storage. Peak viscosity increased from .01 Pa-s for the unpasteurized LWE day zero to 1.1 Pa-s for the unpasteurized LWE day 120. Thixotropic behavior was observed in all treatments of frozen LWE. Apparent viscosity increased with frozen storage from 0.01 Pa-s for the unpasteurized LWE day zero to 0.08 Pa-s for the unpasteurized LWE day 120. The unpasteurized LWE was shear independent throughout storage. The other LWE treatments were shear dependent after 80 days of storage. Pie filling surface aberrations occurred in all the unpasteurized LWE treatments throughout storage. The 68°C LWE did not exhibit aberrations, but the surface was rough and crusty. The unpasteurized LWE pie filling was light in color compared to the 68°C. The unpasteurized LWE had a glossy surface and adhered to the dish during the first 30 days of frozen storage. After this time, the glossiness decreased and the pie filling pulled away from the dish similar to the 68°C LWE which had these attributes throughout the experimental. The combined effect of pasteurization and frozen storage may be more detrimental to LWE quality than either effect alone.

Suggestions for Further Research

The observations and findings associated with the present research raised questions to LWE quality due to pasteurization and frozen storage.

1. The effect of homogenization of LWE on rheological properties and shear denaturable proteins.
2. Studies correlating rate of thawing and freezing to the quality of LWE.
3. Studies of rheological properties of selected LWE proteins that contribute to visco-elastic behavior.

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APPENDIX

FLUID MODEL DESIGN

Table 12a. Effect of pasteurization on fluid model behavior of LWE.

Model	Yield Stress (B_0)	Flow Behavior Index (B_1)	Correlation r	F-value	Treatment Temperature
1	0.01	0.00	0.99	999.9	Unpasteurized
2	0.02	0.01	0.98	700.0	Unpasteurized
3	0.01	1.20	0.90	115.2	Unpasteurized
1	0.03	0.00	0.99	999.9	60°C
2	0.35	0.02	0.99	999.9	60°C
3	0.17	0.77	0.95	330.9	60°C
1	0.02	0.00	0.99	999.9	64°C
2	0.11	0.17	0.99	999.9	64°C
3	0.04	0.80	0.99	999.9	64°C
1	0.06	0.00	1.00	999.9	68°C
2	0.16	0.05	0.99	999.9	68°C
3	0.09	0.86	0.99	999.9	68°C

Model 1 is Newtonian, Model 2 is Bingham Plastic and Model 3 is Power Law. The value of the F ratio has a maximum of 999.9

Table 12b. Effect of pasteurization and storage time at -24°C for 1 day on fluid model behavior of LWE.

Model	Yield Stress (B_0)	Flow Behavior Index (B_1)	Correlation r	F-value	Treatment Temperature
1	0.03	0.00	0.99	999.9	Unpasteurized
2	0.11	0.04	0.99	999.9	Unpasteurized
3	0.06	0.09	0.99	999.9	Unpasteurized
1	0.10	0.00	0.99	999.9	60°C
2	0.48	0.09	0.99	926.2	60°C
3	0.20	0.82	0.99	999.9	60°C
1	0.12	0.00	0.99	999.9	64°C
2	0.76	0.10	0.99	999.9	64°C
3	0.30	0.76	0.99	999.9	64°C
1	0.18	0.00	0.99	847.5	68°C
2	2.26	0.12	0.99	999.9	68°C
3	0.94	0.65	0.99	999.9	68°C

Model 1 is Newtonian, Model 2 is Bingham Plastic and Model 3 is Power Law. The value of the F ratio has a maximum of 999.9.

Table 12c. Effect of pasteurization and storage time at -24°C for 15 days on fluid model behavior of LWE.

Model	Yield Stress (B_0)	Flow Behavior Index (B_1)	Correlation r	F-value	Treatment Temperature
1	0.02	0.00	0.99	999.9	Unpasteurized
2	0.18	0.02	0.99	999.9	Unpasteurized
3	0.06	0.75	0.99	999.9	Unpasteurized
1	0.10	0.00	0.99	999.9	60°C
2	0.88	0.08	0.99	999.9	60°C
3	0.33	0.71	1.00	999.9	60°C
1	0.10	0.00	0.99	430.0	64°C
2	1.20	0.07	0.99	999.9	64°C
3	0.52	0.57	0.99	999.9	64°C
1	0.22	0.00	0.96	161.8	68°C
2	5.38	0.09	0.99	999.9	68°C
3	3.48	0.26	0.93	175.1	68°C

Model 1 is Newtonian, Model 2 is Bingham Plastic and Model 3 is Power Law. The value of the F ratio has a maximum of 999.9.

Table 12d. Effect of pasteurization and storage time at -24°C for 30 days on fluid model behavior of LWE.

Model	Yield Stress (B_0)	Flow Behavior Index (B_1)	Correlation r	F-value	Treatment Temperature
1	0.11	0.00	0.99	775.7	Unpasteurized
2	1.14	0.08	0.99	999.9	Unpasteurized
3	0.27	0.63	0.99	999.9	Unpasteurized
1	0.14	0.00	0.99	583.5	60°C
2	1.88	0.09	0.99	999.9	60°C
3	0.80	0.54	0.99	999.9	60°C
1	0.15	0.00	0.99	997.5	64°C
2	1.49	0.12	0.99	999.9	64°C
3	0.59	0.65	0.99	999.9	64°C
1	0.21	0.00	0.96	175.7	68°C
2	4.56	0.01	0.98	715.6	68°C
3	3.07	0.28	0.85	77.5	68°C

Model 1 is Newtonian, Model 2 is Bingham Plastic and Model 3 is Power Law. The value of the F ratio has a maximum of 999.9.

Table 12e. Effect of pasteurization and storage time at -24°C for 45 days on fluid model behavior of LWE.

Model	Yield Stress (B_0)	Flow Behavior Index (B_1)	Correlation r	F-value	Treatment Temperature
1	0.08	0.00	0.99	999.9	Unpasteurized
2	0.54	0.06	0.99	999.9	Unpasteurized
3	0.20	0.74	0.99	999.9	Unpasteurized
1	0.13	0.00	0.99	908.5	60°C
2	1.35	0.10	0.99	999.9	60°C
3	0.56	0.61	0.99	999.9	60°C
1	0.16	0.00	0.99	846.4	64°C
2	1.69	0.12	0.99	999.9	64°C
3	0.68	0.61	0.99	999.9	64°C
1	0.24	0.00	0.96	194.4	68°C
2	5.92	0.10	0.99	999.9	68°C
3	3.68	0.28	0.94	218.4	68°C

Model 1 is Newtonian, Model 2 is Bingham Plastic and Model 3 is Power Law. The value of the F ratio has a maximum of 999.9.

Table 12f. Effect of pasteurization and storage time at -24°C for 80 days on fluid model behavior of LWE.

Model	Yield Stress (B_0)	Flow Behavior Index (B_1)	Correlation r	F-value	Treatment Temperature
1	0.13	0.00	0.99	999.9	Unpasteurized
2	1.56	0.09	0.99	999.9	Unpasteurized
3	0.65	0.59	0.99	999.9	Unpasteurized
1	0.17	0.00	0.99	693.0	60°C
2	2.09	0.12	0.99	999.9	60°C
3	0.92	0.56	0.99	999.9	60°C
1	0.18	0.00	0.98	588.8	64°C
2	2.35	0.13	0.99	999.9	64°C
3	0.99	0.55	0.99	999.9	64°C
1	0.18	0.00	0.95	138.7	68°C
2	4.81	0.07	0.99	999.9	68°C
3	3.20	0.23	0.94	227.3	68°C

Model 1 is Newtonian, Model 2 is Bingham Plastic and Model 3 is Power Law. The value of the F ratio has a maximum of 999.9.

Table 12g. Effect of pasteurization and storage time at -24°C for 120 days on fluid model behavior of LWE.

Model	Yield Stress (B_0)	Flow Behavior Index (B_1)	Correlation r	F-value	Treatment Temperature
1	0.08	0.00	0.99	999.9	Unpasteurized
2	0.54	0.06	0.99	999.9	Unpasteurized
3	0.22	0.73	0.99	999.9	Unpasteurized
1	0.22	0.00	0.98	503.7	60°C
2	3.17	0.15	0.99	999.9	60°C
3	1.45	0.50	0.99	866.3	60°C
1	0.26	0.00	0.98	519.0	64°C
2	4.11	0.25	0.99	999.9	64°C
3	1.71	0.56	0.99	999.9	64°C
1	0.28	0.00	0.93	102.5	68°C
2	8.63	0.07	0.96	256.7	68°C
3	6.65	0.15	0.86	82.0	68°C

Model 1 is Newtonian, Model 2 is Bingham Plastic and Model 3 is Power Law. The value of the F ratio has a maximum of 999.9.