OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY
OF SPONGE CAKE FORMULATIONS
CONTAINING PEA PROTEIN CONCENTRATE AS AN EGG ALBUMEN REPLACER

by

Sheryl Elizabeth Betker

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ABSTRACT

The feasibility of partial egg albumen replacement with
pea protein concentrate (PPC), in a sponge cake system, was
evaluated.   Response surface methodology was used for
sequential optimization of a sponge cake formulation in which
at least 30 percent (weight/weight) of the egg albumen could
be replaced with PPC. In the first study, a 5 factor, 5 level
design was used to determine the importance of PPC, cream of
tartar, water and emulsifier levels, and of whip times, to
sponge cake batter specific gravity, cake volume and Instron
texture characteristics. Pea protein concentrate, whip time
and cream of tartar had the greatest influence on sponge cake
quality, while water and emulsifier were less important. The
appropriateness of the independent variable levels for further
optimization studies was also evaluated. PPC levels (0-60%)
and whip times (4-12 min.) used were appropriate, while cream
of tartar levels needed to be increased from 0-1.9 to 1.0-
2.9 grams per 100 grams flour. Appropriate constant levels
for water and emulsifier were respectively, 185 grams and 0.9
grams per 100 grams of flour.

In a second response surface study, the effects of PPC,
whip time and cream of tartar on physical and sensory sponge
cake characteristics were thoroughly evaluated, and their
levels optimized. Combinations were identified which produced
cakes comparable to a 100 percent egg albumen reference sponge
cake. All measured responses were influenced more by PPC and

whipping than by cream of tartar. PPC did not adversely affect aroma or flavor. As PPC increased, longer whip times were necessary to produce cakes of comparable volume and texture to the REF cake. Increasing cream of tartar permitted higher PPC replacement levels or faster whip rates. Batter specific gravity and cake specific volume were the most important dependent variables for assessing sponge cake quality. Acceptable cakes (Instron hardness $\leq$32.6 N; Instron gumminess $\leq$21.3 N; SG $\leq$.45; sensory springiness $\geq$6.8) could be produced when 30 or 40 percent of the albumen was replaced with PPC, by adding 2.9 grams cream of tartar per 100 grams flour, and whipping for 8 and 11 minutes, respectively. Based upon these results, recommendations were made for the development of a co-spray-dried PPC-whole egg mix for commercial use in sponge-type snack cakes.

The effects of Instron testing conditions (plunger size, degree of sample compression, cross-head speed) on the measurements of sponge cake hardness, cohesiveness, gumminess and springiness were also evaluated. Sample compression and plunger area strongly influenced texture measurements while cross-head speed had much less effect. Instron conditions which best discriminated textural differences between sponge cakes were determined. As well, conditions most appropriate for the simultaneous detection of all four textural parameters were identified as being 75 percent sample compression, 616 mm^2 plunger and 5.1 centimetres/minute cross-head speed.

ACKNOWLEDGEMENTS

This thesis is dedicated to my Mom, who taught me to believe in myself.

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CHAPTER 1

Introduction and Review of Literature

INTRODUCTION

The fractionation of field peas (Pisum sativum) into fibre, starch and protein fractions has increased the utility of a crop which has become increasingly important to Western Canada (Nielsen et al., 1980). In Manitoba alone an estimated 130,000 acres were planted in 1989 making it a substantial special crop for this province (E. Lewis, personal communication, 1989). Woodstone Foods Ltd. (Portage La Prairie, Manitoba) holds one patent for this fractionation process (Nickel, 1981). While the fibre and starch fractions are highly utilized, the protein fraction has found limited use as an animal feed fortifier and a feed milk replacer (M. Lamb, personal communication, 1987). Pea protein is, therefore, found in excess supply as a result of the high demand for the fibre and starch components and consequently, is a relatively inexpensive protein source ($1.35/lb, dry weight) (E. D. Murray, personal communication, 1989). Pea protein concentrate (PPC) would provide an economical alternative to a more expensive protein source.

The production of snack cakes has become a billion dollar business and the market potential for such cakes continues to grow (Wells, 1989). Egg albumen is an expensive component of the whole egg mix generally used to produce snack cakes such

as sponge cake, cake rolls and other sponge type products. Its replacement with an alternative, less expensive protein source would, therefore, be economically advantageous to the cake manufacturer. Export Packers Co. Ltd. (Winnipeg, Manitoba) is a major producer of dried albumen, yolk and whole egg products. Egg albumen is in great demand due to its widespread food applications (L. Carvalho, personal communication, 1987). Export Packers would, therefore, benefit economically if the albumen in their whole egg sponge cake base could be partially replaced by a less expensive protein, thereby increasing the availability of egg albumen to meet the growing demand for this product.

Pea protein concentrate could provide an economical alternative to egg albumen in a sponge cake system. A sponge cake system is a particularly suitable system for evaluating the effects of such a replacement since overall cake quality is highly dependent upon egg quality. The effects of egg albumen replacement with PPC should therefore, be emphasized in a sponge cake system. The potential benefits of successful substitution of PPC for egg albumen are outlined in Figure 1.1. Woodstone Foods Ltd. would benefit by utilizing the excess PPC and establishing a balance between the supply and demand of pea fibre, starch and protein fractions. Export Packers Co. Ltd. would benefit by freeing up albumen which is in high demand, while still providing a less expensive whole egg product. Finally, if albumen can be successfully replaced

FIBRE   YOLK

FIELD PEA ——▶ STARCH ——▶ FLOUR   WHOLE EGG ◀—— ▶EGG

PROTEIN   ALBUMEN
CONCENTRATE ——

-inexpensive   -expensive
-excess supply   -high demand

PARTIAL REPLACEMENT OF EGG ALBUMEN
WITH PEA PROTEIN CONCENTRATE
IN A SPONGE CAKE

-utilize excess pea protein concentrate
 and restores balance among pea fractions.

-frees up egg albumen for additional sale.

-opens the door to widespread use of pea
 protein in a variety of baked products.

Figure 1.1   Potential benefits of successful substitution of
pea protein concentrate for egg albumen in a sponge cake
system.

in a product as sensitive as a sponge cake, there should be potential for its use in a variety of other baked products. The overall objectives of this research were to first establish the feasibility of using PPC as a functional replacement for egg albumen in a sponge cake, then through the use of response surface methodology, to develop an acceptable sponge cake formulation in which at least 30 percent of the albumen is replaced with PPC. This thesis is organized in the form of three separate papers, preceded by a general review of literature and succeeded by a general summary, general conclusions and future research needs. The specific objectives of each experiment have been compiled here, and are restated in each paper.

The first experiment does not deal with the overall objective of this research, but rather, investigates the effects of Instron testing conditions on the detection of textural differences in sponge cakes. The specific objectives of this experiment were:

1. To determine the effect of plunger area, degree of sample compression and cross-head speed on sponge cake hardness, cohesiveness, gumminess and springiness measurements.

2. To determine the operating conditions best able to detect differences between sponge cake formulations for each of the four textural parameters.

3. To identify the operating conditions most appropriate for the simultaneous evaluation of the four textural parameters.

The second experiment is a preliminary step towards the optimization of a PPC-egg albumen sponge cake formulation in which the feasibility of successful substitution is established. The specific objectives of this research were:

1. To use response surface methodology to determine the importance of five independent variables (levels of PPC, cream of tartar, water and emulsifier, and length of whip time) to sponge cake batter specific gravity, specific volume and Instron texture characteristics, in order to identify the most critical variables for further product optimization.

2. To select best fitting regression models from full second-order models, to predict the effects of all five independent variables on sponge cake quality characteristics.

3. To generate contour plots from the best fitting predictive models to gain insight into the relationships between the most influential independent variables and their effects on sponge cake quality.

4. To identify independent variable levels appropriate for further product optimization.

The final optimization experiment was based upon the results obtained from the preliminary study. The specific objectives of this study were:

1. To select best fitting regression models from full second-order models, to predict the effects of PPC, whip time and cream of tartar on each physical and sensory sponge cake characteristic evaluated.

2.   To use the best fitting predictive models to produce contour and response surface plots, in order to visually evaluate the relationships between PPC, whip time and cream of tartar and clarify their effects on the physical and sensory characteristics of sponge cakes.

3.   To identify sponge cake formulae with at least 30 percent of the albumen replaced with PPC, that are comparable to 100 percent egg albumen sponge cakes.

4.   To provide recommendations for the development of a co-spray-dried PPC-whole egg mix for commercial use in sponge-type snack cakes.

LITERATURE REVIEW

The use of PPC as a functional replacement for egg albumen in sponge cake, or any other food product, has never been investigated. The feasibility of such a replacement can, however, be evaluated from both a theoretical and a practical perspective. Theoretically, the feasibility of successful replacement will be evaluated by comparing the molecular and functional properties of pea proteins with egg albumen proteins, thus highlighting similarities and differences which may ultimately influence sponge cake quality. Practical evaluation will be based upon a review of the effects of pea protein on the quality of various baked products, as well as the effects of partial egg albumen replacement with bovine plasma protein, on the quality of cakes. This practical perspective should provide a more realistic view of what to expect with the partial substitution of PPC for egg albumen in a sponge cake.

Following the feasibility assessment, commonly used methods for sensory and physical evaluation of sponge cake quality will be reviewed with particular attention paid to the use of the Instron Universal Testing Machine for instrumental texture evaluations. Finally, the use of response surface methodology as a statistical method to optimize ingredient levels and procedures will be presented.

Comparison of Pea Protein and Egg Albumen Molecular and Functional Properties

Successful substitution of PPC for egg albumen in a sponge cake system will ultimately depend on the similarities between the two protein systems. Comparison of molecular and functional properties will highlight some of the similarities and differences which exist between the two protein systems. Unstandardized experimental procedures, methodologies and reporting of results have made comparisons difficult, therefore only general observations and conclusions have been attempted.

<u>Molecular Properties</u>

i) Pea Proteins

The protein in legume seeds typically ranges from 20-25 percent (Derbyshire et al., 1976), although the protein content in field peas has been reported to vary from 22-32 percent (Ali-Khan and Youngs, 1973). The globulins legumin and vicilin are the two major storage protein fractions identified in field peas (Danielsson, 1949) and account for approximately 80 ± 10 percent of the total seed protein (Pernollet and Mossè, 1983). Legumin is usually found in greater amounts than vicilin depending on the pea variety (Gatehouse et al., 1981). An excellent review of legumin and vicilin is provided by Derbyshire et al. (1976). Water soluble albumins make a smaller, yet important contribution, accounting for approximately 13-14 percent of the pea seed

protein (Grant et al., 1976). The molecular properties of legumin, vicilin and albumins are summarized in Table 1.1.

Pea legumin has been characterized as a hexameric protein composed of six pairs of subunits, one large acidic unit (Mw 40,000d; pI 4.5-4.9) and one smaller basic unit (Mw 20,000d; pI 8.5-8.8) (Gatehouse et al., 1980; Krishna et al., 1979). The six subunit pairs are held together by non-covalent bonds while the acidic and basic units are often held together by disulfide bonds. The subunits are said to be heterogeneous suggesting that more than one form of pea legumin may exist (Derbyshire et al., 1976; Krishna et al., 1979).

Vicilin, the second major globulin fraction in field peas, is a glycoprotein which is smaller and more soluble in salt solutions than legumin (Pernollet and Mossè, 1983). No well accepted structural model of vicilin exists, however a general model has been proposed by Gatehouse et al. (1981). This protein fraction is probably a trimer composed of three heterogeneous subunits with molecular weights approximating 50,000 daltons. Unlike legumin, there is no apparent disufide bonding. It has been speculated that another form of vicilin exists (Higgins and Spencer, 1977; Thomson et al., 1978; Thomson et al., 1980), however Croy et al. (1980) believe it may be a component of convicilin, a newly isolated storage protein.

The albumin components of field peas make only a minor contribution to seed protein and are therefore not well

Table 1.1 Molecular Properties of the Major Field Pea Proteins

Protein Fraction	Approximate % of Total Protein	Molecular Weight (daltons)	Sedimentation Coefficient ($S_{20,w}$)	Isoelectric Point (pH)	References
Legumin	80 ± 10	350,000-400,000	12.6	4.7 - 4.8	1-3, 5-7, 9,10
Vicilin		150,000-190,000	7-8	5.5	1-3, 8, 9
Albumins	13-14	<legumin and vicilin	<legumin and vicilin	n/a[1]	4, 9, 10

[1]Not available

1. Danielsson, 1949.
2. Danielsson, 1950.
3. Derbyshire et al., 1976.
4. Grant et al., 1976.
5. Croy et al., 1979.
6. Blagrove et al., 1980.
7. Gatehouse et al., 1980.
8. Gatehouse et al., 1981.
9. Pernollet and Mossé, 1983.
10. Gueguen et al., 1984.
11. Schroeder, 1984.

characterized. Grant et al. (1976) used various isolation techniques which indicated the presence of two components with sedimentation coefficients of 0.53 and 3.55 using ultracentrifugation, ten components using polyacrylamide gel electrophoresis (PAGE), three components (Mw≈78,000, 47,700, and 26,000 d) using chromatography, and two major components (Mw≈25,000 and 15,000 d) and six minor components using SDS-PAGE. Schroeder (1984) also used SDS-PAGE and found two major components (Mw≈22,000 and 8,000 d) which were thought to equal approximately 34 percent of the pea albumins. Some minor components were also resolved. Thus the albumins appear to partially consist of two major components of relatively low molecular weights plus some minor components.

ii) Egg Albumen Proteins

Egg albumen is composed of approximately 88 percent water and 11 percent protein, with the remaining 1 percent attributed to carbohydrate and fat (Vadehra and Nath, 1973). The major proteins known to contribute to egg albumen functionality are ovalbumin, conalbumin, ovomucoid, ovomucin, and the globulins lysozyme (G1), G2 and G3 (Parkinson, 1966). Excellent reviews of egg albumen proteins are provided by Parkinson (1966), Vadehra and Nath (1973) and Powrie and Nakai (1986). Lysozyme has been extracted from the egg albumen to be used in this study (exported for use as an antibacterial agent) therefore, this protein fraction will be omitted from

the review. The molecular properties of the other egg albumen proteins are summarized in Table 1.2.

Ovalbumin is the most abundant protein in egg albumen accounting for 54 percent of the total protein (Powrie and Nakai, 1986). It is a phosphoglycoprotein which is composed of three components A1, A2 and A3 which differ in their phosphorous content (Parkinson, 1966; Powrie and Nakai, 1985). Ovalbumin has four sulfhydryl groups and one disulfide group (Nisbet et al., 1981) and is said to be the only egg albumen protein with free sulfhydryl groups (Vadehra and Nath, 1973). In solution, ovalbumin is easily denatured when exposed to new surfaces but is quite resistant to heat denaturation (Powrie and Nakai, 1985). S-ovalbumin is a more heat stable form of ovalbumin which is formed during storage (Smith and Black, 1965).

Conalbumin (ovotransferrin) is the second most abundant protein in egg albumen providing 12-13 percent of the protein (Powrie and Nakai, 1985; 1986). It is a glycoprotein with no phosphorous or sulfhydryl groups (Powrie and Nakai, 1986). This protein is more heat sensitive than ovalbumin but is not as sensitive to surface denaturation (Powrie and Nakai, 1985).

Ovomucoid is a glycoprotein comprising 11 percent of the egg albumen proteins (Powrie and Nakai, 1986). This protein has been described as a single polypeptide with portions of helix and random coil and contains eight disulfide links in every chain (Powrie, 1977). This protein is quite heat

Table 1.2   Molecular Properties of the Major Egg Albumen Proteins

Protein Fraction	Approximate % of Total Protein	Molecular Weight (daltons)	Sedimentation Coefficient ($S_{20,w}$)	Isolectric Point (pH)	References
Ovalbumin	54	45,000	3.27	4.5 - 4.8	2,4,5, 8-10
Conalbumin	12-13	76,000-80,000	5.05	6.1 - 6.6	2,5,7,9,10
Ovomucoid	11	28,000	2.62	3.9 - 4.3	1,2,5,10
Ovomucin	1-3.5	very large (varies with isolation form)	6.4 (reduced condition)	4.5 - 5.0	5,8,9
G2 globulin	4?	30,000-45,000	n/a[1]	5.5	6
G3 globulin	4?	45,000	n/a	5.8	3,6

[1]Not available.

1.   Rhodes et al., 1958.
2.   Parkinson, 1966.
3.   Baker, 1968.
4.   Vadehra and Nath, 1973.
5.   Osuga and Feeney, 1977.
6.   Powrie, 1977.
7.   Pyler, 1982.
8.   Gossett et al., 1984.
9.   Powrie and Nakai, 1985.
10. Powrie and Nakai, 1986.

resistant (Powrie and Nakai, 1985).

Ovomucin accounts for approximately 1-3.5 percent of the egg albumen proteins (Baker, 1968; Gosset et al., 1984), and is believed to account for the jelly-like appearance of egg white (Kato et al., 1985). It is a very large glycoprotein which is insoluble in neutral or acidic solutions (Pyler, 1982) but dissolves in dilute salt solutions at neutral or alkaline pH (Vadehra and Nath, 1973). This protein is highly glycosylated with approximately 30 percent carbohydrate content (Powrie and Nakai, 1985) and is described as fibrous and flexible with disulfide bridges joining the fibres (Vadehra and Nath, 1973).

G2 and G3 globulins comprise approximately 8 percent of the egg albumen proteins (Vadehra and Nath, 1973; Powrie and Nakai, 1985) and are said to exhibit great genetic variability (Osuga and Feeney, 1977).

iii) General Comparison of Molecular Properties

Although amino acid compositions were not presented for individual pea and egg albumen proteins, the amino acid compositions of PPC (PROPULSE 985B to be used in this study) and dried egg albumen are compared in Table 1.3. Overall, pea protein and egg albumen amino acid compositions are quite similar with the exception of the sulfur containing amino acids (cystine and methionine). Typical of legume proteins, pea proteins are nutritionally deficient in the sulfur

Table 1.3   Amino Acid Compositions[1] of Pea Protein Concentrate[2] and Dried Egg Albumen[3]

Amino Acid	Pea Protein Concentrate	Egg Albumen
Aspartic Acid	12.43	8.85
Glutamic Acid	13.74	13.95
*Lysine	6.82	6.16
Arginine	8.71	5.84
Histidine	2.52	2.27
Glycine	4.64	3.74
Serine	4.80	7.38
*Threonine	4.34	4.45
Cystine	0.76	2.48
Tyrosine	3.12	4.02
Asparagine[4]		
Glutamine[4]		
Alanine	5.04	6.45
*Valine	5.27	7.49
*Leucine	8.44	8.70
*IsoLeucine	5.59	6.10
Proline	5.29	3.76
*Phenylalanine	6.13	6.29
*Tryptophan	1.06	1.50
*Methionine	1.31	3.89

[1]Grams of amino acid/100 grams protein.
[2]Woodstone Foods Ltd., 1986.
[3]Adapted from Posati and Orr, 1976.   Converted from 100g egg white (82.4% protein)
 to 100g protein.
[4]Combined with Aspartic and Glutamic acids.

*Essential amino acid for adults.

containing amino acids. Other researchers have noted the resemblance of legume storage proteins to egg proteins with the exception of the sulfur amino acids (Pernollet and Mossè, 1983). It should be noted however, that pea proteins are high in lysine which make them complementary to wheat proteins which are low in lysine (Kreutler, 1980). Therefore, while the partial replacement of egg albumen with PPC may result in a slight decrease in the nutritional value of the product, the presence of proteins from other sources may complement the pea proteins.

While knowledge of the amino composition provides some nutritional information, it does not indicate the sequence of amino acids which has an influence on protein conformation. Kinsella (1982) noted that unless a particular type of amino acid predominates, the knowledge of amino acid composition is not very valuable for predicting protein conformation and physical characteristics. In the case of pea and egg albumen proteins, while their amino acid compositions were quite similar, their molecular properties were very different.

A comparison of Tables 1.1 and 1.2 highlights some of the major molecular differences between pea and egg albumen proteins. Firstly, and most notably, field pea proteins are much larger and possess a more highly ordered structure than egg albumen proteins. Seed storage proteins often associate into compact arrangements in order to store amino acids in small volumes (Pernollet and Mossè, 1983), thus accounting for

the complex structures of legumin and vicilin. Secondly, egg albumen is, overall, a more diverse system of proteins with a wider range of isoelectric points. The diversity of proteins in egg albumen provides the polyfunctionality necessary for many food products, and is what makes this system so difficult to replace. Thirdly, most of the pea proteins are globulins (soluble in dilute salt solutions) whereas egg albumen proteins are primarily albumins (soluble in water). Finally, all the egg albumen proteins evaluated, with the exception of the globulins (G2 and G3), are glycoproteins, whereas vicilin is the only glycoprotein in the pea protein system.

The molecular properties of a protein will ultimately influence functionality. Field pea proteins and egg albumen proteins were found to differ only slightly in their amino acid compositions, however other molecular properties, in particular size and structure, were quite different. It is expected, therefore that the functionality of the two protein systems will also differ, reflecting the molecular differences already discussed.

Functional Properties

Comparison of pea and egg albumen functionality is an important comparison to be made when assessing the feasibility of egg albumen replacement with PPC. According to Hermansson (1979a), the functionality of a new food protein is probably

the most important prerequisite to commercial success. Therefore, if pea protein is to successfully replace some the egg albumen in a sponge cake, it should possess similar functionality characteristics.

The functional characteristics believed to be important to sponge cake quality include protein solubility, foaming and coagulating properties. Sponge cakes are foam cakes which contain no chemical leavening agent or shortening (McWilliams, 1979). Instead, egg albumen provides the foaming, foam stabilizing and heat setting proteins necessary to produce sponge cakes which are tender and of good volume (Kamat et al, 1973; McWilliams, 1979). Thus egg albumen is a polyfunctional protein system. A thorough review of egg albumen functionality is provided by Baldwin (1986). Although standardized methods for functionality testing are lacking, general conclusions can be drawn from comparisons of solubility, foaming and heat coagulating properties of pea protein and egg albumen.

i) Solubility

Solubility is generally one of the first functionality tests conducted since the solubility of the protein may influence other functional properties (Regenstein and Regenstein, 1984). Solubility is especially important for dried protein products if they are to function in foods (Morr et al., 1985). Nitrogen solubility, protein solubility and

total solubility are ways of reporting this characteristic (Regenstein and Regenstein, 1984).

a. Pea Protein Solubility

Table 1.4 summarizes some of the relevant research on pea protein solubility. In general, pea protein concentrates and isolates were found to be least soluble in their isoelectric range of pH 4-5, and exhibited low solubility also around their natural pH (approximately 6.5). Pea proteins were most soluble at pH 9-11 but were also very soluble at low pH values, that is, away from their isoelectric points. The addition of 2.5 percent salt increased pea protein solubility and minimized the effect of pH.

Heat treatments, drying procedures and isolation techniques were found to influence the solubility of the proteins, therefore the results reported by Naczk et al. (1986) and Sosulski and McCurdy (1987) may be the most relevant. These studies reported the solubility properties of pea protein products supplied by Woodstone Foods Ltd., the supplier of PPC for this study (Table 1.4).

Studies on the solubility of the major pea proteins (legumin, vicilin and albumins) have also been conducted. Koyoro and Powers (1987) investigated the effect of pH on the solubility of legumin, vicilin and a mixed globulin fraction. Minimum solubility of all fractions was at pH 5-6, while maximum solubility of the legumin and mixed globulin fractions

Table 1.4   Summary of Research on Pea Protein Solubility

Reference	Form of Pea Protein[1]	Solubility Measurement	Treatment	Results
Vose et al., 1976	PPC	Nitrogen Solubility	Effect of pH (2-10)	- Least soluble at pH 4.2 and most soluble (approx. 90%) at pH 9-10.
Sumner et al., 1981	PPI	Nitrogen Solubility	Effect of isolation method (isoelectric and sodium precipitated) and drying procedure (drum, freeze, spray).	- All treatments least soluble at pH 4.5 and most soluble at pH 10. - Isoelectric PPI more soluble than sodium PPI. - Drum dried isolates (harshest technique) were least soluble.
Megha and Grant, 1986	PPC	Nitrogen Solubility	Effect of heat treatment (control, 50, 60, 70, 80, 98°C) and pH (1-10)	- All treatments least soluble (approx. 0-3%) at pH 4.5 and most soluble at low or high pH (away from the isoelectric region). - Isoelectric range increased with severity of heat treatment.
Hsu et al., 1982	PPI	Nitrogen Solubility	Effect of pH (2-12) and germination.	- Broad isoelectric pH range between pH 4-6. - Germination had little effect on solubility.
Naczk et al., 1986	PPC[2]	Protein Solubility	Solubility of 3 batches at their natural pH (6.50, 6.45, 6.79).	- Solubilities = 30.3, 37.7 and 41.9%.
Sosulski and McCurdy, 1987	PPI[2]	Nitrogen Solubility	Effect of pH (2-11)	- Least soluble at pH 4-5 and most soluble at pH 10-11. - Low solubility between pH 6-9.
Christensen, 1989	Pea Protein[3]	Protein Solubility	Effect of pH (2-8) and salt (2.5%)	- Solubility increased and was less affected by variations in pH with addition of salt.

[1]PPC = pea protein concentrate; PPI = pea protein isolate.
[2]Pea protein supplied by Woodstone Foods Ltd.
[3]Protein content unknown.

was at pH 3. Vicilin showed maximum solubility at pH 7, and was more soluble at all pH levels than legumin. Grant et al. (1976) investigated the solubility of pea albumins and found that this fraction was very water soluble and was only minimally influenced by pH.

b. Egg Albumen Solubility

Table 1.5 summarizes the results from two studies reporting the solubility of dried egg albumen. Because the albumen to be used in this thesis research is of the spray-dried form, studies of dried egg albumen solubility are most relevant. In general, egg albumen was very soluble at all tested pH values, regardless of drying procedure or presence of salt.

c. Comparison of Pea Protein and Egg Albumen Solubility

A comparison of the two protein systems indicates that, in general, egg albumen proteins were more soluble than pea proteins at all levels of pH investigated. Pea proteins were most soluble at a pH of 9-10 but were also soluble at very low pH values. Limited solubility was exhibited around pH 4-5, the isoelectric range of the proteins. Egg albumen proteins were highly soluble at all pH values, including around the isoelectric pH of the protein system (pH 4.65) (Ma and Holme, 1982). For both protein systems, salt minimized the effect of pH on protein solubility. This positive effect was,

Table 1.5   Summary of Research on Dried Egg Albumen Solubility

Reference	Solubility Measurement	Treatment	Results
Morr et al., 1985	Protein Solubility	Effect of pH (3 and 7)	- Solubility was 98% at pH 3 and 100% at pH 7.
Kakalis and Regenstein, 1986	Protein Solubility	Effect of drying procedure (freeze and spray), pH (3,5,7,9,) and NaCl.	- In water, both proteins showed minimum solubility (96%) at pH 5 and maximum solubility (99%) at pH 3. - NaCl generally increased solubility at higher pH values. - Increasing ionic strength decreased the effect of pH on solubility.

however, more apparent for pea protein than egg albumen.

Protein solubility is affected by protein composition, conformation, size and environmental conditions such as pH, ionic strength and temperature (Kinsella, 1982). Observed differences in solubility between egg albumen and pea proteins could be due to any or all of these factors. The major components of pea protein are globulins which are soluble in dilute salt solutions and show limited solubility in water. Conversely, the majority of proteins in egg albumen are albumins which are readily soluble in water. Only one study evaluated the effect of salt on pea protein solubility, and in this study, solubility increased with the addition of low levels of salt (Christenson, 1989). Solubility studies should consider the effects of controllable conditions such as pH, ionic strength and temperature if the results are to be more relevant to particular food systems. Protein size is another obvious difference between pea and egg albumen proteins which would influence solubility. Pea proteins are larger and more highly ordered than egg albumen proteins and in general, the larger the molecule, the less soluble it becomes (Kinsella, 1982). The moderately inferior solubility characteristics of pea protein may ultimately be reflected in the foaming and heat coagulating properties of the pea protein system.

ii) Foaming Properties

The foaming properties of a protein are critical when the

intended food application requires that a stiff, stable foam be formed. Because a sponge cake does not contain chemical leavening agents or shortening, the air incorporated into the egg foam is the sole leavening agent. Therefore, the foaming properties (foaming capacity and foam stability) of egg albumen are probably the most critical functional properties which PPC must simulate in order to achieve successful substitution in a sponge cake system.

Measures of foaming capacity (foam expansion, whippability) and foam stability have traditionally been used to characterize protein foams (Waniska and Kinsella, 1979). Common methods of measurement have been summarized by Waniska and Kinsella (1979). Foaming capacity can be measured by whipping, shaking or gas sparging (bubbling) procedures. Whipping, the most common method, requires 3-40 percent protein for foam production. For this method, foaming capacity is measured as foam volume increase, foam specific gravity, or viscosity. The shaking method requires only 1 percent protein and foam volume is the usual measure of foaming capacity. Gas sparging requires 0.01-2 percent protein for foam production. Foam capacity is calculated as the ratio of the volume of gas in the foam to the volume of gas sparged, or as the ratio of maximum foam volume to gas flow rate. For all three methods, foam stability is usually measured as the amount of liquid drained from the foam after specific periods of time, or alternatively, as the volume of

foam remaining after specific lengths of time.

The variety of methods available and lack of standardization have made comparison of data from different sources difficult. Consequently, the characterization of pea protein and egg albumen foams is quite general and comparisons have been made cautiously.

a. Pea Protein Foaming Capacity and Foam Stability

Table 1.6 summarizes the most relevant research investigating the foaming properties of pea proteins. While this table clearly illustrates the variety of procedures used to evaluate foaming capacity and foam stability, a general picture of pea protein foamability can be drawn.

The foaming properties of pea protein were influenced by heat treatment, drying procedure, isolation procedure and by the addition of skim milk powder and wheat flour. Heat treatments adversely affected the whippability and foam stability of PPC except for samples heated to 70°C. Megha and Grant (1986) suggested that partial denaturation of the pea proteins may have been advantageous to foam formation and stabilization. The study by Vose (1980) illustrated the importance of considering the effect of additional ingredients on the ultimate foaming properties of a protein. While the functionality of a protein in a simple model system may indicate its use in a food system, it may not indicate its functionality in the presence of carbohydrates, fats, other

Table 1.6   Summary of Research on Foaming Properties of Pea Protein

Reference	Form of Pea Protein[1]	Foaming Method	Measurement of Foaming Capacity[2]	Measurement of Foam Stability	Treatment	Results
Sosulski and Youngs, 1979	High protein fraction (3% w/v)	Whipping (6 min.)	Initial foam vol. after whip.	Final foam vol. after 120(?) min.	Used as a comparison to other legume high protein fractions.	- Initial foam vol. = 815 ml; final foam vol. = 10 ml. - Field pea was the only legume to show poor stability.
Vose, 1980	PPI (1% w/v)	Shaking (1 min.)	% Increase in foam vol.[3]	Foam vol. after 30 min.	Effect of isolation procedure (isoelectric precipitation, ultrafiltration) and addition of skim milk powder and wheat flour.	- Isoelectric PPI had inferior foaming properties to the ultrafiltered PPI. - Skim milk improved, and wheat flour decreased, foaming properties.
Sumner et al., 1981	PPI (6% w/v)	Whipping (8.5 min.)	% Increase in foam vol.	Leak determined over 90 min.	Effect of isolation method (isoelectric precipitated) and sodium precipitated) and drying procedure (drum, freeze, spray).	- Spray-dried isolates produced the greatest % increase in foam vol. (412% - isoelectric PPI; 433% - sodium PPI) and were the most stable. - Freeze-dried isolates exhibited the poorest foaming properties.
Hsu et al., 1982	PPI (3.5% w/v)	Whipping (3.5 min.)	% Increase in foam vol.	Vol. of liquid determined after 30, 60, 120 min.	Effect of legume germination.	- Germinated PPI showed greater foam expansion (212% vs 116%) than ungerminated PPI but less foam stability. - Foams had coarse air cells.
Megha and Grant, 1986	PPC (3% w/v)	Whipping (8 min.)	% Increase in foam vol.	Foam vol. after 1, 10, 30, 60, 120 min.	Effect of heat treatment (control, 50, 60, 70, 80, 98°C).	- % foam vol. increase of unheated PPI = 425%. - Foam vol. and stability decreased as temperature increased except for the 70°C treatment where foam volume and stability increased. - Stability of all treatments was poor after 30-60 min.
Sosulski and McCurdy, 1987	PPI[4] (3% w/v)	Whipping (6.5 min.)	Foam vol. (ml) immediately after whipping.	Foam vol. after 10, 30, 60, 120 min.	Effect of isolation procedure (air class-ified, acid extracted[4]).	- Air classified PPI exhibited better foaming properties than the acid extracted PPI. - Both PPIs had high initial volumes which decreased approximately 50% after 120 min. standing. - The air classified PPI produced a coarse textured foam while the acid extracted PPI produced a medium textured foam.

[1] PPC = pea protein concentrate; PPI = pea protein isolate.

[2] Also referred to as "foam expansion" and "whippability".

[3] % foam volume increase = $\dfrac{\text{vol. after whip} - \text{vol. before whip}}{\text{vol. before whip}} \times 100$

[4] Supplied by Woodstone Foods Ltd.

proteins, acids, salts etc. (Hermansson, 1973).

Because isolation and drying procedures influenced the foaming properties of pea proteins, the results from the study by Sosulski and McCurdy (1987) are perhaps the most relevant (Table 1.6). This study reported the foaming properties of pea protein isolate (PPI) supplied by Woodstone Foods Ltd., the supplier of PPC for the proposed thesis research. The acid precipitated PPI exhibited high initial volumes which were fairly stable throughout the two hour standing period. The foam was described as having a "medium" texture. These authors concluded that PPI had promising functional properties and should be evaluated as a potential ingredient in baked and other food products.

The foamability of pea legumin, vicilin and albumins has also been investigated. Koyoro and Powers (1987) used the gas sparging method to characterize the foaming properties of pea globulins at pH 3 and 7. At pH 4 (around the isoelectric range of the proteins) foams with small bubbles were initially formed, however once sparging stopped, the foams quickly collapsed. At pH 3, all protein fractions foamed but legumin and the mixed globulin fraction had significantly greater foaming capacity than vicilin. The vicilin fraction did, however, produce much more stable foams. At pH 7, foams had large air bubbles which broke as they were formed. Interestingly, heating the protein solution in a 90°C water bath for five minutes produced higher foaming capacities than

those observed for the unheated and pH 3 solutions. After heating, the foaming capacity of the mixed globulin fraction was significantly greater than that of legumin and vicilin, whose foaming capacities were no longer significantly different. Heating improved the foam stability of the mixed globulin and legumin fractions and decreased the foam stability of vicilin. Vicilin foam stability, although decreased, was still greater than that of the mixed globulin and legumin fractions. Thus heating apparently improved the foaming ability of vicilin but decreased foam stability, while both foaming capacity and stability of the mixed globulin and legumin fractions were improved. Heating increased surface hydrophobicity of the proteins indicating that the proteins unfolded to expose hidden hydrophobic regions. Surface hydrophobicity was not, however, predictive of the foaming capacity of the pH 3 unheated fractions. Townsend and Nakai (1983) did not find surface hydrophobicity to be a good predictor of foaming capacity, instead, proteins which were flexible, unordered and hydrophobic (not just on the surface) illustrated good foaming properties.

Grant et al. (1976) investigated the foaming properties of a concentrated albumin rich pea protein concentrate (ARPC) at pH 4, 5.4 (natural pH) and 8 using a stirring method. Unlike the other studies, the foaming properties of the pea albumins were compared to those of egg albumen. The pea albumins produced large amounts of foam which could not be

differentiated from the egg albumen foams. Thus the albumin
fraction in field peas, although minor, may play an important
role in foam formation and stabilization.

b. Foaming Capacity and Foam Stability of Egg Albumen

Table 1.7 summarizes the results from the more relevant
investigations of egg albumen foamability. While the
variability of measurement has made cross-study comparisons
difficult, the results presented illustrate the unique ability
of egg albumen to form voluminous foams which are also stable,
particularly with the addition of sucrose. These unique
qualities have made egg albumen one of the most commonly used
food foaming agents (Kinsella, 1981).

The foaming properties of egg albumen proteins have also
been evaluated in more complex angel food cake systems
(MacDonnell et al., 1955; Johnson and Zabik, 1981a).
MacDonnell et al. (1955) found that the globulins were
responsible for the foaming capacity of egg albumen while
ovomucin stabilized the foam in short whip times. Ovomucin
is a very large, highly glycosylated protein (Powrie, 1977)
and the glycoresidues are believed to help structure water and
therefore improve stability (Kinsella, 1981). It has also
been suggested that ovomucoid, a very heat stable protein, may
also increase foam stability by adding visco-elasticity to
films during heating (Kinsella, 1981). Johnson and Zabik
(1981a) also found that the globulins exhibited the greatest

Table 1.7 Summary of Research on Foaming Properties of Egg Albumen

Reference	Form of Egg Albumen	Foaming Method	Measurement of Foaming Capacity	Measurement of Foam Stability	Treatment	Results
Zabik and Brown, 1969	Frozen and dried (amount of water added dependent upon moisture content of egg)	Whipping (5½ min.)	Foam specific gravity.	Foam drainage (ml) after 30 min.	Effect of drying procedure (defrosted, foam-spray-dried, freeze-dried, spray-dried), pH (natural - 7.0, 6.2, 5.6), and addition of SLS (whipping agent).	- Drying procedure had little effect on foaming properties except for the foam-spray-dried treatment which was significantly less stable. - pH had no significant effect on foaming properties, but pH 5.6 produced slightly lower specific gravities and greater foam stability. - SLS improved foaming properties.
Thompson et al., 1982	Fresh (11% protein)	Whipping (6 min.)	% Increase in foam vol.	Volume remaining after 0, 20,40,60,120 min.	Egg albumen used as a control for evaluation of a rapeseed protein concentrate.	- Excellent foam capacity and stability (% increase in foam vol. = 687%; volume of foam decreased 30 ml after 120 min.)
Hsu et al., 1982	Fresh (30 ml)	Whipping (to form soft peaks - 30 sec)	% Increase in foam vol.	Vol. of liquid determined after 30, 60, 120 min.	Egg albumen used as a control for evaluation of germinated and ungerminated pea protein isolate.	- % Increase in foam vol. = 330%. - Stability similar to ungerminated pea protein isolate.
Poole et al., 1984	Spray-dried (0.5% w/v)	Whipping (5 min.)	% foam expansion[2]	Foam liquid stability[3]	Effect of sucrose (100g/L) addition.	- Sucrose increased foam expansion and stability (expansion in water = 240%; with sucrose = 440%).
Ma et al., 1986	Spray-dried[4] (1% w/v)	Shaking (1 min.)	% Increase in foam vol. (?)	% foam remaining after 60 min.	Egg albumen used as a control for evaluation of chemically modified egg albumen.	- Volume increase = 200% and 33% of the foam remained after 60 min.

[1] % foam volume increase = $\dfrac{\text{vol. after whip} - \text{vol. before whip}}{\text{vol. before whip}}$ X 100

[2] % foam expansion = $\dfrac{\text{foam vol. (ml)}}{\text{initial liquid vol. (ml)}}$ X 100

[3] Foam liquid stability = $\dfrac{\text{vol. of liquid (ml) retained in foam after 30 min.}}{\text{initial liquid vol.}}$ X 100

[4] Albumen supplied by Export Packers Ltd.

foaming capacity. Ovomucin, ovomucoid, lysozyme and conalbumin showed minimal foaming capacity.

c. Comparison of Pea Protein and Egg Albumen Foaming
   Properties

The variability of testing procedures used to evaluate the foaming properties of pea protein and egg albumen have made comparison of this functional quality difficult. In general, however, egg albumen appears to exhibit moderately greater foaming capacity and much greater foam stability. This observation is best illustrated by comparing the results from Megha and Grant (1986) and Thompson et al. (1982) (Table 1.6 and 1.7). Similar methods were used to evaluate the foaming properties of PPC and egg albumen, except for whip times (8 min. vs 6 min.) and the times at which foam volume was measured to indicate stability (1,10,30,60,120 min. vs 0, 20, 40, 60, 120 min.). The increase in foam volume for the unheated PPC sample was 425 percent and foam stability after 30-60 minutes was quite poor. Egg albumen produced an increase in foam volume of 687 percent and the foam stability as excellent even after 120 minutes. Differences in foam texture may also exist, that is, egg albumen foams are typically fine textured whereas foams produced from a pea protein isolate were medium-to-coarse textured (Sosulski and McCurdy, 1987). These differences in foam texture may ultimately be reflected in the baked sponge cake crumb.

Hsu et al. (1982) used egg albumen as a reference against

which the foaming capacity and stability of pea protein isolates (PPI) from germinated and ungerminated field peas were compared (Table 1.6 and 1.7). This study appears to be the only one in which the foaming properties of egg albumen and pea proteins are directly compared. While the foaming capacity of egg albumen was moderately higher, foam stability was comparable to the ungerminated PPI, and much better than the germinated PPI. It is possible that egg albumen foam stability was influenced by the degree of whipping, that is, the egg albumen was only whipped to a soft peak stage. If whip times had been increased and stiff peaks formed, perhaps foam stability would have increased.

The globulins in egg albumen have been identified as the fraction responsible for foam formation while ovomucin appears to be responsible for foam stabilization. It is difficult to determine the exact role of each fraction in field peas. It appears, however, that the albumin and legumin fractions may be responsible for foam formation while all three fractions, particularly vicilin, seem important to foam stabilization.

According to Kinsella (1981), factors which influence the foaming properties of proteins include molecular properties such as protein size, composition, conformation, compactness, rigidity, and charge. These inherent properties may then be influenced by processing and environmental conditions. Any or all of these factors could be responsible for the apparent differences in the foaming properties of egg albumen and pea

proteins. A brief review of foam formation and stabilization theory may provide some explanation for these differences.

Foam formation involves the diffusion of soluble proteins towards the air/water interface, where they unfold, concentrate, and quickly spread to lower the surface tension of the solution (Cheftel et al., 1985). Townsend and Nakai (1983) noted that proteins should be flexible, structurally less ordered and hydrophobic in order to quickly concentrate at the air/water interface. Supporting this, Kitabatake and Doi (1982) found that proteins which could quickly lower surface tension were found to have high foaming power and were flexible in nature. Rigid globular proteins took more time to lower surface tension due to the increase in time necessary to unfold their ordered structures. Flexible proteins, on the other hand, easily penetrated the air/water interface and quickly lowered surface tension. Protein size may also influence the rate of reaching the air/water interface, that is, small proteins diffuse more quickly than larger proteins (German et al., 1985).

Formation of a stable foam requires that the adsorbed proteins interact among themselves to form continuous visco-elastic and cohesive films around the air bubbles (Kinsella, 1981; Cheftel et al., 1985). Protein flexibility also appears to be important to foam stability. It has been suggested that flexible proteins are more easily denatured (ie., unfolded) at the air-water interface than rigid protein molecules (Kato

et al., 1985). Surface denaturation increases the protein-protein interactions necessary to form the thick cohesive films for foam stabilization (Kinsella, 1981). Viscous films help decrease the rate of water drainage from the lamellae surrounding the air bubbles (Phillips, 1981). Excessive surface denaturation will, however, result in a loss of film visco-elasticity and consequently, a loss in foam stability (Kinsella, 1981; Townsend and Nakai, 1983). Kato et al. (1986) illustrated the importance of protein flexibility to foam formation and stability by cross-linking a rigid protein (lysozyme) with a flexible protein (bovine serum albumin). Results indicated that foaming power and stability of both proteins were decreased as a result of the cross-linking. In summary, for good foam formation and foam stability, proteins should be soluble, small, flexible, hydrophobic, and surface denaturable, in order to quickly reach the air-water interface, concentrate, unfold, and re-orient to form viscous protein films around the newly formed air bubbles.

Differences in the molecular and solubility characteristics of pea and egg albumen proteins may, in part, explain the inferior foaming capacity and foam stability of pea proteins. Egg albumen proteins were more soluble at all pH levels and solubility has been considered to be an important prerequisite to foam formation (Kinsella, 1981). Some insoluble proteins may however, be necessary for foam stabilization (Schoen, 1977). McWatters and Cherry (1977)

found that protein solubility was more closely related to foam consistency and air cell size than to the increase in foam volume. Pea proteins are also larger and more highly ordered than egg albumen proteins, consequently, are likely slower to diffuse to the air/water interface and to unfold in order to lower surface tension. Possible differences in hydrophobicity may also account for differences in foamability.

The comparison made between the foaming properties of pea protein and egg albumen has been based upon a variety of studies which differ substantially in their methodologies. There is a need for a direct comparison between the two protein systems, such as the one made between the foaming properties of pea albumins and egg albumen (Grant et al., 1976). The apparently inferior foaming properties of pea protein may ultimately be manifested in the quality of the sponge cakes produced with PPC. It must be stressed however, that the effect of pH on the foamability of pea proteins was never investigated, nor was the effect of salt. These two factors influenced pea protein solubility, therefore they may also have had an effect on pea protein foaming properties. Additionally, the presence of other sponge cake ingredients may have an influence on the foaming properties of both protein systems.

iii) Heat Coagulating Properties

The heat coagulating or gelling properties of a protein

system are important to the appearance and texture of many food products (Holt et al., 1984). The coagulation of egg proteins is critical to the structure and crumb strength of a sponge cake (Toney and Bergquist, 1983), therefore, it is important that pea proteins exhibit the ability to coagulate if a successful substitution is to be made.

Gelation and coagulation are often used interchangeably, yet they differ slightly in their definitions (Schmidt, 1981). Coagulation is the random aggregation of denatured proteins while gelation is an orderly aggregation of denatured or undenatured proteins (Hermansson, 1979b). Coagulation involves protein-protein interactions whereas gelation involves a balance of protein-protein and protein-solvent interactions as well as attractive and repulsive forces (Schmidt, 1981). Coagulation is characterized by a less elastic, less hydrated structure than that of a protein gel which is characterized by a highly ordered tertiary network (Schmidt, 1981). Despite these differences, gelation is often used as an index to coagulation (Baldwin, 1977). Cheftel et al. (1985) and Baldwin (1986) have provided reviews of protein gelation.

A compilation of methods used to evaluate the coagulating/gelling properties of proteins was made by Gossett et al. (1984). Methods have included the measurement of gel strength, changes in solubility, gravimetric analysis (amount of protein left in the supernatant after centrifugation),

formation of disulfide links, electrophoresis (disappearance
or appearance of protein bands), and evaluation of structural
changes using differential scanning calorimetry. Viscosity
measurements have also been used to evaluate gelation (Fleming
and Sosulski, 1975; Hsu et al., 1982).

a. Heat Coagulating/Gelling Properties of Pea Proteins

Table 1.8 summarizes some of the limited research which
has reported the coagulating/gelling properties of pea
proteins. In general, pea proteins exhibited the ability to
gel when temperatures were 90°C and heating times were long
enough. Pea protein isolate (PPI) solutions heated for only
ten minutes did not form gels while those heated 45 to 60
minutes did. Fleming and Sosulski (1975) found that PPC
required 60 minutes of heating to form a gel. Differential
scanning calorimetry indicated that the denaturation
temperatures (Td) of an isoelectric PPI and a micelle PPI were
94.5 and 88.9°C, respectively (Murray et al., 1985). Because
denaturation is a prerequisite to coagulation (Ma and Holme,
1982), temperatures of 90-95°C will be necessary to coagulate
the pea proteins. Table 1.7 indicated that pea proteins
gelled when heated to 90°C. Unfortunately, no studies were
done to evaluate the effect of lower heating temperatures on
pea protein coagulating/gelling properties.

Sosulski and Youngs (1979), in addition to investigating
the gelling properties of a high pea protein fraction,

Table 1.8 Summary of Research on the Heat Coagulating/Gelling Properties of Pea Protein

Reference	Form of Pea Protein[1]	Method	Measurement of Coagulation/Gelation	Treatment	Results
Fleming and Sosulski, 1975	PPC and PPI (10% w/v)	Heated to 90°C for 45 min. (PPI) and 60 min. (PPC); cooled.	Viscosity	Effect of pH (7,12) and NaCl (5% solution).	- PPC - at pH 7 and 12 viscosity exceeded 166,000 cps and medium gels formed. - PPI - at pH 7 viscosity = 120,000 cps and a soft gel formed. No evaluation made at pH 12. - NaCl had little effect on gelation properties.
Sosulski and Youngs, 1979	High protein fraction (15% w/v)	Heated to 90°C for 45 min.; cooled.	Volume of pourable slurry. Gel remaining used to calculate % of the slurry which had gelled.	Compared to other legume high protein fractions.	- 100% of the protein gelled.
Hsu et al., 1982	PPI (10% w/v)	Heated to 90°C for 10 min.; cooled	Viscosity	Effect of legume germination.	- Germinated and ungerminated PPIs did not form gels. - Ungerminated PPI formed a smooth paste (viscosity = 34,400 cps). - Germinated PPI formed a granular curd (viscosity = 24,000 cps).
Murray et al., 1985	PPI (10% w/w)	DSC (heating rate = 10°C/min.)	Temperature of denaturation (Td)	Effect of isolation procedure (isoelectric isolation and NaCl extraction).	- Only 1 endotherm was apparent, probably due to the close proximity of the denaturation temperatures of the proteins. - Isoelectric PPI Td = 94.5°C. - NaCl extracted PPI Td = 88.9°C

[1]PPC = pea protein concentrate; PPI = pea protein isolate.

evaluated the gelling properties of the pea globulin and albumin fractions. The fractions (10 % protein) were dispersed in a 0.5 M NaCl solution, heated at 90°C for 45 minutes, and cooled. The globulin fraction formed a gel structure which was smooth, homogeneous and clear. An increase in the protein fraction from 10 to 15 percent produced a firm gel. The albumin fraction did not produce a gel, consequently, it was concluded that pea globulins were responsible for the gelation properties observed in the high protein fraction.

b. Heat Coagulating/Gelling Properties of Egg Albumen

Extensive research has been conducted on the coagulability of egg albumen and its component proteins. Much of the research has focused on ovalbumin in attempts to understand the mechanism of protein coagulation (Hayakawa and Nakai, 1985; Shimada and Matsushita, 1980; Ma and Holme, 1982). Of interest to this review, however, is the research characterizing the coagulating/gelling ability of egg albumen so that comparisons to the pea proteins can be attempted. Table 1.9 summarizes the more relevant research on egg albumen coagulability. The primary heat coagulating proteins in egg albumen have been identified as conalbumin and ovalbumin which, under neutral conditions, coagulate at temperatures of 60-65°C and 80-85°C, respectively (Donovan et al., 1975; Ma et al., 1986). MacDonnell et al. (1955) found that ovalbumin

Table 1.9 Summary of Research on the Heat Coagulating/Gelling Properties of Egg Albumen

Reference	Form of Egg Albumen	Method	Measurement of Coagulation/Gelation	Treatment	Results
Donovan et al., 1975	Fresh	DSC (heating rate = 10°C/min.)	Temperature of denaturation (Td).	Effect of pH (7-9) and sucrose.	- At pH 7 endothermic peaks were apparent at 65°C (denaturation of conalbumin) and 84.5°C (denaturation of ovalbumin). At pH 9 the conalbumin andotherm was shifted to a higher Td (69.5°C) while the ovalbumin endotherm was shifted to a slightly lower Td (84°C). - Sucrose increased Tds 2°C thereby increasing heat stability.
Holt et al., 1984	Fresh (diluted to 8% protein)	Heated in water bath (varying temps.) for 60 min.	Viscosity, gel strength and elasticity.	Effect of temperature (65, 69.8, 77.5, 85.2, 95°C), pH (6.4, 7, 8, 9, 9.6) and NaCl concentration (0, .02, .05, .08, .10 M).	- Temperature had the greatest effect on albumen texture. No measureable gel formed at 69.5°C regardless of pH or NaCl concentration. Gel rigidity increased with temperature up to 80°C after which viscosity, strength and elasticity decreased. - pH had a minimal effect on gelling properties. Weaker gels were formed at acidic pH. - NaCl concentration produced only minor increase in gel strength and elasticity.
Ma et al., 1986	Egg white solids (10% w/v)	Heated in boiling water bath (100°C) for 5 min.; and removed by centrifugation and temperature of denaturation (Td). cooled. DSC (heating rate = 10°/min.)	Amount of protein aggregated and sucrose.	Effect of pH (5.8, 7.2) and sucrose.	- An opaque solid gel formed at pH 5.8 (94-95% protein coagulated). - An opaque gel with a weaker structure formed at pH 7.2 (95% protein coagulated). - DSC - endothermic peaks apparent at 65°C and 85°C (denaturation of ovalbumin). - Addition of 50% sucrose increased Tds. (denaturation of conalbumin)

was the main heat-denaturable protein responsible for the setting of angel food cakes.

The coagulability of egg albumen is affected by many factors such as pH, time and temperature of heating, presence of NaCl and/or other salts, protein concentration and the addition of sucrose (Donovan et al., 1975; Shimada and Matsushita, 1980; Matsuda et al., 1981; Holt et al., 1984; Watanabe et al., 1985; Baldwin, 1986; Ma et al., 1986; Woodward and Cotterill, 1986). The effect of each factor on gelation generally seems to depend upon the other conditions present in the system. Woodward and Cotterill (1986) investigated the effects of pH, protein concentration, and NaCl level at various temperatures and times of heating, on egg white gels. In general, gel strength increased as temperature, time of heating, pH and protein concentration increased, and decreased with the addition of salt. Holt et al. (1984) found that gel strength increased with increasing temperatures up until 80°C but then decreased when pH was less than 8. Unlike the findings of Woodward and Cotterill (1986), increasing NaCl concentration only slightly affected gel strength (a minor increase with increasing NaCl). However, the highest NaCl concentration used in this study was 0.1 M versus 1.0 M used in the Woodward and Cotterill study. It is apparent that the experimental setting in which egg albumen coagulability was evaluated determined the ultimate results obtained.

c. Comparison of Pea Protein and Egg Albumen Coagulation/Gelation Properties

The comparison of pea protein and egg albumen coagulation/gelation properties is especially difficult due not only to the variability of methods, but also due to the limited research on pea proteins and detailed evaluation of egg albumen coagulability. However, the literature does suggest the capability of pea proteins to form gels provided temperatures are high and long enough to denature the proteins.

The most obvious difference between the pea protein and egg albumen systems was the temperature of denaturation associated with the major proteins in each system. Denaturation temperatures for conalbumin and ovalbumin have been reported to be 60-65°C and 80-85°C respectively, while pea proteins were found to denature at 89-95°C, depending upon the isolation procedure. Only one endotherm was apparent for the pea protein isolates suggesting that the denaturation temperatures of the constituent proteins were in close proximity to one another (Murray et al., 1985). It appears, however, that the globulins, not the albumins, are responsible for pea protein gelation. The higher denaturation temperatures for pea proteins were reflected in the slightly higher temperatures required for gelation. A temperature of 90°C was used in all pea protein gelation studies.

The higher temperatures required to denature pea proteins may ultimately influence sponge cake structure if a

significant amount of egg albumen is replaced by PPC.   If temperatures are not high enough or long enough to denature the pea proteins, there may be insufficient heat coagulating protein to support the sponge cake structure.   Bell et al. (1975) reported that setting of the sponge cake batter occurred in the temperature range of 85 to 96°C, while Mizukoshi et al. (1980) reported end batter temperatures of 90 to 98°C.   Final cake batter temperature will ultimately depend upon the constituents of the batter, oven temperature and length of baking time.   In particular, the presence of acids,   salts   and   sugar   may   influence   the   coagulation properties of the proteins.

Although   the   mechanisms   of   gelation   are   not   well understood, several models have been proposed which identify two major steps (Shimada and Matsushita, 1980; Cheftel et al., 1985;   Ma   and   Holme,   1982).   The   first   step   involves   heat denaturation of the native protein, that is, breakage of hydrogen bonds, formation of disulfide links, and dissociation or unfolding of the polypeptide chains to expose hydrophobic groups.   The second step involves the interaction of denatured proteins   to   form   aggregates   which   may   participate   in   the formation of a three dimensional network.   Ma and Holme (1982) found   that   formation   of   the   coagulum   involved   hydrophobic interactions   between   the   heat   denatured   proteins   which decreased the number of exposed hydrophobic groups.   It was also noted that gelling required a balanced electrostatic

attraction between protein molecules, and that sulfhydryl and disulfide interchange may be of importance.

Molecular differences between pea and egg albumen proteins will, in part, be responsible for some of the observed differences in coagulating/gelling properties. Pea legumin and vicilin are much larger and more compact than the heat coagulating egg albumen proteins ovalbumin and conalbumin. Proteins which are structurally compact and contain several disulfide bonds (such as legumin) generally show high resistance to denaturation (Johnson and Zabik, 1981c). Intermolecular bonds such as hydrogen, hydrophobic and covalent bonds stabilize the protein structure and therefore determine the stability towards denaturation (Wright, 1983). Differences in the proportions of these bonds would influence protein denaturation and subsequent coagulation/gelation properties. The higher denaturation temperatures observed for the pea proteins may, therefore, have resulted from structural and compositional differences between the major heat coagulating proteins in each system.

A variety of methods and testing conditions have made the comparison of pea protein and egg albumen coagulating properties difficult. There is the need for a direct comparison under identical conditions in order to draw more reliable conclusions. More importantly, because environmental conditions such as pH, time and temperature of heating, and the addition of sucrose have been found to strongly influence

the coagulating properties of egg albumen, there is a need to evaluate these effects on the gelling properties of pea protein. Results from these studies may provide a better idea of how pea protein may function in a complex food system.

Summary of the Comparison of Pea Protein and Egg Albumen Molecular and Functional Properties

The successful substitution of PPC for egg albumen in a sponge cake system will ultimately depend on the similarity between the two protein systems. The comparisons of structure, amino acid composition, and functional properties highlighted some of the similarities and differences between pea and egg albumen proteins.

As expected, differences were found to exist between the animal based egg albumen proteins and the plant based pea proteins. Egg albumen proteins were smaller, less ordered, more highly glycosylated than pea proteins. Egg albumen was also a more diverse system of proteins with a wider range of isoelectric points. The differences found at the molecular level were a good indication that functionality characteristics would also differ. Functionality tests showed that in general, egg albumen proteins were more soluble, exhibited moderately higher foam capacity, much greater foam stability, and denatured at lower temperatures than pea proteins. Pea proteins were soluble away from their isoelectric pH range (4-6) and exhibited the ability to foam and coagulate under certain testing conditions. Therefore,

from a theoretical perspective, it appears feasible that pea proteins may be able to partially replace egg albumen in a sponge cake formulation.

Schoen (1977) cautioned that the results from simple functionality tests may not be indicative of the performance of the protein in a more complex food system. The observed effects of several environmental factors (pH, ionic strength, temperature, sucrose, etc.) on protein functionality supports this conclusion. Consequently, the best indication of protein functionality and potential use in a food product is to consider the effect of the protein in a more complex food system.

Evaluation of Pea Protein Use and Egg Albumen Replacement in Baked Products

The feasibility of partial egg albumen replacement with PPC in a sponge cake can be evaluated from a more practical perspective based on two rather limited areas of research. Firstly, the evaluation of pea protein use in baked products should highlight some of the effects it has had on product quality. Secondly, evaluation of partial egg albumen replacement with an alternate protein source, in a cake system, would indicate the feasibility of replacing this highly functional cake component.

Applications of Pea Protein in Baked Products

Much of the research to date has focused upon the use of

pea protein products to improve the nutritional quality of baked goods, in particular, bread. Pea proteins are high in lysine and therefore, are nutritionally complementary to wheat proteins (Kreutler, 1980). Consequently, several researchers have attempted to fortify various baked products by replacing some of the wheat flour with pea flour (Jeffers et al., 1978; Repetsky and Klein, 1981; McWatters, 1978; Raidl and Klein, 1983) or more highly concentrated pea fractions (Fleming and Sosulski, 1977; Hsu et al., 1982). The effects on product quality resulting from the partial replacement of wheat flour with pea protein are summarized in Table 1.10. The volume depressing effects and flavor problems associated with pea protein substitution are most obvious from these studies. Some deleterious effects on texture are also apparent. Nevertheless, acceptable products were produced with 10 to 15 percent of the wheat flour replaced with pea flour or pea protein concentrate. In some cases, procedural or formulation changes were recommended in order to produce acceptable products.

These studies highlight the importance of sensory evaluation to the development of acceptable food products, particularly where flavor may be of concern. Repetsky and Klein (1981) found that physical measurements did not result in significant differences between breads made with varying levels of pea flour, while sensory measurements indicated that the texture, color and flavor of the high pea flour breads

Table 1.10 Summary of Pea Protein Use as a Wheat Flour Replacement in Baked Products

Reference	Treatment	Results
Jeffers et al., 1978	Raw pea and cooked pea flour substituted for 5, 10, 15 and 20% of wheat flour in bread.	- Loaf volume, crumb grain and texture generally decreased with increasing PF level. - Crumb and crust color darkened as PF level increased. - Panelists were unable to distinguish between samples at a statistically significant level. - Breads of near standard quality were produced with 15% PF.
McWatters, 1978	Pea flour substituted for 10, 20 and 30% of wheat flour in sugar cookies.	- Physical and baking performance of the dough was similar to the control. - Texture score decreased slightly with increasing PF level. - Undesirable "beany" flavor detected in cookies containing high levels of PF.
Fleming and Sosulski, 1977	Air classified pea protein concentrate substituted for 5, 10 and 15% of wheat flour in bread.	- Loaf volume, texture, crumb grain, color and crust scores decreased with increasing PPC. - 15% PPC breads rated lower in aroma than the 100% whole wheat bread. - 15% PPC substitution produced acceptable breads if 2% vital wheat gluten and dough conditioners were added.
Repetsky and Klein, 1981	Pea flour substituted for 2.5, 5 and 10% of wheat flour in bread.	- Loaf volume, shape, crust, grain and aroma were not significantly affected by PF substitution. - Texture, color and flavor were adversely affected with increasing PF. - 10% replacement produced breads with a yellow off-color and "beany" off-flavor which were tough and dry. These breads were, however, considered acceptable. - 2.5% breads scored similarly or better than the control.
Hsu et al., 1982	Germinated and ungerminated pea protein isolates substituted for 3, 5 and 8% of wheat flour in bread.	- Loaf volume decreased, crusts were pale and unsatisfactory crumb grain resulted with 8% replacement. - 5% and 8% PPI replacement had deleterious effects on bread quality. - Germination did not affect baking quality of PPI.
Raidl and Klein, 1983	Pea flour substituted for 5, 10 and 15% of wheat flour in a quick bread loaf.	- Loaf volume decreased (not significantly), crust color darkened, crumb yellowed and the number of air cells decreased as PF level increased. - PF substitution had no effect on crumb, crust and surface texture, loaf shape, crust thickness, tunnels and mouthfeel. - 10% and 15 loaves were found to have "nutty" or "pea" odors and a stronger aftertaste than the 5% PF or control loaves.

were not very acceptable. Similarly, McWatters (1978) found that the baking performance and physical characteristics of sugar cookies were not significantly altered by the substitution of 30 percent pea flour for wheat flour, yet sensory evaluation indicated that undesirable beany flavors were present at this level of replacement. Ultimately, consumer acceptability will depend upon the appearance, texture and flavor of the product, therefore the use of both physical and sensory evaluation in the assessment of a product, is critical.

The studies presented in Table 1.10 have all dealt with the partial replacement of wheat flour with pea protein flour, or concentrates, for the purpose of fortification. While the potential problems associated with pea protein substitution, such as loss of volume and presence of off-flavors, were highlighted, the relevance of these results to the functional replacement of egg albumen with pea protein (the objective of the proposed research) is somewhat questionable. McWatters (1980), on the other hand, evaluated pea protein flour and concentrate as functional replacements for milk proteins in baking powder biscuits. Pea flour, pea protein concentrate and water were adjusted to simulate the protein and moisture content of milk. Pea protein biscuits were lighter and less yellow in crust color, lost less weight during baking, had higher volumes and more uniform contour than the reference biscuits. Sensory evaluation of the biscuits indicated that

substitution of the pea products adversely affected the aroma and flavor qualities of the biscuits. Common descriptors included "harsh", "beany", and "strong". Appearance, color and texture were not so strongly influenced, but scores were lower than those for the control biscuit. Biscuits were described as being "dull" and "less brown" with a "doughy" and "slightly heavy" texture. Longer baking times were recommended to improve the color and texture of the pea biscuits. The authors concluded that aroma and flavor would have to be improved or alterations made to the formulation or baking procedure, to make the biscuits acceptable. Once again, the addition of pea protein resulted in flavor and textural problems.

Overall, the literature indicated the potential for low levels of pea protein use in baked products. While pea proteins were never substituted for a complex protein system such as egg albumen, or evaluated in a cake system, their substitution for wheat and milk proteins provided some general indication of their functionality in baked products. Unfortunately, research on the use of pea protein as a functional replacement for alternative proteins in baked products is lacking. Such research would provide a better indication of the functional potential of pea protein.

Although the addition of pea protein adversely affected some of the physical and sensory characteristics of the products, there were never any attempts made to optimize

procedures and/or ingredient levels which could have improved pea protein functionality. The importance of environmental conditions to protein functionality have already been demonstrated and they must be considered if a more realistic evaluation of functionality is to be made.

Use of Bovine Plasma Protein for Egg Albumen Replacement in Cakes

Like pea protein, livestock blood is an under-utilized source of protein (Johnson et al., 1979). Bovine plasma exhibits functional properties similar to egg albumen therefore, the feasibility of its use as a replacement for egg albumen in cakes has been assessed (Johnson et al., 1979; Khan et al., 1979).

Johnson et al. (1979) evaluated the substitution of plasma protein concentrates at equivalent protein levels for egg white in a high-ratio white layer cake. Cakes made with the plasma protein were comparable in volume to, and shrank less than, the control cakes. Plasma cakes had good structure and crumb texture. In a subsequent study, the functionality of plasma protein was improved by optimizing water levels and mixing times. Bovine plasma protein could be used successfully to replace egg white solids in white layer cakes.

Khan et al. (1979) evaluated the potential of plasma protein isolate (PPI) as a replacement for dried egg white solids in angel food cake, and as a replacement for wheat flour in bread. While the foaming capacity of PPI was similar

to egg white solids, in combination with the other cake ingredients, results were very different. A 50 percent replacement of egg white solids with PPI produced cakes with significantly lower volumes and coarser crumb structures than the 100 percent egg white cakes. Acceptable cakes resulted from 25 percent substitution, although volumes were slightly lower and crumbs coarser than the control. The partial replacement of wheat flour with PPI (0-10%) in bread produced results similar to those observed for pea protein replacement of wheat flour in bread. Substitution levels of 8 to 10 percent produced loaves with reduced volumes, darker crumb and crust colors, and a more open texture. This is most interesting since the protein was successfully used to replace 25 percent of the egg white in a very sensitive angel food cake. It is possible, therefore, that pea protein will exhibit better functionality in a sponge cake system than was indicated in a bread system.

These two studies, in addition to having illustrated the potential for egg albumen replacement with an alternative protein in a cake system, have brought up two important points. Firstly, although a protein may illustrate very similar functional properties when tested in isolation, the results may differ significantly when tested in combination with other ingredients. Secondly, it may be important to optimize the cake formulation and mixing procedures in order to improve the functionality of a particular protein system.

Summary of the Evaluation of Pea Protein Use and Egg Albumen
Replacement in Baked Products

The literature has demonstrated the potential for partial egg albumen replacement with PPC in a sponge cake. Pea proteins (flours, concentrates and isolates) successfully replaced 10 to 15 percent of the wheat flour in a variety of baked products, and functioned as a milk replacement in biscuits. Although higher replacement levels of pea protein produced adverse changes in the physical and sensory characteristics of the products, it is possible that formulation and procedural changes could reduce the negative effects. Egg albumen was successfully replaced with bovine plasma protein to produce acceptable layer and angel cakes. This result suggests that egg albumen is not indispensable and may be successfully replaced in a cake system. Overall, pea proteins functioned in baked products, and part of the egg albumen was successfully replaced by an alternate protein source in a cake system. Consequently, the potential exists for the partial replacement of egg albumen with PPC in a sponge cake system.

Sensory Evaluation of Sponge Cake

Sensory evaluation of food quality is an essential component of food experimentation since no one instrument, as of yet, has been capable of replicating or replacing the responses of humans (Campbell et al., 1979; Watts et al., 1989). The development of a new food product requires that

certain quality attributes be identified and measured using a trained sensory panel (Watts et al., 1989). Such panels usually consist of 5-15 panelists who are specially trained to identify differences between food products, or to measure the intensities of selected quality characteristics such as flavor, texture and appearance. Trained panels should not be used to evaluate product acceptability due to their increased sensitivity to the product.

Quantitative descriptive analysis is one method of sensory evaluation which is particularly suited to product development (Stone et al., 1974). Panel training typically involves identification of sensory attributes which describe the appearance, flavor and texture of the product, followed by the development of appropriate terminology to describe these attributes. The scale used to rate the intensity of each attribute is a 15 centimetre line scale with anchor points 1.5 centimetres from each end. Descriptors determined during panel training are used to anchor each end point. Panelists rate the intensity of the attribute by making a mark on the line at the point which best describes their perceived intensity of the attribute.

Little sensory evaluation has been completed on sponge cakes. Descriptive analysis (7 point numerical scale with descriptive terms) was used by Zabik et al. (1969) to evaluate the texture, tenderness, moistness, color and flavor of sponge cakes. Deethardt et al. (1965a, 1965b) used 5 point scales

to evaluate sponge cake moisture, texture, tenderness, color intensity, flavor and acceptability. Scoring has also been used to evaluate sponge cake quality. Miller et al. (1946) scored crumb color, texture, tenderness, moistness and flavor. Uchida (1982) suggested that scoring of sponge cake quality should include evaluations of external characteristics such as shape, crust color and crust appearance, and internal characteristics such as cell uniformity, cell size, thickness of cell walls, grain, texture (moisture, tenderness, and softness), crumb color, and flavor. Pierce and Walker (1987) scored (excellent, good, fair, poor) the overall crumb color, shape, and crumb texture (cell size, evenness and density) of sponge cakes.

According to McWilliams (1979), a good sponge cake has fairly fine, uniform cells and thin cell walls. The cake should be of high volume and tender with a pleasing golden brown exterior. Consequently, the cake characteristics which may be important to evaluate sensorily would be those associated with a high quality sponge cake. These would include evaluations of volume, texture, grain and color. Reports of sponge cake sensory evaluation seem to have evaluated the important cake quality characteristics. Because the incorporation of pea protein into various baked products resulted in altered texture, grain and flavor, particular attention should be paid to these characteristics.

Physical/Instrumental Evaluation of Sponge Cake

Instrumental methods of evaluation are said to be more precise and less subject to error than sensory evaluation because they do not depend upon human judgment (Funk et al., 1969; Campbell et al., 1979). The validity of instrumental tests is, however, dependent upon their agreement with the results from sensory evaluation.

Physical measurements which have commonly been used to evaluate sponge cakes include batter specific gravity, cake volume and texture. Specific gravity can be used to indicate the amount of air incorporated into the cake batter (Funk et al., 1969) and is measured by dividing the weight of the cake batter by the weight of an equal volume of water (Campbell et al., 1979). Low specific gravity values, indicating good air incorporation and retention, have been associated with good cake volume and other desirable cake characteristics (Funk et al., 1969; Dunn and White, 1939).

Cake volume is an important physical measurement because it relates to the underlying structural development of the cake (Cloke et al., 1984). Pierce and Walker (1987) noted that while high volume does not necessarily indicate a desirable cake, a cake of low volume is usually of low quality. Volume measurements of sponge cakes were most often determined by rapeseed displacement (Jordan and Pettijohn, 1946; Miller et al., 1946; Uchida, 1982; Mizukoshi, 1985; Sato et al., 1987; Pierce and Walker, 1987). Specific volume can

be calculated by dividing the volume of the cake by its weight (cc/g) and allows comparison of products having different weights (Campbell et al., 1979).

Sponge cake texture measurements have included evaluations of compressibility and tensile strength (Platt and Kratz, 1933; King et al., 1936; Jordan and Pettijohn, 1946; Miller et al., 1946; Deethardt et al., 1965a; Zabik et al., 1969), as well as elasticity (Platt and Kratz, 1933; King et al., 1936; Deethardt et al., 1965a), firmness (Sato et al., 1987; Pierce and Walker, 1987) cohesiveness (Sato et al., 1987) and crumb fragility (Pierce and Walker, 1987). Measurements were made using a variety of instruments such as the Allo-Kramer Shear Press, penetrometer, tensile strength apparatus and the Instron Universal Testing Machine.

The Instron has been widely used as a method of measuring the textural properties of food but has rarely been used to evaluate sponge cake texture. It is a flexible, multipurpose instrument which can be used with a variety of test cells, plunger sizes, cross-head speeds and compression depths. This has resulted in unstandardized, inconsistent conditions of texture measurement (Breene, 1975). Varying Instron test conditions (cross-head speed, plunger area, and degree of compression) have been shown to influence bread firmness and cake firmness measurements (Boyd and Sherman, 1975; Hibberd and Parker, 1985; Redlinger et al., 1985; Baker et al., 1986; Walker et al., 1987). There is, however, a need to determine

the effects of varying Instron testing conditions on other textural parameters such as cohesiveness, gumminess and springiness. As well, there is a need to determine the Instron testing conditions which will most effectively detect differences in sponge cake texture. Limited research has been done to determine the Instron testing conditions most suitable for the detection of differences in firmness among white breads and cakes (Walker et al., 1987; Baker and Ponte, 1987; Baker et al., 1988). Selection of appropriate testing procedures for the textural evaluation of baked products should be investigated further.

Response Surface Methodology

Response surface methodology (RSM) is an experimental technique which is particularly suitable for product development because it helps to establish optimal ingredient levels and processing conditions in a minimal number of experimental runs (Johnson and Zabik, 1981a). Because the number of experimental formulations is minimized, the costs associated with product development are also minimized (Mullen and Ennis, 1985). Response surface methodology helps to determine how the independent variables singly and in combination, influence the dependent variables (Giovanni, 1983). In addition, interactions between the independent variables can be evaluated, unlike the one-variable-at-a-time approach.

Response surface designs (see Box and Hunter, 1957; Box and Draper, 1987) specify the experimental runs, and the independent variable levels for each run (Thompson, 1982). The experimental data are then collected and used to produce regression equations or models which define relationships between independent variables and responses, and which may be used to predict the effects of variable combinations which were not tested (Mullen and Ennis, 1979; Thompson, 1982; Henika, 1982). Because many model equations are very complex, two dimensional contour plots and three dimensional response surface plots can be generated which help visualize the effects on the responses, of varying independent variable levels (Thompson, 1982). Superimposing of the two dimensional contour plots allows for the simultaneous optimization of several responses (Floros and Chinnan, 1988).

Joglekar and May (1987) have described two typical stages of product development and optimization using RSM. The first, a screening stage, is more concerned with selecting those variables having an important effect on the response variables. At this stage, the identification of key variables is more important than investigating their relationships and interactions (Mullen and Ennis, 1985). The second stage is optimization, the determination of optimal levels of the key variables. It is at this stage that the relationships of the variables and their interactions, with the responses are evaluated thoroughly (Mullen and Ennis, 1985).

Response surface methodology has been successfully used to optimize several cake formulations (Kissell and Marshall, 1962; Kissell, 1967; Johnson and Zabik, 1981a; Lee and Hoseney, 1982; Neville and Setser, 1986; Vaisey-Genser et al., 1987), but has never been used to optimize a sponge cake formulation. In addition, no studies have been found reporting the use of RSM to optimize the use of pea protein in baked products, or the replacement of egg albumen with alternative protein sources in cakes.

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CHAPTER 2

Selection of Instron Testing Conditions for the Detection of Textural Differences in Sponge Cakes

INTRODUCTION

Sponge cake quality is highly dependent upon egg quality making it an ideal system for studying the effects of egg albumen replacement with a field pea protein concentrate (PPC). PPC is a relatively inexpensive protein source which, unlike the fibre and starch fractions, is currently under-utilized (M. Lamb, personal communication, 1987). The development of an acceptable food product incorporating a substantial amount of PPC would aid in establishing a balance in the demand for pea fibre, starch and protein.

Texture, a very important component of cake quality, is expected to be affected by the substitution of PPC for albumen in the sponge cake formulation. The degree to which the texture will be affected, however, is unknown. It is important therefore, to have an objective method of texture measurement which is sensitive to textural differences among cake formulations.

The Instron Universal Testing Machine has been widely used as a method of to measure the textural properties of food due to its convenience, accuracy and flexibility (Finney, 1969). Coupled with a computer, the Instron has proven to be a very efficient method of texture measurement (Buckley et al., 1984). The Instron is a flexible instrument which can

be used with a variety of test cells, plunger sizes, cross-head speeds and compression depths. This has resulted in unstandardized, inconsistent conditions of texture measurement (Breene, 1975). Table 2.1, a compilation of Instron conditions used for texture measurement of baked products, indicates this variability.

Increasing use of the Instron to evaluate food texture has led many researchers to attempt standardization of controllable testing conditions thought to affect texture measurements (Redlinger et al., 1985; Baker et al., 1986a, 1986b, 1988; Walker et al., 1987). The effect of cross-head speed on measurements of bread and cake crumb firmness has been investigated (Boyd and Sherman, 1975; Hibberd and Parker, 1985). As well, the importance of plunger area and of degree of sample compression to bread firmness measurements (Redlinger et al., 1985; Baker et al., 1986a) and cake firmness measurements (Walker et al., 1987) have been shown. The effects of varying Instron conditions on textural parameters other than the firmness of baked products, have not been reported in the literature.

Limited research has been done to determine the Instron testing conditions most suitable for the detection of differences in firmness among white breads and cakes (Walker et al., 1987; Baker and Ponte, 1987; Baker et al., 1988). Once again, no research has considered the Instron testing conditions which will most effectively detect differences in

Table 2.1   Conditions Used for the Texture Measurement of Baked Products
Using the Instron Universal Testing Machine

Product	Sample Size	Plunger Size	Compression (%)	Cross-lead Speed (cm/min.)	Reference
white bread	1 slice 3 cm thick	32 mm dia.	33	5	4
angel food cake	2.5 cm cubes	50 cm² area	40	20	2
rye and french breads	2.5 cm disk 2.5 cm thick	35 mm dia.	80	2	1
Danish tin bread and white bread	11 mm thick slices	25 mm dia.	25	20	3
layer cakes and fermented cakes	3x3x2 cm thick slice	35 mm dia.	10	20	5

1. Brady and Mayer, 1985.
2. Coleman and Harbers, 1983.
3. Joensson and Toernaes, 1987.
4. Neukom and Rutz, 1981.
5. Perez and Juliano, 1988.

other textural parameters among cakes or other baked products. Therefore, this study had the following objectives:

1. To determine the effect of plunger area, degree of sample compression and cross-head speed on sponge cake hardness, cohesiveness, gumminess and springiness measurements.

2. To determine the operating conditions best able to detect differences between sponge cake formulations for each of the four textural parameters.

3. To identify the operating conditions most appropriate for the simultaneous evaluation of the four textural parameters.

MATERIALS AND METHODS

Two sponge cake formulations were baked from standardized recipes, one with 100 percent albumen and the other with PPC replacing 30 percent of the albumen (Appendix 2.A). The 30 percent PPC formulation represented the design centre point for a preliminary response surface experiment. It was predicted to be substantially different from the 100 percent albumen cake thus facilitating the objective of selecting Instron conditions best able to detect textural differences between cakes.

Experimental Design

A 3X3X3X2 factorial design, replicated four times, was used to evaluate the effects of plunger area, cross-head speed, percent sample compression and percent PPC in the formulation (Table 2.2). Thus 108 samples of each sponge cake formulation were required.

Four replications of each plunger size/sample compression/cross-head speed combination were completed over a three day period. Two replications of one plunger size and nine cross-head speed/percent sample compression combinations were completed for both formulations in each half day period. That is, 36 compression tests using one plunger size, were completed per half day. One and one half days were required to evaluate all Instron testing condition combinations twice. Another one and one half days were required for the additional

Table 2.2 Factors Included in the 3x3x3x2 Factorial Design

Factors	Levels
Plunger area (mm^2)	314.0 616.0 1018.0
Cross-head speed (cm/min.)	5.1 10.0 20.0
Degree of compression[1] (%)	25.0 50.0 75.0
Level of PPC[2] in Sponge cake (% replacement of albumen)	0.0 30.0

[1]Samples sliced to 2 cm high.
[2]Pea protein concentrate.

two replications. Plunger size was varied only twice a day due to the stress on the load cell associated with changing the plungers as well as the need to recalibrate with each plunger change. Cake samples, plunger size, speed/compression combinations and formulations were all randomized within each test period. Appendix 2.B contains an example of the randomization within one test period.

Sponge Cake and Sample Preparation

Each formulation yielded three, 15 centimetre round sponge cakes. Eighteen batches were prepared over a two day period, resulting in a total of 27, 0 percent PPC cakes and 27, 30 percent PPC cakes. Batches were randomized for each bake day. Cakes were sealed in polybags and were frozen (-20°C) and stored overnight.

The following day, cakes were removed one at a time (to prevent thawing) for rapid sample preparation. Cake tops were sliced off using a two centimetre high, 15 centimetre square, plexiglass box as a guide. Each cake was then quartered, yielding four, two centimetre high crustless wedges which were immediately re-sealed, randomized, and place back in the freezer until texture testing. This sequence was repeated for the 30 percent PPC cakes resulting in 108 subsamples of both sponge cake formulations (27X4=108). A sample height of two centimetres was chosen after preliminary baking indicated that heights greater than this would not accommodate all cakes

baked in the future optimization studies. Texture evaluations were completed approximately two weeks after sample preparation. Cake samples were thawed in their sealed bags for at least one and one half hours prior to testing.

Instron Testing Procedure

The Instron (Table Model TM) and a Texture Profile data acquisition and analysis program developed for the Apple IIE computer (Agriculture Canada, 1986) were used to evaluate four textural parameters; hardness, cohesiveness, gumminess and springiness. Figure 2.1 represents a typical force-time (also force-distance) curve for the sponge cakes and illustrates how each of the textural parameters was evaluated (Bourne, 1978).

For each Texture Profile (two plunger cycles), the plunger was positioned just above the center of the cake wedge and was then lowered at constant speed until the sample was compressed to the predetermined level.

Statistical Analyses

Analysis of variance was used to determine the significant effects of Instron conditions on texture measurements (SAS, 1985). The t-values were computed (Figure 2.2), and their magnitudes compared, to identify the most discriminating set of conditions for individual parameters. Selection of testing conditions appropriate for evaluation of all four parameters was based on the relative importance of
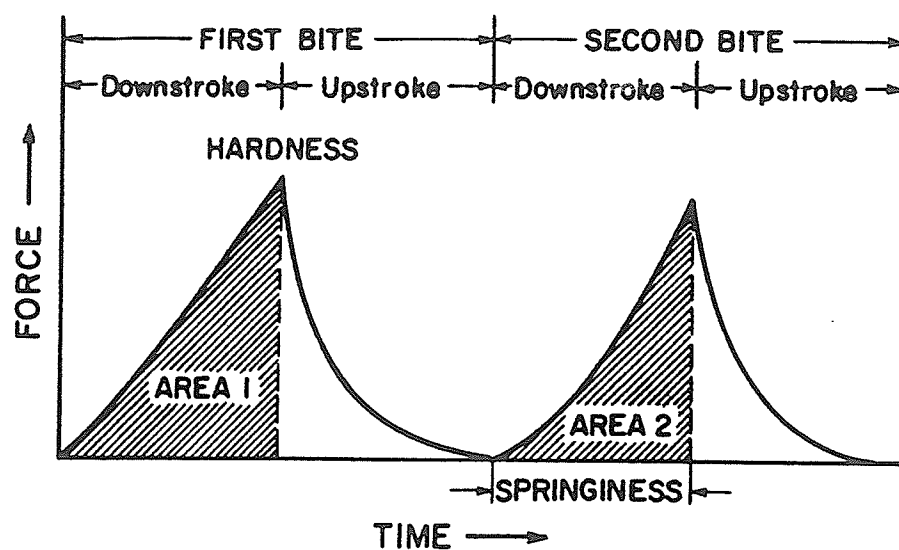
Figure 2.1 Typical force-time (Texture Profile) curve for sponge cake. Cohesiveness=Area 2/Area 1; Gumminess=hardness X cohesiveness.

Adapted from Bourne et al., 1978

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{S_p^2 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

where :

$\overline{X}_1$ = mean of 30% PPC cakes

$\overline{X}_2$ = mean of 0 % PPC cakes

S_p^2 = pooled variance

Figure 2.2 Calculation of t-values.

each textural parameter to cake quality, on the size of the coefficient of variation (CV) and coefficient of determination (R^2) associated with their corresponding analysis of variance, and on the magnitude of the t-values for all parameters.

RESULTS AND DISCUSSION

Prior to the determination of the Instron testing conditions best able to detect differences in sponge cake texture, the effects of plunger size, degree of sample compression, and cross head speed on texture measurements, were considered. The relationships between the testing conditions and hardness, cohesiveness, gumminess and springiness values helped to explain why some Instron conditions resulted in better discrimination of certain parameters. The information derived from this study was used to select one plunger size, degree of compression and cross-head speed for discrimination of all four textural parameters.

Effects of Instron Testing Conditions on Texture Values

The first objective of this study was to determine the effect of plunger area, degree of sample compression and cross-head speed on sponge cake hardness, cohesiveness, gumminess and springiness measurements. Table 2.3 summarizes the significant F-values from the analysis of variance for each parameter and indicates the importance of plunger area, degree of compression and cross-head speed to each Instron condition. Table 2.4 presents the corresponding mean comparisons for each Instron condition and treatment. This table illustrates the differences in mean hardness, cohesiveness, gumminess and springiness values resulting from varying plunger areas, degrees of compression and cross-head

Table 2.3 Significant F-values[1], Coefficients of Determination (R^2) and Variation (CV) from the Analysis of Variance for Four Textural Parameters

Source	df	Textural Parameter			
		Hardness (N)	Cohesiveness	Gumminess (N)	Springiness (mm)
Treatment	1	16.70 (.0001)[2]	15.43 (.0001)	11.77 (.0008)	23.87 (.0001)
Plunger area	2	750.07 (.0001)	5.38 (.0055)	960.09 (.0001)	-
Compression	2	3,165.68 (.0001)	1,097.31 (.0001)	3,124.65 (.0001)	5,451.51 (.0001)
Speed	2	25.66 (.0001)	-	28.81 (.0001)	43.73 (.0001)
Tmt * Compr	4	5.33 (.0057)	11.13 (.0001)	-	-
Tmt * Spd	4	3.78 (.0250)	-	3.90 (.0222)	-
Area * Compr	4	285.79 (.0001)	11.76 (.0001)	334.08 (.0001)	-
Area * Spd	4	4.90 (.0009)	-	5.28 (.0005)	2.60 (.0384)
Compr * Spd	4	11.81 (.0001)	-	11.62 (.0001)	-
Tmt * Compr * Spd	8	-	-	2.47 (.0468)	-
Area * Compr * Spd	8	4.26 (.0001)	4.26 (.0001)	4.06 (.0002)	-
Total Observations		216	216	216	214
R^2		.98	.94	.99	.99
CV (%)		14.0	1.9	12.5	4.4

[1]Associated with partial (Type III) sums of squares.
[2]Probability associated with F-values.

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Table 2.4 Means[1] for the Textural Measurements of Sponge Cakes Made with 0 and 30% Pea Protein Concentrate

Textural Parameter	Treatment[2] (% PPC)		Instron Condition[3]								
			Plunger Area (mm^2)			Degree of Compression (%)			Cross-head Speed (cm/min.)		
	0	30	1018	616	314	75	50	25	20	10	5.1
Hardness (N)	7.43a	8.03b	11.27a	7.66b	4.24c	15.89a	4.87b	2.43c	8.45a	7.55b	7.19b
Cohesiveness	.728a	.721b	.728a	.725ab	.721b	.668a	.731b	.774c	.724	.726	.724
Gumminess (N)	5.21a	5.52b	7.83a	5.34b	2.92c	10.65a	3.55b	1.88c	5.83a	5.26b	5.00c
Springiness (mm)	21.32a	20.71b	21.23a	20.90b	20.92ab	28.90a	21.49b	12.66c	21.78a	20.93b	20.34c

[1]Duncan's Multiple Range Test. Means having different letters within the same row for treatment or Instron condition differ significantly (p≤.05).
[2]Treatment means based on 108 observations.
[3]Instron condition means based on 72 observations.

effects of each Instron condition on texture values are presented in turn.

Effect of Degree of Sample Compression on Instron Texture Values

Compression had by far the most significant effect on all four textural parameters as is indicated by the very large F-values associated with this Instron condition (Table 2.3). The means presented in Table 2.4 indicate that the higher the degree of compression, the higher the values for hardness, gumminess and springiness measurements, while cohesiveness scores decreased with increasing compression. Similar findings were reported when the effect of compression depth on bread firmness measurements was investigated (Redlinger et al., 1985; Baker et al., 1988). Observations recorded by Baker et al. (1988) and Walker et al. (1987) offer a plausible explanation for the increase in hardness force values with increased compression. The total force value may include compression, tension and shearing forces, the relative importance of each depending upon the degree of sample compression (Figure 2.3). Levels of compression chosen for this study were 25, 50 and 75 percent of a two centimetre high cake sample. At a level of 25 percent compression, deformation of the sample was visible but shearing of the crumb was not evident. Hardness values recorded could be attributed to compression forces beneath the plunger, plus
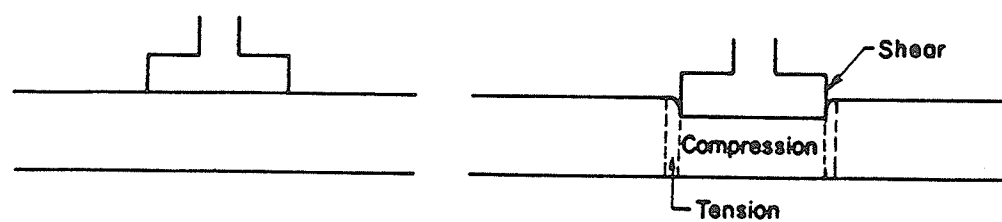
Figure 2.3   Illustration of forces present during cake compression.

some tension forces at the edges of the plunger. At 50 percent compression, there was evidence of some shearing, so that the force required to shear would contribute, with compression and tension forces, to the overall hardness measurement. At the final compression setting of 75 percent, shearing of the sample was clearly evident and packing of the cake crumb resulted in a denser crumb, more resistant to the applied pressure. Higher hardness values would result from increased shear forces and increased resistance of the sample to compression.

While the effect of compression level on hardness values has been well documented in previous studies, the effect of degree of compression on other Texture Profile parameters has not been investigated. Consequently, explanations for the effects of degree of compression on cohesiveness, gumminess and springiness measurements have not been developed. Cake cohesiveness values (ratio of Area 2 to Area 1; Figure 2.1) were found to decrease with increasing levels of compression (Table 2.4). This textural parameter has been defined as "the strength of the internal bonds making up the body of the product" (Szczesniak, 1963). It appears that as the level of compression increased and the cake crumb became more compact, the internal bonds were breaking resulting in lower recorded values for this parameter. Clearly, the higher the level of compression, the greater the effect on the internal structure of the cake and subsequent cohesiveness values, and the

greater likelihood of permanent deformation. Permanent deformation would produce lower Area 2/Area 1 ratios and therefore, lower cohesiveness values (as cohesiveness has been defined).

Cake gumminess values increased with increasing compression (Table 2.4). This positive relationship is not surprising since gumminess is calculated as the product of hardness and cohesiveness (Szczesniak, 1963). The magnitude of the hardness values in comparison to the cohesiveness values resulted in hardness having much greater influence on gumminess values. Effect of degree of compression on gumminess values were, therefore, similar to its effect on hardness.

Table 2.4 indicates that cake springiness measurements also increased with increasing levels of compression. Springiness has been defined by Bourne (1978) as "the distance the food recovers its height between the end of the first rise in force above zero in the second bite, and the point of maximum compression", but is better illustrated in Figure 2.1. The key to understanding the observed effect of compression on springiness measurements lies in the phrase, "point of maximum compression." It takes more time to reach 75 percent compression than 25 percent compression, therefore, the distance between the first rise above zero in the second bite and maximum compression is greater, with greater compression. Consequently, springiness values were larger for higher

degrees of compression. Logically it seems that the more a sample is compressed and therefore damaged, the less it would tend to spring back to its original height. That is, greater levels of compression theoretically should not yield a higher springiness measurement. Perhaps the definition and method of measuring springiness with the Instron needs to be re-evaluated so that the values recorded represent the true springiness of the sample.

Effect of Plunger Area on Instron Texture Values

Plunger area had a very significant effect on hardness and gumminess measurements and a small, but significant effect on cohesiveness and springiness measurements (Table 2.3). As plunger area increased, so did the values for all four textural parameters (Table 2.4). Walker et al. (1987) reported similar results for firmness measurements of white and sponge cakes. The larger the plunger area, the greater were all compression, tension and shearing forces associated with the degree of compression. Walker et al. (1987) suggested that plunger area is associated with compression forces while plunger diameter is associated with tension and shearing forces. Shear and tension forces become important components of the total force values when compressions are high.

The effect of plunger area on cohesiveness and springiness was very small, likely due to the way in which

each parameter was calculated. Cohesiveness was calculated
as the ratio of the area under the second peak to the area
under the first peak (Figure 2.1), therefore, the actual
magnitude of the areas is not important. Increasing plunger
area increased the areas of both peaks, therefore its effect
on cohesiveness values would be minimal. Springiness was a
distance measurement, therefore, the size of the plunger would
not influence this parameter.

Effect of Cross-head Speed on Instron Texture Values

The size of the F-values for cross-head speed (Table 2.3)
indicate that this Instron condition was much less important
to textural measurements than degree of sample compression and
plunger area. In Table 2.4, the means for each cross-head
speed show that hardness, gumminess and springiness values
increased slightly with increasing cross-head speeds.
Cohesiveness was not affected by the rate of compression,
probably because the areas under both peaks would be affected
equally, producing similar ratios regardless of cross-head
speed.

Faster cross-head speeds likely increased the forces
present during cake compression, resulting in slightly higher
values for hardness and gumminess. Baker et al. (1986a) also
found that Instron force (firmness) tended to increase
slightly with greater cross-head speeds. Springiness values
also increased slightly with increasing rates of compression.

Because springiness is a distance measurement rather than a force measurement, increasing values cannot be attributed to increases in the forces present as the cake is compressed. Perhaps, because faster cross-head speeds decreased the length of time that the cake crumb was compressed, less permanent crumb deformation occurred. If this were true, the first rise in force above zero for the second bite would occur sooner (since the plunger would touch the cake sooner than if the cake was permanently compressed) resulting in a longer distance from this point to maximum compression, and higher springiness values.

Boyd and Sherman (1975) noted that while cross-head speed was important for the textural measurement of some foods it was not as important for intermediate hardness foods such as Madeira cakes. Peleg (1987) also found that the type of product determined the importance of cross-head speed to textural measurement. The more solid or elastic the food, the less dependent the texture measurements on the cross-head speed. The sponge cakes used in this study were both fairly firm and also elastic, and so fall into the category of foods thought to be less affected by cross-head speed.

Interaction between Plunger Area and Degree of Compression

There were highly significant interactions between plunger area and degree of sample compression for both hardness and gumminess, as shown in Figure 2.4. Hardness
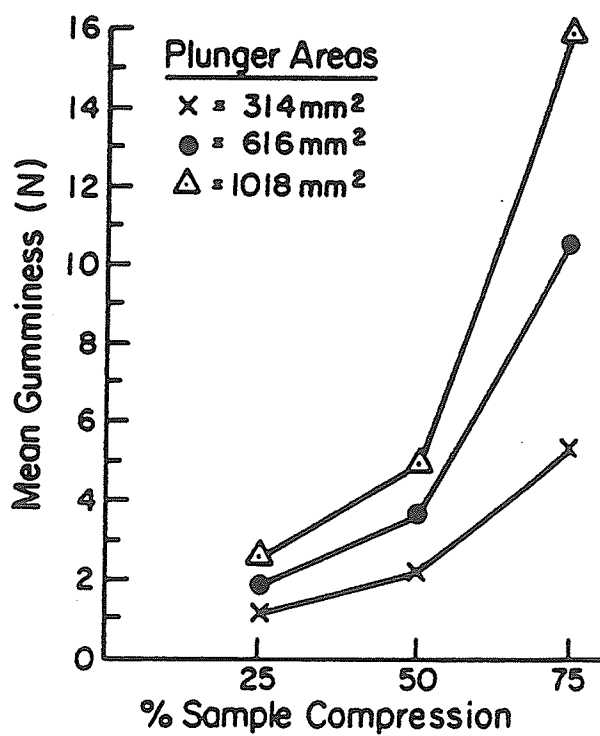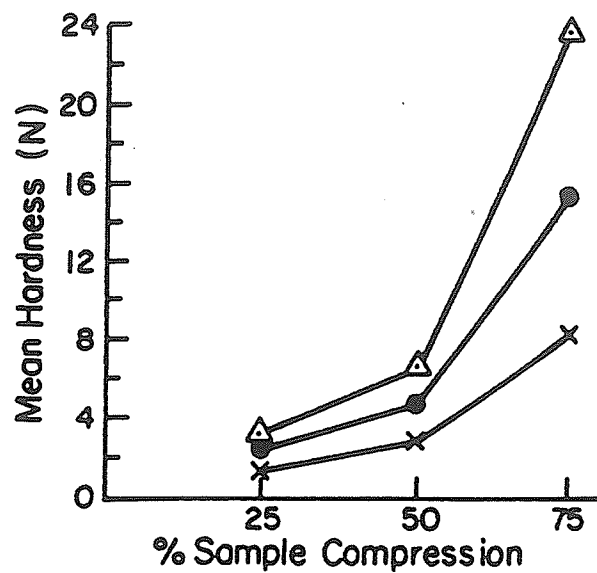
Figure 2.4   Compression X plunger area interactions for Instron hardness and gumminess measurements.

values increased as both compression level and plunger area increased, however, the magnitude of the increase was dependent upon the combination of compression and plunger area. When compressions increased from 25 to 50 percent, the hardness values approximately doubled for all plunger sizes. At 75 percent compression, hardness values doubled again for the smallest plunger but were three times greater for the medium plunger and four times greater for the largest one. Similar results were found for gumminess.

The plunger area*degree of compression interaction indicated that significantly higher hardness and gumminess values were recorded when 75 percent compression and any of the three plungers were used, however, the 1018 mm^2 plunger yielded particularly high values. When samples were compressed to 75 percent of their height, large force values resulted from the presence of compression, shearing and tension forces, in addition to some compacting of the cake crumb. The effect of plunger area would also be more pronounced when the degree of compression was high since high degrees of compression create more forces (compression, shearing and tension) which contribute to the final measurement. At lower degrees of compression, larger plunger areas would only increase compression and possibly tension forces.

Cohesiveness was also affected by a small, but significant, plunger area*degree of sample compression

interaction. The relationship between plunger area and compression depth was not, however, similar to the one observed for hardness and gumminess. That is, while significantly lower cohesiveness values were recorded with increasing depth of compression, the plunger size had very little effect on the cohesiveness values. It is possible that, for this parameter, the interaction between plunger area and compression depth occurred by chance.

Several other interactions (treatment*degree of compression; treatment*cross-head speed; plunger area*cross-head speed; degree of compression*cross-head speed; and plunger area*degree of compression*cross-head speed) were also statistically significant but practically, were not important. F-values were low in comparison to those of the two primary main effects (plunger area and degree of sample compression).

Selection of Operating Conditions for the Individual Detection of Differences in Four Textural Parameters

The second objective of this study was to determine the Instron operating conditions best able to detect differences in each of the four textural parameters; hardness, cohesiveness, gumminess and springiness. Sponge cakes baked from formulations substituting 30 percent PPC for egg albumen were predicted to be substantially different from those baked with 100 percent albumen. In this study, however, differences between treatment means for each textural parameter (Table 2.4) were quite small. While this made the discrimination

task more difficult, it allowed identification of Instron conditions sensitive to small differences.

Absolute t-values have been used to evaluate the ability of each set of Instron conditions to discriminate hardness, cohesiveness, gumminess and springiness between cakes. High t-values indicate that the difference between the means of the two formulations was large and the variability of measurement minimal (Figure 2.2). Thus high t-values would result if the set of Instron conditions detected marked textural differences between the 0 and 30 percent PPC sponge cakes. High t-values would also result, however, if differences between treatments were fairly small but the variability of measurement was very low. For the best discrimination of textural differences, high t-values should result from both differences detected between the treatments and low variability.

Selection of Instron Conditions for the Discrimination of Cohesiveness

Table 2.5 presents the absolute t-values for cohesiveness. There were no apparent relationships between a specific plunger area or cross-head speed and consistently high t-values. However, the higher t-values associated with 75 percent compression suggest that high levels of compression were conducive to greater discrimination of cohesiveness between cakes. With only one exception (1018 mm^2 plunger, 5.1 cm/min. cross-head speed, 75 percent compression),

Table 2.5   Absolute t-values for Instron Cohesiveness

Plunger Area (mm^2)	Cross-head Speed (cm/min.)	Compression (%)		
		25	50	75
1018	20.0	0.91	1.40	3.29
	10.0	0.69	0.44	2.12
	5.1	1.51	2.12	1.73
616	20.0	0.95	1.97	2.46
	10.0	0.15	2.68	4.70
	5.1	0.03	2.05	3.50
314	20.0	0.13	0.33	0.67
	10.0	1.07	0.64	8.09[1]
	5.1	0.96	0.26	2.00

[1]Highest t-value.

compressions of 75 percent produced the highest t-values for each of the nine plunger area/cross-head speed combinations. Table 2.6 presents the mean cohesiveness values for the 0 and 30 percent formulations, and illustrates that the high t-values associated with 75 percent compression were due to the detection of differences between the two cakes and low variability of measurement. Conversely, for 25 percent compression, differences between the means were generally small and variability fairly large, resulting in lower t-values.

The association between high degrees of compression and increased breakdown of internal cake structure may help explain the greater discrimination between the two cakes when 75 percent compression was used. At lower levels of compression, cake structure was likely not altered sufficiently to indicate differences in cake cohesiveness. Combined conditions producing the highest t-value were: 75 percent compression with a 314 mm^2 plunger and a cross-head speed of 10.0 cm/min. This particular combination of Instron conditions produced both a large difference in mean cohesiveness values and demonstrated low variability (Table 2.6).

Selection of Instron Conditions for the Discrimination of Springiness

The absolute t-values for springiness are presented in

Table 2.6   Mean[1] Cohesiveness Values for 0% and 30% PPC[99] Sponge Cakes

Instron Conditions			Treatment	
Plunger Area (mm^2)	Cross-head Speed (cm/min.)	Compression (%)	0% PPC	30% PPC
1018	20.0	75	.679(.01)[2]	.658(.01)
		50	.736(.01)	.722(.02)
		25	.786(.01)	.799(.03)
	10.0	75	.685(.01)	.671(.01)
		50	.734(.01)	.738(.01)
		25	.772(.02)	.764(.01)
	5.1	75	.686(.01)	.672(.01)
		50	.720(.01)	.730(.00)
		25	.764(.02)	.786(.02)
616	20.0	75	.686(.01)	.663(.02)
		50	.729(.01)	.714(.01)
		25	.776(.01)	.767(.01)
	10.0	75	.680(.00)	.666(.00)
		50	.740(.00)	.725(.01)
		25	.773(.02)	.772(.01)
	5.1	75	.692(.01)	.672(.01)
		50	.738(.03)	.724(.01)
		25	.766(.03)	.766(.01)
314	20.0	75	.656(.02)	.648(.02)
		50	.738(.01)	.746(.01)
		25	.764(.02)	.766(.02)
	[3]10.0	75	.682(.00)	.643(.01)
		50	.735(.03)	.726(.00)
		25	.782(.01)	.778(.00)
	5.1	75	.649(.01)	.633(.01)
		50	.740(.01)	.739(.01)
		25	.771(.02)	.782(.01)

[1]Mean of 4 determinations.
[2]Standard deviation associated with the mean.
[3]Conditions producing highest t-value.

Table 2.7. For this parameter, no specific plunger area or level of compression seemed to produce consistently high t-values. However, a relationship was apparent between slower cross-head speeds (5.1 and 10.0 cm/min.) and high t-values. In particular, a cross-head speed of 5.1 centimetres/minute improved discrimination of springiness between the two formulations. With two exceptions (616 mm^2 plunger, 10.0 cm/min. cross-head speed, 50 and 75% compression), this slow rate of compression resulted in consistently high t-values. Table 2.8 presents the mean springiness values for the two cake formulations for each set of Instron conditions evaluated. Higher t-values associated with slower cross-head speeds, resulted from both low variability of measurement and differences between springiness means for the two cakes. At a cross-head speed of 20.0 centimetres/minute, less difference in springiness was detected. Measurements were generally more variable, resulting in lower t-values.

Slow cross-head speeds may have permitted more "spring" back after cake deformation, resulting in greater detection of differences between cakes. Conditions which produced the highest t-value were: 50 percent compression combined with a 314 mm^2 plunger and a cross-head speed of 5.1 centimetres/minute. The high t-value produced for this combination of conditions was a result of substantial differences in springiness between the two formulations and low variability of measurement (Table 2.8).

Table 2.7   Absolute t-values for Instron Springiness

Plunger Area (mm^2)	Cross-head Speed (cm/min.)	Compression (%) 25	50	75
1018	20.0	0.44	0.49	0.56
	10.0	1.32	0.35	0.58
	5.1	1.46	3.25	2.89
616	20.0	0.73	0.29	0.00
	10.0	0.86	2.47	2.05
	5.1	1.14	0.57	1.04
314	20.0	1.10	1.85	0.00
	10.0	1.23	1.55	0.90
	5.1	3.14	4.18[1]	3.63

[1]Highest t-value.

Table 2.8   Mean[1] Springiness Values for 0% and 30% PPC
             Sponge Cakes

Instron Conditions			Treatment	
Plunger Area (mm[2)]	Cross-head Speed (cm/min.)	Compression (%)	0% PPC	30% PPC
1018	20.0	75	29.2(.8)[2]	29.6(.8)
		50	23.0(1.0)	22.5(1.5)
		25	13.6(1.1)	13.0(1.1)
	10.0	75	29.3(.8)	28.9(1.3)
		50	22.3(1.2)	22.0(1.2)
		25	12.8(.5)	13.2(.2)
	5.1	75	29.2(.9)	27.8(.3)
		50	21.4(.6)	20.4(.2)
		25	12.3(.4)	11.6(.8)
616	20.0	75	29.2(1.6)	29.2(.3)
		50	22.4(.6)	22.6(1.3)
		25	13.8(1.1)	13.0(1.7)
	10.0	75	29.2(.8)	28.2(.6)
		50	21.4(.4)	20.4(.6)
		25	12.1(.9)	11.6(.8)
	5.1	75	28.6(.7)	28.1(.8)
		50	20.8(.1)	20.9(.5)
		25	12.5(.1)	12.2(.7)
314	20.0	75	29.6(1.6)	29.6(1.5)
		50	22.8(1.0)	21.6(.5)
		25	14.1(.8)	13.5(.8)
	10.0	75	29.7(.8)	29.2(.6)
		50	21.7(1.4)	20.2(1.1)
		25	12.7(.5)	12.1(.8)
	5.1	75	28.6(.8)	27.0(.3)
		[3]50	21.4(.6)	19.7(.6)
		25	12.5(.3)	11.2(.7)

[1]Mean of 4 determinations.
[2]Standard deviation associated with the mean.
[3]Conditions producing highest t-value.

Selection of Instron Conditions for the Discrimination of Hardness and Gumminess

The absolute t-values for hardness and gumminess are presented in Tables 2.9 and 2.10, respectively. While no specific plunger area or level of compression seemed to produce consistently high t-values for either of these parameters, faster cross-head speeds (10.0 cm./min. and 20.0 cm/min.) generally produced higher t-values. It should be noted, however, that cross-head speeds of 5.1 centimetres/minute did not always produce small t-values suggesting that the relationship between faster cross-head speeds and improved discrimination might not be real. Tables 2.11 and 2.12 present the mean hardness and mean gumminess values for 0 and 30 percent PPC sponge cakes. In most cases, and particularly evident with 25 percent compression, high t-values resulted primarily from low sample variability rather than detection of large differences between treatments. Highest t-values for hardness and gumminess were, however, produced by 25 percent compression combined with a 616 mm^2 plunger and a 10.0 centimetre/minute cross-head speed. It was difficult to be certain that this set of Instron testing conditions was really the best able to discriminate hardness and gumminess.

Table 2.9   Absolute t-values for Instron Hardness

Plunger Area (mm^2)	Cross-head Speed (cm/min.)	Compression (%) 25	50	75
1018	20.0	1.29	4.21	2.12
	10.0	1.40	1.09	0.06
	5.1	0.04	0.99	0.72
616	20.0	6.51[1]	1.06	2.14
	10.0	0.11	0.67	0.43
	5.1	2.77	0.85	1.49
314	20.0	0.09	0.96	2.68
	10.0	3.58	3.06	0.59
	5.1	1.33	1.02	0.29

[1]Highest t-value.

Table 2.10   Absolute t-values for Instron Gumminess

Plunger Area (mm^2)	Cross-head Speed (cm/min.)	Compression (%) 25	50	75
1018	20.0	2.12	3.69	1.92
	10.0	1.51	1.40	0.38
	5.1	0.46	1.16	0.55
616	20.0	6.08[1]	0.73	1.82
	10.0	0.13	0.26	0.03
	5.1	4.70	0.49	1.11
314	20.0	0.04	0.96	2.54
	10.0	3.47	2.01	0.34
	5.1	1.73	1.03	0.03

[1]Highest t-value.

Table 2.11   Mean[1] Hardness Values for 0% and 30% PPC
             Sponge Cakes

Instron Conditions			Treatment	
Plunger Area (mm$^{2)}$)	Cross-head Speed (cm/min.)	Compression (%)	0% PPC	30% PPC
1018	20.0	75	24.9(.7)[2]	29.6(4.4)
		50	6.5(.5)	7.7(.3)
		25	3.3(.1)	3.5(.2)
	10.0	75	22.5(2.2)	22.4(2.5)
		50	6.6(.7)	7.1(.3)
		25	3.1(.3)	3.4(.3)
	5.1	75	21.0(2.5)	22.4(2.9)
		50	6.0(.2)	6.4(.8)
		25	3.2(.5)	3.2(.3)
616	20.0	75	15.7(1.5)	18.1(1.8)
		50	5.1(.5)	5.5(.4)
		25	2.4(.1)	2.9(.1)
	10.0	75	15.2(.8)	15.5(1.1)
		50	4.7(.3)	4.8(.4)
		25	2.3(.2)	2.3(.1)
	[3]5.1	75	13.8(1.3)	15.2(1.3)
		50	4.6(.3)	4.8(.4)
		25	2.3(.2)	2.5(.1)
314	20.0	75	8.2(.3)	9.0(.5)
		50	3.0(.3)	3.3(.4)
		25	1.7(.3)	1.7(.1)
	10.0	75	8.2(.5)	8.5(.9)
		50	2.8(.1)	3.2(.2)
		25	1.4(.1)	1.6(.1)
	5.1	75	7.7(1.0)	7.9(.7)
		50	2.8(.2)	2.7(.2)
		25	1.4(.1)	1.5(.1)

[1]Mean of 4 determinations.
[2]Standard deviation associated with the mean.
[3]Conditions producing highest t-value.

Table 2.12   Mean[1] Gumminess Values for 0% and 30% PPC
              Sponge Cakes

Instron Conditions			Treatment	
Plunger Area ($mm^{2)}$)	Cross-head Speed (cm/min.)	Compression (%)	0% PPC	30% PPC
1018	20.0	75	16.9(.3)[2]	19.5(2.7)
		50	4.8(.3)	5.6(.3)
		25	2.6(.1)	2.8(.1)
	10.0	75	15.4(1.4)	15.0(1.5)
		50	4.9(.5)	5.2(.2)
		25	2.4(.2)	2.6(.2)
	5.1	75	14.4(1.5)	15.0(1.8)
		50	4.3(.1)	4.7(.6)
		25	2.4(.3)	2.5(.2)
616	20.0	75	10.8(1.0)	12.0(1.0)
		50	3.7(.4)	3.9(.2)
		[3]25	1.9(.1)	2.2(.1)
	10.0	75	10.3(.5)	10.3(.8)
		50	3.4(.2)	3.5(.2)
		25	1.8(.1)	1.8(.1)
	5.1	75	9.6(.9)	10.2(.8)
		50	3.4(.2)	3.5(.3)
		25	1.7(.1)	1.9(.1)
314	20.0	75	5.4(.3)	5.8(.2)
		50	2.2(.2)	2.4(.3)
		25	1.3(.2)	1.3(.0)
	10.0	75	5.6(.4)	5.5(.7)
		50	2.1(.2)	2.3(.1)
		25	1.1(.1)	1.3(.1)
	5.1	75	5.0(.7)	5.0(.5)
		50	2.1(.1)	2.0(.1)
		25	1.1(.1)	1.2(.1)

[1]Mean of 4 determinations.
[2]Standard deviation associated with the mean.
[3]Conditions producing highest t-value.

Summary of Instron Testing Conditions for the Individual
Discrimination of Differences in Cohesiveness, Springiness,
Hardness and Gumminess

Differences in cohesiveness were accentuated when depth
of compression was high, while springiness was best
discriminated when cross-head speeds were low. Faster cross-
head speeds generally produced higher t-values for hardness
and gumminess, yet some low cross-head speed combinations also
produced high t-values. The high t-values associated with
cohesiveness and springiness resulted from the detection of
substantial differences between the cakes and low variability
within the samples (standard deviations). For hardness and
gumminess, however, high t-values seemed to result primarily
from low variability within the samples. The relationship
between cross-head speed and high t-values for hardness and
gumminess was much less apparent than the relationships
evident for cohesiveness and springiness. These results
suggest that it may be more important to determine the best
set of Instron testing conditions for detecting differences
in sponge cake cohesiveness and springiness, than hardness and
gumminess.

Graphical presentations of the conditions producing the
greatest discrimination of hardness, gumminess, cohesiveness
and springiness (largest t-values), are presented in Figure
2.5. The t-values associated with the same set of conditions
for the other textural parameters are also shown. These plots
show that while specific combinations of testing conditions

INSTRON HARDNESS & GUMMINESS

INSTRON CONDITIONS: Plunger area – 616 mm²
Cross-head speed – 20 cm/min
Compression – 25%

TEXTURAL PARAMETER

HARDNESS (N) ***
COHESIVENESS
GUMMINESS (N) ***
SPRINGINESS (MM)

1 2 3 4 5 6 7 8 9
ABSOLUTE T-VALUE

*** P<.001

INSTRON COHESIVENESS

INSTRON CONDITIONS: Plunger area – 314 mm²
Cross-head speed – 10 cm/min
Compression – 75%

TEXTURAL PARAMETER

HARDNESS (N)
COHESIVENESS ***
GUMMINESS (N)
SPRINGINESS (MM)

1 2 3 4 5 6 7 8 9
ABSOLUTE T-VALUE

*** P<.001

INSTRON SPRINGINESS

INSTRON CONDITIONS: Plunger area – 314 mm²
Cross-head speed – 5.1 cm/min
Compression – 50%

TEXTURAL PARAMETER

HARDNESS (N)
COHESIVENESS
GUMMINESS (N)
SPRINGINESS (MM) **

1 2 3 4 5 6 7 8 9
ABSOLUTE T-VALUE

** P< .01

Figure 2.5 Instron testing conditions producing the greatest textural discrimination of hardness, gumminess, cohesiveness and springiness between sponge cakes.

allowed successful discrimination for individual textural parameters, only hardness and gumminess shared the same set of conditions. This result is not surprising due to the strong dependence of gumminess measurements on hardness measurements. For these two parameters, the medium sized plunger and highest cross-head speed combined with 25 percent compression were found to produce the highest t-values. Such low levels of compression are not recommended, however since the high t-values associated with 25 percent compression generally resulted from very low sample variability. For the best discrimination of textural differences, high t-values should result from both differences detected in the treatments and low sample variability. In addition, the use of 25 percent compression resulted in very small hardness and gumminess values, suggesting that differences may not be as evident at low degrees of compression than when larger values are produced with higher degrees of compression. Consequently, while high t-values indicated that the greatest discrimination of hardness and gumminess was achieved using 25 percent compression, such a low degree of compression is not recommended. Interestingly, the test conditions recommended to evaluate crumb firmness of white pan bread have been reported to be 25 percent compression, 10.0 centimetres/minute cross-head speed and a round plunger with an area of 600-1150 mm^2 (Baker et al., 1986b). The basis for selection of this compression level was the low variance

associated with the force values. From this study it was apparent that because force values were very low with 25 percent compression, standard deviations were also low. Therefore, the choice of 25 percent compression for white pan bread may be questionable. The differences between the means should also be considered.

These results suggest that one level of compression, plunger size and speed of compression may increase the discrimination of one textural parameter yet simultaneously hinder the discrimination of other textural differences. If only one textural parameter is of interest the choice of Instron conditions is straight forward. If, however, a texture profile analysis consisting of several parameters is planned, then the choice of Instron conditions becomes more complicated.

Selection of Operating Conditions for the Simultaneous Detection of Differences in all Four Textural Parameters

The final objective of this study was to identify the operating conditions most appropriate for evaluating all four textural parameters at once, that is, for a texture profile. Because different conditions were most appropriate for discriminating the textural parameters, the researcher must make some crucial decisions as to the importance of each textural parameter to the quality of the test product. The variability associated with the measurement of each parameter should also be considered.

Table 2.13 presents the absolute t-values associated with each set of Instron conditions for all four textural parameters. This table highlights those conditions which generally discriminated the four parameters on the basis of their absolute t-values.

Figure 2.6 illustrates the set of conditions thought to be most appropriate for the simultaneous evaluation of the four textural parameters. This combination produced t-values exceeding 1.00 for each parameter with cohesiveness and springiness having the highest t-values. Cohesiveness and springiness were felt to be very important characteristics for this sponge cake formulation which is intended for use in swiss rolls. In addition, CV values, indicating the overall variability associated with each textural parameter, were lowest for cohesiveness and springiness, while the R^2 value for springiness, indicating model fit, was highest (Table 2.3). Also, the choice of plunger area, level of compression and cross-head speed may not be so crucial for hardness and gumminess. Therefore, a 616 mm^2 plunger, 75 percent compression, and a cross-head speed of 5.1 centimetres/minute was felt to be most appropriate for the texture profile analysis of these sponge cakes. If, however, all four parameters were not of interest, some other set of conditions might be selected as being most appropriate for the parameters to be tested.

Table 2.13 Absolute t-values for All Textural Parameters and Instron Conditions

Instron Conditions			Textural Parameters[1]			
Plunger Area (mm^2)	Cross-head Speed (cm/min.)	Compression (%)	H	C	G	S
1018	20.0	75	2.12	3.29	1.92	0.56
1018	20.0	50	4.21	1.40	3.69	0.49
1018	20.0	25	1.28	0.91	2.12	0.44
1018	10.0	75	0.06	2.12	0.38	0.58
1018	10.0	50	1.09	0.44	1.40	0.35
1018	10.0	25	1.40	0.69	1.51	1.32
1018	5.1	75	0.72	1.73	0.55	2.89
1018	5.1	50	0.99	2.12	1.16	3.25
1018	5.1	25	0.04	1.51	0.46	1.46
616	20.0	75	2.14	2.46	1.82	0.00
616	20.0	50	1.06	1.97	0.73	0.29
616	20.0	25	6.51	0.95	6.08	0.73
616	10.0	75	0.43	4.70	0.03	2.05
616	10.0	50	0.67	2.68	0.26	2.47
616	10.0	25	0.11	0.15	0.13	0.86
[2]616	5.1	75	1.49	3.50	1.11	1.04
616	5.1	50	0.85	2.05	0.49	0.57
616	5.1	25	2.77	0.03	4.70	1.14
314	20.0	75	2.68	0.67	2.54	0.00
314	20.0	50	0.96	0.33	0.96	1.85
314	20.0	25	0.09	0.13	0.04	1.10
314	10.0	75	0.59	8.09	0.34	0.90
314	10.0	50	3.06	0.64	2.01	1.55
314	10.0	25	3.58	1.07	3.47	1.23
314	5.1	75	0.29	2.00	0.03	3.63
314	5.1	50	1.02	0.26	1.03	4.18
314	5.1	25	1.33	0.96	1.73	3.14

[1]H = hardness; C = cohesiveness; G = gumminess; S = springiness.
[2]Most appropriate set of conditions for discriminating all textural parameters.

INSTRON CONDITIONS: Plunger area — 616 mm^2
Cross-head speed — 5.1 cm/min
Compression — 75 %

TEXTURAL PARAMETER

HARDNESS (N)

COHESIVENESS

GUMMINESS (N)

SPRINGINESS (MM)

ABSOLUTE T-VALUE
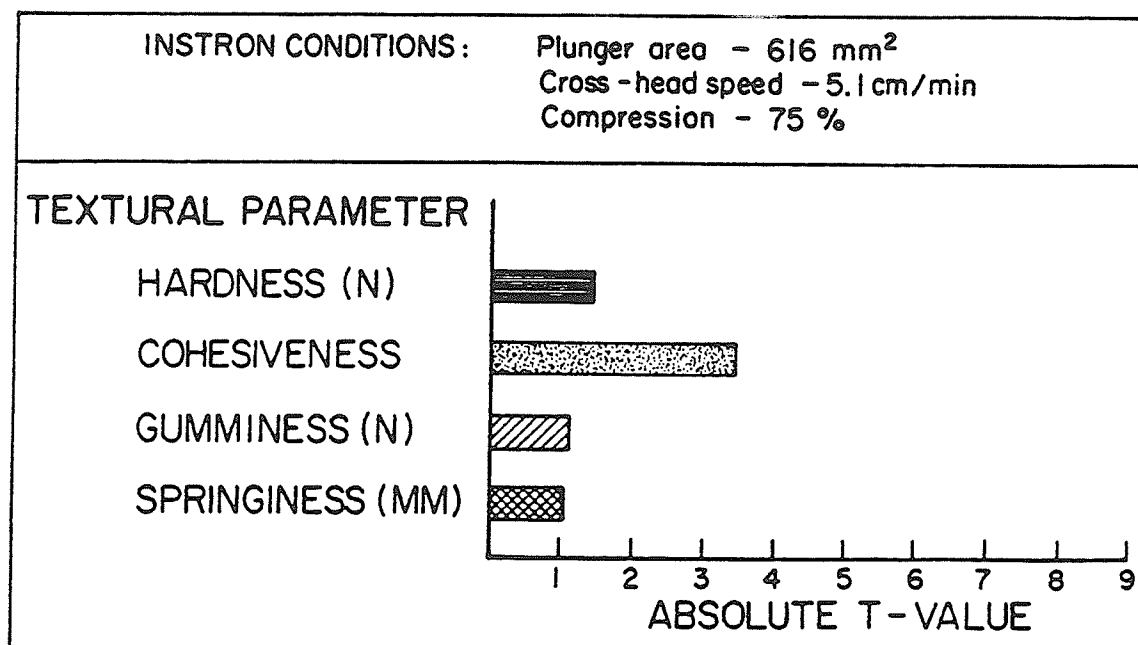
Figure 2.6   Instron testing conditions most appropriate for
the textural discrimination of all parameters between sponge
cakes.

CONCLUSIONS

High F-values from the analyses of variance for each textural parameter indicated that degree of sample compression and plunger area strongly influenced sponge cake texture measurements by the Instron. Therefore, these testing conditions must be carefully selected for texture evaluation studies.

It was possible to discriminate among sponge cakes on the basis of hardness, cohesiveness, gumminess and springiness when conditions appropriate for those individual parameters were selected. High degrees of sample compression were found to improve the discrimination of cohesiveness, while slower cross-head speeds aided the detection of differences in springiness. Discrimination of hardness and gumminess may have been improved with faster cross-head speeds, however, this relationship was not clear. Therefore, the choice of Instron conditions did not seem to be as crucial for hardness and gumminess as for cohesiveness and springiness.

While one set of conditions did not discriminate equally well for all parameters, an appropriate set of conditions was identified on the basis of CV values, R^2 values and t-values as well as the relative importance of the parameters to sponge cake quality.

This study presents a method for standardizing the selection of Instron testing conditions on the basis of discriminatory power. With increasing use of the Instron for

texture profiling, a systematic method of determining the most appropriate set of conditions for detecting differences among the test products is needed. The type of product under investigation will dictate how practical it is to use this method for selection of appropriate Instron testing conditions.

Finally, and most importantly, the relationship of the Instron Texture Profile to sensory Texture Profile evaluation needs to be investigated. Determining the Instron conditions best able to discriminate between cakes becomes of little value if these differences are not detected by sensory analysis.

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CHAPTER 3

Pea Protein Concentrate for Partial Replacement of Egg Albumen in Sponge Cakes - Identification of Critical Independent Variables Using Response Surface Methodology

INTRODUCTION

The fractionation of field peas (Pisum sativum) into fibre, starch and protein components has increased the utility of a crop which has become increasingly important to Western Canada (Nielsen et al., 1980). In Manitoba alone an estimated 130,000 acres were planted in 1989 making it a substantial special crop for this province (E. Lewis, personal communication, 1989). Woodstone Foods Ltd. (Portage La Prairie, Manitoba) holds one patent for this fractionation process (Nickel, 1981) and notes that while the fibre and starch components find widespread use, the protein fraction, a by-product of the process, is currently under-utilized (M. Lamb, personal communication, 1987). Production of an acceptable food product incorporating pea protein concentrate (PPC) would aid in establishing a balance between the supply and demand of the three pea fractions.

Pea proteins have been found to be an acceptable alternative to soy protein in such products as pasta (Nielsen et al., 1980), a tofu type protein curd (Gebre-Egziabher and Sumner, 1981) and a meat extender in ground beef (Vaisey et al., 1975). It has also been found to exhibit potential as a replacement for non-fat dry milk in bread (Patel et al., 1981). Most research, however, has focused on the replacement

of wheat flour with pea flour, or other pea protein products, to improve nutritional quality of breads and other baked products. Pea proteins are high in lysine (Holt and Sosulski, 1979) and therefore possess an amino acid composition nutritionally complimentary to wheat proteins (Kreutler, 1980). Successful incorporation of pea protein flours, concentrates and isolates in yeast breads, quick breads and cookie formulations have been reported by Fleming and Sosulski (1977), Jeffers et al. (1978), Repetsky and Klein (1981), Hsu et al. (1982), Raidl and Klein (1983), and McWatters (1978). In addition, several investigations of pea protein functionality have indicated that the proteins generally possess good functional properties (Vose, 1980; Sosulski and Youngs, 1979; Sumner et al., 1981; Hsu et al., 1982; Fleming and Sosulski, 1975; Megha and Grant, 1986; Naczk et al., 1986). Thus, the protein may be a good candidate for the partial replacement of a more expensive protein source.

Egg albumen is one such protein source for which a high demand exists (L. Carvalho, personal communication, 1987) due to its widespread food application. Partial replacement of egg albumen with pea protein in a food product would increase the market supply of egg albumen, utilize excess pea protein and, at the same time, lower ingredient costs. A sponge cake system is a particularly suitable system for testing the effects of such a replacement since overall cake quality is highly dependent upon egg quality. A sponge cake system

should, therefore, emphasize the effects of egg albumen replacement with PPC.

Successful attempts have been made to replace egg albumen with bovine plasma protein in cakes. Khan et al. (1979) evaluated the potential of plasma protein isolate as a replacement of egg white solids in an angel food cake. While the foaming capacity of the plasma protein isolate was very similar to that of the egg white solids, results were significantly different when tested in combination with the other cake ingredients. Acceptable cakes, however, were made with a 30 percent substitution. Johnson et al. (1979) successfully substituted plasma protein concentrate for egg white in a high ratio white layer cake after optimizing water levels and mixing times.

These two studies emphasize two important points, firstly, although a protein may exhibit very similar functional properties to egg albumen when tested alone, results may differ significantly when tested with other product ingredients. Secondly, it may be necessary to optimize both cake ingredients and mixing procedures, in order to suit the pea protein system. Thus, successful substitution of PPC for egg albumen will likely depend upon formulation and procedural changes which enhance pea protein functionality.

Response surface methodology (RSM) is an experimental design which is especially suitable for product development as is helps to achieve optimal ingredient levels and

conditions in a minimal number of experimental formulations (Johnson and Zabik, 1981). This minimizes the costs associated with product development. Unlike the one-variable-at-a-time approach, RSM helps to determine how the independent variables, singly and in combination, affect the dependent variables (Giovanni, 1983). Additionally, interactions among variables can be determined. Response surface methodology has been successfully used to optimize a variety of cake formulations (Johnson and Zabik, 1981; Vaisey-Genser et al., 1987; Neville and Setser, 1986; Lee and Hoseney, 1982; Kissell and Marshall, 1962; Kissell, 1967).

Joglekar and May (1987) have described two typical stages of product development and optimization using RSM. The first, a screening stage, has the objective of identifying independent variables having the most important effect on chosen response variables. At this stage, the identification of key variables is more important than investigating their relationships and interactions (Mullen and Ennis, 1985). The second stage is referred to as the optimization stage where optimal levels of the key variables selected in the screening stage, are determined. It is during this stage that relationships between the variables are investigated in detail (Mullen and Ennis, 1985).

This study falls into the first stage of the product development process using RSM. Giovanni (1983) suggests that, for simplicity, the number of independent variables

investigated should be limited to two or three, and that any greater that three makes the interpretation of results difficult. In addition, for those variables under the control of the researcher, a fairly broad range should be set. If necessary, additional RSM studies could be conducted using narrower ranges of levels so as to more closely approach the optimum. Therefore, identification of the critical independent variables influencing sponge cake quality and their appropriate test levels are important steps towards the optimization of a formulation in which egg albumen is partially replaced with PPC. The objectives of this preliminary study were:

1. **To use RSM to determine the importance of five independent variables (levels of PPC, cream of tartar, water and emulsifier, and length of whip time) to sponge cake batter specific gravity, specific volume and Instron texture characteristics, in order to identify the most critical variables for further product optimization.**

2. **To select best fitting regression models from full second-order models, to predict the effects of all five independent variables on sponge cake quality characteristics.**

3. **To generate contour plots from the best fitting predictive models to gain insight into the relationships between the most influential independent variables and their effects on sponge**

cake quality.

4.   To identify independent variable levels appropriate for further product optimization.

MATERIALS AND METHODS

Materials

Pea protein concentrate (PPC) was supplied by Woodstone Foods Ltd., Portage La Prairie, Manitoba. The protein, trade named Propulse 985B, was prepared from yellow field peas by an acid extraction method developed by Nickel (1981). Enough pea protein was supplied to complete the entire experiment. A typical analysis supplied by Woodstone Foods Ltd. is presented in Table 3.1. Spray-dried egg albumen containing whipping agents (triethyl citrate and sodium lauryl sulphate) was supplied by Export Packers Co. Ltd., Winnipeg, Manitoba. Lysozyme was extracted prior to drying for commercial sale. A typical analysis for the dried albumen is presented in Table 3.2. A commercial cake flour, 7.5 percent protein (14% moisture basis), was obtained from Reid Milling, Mississauga, Ontario. The emulsifier, TOP-SCOR S Powder (sodium stearoyl lactylate), was provided by Breddo Food Products Corp., Kansas. All other ingredients were purchased locally when the experiment was begun, in amounts sufficient to complete the entire experiment.

Sponge Cake Preparation

Cakes were prepared, with minor changes, according to the procedure outlined in Appendix 2.A. Water was measured in grams instead of millilitres to improve accuracy, and the amount used to re-hydrate the highest PPC level was increased

Table 3.1   Typical Analysis of Propulse 985B Pea
             Protein Concentrate

Protein (Kjeldahl - N X 6.25)db	83%
Moisture (dry 16 hours at 100°C)	5%
Fat (AOAC 7.056, 13th ed.)db	2%
Crude Fiber (Modified AOAC 7.068, 13th ed.)db	0.4%
pH (10% solution)	6.5
Ash (AOAC 14.006, 13th ed)db	4.0%

                                   Woodstone Foods Ltd.

Table 3.2   Typical Analysis of Spray-Dried Egg Albumen

Protein	80% max.
Moisture	8% max.
Fat	negligible
Carbohydrate	0.1%
pH	6.5 - 7.5
Ash	5% max.

                                   Export Packers Ltd.

from 50 to 100 grams (50 grams of water was then deducted from the water added to the albumen batter), facilitating PPC hydration. Cakes were weighed in their pans after cooling for 20 minutes and cake weights calculated (weight of cake in pan - weight of pan) for use in specific volume measurements. After cooling an additional 20 to 30 minutes, cakes were placed into polybags and the air removed for 10 seconds prior to sealing. Cakes were frozen (-20°C) for exactly eight days prior to texture measurement. The revised procedure is presented in Appendix 3.A.

Experimental Design

A central composite, rotatable, response surface design was chosen to determine which independent variables were most critical to selected sponge cake characteristics (Box and Hunter, 1957). The experimental design consisted of five variables (PPC, whip time, cream of tartar, water, emulsifier) at five levels and required 32 test runs. Table 3.3 lists the actual and coded levels of PPC, albumen, cream of tartar, whip time water and emulsifier. Table 3.4 illustrates in coded form, the entire experimental design. A traditional 5^5 factorial would have required 3,125 test runs, therefore the efficiency of response surface methodology is illustrated. The 32 cake treatments were prepared in a randomized order over four consecutive days. The study was not replicated due to the screening nature of the experiment.

Table 3.3 Actual and Coded Independent Variable Levels[1] Chosen for Sponge Cake Preparation

Independent Variables	Levels				
PPC (g)[2]	0 (0%)	3.96 (15%)	7.92 (30%)	11.88 (45%)	15.84 (60%)
Albumen (g)	26.40 (100%)	22.44 (85%)	18.48 (70%)	14.52 (55%)	10.56 (40%)
Cream of Tartar (g)	0.00	0.15	1.50	2.25	3.00
Emulsifier[3]	0.00	0.66	1.31	1.96	2.62
Whip time (min)	4.00	6.00	8.00	10.00	12.00
Water (% flour basis)	185.00	190.00	195.00	200.00	205.00
Coded levels	-2	-1	0	+1	+2

[1]Determined by the experimental design.
[2]Pea protein concentrate; weight/weight replacement of egg albumen.
[3]Top-Scor S powder (Sodium Stearoyl Lactate); Breddo Food Products Corp., Kansas. Weights approximate 0.000%, 0.125%, 0.250%, 0.375% and 0.500% dry weight of a cake mix.

Table 3.4   Experimental Design

Treatment[1]	X_1 (PPC)	X_2 (Whip time)	X_3 (cream of tartar)	X_4 (Water)	X_5 (Emulsifier)
1	1	1	1	1	1
2	1	1	1	-1	-1
3	1	1	-1	1	-1
4	1	1	-1	-1	1
5	1	-1	1	1	-1
6	1	-1	1	-1	1
7	1	-1	-1	1	1
8	-1	1	1	1	-1
9	-1	1	1	-1	1
10	-1	1	-1	1	1
11	-1	-1	1	1	1
12	1	-1	-1	-1	-1
13	-1	1	-1	-1	-1
14	-1	-1	1	-1	-1
15	-1	-1	-1	1	-1
16	-1	-1	-1	-1	1
17	2	0	0	0	0
18	-2	0	0	0	0
19	0	2	0	0	0
20	0	-2	0	0	0
21	0	0	2	0	0
22	0	0	-2	0	0
23	0	0	0	2	0
24	0	0	0	-2	0
25	0	0	0	0	2
26	0	0	0	0	-2
27-32[2]	0	0	0	0	0

[1]Treatments were randomized.
[2]Treatments 27-32 are design center points.

Preliminary baking experiments indicated that the five independent variables chosen for this study were likely to account for most of the variability in sponge cake quality. The experiments also led to the development of a acceptable 30 percent PPC sponge cake which was used for the design center point. The other four levels of the variables were then chosen such that a fairly broad, but practical, range was covered. Therefore, because of the screening nature of this study, the ranges of PPC, cream of tartar and water levels have been set to be fairly broad. The levels of emulsifier were set such that the highest level approaches the limit permitted by Government regulations (0.5% sodium stearoyl lactylate of the weight of the dry ingredients). The highest level of whip time was set at 12 minutes because longer whip times are not likely to be commercially practical.

Instrumental/Physical Measurements

Batter specific gravity, cake specific volume, and crumb hardness, cohesiveness and gumminess were determined for each treatment.

i) <u>Batter specific gravity</u> (SG) was measured by dividing the weight of a 50 millilitre metal cup filled with water by the weight of the same cup filled with batter (Campbell et al., 1979). Duplicate measures were taken immediately after batter preparation.

ii) <u>Specific volume</u> of the baked cake was calculated by

dividing the volume of the cake (as determined by rapeseed displacement) by its weight (Campbell et al., 1979). Volume measurements were performed on the two cakes baked on the right and left hand sides of the oven. Duplicate measurements were made on frozen cakes to prevent crumb indentations. Cakes were wrapped in a thin film of plastic.

iv)   <u>Hardness, cohesiveness, gumminess and springiness</u> of the cake crumb were determined using the Instron Universal Testing Machine (Table model TM) and a Texture Profile data acquisition and analysis program developed for the Apple IIE computer (Agriculture Canada, 1987). Operating conditions were selected based upon preliminary experimentation (Chapter 2) in which a 616 mm^2 round plunger, 5.1 centimetres/minute crosshead speed and a 75 percent sample compression yielded good detection of textural differences for all four parameters. All Instron Texture Profiling was performed on cakes baked in the center of the oven. After eight days of frozen storage, the tops of each center cake was sliced off with the guidance of a two centimetre high, 15 centimetre square, plexiglass box. The cake bottom was then sliced into four equal wedges. Samples were immediately re-sealed and thawed for an additional two to three hours prior to testing. Sampling was done one cake at a time so that cakes did not thaw, in order to prevent the crumb from tearing. Thus texture measurements were made on four samples from each of 32 treatments. Chapter 2 describes, in detail, cake sample

preparation and Texture Profile method using the Instron.

Statistical Analyses

The RSREG procedure of the Statistical Analysis System (SAS, 1985) was used to conduct a joint test which indicated the overall effect (linear, quadratic, and interaction combined) of each of the 5 independent variables on the 6 dependent variables. This procedure was also used to test model adequacy (lack-of-fit).

A second order regression equation was fitted for each dependent variable using the GLM procedure (SAS, 1985a). Analyses were carried out on means of two readings for specific gravity and specific volume, and four readings for Instron texture measurements. For some treatments, problems with the Instron resulted in only two or three texture readings.

The GLM procedure produced Analysis of Variance tables for each dependent variable. Best fitting models were then selected based on these tables by eliminating variables of low significance (p<.05). If a linear effect was not significant but its quadratic or interaction term was, the linear term was also retained in the model. New models were re-analyzed using the GLM procedure (SAS, 1985a) in order to generate new parameter estimates for the final best fitting regression equations.

Contour plots were generated from the best fitting

equations using the GCONTOUR procedure (SAS, 1985b).

RESULTS AND DISCUSSION

Overview

The importance of PPC, whip time, cream of tartar, water and emulsifier to sponge cake quality, was first determined from a joint test and then by considering individual analyses of variance. For each independent variable, significant linear, quadratic and interaction effects were selected for best fitting models in order to predict their effects on sponge cake quality. These models were subsequently used to produce contour plots of the cake quality characteristics which best illustrated the effects of the most influential independent variables. These plots helped gain some preliminary understanding of the relationships between critical independent variables and their effects on sponge cake quality. Levels of PPC, whip time, cream of tartar, water and emulsifier, appropriate for further product optimization, were then set based upon their importance to, and effect on, sponge cake quality. Finally, recommendations have been made for further optimization of a PPC-albumen sponge cake formulation.

Importance of Independent Variables to Sponge Cake Characteristics

The first objective of this study was to determine the importance of PPC, whip time, cream of tartar, water and emulsifier to selected sponge cake quality characteristics, in order to identify the most critical variables for further

product optimization. Table 3.5 summarizes the F-values and corresponding levels of significance (probability values) from a joint test showing the combined linear, quadratic and interaction effects of each dependent variable on each dependent variable. For example, the F-value for the overall effect of PPC on batter SG (15.87) reflects the combined effects of PPC, PPC^2, PPC*whip time, PPC*cream of tartar, PPC*water, and PPC*emulsifier. The F-values and their corresponding probability values indicate the importance of the independent variables to the prediction of each dependent variable. It is clear from this table that PPC, whip time and cream of tartar were the most important independent variables influencing all sponge cake characteristics except springiness. Springiness was not significantly influenced by any of the variables evaluated and therefore, was eliminated from further analysis. Pea protein concentrate had the greatest effect on batter SG, cake specific volume, and crumb cohesiveness, while length of whip time was most critical to Instron hardness and gumminess. Cream of tartar, although significant for all characteristics, was less influential. Water level had a slight, but significant effect on specific volume and cohesiveness, while the combined linear, quadratic and interaction effects of emulsifier were not significant for any characteristic.

Table 3.6, an expansion of Table 3.5, provides the F-values (from the full model analyses of variance) for the

Table 3.5   F-values for a Joint Test[1] on all Parameters Involving Each Dependent Variable Physical Measurements

Independent Variables	Dependent Variable (yi)					
	Specific Gravity	Specific Volume	Hardness	Cohesiveness	Gumminess	Springiness
PCC	15.87 (.0001)[2]	51.84 (.0001)	9.41 (.0008)	46.78 (.0001)	5.67 (.0066)	1.85 (.1788)
Whip time	13.65 (.0002)	23.27 (.0001)	16.90 (.0001)	33.77 (.0001)	13.05 (.0002)	0.97 (.4852)
Cream of tartar	3.94 (.0239)	4.68 (0133)	6.31 (.0044)	5.52 (.0073)	5.56 (0071)	0.65 (.6877)
Water	2.50 (.0892)	3.30 (.0416)	2.30 (.1100)	3.38 (.0384)	1.77 (.1942)	0.79 (.5937)
Emulsifier	1.82 (.1848)	1.03 (.4568)	1.11 (.4132)	1.73 (.2047)	0.93 (.5081)	0.19 (.9735)

[1]Egs. PPC, PPC², PPC*WHIP, PPC*TARTAR, PPC*WATER, PPC*EMULSIFIER. RSREG procedure (SAS, 1985).
[2]Probability associated with F-value.

Table 3.6 F-values[1] from the Full Model Analysis of Variance[2] for All Dependent Variables[3]

Effects of Independent Variables		Dependent Variable (y_i)			
	Specific Gravity	Specific Volume	Hardness	Cohesiveness	Gumminess
Linear					
X_1 (PPC)	59.24(.0001)[4]	267.06(.0001)	25.11(.0004)	199.98(.0001)	12.72(.0044)
X_2 (Whip time)	27.26(.0003)	71.94(.0001)	53.18(.0001)	83.05(.0001)	43.91(.0001)
X_3 (Cream of tartar)	4.87(.0496)	4.14(.0666)	17.32(.0016)	0.10(.7592)	18.45(.0013)
X_4 (Water)	4.04(.0696)	15.67(.0022)	3.32(.0959)	0.03(.8687)	2.59(.1355)
X_5 (Emulsifier)	1.27(.2835)	2.00(.1851)	0.28(.6091)	4.22(.0644)	0.55(.4731)
Quadratic					
X_1^2	7.10(.0220)	7.12(.0219)	1.82(.2048)	12.44(.0047)	0.96(.3486)
X_2^2	14.46(.0029)	22.38(.0006)	16.74(.0018)	34.25(.0001)	12.11(.0052)
X_3^2	0.18(.6764)	4.43(.0590)	0.21(.6538)	0.64(.4397)	0.24(.6305)
X_4^2	0.91(.3609)	0.38(.5518)	0.62(.4467)	4.35(.0612)	1.14(.3092)
X_5^2	0.45(.5174)	0.63(.4447)	0.60(.4539)	0.03(.8755)	0.64(.4416)
Interactions					
X_1X_2	24.84(.0004)	29.18(.0002)	19.47(.0010)	54.35(.0001)	13.43(.0037)
X_1X_3	3.93(.0731)	4.91(.0487)	8.43(.0144)	8.25(.0152)	5.90(.0335)
X_1X_4	0.09(.7696)	1.39(.2639)	1.63(.2278)	4.93(.0483)	1.02(.3343)
X_1X_5	0.01(.9299)	1.39(.2639)	0.00(.9458)	0.74(.4089)	0.01(.9366)
X_2X_3	14.08(.0032)	14.51(.0029)	10.55(.0078)	23.29(.0005)	7.72(.0179)
X_2X_4	0.98(.3431)	0.94(.3531)	1.28(.2811)	6.33(.0287)	0.78(.3970)
X_2X_5	0.29(.5997)	0.69(.4236)	0.16(.6993)	1.33(.2727)	0.33(.5785)
X_3X_4	0.33(.5799)	0.02(.8923)	1.30(.2781)	0.74(.4089)	1.05(.3280)
X_3X_5	0.23(.6404)	0.08(.7869)	0.02(.8997)	0.11(.7508)	0.02(.9000)
X_4X_5	8.66(.0134)	1.39(.2639)	5.63(.0370)	3.93(.0728)	4.06(.0689)

[1]Associated with partial (Type III) sums of squares.
[2]GLM procedure (SAS, 1985). Analysis based on the mean of 2 determinations for specific gravity and specific volume, and 4 determinations for hardness, cohesiveness and gumminess.
[3]Springiness omitted from analysis due to lack of significance.
[4]Probability associated with F-values.

Full model = $y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 + b_{55}X_5^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{15}X_1X_5 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{25}X_2X_5 + b_{34}X_3X_4 + b_{35}X_3X_5 + b_{45}X_4X_5$

linear, quadratic and interaction effects associated with each independent variable. The significant overall effect of PPC on sponge cake quality (Table 3.5) appears to have come from the strong linear and interaction effects evident for all characteristics, and to a lesser extent, from quadratic effects (SG, specific volume, cohesiveness). For whip time, however, the presence of strong linear, quadratic and interaction effects for all characteristics, accounted for its significant overall effect on sponge cake quality. The most notable interaction for both PPC and whip time, were their interactions with each other, strengthening the importance of these two variables to sponge cake quality.

The linear effects of cream of tartar for SG, hardness and gumminess, and fairly strong interactions with PPC and whip time for all characteristics but SG, account for its small, but significant overall effect on sponge cake quality. The slight, but significant effect of water on specific volume was due to a significant linear effect, while the small effect on cohesiveness was due to interactions with both PPC and whip time. A small but significant interaction of water with emulsifier was also present for both SG and hardness, however, the overall effect of water or emulsifier on these two characteristics, was not significant (Table 3.5).

The foregoing analyses have clearly indicated that the level of PPC and length of whip time were the independent variables most important to sponge cake quality, followed by

the level of cream of tartar and water. Emulsifier level was found to have very little influence on final cake quality. Emulsifiers have been used in sponge cakes as egg saving ingredients and to provide faster whipping rates, improved volume and texture (Flack, 1983). Distilled saturated monoglycerides (liquid crystalline dispersion or gel form) are said to be the most effective aerating agents for sponge cakes (Krog, 1977; Flack, 1983), yet some sucrose fatty acid esters have also been found to improve sponge cake volume, tenderness and texture (Pierce and Walker, 1987). Andres (1979) described a spray dried emulsifier, composed of corn syrup solids, monoglycerides, propylene glycol of fatty acids, sodium stearate and diacetylated tartaric acid esters of monoglycerides, which allowed flexible production of high quality sponge cakes. Perhaps sodium stearoyl lactylate was not appropriate for a sponge cake application in which foam formation and stability were critical. Sodium stearoyl lactylate is a hydrophillic emulsifier used primarily as a dough conditioner in baked products (Anonymous, 1988). Dough conditioners mainly interact with the gluten proteins during mixing (Krog, 1977) and therefore, may be more appropriate for bread products than sponge cakes.

It has been suggested that, when using RSM, the number of independent variables investigated be limited to no more than three (Giovanni, 1983). Therefore, further optimization of this formulation should include evaluation of PPC level,

whip time, and cream of tartar level. Although water and emulsifier levels had only slight effects on sponge cake quality, they should still be set at levels most beneficial to the sponge cake formulation. This will be addressed during the discussion of independent variable levels appropriate for further product optimization.

Selection of Best Fitting Models for Each Sponge Cake Characteristic

The second objective of this study was to select best fitting models to predict the effects of PPC, whip time, cream of tartar, water and emulsifier on each sponge cake characteristic. Because lack-of-fit tests, indicating the adequacy of the second-order models, were not significant for any dependent variables, analyses could proceed.

The goal of a best fit model is to provide a useful prediction equation which is composed of only the most important explanatory variables. Selection of the best fitting models was based upon the significance of linear, quadratic and interaction effects found in Table 3.6. Statistically significant effects ($p<.05$) were retained in each model while those having little effect were eliminated. Linear effects which were not significant but appeared in significant quadratic or interaction effects, were also retained in each model.

Best models were re-analyzed to produce regression coefficients for use in predictive equations. The regression

coefficients, coefficients of determination (R^2), coefficients of variation (CV) and overall model significance for each best selected model are summarized in Table 3.7. Joglekar and May (1987) have suggested that R^2 values, CV values and overall model significance are three measures to evaluate the goodness of a selected model. R-square value is the percentage of the variation in the dependent variable explained by the model, CV value describes the amount of variation in a population relative to the mean, and model significance indicates the level of confidence that the selected model cannot be due to experimental error. According to these authors, for good model fit, R^2 values should be at least 80 percent, CV values should not exceed 10 percent and model significance should be at least p<.05. In this study, all three requirements were met for each dependent variable with the exceptions of Instron hardness and gumminess whose CV values (14.4% and 14.0%, respectively) exceeded 10 percent, indicating fairly high variability for these two characteristics. Sampling procedures, or variability within the cake crumb itself, could possibly account for the observed variability. R-square values remained high (.82 to .95) when full models were reduced to include only those variables making a significant contribution. Thus, the models for batter SG, cake specific volume and Instron cohesiveness were considered to be highly adequate for predicting sponge cake quality, while the models for Instron hardness and gumminess possessed less predictive

Table 3.7 Regression Equation Coefficients, R-Square Values and Coefficients of Variation Associated with Selected Best Fitting Models for Each Dependent Variable

Effects of Independent Variables	Dependent Variable (y_i)				
	Specific Gravity	Specific Volume	Hardness	Cohesiveness	Gumminess
b_o	0.449(.0001)[2]	3.28(.0001)	16.21(.0001)	0.65(.0001)	10.44(.0001)
Linear					
b_1 (PPC)	0.026(.0001)	-0.24(.0001)	2.76(.0001)	-0.02(.0001)	1.13(.0018)
b_2 (Whip time)	-0.018(.0001)	0.13(.0001)	-4.01(.0001)	0.02(.0001)	-2.09(.0001)
b_3 (Cream of tartar)	-0.008(.0283)	0.03(.0663)	-2.29(.0003)	-0.00(.7910)	-1.36(.0003)
b_4 (Water)	-0.007(.0437)	-0.06(.0011)	1.00(.0689)	0.00(.8865)	-
b_5 (Emulsifier)	0.004(.2420)	-	-0.29(.5853)	-	-
Quadratic					
b_{11}	0.008(.0074)	-0.03(.0253)	-	-0.01(.0053)	-
b_{22}	0.012(.0004)	-0.06(.0002)	2.03(.0003)	-0.01(.0001)	1.00(.0018)
Interactions					
b_{12}	-0.021(.0001)	0.10(.0001)	-2.97(.0001)	0.02(.0001)	-1.42(.0014)
b_{13}	-	0.04(.0470)	-1.96(.0060)	0.01(.0234)	-0.94(.0247)
b_{14}	-	-	-	-0.00(.0722)	-
b_{23}	0.016(.0006)	-0.07(.0015)	2.19(.0026)	-0.01(.0005)	1.07(.0113)
b_{24}	-	-	-	0.01(.0439)	-
b_{45}	0.012(.0049)	-	1.60(.0208)	-	-
R^2	.90	.95	.89	.94	.82
CV (%)	3.4	2.4	14.4	1.5	14.0
Model Significance	.0001	.0001	.0001	.0001	.0001

[1]Selection based on significant F-values ($p \le .05$) from Table 3.2. For any independent variable appearing in a significant quadratic or interaction term, the linear effect, although not significant, was retained in the model.
[2]Probability associated with the coefficients.

ability due to variability of the measurements.

While F-values from the full model analyses of variance indicated which independent variables significantly affected the dependent variables, the regression coefficients represent the incremental change in the dependent variable (y_i) associated with a unit change in the independent variable (x_i), while all other regressors remain constant (Wonnacott and Wonnacott, 1982). The sign preceding the coefficient indicates the direction in which to change the variables to improve the response. The size of the coefficients and their associated probability values denotes the relative importance of the independent variable to the prediction of the dependent variable.

The influence of both PPC level and length of whip time on all dependent variables was again apparent. Based on the coefficients for the linear effects, a decrease in PPC level, holding all other regressors constant, should decrease batter SG, increase cake volumes, and produce a softer, less gummy, more cohesive sponge cake. An increase in the length of whip time should achieve similar results. An increase in the level of cream of tartar should also improve (lower) batter SG, and produce a softer, less gummy cake texture. These interpretations have only considered the linear effects of PPC, whip time and cream of tartar assuming that all other regressors have been held constant, and are, therefore, oversimplified. The presence of strong interactions and

quadratic effects make it difficult to interpret the actual relationships specified by the prediction equations. Consequently, contour plots were generated to help visualize the information contained in the best fitting prediction equations.

Interpretation of Results Based upon Contour Plots

The third objective of this study was to use the best fitting models to produce contour plots which would give information on the relationships between the most influential independent variables, PPC, whip time and cream of tartar, and their effects on sponge cake quality. The number of contour plots possible from this study were too numerous to produce, therefore only those necessary to convey critical information were generated.

Effects of PPC, Whip Time and Cream of Tartar on Sponge Cake Characteristics

Best fitting models for batter SG, cake specific volume, Instron hardness, cohesiveness and gumminess indicated that all five variables were significantly affected by PPC, whip time and cream of tartar. Therefore, for all contour plots, PPC and whip time, the two most important variables, were chosen for the plot axes. Cream of tartar, the least important of the three, was held constant at low (0.0g), medium (1.5g), and high (3.0g) levels in order to evaluate its effect on the dependent variables. Water and emulsifier

levels were held constant at their center point levels (195%
and 1.31g, respectively) since the effect of these two
variables on sponge cake quality was small.

Highly significant interactions between PPC and whip time
were present for all sponge cake characteristics indicating
that the effects of these two variables on sponge cake quality
depended upon the specific PPC-whip time combination used.
Therefore, the effect of PPC at low and high whip times, and
the effect of whip time at low and high PPC levels, will be
discussed for each sponge cake characteristic. The effect of
increasing PPC will be evaluated by moving along the Y (% PPC)
axis at 4 and 12 minutes of whip time (Figure 3.1). The
effect of increasing whip time will be discussed by moving
along the X (whip time) axis at 0% and 60% PPC levels (Figure
3.2). Significant interactions of both PPC and whip time with
cream of tartar (PPC*tartar; whip time*tartar) were also
present for all dependent variables (except batter SG for
which the PPC*tartar interaction was not significant),
indicating that the effects of PPC and whip time were also
dependent on the level of cream of tartar. These
relationships will be briefly explored for each dependent
variable by evaluating cream of tartar levels.

The levels presented in the plots represent the actual
levels evaluated and were based upon the range of coded levels
determined by the experimental design (-2, -1, 0, +1, +2).
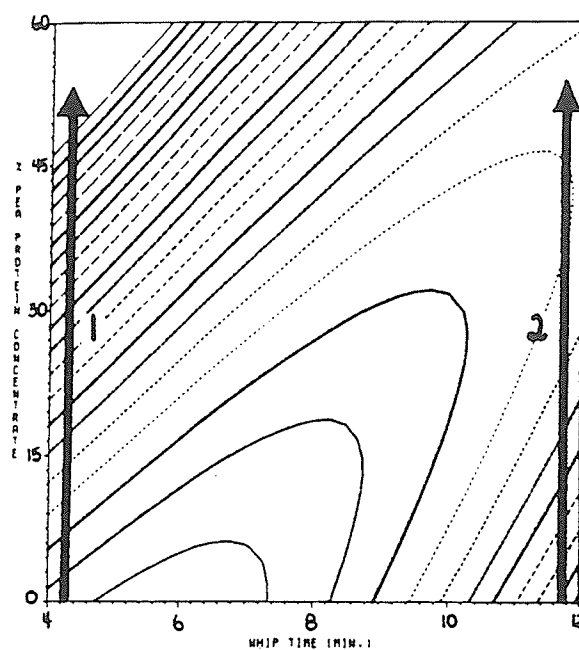Contour lines on the plots show how levels of the dependent

Figure 3.1 Method for discussion of effects of increasing PPC level at low (4 min.) and high (12 min.) whip times.
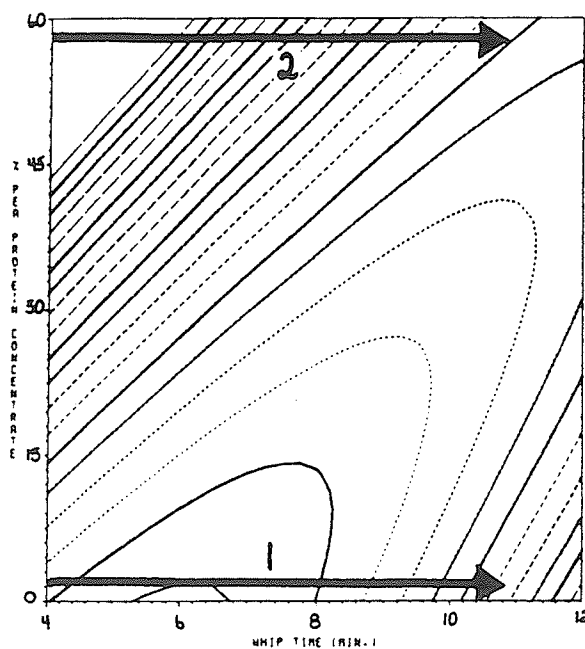

Figure 3.2   Method for discussion of effects of increasing whip time at low (0%) and high (60%) PPC levels.

variable changed as the levels of PPC, whip time and cream of tartar were varied.

i) Effects of PPC, Whip Time and Cream of Tartar on Batter SG

Figure 3.3 presents batter SG as a function of PPC level and whip time, when cream of tartar was held constant at 0.0, 1.5, and 3.0 grams. For these plots, water and emulsifier were held constant at their center point levels.

At low whip times, increasing PPC resulted in an increase in batter SG, indicating that less air was incorporated into the batter. This effect was evident at all cream of tartar levels, however, at high cream of tartar levels SG values were lower for each level of PPC.

At high whip times, higher proportions of PPC first decreased SG, and then, as PPC levels approached the 60 percent level, increased it slightly. Once again, this effect was evident at all cream of tartar levels. Specific gravity values were, however, higher as cream of tartar levels increased. Low cream of tartar levels and long whip times were required to achieve low SG values with high amounts of PPC.

At low PPC levels and low to medium levels of cream of tartar, specific gravities first decreased and then increased as whip times increased. However, at the intermediate cream of tartar level (1.5g), SG values began to increase at a shorter whip time than the lowest level (0.0g). At the

BATTER SPECIFIC GRAVITY

Cream of tartar = 0.0g   Cream of tartar = 1.5g
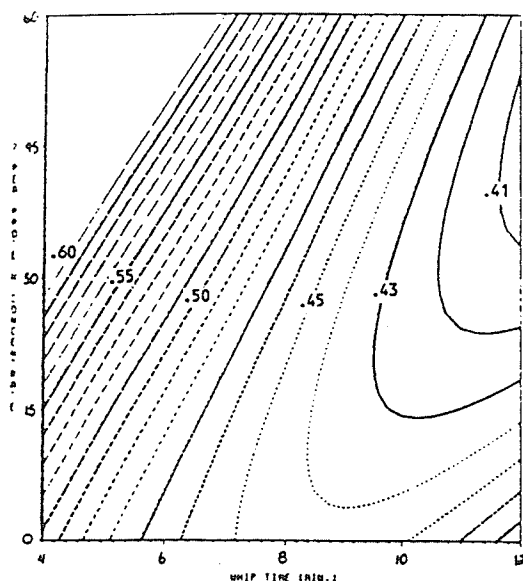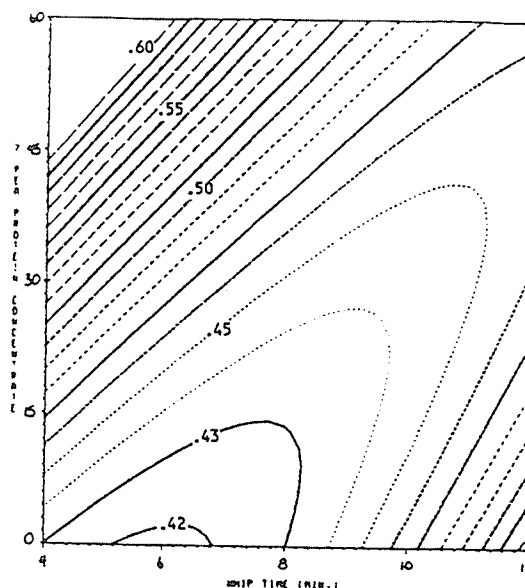
Cream of tartar = 3.0g

Figure 3.3 Contour plots for the effects of PPC and whip time on batter specific gravity (SG) at low, medium and high cream of tartar levels. Water and emulsifier were held constant at their center point levels. Higher values represent increasing SG. Levels of PPC, whip time and cream of tartar are actual values.

highest cream of tartar level, increasing whip time resulted in an increase in SG.

At high PPC levels, increasing whip time decreased SG values for all cream of tartar levels. The SG lowering effect of increasing whip time was not, however, as pronounced at the highest cream of tartar level, as indicated by the flattened lines.

In general, when PPC levels were high, longer whip times were required to incorporate more air into the batter (ie., decrease batter SG values). Conversely, when PPC levels were low, shorter whip times were required to keep batter SG values low since extended whip times increased SG values. Increasing the level of cream of tartar shortened the whip time necessary to achieve low specific gravities with moderately high PPC formulations.

ii) Effects of PPC, Whip Time, and Cream of Tartar on Cake Specific Volume

Figure 3.4 presents specific volume as a function of PPC level and whip time, with cream of tartar held constant at 0.0, 1.5, and 3.0 grams, respectively. Water and emulsifier were held constant at their center point levels.

At low whip times, increasing PPC had a negative effect on specific volume, that is, volumes decreased. This effect was evident at all cream of tartar levels, however, the volume depressing effect of PPC was minimized as cream of tartar levels increased.

SPECIFIC VOLUME (CC/G)

Cream of tartar = 0.0g   Cream of tartar = 1.5g
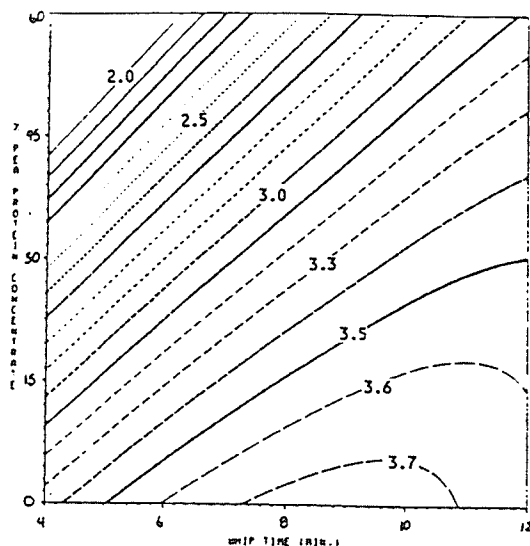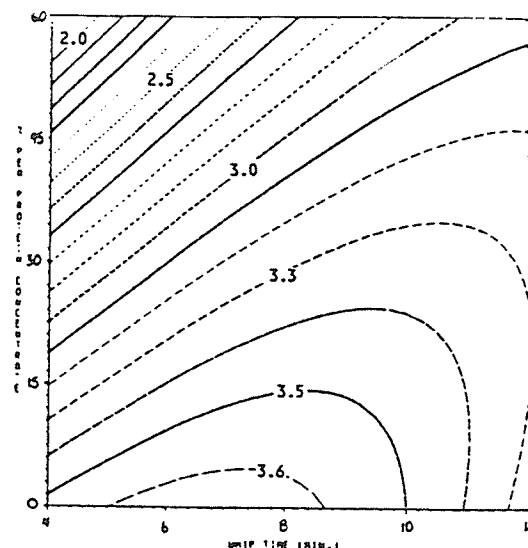
Cream of tartar = 3.0g
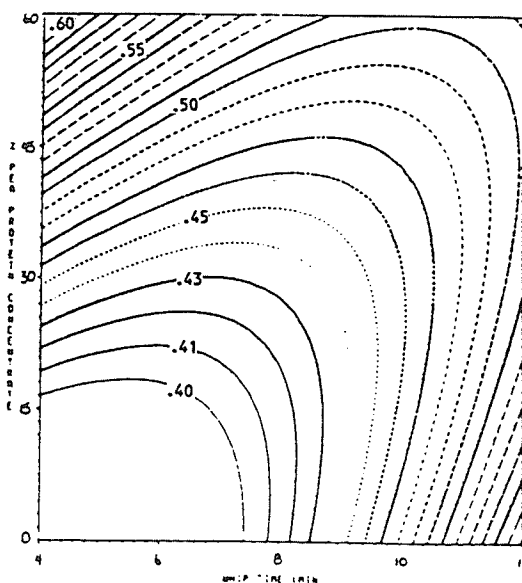
Figure 3.4 Contour plots for the effects of PPC and whip time on cake specific volume at low, medium and high cream of tartar levels.   Water and emulsifier were held constant at their center point levels. Higher values represent increasing specific volume. Levels of PPC, whip time and cream of tartar are actual values.

At high whip times, specific volumes increased with increasing amounts of PPC, particularly at the low cream of tartar level. As cream of tartar levels increased, whip time had little effect on volume.

At low PPC levels and low to medium cream of tartar levels, specific volumes first rose and then fell as whip times increased. However, at the medium cream of tartar level, volumes began to fall at a shorter whip time than the lowest cream of tartar level. At the highest cream of tartar level, shorter whip times produced only slight decreases in volume, however continued whipping resulted in more dramatic volume losses.

At high PPC levels, increasing whip time improved specific volumes regardless of cream of tartar level. The effect of whip time on specific volume was, however, more pronounced when cream of tartar levels were low.

In general, when PPC levels were increased without increasing whip times, cake volumes fell. Increasing the length of whip time was necessary if volumes were to remain high with the addition of moderate amounts of PPC. While high specific volumes were possible at low cream of tartar levels, low PPC levels and long whip times were necessary to achieve this result. Increasing the level of cream of tartar also helped minimize the volume depressing effects of PPC incorporation. That is, comparable specific volumes could be achieved using high PPC levels and shorter whip times when

cream of tartar levels were high, rather than low.

iii) Effects of PPC, Whip Time and Cream of Tartar on Instron Hardness

Figure 3.5 illustrates Instron hardness as a function of PPC level and whip time, with cream of tartar held constant at 0.0, 1.5, and 3.0 grams, respectively. Water and emulsifier were held constant at their center point levels.

At low whip times, increasing the level of PPC increased crumb hardness at low and medium levels of cream of tartar. At the highest cream of tartar level, increasing PPC had a slight crumb softening effect.

At high whip times, increasing PPC softened the cake crumb. This was evident at all cream of tartar levels, however was most apparent when cream of tartar levels were high.

At low PPC levels, increasing the whip time of low and medium cream of tartar formulations first decreased hardness and then increased it. At the intermediate cream of tartar level, however, the increase in hardness occurred at a shorter whip time than when no cream of tartar was used (lowest level). At the highest cream of tartar level short whip times produced only slight increases in hardness, however the effects of additional whipping on hardness, were more pronounced.

At high PPC levels, increasing the length of whip time produced softer cakes, regardless of cream of tartar level.

INSTRON HARDNESS (N)

Cream of tartar = 0.0g
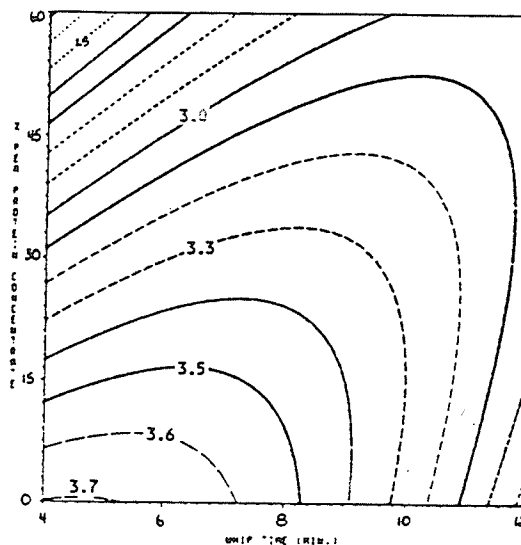
Cream of tartar = 1.5g

Cream of tartar = 3.0g

Figure 3.5 Contour plots for the effects of PPC and whip time on Instron hardness at low, medium and high cream of tartar levels. Water and emulsifier were held constant at their center point levels. Higher values represent increasing hardness. Levels of PPC, whip time and cream of tartar are actual values.

The crumb softening effect was, however, more pronounced when cream of tartar levels were low.

In general, increasing PPC without increasing whip time resulted in cakes which were harder than those produced when whip times had been increased. Increasing PPC levels combined with long whip times actually produced a crumb softening effect. The effects of cream of tartar were also quite apparent from these plots. The high cream of tartar level allowed the production of soft cakes from high PPC formulations using shorter whip times than cakes made with lower levels of cream of tartar.

iv)   Effects of PPC, Whip Time and Cream of Tartar on Instron Cohesiveness

Figure 3.6 presents Instron cohesiveness as a function of PPC level and whip time, with cream of tartar held constant at 0.0, 1.5, and 3.0 grams, respectively. Water and emulsifier were held constant at their center point levels.

At low whip times, increasing PPC decreased cake cohesiveness across all cream of tartar levels. The greater width between the contour lines of the highest cream of tartar plot indicates that the effect was minimized when cream of tartar levels were high.

At high whip times and low cream of tartar level, increasing PPC had little effect on crumb cohesiveness until protein levels exceeded approximately 40 percent, after which time cohesiveness began to decrease. When intermediate levels
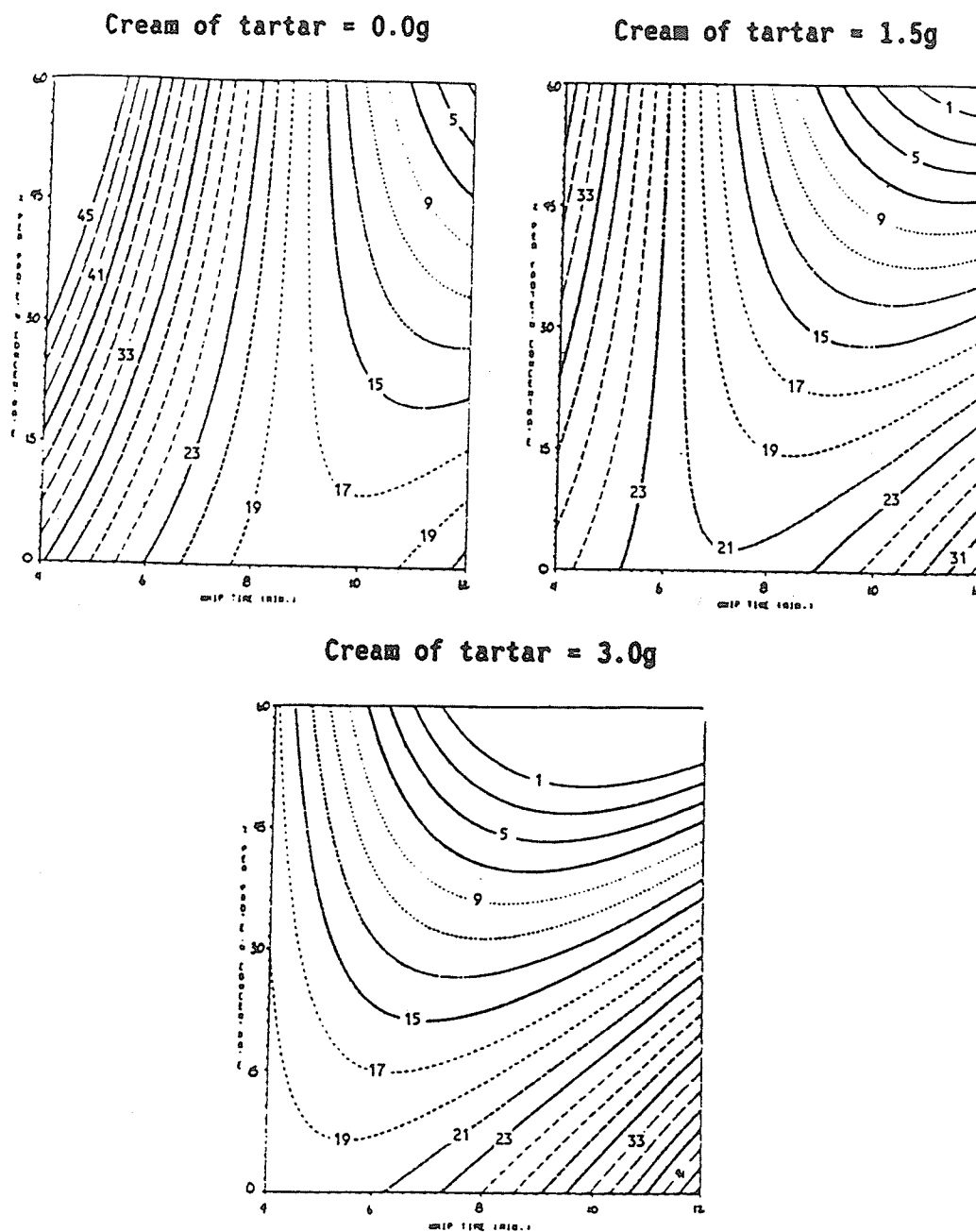
INSTRON COHESIVENESS
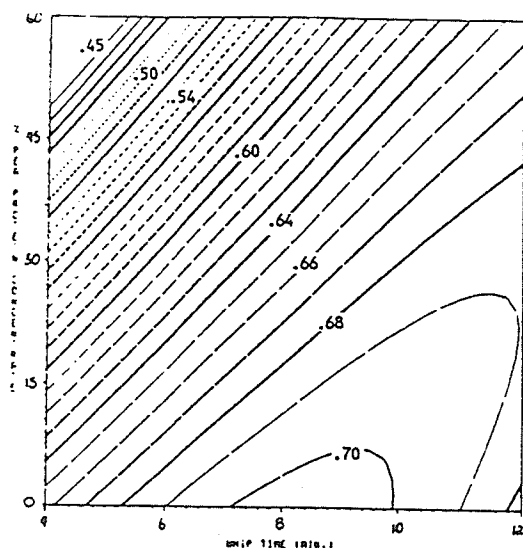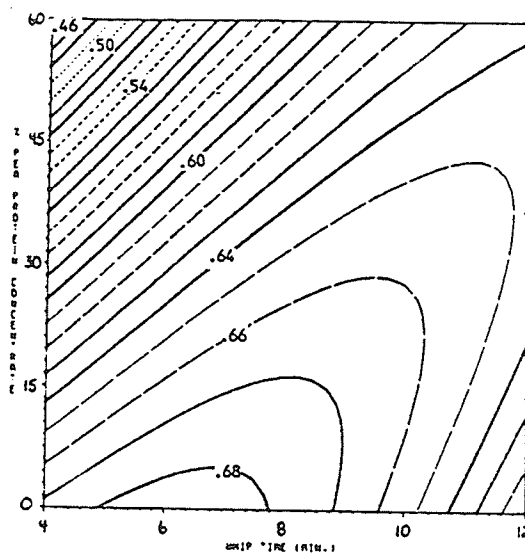
Figure 3.6 Contour plots for the effects of PPC and whip time on Instron cohesiveness at low, medium and high cream of tartar levels. Water and emulsifier were held constant at their center point levels. Higher values represent increasing cohesiveness. Levels of PPC, whip time and cream of tartar are actual values.

of cream of tartar were combined with high whip times, increasing PPC had little effect on crumb cohesiveness. Cohesiveness increased slightly, remained constant, and then began to decrease once PPC levels reached 60 percent. The greatest effect of increasing PPC, when whip times were high, was apparent when the level of cream of tartar was also high. Cohesiveness values increased quite dramatically when PPC levels increased. This was particularly true when PPC levels were low.

At low PPC levels, it can be seen that for low and medium cream of tartar levels, cohesiveness first rose and then fell as whip times increased. The critical whip time at which cohesiveness began to fall, however, was shorter for the intermediate cream of tartar formulation (8 vs 10 min.). At the highest cream of tartar level, increasing whip time produced a decrease in cake cohesiveness, which was particularly evident at the higher whip times.

At high PPC levels, increasing whip time caused crumb cohesiveness to increase across all cream of tartar levels. This effect was most apparent at low cream of tartar levels.

In general, cake cohesiveness was seen to decrease when short whip times were accompanied by increasing levels of PPC. Increasing the length of whip time minimized the negative effect of PPC on cake cohesiveness. While low cream of tartar levels produced the highest cohesiveness values, low PPC levels and long whip times were necessary to achieve this end.

When whip times were short (4-6 min.), high cream of tartar levels made it possible to incorporate more PPC and achieve similar cake cohesiveness. However, once whip times increased, low cream of tartar levels permitted greater PPC incorporation while achieving similar cake cohesiveness.

v) Effects of PPC, Whip Time and Cream of Tartar on Instron Gumminess

Figure 3.7 presents Instron gumminess as a function of PPC level and whip time, with cream of tartar held constant at 0.0, 1.5, and 3.0 grams, respectively. Water and emulsifier were held constant at their center point levels.

At low whip times, increasing PPC increased gumminess across all cream of tartar levels, however, this negative effect was lessened as cream of tartar levels were increased.

At high whip times, increasing PPC had varying effects across cream of tartar levels. At the lowest cream of tartar level (0.0g), increasing PPC had very little effect on gumminess. As cream of tartar level was increased, however, increasing PPC had greater effects on gumminess. For both the intermediate and high cream of tartar levels (1.5g and 3.0g), gumminess was seen to decrease as PPC was increased. This effect was most pronounced at the high level of cream of tartar.

At low PPC levels and low to intermediate cream of tartar levels, increasing the length of whip time caused gumminess to first decrease and then increase once again after exceeding

INSTRON GUMMINESS (N)

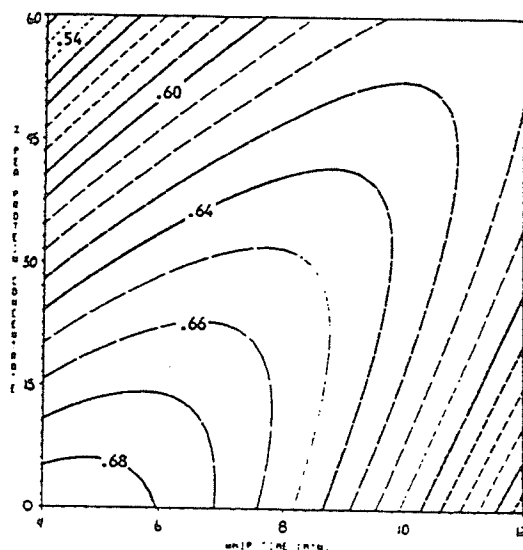## Cream of tartar = 0.0g   Cream of tartar = 1.5g
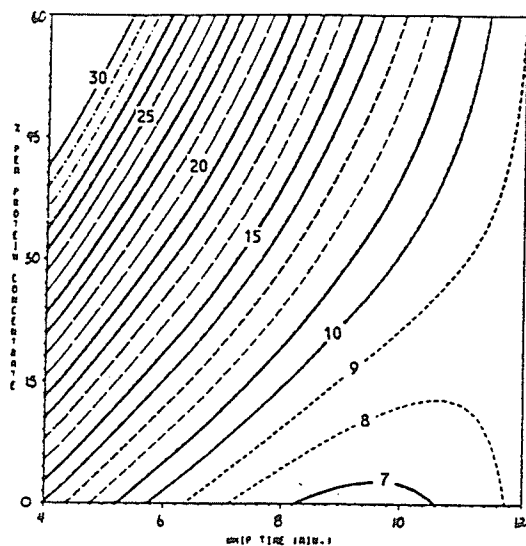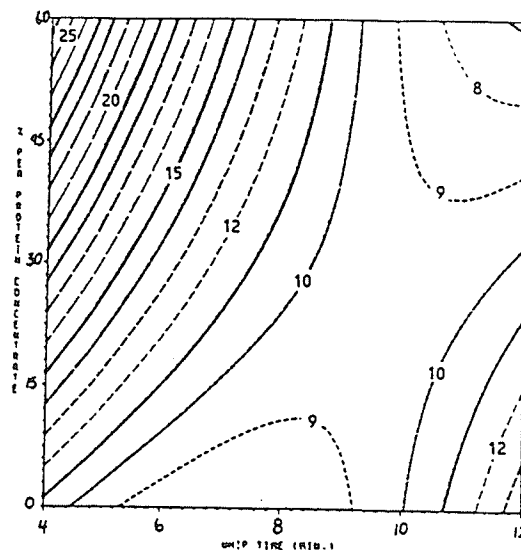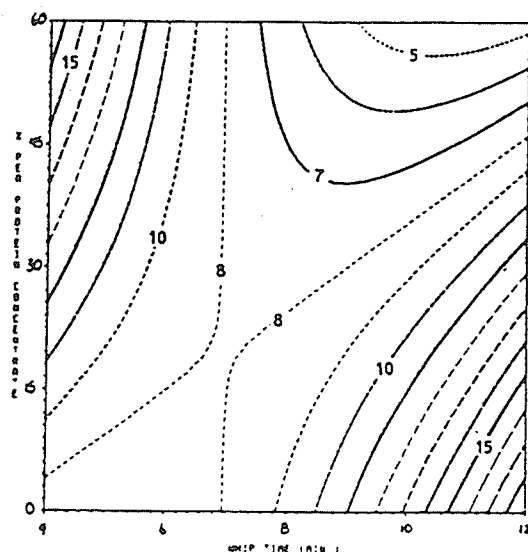
Cream of tartar = 3.0g

Figure 3.7 Contour plots for the effects of PPC and whip time on Instron gumminess at low, medium and high cream of tartar levels. Water and emulsifier were held constant at their center point levels. Higher values represent increasing gumminess. Levels of PPC, whip time and cream of tartar are actual values.

a critical whip time. This critical whip time was much shorter when cream of tartar was increased from 0.0 grams to 1.5 grams. At the highest level of cream of tartar, increasing whip time to approximately 8 minutes had little effect on gumminess. Longer whipping, however, produced quite rapid increases in gumminess.

At high PPC levels, increasing whip time decreased cake gumminess at all cream of tartar levels. As cream of tartar levels increased, long whip times were seen to have less effect on gumminess values. This is particularly evident at the highest cream of tartar level.

In general, cake gumminess increased when short whip times were coupled with increasing PPC levels. However, increasing the length of whip time helped improve crumb texture by decreasing gumminess. The high cream of tartar level made it possible to achieve reduced cake gumminess from high PPC formulations using shorter whip times than those required by cakes made with lower cream of tartar levels.

vi) Summary of Effects of PPC, Whip Time, and Cream of Tartar on Sponge Cake Quality

Contour plots generated from the best fitting regression equations have helped provide a preliminary understanding of the relationships between PPC, whip time and cream of tartar, and their effects on sponge cake quality. Because substitution of substantial amounts of PPC for egg albumen is an ultimate goal of the final sponge cake formulation, it is

important to understand the effects of this substitution on sponge cake quality, and how it may be influenced by the length of whip time and level of cream of tartar.

Generally, when whip times were low, increasing the level of PPC had a negative effect on sponge cake quality. Batter SG, crumb hardness, and gumminess increased with PPC addition while cake volumes and crumb cohesiveness decreased. However, the negative effect of substantial PPC substitution could be minimized and, in some cases eliminated, by simply increasing the length of whip time. The length of whip time necessary to improve PPC sponge cake quality was, however, dependent upon both the levels of PPC and cream of tartar used in the formulation. The higher the level of PPC, the longer the whip time necessary to improve cake quality. This length of whip time was then shortened as cream of tartar level was increased. That is, for moderately high PPC formulations, increasing the level of cream of tartar shortened the whip time necessary to achieve cakes of comparable quality. Conversely, if a specific whip time was selected, increasing the level of cream of tartar would permit greater PPC incorporation while achieving comparable cakes.

The importance of PPC, whip time and cream of tartar to sponge cake quality has been established. Contour plots illustrated that, while the substitution of PPC for egg albumen lowered the quality of sponge cakes, the negative effects could be virtually eliminated with the appropriate

adjustment of whip times and cream of tartar levels. This study has, however, only provided a preliminary look at the relationships between PPC, whip time and cream of tartar, and their effects on sponge cake quality. Further investigation is required to more fully understand the consequences of PPC substitution for egg albumen in a sponge cake system. In order to do so, it is important to determine whether the range of independent variable levels chosen for this study is appropriate for further product optimization, or whether they need to be revised to improve more upon cake quality.

Identification of Independent Variable Levels Appropriate for Further Product Optimization

The final objective of this study was to identify the best range of PPC levels, cream of tartar levels, and whip times, to use in final optimization of the sponge cake formulation. Water and emulsifier levels were found to have very little effect on sponge cake quality, yet in further work it would be beneficial to set these at levels most advantageous to the sponge cake formulation. In all contour plots presented, water and emulsifier levels were conveniently held constant at their center point levels (195% and 1.31g, respectively) while the effects of PPC, whip time and cream of tartar were evaluated. It is possible that these levels were not the most beneficial levels for the sponge cake formulation. The setting of water and emulsifier levels will be considered after the levels of the more critical

independent variables have been evaluated.

Evaluation of Cream of Tartar Levels

The cream of tartar levels evaluated in this study ranged from 0.0 grams to 3.0 grams. The positive influence of cream of tartar on whip times and PPC incorporation has already been shown. It is possible that with even higher levels of cream of tartar whip times could be reduced even further, or PPC levels increased, without any loss of cake quality. Because shorter whip times and increased PPC incorporation are goals of the ultimate sponge cake formulation, future investigations should evaluate higher cream of tartar levels. A practical level for the design center point of an optimization RSM experiment would be 3.0 grams, with lower and higher levels defined by the experimental design. This range of levels would test whether higher amounts of cream of tartar were more beneficial to the formulation, while still evaluating levels similar to those tested in this screening study.

Evaluation of Whip Times

In this study, the whip times tested ranged from 4 minutes to 12 minutes in length. Contour plots indicated that, depending upon the levels of PPC and cream of tartar, all whip times were capable of producing cakes with low SG values, high specific volumes, low hardness and gumminess values and high cohesiveness values. It was clear that when

PPC levels were high, longer whip times were required to produce cakes comparable to low PPC cakes. Conversely, when PPC levels were low, whip times had to be shortened to produce high quality cakes. Thus the range of whip times tested in this study would be suitable for evaluation in a future optimization study, with a center point whip time of 8 minutes. If whip times were increased beyond 12 minutes, it might facilitate incorporation of more PPC, however, this extended whipping time would not be practical from a commercial standpoint.

Evaluation of PPC Levels

A broad range of PPC levels was evaluated in this study spanning from 0 percent PPC (100% albumen) to 60 percent PPC (40% albumen). Although cake quality was very poor when high PPC levels were combined with short whip times, quality was improved substantially when whip times were longer. Higher cream of tartar levels might also make possible successful incorporation of greater amounts of PPC. Therefore, the range of PPC levels chosen for this study should be appropriate for further optimization work, with a center point egg albumen replacement level of 30 percent.

Evaluation of Water and Emulsifier Levels

The levels of the two less critical independent variables, water and emulsifier, should be set at levels most

advantageous to the sponge cake formulation. Water level significantly affected specific volume measurements while emulsifier alone did not significantly influence any of the dependent variables (Table 3.6). Water and emulsifier did, however, interact to produce a slight but significant effect on SG and hardness measurements.

Although water and emulsifier had small effects on sponge cake quality, their slight effect on specific volume, SG and hardness should be considered when determining the levels most likely to improve the sponge cake formulation. Four sequential steps were taken to identify the levels of water and emulsifier most likely to result in the most acceptable specific volumes, SG and hardness values. The first two steps involved, 1) identification of water/emulsifier combinations best able to improve (decrease) SG and hardness, and 2) water levels most likely to produce the highest specific volumes. In the final steps, the selection of the most appropriate water and emulsifier levels were made based upon the results from the first two steps.

Figure 3.8 illustrates the effects of varying water and emulsifier levels on batter SG and Instron hardness. Pea protein concentrate, whip time and cream of tartar were held constant at their center point levels (30%, 8 min. and 1.5g, respectively). These plots indicate that low SG values and soft cakes were achieved with medium-to-high water levels and low-to-medium emulsifier levels, or low-to-medium

BATTER SPECIFIC GRAVITY
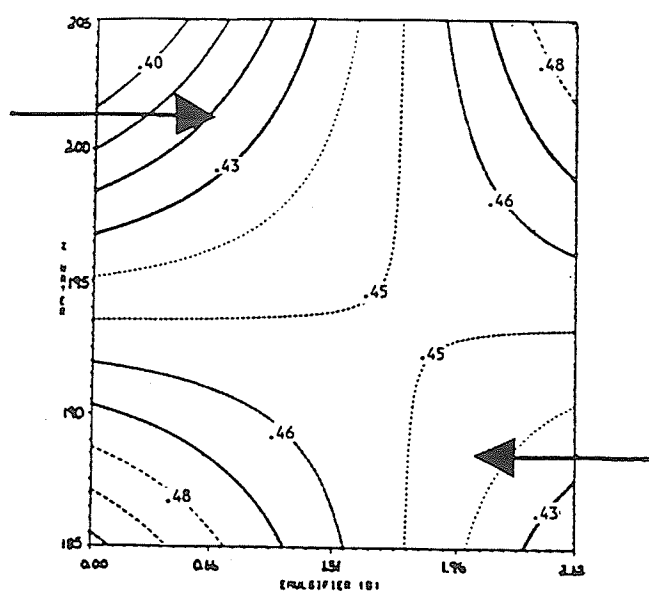
INSTRON HARDNESS (N)
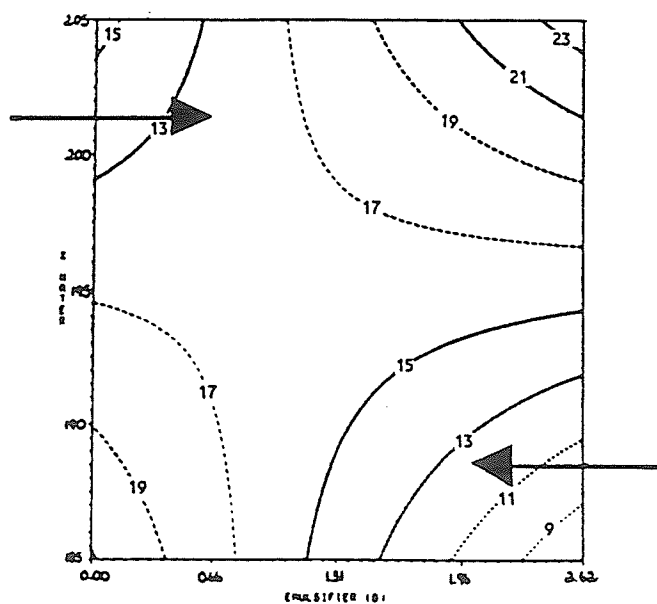
Figure 3.8   Contour plots for the effects of water and emulsifier on batter specific gravity (SG) and Instron hardness.   PPC, whip time and cream of tartar were held constant at their center point levels.   Higher values represent increasing SG and hardness.   Levels of water and emulsifier are actual values.

water levels and medium-to-high emulsifier levels. Intermediate levels of both emulsifier and water also produced fairly low SG and hardness values. Thus, higher water levels were most beneficial to SG and hardness when emulsifier levels were low, and lower water levels were best when emulsifier levels were high. This inverse relationship between water and emulsifier levels accounts for the significant interaction observed for SG and hardness.

Figure 3.9 presents specific volume as a function of PPC level and whip time, with water levels held constant at 185, 195 and 205 percent (% flour basis), respectively. Cream of tartar and emulsifier were held constant at their center point levels (3.0g and 1.31g, respectively). These plots illustrate the negative effect of increasing water level on cake specific volume, that is, volumes decreased with increases in water level. The lowest water level (185%, flour basis) was most beneficial since it allowed comparable volumes to be achieved with higher levels of PPC.

While several water and emulsifier level combinations improved SG and hardness, low water levels resulted in the highest specific volumes. Therefore a combination of low-to-medium water level and medium-to-high emulsifier level would likely produce the best results in the optimization study, and the specific levels to use should be identified.

Based upon these results, the combined effects of various low-to-medium water levels (185%, 190%, 195%) and medium-to-

SPECIFIC VOLUME (CC/G)

Water = 185%   Water = 195%

Water = 205%
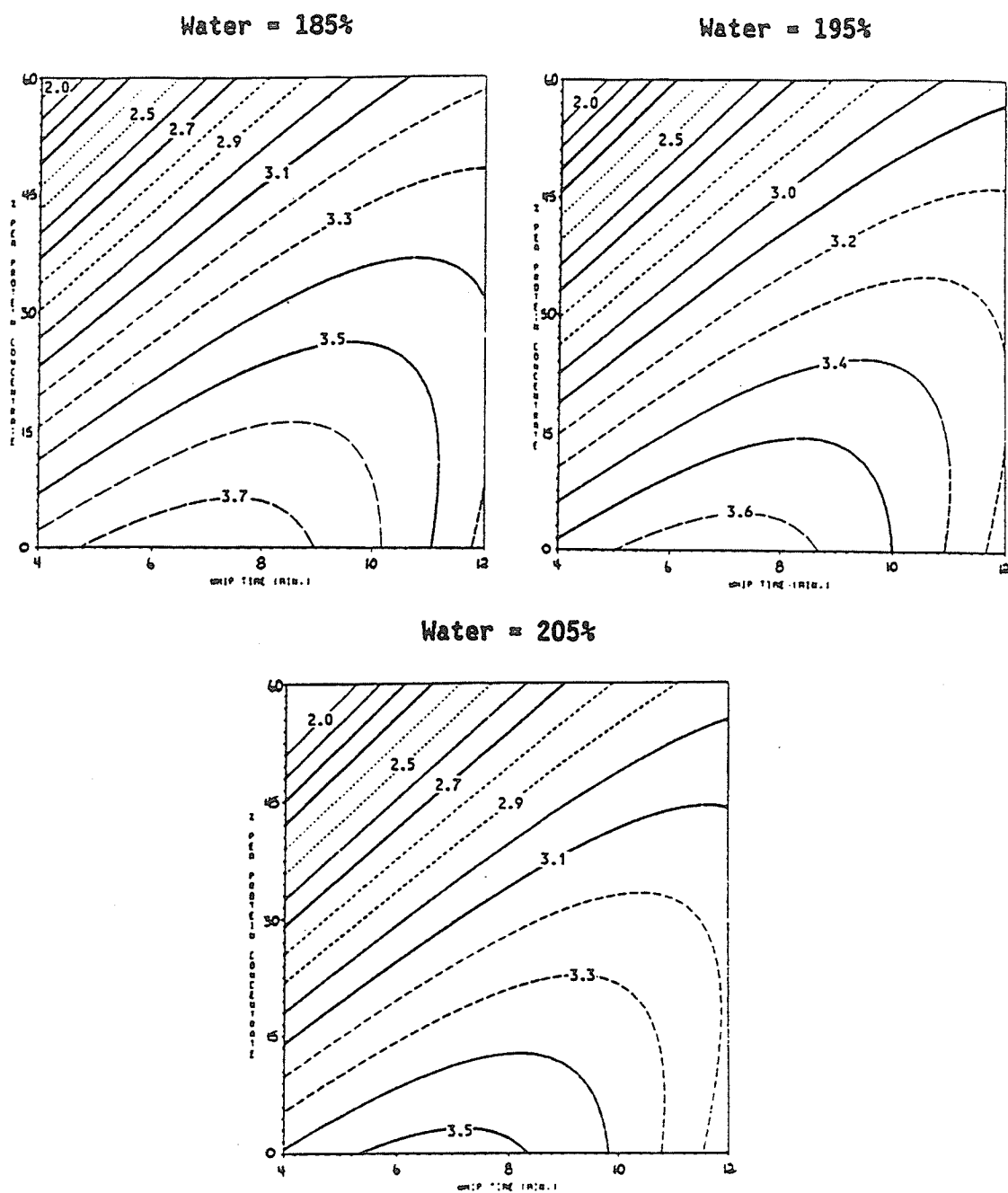
Figure 3.9 Contour plots for the effects of PPC and whip time on cake specific volume at low, medium and high water levels (% flour basis). Cream of tartar and emulsifier were held constant at their center point levels. Higher values represent increasing specific volume. Levels of PPC, whip time and water are actual values.

high emulsifier levels (1.31g, 1.96g, 2.62g) were evaluated more closely (step 3). Contour plots of batter SG were used to evaluate the nine possible combinations of water and emulsifier levels because this independent variable was significantly affected by a water*emulsifier interaction, and a nearly significant water effect (p=.0696) (Figures 3.10-3.12).

PPC and whip time formed the axes of the contour plots due to the importance of these variables to SG. Cream of tartar was held constant at its highest level (3.0g) since this level was found to be most beneficial, while water and emulsifier levels were systematically varied. Selection of the best water/emulsifier combination was based upon SG values achieved after whipping 30 percent PPC formulations for 8 minutes (these were the design center point levels for PPC and whip time). For sponge cake batters, a SG of approximately 0.45 is the commercially acceptable value set by Export Packers, Ltd., therefore, the water/emulsifier combination producing such specific gravities for 30 percent PPC formulations, after 8 minutes of whip time, were considered to be most appropriate. From these plots, three potential combinations of water and emulsifier were identified; 185 percent water and 1.31 grams emulsifier (Figure 3.10); 190 percent water and 1.31 grams emulsifier (Figure 3.10); and 195 percent water and 2.62 grams emulsifier (Figure 3.12). Each of these water/emulsifier combinations produced SG values of

BATTER SPECIFIC GRAVITY
Emulsifier = 1.31g
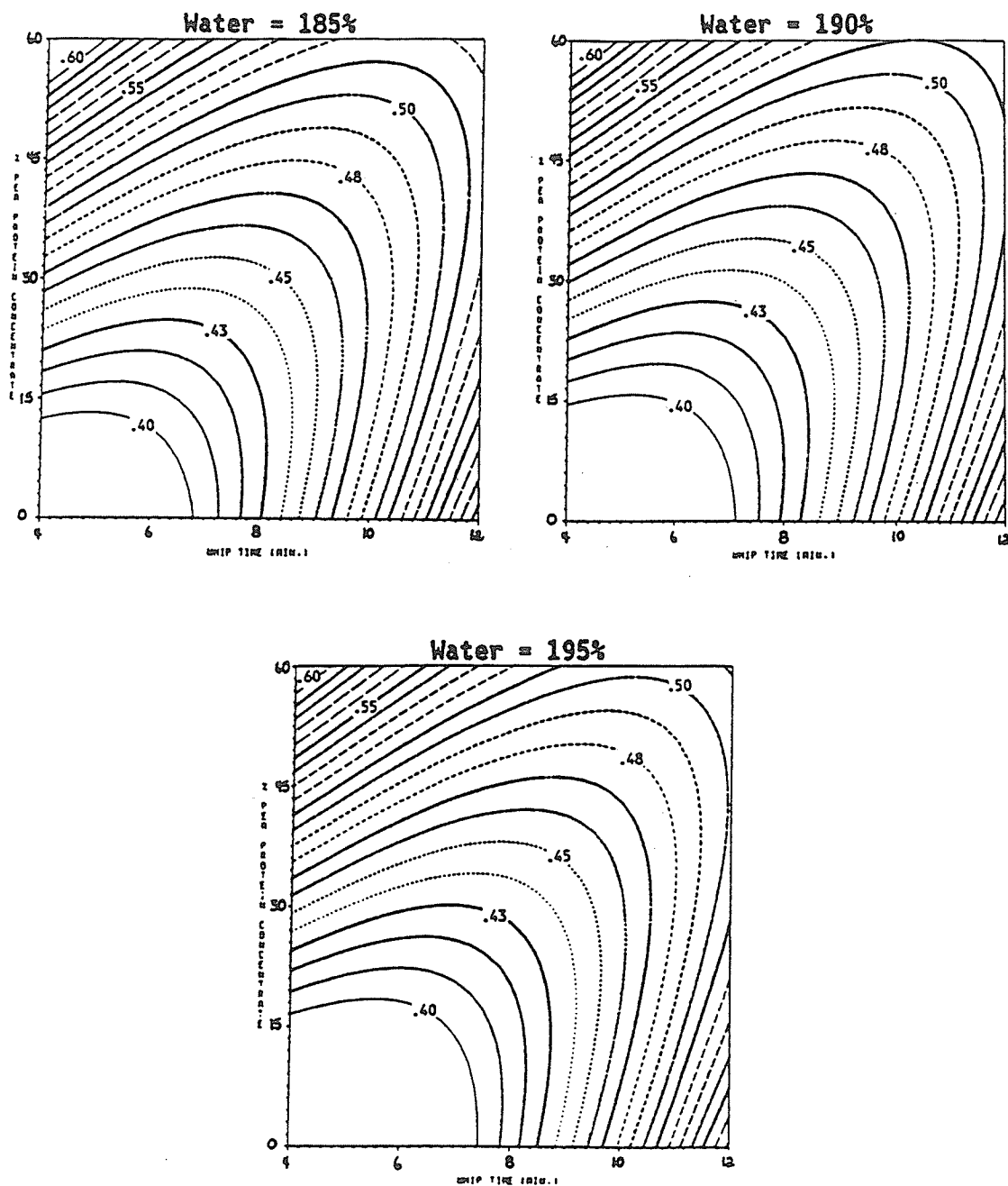
Figure 3.10   Contour plots for the effects of low-to-medium water levels (% flour basis) and 1.31 grams of emulsifier on batter specific gravity (SG). Cream of tartar was held constant at 3.0 grams. Higher values represent increasing SG. Levels of PPC, whip time, and water are actual values.

BATTER SPECIFIC GRAVITY
Emulsifier = 1.96g
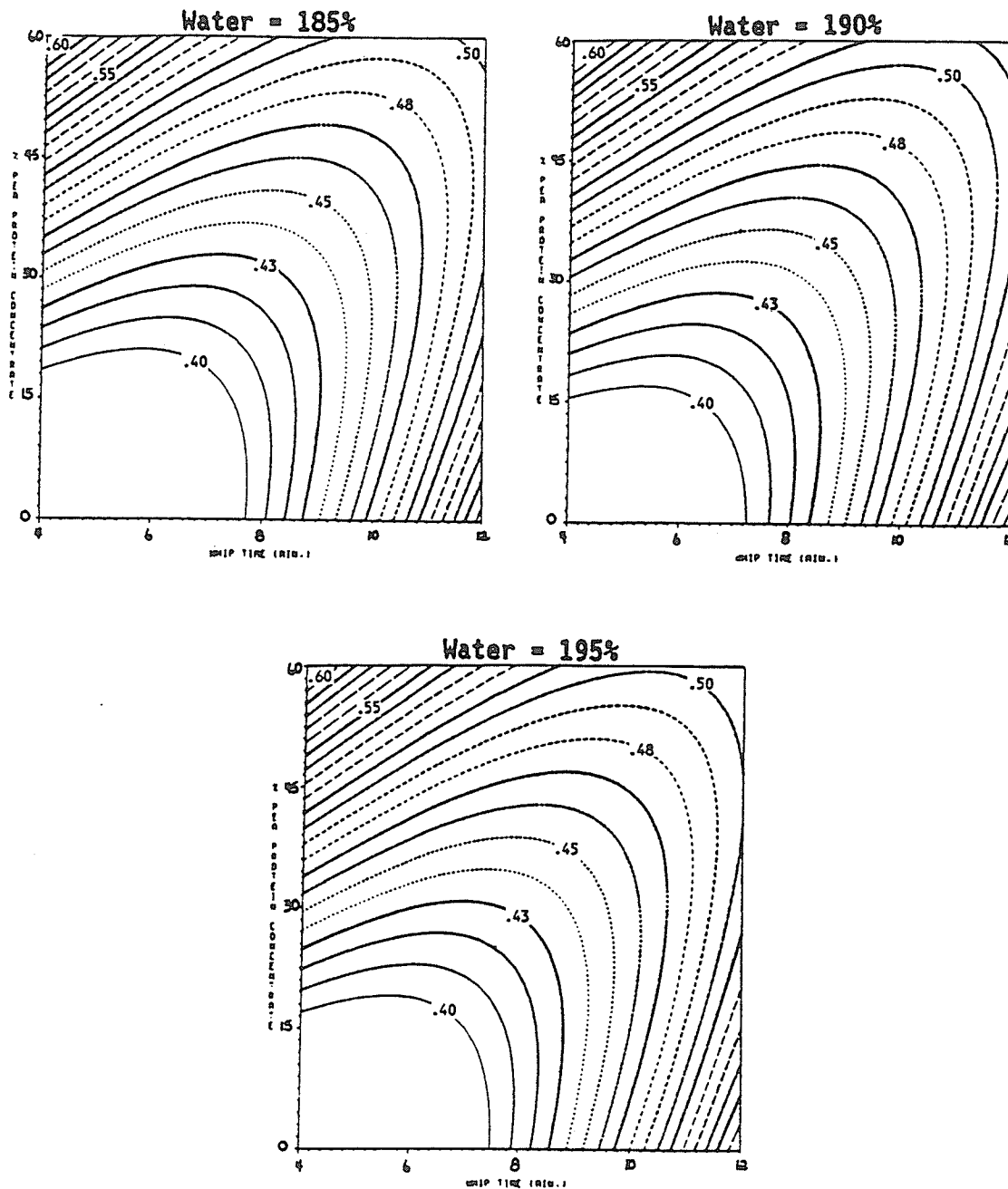
Figure 3.11 Contour plots for the effects of low-to-medium water levels (% flour basis) and 1.96 grams of emulsifier on batter specific gravity (SG). Cream of tartar was held constant at 3.0 grams. Higher values represent increasing SG. Levels of PPC, whip time, and water are actual values.

BATTER SPECIFIC GRAVITY
Emulsifier = 2.62g
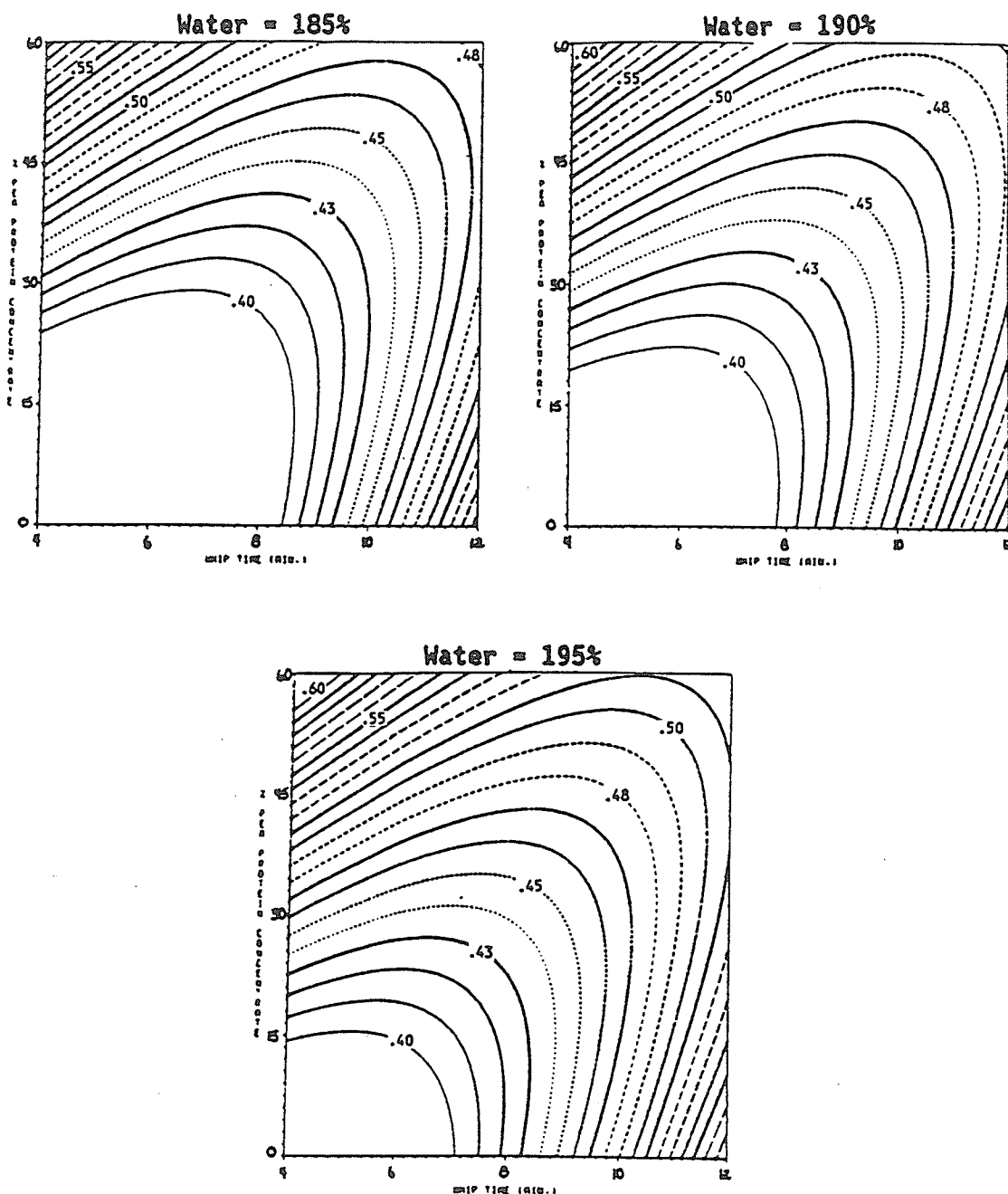
Figure 3.12   Contour plots for the effects of low-to-medium water levels (% flour basis) and 2.62 grams of emulsifier on batter specific gravity (SG). Cream of tartar was held constant at 3.0 grams. Higher values represent increasing SG. Levels of PPC, whip time, and water are actual values.

approximately 0.45 when PPC levels were approximately 30 percent and whip times less than 8 minutes.

Finally (step 4), contour plots for specific volume and hardness (the two other characteristics affected by water and/or emulsifier) were generated for each of the three water/emulsifier combinations identified to be most appropriate, in order to determine which was most beneficial to these cake characteristics (Figures 3.13 and 3.14). The lowest water level (185%) combined with 1.31 grams of emulsifier produced cakes with the highest specific volumes and softest crumb. Therefore, for future optimization of this sponge cake formulation, water levels should be set at 185 percent (% flour basis) and 1.31 grams of emulsifier should be used.

SPECIFIC VOLUME (CC/G)

Water = 185% Emulsifier = 1.31g   Water = 190% Emulsifier = 1.31g
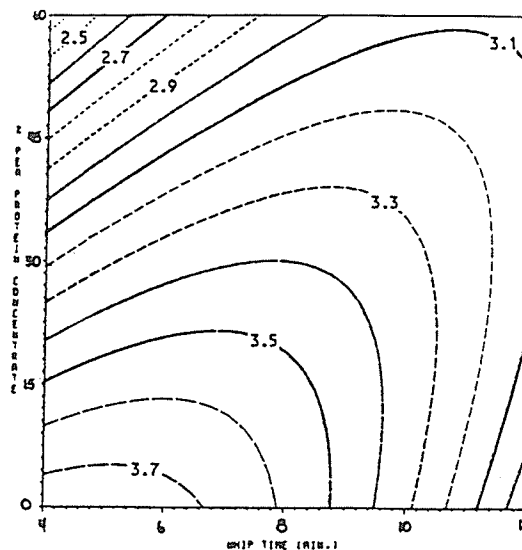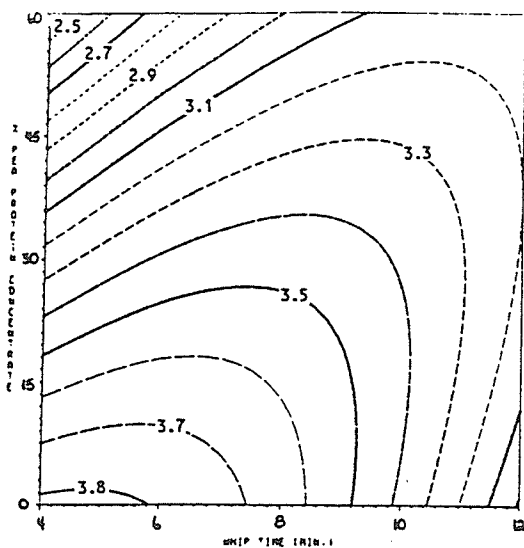
Water = 195% Emulsifier = 2.62g
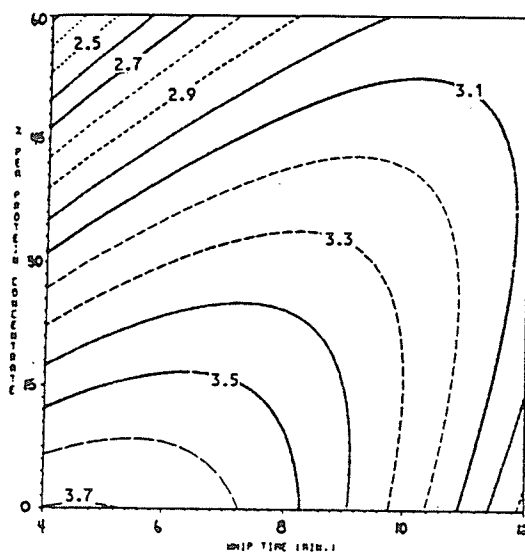
Figure 3.13 Contour plots for the effects of the three most appropriate water/emulsifier combinations on specific volume. Cream of tartar was held constant at 3.0 grams. Higher values represent increasing specific volume. Levels of PPC, whip time, water and emulsifier are actual values.

INSTRON HARDNESS (N)

Water = 185% Emulsifier = 1.31g   Water = 190% Emulsifier = 1.31g
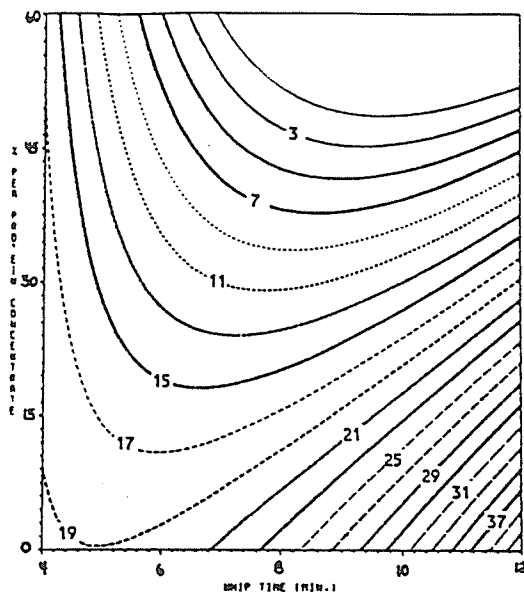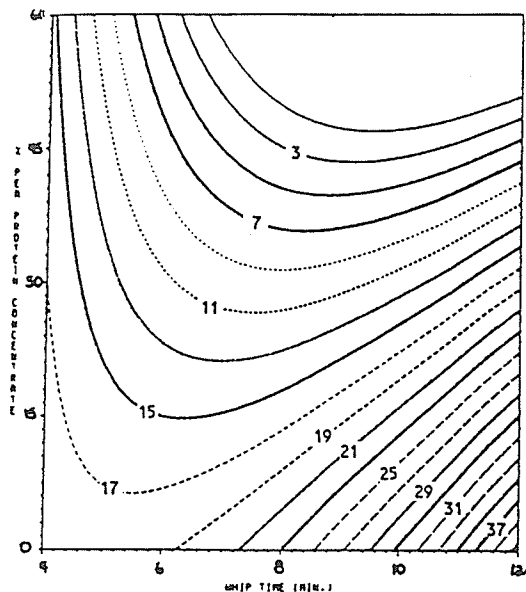
Water = 195% Emulsifier = 2.62g
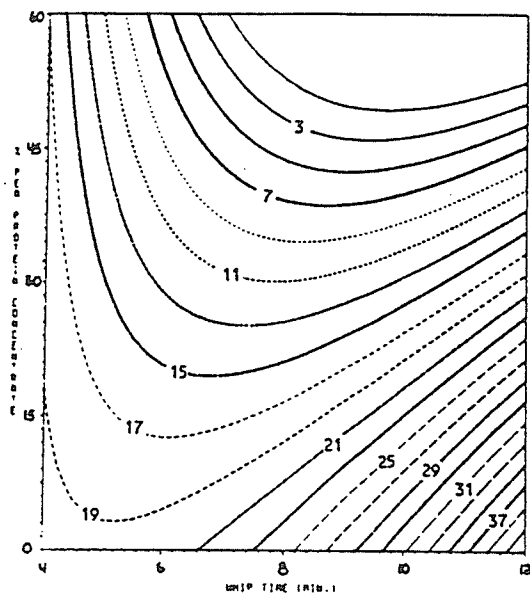
Figure 3.14   Contour plots for the effects of the three most appropriate water/emulsifier combinations on Instron hardness. Cream of tartar was held constant at 3.0 grams. Higher values represent increasing specific volume.   Levels of PPC, whip time, water and emulsifier are actual values.

SUMMARY AND CONCLUSIONS

This study represented a preliminary step towards the optimization of a sponge cake formulation in which egg albumen, an expensive protein source, was partially replaced with less expensive PPC. Response surface methodology was efficiently and successfully used to identify three critical independent variables influencing sponge cake quality, as well as to gain some understanding of their effects on quality. The level of PPC and length of whip time were by far the most important independent variables influencing sponge cake quality. The effect of cream of tartar was also significant, but to a much lesser degree. Water and emulsifier levels had the least effect on overall sponge cake quality.

Contour plots generated from best fitting regression equations for each dependent variable indicated that increasing PPC levels produced a decrease in specific volume and cohesiveness and an increase in specific gravity, hardness and gumminess. These negative effects on sponge cake quality were particularly evident at low whip times and low cream of tartar levels. While the substitution of PPC for egg albumen decreased sponge cake quality, the negative effects could, to a large extent, be overcome by adjusting cream of tartar levels and whip times. The higher the level of PPC, the longer the whip time necessary to improve cake quality. When PPC levels were moderately high (or high), for a set amount of PPC, an increase in cream of tartar generally shortened the

whip time necessary to achieve cakes of good quality. Conversely, for a set whip time, increasing cream of tartar level permitted greater PPC incorporation.

Contour plots were also used to determine whether the levels of PPC, whip time, and cream of tartar used in this study were appropriate for an additional optimization study. Because cream of tartar positively influenced PPC incorporation and length of whip time, it was concluded that higher levels should be evaluated in the final optimization study. The whip times used in this study were all capable of producing cakes of good quality, and longer whip times would probably have permitted greater PPC incorporation. Whip times longer than 12 minutes were, however, thought to be commercially impractical. Consequently, it was concluded that the range of whip times should remain the same. It was also concluded that the range of PPC levels should remain unchanged. Although the quality of the 60 percent PPC cakes was generally low, increasing cream of tartar levels may improve the quality of such high PPC formulations.

Although water and emulsifier were of minor importance to sponge cake quality, it was determined that water levels of 185 percent (% flour basis) and emulsifier levels of 1.31 grams were most beneficial to the high cream of tartar formulations.

To summarize, PPC, whip time and cream of tartar were identified as the three independent variables which should be

evaluated in the next optimization study, with the levels of PPC ranging from 0 to 60 percent and whip times from 4 to 12 minutes. It was decided that cream of tartar levels should be increased, with the highest level used in this study (3.0g) serving as the center point level in the next study, and that water and emulsifier levels should be set at 185 percent and 1.31 grams, respectively. Successful replacement of at least 45 percent egg albumen with PPC (weight/weight) appeared feasible using appropriate cream of tartar levels and whip times.

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CHAPTER 4

Pea Protein Concentrate for Partial Replacement of Egg Albumen in Sponge Cake - Optimization of Critical Independent Variables Using Response Surface Methodology

INTRODUCTION

The production of snack cakes has become a billion dollar business and the market potential for such cakes continues to grow (Wells, 1989). Egg albumen is an expensive component of the whole egg mix generally used to produce snack cakes such as sponge cake, cake rolls and other sponge-type products. Its replacement with an alternative, less expensive protein source would, therefore, be economically advantageous to the cake manufacturer.

Export Packers Co. Ltd. (Winnipeg, Manitoba) is a major producer of dried albumen, yolk, and whole egg products. Egg albumen is in great demand due to its widespread food applications (L. Carvalho, personal communication, 1987). Export Packers would, therefore, benefit economically if the albumen in their whole egg sponge cake base could be partially replaced by a less expensive protein, thereby increasing the availability of egg albumen to meet the growing demand for this product.

Pea protein concentrate (Woodstone Foods Ltd., Portage La Prairie, Manitoba) is an under-utilized by-product of field pea (Pisum sativum) fractionation (E. D. Murray, personal communication, 1989). A high demand exists for the pea fibre

and starch fractions, and meeting these demands ultimately results in an excess supply of the protein. A result of the excess supply is a relatively inexpensive protein source ($1.35/lb, dry weight) (E. D. Murray, personal communication, 1989). Pea protein concentrate (PPC) could provide an economical alternative to egg albumen in a sponge cake system.

Research investigating the replacement of egg albumen with alternative protein sources in a cake system is very limited but does suggest that egg albumen is not indispensable. Khan et al. (1979) successfully replaced 30 percent of the egg white solids with bovine plasma protein isolate in an angel food cake. Johnson et al. (1979) found that bovine plasma protein concentrate could successfully replace egg white solids in white layer cakes if the water and mixing times were adjusted. Thus it is possible to replace the albumen in a cake system with an alternative protein source and still achieve an acceptable product.

Pea protein flours, concentrates and isolates have been investigated in a variety of baked products, although, there have been no reports of their use in sponge or other cake systems. Research has been focused on the replacement of wheat flour with pea flour, or other pea protein products, to improve nutritional quality. Pea proteins are high in lysine (Holt and Sosulski, 1979) and therefore complement the amino acid composition of wheat proteins (Kreutler, 1980). Successful incorporation of pea protein flours, concentrates

and isolates in yeast breads, quick breads and cookie formulations have been reported by Fleming and Sosulski (1977), Jeffers et al. (1978), Repetsky and Klein (1981), Hsu et al. (1982), Raidl and Klein (1983) and McWatters (1978).

The potential of pea proteins as milk protein replacers has also been investigated. McWatters (1980) attempted to partially replace milk proteins with pea flour and pea protein concentrate in a baking powder biscuit. Formulation changes and flavor improvement were recommended to produce an acceptable product. Patel et al. (1981) co-spray-dried pea protein concentrate with cheddar cheese whey in an attempt to produce a product similar to non-fat dry milk. The product, then tested in a bread formulation, was found to have potential as a replacement for non-fat dry milk in bread.

The literature, in general, illustrates great potential for pea protein use in baked products. Research is lacking, however, on the use of pea protein as a functional replacement for egg albumen in baked products, particularly sponge cakes.

Several investigations of pea protein functionality have indicated the very good functional properties of the proteins (Fleming and Sosulski, 1975; Sosulski and Youngs, 1979; Vose, 1980; Sumner et al., 1981; Hsu et al., 1982; Megha and Grant, 1986; Naczk et al., 1986). Good functional properties, coupled with successful use in some baked products, suggest the possibility that pea proteins might be successfully used in sponge cake type products for albumen replacement. The

use of a sponge cake system should have the additional benefit of emphasizing the effects of albumen replacement with PPC due to the high dependence of cake quality upon egg quality.

The benefits of successfully developing a sponge cake formulation, incorporating PPC are, therefore, two-fold. Woodstone Foods Ltd. would benefit by utilizing the excess PPC and establishing a better balance between the protein, starch and fibre fractions. Export Packers Ltd. would benefit by freeing up albumen which is in high demand, while still providing a less expensive whole egg product. In addition, if albumen can be successfully replaced by PPC in a product as sensitive as a sponge cake, there should be potential for its use in a variety of other baked products.

Optimization of a sponge cake formulation can be done efficiently by using response surface methodology (RSM). This is a statistical technique for establishing optimal formulation conditions in a minimal number of experimental runs (Johnson and Zabik, 1981a). Several investigators have successfully used RSM to optimize cake formulations (Johnson and Zabik, 1981a; Vaisey-Genser et al., 1987; Neville and Setser, 1986; Lee and Hoseney, 1982; Kissell and Marshall, 1962).

According to Joglekar and May (1987), product development using RSM typically involves two stages. The first stage involves identification of key independent variables through screening investigations. This stage has been completed and

is described in Chapter 3. This study will focus upon the second stage where optimal levels of the key variables are determined in order to simultaneously satisfy a specific set of desirable product characteristics. Finally, relationships between the variables can be examined more thoroughly at this stage of investigation (Mullen and Ennis, 1985).

In summary, PPC is an inexpensive, under-utilized fraction of the field pea fractionation process. This protein fraction is found in excess supply as a result of the high demand for the fibre and starch components. Consequently, there is a need for a food product to help utilize the excess supply of PPC. Egg albumen, on the other hand, is an expensive, highly utilized protein source for which there is a great demand. This high demand has created a need for an alternative protein source which could successfully replace part of the albumen in products such as a whole egg mix. Pea protein concentrate has been shown to exhibit good functional properties and potential as a food ingredient, making it an ideal choice for evaluation as an egg albumen replacement in a food product. A sponge cake is a logical product choice for both commercial and experimental reasons, as discussed. Partial replacement of egg albumen with PPC in a sponge cake would accomplish three things; i) it would help utilize the excess PPC and restore balance among the three pea fractions; ii) it would free up egg albumen for additional sale; and finally, iii) it could open the door to widespread use of the

pea protein in a variety of baked products. Therefore, this study had the following objectives:

1. To select best fitting regression models from full second-order models, to predict the effects of PPC, whip time and cream of tartar on each physical and sensory sponge cake characteristic evaluated.

2. To use the best fitting predictive models to produce contour and response surface plots, in order to visually evaluate the relationships between PPC, whip time and cream of tartar and clarify their effects on the physical and sensory characteristics of sponge cakes.

3. To identify sponge cake formulae with at least 30 percent of the albumen replaced with PPC, that are comparable to 100 percent egg albumen sponge cakes.

4. To provide recommendations for the development of a co-spray-dried PPC-whole egg mix for commercial use in sponge-type snack cakes.

MATERIALS AND METHODS

Materials

Pea protein concentrate was supplied by Woodstone Foods Ltd., Portage La Prairie, Manitoba. The protein, trade named Propulse 985B, was prepared from yellow field peas by an acid extraction method developed by Nickel (1981). Enough pea protein was supplied to complete the entire experiment. A typical analysis supplied by Woodstone Foods Ltd. is presented in Table 4.1. Spray-dried egg albumen containing whipping agents (triethyl citrate and sodium lauryl sulphate) was supplied by Export Packers Co. Ltd., Winnipeg, Manitoba. Lysozyme was extracted prior to drying for commercial sale. A typical analysis for the dried albumen is presented in Table 4.2. A commercial cake flour, 7.5 percent protein (14% moisture basis), was obtained from Reid Milling, Mississauga, Ontario. The emulsifier, TOP-SCOR S Powder (sodium stearoyl lactylate), was provided by Breddo Food Products Corp., Kansas. All other ingredients were purchased locally when the experiment was begun, in amounts sufficient to complete the entire experiment.

Sponge Cake Preparation

Sponge cakes were prepared according to the procedure outlined in Appendix 4.A. Reference (REF) cakes were prepared with 100 percent albumen (0% PPC) from the same formulation as treatment cakes.

Table 4.1   Typical Analysis of Propulse
                   985B Pea Protein Concentrate

Protein (Kjeldahl - N X 6.25)db	83%
Moisture (dry 16 hours at 100°C)	5%
Fat (AOAC 7.056, 13th ed.)db	2%
Crude Fiber (Modified AOAC 7.068, 13th ed.)db	0.4%
pH (10% solution)	6.5
Ash (AOAC 14.006, 13th ed)db	4.0%

Woodstone Foods Ltd.

Table 4.2   Typical Analysis of Spray-Dried Egg Albumen

Protein	80% max.
Moisture	8% max.
Fat	negligible
Carbohydrate	0.1%
pH	6.5 - 7.5
Ash	5% max.

Export Packers Ltd.

Experimental Design

A central composite, orthogonally blocked, rotatable response surface design was used (Box and Draper, 1987) consisting of three variables (PPC, whip time, cream of tartar) at five levels (Table 4.3). Table 4.4 lists the actual and coded values of egg albumen and the three independent variables. The design required 24 test runs, significantly fewer than the 125 test runs necessary to evaluate the same range of combinations using a traditional 5^3 factorial design. One block of six treatments was completed for four consecutive days. Blocks, and treatments within blocks, were randomized. Center points and star points were replicated within the experimental design, as well, the entire design was replicated.

Allocation and Preparation of Samples for Sensory and Physical Tests

Because the sponge cake formulation produced only three cakes, a detailed sampling procedure was necessary to ensure enough sample for all physical and sensory tests. Figure 4.1 illustrates the allocation and preparation of both treatment and 100 percent egg albumen (0% PPC) REF cake samples for physical and sensory measurements. Left, center, and right represent the cake position in the oven.

Table 4.3   Experimental Design[1]

Blocks	Treatment[2]	Independent Variables Coded Levels		
		X_1 (PPC)	X_2 (Whip time)	X_3 (Cream of Tartar)
1	1	-1	-1	1
	2	1	-1	-1
	3	-1	1	-1
	4	1	1	1
	5	0	0	0
	6	0	0	0
2	7	-1	-1	-1
	8	1	-1	1
	9	-1	1	1
	10	1	1	-1
	11	0	0	0
	12	0	0	0
3	13	$-\sqrt{2}$	0	0
	14	$\sqrt{2}$	0	0
	15	0	$-\sqrt{2}$	0
	16	0	$\sqrt{2}$	0
	17	0	0	$-\sqrt{2}$
	18	0	0	$\sqrt{2}$
4	19	$-\sqrt{2}$	0	0
	20	$\sqrt{2}$	0	0
	21	0	$-\sqrt{2}$	0
	22	0	$\sqrt{2}$	0
	23	0	0	$-\sqrt{2}$
	24	0	0	$\sqrt{2}$

[1]Blocks and treatments within blocks were randomized.
[2]Treatments 1-4 and 7-10 are cube points; treatments 5,6,11,12 are center points; treatments 13-24 are star points.

Table 4.4   Actual and Coded Independent Variable Levels[1] Chosen for Sponge Cake Preparation

Independent Variables	Levels				
PPC (g)[2]	0.00 (0%)	2.32 (8.78%)	7.92 (30%)	13.52 (51.22%)	15.84 (60%)
Albumen (g)	26.40 (100%)	24.08 (91.22%)	18.48 (70%)	12.88 (48.78%)	10.56 (40%)
Cream of Tartar (g)	1.50	1.94	3.00	4.06	4.50
Whip time (min)	4.00	5.17[3]	8.00	10.83[3]	12.00
Coded levels	$-\sqrt{2}$	-1	0	+1	$+\sqrt{2}$

[1] Determined by the experimental design.
[2] Pea protein concentrate; weight/weight replacement of egg albumen.
[3] 5.17 min = 5 min., 10 sec.; 10.83 min = 10 min., 50 sec.
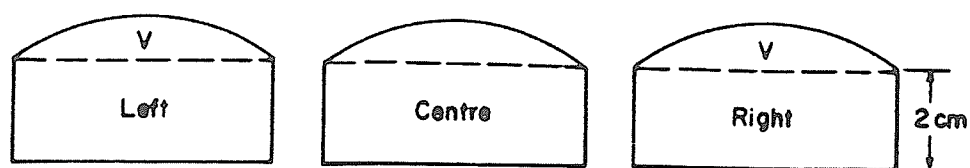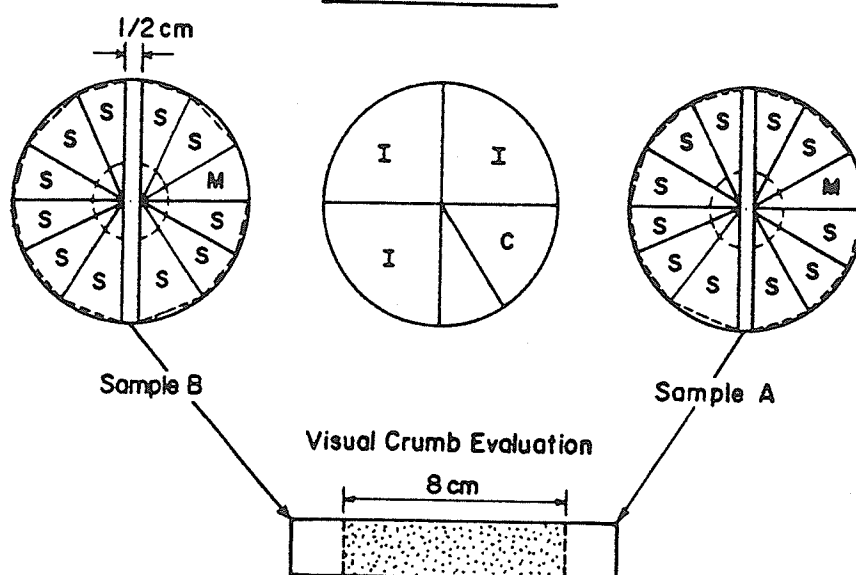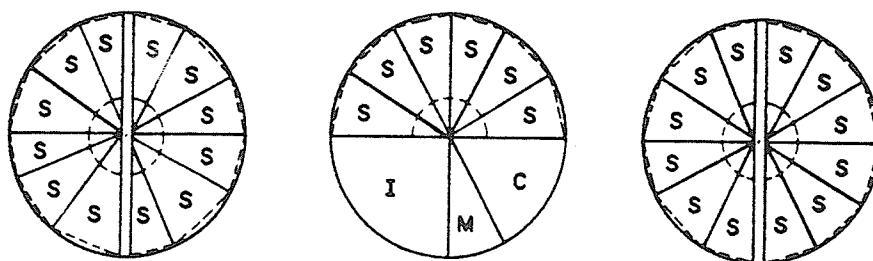
Figure 4.1 Sponge cake sample preparation and allocation for physical and sensory measurements. V=volume measurement, I=instrumental texture measurement, M=moisture measurement, C=color measurement, S=sensory evaluation.

Allocation of Samples for Physical and Sensory Tests

i) Treatment Cakes: Left and right cakes were used for volume, moisture and all sensory measurements. It was necessary to designate two sample pieces for the sensory evaluation of each treatment because more than one cake piece was needed to complete the sensory task. Samples from the right cake were designated 'A' and were used to evaluate the intensity of aroma and flavor, as well as cake firmness. Samples from the left cake, designated 'B', were used to evaluate springiness, cohesiveness, and moistness. This ensured that any differences due to oven position would not influence the evaluations. For example, cake moistness was always evaluated on cakes baked in the left oven position (sample B). Cake strips for visual crumb evaluation were taken from both the right and left cakes in order to consider variability within cakes from the same treatment batch. The center cake was used for instrumental texture and color evaluations.

ii) 100 Percent Egg Albumen REF Cakes: Left and right cakes were used for volume and sensory measurements exclusively. REF samples were not designated A or B, but rather, were assigned an "R" and were randomly assigned to each judge. That is, unlike the treatment samples, it was not specified which REF sample to use for each judgement. The remainder of the center cake was used to evaluate instrumental texture, color and moisture.

Preparation of Samples for Physical and Sensory Tests

Volume measurements for treatment and REF cakes were performed on whole right and left cakes just prior to sample preparation for the remaining physical and sensory tests. Volumes were performed on frozen cakes to prevent crumb indentations. Additionally, cakes were wrapped in a thin film of plastic permitting use for sensory analysis. Following volume measurements, cake tops were sliced off with the guidance of a two centimetre high, fifteen centimetre square, plexiglass box. Sampling was done one cake at a time so that cakes did not thaw, in order to prevent the crumb from tearing.

Cakes were sliced according to the scheme presented in Figure 4.1. Thin strips of cake (approximately 0.5cm) were removed from the middle of the left and right cakes, trimmed to eight centimetres long, and used for visual crumb assessments. Both treatment and REF cakes were prepared this way. The remainder of each cake was divided into twelve equal wedges and allocated to sensory and moisture tests for treatment cakes, and sensory tests only, for REF cakes. The tips and rounded crust edges of each wedge were trimmed so that samples would fit into their sample cups. Samples used for moisture determinations were further trimmed to fit the moisture determination cups.

The center treatment and REF cakes were first cut into four equal wedges. One half of the REF cake was further

divided into six wedges which were allocated for additional sensory REF samples. The other half of the cake was divided, as illustrated in Figure 4.1, to produce samples for instrumental texture, moisture and color measurements. Limited REF sample permitted only one measurement of texture and moisture. The entire treatment center cake was subdivided to produce samples for instrumental texture and color measurements, identical to those of the REF cake. However, three cake wedges were available for texture measurement.

After each cake was prepared, pieces were resealed in polybags and placed back into the freezer until the next morning. The morning of testing, cake samples for sensory and physical tests were removed from the freezer. Samples for instrumental texture, color and moisture measurements remained sealed and were equilibrated to room temperature (22° C) before testing. Samples for the first sensory panel were thawed approximately one half hour in their polybags before being placed into sample cups where they continued to thaw to room temperature (22°C). Samples for the afternoon panel, already at room temperature, were placed into sample cups approximately one half hour before the panel.

Cake strips for the morning visual crumb evaluation were placed flat into a petri dish which had been lined with black felt to produce greater contrast. The petri dish was covered and then placed into a plastic zip-lock bag to prevent any drying. Samples for the afternoon panel were similarly

prepared.

Instrumental/Physical Measurements

Batter specific gravity (SG) and pH, cake specific volume, and crumb hardness, cohesiveness, gumminess, moisture and color were measured on treatment and REF batters and cakes.

i) Batter SG was measured by dividing the weight of a 50 millilitre metal cup filled with water by the weight of the same cup filled with batter (Campbell et al., 1979). Duplicate measures were taken immediately after batter preparation.

ii) Batter pH measurements (one per cake batch) were made using a Fisher Accumet pH meter (Model 810).

iii) Specific volume of the baked cake was determined by dividing the volume of the cake by its weight (Campbell et al., 1979). Volume measurements were made using a volumeter and rapeseed displacement. Cake weights were recorded 20 minutes after removal from the oven.

iv) Hardness, cohesiveness and gumminess of the cake crumb were determined using the Instron Universal Testing Machine (Table model TM) and a Texture Profile data acquisition and analysis program developed for the Apple IIE computer (Agriculture Canada, 1987). Operating conditions were selected based upon preliminary experimentation (Chapter 2) in which a 1018 mm^2 round plunger, 20 centimetres/minute

crosshead speed and a 75 percent sample compression yielded good detection of textural differences for all three parameters. These conditions differ from those used in the previous screening study due to the omission of springiness from the texture profile analysis. Chapter 2 details the Instron Texture Profile method used.

v) <u>Percent moisture content</u> was determined by a modification of the standard air oven method, AACC Method 44-15A (1983). Small cake samples were dried for 24 hours at 103°C.

vi) <u>Crumb color</u> was determined using a Hunterlab Tristimulus Colorimeter (Model D25M-9; Hunter Associates Laboratory, Inc., Reston, Virginia). The meter was standardized using the white standard tile (L=92.4, a=-1.2, b=0.5) and values for lightness (L), red-green (a), and blue-yellow (b) were obtained. Each cake wedge was placed into a petri dish cut side down. The center of the wedge was positioned over the port and the reading taken. The sample was rotated 180 degrees and a second reading was made. Color measurements were taken only to determine if the addition of PPC created any serious color problems. To evaluate this, color measurements were also taken of cakes available in the commercial market which were similar to the experimental sponge cakes.

Sensory Descriptive Analysis

Sensory evaluations were performed by a ten member trained panel (nine female and one male; students and staff

in the Department of Foods and Nutrition). For each replication, a total of 24 treatments were evaluated over eight sessions. Two sessions were held daily (morning and afternoon) over four consecutive days.

Panel Training

Eleven training sessions were conducted over a three week period: sessions one to five were used to introduce the product, define important characteristics for evaluation, standardize handling procedures, and develop acceptable ballots; sessions six to nine focused upon the introduction and use of REF samples and the introduction of visual crumb evaluations; the last sessions familiarized panelists with the final ballots to be used experimentally and also served as practice sessions.

The first session involved a general orientation to the product, and predominant sponge cake characteristics were identified for scaling. Sponge cake samples of varying quality were chosen for training in order to represent the variety of products to be expected during the experiment.

The objectives of the next four sessions were to refine and clarify definitions of the characteristics to be judged, determine and standardize handling and evaluation procedures, and construct a ballot with which all panelists agreed. A 15.0 centimetre unstructured line scale was chosen due to the panelists' familiarity with this scaling technique. The line

scale was anchored 1.5 centimetres from each end with appropriate descriptors. A value from 0.0 to 15.0 was determined by measuring the distance, in centimetres, from the left end point to the panelist's mark. In order to define and clarify endpoint descriptors, products representing the endpoints were provided. Information on the endpoints used is provided in Appendix 4.B. References to help identify predominant aromas and flavors were also provided (eg. eggy, egg albumen, PPC, sweet, etc.), however, panelists were unable to scale any of the aroma and flavor attributes identified in the first training session, consistently. For this reason, only the overall intensity of aroma and flavor were evaluated.

Results of the previous day's panel were briefly discussed at the beginning of each session and group discussions were held at the end of the sessions. At this time, panelists were encouraged to discuss evaluation techniques, sensory definitions and any problems and/or suggestions they might have. The set of characteristics selected for the evaluation of sponge cakes were overall intensity of aroma and flavor, firmness, springiness, cohesiveness, moistness, predominant cell size, cell uniformity and cell wall thickness.

The next three sessions were conducted in the sensory panel room where evaluations were made in partitioned booths under yellow lights. An identified reference (REF) sample (0% PPC/100% albumen sponge cake) was introduced and panelists

were instructed to score the REF and three other samples according to the REF.

Three visual crumb characteristics (predominant cell size, cell uniformity and cell wall thickness) and a second ballot for scaling these characteristics, were also introduced and discussed during these sessions. Reference cards with air dried cake samples representing the end points of the scales (predominant cell size- very small, large; cell size uniformity- very uniform, moderately irregular; cell wall thickness- very thin, very thick) were provided for clarification of the characteristics and as a frame of reference for making evaluations. The air dried samples were sliced according to Figure 4.1 ("visual crumb evaluation"), and were selected from sponge cakes baked during preliminary experimentation. Panelists were, again, asked to score the REF and three other samples according to the REF.

The last sessions introduced the panelists to, and familiarized them with, the final ballots to be used for the experiment. These ballots are shown in Figures 4.2 and 4.3. Because evaluation of all treatments on one ballot seemed to result in comparisons among the treatments, ballots were changed such that only one treatment was evaluated per ballot. The REF scores, having been determined by taking the group mean for the REF cake from three training sessions, were permanently positioned on the line scale.

Judge: _____   Sample #: _____

Date: _____

SPONGE CAKE EVALUATION

For each characteristic, place a vertical line across the horizontal line at the point that best describes that characteristic in the sample. Compare the sample to the reference (R), which has been positioned on the line. Evaluate each treatment independently for all of the characteristics before moving on the the next treatment. When you have evaluated all the samples move on to the MacBeth Booth to evaluate the cake crumb structure.

Overall Intensity of Aroma

```
┬─────────────────────────────┬──────────────────────┬──
   weak                        R              intense
```

Comments:

Overall Intensity of Flavor (evaluate just prior to swallowing).

```
┬───────────────────┬──────────────────────────────┬──
   weak              R                        intense
```

Comments:

Firmness

```
┬───────────────────┬──────────────────────────────┬──
 extremely           R                    moderately
   soft                                         firm
```

Springiness by Touch

```
┬───────────────────┬──────────────────────────────┬──
 moderately          R                     extremely
   springy                                   springy
```

Cohesiveness

```
┬───────────────────┬──────────────────────────────┬──
 slightly            R                     extremely
 cohesive                                   cohesive
```

Moistness

```
┬───────────────────┬──────────────────────────────┬──
 moderately          R                         moist
   dry
```

Figure 4.2   Ballot used for sensory evaluation of sponge cakes.

Judge: _____   Sample #: _____

Date: _____

SPONGE CAKE CRUMB EVALUATION

For each characteristic listed, place a vertical line across the
horizontal line at the point that best describes that
characteristic in the sample. Compare each treatment to the
reference, which has been positioned on the line. Evaluate each
treatment independently for all of the characteristics before
moving on to the next treatment.

Predominant Cell Size

```
|_____|_____|____
  very                   R                          large
  small
```

Cell Size Uniformity

```
|_____|_____|____
  very                   R                      moderately
  uniform                                        irregular
```

Cell Wall Thickness

```
|_____|_____|____
  very                 R                             very
  thin                                               thick
```
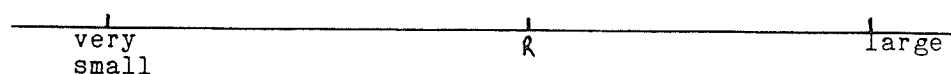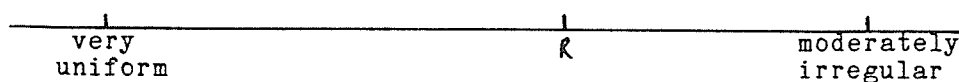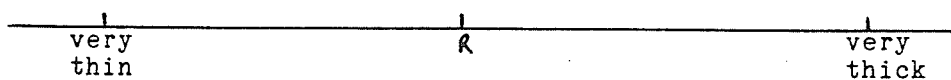

COMMENTS:

Figure 4.3 Ballot used for sensory evaluation of sponge cake
crumb.

Sample Presentation

Panelists completed aroma, flavor and texture evaluations of the cake samples prior to the visual evaluation of crumb characteristics. The 48 treatments (24 x 2 reps) were presented in the same randomized order used for baking. All panelists received the same three treatments at any one session but the order of presentation was randomized.

Aroma, flavor and texture evaluations were made in individual sensory booths under yellow lights. Instructions for evaluating each parameter (Appendix 4.C) were posted in each booth and distilled water, unsalted crackers and toothpicks were provided for rinsing and clearing the teeth between treatments. Each panelist received three REF samples plus two samples (A and B) of each of three treatments. A plastic knife was provided for cutting the wedges into specified pieces for evaluation. A set of reference end point samples were provided for the first panel of each replication in order to reorient panelists with the scale. Treatment and REF samples were presented in 60 millilitre lidded plastic souffle cups and were coded with three digit random numbers. All evaluations were make in comparison to the REF sample.

Visual evaluations of predominant cell size, cell uniformity and cell wall thickness were conducted in a Macbeth booth (Model EBX-222) under incandescent light. Three treatment samples and a REF sample were placed in the booth, and evaluated by each panelist. The samples, presented in

petri dishes lined with black felt, were coded with three digit random numbers and an 'A' or 'B'. Five panelists were randomly assigned to judge set 'A' and the other five panelists judged set 'B'. Panelists evaluated each sample by bringing it to the center of the Macbeth booth, which was marked by a black felt circle, and removing the lid of the petri dish. Instructions were provided to the panelists (Appendix 4.D) along with visual reference cards representing the end points of the three scales. All evaluations were made in comparison to the REF sample.

Statistical Analysis

A second-order regression equation was fitted for each dependent variable using the GLM procedure of the Statistical Analysis System (SAS, 1985). For each replication, analyses of physical measurements were carried out on means of two readings for specific gravity, specific volume, and moisture determinations, and three readings for Instron texture measurements. Only one reading was made for pH. Regression equations for sensory data were based on the panel means of ten judges.

The GLM procedure produced full model analysis of variance tables for each dependent variable, from which best fitting models were selected by eliminating variables of low significance (p<.05). If a linear effect was not significant but its quadratic or interaction term was, the linear term

was retained in the model. Replicate and block effects were removed from the models. The new models were re-analyzed using GLM in order to generate new parameter estimates for the final best fitting regression equations. Contour plots were then generated from the best fitting regression equations (STSC, Statgraphics, 1986).

RESULTS AND DISCUSSION

Overview

Prior to any interpretation of the results, residual analysis was conducted as a diagnostic check of the assumptions underlying the regression analysis (Joglekar and May, 1987). Following residual analysis, the importance of PPC, whip time and cream of tartar to sponge cake quality, was determined from the individual analyses of variance. Significant linear, quadratic and interaction effects of PPC, whip time and cream of tartar, were selected for best fitting models and subsequently used to produce three-dimensional response surface plots and two-dimensional contour plots. These plots helped to visualize the relationships between PPC, whip time and cream of tartar, and clarify their effects on each of the physical and sensory sponge cake characteristics tested. Contour plots for selected sponge cake characteristics were then superimposed to define an area of PPC, whip time and cream of tartar levels which, predictably, would produce cakes comparable to the REF sponge cakes. Finally, formulation recommendations for the production of a co-spray-dried PPC-whole egg mix were made based upon the results of the superimposed contour plots.

Evaluation of Assumptions Underlying Regression Analysis

The residuals (observed value - predicted value) for each dependent variable were plotted against the predicted values

generated from the full model in order to check the assumptions underlying the analysis (egs. normality and constant variance). An example of a residual plot for batter SG is given in Figure 4.4. The random distribution of the residuals about the zero mark suggest that no assumptions of the statistical analysis were violated (Joglekar and May, 1987). The remaining residual plots (not shown) also indicated that no assumptions were violated.

Selection of Best Fitting Models for Each Physical and Sensory Sponge Cake Characteristic

The first objective of this study was to select best fitting models to predict the effects of PPC, whip time and cream of tartar on each of the physical and sensory responses evaluated. The goal of a best fit model is to provide a prediction equation that is composed of only the most important explanatory variables. For each physical and sensory response, selection of the best fitting models was based upon the results of the full model analysis of variance.

Summary of Results from the Analyses of Variance

Tables 4.5 and 4.6 summarize the F-values and corresponding levels of significance (probability values) from the full model analysis of variance for each physical and sensory response, respectively.

The importance of PPC and whip time to physical and sensory sponge cake quality is illustrated by the presence of
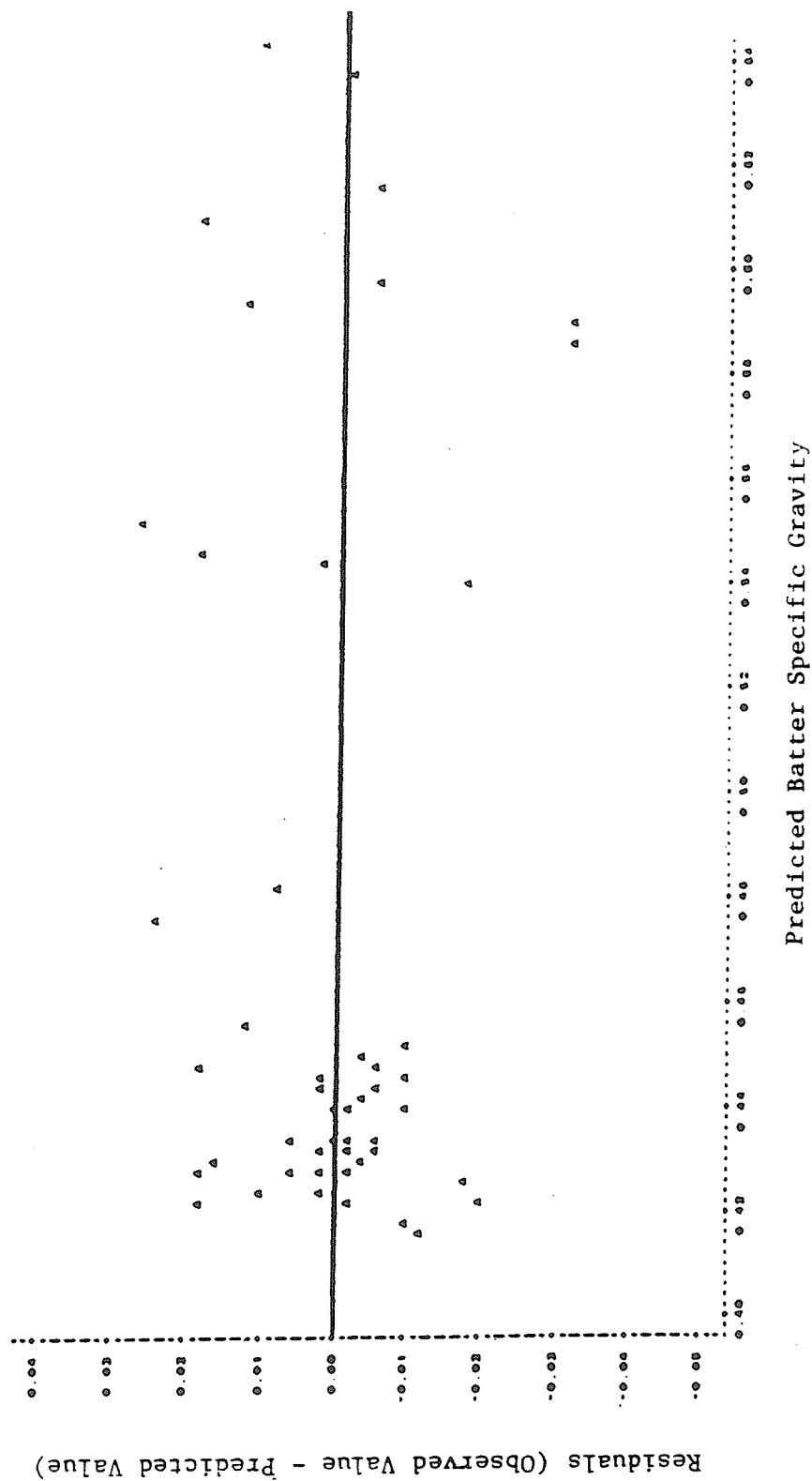
Figure 4.4   Plot of residuals (observed value - predicted value) versus predicted values for batter specific gravity.

Table 4.5   F-values[1] from the Full Model Analysis of Variance[2] for Physical Measurements

Effects of Independent Variables[3]	Dependent Variable (yi)						
	Specific Gravity	Specific Volume	Hardness	Cohesiveness	Gumminess	Moisture	pH
Linear							
X_1 (PPC)	467.72(.0001)[4]	176.34(.0001)	62.36(.0001)	88.48(.0001)	53.68(.0001)	0.29(.5921)	237.82(.0001)
X_2 (Whip time)	175.77(.0001)	74.97(.0001)	147.21(.0001)	57.39(.0001)	153.21(.0001)	3.40(.0741)	15.25(.0004)
X_3 (Cream of tartar)	4.94(.0330)	0.32(.5759)	2.79(.1044)	0.75(.3916)	3.65(.0648)	0.17(.6806)	226.60(.0001)
Quadratic							
X_1^2	124.18(.0001)	31.06(.0001)	18.19(.0002)	14.08(.0007)	17.60(.0002)	0.01(.9378)	5.59(.0239)
X_2^2	77.37(.0001)	7.17(.0113)	37.03(.0001)	11.70(.0017)	37.88(.0001)	0.20(.6540)	4.75(.0363)
X_3^2	0.59(.4491)	0.24(.6266)	0.23(.6367)	1.33(.2577)	0.30(.5896)	0.42(.5231)	6.19(.0179)
Interactions							
X_1X_2	143.70(.0001)	41.43(.0001)	44.78(.0001)	28.22(.0001)	39.94(.0001)	2.90(.0978)	1.01(.3208)
X_1X_3	4.16(.0491)	6.03(.0193)	3.79(.0602)	1.97(.1699)	3.48(.0709)	0.12(.7353)	3.29(.0786)
X_2X_3	0.15(.7002)	0.09(.7713)	2.86(.1002)	0.30(.5867)	2.91(.0973)	0.07(.7996)	0.65(.4259)

[1]Associated with partial (Type III) sums of squares.
[2]GLM procedure (SAS, 1985). Analysis based on the mean of 2 determinations for specific gravity, specific volume and moisture, 3 determinations for hardness, cohesiveness and gumminess, and 1 pH determination.
[3]There were no significant block (day) or rep effects except for moisture, where there was a small, but significant, block effect.
[4]Probability associated with F-values.

Full model = yi = $b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$

Table 4.6   F-values[1] from the Full Model Analysis of Variance[2] for Sensory Measurements

Effects of Independent Variables[3]	Dependent Variable (y1)								
	Aroma Intensity	Flavor Intensity	Firmness	Springiness	Cohesiveness	Moistness	Predominant Cell Size	Cell Uniformity	Cell Wall Thickness
Linear									
X_1 (PPC)	0.11(.7423)[4]	0.13(.7221)	52.59(.0001)	97.09(.0001)	33.99(.0001)	64.62(.0001)	36.24(.0001)	62.10(.0001)	10.25(.0025)
X_2 (Whip time)	0.26(.6160)	2.58(.1178)	96.59(.0001)	33.14(.0001)	55.80(.0001)	9.02(.0050)	7.98(.0069)	20.89(.0001)	31.60(.0001)
X_3 (Cream of tartar)	0.72(.4012)	10.00(.0033)	0.34(.5641)	2.80(.1037)	0.14(.7069)	0.34(.5635)	0.80(.3745)	3.52(.0668)	0.25(.6186)
Quadratic									
X_1^2	0.15(.6974)	0.97(.3314)	22.39(.0001)	11.94(.0015)	31.81(.0001)	10.65(.0025)	29.99(.0001)	2.89(.0959)	0.18(.6727)
X_2^2	1.04(.3158)	0.20(.6539)	10.90(.0023)	5.27(.0280)	9.81(.0036)	0.88(.3546)	27.75(.0001)	26.02(.0001)	2.71(.1064)
X_3^2	1.27(.2684)	7.74(.0087)	0.70(.8000)	0.00(.9605)	0.08(.7844)	0.24(.6300)	0.65(.4239)	0.12(.7294)	0.95(.3348)
Interactions									
X_1X_2	0.88(.3545)	1.16(.2886)	21.02(.0001)	25.35(.0001)	17.13(.0002)	2.11(.1551)	25.87(.0001)	7.31(.0095)	3.46(.0693)
X_1X_3	0.00(1.000)	7.24(.0110)	1.16(.2901)	1.16(.2881)	0.79(.3818)	0.10(.7481)	8.17(.0063)	1.98(.1657)	4.33(.0428)
X_2X_3	0.05(.8285)	0.09(.7610)	0.40(.5334)	0.00(.9621)	1.29(.2645)	0.58(.4509)	0.03(.8666)	1.27(.2654)	3.31(.0750)

[1]Associated with partial (Type III) sums of squares for first 6 dependent variables and sequential (Type I) sums of squares for the last 3 variables.
[2]GLM procedure (SAS, 1985). Analysis based on the mean of 10 judgements.
[3]There were no significant block (day) or rep effects except for cell uniformity, where there was a significant rep effect.
[4]Probability associated with F-values.

Full model = y1 = $b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$

significant F-values for linear, quadratic and interaction effects involving these two factors. There were significant linear and/or quadratic and/or interaction effects of PPC on all physical cake characteristics except moisture content, and on all sensory characteristics except aroma intensity. The length of whip time affected the same physical and sensory characteristics as PPC, except flavor which was slightly affected by PPC, but not by whip time.

Moisture content and aroma intensity were not significantly affected by any of the independent variables investigated, while flavor intensity was slightly affected by PPC in the form of a significant interaction with cream of tartar. Measured moisture content varied slightly from day to day (significant block effect), probably due to fluctuating oven temperatures during drying, therefore no further analysis was conducted on this data. Similarly, aroma intensity was excluded from further analysis because only small differences were observed among sponge cake treatments. Mean sensory scores (n=10) for aroma intensity ranged from 6.0 to 8.0 on a scale from 0.0 to 15.0.

It was interesting that PPC did not seem to influence the aroma of the sponge cakes and only slightly affected the flavor intensity. Many researchers attempting to incorporate pea flour or pea protein into various baked products have documented flavor and aroma problems (McWatters, 1978; McWatters, 1980; Repetsky and Klein, 1981; Raidl and Klein,

1983). The PPC used in this study did not appear to contribute any of the "beany", "harsh", "pea", "nutty" or "strong" aroma and flavor notes described by other researchers. If such flavor notes had been present, even at low intensity levels, they should have been detectable in the bland sponge cake formulation. This is a very positive finding due to the importance of flavor to ultimate product acceptability.

Cream of tartar was found to have much less effect than PPC and whip time on physical and sensory cake characteristics. Batter pH was significantly affected but batter SG and cake specific volume were only slightly affected by cream of tartar, while of the sensory characteristics, only flavor intensity and cell wall thickness were significantly affected. The effect of cream of tartar on flavor intensity was much more apparent than its affect on cell wall structure, with significant linear, quadratic and interaction effects. Snack cakes are often flavored with lemon and the slightly acidic flavor evident with higher levels of cream of tartar would likely be masked with this flavoring.

These results strongly support the findings of the preliminary study presented in Chapter 3, in which PPC and whip time were found to be critical factors affecting the sponge cake characteristics evaluated. The influence of cream of tartar on cake quality was significant, but much less pronounced.

Selection of Best Fit Models

Best fitting models to predict each of the physical and sensory cake characteristics were determined based upon the significance of linear, quadratic and interaction effects found in Tables 4.5 and 4.6. Statistically significant effects (p<.05) were retained in each model while non-significant effects were omitted. Linear effects which were not significant but appeared in significant quadratic or interaction effects, were also retained in each model.

Best models were re-analyzed to produce regression coefficients for use in predictive equations. The regression coefficients, R^2 values and coefficients of variation for each best selected model for physical and sensory characteristics are summarized in Tables 4.7 and 4.8 respectively. While F-values from the full model analyses of variance indicated which independent variables significantly affected the dependent variables, the regression coefficients represent the incremental change in the dependent variable (y_i) associated with a unit change in the independent variable (x_i), while all other regressors remain constant (Wonnacott and Wonnacott, 1982). The sign preceding the coefficient indicates the direction of the change. For example, increasing the level of PPC from 30 to 51.22 percent (a one unit change), holding all other terms constant, would result in a linear increase in batter specific gravity of approximately 0.06. Increasing the whip time from 8 to 10.83 minutes would produce a linear

Table 4.7   Regression Equation Coefficients, R-Square Values and Coefficients of Variation Associated with Selected Best Fitting Models[1] for the Physical Measurements

Effects of Independent Variables	Dependent Variable (y_i)					
	Specific Gravity	Specific Volume	Hardness	Cohesiveness	Gumminess	pH
b_o	0.433 (.0001)[2]	3.62 (.0001)	28.06 (.0001)	0.65 (.0001)	18.15 (.0001)	5.44 (.0001)
Linear						
b_1 (PPC)	0.058 (.0001)	-0.29 (.0001)	8.01 (.0001)	-0.02 (.0001)	4.04 (.0001)	0.14 (.0001)
b_2 (Whip time)	-0.036 (.0001)	0.19 (.0001)	-12.43 (.0001)	0.17 (.0001)	-6.89 (.0001)	-0.03 (.0007)
b_3 (Cream of tartar)	-0.006 (.0441)	-0.01 (NS)	-	-	-	-0.13 (.0001)
Quadratic						
b_{11}	0.035 (.0001)	-0.14 (.0001)	5.10 (.0002)	-0.01 (.0002)	2.74 (.0003)	0.02 (.0325)
b_{22}	0.028 (.0001)	-0.07 (.0144)	7.21 (.0001)	-0.01 (.0004)	3.97 (.0001)	0.02 (.0478)
b_{33}	-	-	-	-	-	0.03 (.0248)
Interactions						
b_{12}	-0.045 (.0001)	0.20 (.0001)	-9.80 (.0001)	0.02 (.0001)	-5.03 (.0001)	-
b_{13}	-0.008 (NS)[3]	0.08 (.0358)	-	-	-	-
b_{23}	-	-	-	-	-	-
R^2	.96	.87	.87	.83	.86	.91
CV (%)	3.4	4.0	17.0	1.9	15.2	1.0
Model Significance	.0001	.0001	.0001	.0001	.0001	.0001

[1]Selection based on significant F-values (P≤.05) from Table 4.5. For any independent variable appearing in a significant quadratic or interaction term, the linear effect, although not significant, was retained in the model.
[2]Probability associated with coefficients.
[3]Not significant.

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Table 4.8 Regression Equation Coefficients, R-Square Values and Coefficients of Variation Associated with Selected Best Fitting Models[1] for Sensory Measurements

Effects of Independent Variables[3]	Dependent Variable (y_i)							
	Flavor Intensity	Firmness	Springiness	Cohesiveness	Moistness	Predominant Cell Size	Cell Uniformity	Cell Wall Thickness
b_o	6.4 (.0001)[2]	6.2 (.0001)	7.0 (.0001)	7.5 (.0001)	7.5 (.0001)	7.7 (.0001)	8.6 (.0001)	7.4 (.0001)
Linear								
b_1 (PPC)	0.0 (NS)[3]	0.8 (.0001)	-0.8 (.0001)	0.5 (.0001)	0.6 (.0001)	-0.8 (.0001)	-1.7 (.0001)	0.3 (.0059)
b_2 (Whip time)	-	-1.1 (.0001)	0.5 (.0001)	-0.6 (.0001)	-0.2 (.0062)	0.4 (.0097)	1.0 (.0001)	-0.5 (.0001)
b_3 (Cream of tartar)	0.3 (.0025)	-	-	-	-	-0.1 (NS)	-	-0.1 (.6601)
Quadratic								
b_{11}	-	0.6 (.0001)	-0.3 (.0001)	0.5 (.0001)	0.3 (.0025)	-0.9 (.0001)	-	-
b_{22}	-	0.4 (.0007)	-0.2 (.0190)	0.3 (.0012)	-	-0.8 (.0001)	-1.1 (.0001)	-
b_{33}	-0.3 (.0020)	-	-	-	-	-	-	-
Interactions								
b_{12}	-	-0.7 (.0001)	0.6 (.0001)	-0.5 (.0001)	-	0.9 (.0001)	0.8 (.0115)	-
b_{13}	-0.3 (.0090)	-	-	-	-	0.5 (.0089)	-	-0.3 (.0700)
b_{23}	-	-	-	-	-	-	-	-
R^2	.40	.84	.81	.80	.64	.58	.53	.28
CV (%)	8.0	8.8	6.9	5.5	6.2	16.6	22.7	11.3
Model Significance	.0001	.0001	.0001	.0001	.0001	.0001	.0001	.0001

[1]Selection based on significant F-values (P≤.05) from Table 4.6. For any independent variable appearing in a significant quadratic or interaction term, the linear effect, although not significant, was retained in the model.
[2]Probability associated with coefficients.
[3]Not significant.

decrease in batter SG (as indicated by the negative coefficient) of approximately 0.04. The size of the coefficients and their associated p-values denotes the relative importance of the independent variable to the prediction of the dependent variable. Thus, the trends observed in Tables 4.5 and 4.6 are also apparent in Table 4.7 and 4.8. That is, PPC and whip time were the two most important factors affecting both physical and sensory sponge cake characteristics.

Criteria for Evaluation of Model Adequacy

Joglekar and May (1987) have suggested three measures to evaluate the goodness of a selected model: overall model significance, coefficient of multiple determination and coefficient of variation. In the following discussion, these three criteria have been applied to judge how well the models fit the data.

Model significance is the level of confidence that the selected model cannot be due to experimental error. Joglekar and May (1987) have suggested that for a good model, model significance should be at least $p<.05$. The model significance for all dependent variables in this study was $p<.0001$.

Coefficient of multiple determination (R^2) is the percentage of the variation in the dependent variable explained by the model. According to Joglekar and May (1987), in general, R^2 values should be at least 80 percent. For nine

out of fourteen dependent variables evaluated, the selected models explained at least 80 percent of the variability. R-square values for sensory judgements were, in general, much lower than those for physical measurements. Most notably, R^2 values for flavor and crumb quality evaluations (predominant cell size, cell uniformity, and cell wall thickness) were quite low explaining only 40, 58, 53 and 28 percent of the variation, respectively. Vaisey-Genser et al. (1987) noted similar results for flavor and crumb quality judgements of layer cakes. Unexplained variability in flavor judgements may be due to the complex task of judging overall flavor intensity in a product that is rather bland in flavor. Alternatively, perhaps, PPC, whip time and cream of tartar do not adequately explain the differences in flavor judgements. Unexplained variability in the sensory evaluation of cake crumb quality may be due to the difficulty of the judgement task, or to variability within the cake samples presented. Judgements could differ depending upon where, within the cake sample, the panelist based his/her judgement.

Coefficient of Variation (CV) is equal to the standard error of estimate/mean of dependent variable X 100, and describes the amount of variation in a population relative to the mean. In general CV levels for model adequacy should be no greater than 10 percent (Joglekar and May, 1987), however, experience with similar data is necessary to know whether CV values are unusually high (Steel and Torrie, 1980).

Coefficients of variation greater than 10 percent were observed for Instron hardness and gumminess, and all three sensory crumb evaluations. The remaining responses showed limited variability with CV values ranging from 1.0 percent for pH to 4.0 percent for specific volume. Variability among Instron hardness and gumminess scores could possibly be due to slight differences in the heights of the tested samples. During texture testing, it was noted that some cake wedges "sprung" back higher than 2.0 centimetres after thawing, resulting in sample heights ranging from about 2.0 to 2.3 centimetres. Additionally, there could be variability within the cake crumb itself. Variability among judgements of crumb quality as discussed, is most likely due to a combination of sample variability and the difficulty of the judgement task.

Model significance, R^2 values and CV values were used to judge model adequacy in order to identify the dependent variables that should be further investigated via contour and response surface plots.

Interpretation of Results Based upon Contour and Response Surface Plots

The second objective of this study was to use the best fitting models, which showed good fit to the data, to produce contour and response surface plots. These graphical presentations of the models, in two and three dimensions, help to visualize the linear, quadratic and interaction

relationships between PPC, whip time and cream of tartar. More importantly, contour and response surface plots help to clarify the effects of PPC, whip time and cream of tartar on the dependent variables, and facilitate the location of optimal/acceptable physical and sensory cake characteristics.

Selected best models meeting all three good fit criteria (model significance <.05, R^2 >80%, or CV <10%), were used to produce contour and response surface plots. Good fit models included batter specific gravity, cake specific volume, Instron cohesiveness, batter pH, sensory firmness, springiness and cohesiveness. Plots were also generated from models meeting only two of the good fit criteria (Instron hardness, gumminess, sensory flavor, moistness), however results were interpreted with caution. The models for predominant cell size, cell uniformity, and cell wall thickness, were not used to produce plots since all three crumb characteristics yielded low R^2 values and high CV values indicating limited predictive ability.

In the following discussion, contour and response surface plots of the physical cake characteristics will be presented first, followed by the sensory characteristics. Acceptance regions have been highlighted (shaded) on all contour plots, except flavor and pH, in order to identify sponge cake formulations which have met the REF sponge cake standards (Table 4.9). That is, the shaded regions indicate the sponge cake formulae which are predicted to produce cakes of

Table 4.9   Reference Values and Standards for Physical and
             Sensory Characteristics of Sponge Cakes

Dependent Variable	Mean Response[1] for REF Sponge Cake (100% albumen)	REF Standard[2]
Physical Characteristics		
Specific Volume (cc/g)	3.83	≥ 3.45
Hardness (N)	29.60	≤ 32.60
Cohesiveness	0.66	≥ 0.59
Gumminess (N)	19.40	≤ 21.30
Specific Gravity	0.40	≤ 0.45
Sensory Characteristics		
Firmness	6.3	≤ 6.9
Springiness	7.6	≥ 6.8
Cohesiveness	7.0	≥ 6.3
Moistness	6.8	≥ 6.1

[1]Physical Characteristics - n=16 (8 x 2 reps) for hardness, cohesiveness, gumminess; n=32 (8 x 2 observations x 2 reps) for specific volume and specific gravity.
Sensory Characteristics - n=30 (10 judges x 3 training sessions); mean value is the reference score positioned on a 15 cm line scale where 1 = low level of the characteristic and 15 = a high level of the characteristic.

[2]Limit set as a minimum (10% less than the REF sponge cake mean response) for specific volume, Instron cohesiveness, sensory springiness, cohesiveness, and moistness. Limit set as a maximum (10% greater than the REF sponge cake mean response) for Instron hardness, gumminess and sensory firmness. Limit for batter specific gravity was based upon acceptable commercial value set by Export Packers, Ltd.

comparable quality to the 100% albumen REF cakes. For each sponge cake characteristic REF standards were set to come within ten percent of the mean value obtained from evaluation of the 100 percent albumen REF cakes. REF standards were not set for overall flavor intensity due to the difficulty of identifying a specific acceptable/unacceptable cut-off for this very general flavor parameter. Plots illustrating the effects of PPC, whip time and cream of tartar on batter pH were used only to help explain their effects on other dependent variables. Finally, where possible, comparisons between physical and sensory responses have been made.

Physical Sponge Cake Characteristics

The effects of PPC, whip time and cream of tartar on batter SG and cake specific volume will be examined first due to the high predictive ability of these two models. The importance of SG and volume to overall cake quality has been suggested by other researchers. Wells (1989) noted the importance of carefully controlling the SG of snack cake batter because of its direct effect on the final volume, grain and texture of baked cakes. Pierce and Walker (1987) suggest that while high volume cakes do not always represent a good quality cake, cakes of low volume are generally of low quality. Funk et al. (1969) found that volume was a valid assessor of angel cake texture. Thus, SG and volume are probably the two most important physical characteristics

reflecting overall sponge cake quality. In the second section, the effects of PPC and whip time on Instron cohesiveness, hardness and gumminess will be discussed.

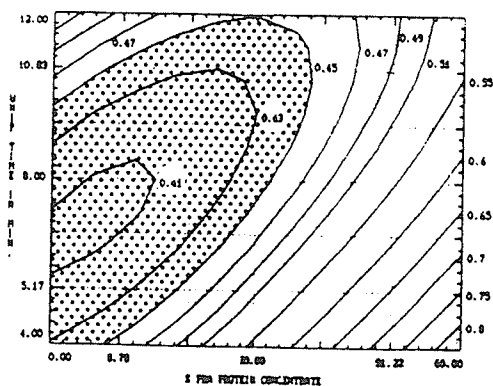i) Batter SG and Cake Specific Volume

Best fitting models for batter SG and specific volume indicated that both variables were significantly affected by PPC, whip time and cream of tartar. Since it is possible to simultaneously vary only two variables at one time in response surface and contour plots, the two most influential variables, PPC and whip time, were chosen for the axes. Cream of tartar was held constant at low (1.5g), medium (3.0g) and high (4.5g) levels in order to evaluate its effect on the dependent variables. Thus, cream of tartar, having the least effect on the response, was held at a constant value, while PPC and whip time were varied.

Figures 4.5 and 4.6 present SG and specific volume as functions of PPC level and whip time, with cream of tartar held constant at 1.5, 3.0 and 4.5 grams, respectively. Levels presented in the plots represent the actual levels evaluated and were based upon the range of coded levels determined by the experimental design ($-\sqrt{2}$, -1, 0, +1, $+\sqrt{2}$). Reference standards have been highlighted on each contour plot indicating the sponge cake formulations that have met these REF standards. Shaded regions represent SG values $\leq.45$ and specific volumes ≥ 3.45 cubic centimetres/gram. Acceptance
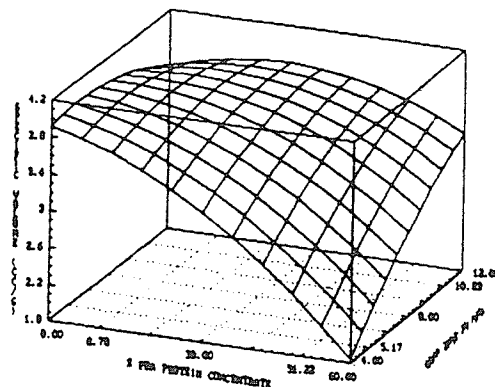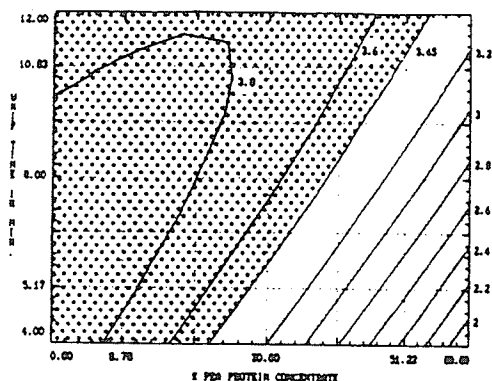
Cream of tartar = 1.5g
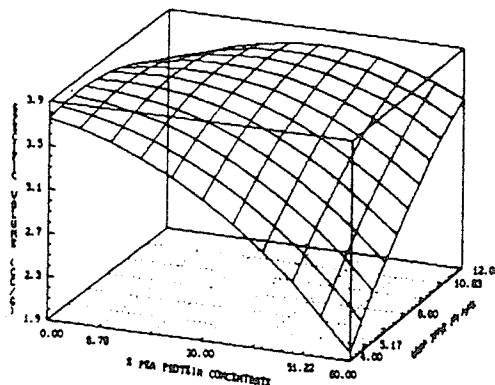

Cream of tartar = 3.0g

Cream of tartar = 4.5g

Figure 4.5 Contour and response surface plots for the effects of PPC and whip time on batter specific gravity (SG) at low, medium and high cream of tartar levels. Shaded regions met the REF standard. Higher values represent increasing SG. Levels of PPC, whip time and cream of tartar are actual values.
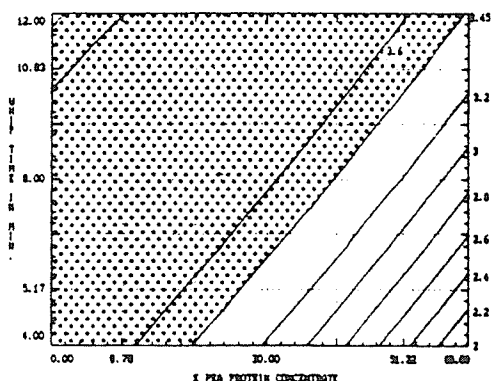
Cream of tartar = 1.5g

Cream of tartar = 3.0g
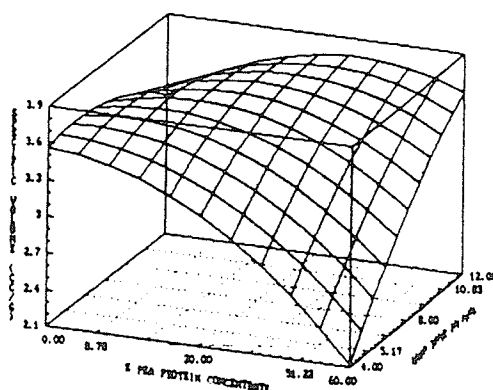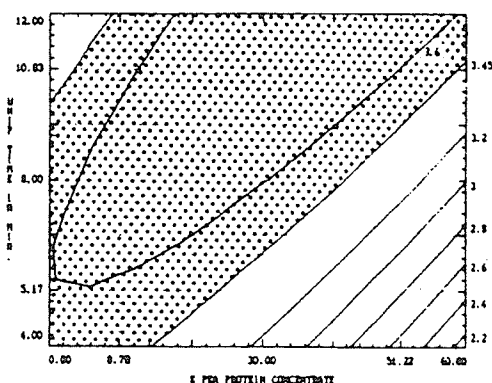

Cream of tartar = 4.5g

Figure 4.6 Contour and response surface plots for the effects of PPC and whip time on cake specific volume at low, medium and high cream of tartar levels. Shaded regions met the REF standard. Higher values represent increasing specific volume. Levels of PPC, whip time and cream of tartar are actual values.

regions for both SG and specific volume are quite large offering a number of PPC and whip time combinations which met the REF standards. In general, low quality cakes, characterized by high SG values and low specific volumes, resulted when high PPC levels were accompanied by low whip times and when low PPC levels were accompanied high whip times. The level of cream of tartar did not effectively change this relationship.

The shape of the response surface plots clearly illustrates the significant linear, quadratic (curvilinear) and interaction effects found in the best fitting models for SG and specific volume. Because of highly significant interactions between PPC and whip time, it is impossible to discuss the effect of PPC on SG and volume without considering the length of whip time, and vice versa. Therefore, the effect of increasing PPC at low and high whip times will be discussed by moving along the X (% PPC) axis at 4 and 12 minutes of whip time (Figure 4.7). Conversely, the effect of increasing whip time at low and high PPC levels will be discussed by moving along the Y (whip time) axis at 0 percent and 60 percent PPC levels (Figure 4.8).

At low whip times, increasing PPC had a negative effect on batter SG and cake specific volume, that is, batter SG increased and specific volumes decreased (Figures 4.5 and 4.6). This effect was evident at all cream of tartar levels, however, SG values were lower and specific volumes higher, at
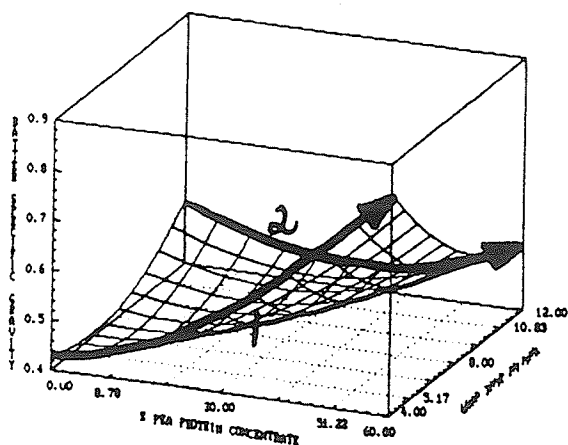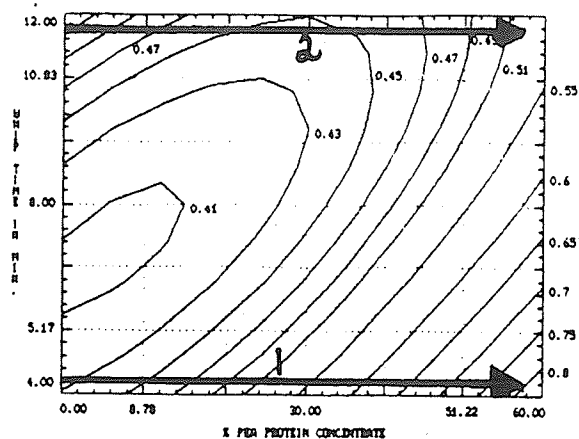
Figure 4.7 Method for discussion of effects of increasing PPC
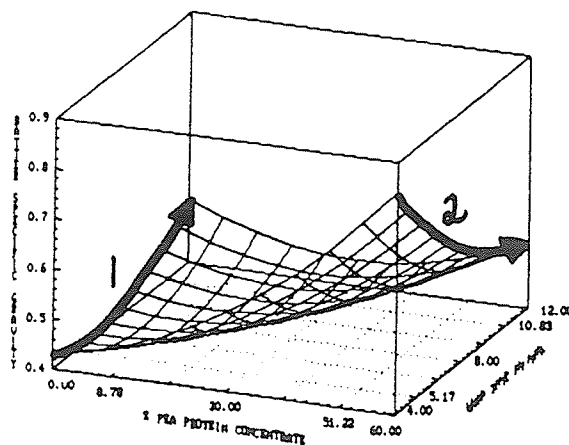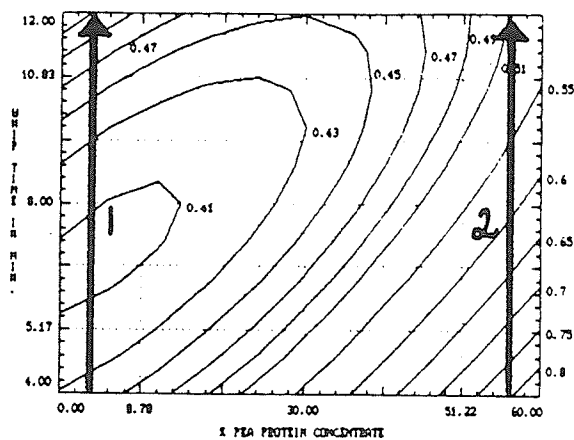level at low (4 min.) and high (12 min.) whip times.

Figure 4.8   Method for discussion of effects of increasing
whip time at low (0%) and high (60%) PPC levels.

all but the lowest PPC levels, as cream of tartar increased.

Recall that high SG values indicate less air incorporation into the batter and that sponge cakes depend entirely upon this air for leavening. Therefore the fact that high SG values were accompanied by low volumes, is not surprising. Other researchers have found similar relationships between batter SG and finished cake volume (Dunn and White, 1939; Rolfes et al., 1955; Zabik et al., 1969).

At high whip times, increasing PPC first lowered SG, and then, once beyond a critical protein level, SG values were seen to rise again. The point at which SG values began to rise, however, depended upon the level of cream of tartar. High cream of tartar levels shifted this critical protein level (where SG values began to rise again) such that it was possible to incorporate greater levels of PPC before causing SG values to increase. Unlike SG, volume was only slightly affected by increasing PPC level when whip times were high. Volumes initially increased slightly, and then decreased, however, little change occurred across all PPC levels. Low cream of tartar levels produced the highest volumes, at low PPC levels, but high cream of tartar levels were necessary to achieve good volumes with high levels of PPC. Thus negative effects of high PPC levels on SG and specific volume were reduced by long whip times and high cream of tartar levels.

Moving along the Y-axis, at low PPC levels, it can be seen that increasing whip time first decreased SG and then

increased it. While this effect was evident at all cream of tartar levels, the SG at any given whip time was higher as cream of tartar levels increased. Volume, on the other hand, was only slightly affected by whip time when PPC levels were low. Volumes were seen to fall slightly at high whip times, particularly when cream of tartar levels were high. Thus low cream of tartar levels appeared to be most beneficial when PPC levels were also low.

At high PPC levels, increasing whip time improved both batter SG and specific volume. Batter SG decreased and specific volumes increased. This effect was evident at all cream of tartar levels, however, the SG values and specific volumes at any given PPC level were lower and higher, respectively, as cream of tartar increased.

Some general relationships between PPC, whip time and cream of tartar, and their effects upon batter SG and cake specific volume, have emerged from the response surface and contour plots. In general, when PPC levels were high, longer whip times were necessary to improve batter SG and cake specific volume. Higher SG values and lower specific volumes were a result of short whip times combined with high PPC cake formulations. Conversely, when PPC levels were low, whip times had to be shortened since long whip times were seen to increase SG values and decrease specific volumes.

The subtle effects of cream of tartar on SG and specific volume have been clarified in Figure 4.9. The REF standard

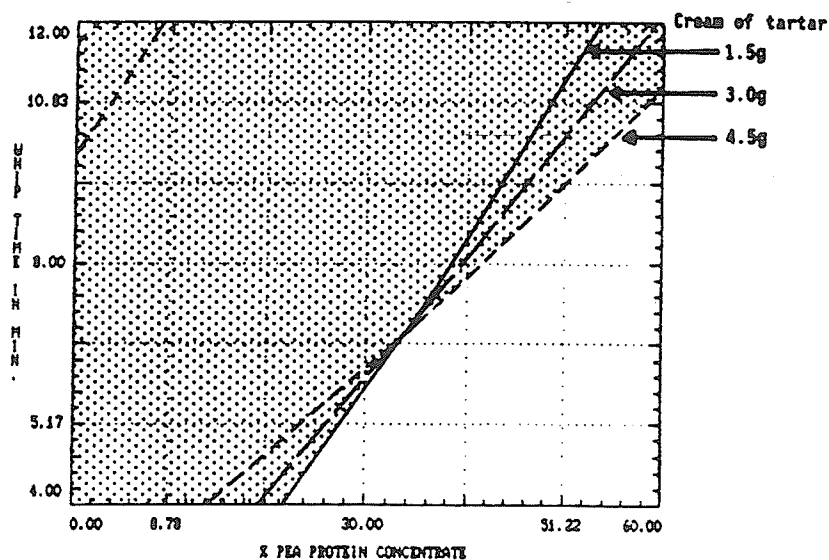## BATTER SPECIFIC GRAVITY
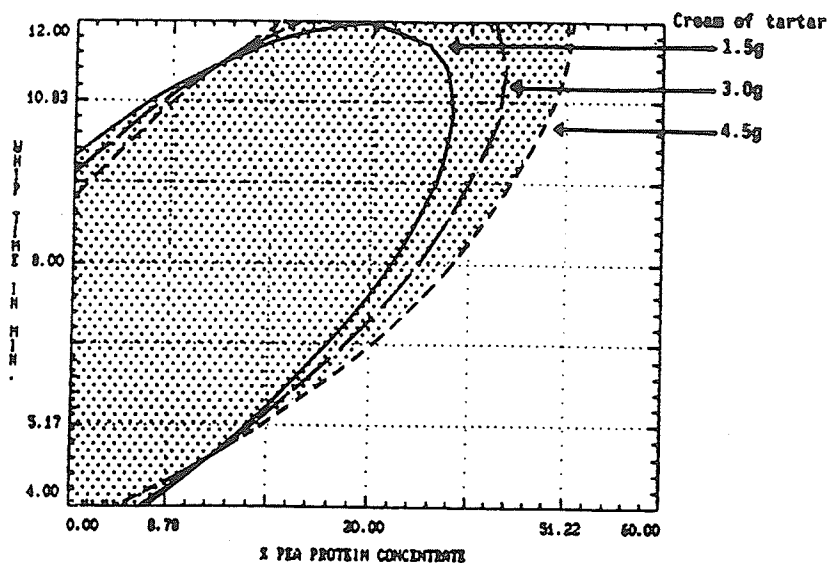
SPECIFIC VOLUME (CC/G)

Figure 4.9   Effect of cream of tartar level on the acceptability regions (shaded) for batter specific gravity and cake specific volume.

contour lines for SG (≤0.45) and specific volume (≥3.45), for 1.5g, 3.0g, and 4.5 grams of cream of tartar, have been superimposed on one contour plot to highlight differences. These plots indicate that high cream of tartar levels were most beneficial for high PPC formulations. At high PPC levels, increasing cream of tartar decreased the whip time necessary to meet the REF standard. Similarly, when PPC levels and whip times were relatively high (ie. exceed approximately 30% and 8 min., respectively), high cream of tartar levels permitted greater PPC incorporation for a given whip time, while still meeting the REF standards. The best cream of tartar level depended, however, upon the specific level of PPC and whip time chosen for the sponge cake formulation.

Sponge cake batter SG and finished cake volume both relate to the amount of air incorporated and retained during mixing and baking, and therefore, are affected by the foaming and coagulating abilities of the proteins in the system. Because cakes did not fall when removed from the oven, and there was no evidence of uncooked batter, the higher denaturation temperatures of pea proteins (approximately 90° C, Murray et al., 1985) versus egg albumen proteins (approximately 65 and 84°C, Donovan, 1977; Guy and Pithawala, 1981), did not appear to be critical. Thus, heating times must have been long enough and/or temperatures high enough to produce sufficient amounts of heat denatured proteins to

stabilize the cake structure. Thompson et al. (1982) found that while a rapeseed concentrate produced foams similar to those of egg albumen, the concentrate did not coagulate upon heating like egg albumen. Consequently, once baked as a meringue, volume was not maintained.

Differences in the foaming abilities of the two protein systems must, therefore, be the major reason for the observed effects on SG and specific volume. Small, soluble, highly flexible proteins with exposed hydrophobic surfaces are postulated to make good foaming agents (German et al., 1985). Such molecules are thought to quickly reach the air-water interface, concentrate, unfold, and re-orient to form viscous protein films around the newly formed air bubbles (Kinsella, 1981; Phillips, 1981; Cheftel et al., 1985). The globulins legumin and vicilin, which comprise 70 to 90 percent of the protein in peas, are very large proteins (360,000-400,000d and 145,000-200,000d, respectively) with highly ordered, complex structures (Table 1.1, Chapter 1). Pea proteins are less soluble than egg albumen proteins (Tables 1.3 and 1.4) however, solubility may be increased by the addition of salt via a salting in effect (Christensen, 1989; Gueguen, 1980). Thus, pea proteins appear to lack the properties thought to produce a good foam. However, the literature indicated that, while pea protein foam capacity and stability were inferior to that of egg albumen, pea proteins did exhibit some foamability (Table 1.6, Chapter 1). This study showed that

the pea proteins did foam when whip times were lengthened, suggesting that additional energy was necessary to unfold them to permit association and film formation at the interface (German et al., 1985). Sunflower and jojoba proteins have also been shown to require long whip times for foam formation and stabilization (Huffman et al., 1975; Wiseman and Price, 1987). Cream of tartar appeared to reduce the energy necessary to unfold the proteins, thus reducing the whip time necessary to achieve acceptable SG values. Increased cream of tartar lowered the pH so that it approached the isoelectric points (pI) of legumin (pI=4.8; Table 1.1) and, in particular, vicilin (pI=5.5; Table 1.1). Near the isoelectric point of the protein, the rate of protein coagulation is sometimes increased (Halling, 1981), which may help explain the faster rate of whip time observed at high cream of tartar levels. Rhodes et al. (1960) found that addition of an acid ingredient (lemon juice) to duck egg albumen decreased the time required for whipping.

Longer whip times produced PPC foams that appeared of similar stiffness to egg albumen foams, however, the volumes of the PPC foams were less. This suggests that while the foaming capacity of the PPC/albumen mixture was depressed due to the presence of PPC, the stability of the foam may have been retained, and even improved in the case of long whip times. It has been suggested that large globular proteins which do not unfold quickly at the air/water interface form

thick films which may contribute to foam stability (Cheftel et al., 1985). Cream of tartar may also have helped to increase the stability of the formed foams by lowering the batter pH closer to the isoelectric points of the pea proteins. It has been postulated that foam stability is maximum at the isoelectric point of the protein due to increased viscosity and rigidity of the protein films (Halling, 1981; Waniska and Kinsella, 1979; Cheftel et al., 1985; Phillips, 1981). The addition of cream of tartar to defatted sunflower meal increased foam volume and stability when sugar was also present (Huffman et al., 1975). Foam stability is important to final cake structure as it prevents the foam from collapsing while the heat coagulable proteins are denatured (Baldwin, 1986). This may be particularly important for the high PPC cakes due to the higher coagulation temperatures of legumin and vicilin compared to the heat coagulable egg albumen proteins.

The loss of foam stability due to overwhipping of protein solutions is believed to result from excessive protein unfolding and aggregation (Kinsella, 1981). In this study, the overwhip effect (denoted by a decrease in SG followed by an increase, as whip times were increased), was most apparent when low PPC/high cream of tartar formulations were whipped for greater than approximately 9 minutes. Egg albumen is known to be susceptible to overwhipping and destabilization, caused, it is believed, by excessive surface denaturation of

ovalbumin (Kinsella, 1981). Because low PPC formulations are also high egg albumen formulations, the observed overwhip effect was probably due to the excessive denaturation of ovalbumin. Cream of tartar appeared to accelerate the overwhip effect probably due to increased surface denaturation.

Halling (1981) noted that overwhip effects are not as severe with other proteins, as with albumen, and this appeared to be true for the pea protein. At higher levels, PPC seemed to exert a protective effect over excessive ovalbumin denaturation when whip times were high, that is, the overwhip phenomenon was less apparent. The lower level of egg albumen proteins in the high PPC formulations accompanied by the "slow-to-denature" pea proteins, probably accounts for this observation.

The loss in foam stability resulting from overwhipped protein systems is ultimately reflected in lower cake volumes. The cell walls enclosing the air in such foams lose elasticity and do not expand as the cake is baked, consequently cake volumes decrease (McWilliams, 1979). Similar results have been reported for sunflower meal and jojoba protein concentrate (Huffman et al., 1975; Wiseman and Price, 1987).

In summary, the observed effects of partially replacing egg albumen with PPC, on SG and specific volume, appear to be due to differences in the foaming properties of these proteins. When the level of PPC substitution increased, whip

times had to be increased as well, to provide the additional energy required to unfold the pea proteins so that they could participate in foam formation and stabilization. Excessive whip times were seen to result in a loss of foam stability which manifested itself in the form of high SG values and low specific volumes. Low PPC/high albumen formulations were most sensitive to this overwhip effect probably due to the susceptibility of egg ovalbumin to overwhipping. Cream of tartar decreased the whip times necessary to achieve acceptable SG values probably by increasing the rate of protein denaturation. The foam stabilizing effect of cream of tartar can be attributed to the lowering of batter pH so that it approached the isoelectric points of the pea and albumen proteins.

ii) Instron Cohesiveness, Hardness and Gumminess

Best fitting models for Instron cohesiveness, hardness and gumminess indicated that all three parameters were strongly affected by PPC and whip time. Unlike the preliminary study, cream of tartar level did not significantly affect any of the texture measurements in this study. Gates (1976), however, found that cream of tartar helped produce softer, more tender sponge cakes.

Figure 4.10 presents Instron cohesiveness, hardness, and gumminess as functions of PPC level and whip time. Because cream of tartar did not significantly affect these parameters

INSTRON COHESIVENESS
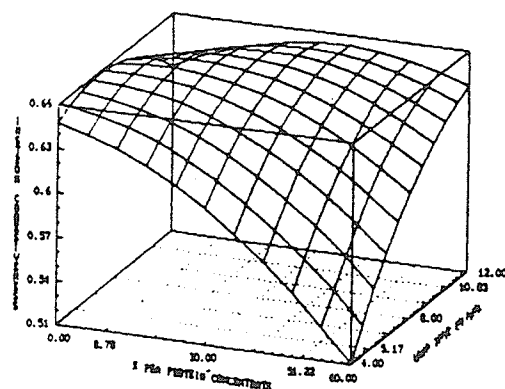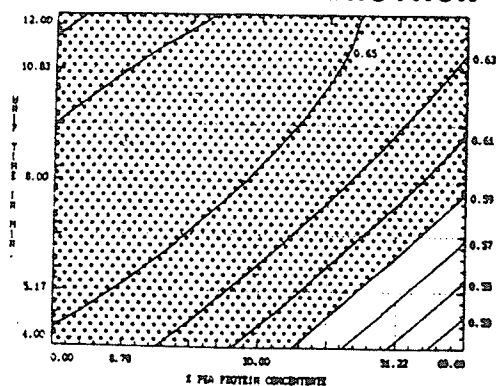
INSTRON HARDNESS (N)
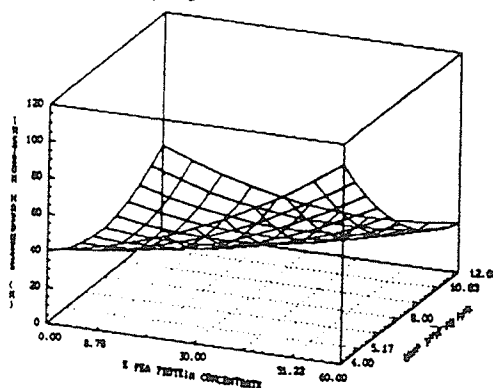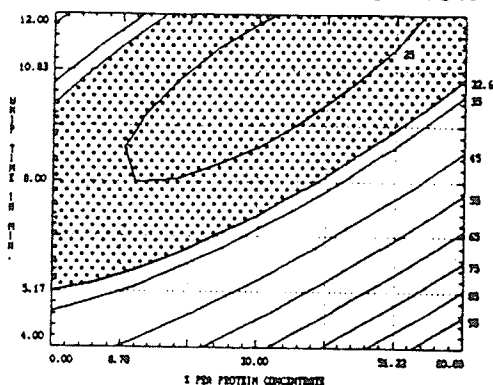
INSTRON GUMMINESS (N)
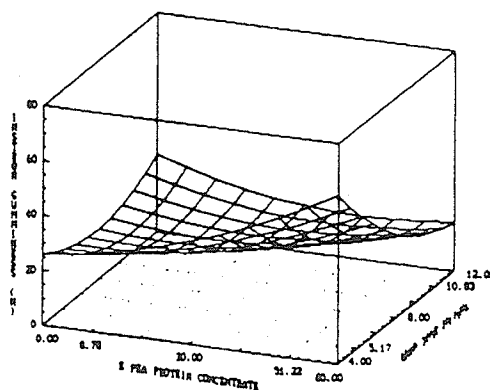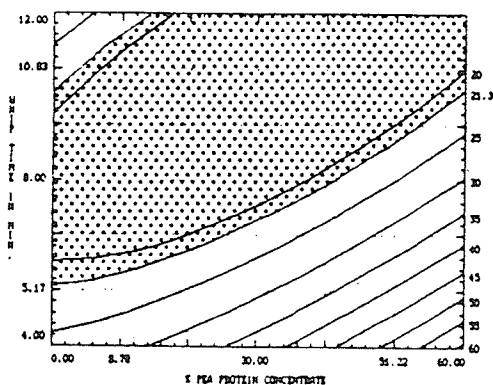
Figure 4.10 Contour and response surface plots for the effects of PPC and whip time on Instron cohesiveness, hardness and gumminess. Shaded regions met the REF standards. Higher values indicate increasing cohesiveness, hardness and gumminess. Levels of PPC and whip time are actual values.

it was not included in the regression equations used to generate the contour and response surface plots. Consequently, separate plots for low, medium and high cream of tartar levels were not produced, since one plot would represent any level of cream of tartar. The conclusions drawn from the hardness and gumminess plots should be considered as trends due to the variability associated with these measurements (high CV values). Once again, acceptance regions for cohesiveness, hardness and gumminess were quite large with high quality cakes produced by a large number of PPC levels and whip time combinations. Low quality cakes would result from a combination of high PPC levels and low whip times as well as low PPC levels combined with high whip time. The loss of cake quality due to the latter combination, however, was not as severe.

The significant linear, quadratic and interaction effects of PPC and whip time found in the best fitting models, are clarified in the three dimensional response surface plots. In the contour plots, slopes of the lines indicate that whip time had a greater effect than PPC on Instron cohesiveness, hardness and gumminess. Interactions between PPC and whip time were highly significant, therefore, their effects could not be considered independently. The effects of PPC and whip time on Instron cohesiveness, hardness and gumminess will therefore be discussed in the same manner as was done for SG and specific volume.

At low whip times, increasing PPC decreased cake cohesiveness, and increased cake hardness and gumminess. At high whip times, the effect of PPC on Instron texture measurements was much less pronounced, particularly for gumminess. Differences in slopes of lines for low and high whip times emphasize this result (Figure 4.10). At first as PPC increased cake cohesiveness also increased, while hardness and gumminess decreased. Once past a critical PPC level, however, high levels of PPC adversely affected cake texture producing less cohesive cakes which were harder and gummier.

At low PPC levels, increasing whip time had little effect on cake cohesiveness, hardness and gumminess, that is, texture values did not change a great deal with increasing whip times. Cohesiveness was increased slightly, and then fell again when whip times exceeded approximately 10 minutes. Similarly, hardness and gumminess decreased slightly, remained constant, and then increased when whip times approached approximately 10 minutes.

At high PPC levels, increasing whip time significantly increased cake cohesiveness and decreased hardness and gumminess. The marked effect of longer whip times on Instron texture measurements, when PPC levels were high, is shown by the closeness of the contour lines on the contour plots (Figure 4.10). Only small increases in whip times were required to increase cohesiveness and decrease hardness and gumminess.

The response surface and contour plots illustrate some important relationships between PPC and whip time, and their effects on Instron cohesiveness, hardness and gumminess. In general, when PPC levels were high, longer whip times were necessary to increase cake cohesiveness and decrease cake hardness and gumminess. High PPC levels combined with low whip times produced cakes of low quality. Low whip times were, however, necessary when PPC levels were low since excessive whip times negatively affected cake texture.

These results tend to mirror those observed for batter SG and cake specific volume suggesting that cake texture was strongly determined by initial batter SG and final cake volume. Other researchers have noted similar relationships (Wells, 1989; Johnson and Zabik, 1981b; Funk et al., 1969). A comparison of the response surface plots for SG and specific volume (Figures 4.5 and 4.6) with those for cohesiveness, hardness and gumminess (Figure 4.10) emphasizes their relationship. This relationship is not surprising when the method of sample preparation for Instron testing is considered. Cake samples were cut to identical heights, regardless of initial cake height. Cakes of lower volume were, therefore, much more compact and dense than higher volume cakes which resulted in higher hardness and gumminess values. Interestingly, although cakes of high volume were produced from low PPC/high whip time formulations, hardness and gumminess measurements were quite high. Such cakes were

overwhipped and the batter produced was meringue-like in appearance and texture. Consequently, once baked, the cake also took on a firm, meringue-like texture.

Loss of crumb cohesiveness did not appear to present a problem unless high PPC cakes were whipped for a very short time. In this case, cakes were very dense and when compressed by the Instron plunger, tended to stay indented rather than springing back. Cohesiveness is defined as the ratio of the areas under the curves produced by two compressions (Figure 2.1, Chapter 2), and a low ratio represents low cohesiveness.

Sensory Sponge Cake Characteristics

Sensory evaluations of cake crumb firmness, springiness, cohesiveness and moistness were all significantly affected by PPC and whip time, therefore, these parameters will be discussed together. Overall flavor intensity, which was significantly influenced by the level of cream of tartar and its interaction with PPC, will then be discussed.

i) Sensory Firmness, Springiness, Cohesiveness and Moistness

Response surface and contour plots for sensory moistness and the three textural parameters are presented in Figures 4.11 and 4.12, respectively. Cream of tartar did not significantly affect any of these parameters, therefore, for each parameter, only one contour and one response surface plot was necessary to evaluate the PPC and whip time effects.

SENSORY MOISTNESS
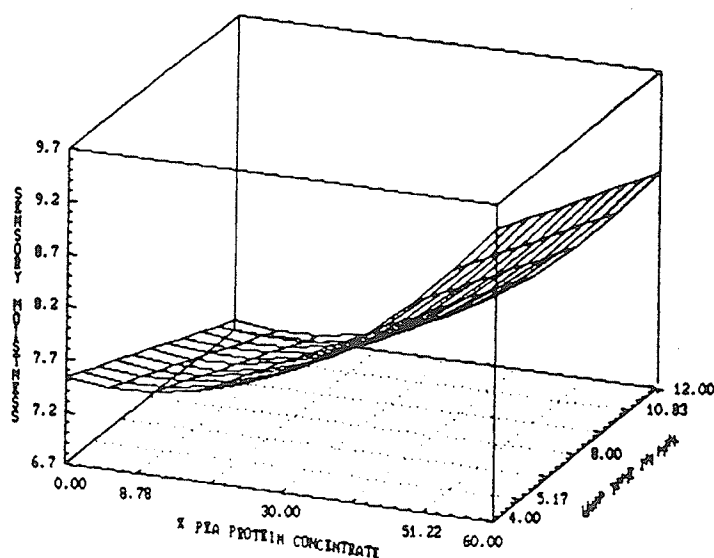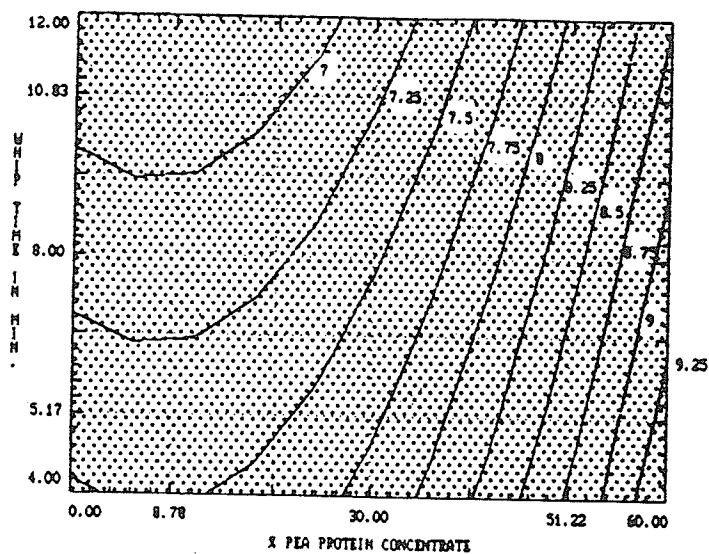
Figure 4.11 Contour and response surface plots for the effects of PPC and whip time on sensory moistness. The shaded region met the REF standard. Higher values indicate increasing moistness. Score range=0 to 15; 1.5=moderately dry, 13.5=moist. Levels of PPC and whip time are actual values.
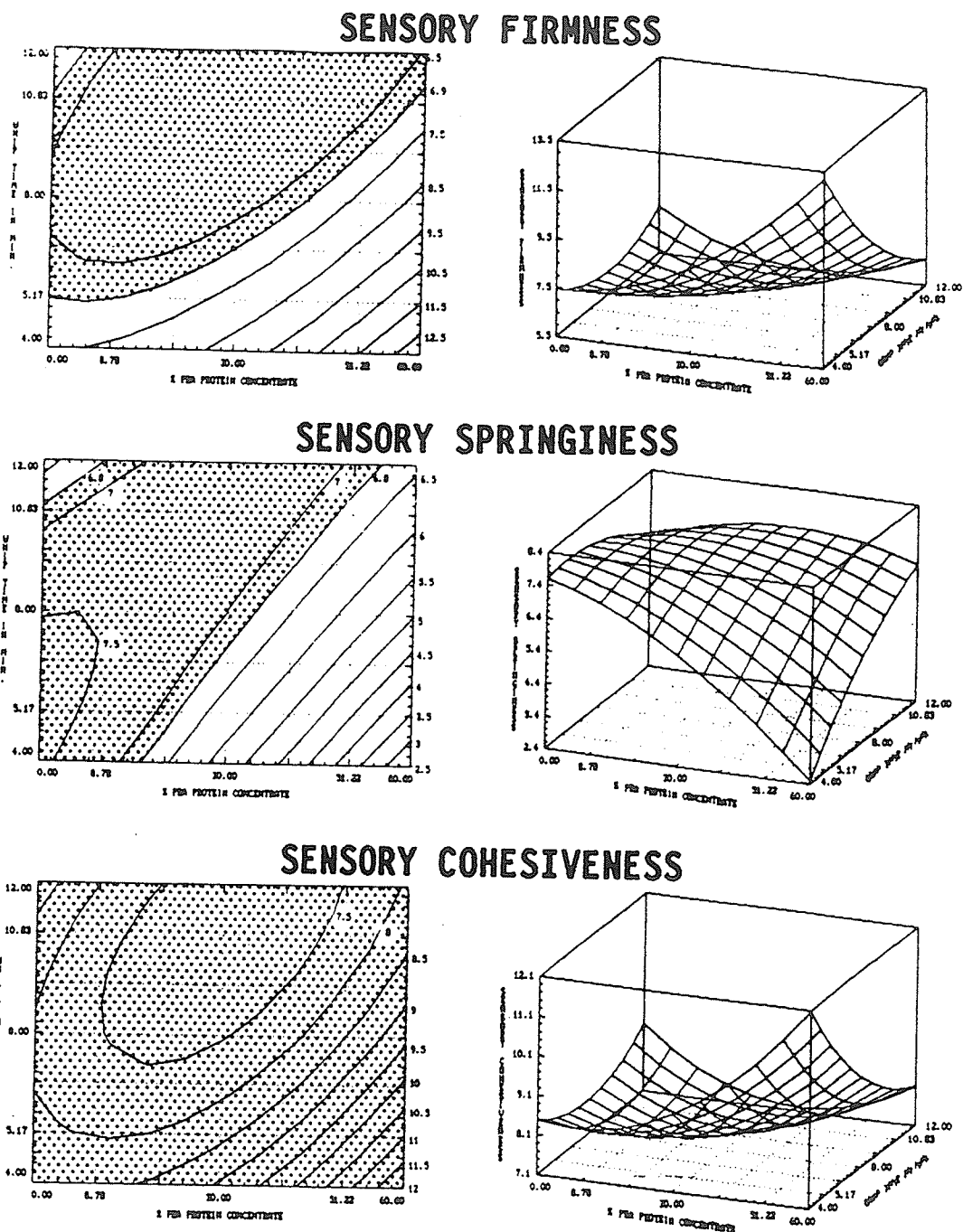
Figure 4.12 Contour and response surface plots for the effects of PPC and whip time on sensory firmness, springiness and cohesiveness. Shaded regions met the REF standards. Higher values indicate increasing firmness, springiness and cohesiveness. Score range=0 to 15; 1.5=extremely soft, moderately springy, slightly cohesive, 13.5=moderately firm, extremely springy, extremely cohesive. Levels of PPC and whip time are actual values.

Shaded regions (acceptance regions) indicate sponge cake formulations which met the REF standards for firmness, springiness, cohesiveness and moistness. Acceptance regions for firmness and springiness were similar to those observed for all physical measurements. Unacceptable values for these two attributes occurred in the high PPC/low whip time regions, and to a lesser extent, in the low PPC/high whip time regions. The acceptance regions for cohesiveness and moistness included the entire acceptance region, therefore, these two attributes do not appear to be restrictive characteristics for the optimization of this sponge cake formulation.

The significant linear, quadratic and interaction effects of PPC and whip time are, once again, best illustrated in the three dimensional response surface plots. Most notably, highly significant interactions between PPC and whip time existed for all parameters except moistness. Increasing PPC level resulted in an increase in the perception of crumb moistness, while whip time had very little influence on this attribute. These results should be interpreted as trends, however, since moistness met only two of the "good fit" criteria.

Pea protein isolates and concentrates generally have been found to possess very good water binding properties (Naczk et al., 1986) which may account for the higher moistness perception with increasing levels of PPC. However, instrumental moisture determinations, although subject to a

day effect, were not significantly affected by PPC, whip time or cream of tartar. This suggests that, for high PPC formulations, increased perceptions of moistness were not likely due to the ability of PPC to bind water. Funk et al. (1969) evaluated angel cake moistness sensorily and instrumentally and found that moistness differences detected by panelists were not detected instrumentally. This difference in results was attributed to the fact that samples for sensory evaluation were of constant size while samples for moisture determination were based upon weight. Consequently, low volume, denser cakes were perceived as moister than high volume cakes. In this study, increasing whip time, known to improve cake volume for high PPC formulations, did not extensively change the perception of moistness. That is, low volume cakes (resulting from high PPC/low whip time formulations) were not perceived to be much moister than cakes of higher volume. The explanation put forth by Funk et al. (1969), therefore, does not really apply in this study. Perhaps, then, the heightened perception of crumb moistness with increasing PPC level resulted from differences in moisture release from the cake crumb during mastication.

Unlike moistness, highly significant interactions between PPC level and length of whip time meant that their effects on firmness, springiness and cohesiveness could not be discussed independently. At low whip times, increasing PPC level increased sensory firmness and cohesiveness and decreased

springiness. <u>At high whip times</u>, firmness and cohesiveness first decreased, then remained constant, and finally increased as PPC levels rose from 0 to 60 percent. Springiness was inversely affected, that is, values increased, remained constant, and then decreased as PPC levels increased. When whip times were high there were large areas of constant firmness, springiness and cohesiveness reflecting a minimal effect of PPC on sensory texture.

<u>At low PPC levels</u>, increasing whip time was seen to first decrease, and then increase firmness and cohesiveness. Springiness was similarly, but inversely, affected. Whip time had little effect on texture when PPC levels were low, as indicated by the limited change in firmness, cohesiveness and springiness, over the whip times evaluated.

<u>At high PPC levels</u>, firmness and cohesiveness decreased and springiness increased as whip times were lengthened. The important effect of whip time, when PPC levels were high, is evident from the steep slopes of the lines in the response surface plots and by the closeness of the contour lines in the contour plots. Small increases in whip time resulted in marked decreases in firmness and cohesiveness, and increases in springiness.

Judgements of cohesiveness, firmness and springiness were most likely influenced by the method of sample preparation. Because samples were of similar size, those cut from low volume cakes contained a greater proportion of crumb than

samples cut from higher volume cakes, and therefore, were more compact and dense. Thus samples from low volume cakes (such as from high PPC/low whip time formulations) were perceived to be firmer, more cohesive and less springy than samples from higher volume cakes (ie., cakes having received sufficient whipping for the amount of PPC incorporated). Cohesiveness was judged as the degree to which the sample held together after five chews, therefore, denser samples could give the impression of holding together better than light, airy samples. Firmness was judged as the force required to compress the sample against the palate. Dense cakes would require more force to compress. Finally, springiness was judged as the quickness of crumb recovery after compressing the sample to one half its height with the finger, holding for two seconds, and then releasing. The compact cell structure of the high PPC formulations probably reduced the elasticity of the cell walls, thereby slowing the rate of recovery.

In summary, increasing PPC level caused an increase in moistness, firmness and cohesiveness and a decrease in springiness. Increased perception of moistness was possibly due to more moisture release from the cake crumb. Panelists' perceptions of crumb texture could have been influenced by the method of sample preparation. Increasing PPC levels had a negative effect on cake firmness and springiness yet the resultant increase in moistness and cohesiveness could be viewed positively. Higher amounts of PPC produced firmer,

less springy cakes, but cakes that were moister and more cohesive. Therefore, all PPC levels within the acceptance region could produce cakes of acceptable moistness and cohesiveness, and by lengthening the whip time of high PPC formulations, cakes of acceptable firmness and springiness could be produced. On the other hand, whip time had little effect on the texture of low PPC sponge cake formulations. The influence of PPC and whip time were, therefore, similar to those reported for SG and specific volume, suggesting that sensory texture was dependent upon initial SG and finished cake volume.

ii) Overall Flavor Intensity

Cream of tartar was the most significant variable affecting overall flavor intensity followed by PPC, therefore, these two variables formed the axes of the response surface and contour plots. Because whip time did not significantly influence flavor, only one set of plots was necessary, and they are presented in Figure 4.13.

The significant linear and quadratic effects of cream of tartar and its interaction with PPC are most apparent in the response surface plot. The interaction between cream of tartar and PPC is of particular interest. At low cream of tartar levels, increasing PPC level increased the overall flavor intensity of the sponge cake. At high cream of tartar levels, however, increasing PPC level was seen to decrease

OVERALL FLAVOR INTENSITY
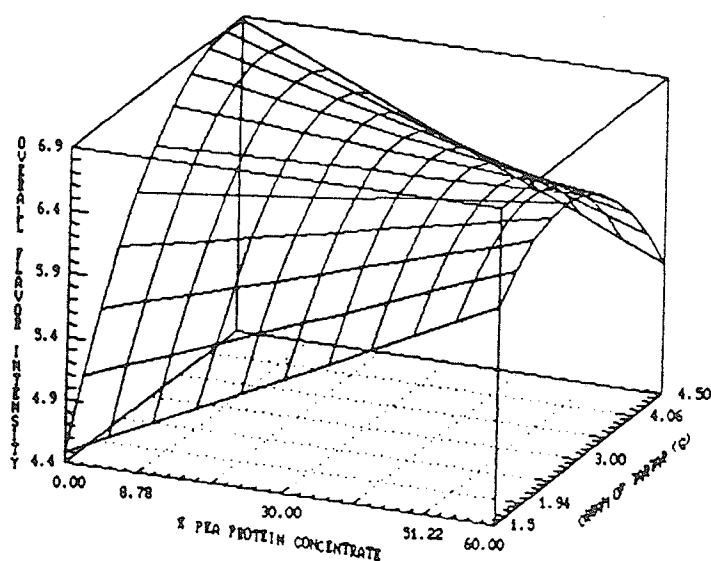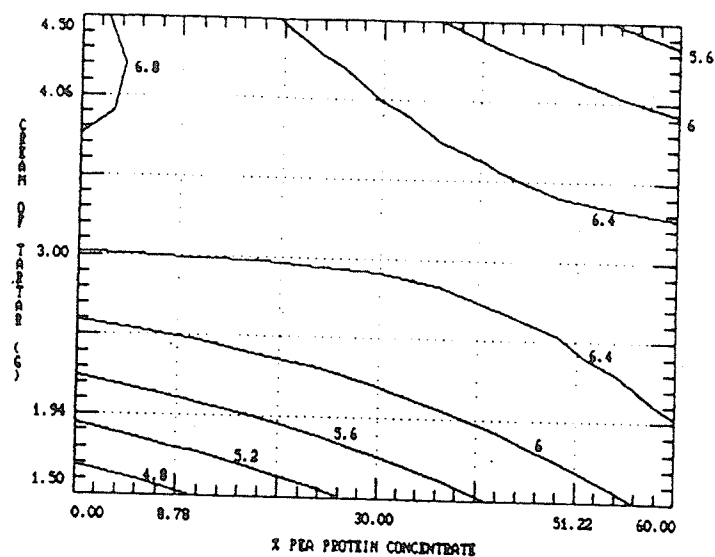
Figure 4.13 Contour and response surface plots for the effects of cream of tartar and whip time on overall flavor intensity. Higher values indicate increasing flavor intensity. Score range=0 to 15; 1.5=weak, 13.5=intense. Levels of cream of tartar and whip time are actual values.

overall flavor intensity. <u>At low PPC levels</u>, increasing cream of tartar increased the flavor intensity but when <u>PPC levels were high</u>, increasing cream of tartar first increased flavor intensity and then decreased it.

In general, flavor intensity was weak when both cream of tartar and PPC levels were low, or when both levels were high. The perception of flavor intensified when high cream of tartar levels were combined with low PPC levels, when low cream of tartar levels were combined with high PPC levels, and when intermediate levels of the two ingredients were combined. Kissell (1978) found that the flavor quality of angel cakes was improved when foams were prepared with less acid, apparently by decreasing the acidic aftertaste. Therefore, to determine whether flavor intensity was related to acidity, the contour plot for batter pH was superimposed on the flavor intensity contour plot (Figure 4.14). Interestingly, the highest flavor intensity occurred when batter pH was lowest, that is, when cream of tartar levels were high and PPC levels low. High acidity may, therefore, be the reason for the perception of high flavor intensity. Figure 4.14 also indicates, however, that acidity cannot be solely responsible for high flavor intensity. Low levels of cream of tartar combined with high PPC levels also produced fairly high measures of flavor intensity. Perhaps when cream of tartar levels were fairly low a flavor associated with the PPC predominated. At intermediate cream of tartar and PPC levels,
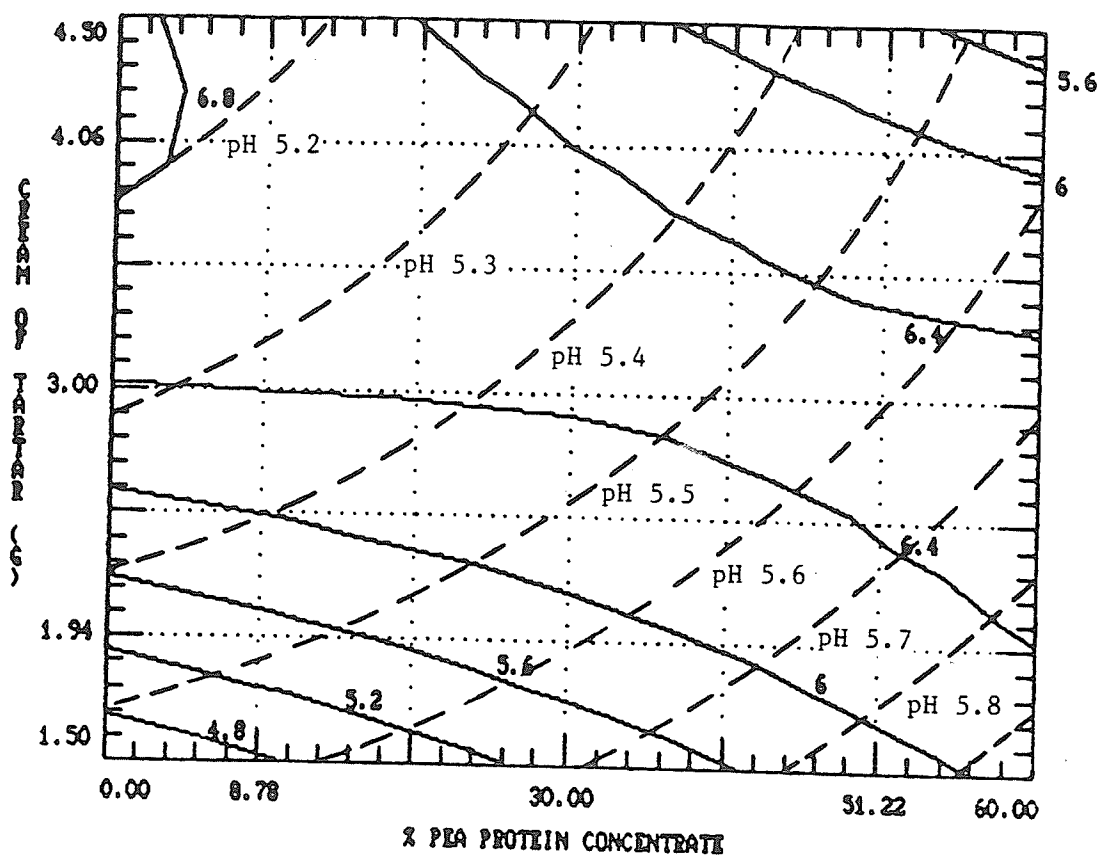
Figure 4.14 Superimposed contour plots of overall flavor intensity and batter pH. Higher values indicate increasing flavor intensity and pH.

a combination of acidic and PPC flavors may have increased panelists' perceptions of overall flavor intensity. These results must be interpreted cautiously, however, since it is questionable how well the best fit model predicted the responses presented graphically (R^2=.40; CV=8.0%). Additionally, sponge type products are typically flavored with lemon and are served with jam or cream fillings. Therefore slight differences is flavor intensity should not present a problem.

Comparison of Physical and Sensory Characteristics

The importance of batter SG and final cake volume to the ultimate perception of crumb texture was quite apparent. The instrumental measurement of crumb texture was also highly dependent upon these two characteristics, therefore instrumental and sensory measurements claiming to measure the same characteristic (ie., Instron hardness and sensory firmness, and Instron cohesiveness and sensory cohesiveness), should also be related. The similarity between the instrumental measurement of hardness and sensory measurement of firmness is best illustrated by comparing response surface plots (Figure 4.15). The shapes of the plots are very similar suggesting that Instron hardness measurements simulated the sensory judgements of cake firmness. According to both instrumental and sensory measurements, high PPC/low whip time formulations produced the hardest cakes.
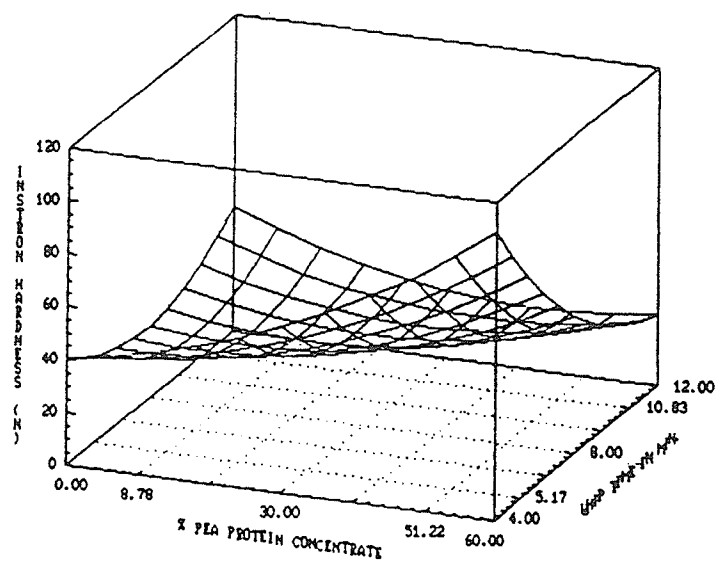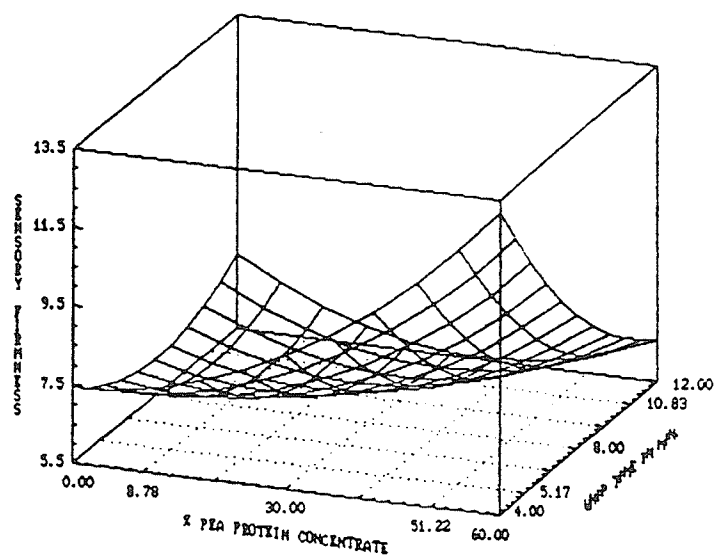
Figure 4.15 Comparison of response surface plots for Instron hardness and sensory firmness.

Figure 4.16 presents the response surface plots for instrumental cohesiveness, sensory cohesiveness and sensory springiness. Comparison of instrumental and sensory cohesiveness plots indicates that the panelists' perception of cohesiveness was not simulated instrumentally. The lowest instrumental measurement of cake cohesiveness was detected for high PPC/low whip time formulations while sensorily, this formulation was perceived to be the most cohesive. Thus instrumental and sensory cohesiveness appear to be inversely related, as is apparent from the response surface plots.

Differences in the sensory and instrumental definitions for cohesiveness are likely responsible for this observed relationship. The calculation of instrumental cohesiveness is illustrated in Figure 2.1 (Chapter 2). Using this definition, a cake sample which does not fracture, and springs back to its original size would illustrate a high degree of cohesiveness because area 2 would be very similar to area 1 (ie., Area2/Area1 would be close to 1). Conversely, a cake sample which remains compressed would illustrate a low degree of cohesiveness because Area 2 would be much smaller than Area 1.

The sensory definition of cohesiveness used in this study was the degree to which the sample held together after five chews, therefore, "cohesiveness of the cake mass" (Munoz, 1986) was actually measured as opposed to the degree of cake crumb compression before breaking (Civille and Szczesniak,

INSTRON COHESIVENESS
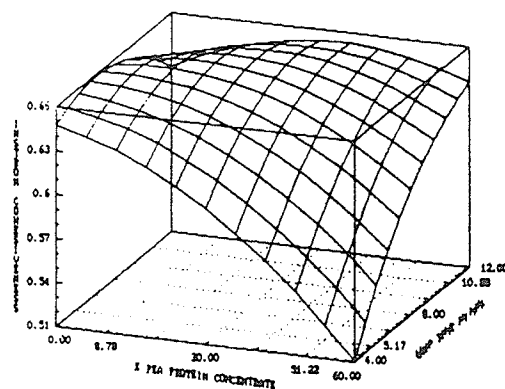
SENSORY COHESIVENESS
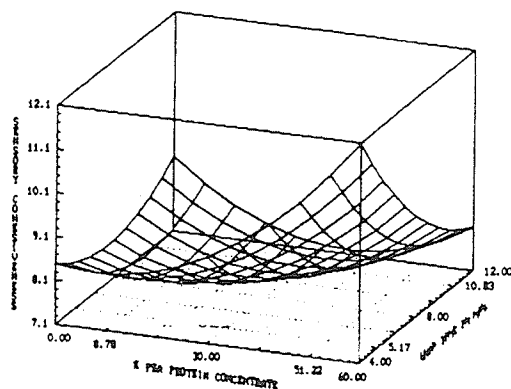
SENSORY SPRINGINESS
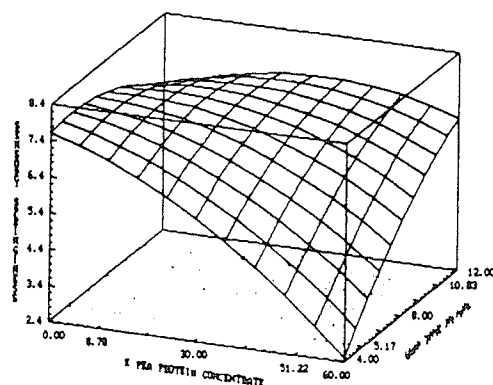
Figure 4.16 Comparison of response surface plots for Instron cohesiveness, sensory cohesiveness and sensory springiness.

1973), such is measured by the Instron. A brownie represented the "extremely cohesive" endpoint of the sensory cohesiveness scale because it rated high on the cohesiveness of mass reference scale proposed by Munoz (1986). Instrumentally, however, a brownie would exhibit a low degree of cohesiveness because, although it would not fracture, it would remain compressed thereby producing a low Area2/Area1 cohesiveness ratio. It appears that the inverse of Instron cohesiveness may be a better representation of sensory cohesiveness.

Instrumental cohesiveness seems to more closely reflect the springiness of the sponge cake crumb because of its high dependence upon the spring back of the crumb. Comparison of the plots for instrumental cohesiveness and sensory springiness supports this observation (Figure 4.16). It is questionable, therefore, whether Instron cohesiveness actually relates to the human perception of cohesiveness, particularly when human perception involves the presence of saliva, heat and much more complex mechanical breakdown. In Chapter 2, the Texture Profile definition of springiness was also challenged. More research is necessary to understand exactly what textural parameters are measured by the Instron, and how these relate to human perception.

Identification of Acceptable Sponge Cake Formulae

The third objective of this study was to identify sponge

cake formulae, with at least 30 percent of the albumen replaced with PPC, that were comparable to 100 percent albumen/0 percent PPC REF sponge cakes. This was accomplished by superimposing the contour plots of the sponge cake characteristics illustrating good predictive ability, and for which REF standards could be set. Therefore, the cake characteristics included were batter SG, cake specific volume, Instron cohesiveness, hardness, and gumminess as well as sensory firmness, springiness, cohesiveness and moistness. Figure 4.17 illustrates the superimposed contour plot of the REF standard lines for each of these physical and sensory responses. The shaded region represents the combinations of PPC and whip time which are expected to, on average, produce sponge cakes of comparable quality to the 100 percent albumen REF cake. The darker shaded region indicates the formulations incorporating at least 30 percent PPC. For this plot, cream of tartar was held constant at its highest level (4.5g) due to the positive influence of this variable on the acceptance regions for high PPC formulations (Figure 4.18). Increasing cream of tartar from 1.5 to 4.5 grams substantially increased the region of acceptance.

According to Figure 4.17, whip times would have to exceed approximately 7 minutes in order to successfully replace 30 percent or more of the albumen with PPC. Increasing PPC levels beyond 30 percent would necessitate even longer whip times. The longest whip time (12 min.), however, was not
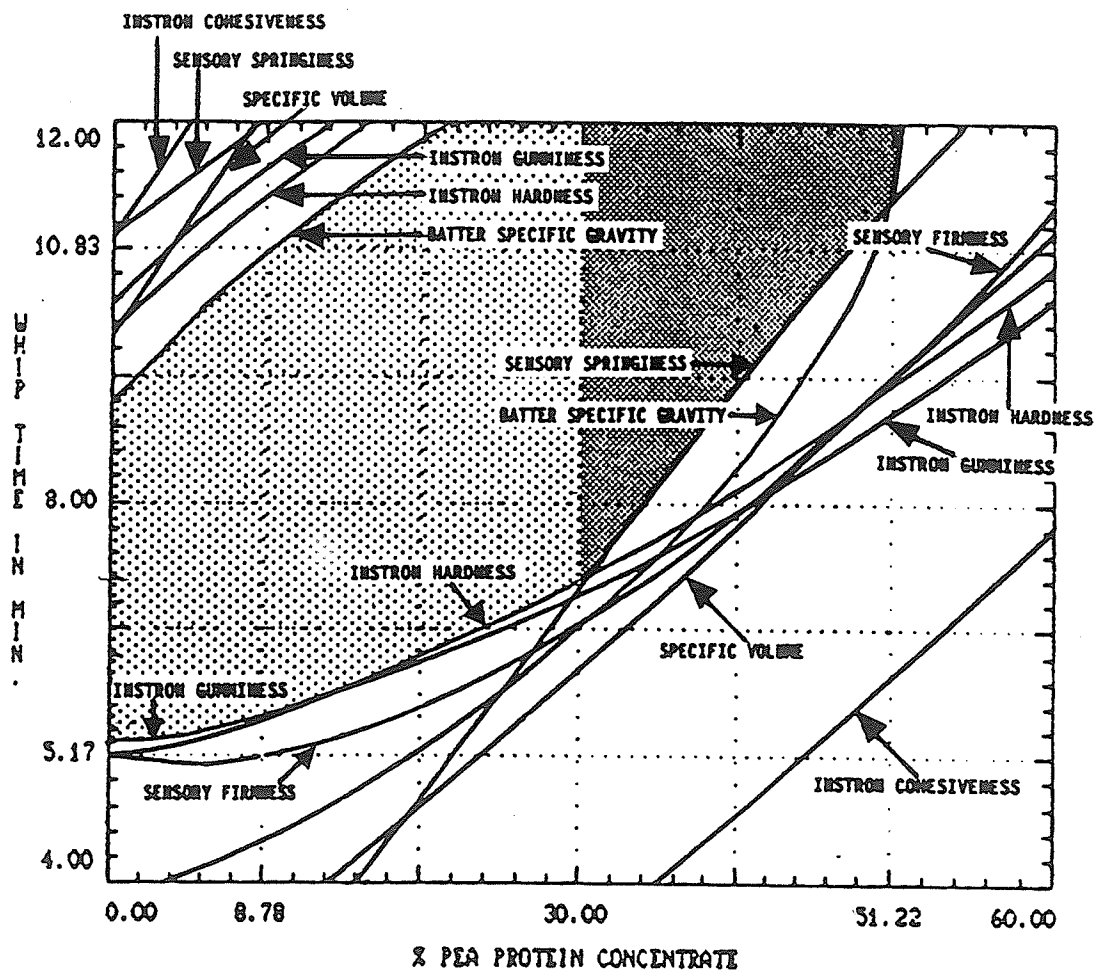
Figure 4.17 Superimposed contour plot for physical and sensory responses at 4.5 grams cream of tartar. The shaded region met REF standards for batter SG, cake specific volume, Instron cohesiveness, hardness and gumminess, sensory firmness, springiness, cohesiveness and moistness. The darker region represents formulations incorporating at least 30 % PPC. Levels of PPC and whip time are actual values.
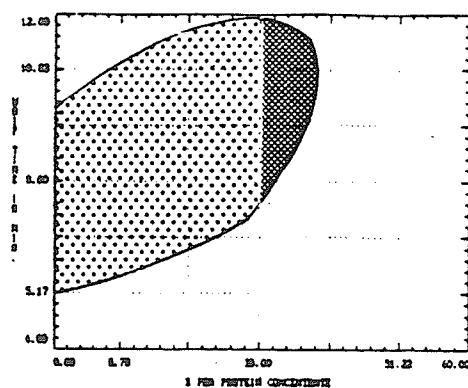
Cream of tartar = 1.5g
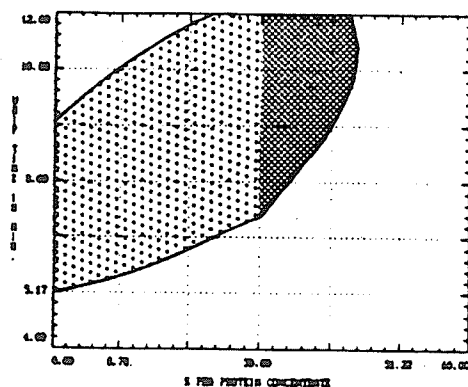
Cream of tartar = 3.0g
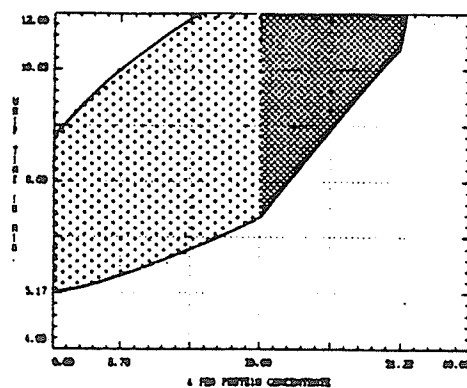
Cream of tartar = 4.5g

Figure 4.18 Acceptance regions from the superimposed contour plots for physical and sensory responses at low, medium and high cream of tartar levels. Shaded regions met REF standards for batter SG, cake specific volume, Instron cohesiveness, hardness and gumminess, sensory firmness, springiness, cohesiveness and moistness. Darkest regions represent formulations incorporating at least 30 % PPC. Levels of PPC, whip time and cream of tartar are actual values.

sufficient to produce a 60 percent PPC sponge cake comparable to the 100 percent albumen REF sponge cake. Had whip times exceeded 12 minutes, perhaps an acceptable cake could have been produced with 60 percent PPC. However, it is doubtful that such a long whip time would be acceptable from a commercial production stand point.

The results of this study indicate that PPC levels should not exceed the 50 percent replacement level. The conclusions drawn from this superimposed contour plot must be interpreted cautiously, particularly when the conclusions are drawn from the boundaries of the acceptance region, as in this case. Regression analyses were performed using mean values, therefore while the average response for a cake formulation may have fallen within the acceptance boundaries, individual cakes may fall outside. The risk of this occurring is dependent upon the variability associated with the particular cake characteristic. In order to determine the variability about each REF standard contour line, 95 percent confidence intervals (intervals of values likely to contain the true value of that response) were generated for specific PPC/whip time combinations which fell close to the boundaries of the acceptance region. In general, the confidence limits generated (results not shown) included some values which fell outside the acceptance boundary. This suggests that, while some cakes produced from a certain PPC/whip time formulation may compare to the REF sponge cake, others may not.

Therefore, conclusions should not be based upon PPC/whip time combinations occurring close to the boundary of the acceptance region. Interestingly, however, an increase in whip time for a set PPC level usually resulted in confidence intervals which fell entirely within the acceptance region. Thus, an increase in whip time will help to ensure that every sponge cake is likely to be an acceptable one. At high PPC levels, there was little chance of overwhipping the protein, therefore, lengthening whip times to increase the chance of producing an acceptable cake is not likely to adversely affect foam stability.

The importance of batter SG and final cake volume to ultimate cake quality has been stressed throughout the discussion of individual cake characteristics. The superimposed contour plots indicate that specific volume had no influence on the boundaries of the final acceptance region. Specific gravity, however, limited the acceptance region when PPC levels were low and whip times high, but was only slightly influential when both PPC levels and whip times were high. Instron hardness, gumminess and sensory springiness were the three characteristics limiting the acceptance region for low PPC/low whip time and high PPC/high whip time formulations, respectively. The limiting effect of sensory springiness is more important than Instron hardness since springiness limits the acceptance of higher PPC formulations. The importance of sponge cake springiness to ultimate cake quality is not known,

therefore whether this characteristic should limit the acceptability region is questionable. If the springiness REF standard line were removed, the acceptability region would expand allowing greater PPC incorporation. Batter SG, which has greater predictive ability and is known to influence overall sponge cake quality, would then become the limiting characteristic for higher PPC formulations.

Having identified the conditions predicted to produce high PPC sponge cakes comparable to 100 percent albumen REF cakes, the next step should be to experimentally verify that the predicted conditions do produce acceptable cakes. However, because the results from this study were only to be used to recommend feasible levels of albumen replacement with PPC in a whole egg mix, verification was not necessary.

Recommendations for a Co-Spray-Dried PPC-Whole Egg Mix Formulation

Although this research has been based upon the use of egg yolk and albumen in two separate batter systems, a more practical/commercial approach would be to partially replace the egg albumen with PPC in a spray-dried whole egg mix. Sponge-type snack cakes are generally produced from whole egg mixes because it allows an "all in" process as opposed to the two stage process described in this research. From an economic stand point, the egg albumen is generally the most expensive component of the mix. Therefore, the final objective of this study was to provide recommendations for the

development of a co-spray-dried PPC-whole egg mix for commercial use in a sponge-type snack cake. The superimposed contour plot of significant sponge cake characteristics presented in Figure 4.17 illustrates the feasible levels of PPC for albumen replacement, and corresponding whip times necessary to produce acceptable sponge cakes. Co-spray-drying liquid whole egg with a slurry of PPC could alter the manner in which the egg and pea proteins interact as well as change the highly significant protein-whip time interaction so prevalent throughout this study. Patel et al. (1981) found that co-spray drying cheddar cheese whey and PPC resulted in a product with the potential as a non-fat dried milk replacement in bread. Pea protein concentrate/whey blends subjected to light heat treatments (63°C for 30 min.) prior to drying exhibited better functional properties than two commercial non-fat dry milk samples and soy/whey blends. In particular, foam stability was improved. While the effects of co-spray drying PPC and egg albumen on protein functionality has not been determined, recommendations of PPC replacement levels likely to be successful in a co-spray-dried product can still be made. From the superimposed contour plot (Figure 4.17) it is believed that PPC could successfully replace 30 percent of the egg albumen if 4.5 grams of cream of tartar was used and whip times were at least 8 minutes long. Increasing whip times an additional three minutes would permit about a 40 percent replacement level.

Use of these PPC, whip time and cream of tartar levels are most likely to succeed because the confidence intervals for all sponge cake characteristics were contained within the acceptance boundaries. Based upon these results, it is recommended that:

1. Initial attempts to co-spray-dry the two aqueous protein systems should be made with a 30 percent egg albumen replacement level since this level appears to be most feasible. In addition, evaluation with a fairly low egg albumen replacement level will help evaluate the success of adapting this optimized formulation to a co-spray-dried mix.

2. Because cream of tartar had a positive influence on the higher PPC formulations, the co-spray-dried PPC-whole egg mix should be evaluated in an acidified (pH 5.2-5.4) sponge cake system.

3. The effect of whip time should be re-evaluated in order to determine the effects of co-spray-drying on the foaming properties of the proteins. Whip times may have to be adjusted to produce batters of acceptable SG (approximately 0.45).

4. If adaptation to a co-spray-dried mix is successful, and the foamability of the pea and egg albumen proteins improves due to the co-spray-dry process, then PPC replacement levels should be increased beyond the 30 percent replacement level.

SUMMARY AND CONCLUSIONS

This study has illustrated the potential use of PPC, an inexpensive, under-utilized field pea fraction, as an egg albumen replacement in a whole egg mix for use in sponge cake-type products. Response surface methodology was successfully and efficiently used to clarify relationships between PPC, whip time and cream of tartar, as well as to determine their effects on physical and sensory sponge cake characteristics. For every cake characteristic except flavor, a very significant relationship emerged between the amount of PPC incorporated and the length of whip time. As PPC levels increased, longer whip times were necessary to produce sponge cakes of comparable volume and texture to 0 percent PPC REF sponge cakes, probably due to differences in the foaming abilities of PPC and egg albumen. Cream of tartar, although less influential than PPC and whip time, was an important component of the sponge cake formulation, particularly when PPC levels were high. Increasing cream of tartar levels allowed either higher PPC replacement levels (for specific whip times) or faster whip rates (for specific PPC levels), while still achieving acceptable sponge cakes.

Batter SG and cake specific volume were the two most critical dependent variables for assessing ultimate sponge cake quality. Instrumental and sensory texture measurements were, in general, determined by final cake volumes, which, in turn, appeared to depend upon initial batter SG. Cakes of

high SG and low volume were characterized by high instrumental cohesiveness, hardness and gumminess measurements. Only a high degree of cohesiveness could be considered a positive attribute, particularly for cakes which are rolled. Sensorily, panelists perceived low volume cakes as being firmer, more cohesive and less springy than high volume cakes. Preliminary sponge cake experimentation should, therefore, include measurements of SG and specific volume since they were primary indicators of cake quality. Final cell structure should also be monitored since high volumes may result from excessively large, uneven air cells. The additional physical and sensory quality measurements conducted in this study need to be carried out only in the final testing stages of additional studies, unless possible problems with flavor warrants preliminary sensory evaluation of this parameter.

In this study, one of the most important and positive findings was that PPC did not adversely affect sponge cake flavor. Therefore, partial replacement of egg albumen with PPC should not adversely affect the flavor of a sponge cake type product, particularly when such snack-type cakes are traditionally flavored with vanilla, lemon, or chocolate, and served with a jam or cream filling.

Sponge cake color is another quality which would influence consumer acceptability. Color measurements were made on all sponge cake formulations and were compared to measurements made on commercial cakes of similar coloring.

Crumb color was not adversely affected by PPC addition (results not shown), therefore, it is not likely to present a problem in sponge cake-type products.

The methods used to evaluate physical and sensory texture of any product will influence the way in which results are interpreted. If instrumental tests are to be used as reliable tests of quality, they must simulate human perception of quality, since people, not instruments, ultimately determine product acceptability. In this study, a comparison of response surface plots showed that Instron hardness values corresponded to panelists' perceptions of firmness. Instron cohesiveness values did not correspond to sensory cohesiveness assessment, instead, the Instron cohesiveness response surfaces resembled sensory springiness response surfaces. Therefore, for products such as the sponge cake tested in this study, the ratio of Texture Profile curve Area 2/ Area 1 (defined as cohesiveness) appears to be an indicator of sensory springiness rather than sensory cohesiveness.

The ultimate goal of this study was to optimize a sponge cake formulation using PPC substitution levels of at least 30 percent or more. Superimposition of contour plots revealed several PPC, whip time and cream of tartar combinations predicted to produce sponge cakes comparable to the 100 percent albumen REF sponge cake. Based upon these results, it is recommended that the 30 percent substitution be evaluated first, as it is most likely to succeed. The sponge

cake system should be acidified (pH 5.2-5.4) and whip times may have to be adjusted depending upon the effect of the co-spray-drying procedure on pea protein and egg albumen functionality. If co-spray-drying improves the functionality of the pea and egg albumen proteins, then higher levels of PPC, such as a 40 percent replacement, should be attempted.

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CHAPTER 5

Summary, Conclusions and Recommendations for Future Research

SUMMARY

In the first chapter of this thesis, an attempt was made to evaluate the feasibility of successful egg albumen replacement with PPC in a sponge cake formulation. A theoretical assessment was made which compared the molecular and functional properties of pea proteins with egg albumen proteins in order to highlight similarities and differences which would ultimately influence sponge cake quality. Molecular differences were manifested in the form of slightly inferior solubility, foaming and coagulating properties. Nevertheless, the use of pea protein concentrate (PPC) as a partial egg albumen replacement in sponge cake was considered feasible if conditions promoting pea protein functionality were utilized.

A more direct feasibility assessment was made based upon a review of pea protein use in baked products, as well as of partial egg albumen replacement with bovine plasma protein in cakes. An examination of the effects of pea protein incorporation on baked product quality provided some idea of the possible effects to expect with PPC substitution in sponge cake. Evaluation of partial egg albumen replacement with an alternate protein source assessed the feasibility of replacing this highly functional cake component. The literature

indicated the potential for using low levels of pea protein in baked products without adversely affecting sensory and physical quality. As well, acceptable layer and angel cakes were produced with partial replacement of egg albumen with bovine plasma protein, suggesting that egg albumen is not an indispensable cake component.

Overall, the feasibility of successful partial egg albumen replacement with PPC in a sponge cake formulation was established. Sensory and physical testing methods for sponge cake evaluation were also reviewed as well as the use of response surface methodology as a product optimization technique.

The first study of this thesis (Chapter 2) arose out of the need to select Instron testing conditions for the textural evaluation of various PPC-egg albumen sponge cakes. The effects of Instron testing conditions (plunger size, degree of sample compression and cross-head speed) on the measurements of sponge cake hardness, cohesiveness, gumminess and springiness were evaluated. Degree of sample compression and plunger area strongly influenced sponge cake texture measurements and therefore, should be carefully selected for texture evaluation studies. Cross-head speed had much less effect on texture values.

The Instron testing conditions which best discriminated textural differences between sponge cakes were also identified. It was possible to discriminate among sponge

cakes on the basis of hardness, cohesiveness, gumminess and springiness when conditions appropriate for those individual parameters were selected. High degrees of compression improved the discrimination of cohesiveness, while slower cross-head speeds helped detect differences in springiness. Discrimination of hardness and gumminess may have improved with faster cross-head speeds, however, this relationship was not clear. Therefore, the choice of Instron conditions did not seem to be as crucial for hardness and gumminess as for cohesiveness and springiness. Because one set of Instron testing conditions did not discriminate equally well for all parameters, a set of conditions was selected which was most suitable for the simultaneous detection of differences in all four parameters.

Chapter 3 presented the first experiment in the optimization of a PPC-egg albumen sponge cake formulation. Response surface methodology (RSM) was used to identify the critical independent variables influencing sponge cake quality, and to gain some preliminary understanding of their effects on cake quality. The effects of varying levels of PPC, whip time, cream of tartar, water and emulsifier on batter specific gravity (SG), cake specific volume and Instron texture characteristics were evaluated. Of the five independent variables, PPC level and length of whip time were by far the most important independent variables influencing sponge cake quality. The effect of cream of tartar was also

significant, but to a much lesser degree. Water and emulsifier levels had the least effect on overall sponge cake quality.

Contour plots generated from best fitting regression equations depicted the relationships between PPC, whip time and cream of tartar, as they affected each dependent variable. In general, when whip times were low, increasing the level of PPC had a negative effect on sponge cake quality. These negative effects could, to some extent, be overcome by adjusting whip times and cream of tartar levels. The length of whip time necessary to improve sponge cake quality was, however, dependent upon both the level of PPC and of cream of tartar. Higher levels of PPC substitution required longer whip times in order to improve sponge cake quality, while the addition of cream of tartar shortened the necessary whip times. Conversely, for a set whip time, the addition of cream of tartar permitted greater PPC substitution levels.

Because PPC, whip time and cream of tartar were critical to sponge cake quality, it was concluded that these three variables be studied more thoroughly, and their levels optimized, in a second study. Prior to further investigation, however, it was necessary to determine whether the levels tested for each independent variable were appropriate, or whether adjustments were required. The range of PPC levels and whip times used in the preliminary study were appropriate for further optimization, while higher cream of tartar levels

were indicated by the positive influence of this variable on PPC incorporation and length of whip time. Although water and emulsifier were of minor importance to sponge cake quality, levels most advantageous to the sponge cake formulation were also determined.

The final optimization response surface study was presented in Chapter 4. In this experiment, the relationships between PPC, whip time and cream of tartar, and their effects on both physical and sensory cake characteristics, were evaluated and clarified through the use of two dimensional contour plots and three dimensional response surface plots.

Physical sponge cake characteristics were strongly affected by the level of PPC and length of whip time. In general, when PPC levels were high, longer whip times were necessary to improve batter SG, cake specific volume, crumb hardness, cohesiveness and gumminess. These results support those found in the preliminary response surface experiment. High PPC levels combined with low whip times produced cakes of low quality, but low whip times were necessary when PPC levels were also low since excessive whip times negatively affected cake quality. Cream of tartar did not significantly influence the Instron texture characteristics but did have an effect on batter SG and cake specific volume. At high PPC levels, increasing cream of tartar decreased the whip time necessary to meet the 100 percent egg albumen reference (REF) sponge cake standards for SG and specific volume. Similarly,

when PPC levels were relatively high, high cream of tartar
levels permitted greater PPC incorporation for a given whip
time, while still meeting the REF standards. However, the
optimum cream of tartar level depended upon the specific level
of PPC and whip time chosen for the sponge cake formulation.
Differences in the foaming properties of the two protein
systems were believed to be responsible for the observed
effects on SG, specific volume, and ultimate cake texture.

Sensory evaluations of cake crumb firmness, springiness,
cohesiveness and moistness were all significantly affected by
PPC and whip time. Higher amounts of PPC produced firmer,
less springy cakes, but the cakes were more moist and
cohesive. Unacceptable firmness and springiness values
occurred in the high PPC/low whip time regions, and to a
lesser extent, in the low PPC/high whip time regions, whereas
acceptable moistness and cohesiveness values were produced at
any combination of PPC and whip time. It was possible to
achieve acceptable firmness and springiness values for higher
PPC formulations when whip times were increased. This trend
was similar to that observed for SG and specific volume
suggesting that sensory texture (firmness and springiness)
was also dependent upon these two quality characteristics.
Thus both instrumental and sensory texture measurements
indicated the importance of batter SG and cake specific volume
as a measure of quality which generally reflected the ultimate
textural quality of the sponge cake.

Cream of tartar did not significantly influence any of the sensory parameters except overall flavor intensity. Various combinations of cream of tartar and PPC produced slight differences in flavor intensity, however, such differences would not present a problem in sponge-type cakes which are typically flavored and served with jam or cream fillings.

Superimposition of contour plots allowed identification of PPC-albumen sponge cake formulations which were predicted to result in sponge cakes comparable to the 100 percent egg albumen REF sponge cake. The highest cream of tartar level (4.5g) increased the number of formulations which met the objective of at least 30 percent albumen replacement. Acceptable cakes could be produced when 30 or 40 percent of the albumen was replaced with PPC by adding 4.5 grams cream of tartar and whipping for 8 and 11 minutes, respectively. Substitution of up to 50 percent of the albumen with PPC appeared possible.

Based upon the results of this study, recommendations were made for the development of a co-spray-dried PPC-whole egg mix for commercial use in sponge-type snack cakes.

GENERAL CONCLUSIONS

The overall objectives of this research were, first to establish the feasibility of using PPC as a functional replacement for egg albumen in a sponge cake, and then to develop an acceptable sponge cake formulation in which at least 30 percent of the egg albumen was replaced with PPC. Recommendations for the development of a co-spray-dried PPC-whole egg mix were to be made based upon the results from this research.

The feasibility of partial egg albumen replacement with PPC in a sponge cake formulation was confirmed by this research. It should be possible to replace 30 to 40 percent of the albumen with PPC without compromising cake quality, provided that whip times and cream of tartar levels are adjusted appropriately.

Response surface methodology was shown to be an extremely useful and efficient method for first identifying the critical independent variables affecting cake quality, and then establishing optimal ingredient levels and processing conditions to produce cakes of comparable quality to a 100 percent REF sponge cake. Contour and response surface plots illustrated and clarified relationships and interactions between the key independent variables as well as their effects on each dependent variable.

This research showed that sponge cake quality undoubtedly depended upon the level of PPC substitution and the length of

whip time. Higher PPC formulations required longer whip times to produce cakes of comparable volume and texture to the REF cakes, probably due to differences in the foaming properties of the two protein systems. Cream of tartar was less influential than the level of PPC and length of whip time but was seen to either increase the whipping rate of high PPC formulations, or permit greater PPC incorporation for a given whip time. Thus, PPC exhibited great potential for use as a functional egg albumen replacement in sponge cake, provided that whip times and cream of tartar levels were optimized for the level of PPC substitution. PPC levels of 30 to 40 percent should be attempted in a PPC-whole egg co-spray-dried product.

The choice of Instron testing conditions greatly affected the measurements of hardness, cohesiveness, gumminess and springiness. Plunger area and degree of sample compression were shown to be critical to Instron texture measurements while cross-head speed was of less importance. Consequently, plunger area and degree of sample compression should be carefully selected for texture evaluations. Because one set of conditions did not discriminate equally well for all textural parameters, the conditions most appropriate for the evaluation of several parameters should be identified. Instron testing conditions should be chosen based upon their ability to detect textural differences among test products.

RECOMMENDATIONS FOR FUTURE RESEARCH

PPC functioned as an egg albumen replacement in sponge cakes provided whip times were long enough to allow foam formation and stabilization. Research is needed to improve the foaming properties of PPC. This could include the addition of whipping agents and/or leavening agents to improve batter aeration, mild heat treatments (as was shown by Megha and Grant, 1986), and/or chemical modifications of the proteins to improve their functional characteristics. Johnson and Brekke (1983) found that acylation with acetic and succinic anhydride improved the functional properties of pea protein isolates, in particular, foam capacity and stability. Improvement of pea protein functionality would make PPC a more marketable food component, therefore, efforts to improve its functionality should continue.

Pea protein concentrate illustrated great potential as a partial egg albumen replacer in a very sensitive sponge cake system, therefore, great potential exists for its use in a variety of other baked products such as cookies, shortened cakes, biscuits, etc., which do not depend so highly upon egg functionality for success. Pea protein concentrate could also be evaluated in an angel cake system which is highly dependent upon egg albumen functionality for success. Pea protein concentrate should be evaluated as a partial egg albumen replacement in other baked products, and RSM would be an efficient method for this task.

Recommendations were made for the development of a co-spray-dried PPC-whole egg mix for commercial use in sponge-type snack cakes, therefore, research should continue in this area. Once again, RSM would be an appropriate technique for optimizing ingredient levels and processing conditions.

With increasing use of the Instron Universal Testing Machine for texture profiling, a systematic method of determining the most appropriate set of conditions for detecting differences among test products is needed. The research presented in Chapter 2 of this thesis attempted to standardize the selection of Instron testing conditions on the basis of discriminatory power. Research should continue in this area in order to develop a standard procedure for determining the best set of testing conditions for a variety of products. Additionally, the definitions of Instron cohesiveness and springiness need to be reviewed so that they relate more closely to sensory measurements of cohesiveness and springiness.

The last recommendations for future research have arisen out of the literature review rather than the thesis research. There is a need to standardize protein functionality tests in order to facilitate comparisons from one study to the next. It was also apparent that a large gap between protein functionality in a simple system and its functionality in a more complex food system. There needs to be a better understanding of how results from simple functionality tests

relate to the functionality of the protein in a food system. Functionality tests evaluating the foaming and coagulating properties of PPC and egg albumen should be conducted and related to the results obtained from this thesis research.

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Appendix 2.A. Sponge Cake Formula and Procedure
(Adapted from King et al., 1936)

Ingredients	Grams	mls	%Flour
YOLK BATTER:			
Dried yolk	66.4		43.1
Tap water		73.0	47.4
Granulated sugar	40.0		26.0
Icing sugar	54.0		35.1
ALBUMEN BATTER:			
Dried albumen[1]	variable		variable
Granulated sugar	180.0		116.9
Salt	3.5		2.3
Cream of tartar	1.5		1.0
SSL[2]	1.3		0.9
Tap water		227.0[3]	147.4
Pea protein concentrate[4] (PPC)	variable		variable
FLOUR:			
Cake flour, sifted[5]	154.0		100.0

[1] Export Packers Ltd., Winnipeg, Manitoba. (80% protein; NX6.25). 0% PPC cake=26.4g albumen; 30% PPC cake=18.48g albumen.

[2] Top-Scor S Powder; Breddo Food Products Corp., Kansas.

[3] 177.0g + 50g for hydration of 30% PPC; 227.0g for 0% PPC.

[4] Woodstone Foods Ltd., Portage La Prairie, Manitoba. (83% protein; NX6.25). 30% PPC cake=7.92g PPC.

[5] 7.5% protein (14% mb); Reid Milling, Missisauga, Ontario.

PROCEDURE

PPC RE-HYDRATION (for 30% PPC formulation):

1. Weigh out PPC into a plastic container, add 50 ml of water and mix to hydrate all PPC.

2. Cover and re-hydrate overnight.

YOLK BATTER:

1. Shake yolk and sugars in enclosed container to mix.

2. Using the whisk attachment, mix on speed 1 of a Braun Kitchen Machine (Model KM 32) for 30 seconds to thoroughly combine dry ingredients.

3. Add 73.0 ml of water, which has been heated to 80 ± 1°C, to yolk mix.

4. Mix on speed 3 for 30 seconds. Scrape down sides to incorporate any dry ingredients remaining on bottom of bowl.

5. Continue to mix on speed 3 for 30 seconds. Scrape down sides.

6. Mix on speed 3 for 9 minutes.

ALBUMEN/PPC BATTER:

1. In 10 quart Hobart (Model C100) mix bowl combine all dry ingredients. Blend well manually using a wire whisk.

2. Add hydrated PPC and remaining water (add 227 ml of water for the 0% PPC cake).

3. Using the wire whip attachment, mix on speed 1 for 1 minute to combine. Scrape down sides and bottom, making sure the sugar has not accumulated on the bottom of the bowl.

4. Whip on speed 3 for 4 minutes for the 0% PPC cake, and 8 minutes for the 30% PPC cake (these times yield foams of similar stiffness).

*NOTE - Batters should be prepared so that they are ready to be combined at approximately the same time.

COMBINING BATTERS:

1. Add yolk batter carefully to albumen batter.

2. Using the Hobart flat paddle attachment, mix on speed 1 for 10 seconds. Scrape down sides.

3. Sprinkle 1/3 of flour (51.3g) over batter. Mix on speed 1 for 10 seconds. Scrape down sides. Repeat for second 1/3 of flour.

4. Add last of flour to batter and mix on speed 1 for 30 seconds. Scrape down sides.

5. Manually fold batter 3 to 4 times using a spatula.

6. Take 2 specific gravity measurements.

7. Pour 200g batter into each of 3, 15 cm round, ungreased aluminum cake pans.

BAKING:

1. Bake cakes 23 minutes on the middle rack of a 190°C oven. Be sure to standardize the placement of the cakes.

COOLING AND DE-PANNING:

1. Cool cakes in their pans, on a cooling rack, for 20 minutes.

2. Remove cakes from pans and cool on racks for an additional 20 to 30 minutes.

3. Place in plastic bags, seal and freeze (-20°C).

Appendix 2.B   Example of Cake Sample Randomization within One
             Test Period

DAY/TIME	PLUNGER AREA (mm^2)	SPEED / COMPRESSION (cm/min.) (%)		REP	FORMULA (%PPC)
1 / am	616	20 /	50	1	0
		20 /	50	1	30
		20 /	50	2	30
		20 /	50	2	0
		5.1 /	75	1	30
		5.1 /	75	1	30
		5.1 /	75	2	0
		5.1 /	75	2	0
		20 /	75	1	30
		20 /	75	1	0
		20 /	75	2	30
		20 /	75	2	0
		10 /	25	1	30
		10 /	25	1	0
		10 /	25	2	0
		10 /	25	2	30
		20 /	25	1	0
		20 /	25	1	30
		20 /	25	2	30
		20 /	25	2	0
		5.1 /	25	1	30
		5.1 /	25	1	0
		5.1 /	25	2	30
		5.1 /	25	2	0
		5.1 /	50	1	30
		5.1 /	50	1	0
		5.1 /	50	2	0
		5.1 /	50	2	30
		10 /	50	1	0
		10 /	50	1	30
		10 /	50	2	0
		10 /	50	2	30
		10 /	75	1	30
		10 /	75	1	0
		10 /	75	2	30
		10 /	75	2	0

Appendix 3.A. Sponge Cake Formula and Procedure
(Adapted from King et al., 1936)

Ingredients	Grams	mls	%Flour
YOLK BATTER:			
Dried yolk	66.4		43.1
Tap water		73.0	47.4
Granulated sugar	40.0		26.0
Icing sugar	54.0		35.1
ALBUMEN BATTER:			
Dried albumen[1]	variable[2]		variable
Granulated sugar	180.0		116.9
Salt	3.5		2.3
Cream of tartar	variable[2]		variable
SSL[3]	variable[2]		variable
Tap water	variable[2]		variable
Pea protein concentrate[4] (PPC)	variable[2]		variable
FLOUR:			
Cake flour, sifted[5]	154.0		100.0

[1] Export Packers Ltd., Winnipeg, Manitoba. (80% protein; NX6.25).
[2] Refer to Table 3.3 for variable levels.
[3] Top-Scor S Powder; Breddo Food Products Corp., Kansas.
[4] Woodstone Foods Ltd., Portage La Prairie, Manitoba. (83% protein; NX6.25).
[5] 7.5% protein (14% mb); Reid Milling, Missisauga, Ontario.

PROCEDURE

PPC RE-HYDRATION (for PPC formulations):

1. Weigh out PPC into a plastic container, add 50 or 100g water (depending upon amount of PPC) and mix to hydrate all PPC.

2. Cover and re-hydrate overnight.

YOLK BATTER:

1. Shake yolk and sugars in enclosed container to mix.

2. Using the whisk attachment, mix on speed 1 of a Braun Kitchen Machine (Model KM 32) for 30 seconds to thoroughly combine dry ingredients.

3. Add 73.0 mls of water, which has been heated to $80 \pm 1°C$, to yolk mix.

4. Mix on speed 3 for 30 seconds. Scrape down sides to incorporate any dry ingredients remaining on bottom of bowl.

5. Continue to mix on speed 3 for 30 seconds. Scrape down sides.

6. Mix on speed 3 for 9 minutes.

ALBUMEN/PPC BATTER:

1. In 10 quart Hobart (Model C100) mix bowl combine all dry ingredients. Blend well manually using a wire whisk.

2. Add hydrated PPC and remaining water.

3. Using the wire whip attachment, mix on speed 1 for 1 minute to combine. Scrape down sides and bottom, making sure the sugar has not accumulated on the bottom of the bowl.

4. Whip on speed 3 for the experimental length of time outlined in Table 3.3.

*NOTE - Batters should be prepared so that they are ready to be combined at approximately the same time.

COMBINING BATTERS:

1. Add yolk batter carefully to albumen batter.

2. Using the Hobart flat paddle attachment, mix on speed 1 for 10 seconds. Scrape down sides.

3. Sprinkle 1/3 of flour (51.3g) over batter. Mix on speed 1 for 10 seconds. Scrape down sides. Repeat for second 1/3 of flour.

4. Add last of flour to batter and mix on speed 1 for 30 seconds. Scrape down sides.

5. Manually fold batter 3 to 4 times using a spatula.

6. Take specific gravity measurements.

7. Pour 200g batter into each of 3, 15 cm round, ungreased aluminum cake pans.

BAKING:

1. Bake cakes 23 minutes on the middle rack of a 190°C oven. Be sure to standardize the placement of the cakes.

COOLING AND DE-PANNING:

1. Cool cakes in their pans, on a cooling rack, for 20 minutes.

2. Weigh cakes in pans.

3. Remove cakes from pans and cool on racks for an additional 20 to 30 minutes.

4. Place in plastic bags, seal and freeze (-20°C).

Appendix 4.A.   Sponge Cake Formula and Procedure
(Adapted from King et al., 1936)

Ingredients	Grams	mls	%Flour
YOLK BATTER:			
Dried yolk	66.4		43.1
Tap water		73.0	47.4
Granulated sugar	40.0		26.0
Icing sugar	54.0		35.1
ALBUMEN BATTER:			
Dried albumen[1]	variable[2]		variable
Granulated sugar	180.0		116.9
Salt	3.5		2.3
Cream of tartar	variable[2]		variable
SSL[3]	1.3		0.9
Tap water	212.0[4]		137.7
Pea protein concentrate[5] (PPC)	variable[2]		variable
FLOUR:			
Cake flour, sifted[6]	154.0		100.0

[1] Export Packers Ltd., Winnipeg, Manitoba.   (80% protein; NX6.25).

[2] Refer to Table 4.4 for variable levels.

[3] Top-Scor S Powder; Breddo Food Products Corp., Kansas.

[4] 162.0g + 50g for hydration of 8.78% and 30% PPC; 112g + 100g for hydration of 51.22% and 60% PPC; 212g for 0% PPC.

[5] Woodstone Foods Ltd., Portage La Prairie, Manitoba.   (83% protein; NX6.25).

[6] 7.5% protein (14% mb); Reid Milling, Missisauga, Ontario.

PROCEDURE

PPC RE-HYDRATION (for PPC formulations):

1. Weigh out PPC into a plastic container, add 50 or 100g water (depending upon amount of PPC) and mix to hydrate all PPC.

2. Cover and re-hydrate overnight.

YOLK BATTER:

1. Shake yolk and sugars in enclosed container to mix.

2. Using the whisk attachment, mix on speed 1 of a Braun Kitchen Machine (Model KM 32) for 30 seconds to thoroughly combine dry ingredients.

3. Add 73.0 mls of water, which has been heated to $80 \pm 1°C$, to yolk mix.

4. Mix on speed 3 for 30 seconds. Scrape down sides to incorporate any dry ingredients remaining on bottom of bowl.

5. Continue to mix on speed 3 for 30 seconds. Scrape down sides.

6. Mix on speed 3 for 9 minutes.

ALBUMEN\PPC BATTER:

1. In 10 quart Hobart (Model C100) mix bowl combine all dry ingredients. Blend well manually using a wire whisk.

2. Add hydrated PPC and remaining water.

3. Using the wire whip attachment, mix on speed 1 for 1 minute to combine. Scrape down sides and bottom, making sure the sugar has not accumulated on the bottom of the bowl.

4. Whip on speed 3 for the experimental length of time outlined in Table 4.3.

*NOTE - Batters should be prepared so that they are ready to be combined at approximately the same time.

COMBINING BATTERS:

1. Add yolk batter carefully to albumen batter.

2. Using the Hobart flat paddle attachment, mix on speed 1 for 10 seconds. Scrape down sides.

3. Sprinkle 1/3 of flour (51.3g) over batter. Mix on speed 1 for 10 seconds. Scrape down sides. Repeat for second 1/3 of flour.

4. Add last of flour to batter and mix on speed 1 for 30 seconds. Scrape down sides.

5. Manually fold batter 3 to 4 times using a spatula.

6. Take specific gravity measurements and reserve this batter for pH determinations.

7. Pour 200g batter into each of 3, 15 cm round, ungreased aluminum cake pans.

BAKING:

1. Bake cakes 23 minutes on the middle rack of a 190°C oven. Be sure to standardize the placement of the cakes.

COOLING AND DE-PANNING:

1. Cool cakes in their pans, on a cooling rack, for 20 minutes.

2. Weigh cakes in pans.

3. Remove cakes from pans and cool on racks for an additional 20 to 30 minutes.

4. Place in plastic bags, seal and freeze (-20°C).

NOTE - 100 percent egg albumen reference (REF) sponge cakes were made according to the method outlined above. Egg albumen=26.4g; PPC=0.0g; cream of tartar=3.0g (center point level); whip time=3.00 min.

Appendix 4.B. Sensory Reference Endpoints[1]

Firmness:

"extremely soft" - Betty Crocker French Vanilla Supermoist.

"moderately firm" - Banana bread[2].

Springiness by touch:

"moderately springy" - Safeway Generic large marshmallows (approximately 3cm X 3cm).

"extremely springy" - fine foam piece (approximately 4cm X 4cm).

Cohesiveness:

"slightly cohesive" - Supervalue No Name sponge cake layers.

"extremely cohesive" - Monarch Added Touch Brownie Mix.

Moistness:

"moderately dry" - Supervalue No Name sponge cake layers.

"moist" - Monarch Added Touch Golden Cake Mix (light recipe).

[1]Reference endpoint samples were baked in the same pans and prepared in the same way as treatment sponge cakes (Figure 4.1).

[2]Banana Bread

| Ingredients	Grams
margarine	55.0
egg	1 med.
granulated sugar	200.0
all purpose flour	220.0
salt	1.5
mashed ripe bananas	250.0
baking soda	5.0

PROCEDURE:

1. Combine flour and salt in a large bowl. Set aside.

2. Manually cream margarine and sugar in a separate bowl.

3. Add egg and mix well.

4. Mix baking soda into mashed banana. Add to egg-sugar-margarine mix. Mix well.

5. Add flour. Mix.

6. Spoon 380g batter into 2, 15cm round, greased cake pans.

7. Bake 50 minutes at 190°C.

8. Cool 5 minutes in pans. Remove and cool on racks overnight. Freeze (-20°C). Remove, thaw and sample like for sponge cakes.

Appendix 4.C.   Sensory Evaluation Instruction Sheet

SPONGE CAKE EVALUATION INSTRUCTIONS

You have been given two sponge cake samples (A&B) for each treatment.   You have also been given enough reference (R) sample to allow re-evaluation when necessary.

Use the bottom half of the reference samples for firmness and cohesiveness evaluations only and the top half for moistness and flavor evaluations only (as necessary between treatments).
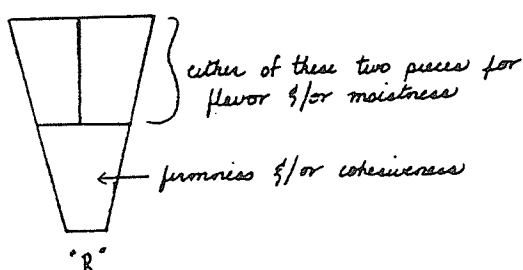

Evaluate each treatment in comparison to the reference which has been positioned on the line scale.   Evaluate each treatment independently for all of the characteristics before moving on to the next treatment.

TREATMENT SAMPLE A: Use this sample to evaluate AROMA INTENSITY. Bring the sample cup up to your nose, lifting the lid slightly at one side and take 2-3 short sniffs.   Following this evaluation, cut the sample in half.   Cut the top half in half again and use it to evaluate FLAVOR INTENSITY first.   Use the entire bottom half to evaluate FIRMNESS. (see below).
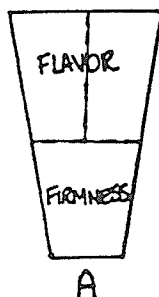
FIRMNESS: Evaluate the firmness of the sample by placing it on your tongue and gently compressing it against your palate. Firmness is the force required to compress the sample against your palate.

TREATMENT SAMPLE B: Use this sample to evaluate SPRINGINESS, COHESIVENESS, and MOISTNESS (see below).

B
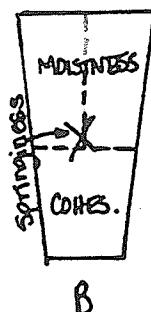
SPRINGINESS BY TOUCH: Use your index finger to gently compress the middle of the sample to approximately 1/2 its height (1 cm); hold for a count of 2 seconds; then quickly release the pressure and evaluate the quickness of recovery.

Following this evaluation, cut the sample in half. Cut the top half in half again and use it to evaluate MOISTNESS. Use the bottom half to evaluate COHESIVENESS. Use the entire piece for each evaluation.

COHESIVENESS: Evaluate cohesiveness as the degree to which the sample holds together after 5 chews.

MOISTNESS: Evaluate moistness as the perceived degree of moisture in the sample after 5 chews.

Appendix 4.D.   Cake Crumb Evaluation Instruction Sheet

SPONGE CAKE CRUMB STRUCTURE EVALUATION

1.   View the visual reference cards which illustrate the line scale anchor points for predominant cell size, cell size uniformity, and cell wall thickness.

2.   Visually evaluate the cake samples in the order indicated on your ballot.

3.   Bring the first sample to the position in the booth marked by a black circle.   Remove the lid of the petri-dish to view the sample, tilting the dish towards yourself to permit optimal light reflectance.   Evaluate predominant cell size, cell size uniformity, and cell wall thickness.

PREDOMINANT CELL SIZE: the cell size that makes up the largest percentage of the sample.

CELL SIZE UNIFORMITY: the cell size uniformity is dependent on the number of different cell sizes present within the sample.   If the cells are all the same size the sample is very uniform.   As the number of different cell sizes within the sample increase, the sample becomes more irregular.

CELL WALL THICKNESS: If a sample is so compact that the cell walls are indistinguishable (you cannot tell whether they are very thick or very thin), score the cell wall thickness at the right endpoint of the scale (15).

4.   Replace the lid on the petri-dish, so the samples do not dry out and return the sample to its original position in the booth. Move the second sample to the viewing position and evaluate it, continuing until all the samples have been evaluated.