# Lipid Extraction Process on Texturized Soy Flour and Wheat Gluten Protein-Protein Interactions in a Dough Matrix

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**ABSTRACT**

Protein-protein interactions between wheat flour and solvent-extracted (SE) or nonsolvent extracted (NSE) texturized soy flours were compared. Doughs were prepared to contain varying ratios of texturized soy flour in combination with wheat flour. Sucrose esters (2.5%) were included in several formulations. Doughs were fractionated into soluble and insoluble fractions at pH 4.7 and pH 6.1. Fractions were dried, powdered, and analyzed using SDS-PAGE and spectrophotometric techniques. Electrophoretic evaluation indicated interactions between wheat gluten proteins and texturized soy proteins in the absence of sucrose esters. Electrophoretic gels of the wheat-soy flour mixtures maintained a characteristic soy protein band after acidification to the soy protein isoelectric point. Inclusion of sucrose esters increased the interaction. Texturization conferred effects similar to that of sucrose ester on both forms of lipid-extracted soy. Sulfhydryl analyses using 7-chloro-4-nitrobenzo-2-oxa-4, 3-diazole (NBD-Cl) revealed no change in the relative amount of sulfhydryl groups present in doughs prepared from either the texturized soy flours or the doughs containing equal amounts of wheat starch. These data indicate that interactions between soy protein from texturized soy flours and wheat proteins are not covalent.

Introducing soybean components to wheat breads has been the focus of research for over 50 years, primarily in an attempt to increase the protein balance of the breads. Previous research into soy protein supplementation determined that the negative effects associated with soy-wheat breads are primarily due to the lack of interaction between soy and gluten proteins (D'Appolonia 1977; Fleming and Sosluski 1977; Silaula et al 1989). Nongluten-forming protein networks are unable to retain carbon dioxide formed during fermentation and unable to temporarily bind water required during starch gelatinization. Theories proposing disulfide (SS) bond disruption and bulk effects have been questioned (Lorimer et al 1991). Electrophoretic studies demonstrate an interaction between soy protein and wheat gluten with the addition of sucrose esters (Hyder et al 1974) or other dough conditioners. Sucrose esters such as sucrose monotallowate cause the insoluble gluten fraction of wheat and the soluble soy fraction (both pH 6.1) to elute in the same column during starch gel electrophoresis. It is hypothesized that the gluten and soy protein interactions provide the dough improvement characteristics. Addition of emulsifiers to low-gluten wheat breads also improves gas retention and crumb texture (Chung et al 1981).

Previous studies focused on traditional solvent-extracted soy protein. A method of nonsolvent extraction (dry-extruded-expelled soy protein) was developed (Nelson et al 1987) and recently applied to the manufacture of nonsolvent-extracted texturized soy flour (NSE). The interaction of NSE texturized soy with the gluten-forming proteins present in wheat flour has yet to be fully elucidated. For this investigation, two types of texturized soy flours where used. Solvent extracted (SE) texturized soy flour is produced by high-temperature extrusion of a hexane-extracted soy flour to produce pelleted products with a fibrous consistency. These pellets were then ground. NSE texturized soy flour is produced by low-temperature expelling of ground, full-fat soybeans followed by extrusion texturization similar to that used in the manufacture of SE. The pellets produced were then ground.

The objectives of this study were to identify the interactions between wheat gluten and SE texturized soy flour (SE) produced by the traditional solvent extraction method and NSE texturized soy flour in bread dough. Sucrose esters (F-160, Montello Corp., Tulsa, OK) were evaluated to confirm the interactions theorized by other research groups (Pomeranz et al 1969; Chung et al 1981) and identify new interactions.

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## MATERIALS AND METHODS

Ground (100 mesh) SE (50.8% protein) and NSE (43.7% protein) were obtained from International Soybean Research Laboratory, UIUC, Urbana, IL, or Archer-Daniels Midland, Decatur, IL, respectively. Bread flour (12.2% protein content) was obtained from North Arkansas Wholesale Co., Bentonville, AK. Sucrose ester specifically for bread dough manufacture (F-160) was provided by Montello. Doughs were prepared by combining bread flour and 12% texturized soy (SE or NSE, dwb). Concentration of soy addition was based on optimized ratios developed for soy flour addition to wheat breads by Kansas State University researchers (Tsen and Tang 1971). Doughs were also prepared containing 2.5% (w/w) sucrose esters, the optimal concentration as described by Hyder et al (1974). The control doughs were 100% wheat flour.

### Dough Preparation

Doughs were prepared as described by Hyder et al (1974). The dry components, bread flour, SE or NSE, and sucrose esters were added to a 250-mL beaker. Distilled water (≈80 mL, 22°C) was added. The mixture was stirred with a glass rod until a cohesive dough formed (≈2 min). Dough was removed from the beaker and kneaded by hand to optimum consistency (≈8 min, 28°C). Dough was washed to obtain the crude gluten fraction by holding under running tap water and kneading until the water ran clear (≈20 min). The crude gluten was cut into pieces (≈4 $cm^3$), placed in a beaker containing 280 mL of 0.005*N* lactic acid and stirred for 60 min with a magnetic stir bar. The mixture was adjusted to pH 4.7 using 85% lactic acid and allowed to stir for a total of 5 hr. The gluten suspension was centrifuged at $1,000 \times g$ for 30 min to remove the insoluble fraction. The supernatant was removed and adjusted to pH 6.1 with 0.1*N* sodium carbonate, then recentrifuged at $1,000 \times g$ for 30 min. The insoluble fraction (pH 4.7), the supernatant (pH 6.1), and the insoluble gluten (pH 6.1) were placed in Pyrex petri plates and dehydrated (SnackMaster Junior, American Harvest, Two Rivers, WI). Samples were dried for 24 hr (87°C) and ground to a powder in a mortal and pestle. Powdered fractions were placed in 30-$cm^3$ plastic cups, covered, and stored at 22°C until analyses (less than two weeks). Prepared doughs contained 12% SE or 12% NSE with and without 2.5% sucrose esters (Table I).

### Protein Preparation

Soy protein was extracted from ground SE and NSE (100 mesh) using isoelectric precipitation as described by Hyder et al (1974).

Ground texturized soy was combined with distilled water (1:10), stirred with a magnetic stir bar for 1 hr (22°C), and centrifuged at 1,000 × *g* for 30 min. The residue was reextracted with distilled water (1:10) and recentrifuged at 1,000 × *g* for 30 min. Supernatants from both extractions were combined. This water-soluble fraction was adjusted to pH 4.5 with lactic acid (85%) and centrifuged (1,000 × *g*) to collect the precipitate. The protein precipitate was dried and ground as previously described.

### Electrophoresis Procedure

Electrophoretic protein separation was conducted on the dried, ground doughs, as well as on two ground, dried doughs prepared using defatted soy flour (ADM, Decatur, IL) and defatted soy flour with sucrose esters. Nontexturized soy flour was evaluated electrophoretically for comparison with previous work by Hyder et al (1974). Electrophoresis was performed using the Xcell SureLock mini-cell system (Novex USA, San Diego, CA). All solutions and procedures were performed according to manufacturer instructions. All electrophoretic gels were prepared by combining 32 µg of sample with 33 µL of buffer (NP007) and 25 µL of ultrapure water in a test tube. Reducing agent (10 µL) (NP004) was then added to the solution and tubes were placed in a 70°C water bath for 10 min. Running buffer (1 L) (NP0002) was prepared and 200 mL was combined with 500 µL of antioxidant (NP005). A 10-well bis-Tris gradient gel (4–12%) (NP032) was placed in the electrophoretic system. The lower chamber was filled with 650 mL of running buffer and the upper chamber was filled with running buffer with added antioxidant. Portions (5 µL) of each solution were underlayed into the gel with a wide-range protein standard (220 to 4 kDa). Samples were electrophoresed using a 200V power source for ≈40 min, removed, and stained using a colloidal blue staining kit (LC6025). After destaining in distilled water (22°C) for 12 hr, gels were photographed using a digital camera (MX-700, Fujifilm, Tokyo, Japan). All gels were run in triplicate.

### Sulfhydryl Analysis

All chemicals used for sulfhydryl (SH) determination were purchased from Fisher Scientific (Pittsburgh, PA). Analysis and determination of free SH was conducted as described by Andrews et al (1995).

Dried, ground dough or flour (40 mg) was suspended in 0.8 mL of a solution of Proteinase K, 1 m*M* $CaCl_2$ (0.16 mg/mL) , and 0.2 mL of 5% (w/v) SDS. This solution was vortexed for 5 min and centrifuged (2,000 × *g*) for 45 min. The supernatant (0.2 mL) was added to 1.0 mL of a buffer of 30 m*M* MOPS (3-[N-Morpholino]-propane sulfonic acid) and 1 m*M* ethylenediaminetetraacetic acid (EDTA) at pH 7 (adjusted with 1*M* NaOH). The resulting solution was transferred to a 1.5-mL cuvette for comparison to a blank containing 1% SDS and prepared buffer. Samples were positioned in a spectrophotometer (Beckman DU Series 600, Fullerton, CA) and the reaction was initiated by adding 20 µL of fresh 7-chloro-4-nitrobenzo-2-oxa-4, 3-diazole (NBD-Cl) in dimethyl sulfoxide (6 mg/mL). Absorbance was determined at 420 nm in 1-min intervals for 70 min with a read time of 2 sec. Absorbance data were fitted to the equation:

$$A(t) = [A_\infty - A_0] \times [1\text{-}\exp(-kt)] + R \times t + A_0$$

where A ($t$) = absorbance at 420 nm at time $t$; $A_0$ = absorbance at $t$ = 0; $A_\infty$ = absorbance at $t = \infty$ (when $R$ = 0); $k$ = first-order rate constant of the reaction between NBD-Cl and sulfhydryl; $R$ = residual rate constant

The values of $R$ and $k$ were obtained using the nonlinear regression program supplied by the spectrophotometer manufacturer (Beckman Instruments). Moles of SH titrated were calculated using the standard curve for SH compounds derivatized with NBD-Cl provided by Andrews et al (1995). The theoretical number of SH groups observed in the wheat-soy dough was calculated using the equation:

$$\text{No. of SH expected} = [0.88(\text{no. of SH in wheat flour})] + [0.12\ (\text{no. of SH in soy flour})]$$

The difference between the expected number of SH observed in the dough and the experimental number is reported as the number of SH participating in dough formation reactions.

## RESULTS AND DISCUSSION

### SDS-PAGE Analysis

Electrophoretic separation of soy flour protein fractions confirmed the findings of Hyder et al (1974). The insoluble gluten fraction (pH 6.1) from dough composed of NSE plus wheat lacked bands of low molecular weight characteristic of soy protein (37–6 kDa)

**TABLE I**
**Soy and Wheat Flour Analysis**

| Attribute | Method of Determination | Sample[a] SETSF | NSETSF | Wheat |
|---|---|---|---|---|
| Water holding capacity (%) | D'Appolonia (1977) | 98.7 ± 1.3 | 99.1 ± 0.41 | 67.3 ± 3.1 |
| Water solublity (%) | Snyder et al (1987) | 40.1 ± 1.2 | 35.9 ± 3.7 | 56.2 ± 2.9 |
| Protein content (%) | Approved Method 46-10 (AACC 2000) | 50.841 | 43.721 | 12.162 |
| Protein dispersiblity index (%) | Approved Method 46-24 (AACC 2000) | 18.4 | 21.05 | 44.94 |
| Lipid content (%) | Mfg. data | 0.2 | 7.9 | 1.7 |

[a] SETSF = solvent-extracted texturized soy flour; NSETSF = nonsolvent-extracted texturized flour.

**TABLE II**
**Sulfhydryl (SH) Content of Prepared Doughs and Flours**

| Sample[a] | Total SH Content (µmol/g)[b] | Expected SH in Dough (µmol/g) | SH in Dough Formation (µmol/g) | % Total[c] |
|---|---|---|---|---|
| NSETSF flour | 2.11 ± 0.14 | nd[d] | nd | nd |
| SETSF flour | 3.47 ± 0.18 | nd | nd | nd |
| Wheat flour | 3.56 ± 0.21 | nd | nd | nd |
| Wheat dough | 3.03 ± 0.11 | 3.56 | 0.53 | 17.53 |
| Wheat + 12% wheat starch | 3.05 ± 0.07 | 3.56 | 0.52 | 16.93 |
| Wheat + 12% NSETSF +SE | 2.97 ± 0.11 | 3.39 | 0.42 | 14.02 |
| Wheat + 12% SETSF +SE | 3.01 ± 0.12 | 3.55 | 0.54 | 18.13 |
| Wheat + 12% NSETSF | 2.92 ± 0.08 | 3.39 | 0.47 | 16.09 |
| Wheat + 12% SETSF | 3.07 ± 0.06 | 3.55 | 0.49 | 15.77 |

[a] SETSF = solvent-extracted texturized vegetable protein; NSETSF = nonsolvent-extracted texturized soy protein; SE = sucrose esters.
[b] Mean values of three observations ± standard deviation.
[c] SH in dough formation/total SH content × 100.
[d] Not determined.

(Fig. 1). However, dough composed of either of the texturized soy flours plus sucrose esters contained the low molecular weight bands. The sucrose esters apparently promoted interaction between the soy flour protein and the gluten proteins in the pH 6.1 insoluble gluten fraction. Electrophoretic separation of SE and NSE without sucrose esters is presented in Fig. 2. Without sucrose esters, both SE and NSE interacted to a limited degree with the insoluble gluten fraction of wheat at pH 6.1 observed as the appearance of the low molecular weight bands characteristic of soy protein. When sucrose esters were introduced (Fig. 3), the interaction increased, observed as increased banding characteristic of isoelectric precipitated soy protein. The increased interaction observed between SE and NSE protein and insoluble gluten is attributed to the differences in the processing of the raw soybeans. Extrusion texturization is a high-temperature short-time (HTST) process. Although the texturization process decreases functionality in some respects (protein dispersability index), in other ways it may increase. Partial denaturation of proteins of the textured soy protein maybe responsible for protein-protein interaction mechanisms similar to those observed with surfactants.

Denaturation of the texturized soy flour proteins may expose formerly hidden hydrophobic groups, which may afford soy proteins the ability to bridge lipid-aqueous interfaces in much the same way as glycolipids. Successful performance of textured soy flours in meat products is evidence of its emulsification ability (Rentfrow et al 1999). The introduction of sucrose esters into doughs may simply serve as a reinforcement of this link.

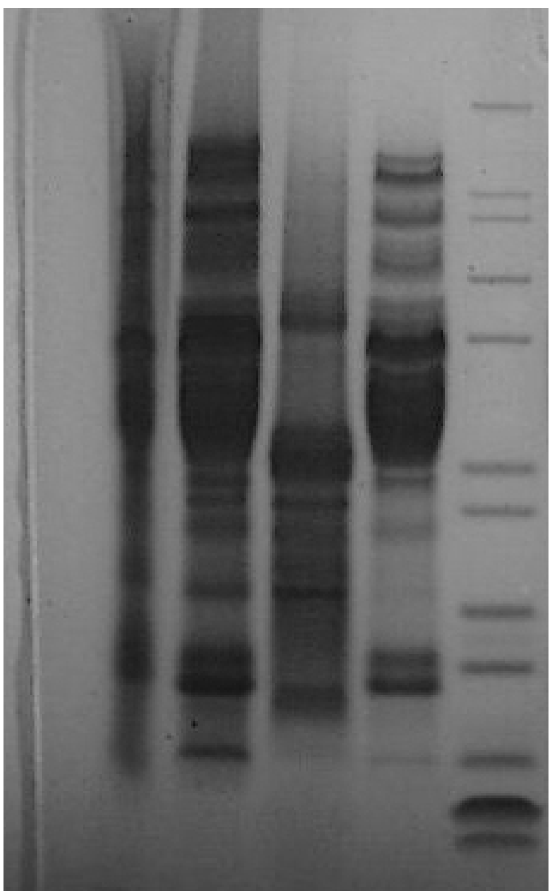


**Fig. 1.** Electrophoretic separaton of solvent-extracted (SE) or nonsolvent extracted (NSE) soy and wheat flour protein fractions. Lanes: 1) wheat + soy flour (12%) with sucrose ester (insoluble gluten pH 6.1); 2) soy flour isoelectric protein (pH 4.7); 3) wheat + soy flour (12%) without sucrose ester (insoluble gluten pH 6.1); 4) marker; B) blank. Molecular masses of standard shown in kDa.

## Spectrophotometric Analysis

Sulfhydryl content of SE was substantially higher than in NSE (Fig. 4). This may be due to higher temperature processing of the soy flours that may sever internal SS bonds in the globulin fraction of the SE protein producing more SH groups. A decreased thiol group number has been reported in high-temperature processed soy products (Wolf 1977). Doughs prepared from textured soy flour and wheat flour contained no more SH groups than did doughs prepared with the same proportion of wheat starch and wheat flour. Neither textured soy flour appeared to contribute a significant number of SH groups (Table II).

These results imply that the deleterious effects (decreased loaf volume, dense crumb, etc.) of textured soy flour is not due to a lack of SH availability. These data are consistent with similar studies by Lorimer et al (1991). However, this does not rule out the possibility that native SS bonds were disrupted. The SH groups present in the soy flour potentially participate in dough development through the process of SS interchange. The apparent lack of such a reaction within the texturized soy-wheat flour doughs may be due to the extremely hydrophilic interaction of the hydrated soy protein and wheat starch. These hydrophilic forces may interfere with the ability of soy protein to approach the gluten complex and initiate SH interchange.

Addition of sucrose esters exhibited no effect on the SH content of the prepared doughs (Fig. 5). These results are consistent with a lack of change in dough rheology reported in earlier research (Pomeranz et al 1969).

Proposed mechanisms regarding the role of sucrose esters in volume and crumb improvement implicate hydrogen and hydrophobic bonding. The main role of sucrose esters is enhancement of noncovalent interactions between gluten and starch.

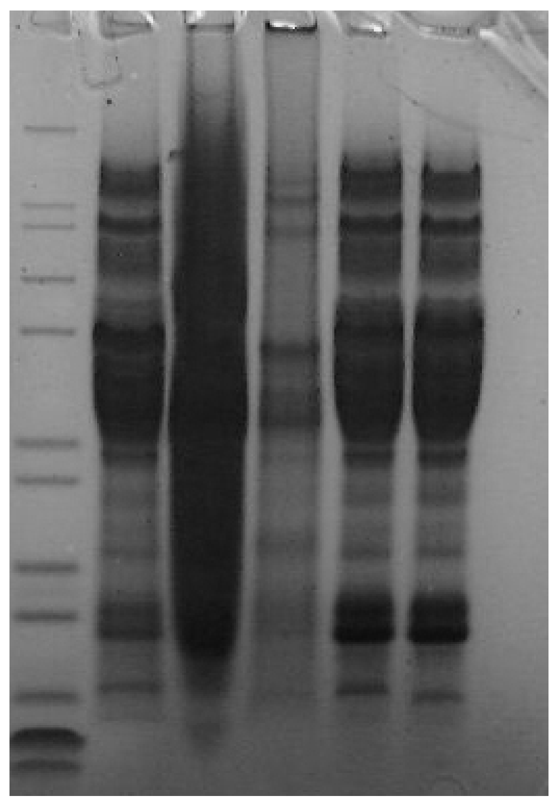


**Fig. 2.** Electrophoretic separation of solvent-extracted (SE) or nonsolvent extracted (NSE) texturized soy and wheat flour protein fractions without sucrose esters. Lanes: 1) marker; 2) wheat (insoluble gluten pH 6.1); B) blank; 3) wheat (insoluble gluten pH 4.7); 4) wheat + SE texturized soy flour (insoluble gluten pH 6.1); 5) wheat + NSE texturized soy flour (insoluble gluten pH 6.1). MW = molecular standards.

The interactions between textured soy flour and gluten-forming proteins is similar to that found with sucrose ester as reported by previous research (Hyder et al 1974; Pomeranz 1980). Texturization of soy flour strengthens the potential for hydrophobic and hydrogen bonding between soy and gluten proteins. Electrophoretic analysis demonstrated that solvent extracted, texturized soy flour and dry extruded, texturized soy flour exhibited similar interactions with gluten proteins with or without sucrose esters.

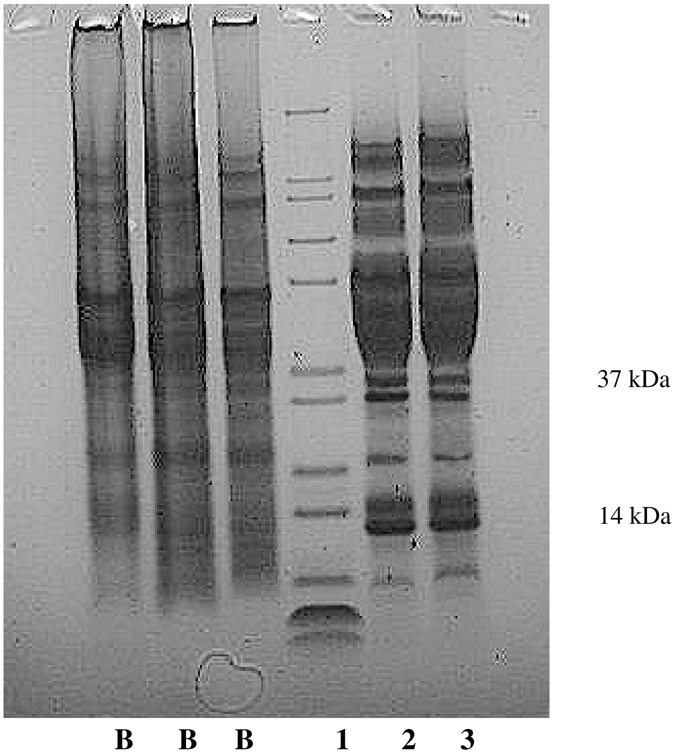


**Fig. 3.** Electrophoretic separation of solvent-extracted (SE) or nonsolvent extracted (NSE) texturized soy and wheat flour protein fractions with sucrose esters. Lanes: B) blank; 1) marker; 2) wheat + SE texturized soy flour + sucrose esters (insoluble gluten pH 6.1); 3) wheat + NSE texturized soy flour + sucrose esters (insoluble gluten pH 6.1). MW = molecular standards.

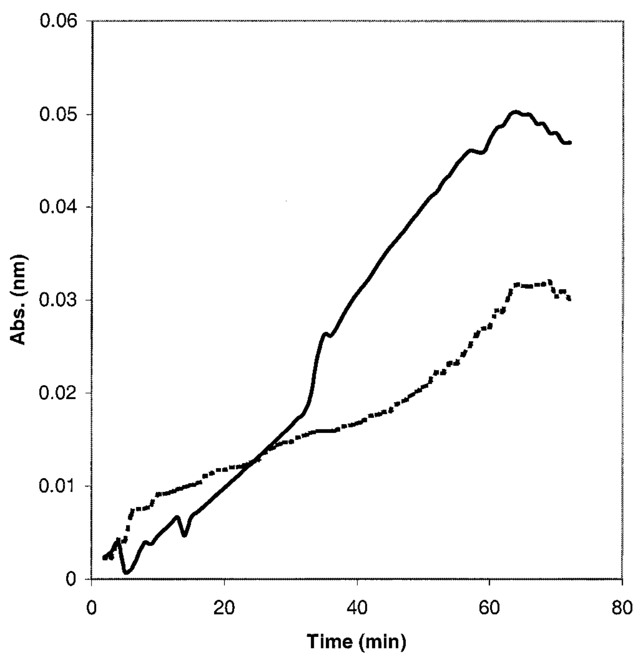


**Fig. 4.** Spectrophotometric analysis of sulfhydryl content of prepared doughs with no sucrose esters. SETSF (——) solvent-extracted texturized soy flour (Lorimer et al 1991); NSETSF (▬ ▬ ▬) nonsolvent-extracted texturized soy protein.

## CONCLUSIONS

Lipid extracted, texturized soy flour (solvent and nonsolvent) demonstrated similar noncovalent protein-protein interactions in a wheat dough system. An increase in noncovalent interactions with the addition of sucrose esters and the lack of change in SH group content suggest an interaction mechanism similar to that proposed for synthetic glycolipids in dough manufacture. The process of texturization improves the functionality of soy proteins in wheat dough through an enhancement of noncovalent interactions. Further development and enhancement of the properties of sucrose esters present in these flours may lead to improved soy-wheat bread characteristics.

### ACKNOWLEDGMENTS

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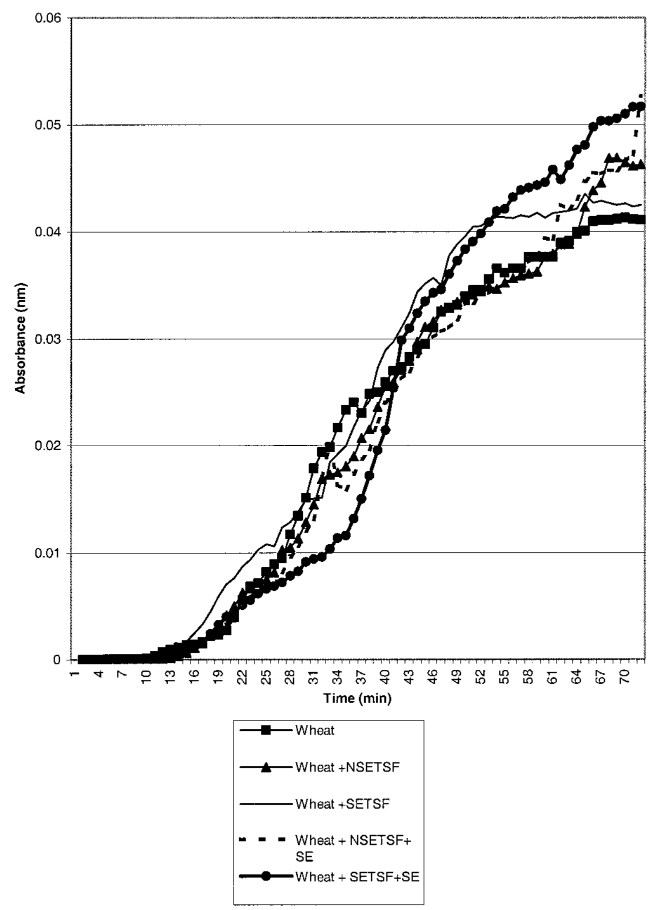


**Fig. 5.** Spectrophotometric analysis of sulfhydryl content of prepared doughs with addition of sucrose esters (SE). SETSF = solvent extracted texturized soy flour; NSETSF = nonsolvent extracted texturized soy flour.

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