THE EFFECT OF DIACETYL TARTARIC ACID ESTERS OF MONOGLYCERIDES (DATEM) UPON WHEAT GLUTEN ANALYZED BY CAPILLARY ELECTROPHORESIS

By

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Title of Study: The effect of Diacetyl tartaric acid esters of monoglycerides(DATEM) upon wheat gluten analyzed by Capillary Electrophoresis

Major Field: Food Science

Abstract

Capillary zone electrophoresis was used to characterize soluble gluten proteins extracted from six different commercial wheat samples (with protein content varying from 8 to 13%). The objective of this study was to describe qualitatively separation patterns of wheat gluten treated with four different levels of DATEM (0, 0.3, 0.6 and 1.0%) and changes in proteins solubility, interactions and charge/mass ratio. Three different groups named A, B and C based on their migration profile were observed where group A represents positively charged peptides with the largest charge to mass ratios, group B represents positively charged peptides with reduced ratios, and finally group C are anions with greater ratios. DATEM's ability to induce changes in protein conformation was observed by (i) decrease in maximum peak intensity and (ii) an increase in resolution of peaks. DATEM decreases protein solubility at a concentration of 0.6% for flour 1C, 4C and 6C and 0.3% for flour 2C. DATEM's ability to increase interactions between gluten proteins, resulting in change in charge/mass ratio is flour dependent. However, flour 6C displayed a reversal in solubility at 1.0%, suggesting that DATEM at critical levels forms micelles, which leads to reduced interactions of DATEM with gluten. A proposed general mechanism by which DATEM influences overall protein spatial conformation is explained by a four and two stage model.

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ABBREVIATIONS

CIEF	Capillary iso electric focusing
CMC	Critical micellar concentration
CZE	Capillary zone electrophoresis
DATEM	Diacetyl tartaric acid ester of monoglycerides
DTT	Dithiothreitol
FZCE	Free zone capillary electrophoresis
HMW-GS	High molecular weight - glutenin subunits
HPCE	High pressure capillary electrophoresis
HPLC	High pressure liquid chromatography
HPMC	Hydroxy propyl methyl cellulose
LMW-GS	Low molecular weight - glutenin subunits
SDS-CE	Sodium dodecyl sulfate - capillary electrophoresis
SDS-PAGE	Sodium dodecyl sulfate - polyacrylamide gel Electrophoresis

CHAPTER I

INTRODUCTION

1.1 Statement of problem

Baking industry has been using dough improvers for many years to improve bread quality. Numerous studies have been done to analyze the effect of surfactants upon wheat's rheological and baking properties. The ability of surfacants like DATEM to induce improvement on loaf volume and crumb texture has been studied extensively. Gluten proteins are held together by various interactions such as hydrophobic, non covalent and disulfide bonds. DATEM helps in stabilizing the interactions between gluten proteins, by its ability to form gluten-DATEM complexes. DATEM's effect upon wheat gluten proteins solubility and its ability in aiding the formation of insoluble gluten polymer has not been studied extensively. It is widely accepted that the higher insolubility of gluten polymer the better the characterisitcs of gluten for yeast fermented products. In this study, we analyzed DATEM's effect (i) upon protein solubility and (ii) changes in proteins **charge/mass** ratio.

1.2 Aim of the study

The aim of the present study is to characterize the separation patterns of wheat gluten from commercial flours with varying protein concentrations (8 – 13%) by free

zone capillary electrophoresis. The ability of DATEM to modify wheat gluten interactions at four different levels (0, 0.3, 0.6 and 1.0%) will be investigated.

CHAPTER II

REVIEW OF LITERATURE

2.1 Introduction

Grain protein composition and concentration are some of the key factors responsible for the baking quality of bread (Singh and Khatkar, 2005). In wheat, gluten proteins are important in determining the quality difference between wheat varieties (MacRitchie, 1984). Glutenins and gliadins are the two major proteins that contribute to the wheat quality. Glutenins are polymeric alcohol insoluble proteins, which when reduced yield two types of subunits classified as low (LMW-GS) and high molecular weight glutenin subunits (HMW-GS) with a molecular weight in the range of 80,000-120,000 Da for high molecular weight and 30,000-50,000 Da for low molecular weight proteins (Shewry et al., 1986). Polymers of gluten are formed by intermolecular disulfide bonds linking the high molecular weight and low molecular weight glutenin subunits.

2.2 Gliadins

Gliadins are alcohol soluble monomeric proteins in which the disulfide bonds when present, link one cysteine of the polypeptide chain to another cysteine of the same chain, i.e., they form intramolecular disulfide bonds.

Gliadins contribute to the extensibility of the dough system (Wall, 1979). Gliadins are classified into 4 classes namely α-, ß-, γ- and ω-gliadins based on the electrophoretic mobility on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Shewry et al., 2003). Gliadin contains mainly repetitive sequences of glutamine (Q) and proline (P) (e.g., PQQPFPQQ) (Wieser, 2007).

ω-gliadins have higher concentration of glutamine and proline compared to α-, ß-, and γ- gliadins. ω-gliadins (49-55 KDa) have molecular weight higher than that of α-, ß-, and γ- gliadins (28-35 KDa). Most of the ω-gliadins lack cysteine residues, hence they are unable to form disulfide bonds (Wieser, 2007). The N-terminal domain of gliadins mainly consists of repetitive sequence of glutamine (Q), proline (P), phenylalanine (F), and tyrosine (Y) (for e.g. QPQPFQQPYP). The C-terminal domains of gliadins are homologous, and have amino acid sequences that are non-repetitive with lower concentration of glutamine and proline when compared to N- terminal. (Wieser, 2007). The α-, ß- gliadins contains six cysteines and the γ- gliadins contain about 8 cysteine residues. The presence of cysteine residues helps in the formation of 3 or 4 homologous intrachain crosslink (Grosch and Wieser, 1999). The structure of α- , γ- gliadins and the position of cysteine are represented in Figure 1.

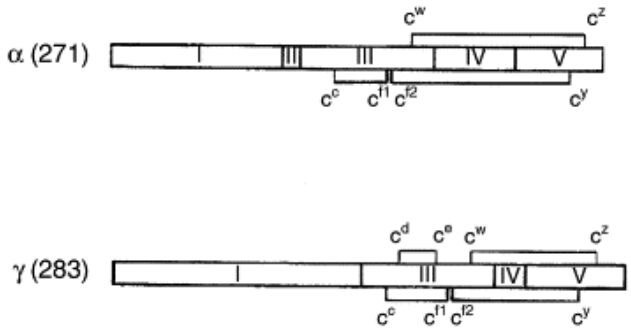


Figure 1. Schematic representation of the position of disulfide bonds structures in α-, γ-gliadins (from Grosch and Wieser, 1999).

2.3 High Molecular Weight glutenin subunits (HMW-GS)

The HMW-GS are encoded at the Glu-1 loci on the long arms of group1 chromosomes (1A, 1B and 1D) (Bietz, 1975; Payne et al., 1980). Each locus comprises of two genes that are linked together encoding two different types of HMW-GS namely x and y-type subunits (Payne and Corfield, 1979; Payne et al., 1987; Shewry et al., 1992). The x-type has slower mobility than the y-type as determined from SDS-PAGE electrophoresis, suggesting that x-type has higher molecular weight compared to y-type subunits. The x-type contains four cysteine residues whereas the y-type contains seven cysteine residues (Shewry et al., 1992). A summary of the molecular weight of x and y-type HMW-GS and their amino acids composition are shown in Table 1 (Shewry et al., 1992).

The HMW-GS consist of 3 major domains namely 1) domain A (a non repetitive N-terminal domain contains 80-105 residues), 2) domain B (repetitive central domain contains 480-700 residues), and 3) domain C (C-terminal domain contains 42 residues)

(Shewry et al., 1992). Domains A and C are hydrophobic in nature whereas domain B is hydrophilic in nature. Domain B is rich in amino acids like proline and glycine. However no sulfur is present in domain B. Most or all of the cysteines are present in either domain A or C (Figure 2) (Wieser, 2007). Cysteine residues form disulfide bonds between HMW-GS and LMW-GS leading to the formation of large protein polymers. Domain A is quite conservative in which the first 16 amino acids are consistent. Only small changes in amino acid sequences can be found such as E (glutamic acid) at position 6 (EGEASEQLQCERELQE) in x-type HMW-GS, which is replaced by R (arginine) in y-type HMW-GS (EGEASRQLQCERELQE). The x-type HMW-GS has 4 cysteines, one in C-terminal and 3 in N-terminal, whereas the y-type HMW-GS subunit has 5 cysteines in N terminal domain and one in B and C terminal domain each (Shewry et al., 1992) (Figure 2). Among the five cysteines present y-type HMW-GS two are linked together by intrachain link (domain A), the other two (domain A and C) are suggested to form intermolecular interaction. In y-type HMW-GS interchain bond formation are found only for adjacent cysteine present in domain A. The cysteine in repetitive domain of y-type HMW-GS is linked to cysteine of LMW-GS (Wieser, 2007; Gianibelli et al., 2001).

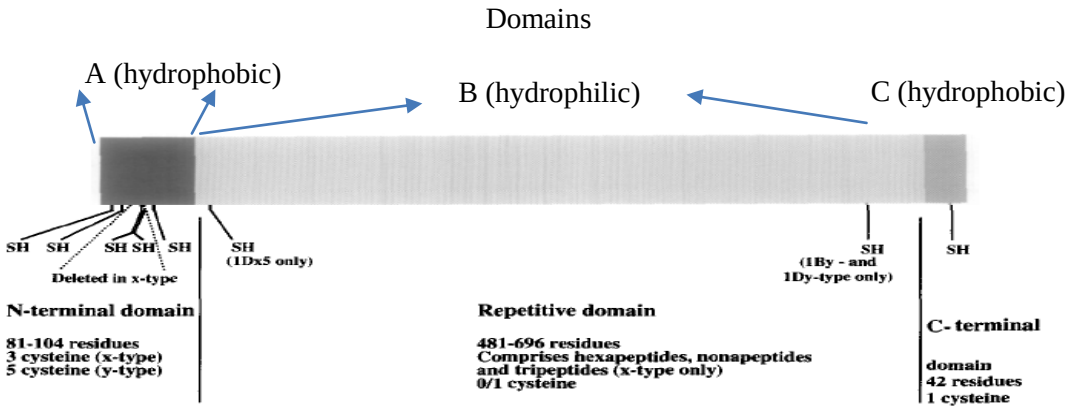


Figure 2. Schematic representation of 3 domains of HMW-GS (from Shewry et al., 2003)

2.4 Low Molecular Weight glutenin subunits (LMW-GS)

LMW-GS are 40% of the wheat gluten proteins (Payne et al., 1984). When glutenins are reduced two types of subunits are released HMW-GS and LMW-GS. The LMW-GS are more complex and the relationship of LMW-GS to wheat quality is still an enigma.

The N-terminal amino acid sequence study on LMW-GS reveals 3 subgroups of typical LMW-GS namely LMW-s, LMW-m and LMW-i., where s, m and i represent the first amino acid present in the sequence (serine, methionine and isoleucine) (Gianibelli et al., 2001). LMW-s type is the most predominant with a molecular weight in the range of 35,000-50,000 Da. LMW-m and LMW-i type have molecular weight in the range of 30,000-40,000 Da (Tao, 1989; Masci et al., 1995). The N-terminal domain of LMW-GS are rich in glutamine and proline and the C-terminal domain of LMW-GS is homologous to α-, ß-, and γ- gliadins. LMW-GS contain 8 cysteines of which 6 residues are present in position similar to α-, ß-, and γ- gliadins and 2 additional cysteine are present in N

terminal and C terminal II domain (Figure 3). The total cysteine and cysteines involved in the formation of inter, intra molecular disulfide bonds are shown in Table 2. The molecular weight and amino acid composition of α-, ß-, and γ- gliadins, LMW-GS and HMW-GS (x and y type) are shown in Table 1.

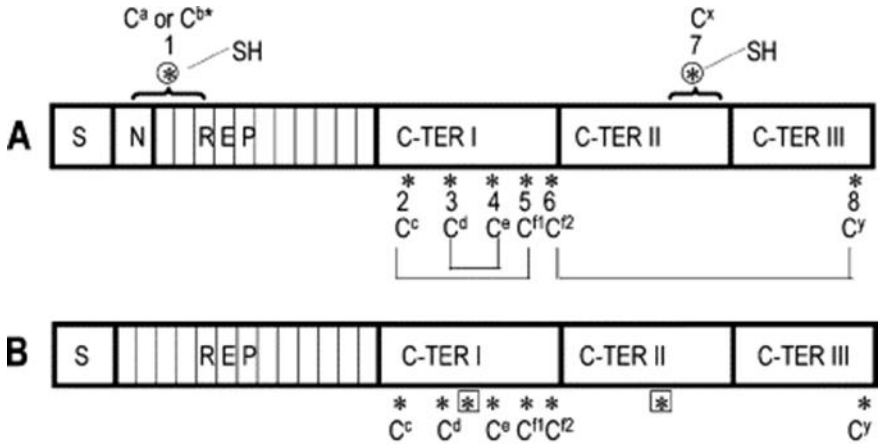


Figure 3. LMW-GS representing the disulfide structure of gluten proteins (A) LMW-m and LMW-s type (B) LMW-i type. S: Signal peptide; cysteine are represented by (*) (from D'Ovidio and Masci, 2004).

Table 1. Molecular weight distribution and amino acid composition of gliadins and glutenins (from Wieser, 2007).

Type	MW *10^3	Proportions (%)	Partial amino acid composition (%)				
	MW *10^{-3}	proportions (%)	Gln	Pro	Phe	Tyr	Gly
ω5-Gliadins	49-55	03-06	56	20	9	1	1
ω1,2 -Gliadins	39-44	04-07	44	26	8	1	1
α, ß Gliadins	28-35	28-33	37	16	4	3	2
γ- gliadins	31-35	23-31	35	17	5	1	3
x-HMW-GS	83-88	04-09	37	13	0	6	19
y-HMW-GS	67-74	03-04	36	11	0	5	18
LMW-GS	32-39	19-25	38	13	4	1	3

Table 2. Total cysteines and cysteines capable of forming intra and intermolecular disulfide bonds (Wieser, 2007)

	Cysteine forming Intra and inter molecular links		
	Total Cysteins	Intra	Inter
ω5-Gliadins	-	-	-
ω1,2 -Gliadins	-	-	-
α, ß Gliadins	6	6	-
γ- gliadins	8	8	-
x-HMW-GS	4	2	2
y-HMW-GS	7	5	2
LMW-GS	8	6	2

2.5 Genetics of wheat gluten proteins

Bread wheat can contain up to six different HMW-GS from 3 chromosomes A, B and D and 2 genes x and y; however only 3 to 5 HMW-GS are commonly expressed. Hexaploid wheats contain at least 1Bx, 1Dx and 1Dy subunits, while some cultivars also contain a 1By and 1Ax subunits as well. LMW-GS genes are found on the short arms of chromosomes 1AS, 1BS and 1DS. These genes are located at Glu-A3, Glu-B3 and Glu-D3 loci (Gianibelli et al., 2001). LMW-GS comprise 40% of wheat proteins (Payne et al., 1984); however they are very poorly studied or understood. Some of the LMW-GS tend to overlap with gliadin subunits (Gianibelli et al., 2001), making them very difficult to study. Numerous researches over the past 30 years have shown that the allelic variation among the HMW-GS and LMW-GS has significantly impacted the wheat baking quality. However the mechanism by which the gluten proteins influence the wheat quality is still yet to be fully understood.

2.6 Qualitative analysis of wheat gluten proteins

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), 2-Dimensional electrophoresis and ion exchange chromatography are some of the many methods of chromatography used to analyze wheat gluten proteins (Bietz and Simpson, 1992). However these methods have serious disadvantages including long separation time and labor intensive. High pressure liquid chromatography (HPLC) seems to be the answer to the above mentioned limitations; however some gluten separations were not satisfactorily achieved (Bietz and Schmalzried, 1995). It has been stated that no one technique would be good enough to provide us with complete information about gluten proteins (Gianibelli et al., 2001).

2.7 Analysis of wheat gluten proteins using Capillary Electrophoresis

Capillary electrophoresis is an analytical technique that separates ions based on their electrophoretic mobility, with the use of applied voltage. It is capable of very fast separations. High performance capillary electrophoresis (HPCE) is a poweful tool used for separating proteins based on their size (sodium dodecyl sulfate-capillary electrophoresis (SDS-CE)), iso electric point (capillary iso electric focussing (CIEF)) and charge /mass ratio (free zone capillary electrophoresis (FZCE)) (Bean et al., 1998). Capillary elecrophoresis has been successfully used in separating proteins from various cereals, including wheat, oat, rice and maize (Bean et al., 1998). Capillary electrophoresis is rapidly becoming the most sought method to analyze proteins in cereal grains because of their ability to analyze samples rapidly and also its ability to render results with low concentration of analytes.

Capillary electrophoresis has been used extensively for separation of wheat proteins and also for analyzing wheat storage proteins since the 1990's. Capillary electrophoresis has been used to analyze gluten and gliadin subunits of wheat gluten proteins (Bean and Lookhart, 2000; Di Luccia et al., 2009; Yan et al., 2004; Piergiovanni, 2013). Capillary electrophoresis along with SDS-PAGE, Acid-Polyacrylamide gel electrophoresis (A-PAGE) has been used to identify and separate wheat proteins as water soluble (Bean and Tilley, 2003), HMW-GS (Gao et al., 2010) and gliadins (Yan et al., 2003; Yan et al., 2004).

Free zone capillary electrophoresis (FZCE) has been the most efficient and developed form of capillary electrophoresis. Many modifications have been done to the methods and instrumentation used for FZCE, including the usage of low pH buffers (phosphate glycine) (Bean and Tilley, 2003), and modifications in the capillary diameters have improved the resolution of peaks in the last 20 years.

2.8 Effect of emulsifiers

Emulsifiers are amphiphilic substances that possess both hydrophilic and hydrophobic properties. They are commonly used in bakery products to enhance dough properties by increasing the dough strength or crumb softness. Due to the amphiphilic nature of emulsifiers, it enables them to migrate to interfaces between two physical phases of the dough and lowering the surface tension. Hydrophobic or lipophilic region of the emulsifier molecule interacts with the non-polar lipid phase, whereas the hydrophilic regions interact with the polar aqueous components (Stauffer, 1999).

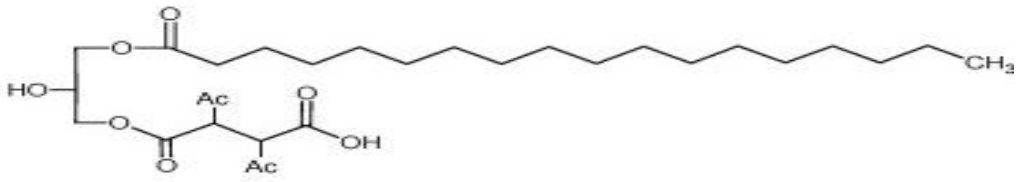


Figure 4. Structure of DATEM (from Gomez et al., 2004)

DATEM is used throughout the world as improver for bread making. It has been shown to improve bread texture and volume (Köhler and Grosch, 1999). Numerous studies have shown that DATEM prevents bread staling and yield better dough properties during proofing (Gomez et al., 2004). DATEM is produced by the reaction of mono and diacetyltartaric acid anhydride with monoacylglycerols or mixture of mono and diacylglycerols (Köhler and Grosch, 1999).

2.9 Protein quality and DATEM

Wheat flour quality plays a key role in determining the quality of baked products. Dough additives have been used for years in food industry to compensate for the non-uniform quality of wheat flours (Ambardekar, 2009). DATEM at a concentration of 0.6% has the maximum ability to improve loaf volume of flours with varying protein concentrations. However, the ability of DATEM to improve loaf volume and elastic nature of gluten is stalled at a concentration of 1.0% DATEM (Ambardekar, 2009). The strength of weak gluten and the visocoelastic properties of flour with different protein content were improved with higher concentration of DATEM (Ambardekar, 2009).

DATEM increased the loaf volume of bread at a concentration of 0.6%, suggesting that DATEM influences protein quality and baking characteristics by its ability to modify the gluten structure. Two flours with similar protein content e.g only a 0.2% difference showed a significant differenec in loaf volumes compared, suggesting that along with protein content quality also influences loaf volumes (Ambardekar, 2009). Contrast in stability time in flours with similar protein concentration, also suggested that protien quality plays an important role in affecting dough mixing properties in wheat flour (Ambardekar, 2009). The diference in protein quality among flour with similar protien concentration could be attributed to the presence of low molecular weight glutenin polymers, which plays a major role in determining wheat dough strength (Scanlon & Dexter, 2003).

Protein aggregation studies observed that at DATEM's ability to induce protein aggregation was observed at a low concentration of flour compared to increased ranges of flour required for samples without DATEM (Lim, 2011). It was suggested that DATEM decreased the surface tension between starch, lipid and protein leading to decrease in gluten strength which is dependent on protein quality (Lim, 2011). Fermentation properties of dough revealed that addition of DATEM increased the dough development height and volume of gas retained. DATEM at a concentration of 0.3 and 0.6% positively impacted maximum height of dough development. However, increaseing the concentration to 1.0% decreased its ability (Visireddy, 2011).

DATEM's ability to influence dough quality has been linked to its components. It contains hydrophilic radicals such as diacetyl radicals and hydroxyl group that interact with water, which in turn is conducive for water retention (Cambell et al., 2001).

DATEM with one carboxyl group component had superior baking performance compared to one with two carboxyl groups, (Kohler, 2001).

DATEM been suggested to interact strongly with gluten thus favoring the formation of the gluten-starch-lipid network. DATEM at a concentration of 0.2% showed an increase in stability time ($p < 0.05$) suggesting the ability of DATEM to increase dough strength. DATEM (0.2%) is suggested to improve the elasticity of wheat dough by influencing dough's resistance to extension. It is suggested that DATEM acts by distributing itself evenly within the gluten network, between the starch and gluten, leading to the formation of gluten film that is more expandable (Ding et al., 2013).

CHAPTER III

CAPILLARY ELECTROPHORESIS ANALYSIS OF THE EFFECT OF DIACETYL TARTARIC ACID ESTER OF MONOGLYCERIDES (DATEM) UPON WHEAT GLUTEN

3.1 Abstract

Capillary zone electrophoresis was used to characterize soluble gluten proteins extracted from six different commercial wheat samples (with protein content varying from 8 to 13%). The objective of this study was to describe qualitatively separation patterns of wheat gluten treated with four different levels of DATEM (0, 0.3, 0.6 and 1.0%) and changes in proteins solubility, interactions and charge/mass ratio. Three different groups named A, B and C based on their migration profile were observed where group A represents positively charged peptides with the largest charge to mass ratios, group B represents positively charged peptides with reduced ratios, and finally group C are anions with greater ratios. DATEM's ability to induce changes in protein conformation is observed by (i) decrease in maximum peak intensity and (ii) an increase in resolution of peaks. DATEM decreases protein solubility at a concentration of 0.6% for flour 1C, 4C and 6C and 0.3% for flour 2C. DATEM's ability to increases interactions between gluten proteins, leading to a change in charge/mass ratio is flour dependent. However, flour 6C displayed an increase in solubility and resolution at 0.3%,

and a reversal in solubility was observed at 1.0%, suggesting that DATEM at critical levels forms micelles, which leads to reduced interactions of DATEM with gluten. A proposed general mechanism by which DATEM influences overall protein spatial conformation is explained by a four and two stage model. Our study suggests that the ability of gluten to form insoluble gluten polymers is dependent on protein quality and concentration.

The objective of the study was to analyze the effect of DATEM treatments upon wheat gluten obtained from flours with varying concentrations and to investigate the ability of DATEM to influence protein interactions, leading to changes in protein solubility. Capillary electrophoresis analysis of the separation pattern of control and treatments suggested that DATEM changes protein solubility, resulting in an electropherogram with increased resolution and changes in proteins charge/mass ratio. However, the concentration of DATEM required to induce changes upon protein solubility is flour dependent.

3.2 Materials and Methods

3.2.1 Wheat sample

Six different commercial wheat flours were used for this study. The wheat flour samples (enriched and malted) had protein content ranging from 8-13%. Four levels of DATEM (Caravan Ingredients, Lenexa, KS 66515) were added to the flour (0, 0.3, 0.6 and 1.0%) (w/w) flour basis.

3.2.2 Sample preparation (Gluten preparation)

Wet gluten was isolated by washing the flour (10g) with 2% NaCl (w/v) for 5 minutes using a Glutomatic 2200 instrument (Perten Instruments, Sweden). DATEM (0.3, 0.6, and 1.0%) was added directly and mixed with flour samples and subjected to gluten separation. Proteins were extracted from 10mg of gluten using 1ml of 50% n-propanol containing 1% dithiothreitol (DTT). Samples were sonicated at 60˚C for 45 minutes, filtered using 0.45 µm filters (Sun Sri, Rockwood, TN) and immediately injected into capillary electrophoresis apparatus.

3.2.3 Sequential extraction of gliadins and glutenins

To distinguish the separation of patterns of gliadins and glutenins on capillary electrophoresis, a sequential method of extraction for gliadins and glutenins is followed based on protocol developed by Singh et al., (1991).

Gliadins were extracted for 30 minutes in 1.0 ml of solution A (50% 1-propanol). Samples were placed in water bath maintained at 65ºC. Samples were vortexed intermittently followed by centrifugation for 15 min at 12,500 rpm. The supernatant is discarded and the extraction is repeated again. The residue is washed with 0.5 ml of solution A and the liquid were removed by aspiration

Glutenins were extracted for 30 minutes in solution B (50% 1-propanol in 0.08 M Tris HCl pH 8.0) containing 1% (w/v) freshly prepared dithiothreitol (DTT). Samples were centrifuged at 12,500 rpm for 5 minutes. 0.1 ml of Solution B containing 1.4% (v/v) vinyl pyridine is added to each tubes and incubated for 15 minutes at 65ºC. Samples were centrifuged for 5 minutes at 12,500 rpm and the supernatant were filtered using 0.45 µm

filters (Sun Sri, Rockwood, TN) and immediately injected into capillary electrophoresis apparatus.

3.2.4 Free Zone Capillary Electrophoresis (FZCE)

Free zone capillary electrophoresis separations were run on a Beckman P/ACE MDQ (Beckman- Coulter, San Ramon, CA) according to protocol described by Patel (2003). Samples were injected for 5 seconds and separated on a 27cm (20 cm to the detector, 50µm I.D) uncoated fused silica capillaries (Polymicro Technologies, Phoenix, AZ). An operating voltage of 15 kV at a temperature of 30˚C was used for analyzing samples using absorbance at 214 nm. Samples were separated using a freshly prepared running buffer containing 100 mM phosphate (pH 2.5), 20% (v/v) acetonitrile, 0.4% (w/v) glycine and 0.05% (w/v) hydroxypropylmethylcellulose (HPMC).

3.3 Results

Protein, moisture and ash (%) content of the six flours used in this study is exhibited in Table 3.

Gluten proteins from six different commercial wheat flours were separated based on their charge/mass ratio using FZCE. Proteins were extracted using phosphate buffer at acidic pH (2.5), as studies have shown that it helps in better resolution and reproducibility (Bean et al., 1998). The extracted proteins represent soluble gluten proteins from both monomeric (mostly gliadins) and polymeric proteins (HMW-GS and LMW-GS). However, the soluble proteins can aggregate easily after extraction even in the presence of 1% DTT, which prevents oxidation to form disulfide bonds.

FZCE separated gluten proteins into multiple peaks (Figure 5a – 10e). The electropherogram represents a fingerprint of the gluten proteins present in flour. The presence of numerous peaks represents the multiple component nature of gluten proteins present in wheat. The peptides migrating earlier represent proteins that contain positively charged peptides with the largest charge to mass ratios, followed by positively charged peptides with reduced ratios, neutral peptides, followed by anions with smaller charge to mass ratios, and finally anions with greater ratios.

It has been shown that FZCE separations of glutens at acidic pH differentiated peaks into 4 different zones. The peaks migrating in the first minutes of the electropherograms were assumed or proposed to be as LMW-GS and the rest of the peaks as HMW-GS (Di Luccia et al., 2009). HMW-GS can further be separated into three different categories. The 1st set of peaks migrating after the LMW-GS were identified to be the y-type HMW-GS, followed by intermediate HMW-GS and finally by x-type HMW-GS (Sutton and Bietz, 1997; Yan et al., 2003; Yan et al., 2004). The x-type HMW-GS has molecular weight greater than that of the y-type (Di Luccia et al., 2009). HMW-GS and LMW-GS separation patterns for hard red spring cultivar Butte 86 suggested that most of the LMW-GS migrated within the first twelve minutes. The electropherogram for HMW-GS indicates that complete separation of HMW-GS was achieved around 18 minutes (Patel, 2003). LMW-GS obtained by sequential method of extraction showed that LMW-GS proteins have similar electrophoretic mobility as HMW-GS proteins, as overlapping protein peaks could be seen (Patel, 2003). Di Luccia et al., (2009), suggested that group A contains peptides that are mostly low in molecular weight with higher positive charge, which can be attributed to LMW-GS as they are

positively charged at pH 2.5. LMW-GS comprises at least 38% glutamine, 13% proline, 4% phenylalanine, 1% tyrosine and 3% glycine (Table 1). It is also possible that gliadins mainly α-, ß- and γ- gliadins could have similar electrophoretic mobility with LMW-GS as they have similar amino acid composition and molecular weight compared to LMW-GS (Table 1). The x-type HMW-GS contains tri, hexa and nona peptide motifs however; the y-type contains only hexa and nona peptide motifs. The amount of arginine present in tri, hexa and nona peptides of x-type HMW-GS glutenin are 6, 4 and 7% compared to y-type HMW-GS that contains 4% in both hexa and nona peptides (Shewry et al., 2003). The higher composition of arginine in x-type HMW-GS compared to y-type LMW-GS leads to a faster migration of y-type compared to x-type (Di Luccia et al., 2009).

In our study, we separated the electropherograms into three different groups A, B and C, where group A represents proteins with highest charge to mass (*z/m*) ratios. Proteins with more positive charge (cations) and small molecular mass migrate faster than group B and C. Group B represents proteins with lower charge to mass ratio compared to group A. Proteins with less positive charge (cations) and larger molecular mass migrate slower than group A towards the cathode. Group C represents proteins with lowest *z/m* ratio. Proteins with less negative charge and large molecular mass and proteins with low molecular weight and higher negative charge could cause them to repeal from migration to cathode.

Our method of extractions were based on whole gluten extract, which also contain gliadins, hence making it difficult to infer whether the proteins are LMW-GS, y-type HMW-GS or x-type HMW-GS as suggested by Di Luccia et al., (2009). The FZCE patterns of 6 flours treated with 0.3, 0.6 and 1.0% DATEM are shown in (Figure 5a-10e).

It has been previously reported that all the gliadins, LMW-GS and HMW-GS migrated within the first 20 minutes (Lookhart and Bean, 1995; Bean et al., 1998; Patel, 2003; Di Luccia et al., 2009), similar to the migration patterns obtained from our samples. The peak heights steadily increased from 8 minutes and started decreasing after 20 minutes which is consistent with most of the samples analyzed (Figure 5a-10e). Some 4-5 peaks migrated well after 30 minutes and no peaks were seen after 50 minutes. Very little information is available regarding gluten proteins profile migrating after 30 minutes as most separation protocols were limited to 30 minutes (Piergiovanni, 2013; Bean and Lookhart, 2000). In order to avoid any proteins that might become undetected, we established a protocol in which gluten separation was carried for a period of 60 minutes.

Sequential extraction protocol was used to separate gliadin and glutenin fraction from flour 3C, without the addition of DATEM. Sequential method of extraction revealed that all the gliadin and glutenins were separated completely around 30 minutes. The gliadin protein extract of this sample showed a group of protein migrating earlier than five minutes, suggesting that this group has highly positive charge species with low molecular weight than the next group of migration peaks (Figure 11b). However, many of the gliadin and glutenin migration patterns overlap suggesting the presence of many proteins with same charge/mass ratio (Figure 11a and 11b).

. The electropherograms are presented in two formats to facilitate comparisons. The first format is individual electropherogram that reveal more detail and the comparison of control and treatments need to be done by individual graph comparison. The second format is a compiled and overlay of electropherograms that facilitates the comparisons of peak resolution and heights.

i**) Flour 1C**

The electropherogram of gluten extract from 1C (control, 7.95% protein content) and treatments exhibited a total of 19 and 18 peak, respectively (Figure 5a-d). The separations were arbitrarily named group A, B and C, where group A represents the major group which were represented by "trade mark" big peaks with higher intensity and wide base, group B represents peaks that separate from the slope of large peaks. Group C represents peaks that migrate after 30 minutes and indicates proteins that have less positive charge and/or higher mass compared to control.

Effect on protein solubility

In Flour 1C, the intensity of maximum peak increased at least five fold for 0.3 and 0.6% DATEM and four fold with 1.0% DATEM (Table 4 and Figure 5a-d) compared to the control (0% DATEM). An extra peak could be noted in 1C control migrating at around 45th minute (Figure 5a), which is absent in the DATEM treatments. The presence of a large peak migrating at around 45th minute indicates presence of large gluten aggregates leading to protein agglomeration. Such aggregates were not formed in the presence of DATEM suggesting the protein must have been insoluble and not extracted with 0.3, 0.6 and 1.0% DATEM.

Effect on protein resolution

Flour 1C treated with 0.3 and 0.6% DATEM, showed an improvement in resolution and separation by new protein (7a), migrating next to protein 7 at around 18 minutes (Figure 5b and 5c). The presence of protein 7a, indicates that DATEM at a concentration of 0.3 and 0.6% induces changes in protein conformation, by its ability to

increase the interactions of proteins by means of hydrophobic, hydrophilic interactions, leading to detection of a new peptide with decreased charge/mass ratio. The decreased intensity of peak 7a in sample treated with 1.0% DATEM (Figure 5d), suggest that DATEM at this concentration, causes conformational changes in proteins, resulting in formation of peptides that has lower charge and higher mass compared to control.

Flour 1C treated with 0.3 and 0.6% DATEM showed an increased resolution in peaks separation compared to control (Figure 5e). However, treatment 1.0% had a decrease in protein resolution. The ability of DATEM to decrease the solubility of gluten proteins, leads to a decrease in amount of soluble gluten proteins (lower absorbance compared to control) migrating in the column, leading to a highly resolved electropherogram. The decrease in amount of protein soluble corresponds to decreased amount of protein migrating in the column at a given time, hence leading to separation of peaks with increased resolution and sharpness.

Summary of the electropherograms of flour 1C control and treatments from Figure 5e suggests

a) An improvement in resolution of peaks with 0.3 and 0.6% DATEM treatment
b) A decrease of amount of extracted soluble proteins with 1.0% DATEM. The latter one is suggested by the reduction in absorbance (214 nm) which is directly proportional to soluble proteins.

ii) Flour 2C

Effect on protein solubility

The electropherogram of gluten extract from 2C (control, 10.4% protein content) and treatments (0.3, 0.6 and 1.0%) exhibited 18, 16, 18 and 17 peaks, respectively (Figure 6a-d). The intensity of maximum peak decreased at least 60% for sample with treatments (0.3, 0.6 and 1.0%) compared to control (Table 4 and Figure 6a-d). A decrease of peak intensity for peak 7 in 0.3% DATEM treated sample, suggest that DATEM influences the protein interactions leading to the formation of gluten polymers with decreased charge/mass ratio. This is visualized as peak "7a" (Figure 6b).

Effect on proteins migrating after 20 minutes

The electropherogram of group C for 0.3% DATEM indicates an increase in the formation of insoluble gluten polymers as the peaks look broader and lower in resolution compared to the peaks migrating before 20 minutes (Figure 6b). The migration profile around 50^{th} minute indicates the beginning of separation of a large peak, which could have been detected with increased time, suggesting that for this flour, DATEM at 0.3% enables gluten interactions to form insoluble polymers in an aggregated form, supported by the peaks migrating with an broad base and shallow heights (Figure 6b).

In sample treated with 0.6 and 1.0% DATEM, an increase in agglomeration of gluten polymers are evident with a migration of a large peak around the 50^{th} minute (Figure 6c and 6d). This confirms the ability of gluten to increase the interaction of gluten proteins leading to a formation of polymers which have a higher negative charge and lower charge/mass ratio compared to control.

Summary of the electropherograms of 2C control and treatments from Figure 6e reveals

a) A decrease of amount of extracted soluble proteins with 0.3, 0.6 and 1.0% DATEM revealed by the decrease of intensity of maximum peak height.

iii) Flour 3C

Effect on protein solubility

The electropherogram of gluten extract from 3C (control, 10.5% protein content) and treatments (0.3, 0.6 and 1.0%) exhibited 16, 19, 19 and 17 peaks respectively (Figure 7a-d). Comparing sample 3C (control) and treatments, the maximum peak intensity decreased for sample treated with 1.0% DATEM (Table 4 and Figure 7a-d).

In flour 3C treated with 0.3 and 0.6% DATEM, the peaks are better resolved compared to control (Figure 7a-c). In 0.3% DATEM treatment, three new peaks are observed in treatments, namely “17”, “18” (migrating as a doublet) (Figure 7a and 7b). This suggests that 3C treated with 0.3 and 0.6% DATEM induced protein interactions leading to formation of peptides with increased negative charge and lower charge/mass ratio compared to control. The peak migrating at 45 minutes compared to control (Figure 7a and 7b), indicates that the DATEM at this concentration induces the formation of protein agglomeration.

Effect on protein resolution

In flour 3C treated with 1.0% DATEM, an electropherogram with a decreased resolution was displayed (Figure 7e). This suggests that at this DATEM concentration

majority of the gluten are present in polymeric or insoluble form leading to a large decrease in population of gluten proteins that are soluble. These observations are supported by a decrease in maximum peak intensity (Table 4) and a poor resolution of peaks separated compared to treatments (0.3 and 0.6% DATEM) (Figure 7e).

Effect of protein migration after 20 minutes

The presence of a large peak migrating around 50^{th} minute suggest the formation of protein agglomeration. Gluten protein formation is accompanied by constant stretching and breaking of bonds between proteins and DATEM plays a key role in stabilizing and facilitating the formation of high molecular weight gluten polymers. The strong agglomeration is a key factor in determining gluten development.

Summary of the electropherograms of 3C control and treatments from Figure 7e reveals.

a) An improvement in resolution of peaks with 0.3 and 0.6% DATEM treatment.

b) A decrease of amount of extracted soluble proteins with 1.0% DATEM.

iv) Flour 4C

Effect on protein solubility and charge/mass ratio

The electropherogram of gluten extract from 4C (control, 11.1% protein content) and treatments (0.3, 0.6%) exhibited a total of 21 peaks compared to 18 peaks for sample treated with 1.0% DATEM (Figure 8a-d). In flour 4C (control) and treatments, the intensity of maximum peak increased at least six fold and three fold for sample treated with 0.3 and 0.6% DATEM respectively (Table 4 and Figure 8a-d).

Flour 4C and treatments (0.3, 0.6 and 1.0% DATEM), exhibited a new peak "5a" (Figure 8a-c). However, the peak intensity for peak "5a" was decreased in 1.0% DATEM (Figure 8d). The decrease in intensity of peak "5a" and the entire protein profile in 1.0% DATEM treated sample, suggest that at this concentration DATEM's ability to form insoluble gluten protein complexes is increased significantly, leading to the decrease in the population of soluble gluten proteins. The decrease in peak intensity also suggest that DATEM at this concentration enables the formation of high gluten polymer formation, which leads to proteins with lower charge/mass ratio (proteins with more negative charge and increased mass), which leads to the formation of protein agglomerates , as seen with a large peak migrating around 50^{th} minute (Figure 8d)

Effect on protein resolution

Sample treated with 0.3 and 0.6% DATEM also showed an increased resolution in peaks separation compared to control (Figure 8e). However, treated with 1.0% DATEM had a decrease in protein resolution (Figure 8e). The ability of DATEM to increase the formation of insoluble gluten polymers, leads to decrease in the amount of soluble gluten proteins migrating in the column, leading to a highly resolved electropherogram for samples treated with 0.3 and 0.6 % DATEM. However DATEM at a concentration of 1.0 % increase the ability of formation of insoluble gluten polymers, leading to large decrease in soluble gluten polymers that can be detected, leading to a poorly resolved electropherogram.

Effect of protein migration after 20 minutes

The presence of a large peak migrating around 50th minute for sample containing 1.0% DATEM (Figure 8d), confirms our suggestion that DATEM at 1.0% increases the formation of insoluble gluten polymers, that has a tendency to aggregate and form protein with higher mass and increased negative charge compared to proteins migrating before 20 minutes. The slow moving proteins could also be aggregates of highly negatively charged proteins of low molecular weight. They move slower because the higher density of negative charges would make them repulse from the cathode.

Summary of the electropherograms of 4C control and treatments from Figure 8e reveals

a) An improvement in resolution of peak with 0.3 and 0.6% DATEM treatment
b) A decrease of amount of extracted soluble proteins with 0.6 and 1.0% DATEM.

v) Flour 5C

Effect on protein solubility

The electropherogram of gluten extract from 5C (control, 11.3% protein content) and treatments exhibited a total of 17 peaks (Figure 9a-d). In sample 5C (control) and treatments, the intensity of maximum peak was similar between control and treatments (Table 4 and Figure 9a-d).

Effect on protein resolution

In 0.3% DATEM treatment, the peaks were well separated and were distinguished with an increase resolution compared to control (Figure 9a). However, a new peak (17a) migrates at around 40^{th} and 50^{th} minute, suggesting the process of agglomeration taking place (Figure 9a-b). The absence of these peaks in control samples confirms the ability of DATEM to induce protein changes leading to increased protein polymer formation which can be attributed to an increase peak seen in electropherogram of 0.3% DATEM treatment (Figure 9a). This suggests that DATEM induces changes in protein interaction leading to formation of high molecular weight gluten polymers with an increase mass and lower negative charge.

An electropherogram with similar resolution and was obtained for sample treated with 1.0% DATEM. However, compared to 0.3% DATEM, a clear separation of agglomerated peaks (17a) (Figure 9b) was not seen. This suggest that DATEM at 1.0% decreases the population of gluten proteins that are soluble, which can further be confirmed with a peak starting to separate around 50^{th} minute, leading to decrease in intensity of peak 17a in 1.0% DATEM treatment (Figure 9d).

In 0.6% DATEM treatment, the peaks were not well resolved compared to control and 0.3% treatment (Figure 9e). The maximum peak intensity shows a trend to decrease, however, the loss of resolution and decreased intensity suggest that DATEM at this concentration induces change in protein conformation. The presence of large peak migrating at 50^{th} minute suggests the formation of protein agglomerates.

Summary of the electropherogram of 5C control and treatments from Figure 9e reveals

a) An improvement in resolution of peak with 0.3 and 1.0% DATEM treatment

b) A decrease of amount of extracted soluble proteins 0.6% DATEM.

vi) Flour 6C

Effect on protein solubility and charge/mass ratio

The electropherogram of gluten extract from 6C (control) and treatments (0.6, 1.0%) exhibited a total of 18 peaks compared to 17 peaks for sample treated with 0.3% DATEM (Figure 10a-d).

In flour 6C (control) and treatments, the maximum peak intensity were similar between control, and 0.3 and 1.0% DATEM. Flour 6C treated with 0.3% DATEM, introduced a new peak "8a" separating in group B (Figure 10b). The presence of peak 8a decreased the intensity of peak 8, suggesting the ability of DATEM to modify protein-protein interactions. Peak 18 migrating in control sample is absent in 0.3% treatment suggesting that DATEM's ability to form insoluble gluten polymers caused an increased charge/mass ratio, suggesting that the peptide might migrate later (Figure 10a and 10b).

Flour 6C treated with 0.6% DATEM, displayed two new peaks "3a" and "3b" compared to control. This suggest that DATEM at this concentration, induces changes in protein conformation and interactions, leading to formation of a peptide that has a lower charge/mass ratio compared to peak "3" (Figure 10a and 10c). Flour 6C treated with 1.0% DATEM, showed new peaks "6a", "9a" and "10a" (Figure 10d). The presence of new peaks suggests the increase in separation of proteins with a lower charge/mass ratio which could be attributed to the ability of DATEM to influence protein interactions.

Effect on protein resolution

Control and treatments 0.3 and 0.6% showed a better resolution compared to control (Figure 10a and 10e). The electropherogram of 1.0% DATEM displayed an improvement in resolution of peaks in group A and a reduction in resolution of peaks in group B compared to control. This suggests that 1.0% DATEM in sample 6C increases interactions between peptides thus modifying their charge to mass ratio. The increase in maximum peak intensity for samples treated with 1.0% DATEM suggests that DATEM at this level has reached a critical level or critical micellar concentration (CMC). At this level, the affinity of DATEM towards formation of micelle is increased, thus leading to decreased interaction with proteins.

Summary of the electropherograms of 6C control and treatments from Figure 10e reveals

a) An improvement in resolution of peak with 0.3 and 0.6% DATEM treatment.
b) An increase in soluble protein with 0.3 and 1.0% and a decrease of amount of extracted soluble proteins with 0.6% DATEM.

3.4 Discussion

(i) Flour 1C, 3C and 4C

In our study with flour 1C and 4C (Figure 15), showed a semi-quantitative increase in protein solubility at 0.3% of DATEM, which is evident from the increase in maximum peak intensity (Figure 15). However, the limitation of this study is the number of samples and representation of protein concentration. This study also observed that

flour 1C and 4C with protein concentration of 7.9 and 11.2% had a similar semi-quantitative solubility characteristic when treated with 0.6 and 1.0% DATEM. Flour 1C and 4C, treated with 0.6 and 1.0% DATEM (Figure 15and Table 4), showed increased protein solubility compared to control and a decrease in protein solubility compared to 0.3% DATEM treatment suggesting the formation of insoluble gluten polymers. Flour 3C when treated with 0.3 and 0.6% DATEM did not change the gluten solubility compared to control. However, at a concentration of 1.0% DATEM, the protein solubility decreased (Figure 15). DATEM's ability to induce changes in protein conformation leading to formation of soluble gluten polymers appears to be independent of protein concentration.

A four stage model is proposed to explain the mechanism of action by which DATEM induces changes in solubility of proteins and induces the complex formation leading to insoluble gluten polymers. Stage 1 (Figure 12), represent gluten proteins in its native state. Stage 2 (Figure 12) represents DATEM at 0.3% indicates a low level of interaction with amino acids side chains. DATEM's interaction with amino acid residues of protein side chains can involve hydrophobic, hydrophilic interactions that are necessary for hydrated gluten leading to an increased solubility (Khatkar, 2004). Stage 3 (Figure 12) indicates that increase in DATEM concentration (0.6%) leads to an increase in interactions with proteins, leading to formation of gluten polymers. However at this concentration the population of proteins that forms insoluble gluten polymers is relatively lower compared to DATEM at a concentration of 1.0%.

Stage 4 (Figure 12) indicates that DATEM at 1.0% increases the interaction with amino acids chains thereby enabling the formation of protein complex. This supports the findings that DATEM has been suggested to interact mainly with hydrophobic domain of

gluten proteins, leads to the formation of protein polymers (which imparts gluten strength) and increased protein aggregation (Orthoefer, 1997; Shiau, 2004; Gomez et al., 2012). It is also suggested that DATEM at a concentration lower than 0.5% leads to formation of hydrophobic interaction of DATEM with amino acids of gluten proteins, however the interaction was weak due to low aggregation, compared to increased aggregation caused when DATEM was present at concentration of 1.0% (Gomez et al., 2012).

DATEM also plays a facilitating role in the formation of disulfide bonds between protein chains which would otherwise have cysteines away from each other, i.e., physically separated (Figure 12, Stage 1 and 2). DATEM's ability to bind to hydrophobic amino acid side chains, along with constant mixing during hydration of flour leads to opening up of the proteins hydrophobic domains. Most likely changes in the secondary structure lead to alignment of sulfhydryl groups in close proximity whereby they can form disulfide bonds, which is represented in our model (Figure 12, Stage 3 and 4), whereby at low DATEM concentrations the -SH groups are aligned away from one another (Figure 12, Stage 1 and 2). The presence of two cysteine residues in y-type HMW-GS capable for forming interchain disulfide bonds with LMW-glutenins (Wieser, 2007) (Table 2) enables the gluten to form large chain polymers with HMW-GS, that are rendered insoluble.

(ii) Flour 2C and 5C

The mechanism of action of DATEM upon wheat gluten proteins exhibited similar mode of action in flour 2C and 5C with a protein concentration of 10.4 and

11.4%. In flour 2C and 5C (Figure 15), DATEM at concentration of 0.3% reduced the amount of gluten that is soluble. The decrease in solubility of gluten proteins suggest the formation of insoluble high molecular weight polymers which cannot be extracted. The electropherogram of samples treated with DATEM also showed an increased resolution compared to control (sharper peaks). This suggest that the decrease in amount of soluble proteins leads to an decrease in population of samples migrating in the column, thus enabling the samples to be separated with a greater resolution. The decrease in solubility of gluten proteins at 0.3% DATEM, confirms our suggestion that the amount of DATEM required to induce changes in protein conformation is dependent mainly on protein quality. Our data confirms the findings by Ambardekar (2009) using the same flour set and DATEM treatments than this study who reported that flours with similar protein concentration have different rheological properties, suggesting protein quality plays an important role compared to quantity.

In order to explain the mechanism of action in relation to flour 2C and 5C (Figure 15), we propose a two stage model. According to this model, unlike for flour 1C, 3C and 4C (Figure 15) the proteins are tightly arranged and are compact in nature (Figure 13, Stage 1). At this molecular arrangement, 0.3% of DATEM is sufficient to induce changes in conformation of proteins, by its ability to induce interactions between amino acid side chains and align sulfhydryl groups in close proximity (Figure 13, Stage 2). At 0.6% concentration, it is also assumed that DATEM has reached its saturation level to induce any effect upon protein conformation, and further increase in DATEM concentration would not yield any significant changes in protein interactions.

(iii) Flour 6C

In Flour 6C, the mechanism of action of DATEM upon wheat gluten proteins exhibited some similar mode of action compared to 1C, 3C and 4C (Figure 15) up to a concentration of 0.6%. An increase in solubility of proteins was observed when DATEM was present at a concentration of 0.3 and 0.6%. However, an increase in DATEM concentration to 1.0%, suggested a reversal in the solubility of proteins. Proteins that remained insoluble were deemed soluble at a DATEM concentration of 1.0%. A decrease in peak resolution for flour treated with 1.0% also suggests that more soluble proteins are extracted at 1.0% DATEM concentration. Similar behavior was observed in other flours (5C) where the non-linear response of protein solubility could be attributed to a property exclusive to surfactants called as critical micellar concentration. It is defined as the concentration of surfactants, beyond which all the additional surfactants added to the system is devoted to forming micelles (Mukerjee et al., 1971). At a concentration greater than 0.6%, for this particular flour, DATEM has reached critical level, beyond which any addition of DATEM yields or forms micelle with themselves, leading to an increased solubility of gluten proteins. The process of micelle formation is explained in (Figure 14).

DATEM and protein solubility based on maximum peak absorbance

From the electropherograms, the maximum peak absorbance was used for a semi-quantitative evaluation of the solubility of gluten proteins when treated with DATEM (Figure 15). The effect of DATEM on solubility was overall not linear except for sample 5C which suggest no change in the maximum peak. The response can be generalized as polynomial (with the exception of 5C) and confirms the practical knowledge of the

baking industry personnel that practice an overall expectation of improvement of specific parameters of quality such as improved volume and crumb texture but acknowledges treatment levels tailored for each particular flour. Taking sample 6C as an example, reflects a particular response more classical polynomial then the rest of the samples. This suggests a more complex interactions of gluten-DATEM compared to the rest of the samples. The effect of DATEM on samples 1C, 4C and 6C is a trend to increase the protein solubility followed by a decrease in solubility. It is expected that when increased gluten solubility is observed, insoluble protein has increased since they are negatively correlated. Higher insolubility of gluten proteins is generally assumed the result of the formation of large polymers that are key to larger aggregates and entanglements with elastic and extensible properties compared to lower insoluble gluten. The effect of DATEM on sample 2C is a decrease in protein solubility (Figure 15) and no effect on sample 5C. Sample 3C shows a decrease in soluble protein with 1% DATEM and no change with 0.3 and 0.6% DATEM.

3.5 Conclusion

Capillary electrophoresis analysis suggests that DATEM's ability to induce changes in protein conformation results in a change in protein solubility. The ability of DATEM to modify protein interactions were observed with both decrease and increase in maximum peak intensity and an increase in resolution of proteins detected. The concentration of DATEM influencing change in protein solubility varied in the set of six commercial flours with varying protein concentration as determined from maximum peak intensity. The mechanism of action and the concentration of DATEM varied with flours with similar protein concentration (Flour 4C and 5C, 2C and 3C). DATEM's ability to

induce changes in protein solubility was evident from improved and decrease resolution of peaks separated. Overall DATEM's ability to induce polymer formation leading to a change in protein solubility is flour dependent. The experiments were performed in relatively small set of samples to conclude that is only due to quality of flour. More studies are needed to elucidate the role of DATEM treatments and the effect on protein profiles and solubility.

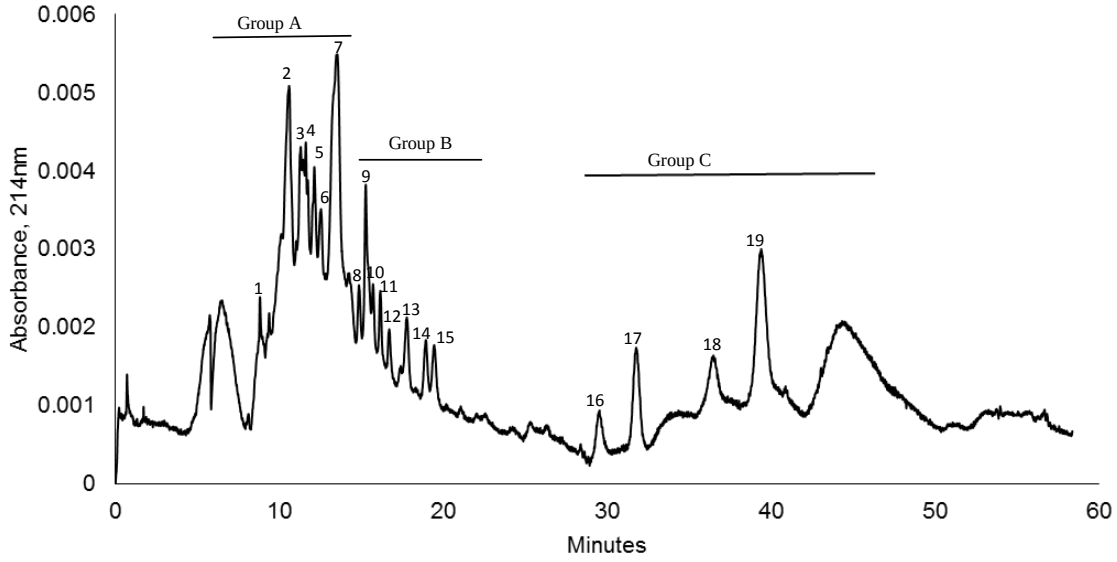


Figure 5a. FZCE patterns of gluten extract obtained from control flour 1C.

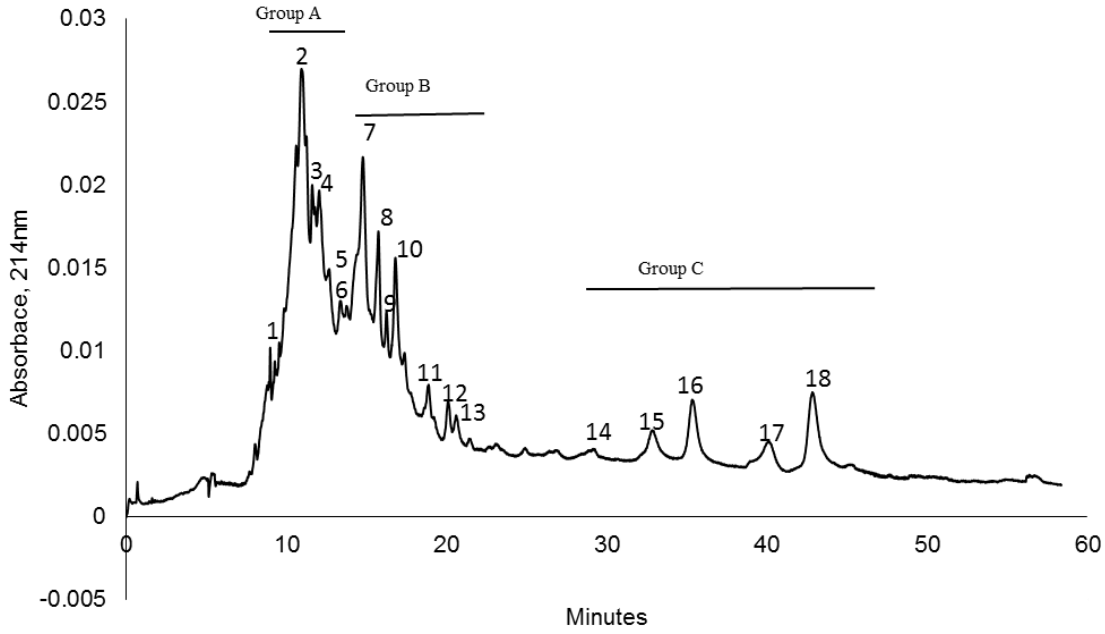


Figure 5b. FZCE patterns of gluten extract obtained from flour 1C treated with 0.3% DATEM.

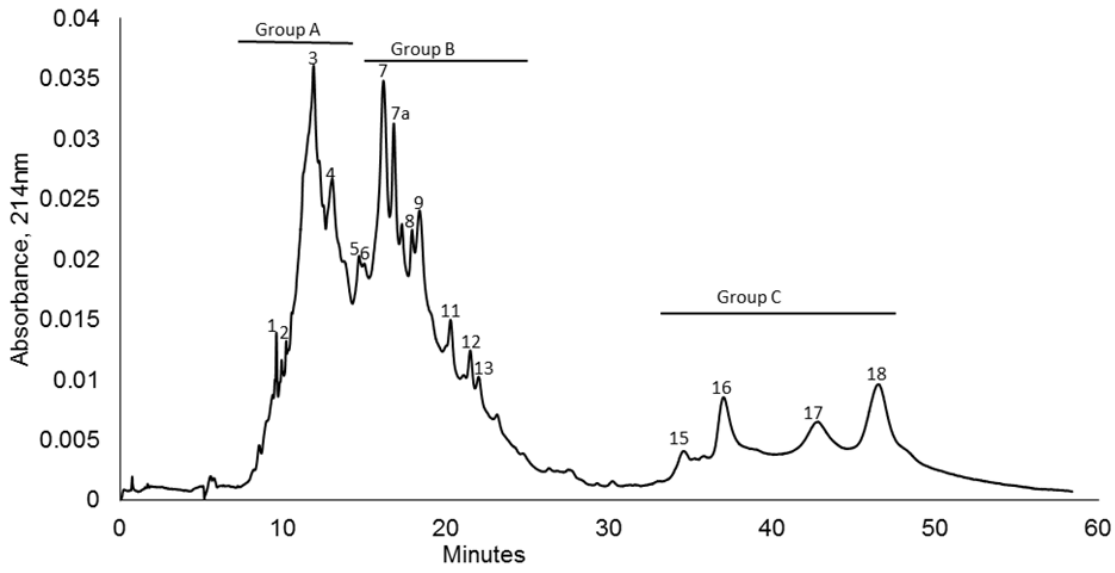


Figure 5c. FZCE patterns of gluten extract obtained from flour 1C treated with 0.6% DATEM.

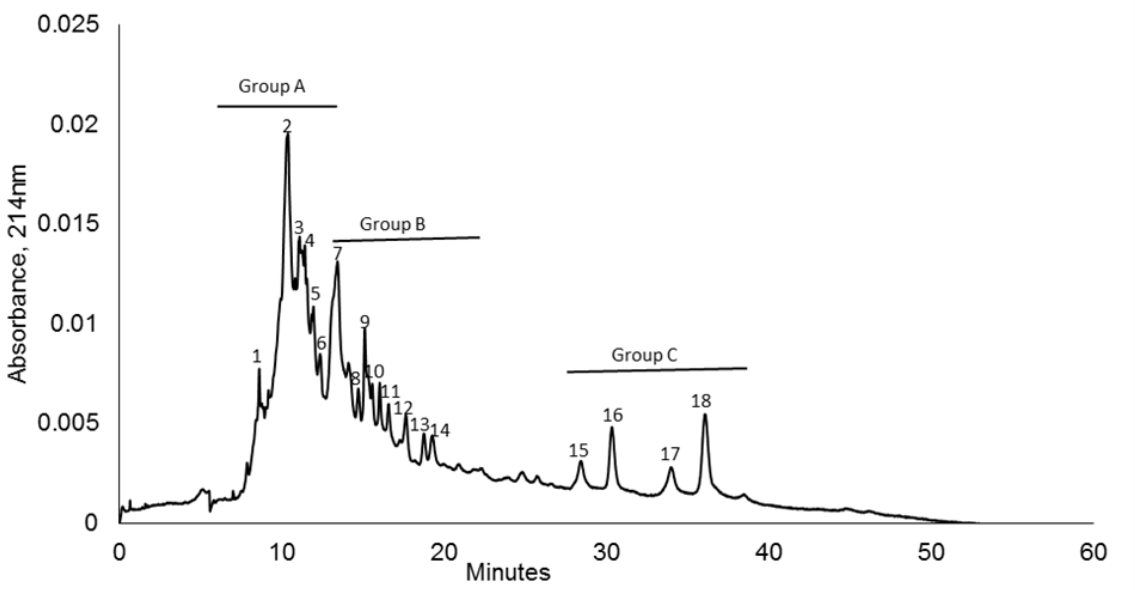


Figure 5d. FZCE patterns of gluten extract obtained from flour 1C treated with 1.0% DATEM.

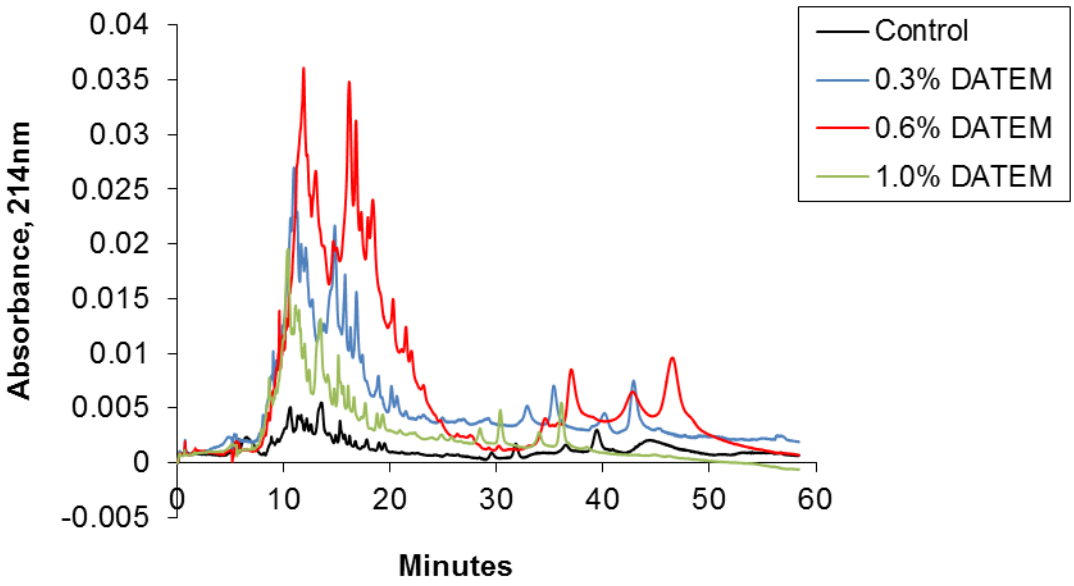


Figure 5e. Compiled and overlay graph of untreated Flour 1C (control) and Flour 1C treated with 0.3, 0.6 and 1.0% DATEM.

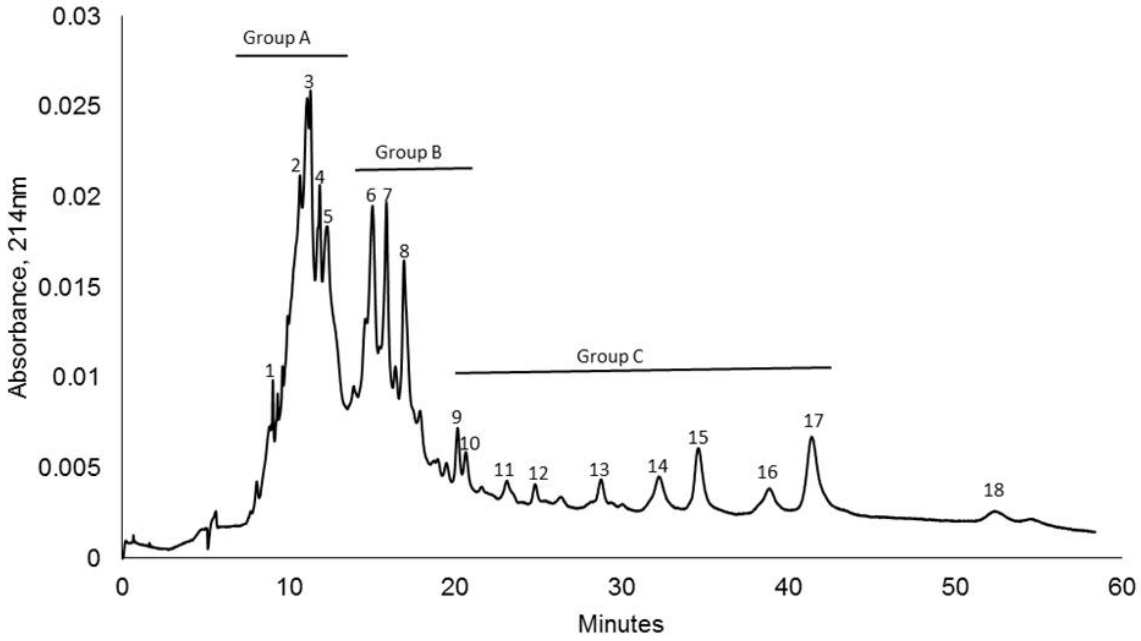


Figure 6a. FZCE patterns of gluten extract obtained from control flour 2C.

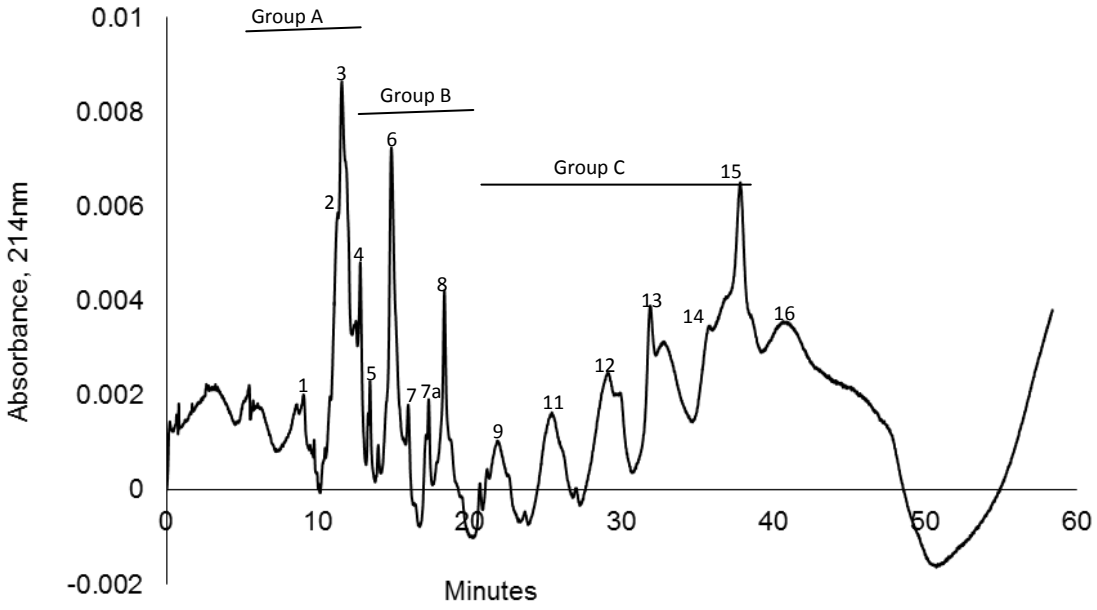


Figure 6b. FZCE patterns of gluten extract obtained from flour 2C treated with 0.3% DATEM.

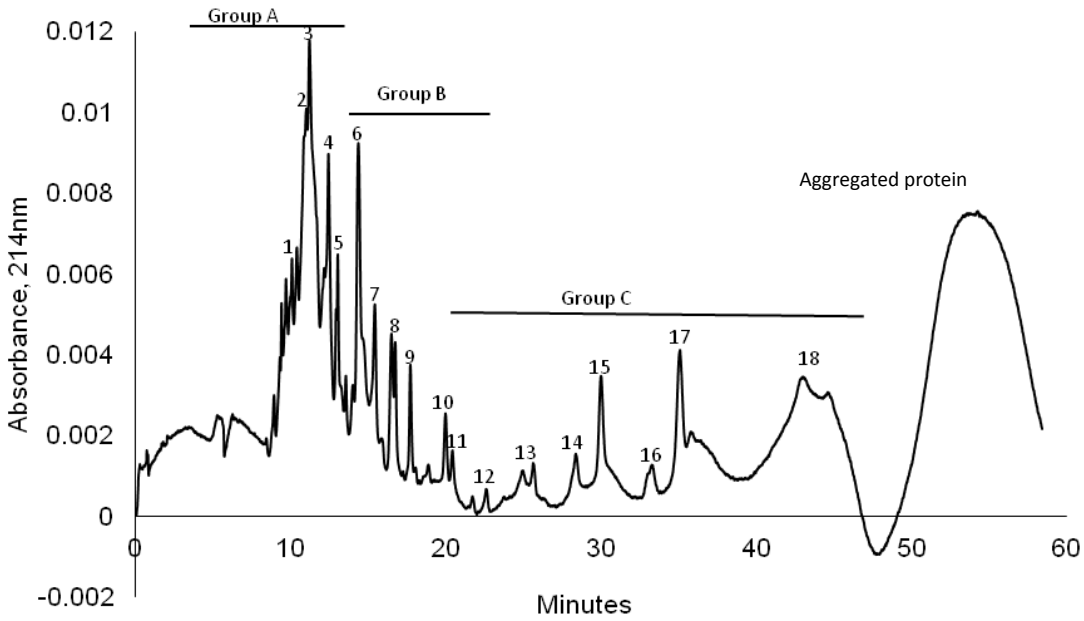


Figure 6c. FZCE patterns of gluten extract obtained from flour 2C treated with 0.6% DATEM.

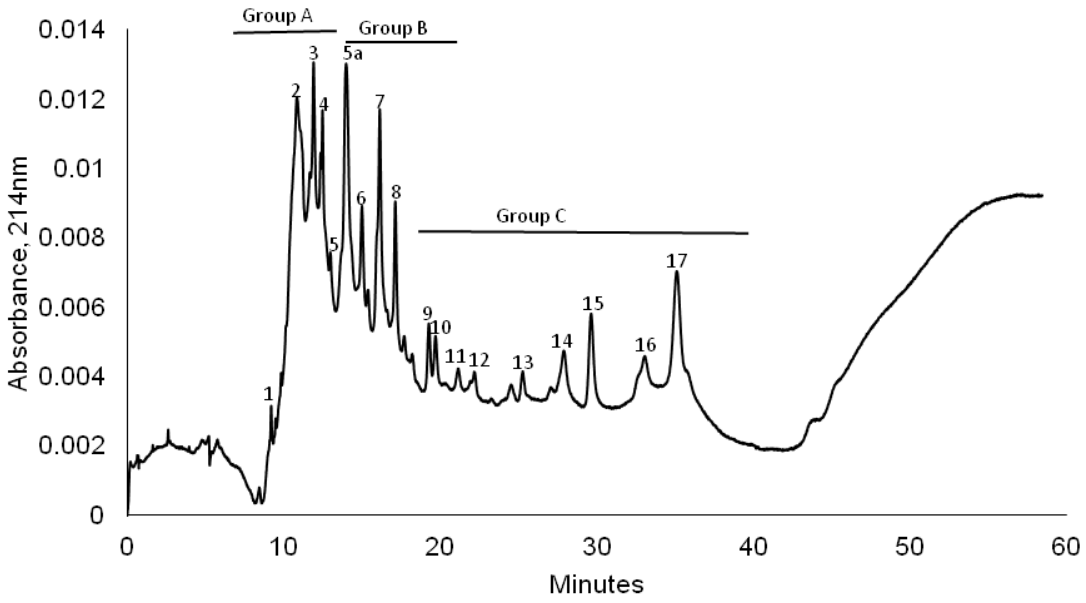


Figure 6d. FZCE patterns of gluten extract obtained from flour 2C treated with 1.0% DATEM.

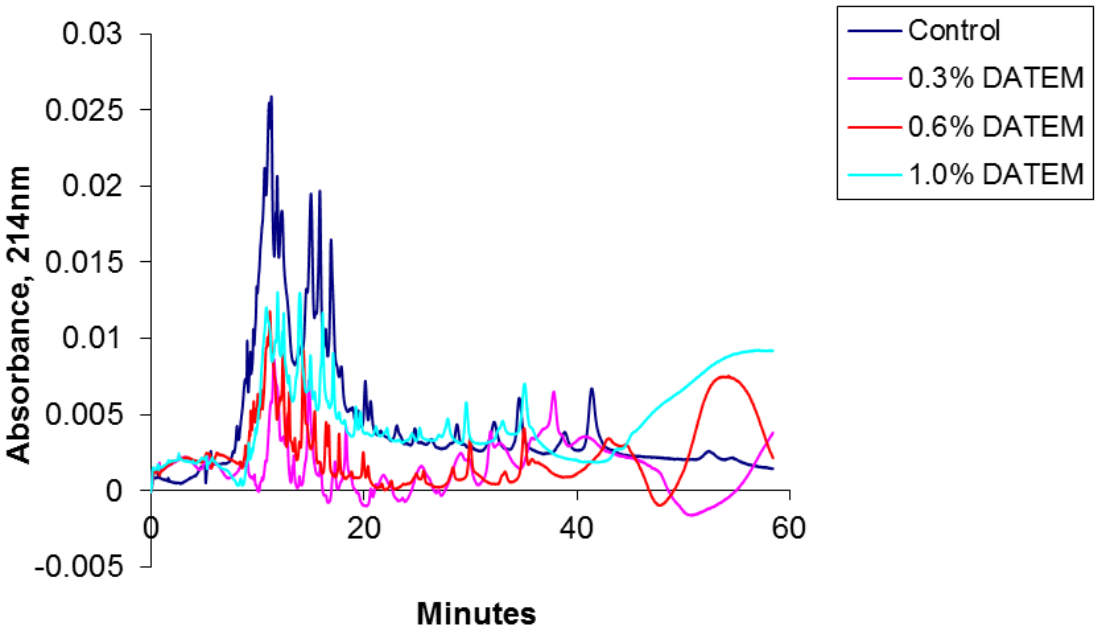


Figure 6e. Compiled and overlay graph of untreated Flour 2C (control) and Flour 2C, treated with 0.3, 0.6 and 1.0% DATEM.

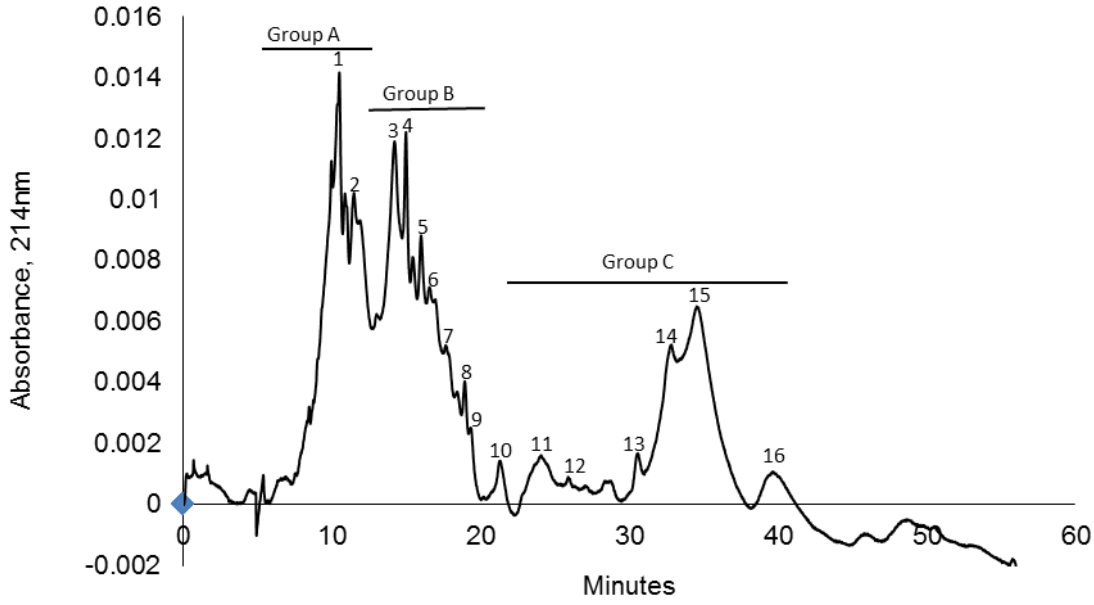


Figure 7a. FZCE patterns of gluten extract obtained from control flour 3C.

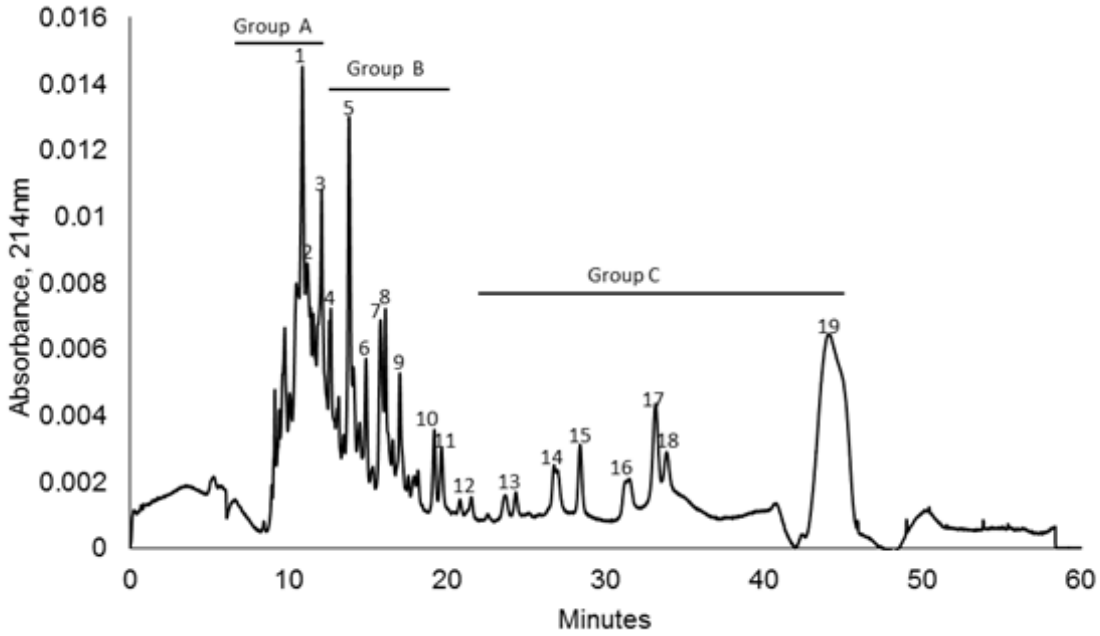


Figure 7b. FZCE patterns of gluten extract obtained from flour 3C 0.3, treated with 0.3% DATEM.

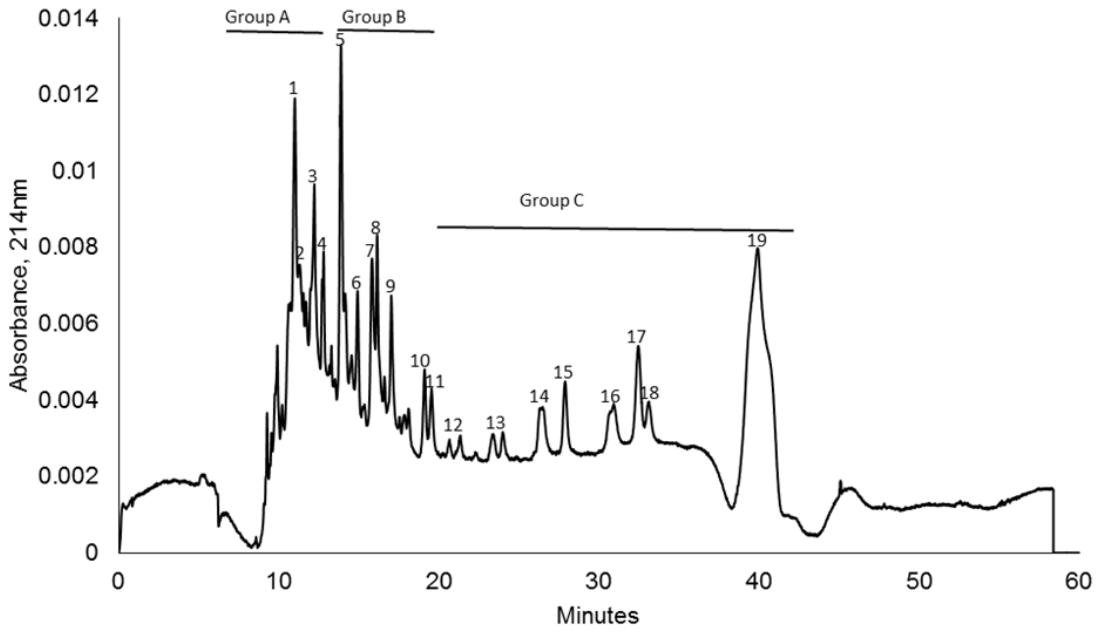


Figure 7c. FZCE patterns of gluten extract obtained from flour 3C, treated with 0.6% DATEM.

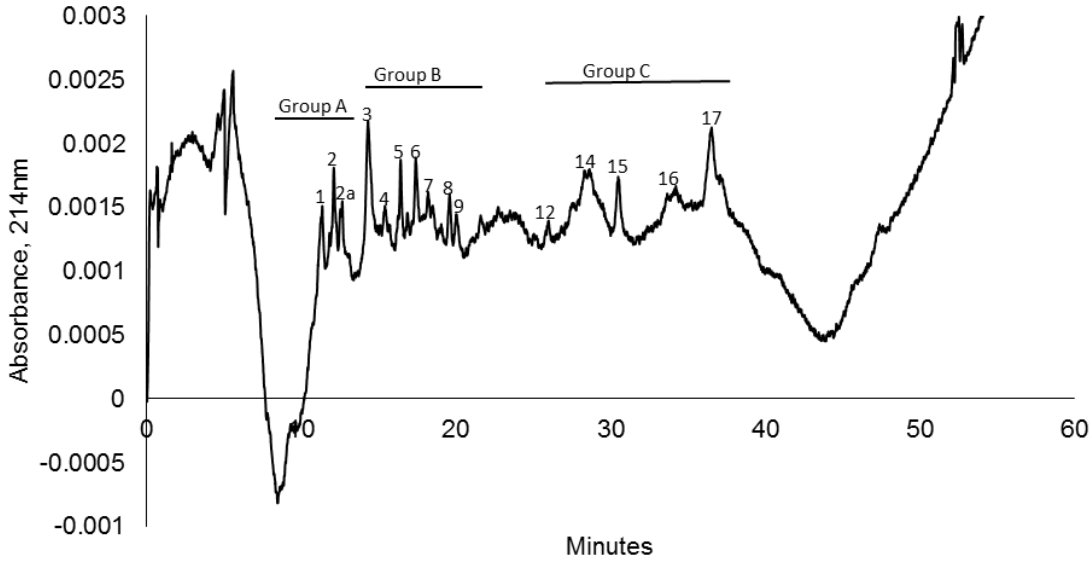


Figure 7d. FZCE patterns of gluten extract obtained from flour 3C, treated with 1.0% DATEM.

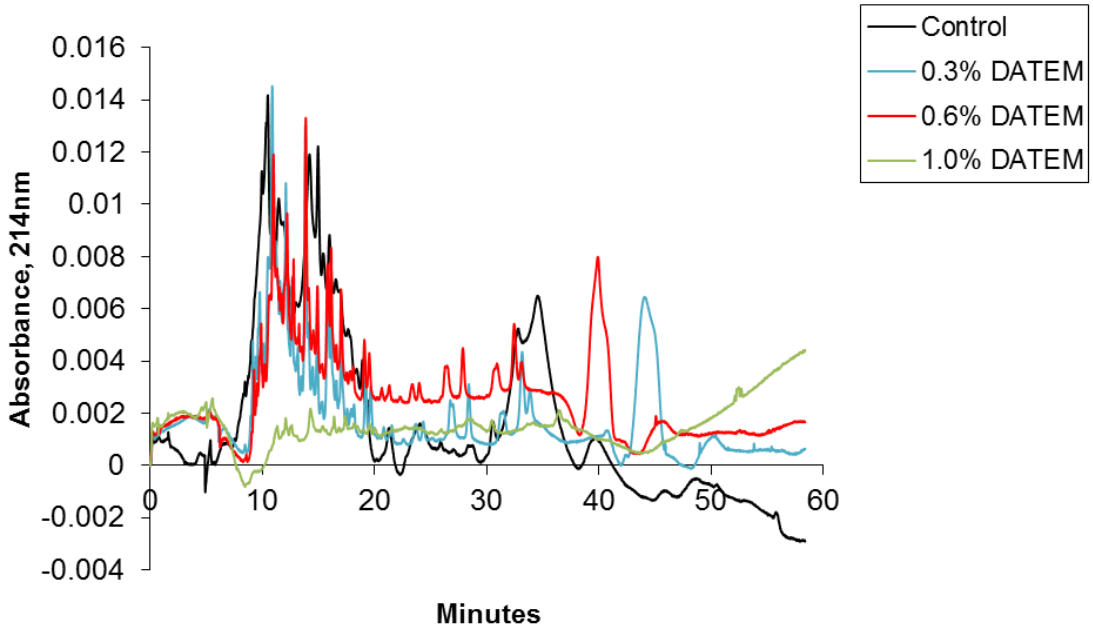


Figure 7e. Compiled and overlay graph of untreated flour 3C (control) and flour 3C, treated with 0.3, 0.6 and 1.0% DATEM.

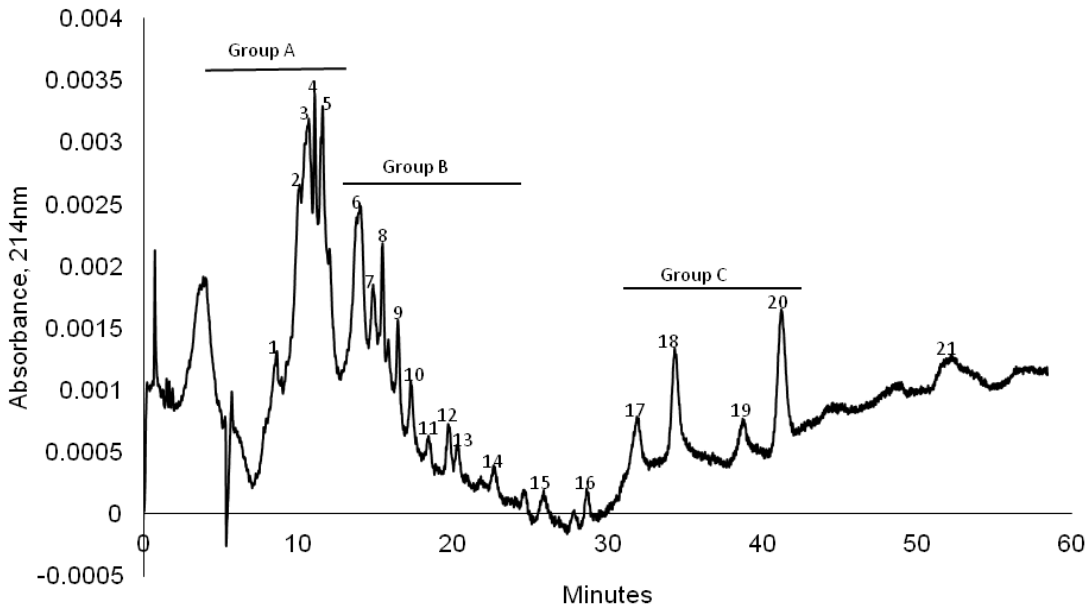


Figure 8a. FZCE patterns of gluten extract obtained from control flour 4C.

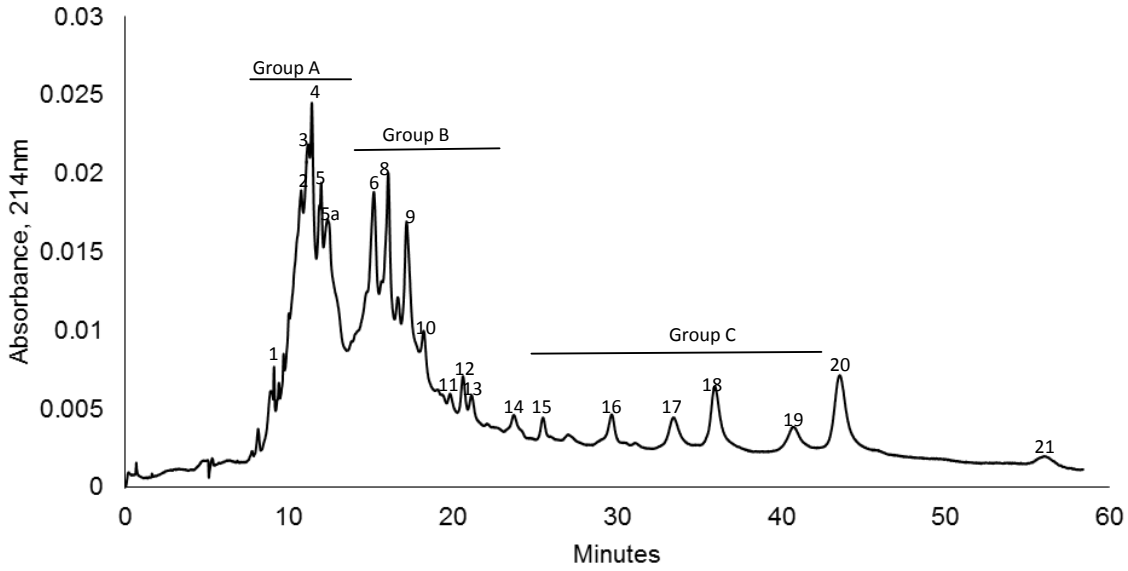


Figure 8b. FZCE patterns of gluten extract obtained from flour 4C treated with 0.3% DATEM.

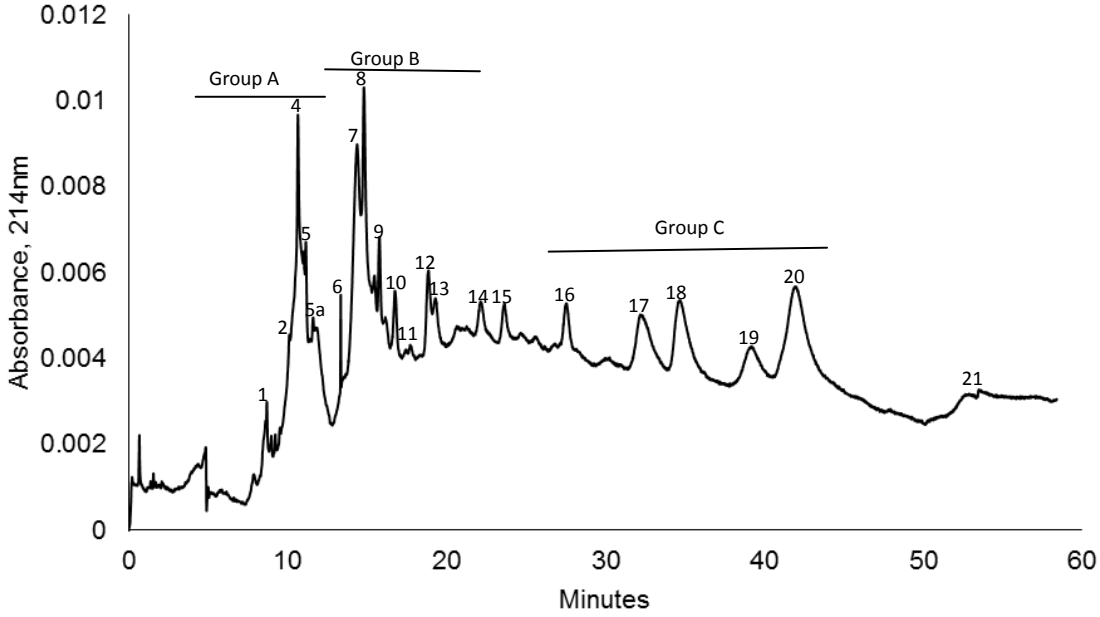


Figure 8c. FZCE patterns of gluten extract obtained from flour 4C treated with 0.6% DATEM.

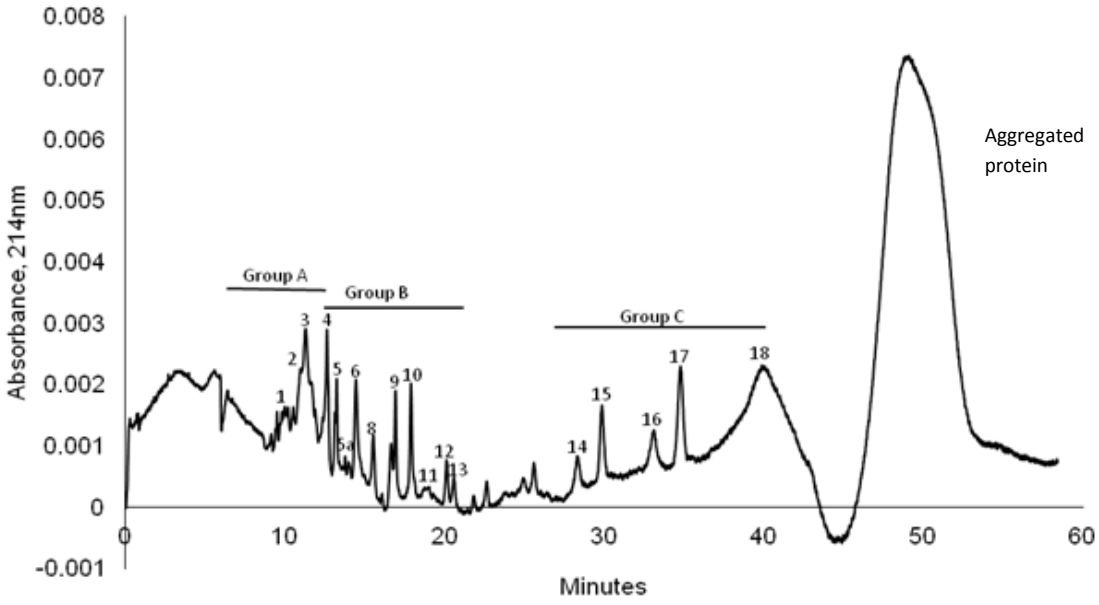


Figure 8d. FZCE patterns of gluten extract obtained from flour 4C treated with 1.0% DATEM.

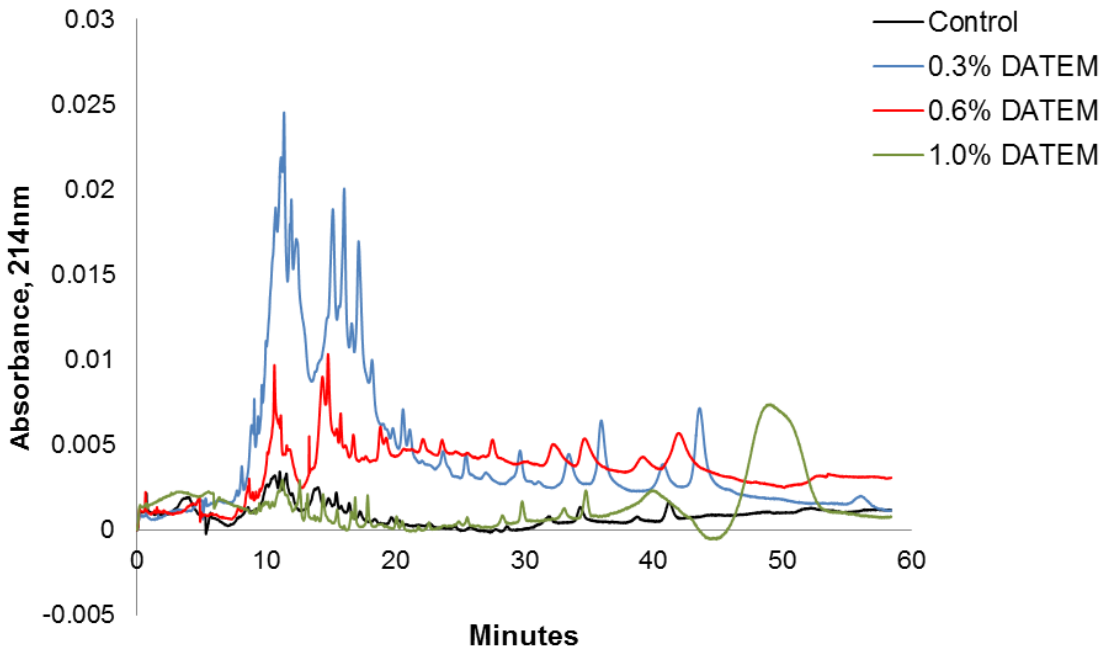


Figure 8e. Compiled and overlay graph of untreated flour 4C (control) and flour 4C, treated with 0.3, 0.6 and 1.0% DATEM.

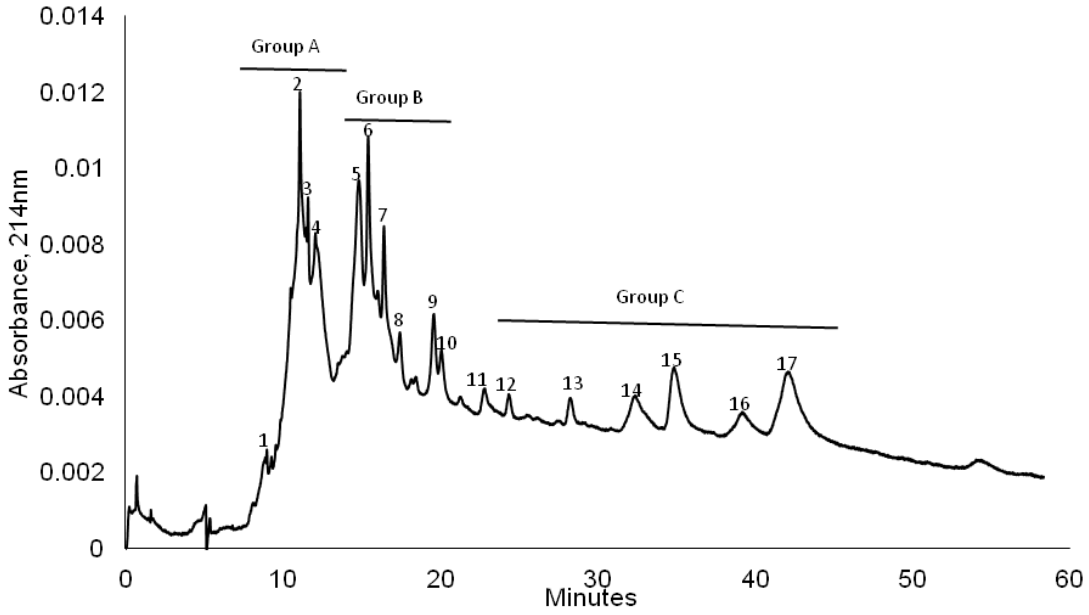


Figure 9a. FZCE patterns of gluten extract obtained from control flour 5C.

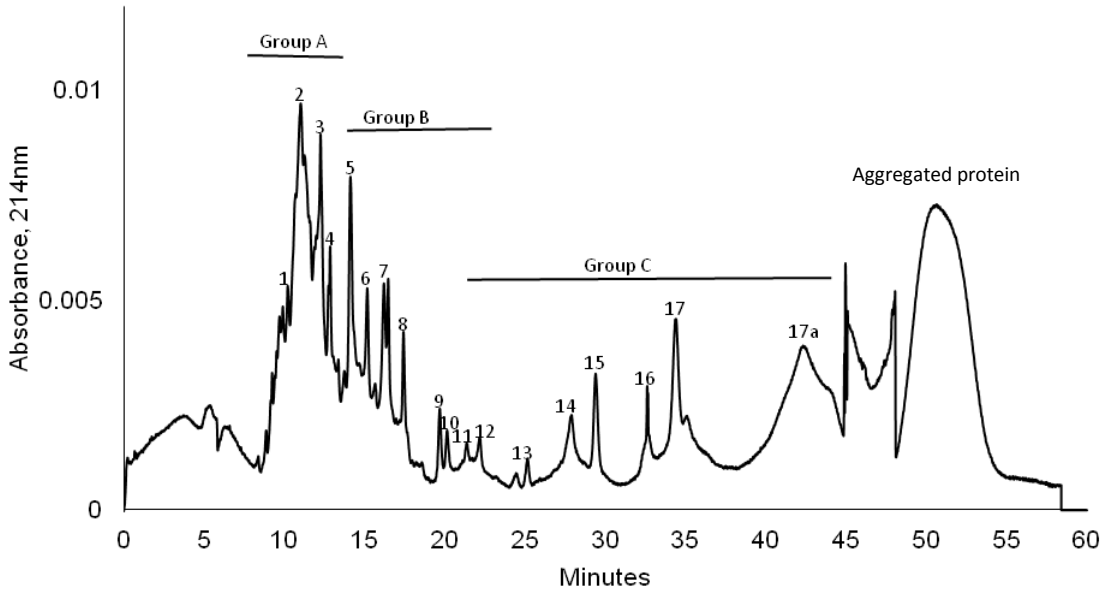


Figure 9b. FZCE patterns of gluten extract obtained from flour 5C, treated with 0.3% DATEM.

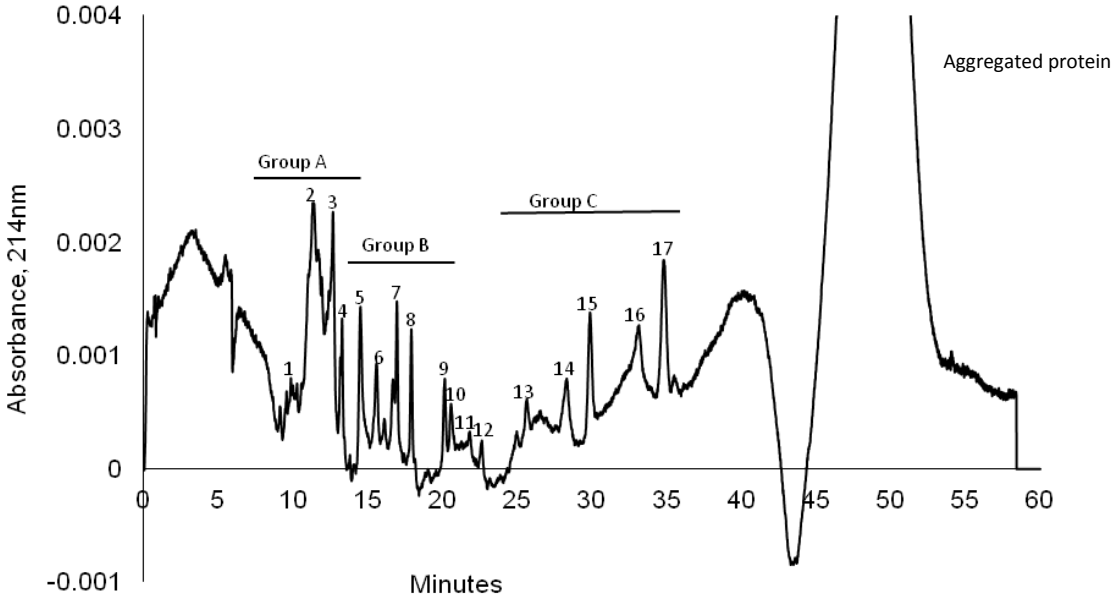


Figure 9c. FZCE patterns of gluten extract obtained from flour 5C treated with 0.6% DATEM.

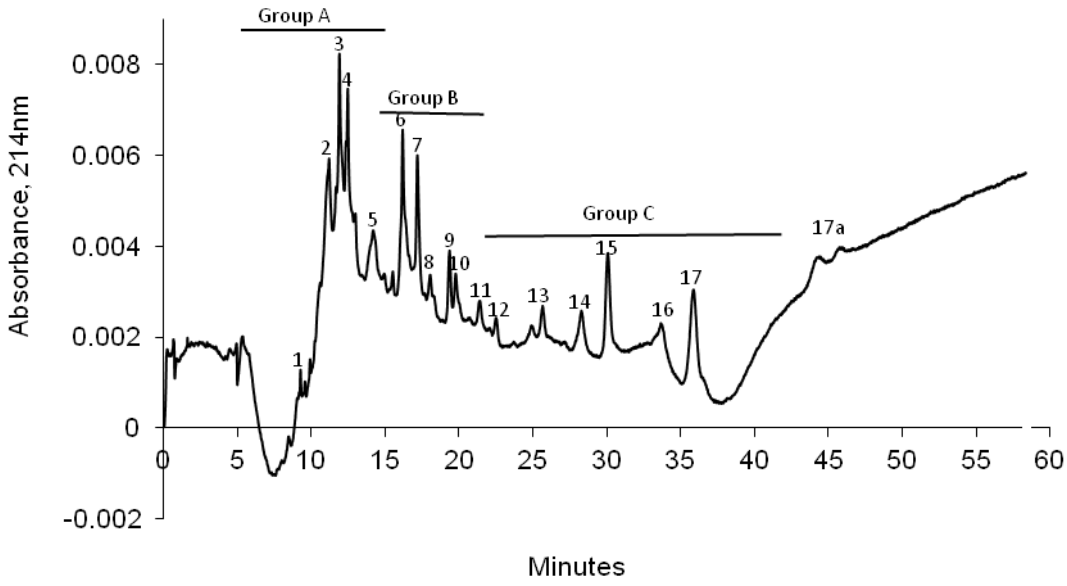


Figure 9d. FZCE patterns of gluten extract obtained from flour 5C treated with 1.0% DATEM.

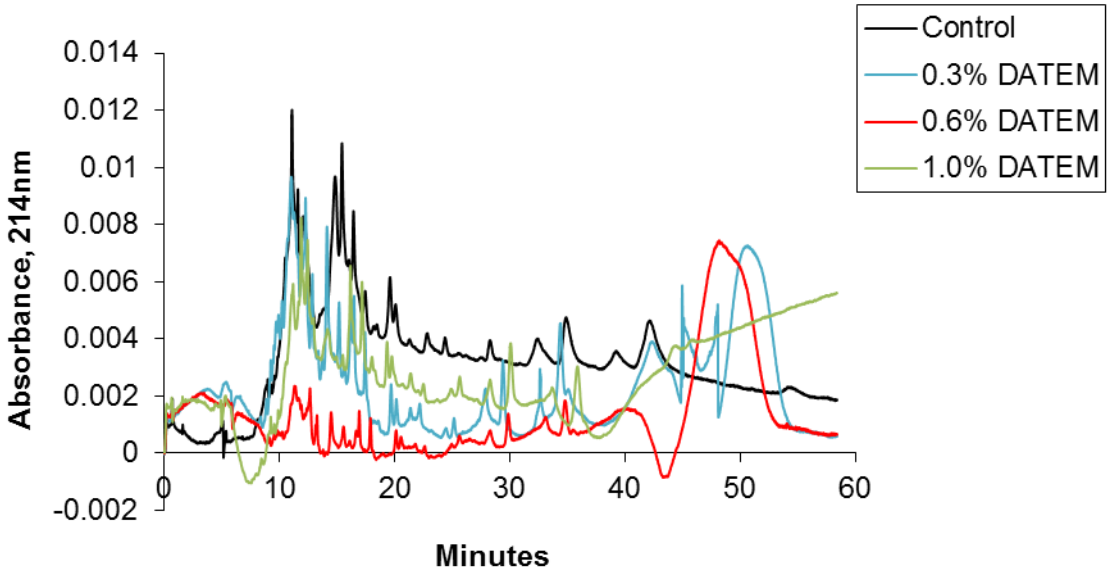


Figure 9e. Compiled and overlay graph of untreated Flour 5C (control) and flour 5C, treated with 0.3, 0.6 and 1.0% DATEM.

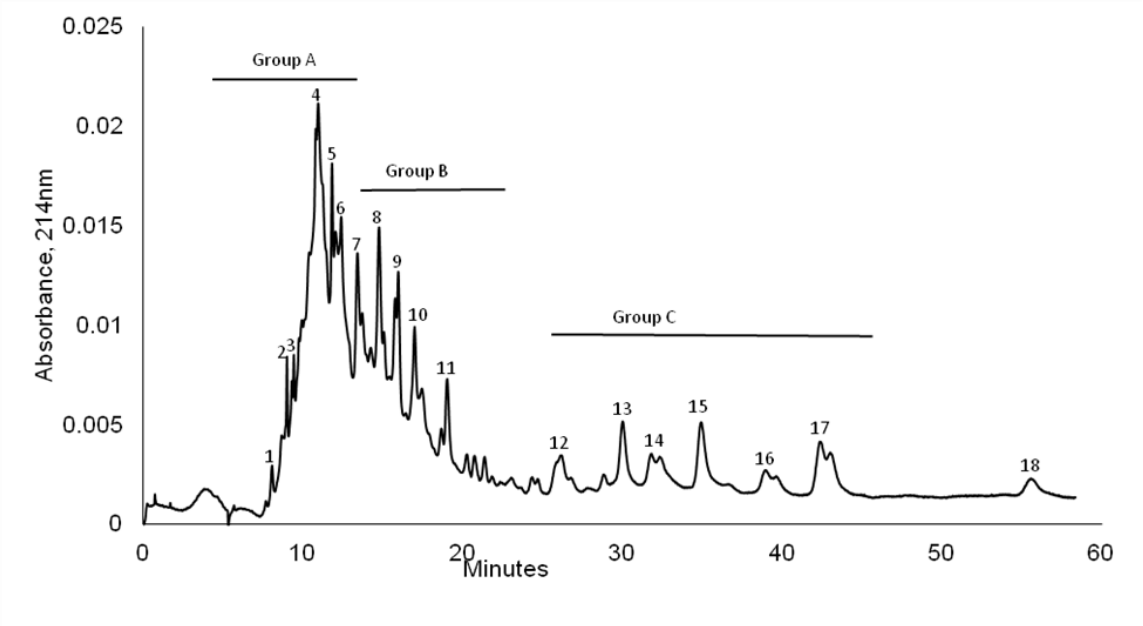


Figure 10a. FZCE patterns of gluten extract obtained from control flour 6C.

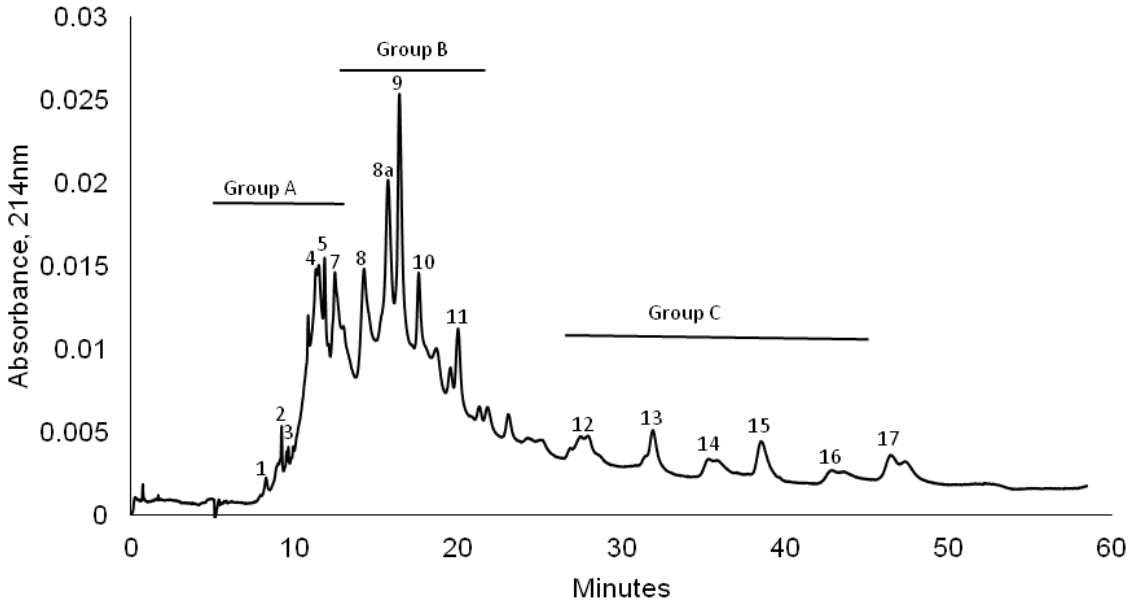


Figure 10b. FZCE patterns of gluten extract obtained from flour 6C treated with 0.3% DATEM.

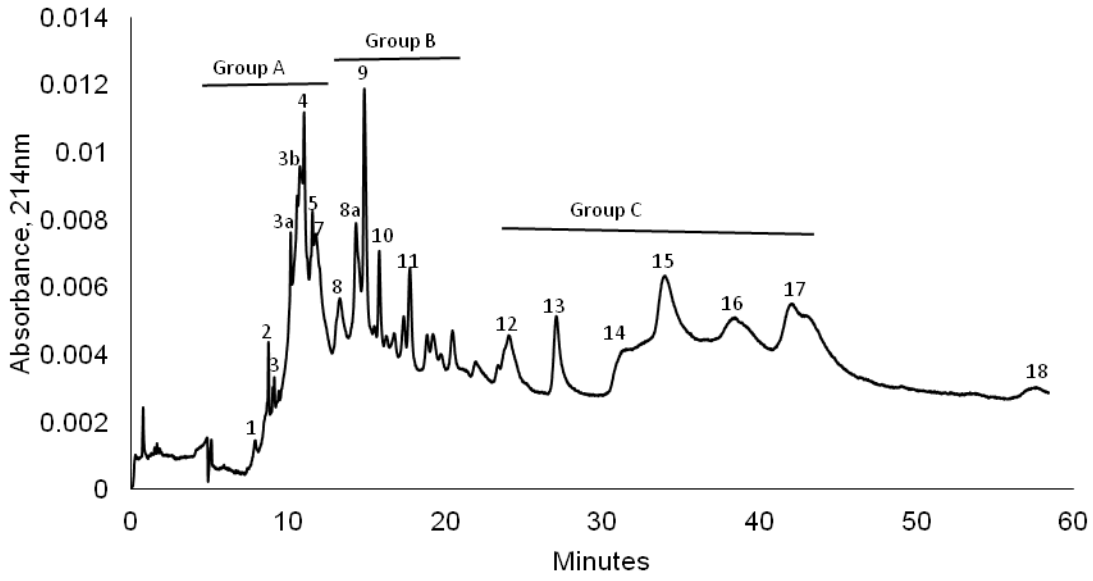


Figure 10c. FZCE patterns of gluten extract obtained from flour 6C treated with 0.6% DATEM.

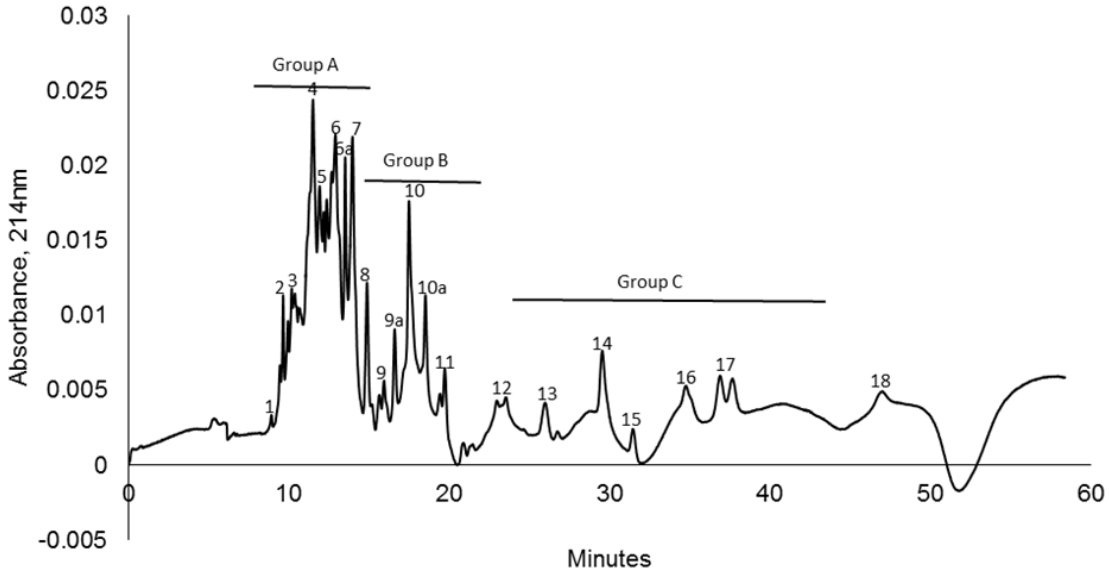


Figure 10d. FZCE patterns of gluten extract obtained from flour 6C treated with 1.0% DATEM.

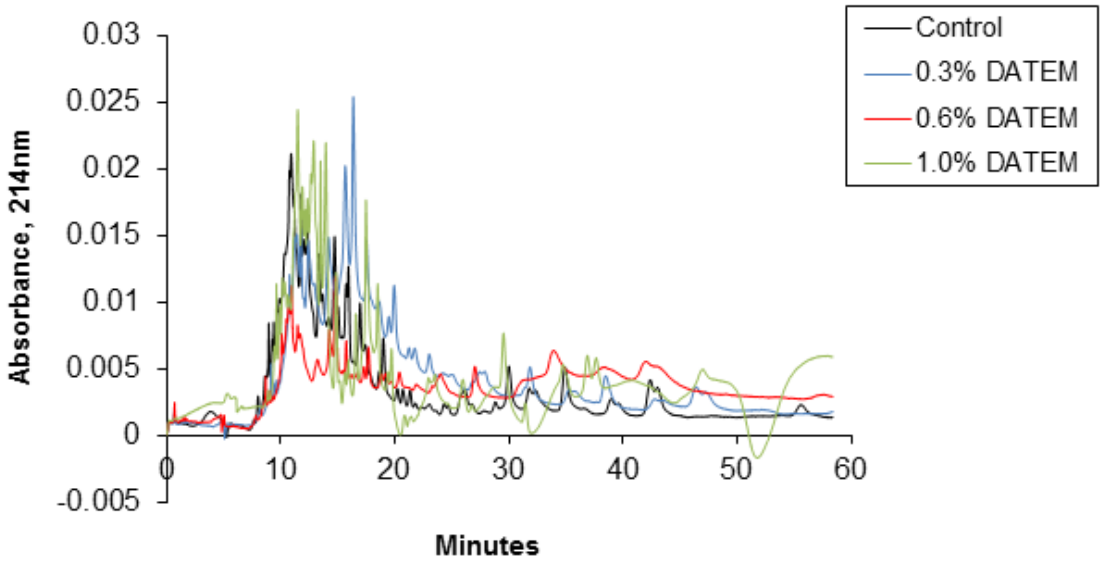


Figure 10e. Compiled and overlay graph of untreated flour 6C (control) and flour 6C, treated with 0.3, 0.6 and 1.0% DATEM.

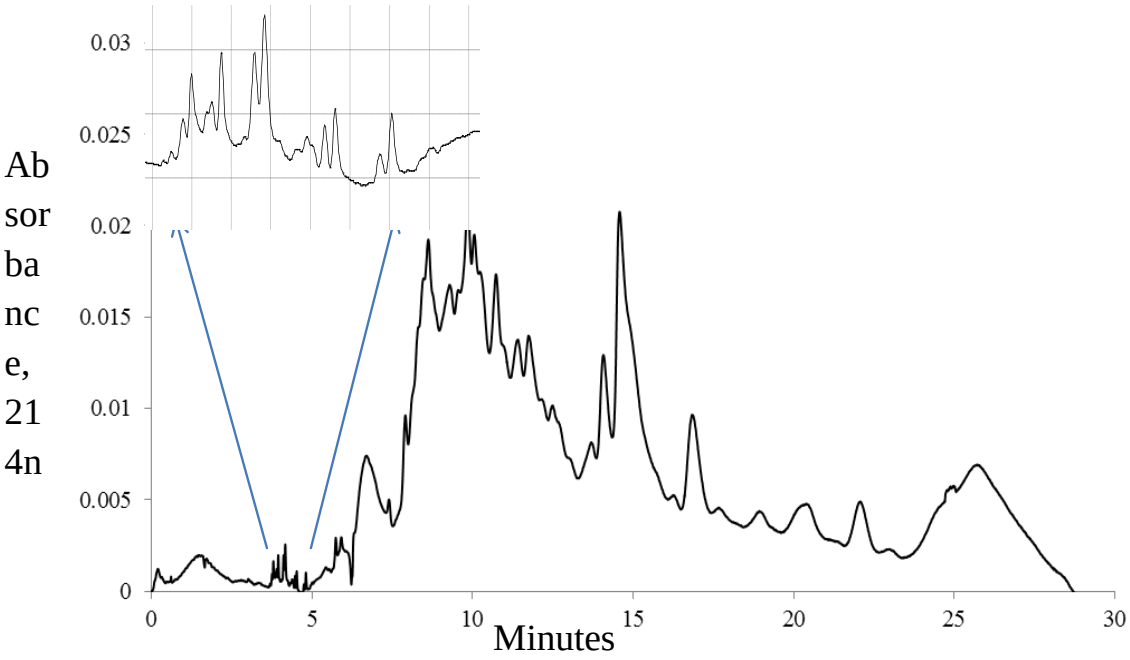


Figure 11a. FZCE patterns of gliadin extract obtained from control flour 3C.

a) ω-gliadins are assumed to migrate earlier than 5 minutes.

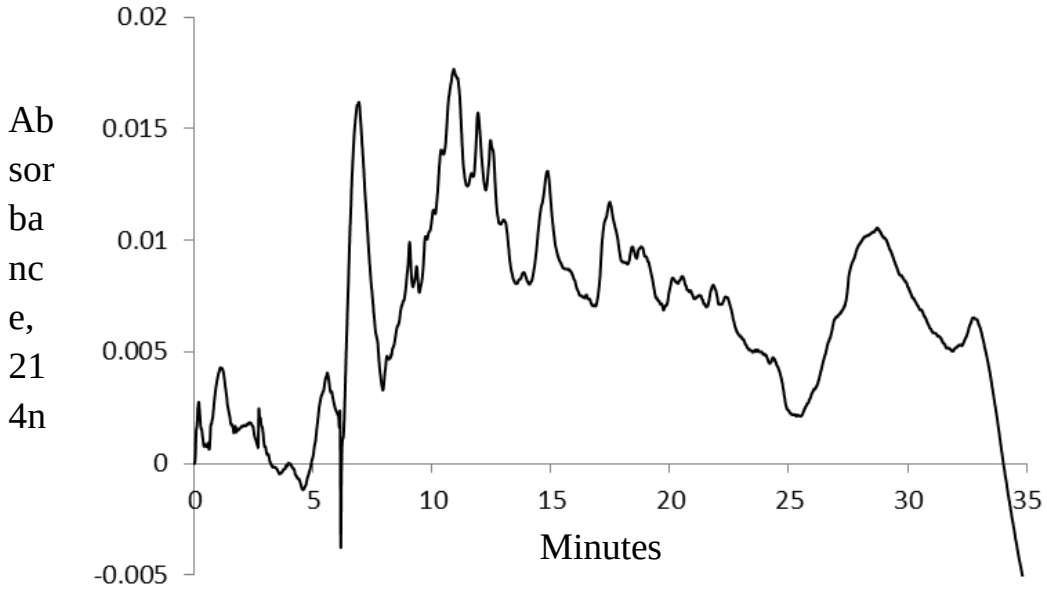
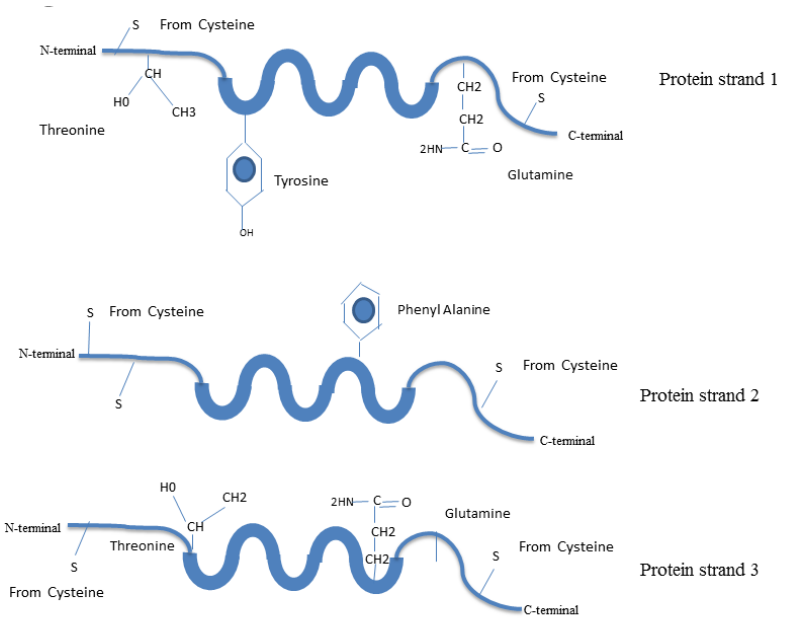
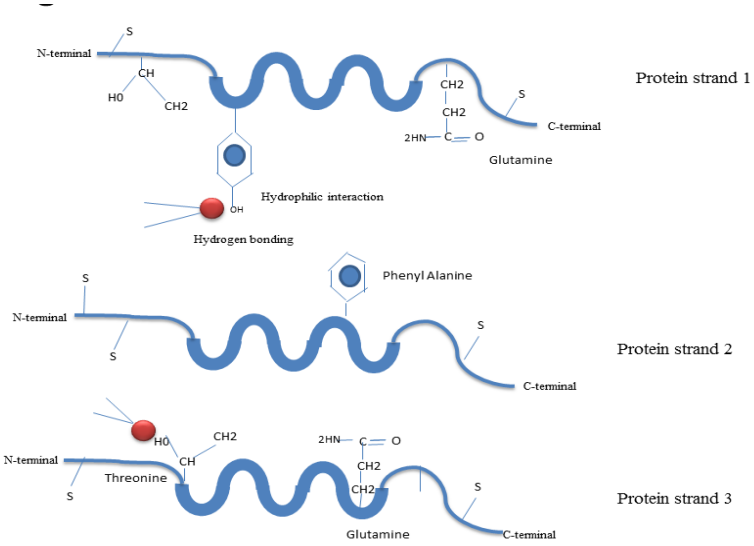


Figure 11b. FZCE patterns of gluten extract obtained from control flour 3C.

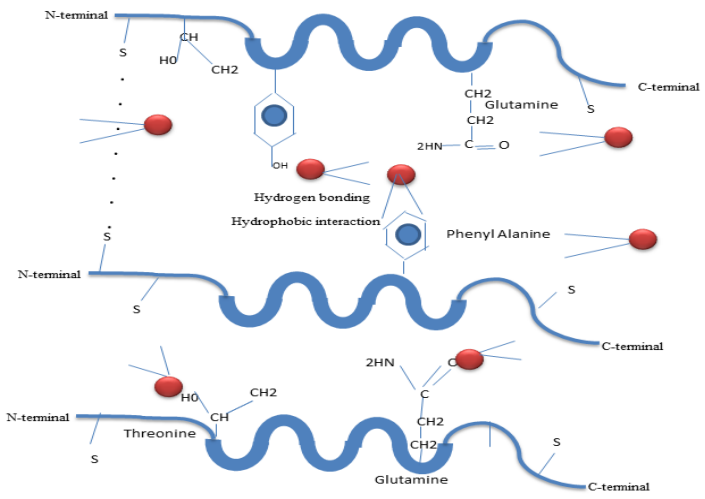
Stage 1. Soluble proteins chains at their native conformations in 50% n-propanol + 1% DTT



Stage 2. Interactions between gluten proteins and DATEM (0.3%) 50% n-propanol + 1% DTT



Stage 3. Interactions between gluten proteins and DATEM (0.6%) 50% n-propanol + 1% DTT



Stage 4. Interactions between gluten proteins and DATEM (1.0%) in 50% n-propanol+1% DTT

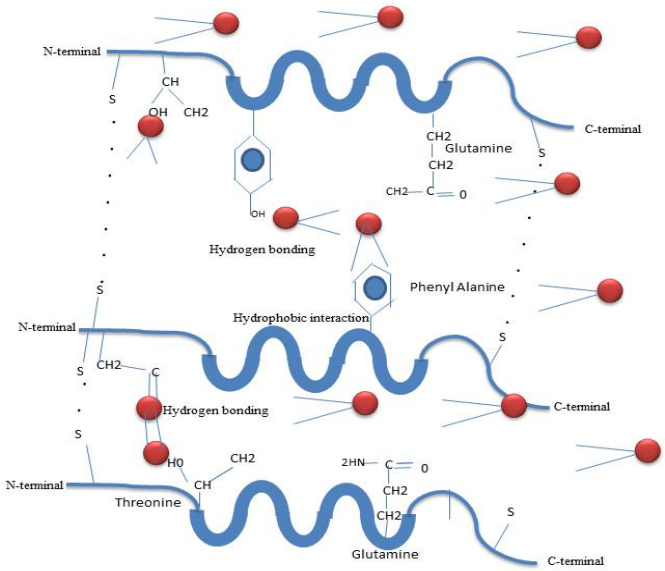
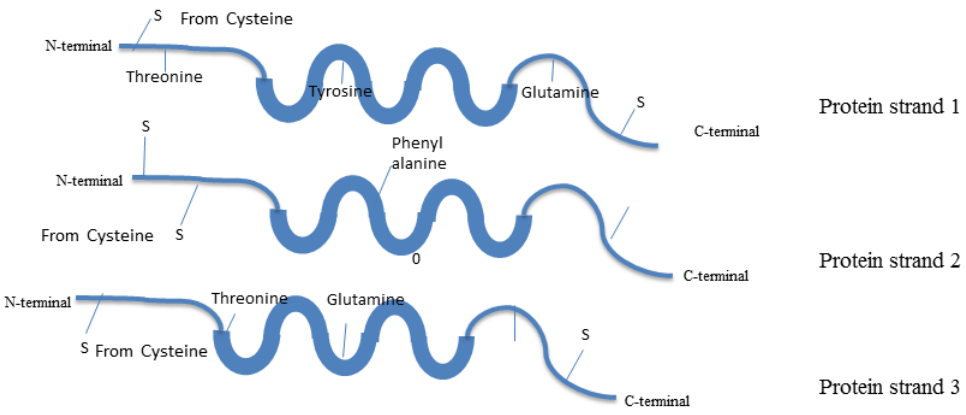


Figure 12. Proposed four stage model for 1C, 3C and 4C flour treated with DATEM

Stage 1. Proteins chains at their native conformations in 50% n-propanol + 1% DTT



Stage 2. Interactions between gluten proteins and DATEM (0.3%) in 50% n-propanol + 1% DTT

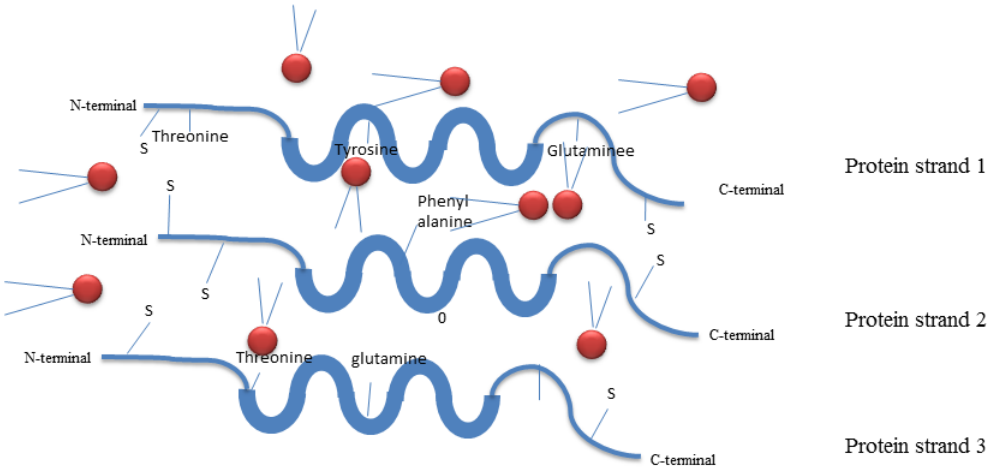


Figure 13. Proposed two stage model for 2C and 5C flour treated with DATEM

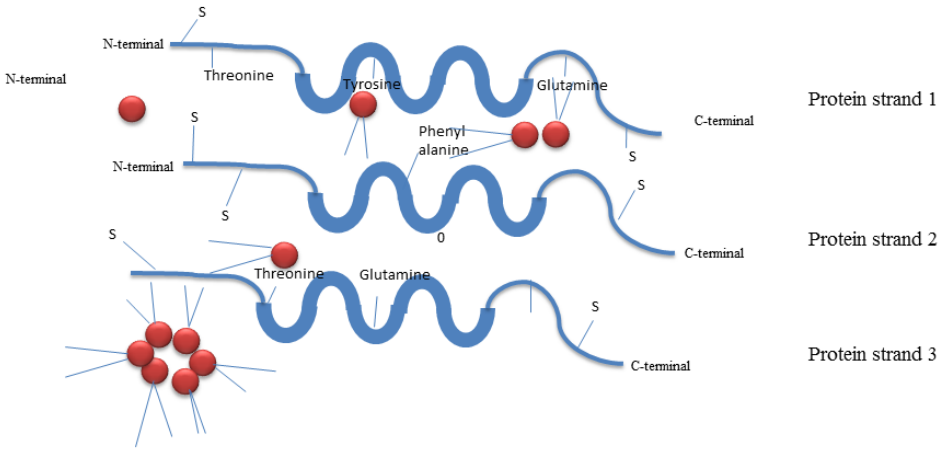


Figure 14. Interactions between gluten proteins and 1.0% DATEM in 50% n-propanol + 1% DTT

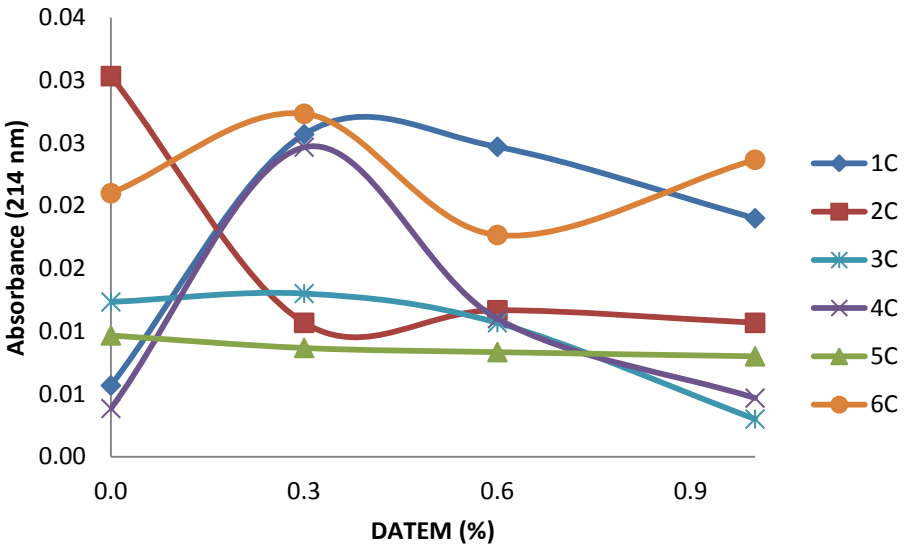


Figure 15. Effect on different levels of DATEM (0, 0.3, 0.6 and 1.0%) upon protein solubility (absorbance, 214 nm) based on maximum peak intensity from CE electropherograms

Table 3. Proximate analysis of flours (means ± 2, n=2). (Adapted from Ambardekar, 2009)

Flours	Protein (%)	Moisture (%)	Ash (%)
1C	7.95 ± 0.05	11.69 ± 0.02	0.29 ± 0.01
2C	10.40 ± 0.10	12.54 ± 0.02	0.47 ± 0.00
3C	10.59 ± 0.07	12.57 ± 0.00	0.48 ± 0.01
4C	11.19 ± 0.07	10.51 ± 0.03	0.38 ± 0.01
5C	11.38 ± 0.01	12.98 ± 0.04	0.58 ± 0.01
6C	13.68 ± 0.02	10.14 ± 0.02	0.41 ± 0.00

Table 4. Maximum peak intensity (absorbance, 214 nm) from electropherogram of gluten samples of commercial flours treated with 0, 0.3, 0.6 and 1.0% DATEM

Flour	DATEM (%)	Rep 1	Rep 2	Rep 3	Mean	S.D
1C	0	7.00E-03	5.00E-03	5.00E-03	5.67E-03	1.15E-03
	0.3	2.50E-02	2.60E-02	2.60E-02	2.57E-02	5.77E-04
	0.6	2.70E-02	2.40E-02	2.30E-02	2.47E-02	2.08E-03
	1.0	1.80E-02	2.00E-02	1.90E-02	1.90E-02	1.00E-03
2C	0	3.00E-02	3.00E-02	3.10E-02	3.03E-02	5.77E-04
	0.3	1.10E-02	1.10E-02	1.00E-02	1.07E-02	5.77E-04
	0.6	1.30E-02	1.20E-02	1.00E-02	1.17E-02	1.53E-03
	1.0	1.00E-02	1.20E-02	1.00E-02	1.07E-02	1.15E-03
3C	0	1.00E-02	1.30E-02	1.40E-02	1.23E-02	2.08E-03
	0.3	1.20E-02	1.40E-02	1.30E-02	1.30E-02	1.00E-03
	0.6	1.20E-02	1.00E-02	1.00E-02	1.07E-02	1.15E-03
	1.0	4.00E-03	3.00E-03	2.00E-03	3.00E-03	1.00E-03
4C	0	4.00E-03	3.50E-03	4.00E-03	3.83E-03	2.89E-04
	0.3	2.40E-02	2.50E-02	2.50E-02	2.47E-02	5.77E-04
	0.6	1.00E-02	1.20E-02	1.10E-02	1.10E-02	1.00E-03
	1.0	4.00E-03	5.00E-03	5.00E-03	4.67E-03	5.77E-04
5C	0	8.00E-03	1.00E-02	1.10E-02	9.67E-03	1.53E-03
	0.3	8.00E-03	8.00E-03	1.00E-02	8.67E-03	1.15E-03
	0.6	8.00E-03	9.00E-03	8.00E-03	8.33E-03	5.77E-04
	1.0	7.00E-03	9.00E-03	8.00E-03	8.00E-03	1.00E-03
6C	0	2.00E-02	2.00E-02	2.30E-02	2.10E-02	1.73E-03
	0.3	2.70E-02	2.80E-02	2.70E-02	2.73E-02	5.77E-04
	0.6	1.30E-02	2.00E-02	2.00E-02	1.77E-02	4.04E-03
	1.0	2.50E-02	2.50E-02	2.10E-02	2.37E-02	2.31E-03

CHAPTER IV

FUTURE STUDIES

To our knowledge, this study was the first in using capillary electrophoresis to examine the effect of DATEM upon wheat gluten interaction and charge/mass ratio. We hypothesize that DATEM's ability to promote protein interactions leading to change in solubility of gluten is dependent on flour with some factors such as protein quality and concentration. This study suggested that DATEM acts by influencing protein interactions promoting interactions among gluten proteins through hydrophobic and hydrophilic interactions.

In this study, DATEM's ability to interact with proteins was enabled during the mixing stages of the glutomatic. It would be of interest to increase the interaction time between DATEM and gluten to test if it affects the properties analyzed. I suggest resting the sample for 5 minutes after initial mixing using glutomatic and compare the protein separations with current data to analyze the effect of interaction time on changes in gluten proteins interaction. Alternatively, a study can be designed to find the optimum time of mixing and or resting that produced maximum changes produced by DATEM.

Sequential extraction protocol to separate gliadins, HMW-GS and LMW-GS could be used to separate proteins into individual fractions. Each fraction obtained could

be analyzed using 2D gel electrophoresis and mass-spectrometry. This would enable us to identify individual proteins based on their pI, and the sequence of individual proteins can then be obtained from mass spectrometry and identification to confirm the type of proteins. DATEM's ability to induce protein interactions would result in a change in molecular mass and pI which can be visualized in 2D when compared to a control.

Recently, HMW-GS has been successfully identified using SDS-CE, whereby the separation is based upon molecular mass and not by charge. Comparing the separation pattern of control with published standards used would enable us to identify individual subunits of HMW-GS and any changes resulting in DATEM treatment could be identified. The studies with LMW-GS separation using SDS-CE is in initial stages due to complexities in analyzing the large number of LMW-GS present in gluten. However, a modified protocol for extraction of LMW-GS and separation of LMW-GS using HPLC-MS would shed more light onto LMW-GS composition, that composition can be related to functionality properties.

Even though four different levels of DATEM were used in this study, it would be essential to identify optimum DATEM concentration required, which could be achieved by statistical modeling and by appropriate experimental design. It is essential to prepare samples for capillary electrophoretic separation fresh before each run, in order to avoid any changes in protein separation pattern caused due to protein agglomeration. Rinsing the column for at least 30 minutes with sodium hydroxide, acetic acid and phosphate buffer before each run will helps to remove any protein left behind in the capillary column. This will help in obtaining an electropherogram with good resolution and consistency.

REFERENCES

Ambardekar A., 2009. Modifications in visco-elasticity of gluten by diacetyl tartaric acid esters of monoglycerides (DATEM), ascorbic acid, urea and dithiothreitol and its effects on mixing and baking properties in commercial wheat flours. PhD Dissertation, Oklahoma State University.

Bean, S.R., Bietz, J.A., Lookhart, G.L., 1998. High-performance capillary electrophoresis of cereal proteins. Journal of Chromatography A 814, 25-41.

Bean, S.R., Lookhart, G.L., 2000. Electrophoresis of cereal storage proteins. Journal of Chromatography A 881, 23-36.

Bean, S.R., Tilley, M., 2003. Separation of water-soluble proteins from cereals by high-performance capillary electrophoresis (HPCE). Cereal Chemistry Journal 80, 505-510.

Bietz, J., Wall JS, 1973. Isolation and characterization of gliadin like subunits from glutelin. Cereal Chemistry 50, 537-547.

Bietz, J., 1975. Single-kernel analysis of glutenin: use in wheat genetics and breeding. Cereal Chemistry 52, 513-532.

Bietz, J.A., Schmalzried, E., 1995. Capillary electrophoresis of wheat gliadin: initial studies and application to varietal identification. Lebensmittel-Wissenschaft und-Technologie, 28, 174–184

Bietz, J.A., Simpson, D.G., 1992. Electrophoresis and chromatography of wheat proteins: available methods, and procedures for statistical evaluation of the data. Journal of Chromatography A 624, 53-80.

Brown, J.W.S., Flavell, R.B., 1981. Fractionation of wheat gliadin and glutenin subunits by two-dimensional electrophoresis and the role of group 6 and group 2 chromosomes in gliadin synthesis. Theoretical and Applied Genetics 59, 349-359.

Campbell, G. M., Herrero-Sanchez, R., Payo-Rodriguez, R., & Merchan, M. L. 2001. Measurement of dynamic dough density and effect of surfactants and flour type on aeration during mixing and gas retention during proofing. Cereal Chemistry Journal 78(3): 272-277.

Conforti, F.D., Smith, P.M., 1998. Effects of selected emulsifiers, enzymes and a carbohydrate-based fat substitute on physical and sensory characteristics in a low-fat muffin. Journal of Consumer Studies & Home Economics 22, 91-96.

Di Luccia, A., Lamacchia, C., Mamone, G., Picariello, G., Trani, A., Masi, P., Addeo, F., 2009. Application of capillary electrophoresis to determine the technological properties of wheat flours by a glutenin index. J Food Sci 74, C307-11.

Ding, S. and J. Yan., 2013. The influence of emulsifiers on the rheological properties of wheat flour dough and quality of fried instant noodles. LWT - Food Science and Technology. 53(1): 61-69.

D'Ovidio, R. and S. Masci., 2004. The low-molecular-weight glutenin subunits of wheat gluten. Journal of Cereal Science 39(3): 321-339.

Gao, L., Ma, W., Chen, J., Wang, K., Li, J., Wang, S., Bekes, F., Appels, R., Yan, Y., 2010. Characterization and comparative analysis of wheat high molecular weight glutenin subunits by SDS-PAGE, RP-HPLC, HPCE, and MALDI-TOF-MS. Journal of Agricultural and Food Chemistry 58, 2777-2786.

Gianibelli, M.C., Larroque, O.R., MacRitchie, F., Wrigley, C.W., 2001. Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. Cereal Chemistry Journal 78, 635-646.

Gómez, M., del Real, S., Rosell, C. M., Ronda, F., Blanco, C, A., & Caballero, P. A., 2004. Functionality of different emulsifiers on the performance of breadmaking and wheat bread quality. European Food Research and Technology 219(2): 145-150.

Gómez, A. V., Evelina Ferrer, Maria C. Anon, Maria C. Puppo., 2012. Analysis of soluble proteins/aggregates derived from gluten-emulsifiers systems. Food Research International 46(1): 62-68.

Grosch, W. and H. Wieser., 1999. Redox reactions in wheat dough as affected by ascorbic acid. Journal of Cereal Science 29(1): 1-16.

Inoue, Y., Sapirstein, H., Bushuk, W., 1995. Studies on frozen doughs. IV. Effect of shortening systems on baking and rheological properties. Cereal Chemistry 72, 221-226.

Khatkar, B. S., 2004 Dynamic rheological properties and breadmaking qualities of wheat gluten: effects of urea and dithiothreitol. Journal of the Science of Food and Agriculture, 85, 337-341.

Kohler, P., 2001. Study of the effect of DATEM. 4: Optimization of DATEM synthesis. Lebensmittel-Wissenschaft und-Technologie 34, 367-373.

Köhler, P., Grosch, W., 1999. Study of the effect of DATEM. 1. Influence of fatty acid chain length on rheology and baking. Journal of Agricultural and Food Chemistry 47, 1863-1869.

Kulp, K., Ponte, J.G., D'Appolonia, B.L., 1981. Staling of white pan bread: Fundamental causes∗. C R C Critical Reviews in Food Science and Nutrition 15, 1-48.

Lim S., 2011. Effect of physical changes on redox state and surface tension on gluten aggregation, viscoelastic, mixing and baking properties of wheat batter. M.S Dissertation, Oklahoma State University.

Lindsay, M.P., Skerritt, J.H., 1999. The glutenin macropolymer of wheat flour doughs: structure–function perspectives. Trends in Food Science & Technology 10, 247-253.

Lookhart, G., Bean, S., 1995. A fast method for wheat cultivar differentiation using capillary zone electrophoresis. Cereal chemistry. 72, 42-47.

Lookhart, G.L., Bean, S.R., 1996. Improvements in cereal protein separations by capillary electrophoresis: resolution and reproducibility. Cereal chemistry. 73, 81-87.

Macritchie, F., 1984. Baking quality of wheat flours. In: C.O. Chichester, E.M.M., Schweigert, B.S. (Eds.), Advances in Food Research. Academic Press, pp. 201-277.

Masci, S., E.J.L.Lew, D.Lafiandra, E. Porceddu, and D.D. Kasarda., 1995. Cjaracterization of low molecular weight glutenin subunits in durum wheat by reversed-phase high-performance liquid chromatography and N-terminal sequencing. Cereal chemistry. 72:100-104

Mukerjee, P., Mysels, K.J, 1971. Critical micelle concentrations of aqueous surfactants systems; National standard reference data system 36. (U.S.), Washington DC.

Nunes, M., Moore, M., Ryan, L., Arendt, E., 2009. Impact of emulsifiers on the quality and rheological properties of gluten-free breads and batters. European food research and technology = Zeitschrift fur Lebensmittel-Untersuchung und -Forschung. A 228, 633-642.

Orthoefer, F. T. 1997. Applications of emulsifiers in baked foods. In G. L. Hasenhuettl & R.W. Hartel (Eds.), Food emulsifiers and their applications (pp. 211–234). New York: Chapman and Hall.

Patel, S., 2003. Characterization of physicochemical and rheological properties of gluten proteins of hard red spring wheat CV. Butte 86. M.S Dissertation, Oklahoma State

University.

Payne, P., Corfield, K., 1979. Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. Planta 145, 83-88.

Payne, P.I., Law, C.N., Mudd, E.E., 1980. Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. Theoretical and Applied Genetics 58, 113-120.

Payne, P.I., Holt, L.M., Jackson, E.A., Law, C.N., Damania, A.B., 1984. Wheat storage proteins: their genetics and their potential for manipulation by plant breeding [and Discussion]. Philosophical Transactions of the Royal Society of London. B, Biological Sciences 304, 359-371.

Payne, P.I., Nightingale, M.A., Krattiger, A.F., Holt, L.M., 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. Journal of the Science of Food and Agriculture 40, 51-65.

Piergiovanni, A.R., 2013. Capillary electrophoresis: a useful tool for the management of plant genetic resources. Anal Bioanal Chem 405, 481-91.

Qi, P., Wei, Y., Yue, Y., Yan, Z., Zheng, Y., 2006. Biochemical and molecular characterization of gliadins. Molecular Biology 40, 713-723.

Ribotta, P.D., Pérez, G.T., León, A.E., Añón, M.C., 2004. Effect of emulsifier and guar gum on micro structural, rheological and baking performance of frozen bread dough.

Food Hydrocolloids 18, 305-313.

Shiau, S. Y. 2004. Effects of emulsifiers on dough rheological properties and the texture of extruded noodles. Journal of Texture Studies, 35, 93–110.

Shewry, P.R., Halford, N.G., Tatham, A.S., 1992. High molecular weight subunits of wheat glutenin. Journal of Cereal Science 15, 105-120.

Shewry, P.R., Tatham, A.S., Forde, J., Kreis, M., Miflin, B.J., 1986. The classification and nomenclature of wheat gluten proteins: A reassessment. Journal of Cereal Science 4, 97-106.

Shewry, P.R., Halford N.G., Tatham, A.S., Popineau, Y., Lafiandra, D., Belton P.S., 2003. The high molecular weight subunits of wheat glutenin and their role in determining wheat processing properties. Adv Food Nutr Res, Academic Press. Volume 45: 219-302.

Shewry, P.R., Halford, N.G., Lafiandra, D., 2003. Genetics of wheat gluten proteins. In: Jeffrey C. Hall, J.C.D., Theodore, F. (Eds.), Advances in Genetics. Academic Press, pp. 111-184.

Singh, N.K., Shepherd, K.W., Cornish, G.B., 1991. A simplified SDS—PAGE procedure for separating LMW subunits of glutenin. Journal of Cereal Science 14, 203-208.

Singh, M., Khatkar, B., 2005. Structural and functional properties of wheat storage proteins : A review. Journal of Food Science and Technology 42, 455-471

Stampfli, L., Nersten, B., Molteberg, E.L., 1996. Effects of emulsifiers on farinograph and extensograph measurements. Food Chemistry 57, 523-530

Stauffer, C. E. Emulsifiers. (St Paul: Eagen Press. 1999), chap. 8 pp 229-267

Sutton, K.H., Bietz, J.A., 1997. Variation among high molecular weight subunits of glutenin detected by capillary electrophoresis. Journal of Cereal Science 25, 9-16.

Tao, H,P.a.D.D.K. 1989. Two-dimensional gel mapping and N-terminal sequencing of LMW-glutenin subunits. Journal of Experimental Botony 40: 1015-1020.

Visireddy H., 2011. Dough fermentation properties as a function of physical and chemical changes. M.S Dissertation, Oklahoma State University.

Wall, J.S., 1979. The role of wheat proteins in determining baking quality. Recent Advances in Biochemistry of Cereals, 275-311.

Wieser, H., 2007. Chemistry of gluten proteins. Food Microbiol 24(2): 115-119.

Xiujin, Z., Jinquan, S., Zaigui, L., 2007. Effects of DATEM on dough rheological characteristics and qualities of csb and bread. Cereal Chemistry Journal 84, 181-185.

Yan, Y., Hsam, S.L.K., Yu, J., Jiang, Y., Zeller, F.J., 2003. Allelic variation of the HMW glutenin subunits in aegilops tauschii accessions detected by sodium dodecyl sulphate (SDS-PAGE), acid polyacrylamide gel (A-PAGE) and capillary electrophoresis.

Euphytica 130, 377-385.

Yan, Y., Zheng, J., Xiao, Y., Yu, J., Hu, Y., Cai, M., Li, Y., Hsam, S.L., Zeller, F.J., 2004. Identification and molecular characterization of a novel y-type Glu-Dt 1 glutenin gene of aegilops tauschii. Theor Appl Genet 108, 1349-58.

APPENDIX I

Table 1. Partial composition analysis (Protein, moisture and ash) flour sample from Ambardekar (2009) to this study

Flours	Ambardekar (2009)	Protein (%)	Moisture (%)	Ash (%)
1C	1A	7.95 ± 0.05	11.69 ± 0.02	0.29 ± 0.01
2C	1B	10.40 ± 0.10	12.54 ± 0.02	0.47 ± 0.00
3C	2B	10.59 ± 0.07	12.57 ± 0.00	0.48 ± 0.01
4C	2A	11.19 ± 0.07	10.51 ± 0.03	0.38 ± 0.01
5C	3B	11.38 ± 0.01	12.98 ± 0.04	0.58 ± 0.01
6C	3A	13.68 ± 0.02	10.14 ± 0.02	0.41 ± 0.00

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