

EFFECT OF PROCESSING ON GAS CELL AREA
AND SPHERICITY OF FOAM DOUGH: A
MICROSCOPY STUDY

By

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CHAPTER I

INTRODUCTION

Statement of Problem

Bubbles in dough have been a problem for the baking industry and can have an effect on the final bread product. In this study, the area of bubbles and their sphericity in nonfermented dough were investigated. The effect of mixing times, laminations (vertical and vertical + horizontal), yeast, and DATEM (diacetyl tartaric ester of mono and diglycerides) concentrations on area and sphericity of the gas cells (bubbles) was studied. The air that is dispersed in the dough during mixing helps to form the nucleation on small regions of a new gas phase that will make the bubbles. Different mixing times have different aeration levels that are incorporated into the dough. At the same time, mixing will develop interaction between gluten molecules. Thus, mixing affects the aeration level and gluten development. In this study, the area of bubbles in dough was studied as bubble size is related to surface tension. The surface tension will increase as the size of the bubbles increases. However, the bubbles will rupture when it reaches a critical size. Laminations in the baking industry are usually done in the one direction. Research of bubble size of two directional laminations in dough has yet to be conducted. This study compares two lamination directions, vertical and vertical + horizontal, and their effects on bubble size. The function of yeast is to ferment sugars and create an aerated product. The sizes of bubbles are important in the creation of the aerated structure of crumb. The fermentation process gives off carbon dioxide and ethanol. These gases diffuse in the

dough and merge in small bubbles which will merge (coalesce) into large bubbles expanding the dough. The effect of yeast on the diameter and sphericity of air bubbles was recorded. DATEM (diacyl tartaric ester of mono and diglycerides) is commonly used in the industry as a dough improver, which helps to strengthen some of the weak characteristics in dough. DATEM improves dough during mixing by reducing the surface tension of the bubbles in dough and stabilizes the foams.

Purpose of the Study

The aim of this study was to measure the diameter size of air bubbles in the dough at different levels of mixing, lamination processes, with given levels of DATEM, and yeast concentrations.

Objective

The objective of this study is to analyze the air bubble size of dough and bubble sphericity as affected by mixing, lamination, yeast and DATEM addition.

Hypotheses

1. Mixing times (7, 10, and 12 minutes) affect the diameter and sphericity of bubbles.
2. DATEM concentration (0 and 0.5%) affect the diameter and sphericity of bubbles.
3. Yeast concentration (0 and 0.4%) affect the diameter and sphericity of bubbles.

4. Lamination processes (vertical and vertical+horizontal) affect the diameter and sphericity of bubbles.
5. Combination of all treatment and processes will affect the diameter and sphericity of bubbles.

Assumptions

Dough is usually mixed at optimum mixing, because research has proven that at this stage of dough development it will produce optimum yeasted bread quality. Over-mixed dough will become stressed to the point of no recovery by breaking up the gluten and forming fragments of small structure (Okada et al. 1987). In contrast, the protein from under-mixed dough is not given the opportunity to fully develop, which causes less aeration incorporated in the dough. Also it does not allow the disulfide bonds to fully develop and thus affecting negatively the gluten matrix. We assume that nucleation or introduction of smaller gas cells during mixing is directly related to the mixing time, and the presence of yeast and additives like surfactants. We also assume that laminating the dough in two directions will assist in maintaining a certain diameter and sphericity of the bubble. Lamination will orient the direction of the protein fibrils to stabilize the size of bubbles.

DATEM is an emulsifier in which diacetyl tartaric acid that is bound with mono and diglycerides. It is used to stabilize dough due to the reduction of surface tension of the oil and water phases. Flour constituents (like protein and starch) and gas cells form different phases (i.e., solid and gas) in the dough. The different phases and gluten structures will be stabilized by the presence of DATEM in the dough by decreasing the surface tension. DATEM stabilizes the nucleation of bubbles and contributes to the

formation of small diameter and high sphericity during mixing. In this study dough was not allowed to ferment by storing it at 0 to 4°C immediately after mixing. This measure was assumed to slow down or prevent yeast activation within the dough in the time frame of this study. We assume that two dimensional images of bubbles in dough obtained by light microscopy are representative images and can be used to estimate the two axes length (L_1 and L_2) needed to calculate the area of bubbles and their sphericity.

CHAPTER II

REVIEW OF LITERATURE

Rheological Properties of Dough

The rheological properties of dough have been used to predict or associate with the quality of the final product (Puppo et al. 2005). Having an understanding of the dough properties is an essential element to making acceptable and consistent wheat bread products. It is important to understand the rheological properties of dough and its effects on bubble stability in dough (Kokelaar and Prins 1995). Bellido et al. (2006) mentioned that the mechanisms of the bread crumb structure needed to be studied further because the process of breadmaking consists of sequence of aeration stages and this can affect gas cells in dough which creates the cellular structure of the bread crumb. These researchers suggested that the study of the mechanisms of cellular structure that is created by gas cells in dough will give scientist a better understanding of the air bubbles during mixing (Bellido et al. 2006). When mechanical mixing is applied to the ingredients in order to make the viscoelastic dough, the dough forms multiple phases with different surface tensions, such as formation of gluten protein, gluten-starch matrix, and the entrainment of air bubbles throughout the gluten matrix (Mills et al. 2003). When the dough is not stable coalescence and disproportionation of air cells occur (Kokelaar and Prins 1995). The bubble size is influenced by the rate of disproportionation (Bellido et al. 2006). In bread

dough, the bubble stability can be affected by surface properties. When the wheat flour undergoes hydration, it leads to the formation of gluten protein forming the viscoelastic dough matrix (Millar 2000). Gliadins and glutenins are two types of proteins that form the gluten protein (Hamer 2009). Glutenins are insoluble proteins and highly polymerized. Glutenins proteins form polymeric structure and shear strain during mixing can cause disruptions of disulfide bonds (Knyaginichev et al. 1977). The strength of bread dough is determined by the formation of the disulfide bonds between gluten polymers (Knyaginichev et al. 1977). The stabilization of the liquid film that surrounds the bubbles is believed to keep the bubbles from rupturing (Mills et al. 2003).

Proofing of dough has been studied to record the effects of the growth of individual bubbles in bread dough (Shah et al. 1998). This particular study used a modeling system to measure the bubble growth rate, surrounded by liquid dough containing dissolved carbon dioxide (Shah et al. 1998). A diffusion theory model was developed to analyze the early stages of proofing and the effects on the diameter and condition of the growth of bubbles (Shah et al. 1998). The model is a mathematical equation that is able to monitor the characteristics in bread dough, such as growth rate of the bubble, shrinkage, diameter, and other conditions in single bubbles in dough that are supersaturated or unsaturated with carbon dioxide (Shah et al. 1998). Shah et al. (1998) reported that during the early stages of proving the dough rheology is incidental and gluten network has yet to stretch in order to maintain the bubble structure within the bread dough, so the surface tension of the dough causes pressure on the bubbles, this may cause coalescence to occur which causes stress on the dough. The mathematical model demonstrates the effects of bubble size and surface tension on bread dough (Shah et al.

1998). The authors suggested that the model can be used to measure the distribution in the size of the complete bubbles as well as atmospheric and spatial variation loss in carbon dioxide (Shah et al. 1998).

Effect of Mixing on Wheat Dough

During mixing, foam structures are formed and the dispersion of gas bubbles is similar to complex coacervate systems in which the quality is determined by the lamella formed around the bubbles (Dickinson 2010). When dough is mixed, foams become unstable due to the rising temperature of the dough as well as the ingredients that have been incorporated into the dough. The ingredients may have an effect on the air bubbles size as well as their distribution in the dough matrix. Studies have shown that the process of mixing dough incorporates different types of characteristic into the dough also affecting the viscoelastic properties. When the dough is mixed the bubbles are entrained in the dough matrix which later in the bread making process the bubbles will be broken down and decrease in size (Mills et al. 2003). The coalescence of gas bubbles is a result of disruption of the lamella which in turn leads to disproportionation of the gas bubble. Coalescence will not occur if the liquid film around the bubble is stabilized. If the bubbles are not stabilized drainage occurs, which means liquid will flow by gravity and result in bubble to bubble combination. Disproportionation will affect the surface tension of the bubbles which causes them to disrupt and destabilize. Research is needed to unequivocally demonstrate the presence of a thin liquid layer lining the bubble surface and characterize the surface properties of the aqueous phase of doughs to understand and manipulate this system more than is currently possible (Mills et al. 2003).

There are different types of mixers that are used to mix or study the mixing properties of dough such as farinograph, mixograph, Hobart mixer, etc. Farinograph is a common instrument that is used throughout the world to test the mixing properties of flour. The farinograph measures the water absorption, arrival time, stability time, peak time, departure time, and mixing tolerance index. The mixograph also determines the mixing properties and gives indicators of gluten strength, development time at the optimum water absorption level, and the mixing tolerance of the dough. The Hobart mixer is generally used in industrial and laboratory settings for obtaining dough to manufacture baking products and has no measuring devices to record mixing properties.

Microscopy Analysis

There are different types of image analysis that are used in scientific research for example microscopy analysis in which digital image processing is also included. Image processing techniques have been used to analyze specimens in two and three dimensional imaging. On a light microscope the low magnification can be 3.5x or 4.0x. The magnification that is commonly used is 10x. High magnifications in light microscopy are 40x, 97x, and 100x. These magnifications can be used to analyze fixed and unfixed samples. Besides light microscopy, other microscopy types used to study the structure of dough, baked products and their components (like starch granules) include scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). In SEM the samples are scanned with a high-energy beam light. The CLSM can look at an image in depth by laser scanning at high resolutions. High resolution produces an image with a high level of detail, i.e., digital image and high definition monitors. CLSM uses a light

source of a mercury lamp and the light passes through a pinhole which helps to control how much resolution is obtained. Even though, a light microscope is a part of the CLSM setting, when a field of interest has been selected the mercury lamp is turned off and the tungsten lamp in the CLSM is turned on.

Microscopy analysis has been used in the food industry to examine food structures for many years. Image analysis can also be used to analyze microstructure in dough such as dimensions of cell walls, starch granules, protein, and other components. Image analyses have also been used to study the porosity of dough during the fermentation process (Shehzad et al. 2010). Perez-Nieto et al. (2010) used image analysis to study changes in dough structure during the baking process. Samples of bread were taken at different time intervals, sliced and scanned. The authors suggest that the first stage of baking is air bubbles that coalesce at < 250 s and the second stage is dough that is transformed from semi elastic sponge structure to a very viscous liquid state that occurs ≤ 400 s (Perez-Nieto et al. 2010).

ImageJ Software

ImageJ software was created at the National Institutes of Health by Wayne Rashband and other individuals who have contributed to the software over time (Collins 2007). This software has been around for over a decade. It is user friendly with online components such as handbooks, wikis, and plugin to help the users navigate through the different applications, as well as to easily understand the software and its different characteristics (Collins 2007). It has been used for measuring the diameter, average of thickness, and area; along with other characteristics that can help the user analyze images

with an imaging processor (Collins 2007). There are many types of research that the ImageJ software is used for such as tissue and cell analysis, and measuring orthogonal dimensions of the eight types of food grains (Collins 2007). Orthogonal dimension is a set of dimensions that cannot be composed from each other.

Influence of DATEM on Wheat Dough

Surfactants are food additives used in many food products. Mono and diglycerides were some of the earliest food additive surfactants used, but they were not used in the United States until after 1929 (Birnbaum 1977). Surfactants are surface active agents that reduce the surface tension of liquids. In dough, surfactants such as mono and diglycerides are known to strengthen the dough and to soften the bread (Birnbaum 1977). The surfactants may also increase the growth rate of the bubbles. One of the problems that occur in dough is coalescence and disproportionation of gas cells. Surfactants help to prevent these two characteristics by breaking up the bubbles into smaller ones, as well as strengthening the dough. DATEM (Di-actyl tartaric acid ester of mono and diglycerides) is an anionic oil-in-water emulsifier that improves the bread-making characteristics (Koehler and Grosch 1999). The interfacial properties are major contributing factors to foam stability. Low molecular weight (LMW) surfactants are more useful in foods because they lower the interfacial tension, but they are less stable against coalescence compared to high molecular weight (HMW) surfactants (Bos and van Vliet 2001). DATEM is used to improve the stability of foam and gas retention; it is also a dough strengthener and softens bread (Koehler and Grosch 1999). There are a variety of surfactants that are used in different types of foods for different purposes. When dough is

frozen, it goes through a depletion of some of its characteristic such as the loss in the dough strength, extended fermentation, difficult in retaining the CO₂, as well as decreased yeast activity, and loss in bread volume (Selomulyo and Weibiao 2007). DATEM is used in a variety of bread doughs whether frozen or unfrozen to improve volume and form finer, more uniform crumb structure.

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CHAPTER III

EFFECT OF PROCESSING ON GAS CELL AREA

AND SPHERICITY OF FOAM DOUGH:

A MICROSCOPY STUDY

Abstract

The formation of air bubbles in dough produce important physical changes that are translated into the aerated texture of different products including yeasted breads. Details of the physical characteristic of air bubbles in dough are not fully understood. The effects of mixing, lamination, yeast, and DATEM on air bubble area and sphericity of unfermented dough were studied. DATEM produced more spherical air bubbles ($P < 0.0001$). Yeast treatment produced larger and less spherical bubbles. In dough with treatment of lamination in vertical + horizontal direction, the bubbles were more spherical than in dough laminated only in vertical direction.

1. Introduction

Mixing of bread dough helps to develop and incorporate air into the dough. The aeration that is incorporated during mixing forms the bubbles which will promote bread cell formation. The growth of bubbles can be a complex phenomenon. The foam

structures consists of a gas disperse phase in a second and continuous phase (Murray and Ettelaie 2004). When foams are formed their system is a high volume fraction, where the gas is dispersed and bubbles are created in a closely packed liquid matrix (Murray 2007). In a closely packed foam, drainage will occur causing coalescence of air cells and this can cause collapse of the foam, affecting the gas retention properties, as well as the structure and texture of the foam (Murray and Ettelaie 2004). The bubbles found in foam have non-spherical shapes, which are also known to be a contributor of bubbles not being easily stabilized (Murray 2007). Coarsening of bubbles leads to disproportionation known as Ostwalds ripening; that causes coarsening which occurs by the migration of the gas between the bubbles (Murray 2007).

DATEM (diacetyl tartaric acid ester of mono and diglycerides) is an anionic oil-in-water emulsifier that is commonly used in the baking industry. DATEM is a low-molecular weight surfactant that helps to strengthen the gluten network, as well as improves the texture and the crust of the final bread product (Dobraszczyk 2008). It has been reported as an effective bread emulsifier in many countries. DATEM is often used to increase the volume of bread and stabilize bubbles in bread dough (Zhang et al. 2007). The function of yeast is to ferment sugar added to dough, after the oxygen and nitrogen have been depleted in the flour. Carbon dioxide gases diffuses into bubbles incorporated during mixing, causing the bubbles to inflate and dough to rise when yeast metabolizes flour sugars into carbon dioxide and ethanol (Chiotellis and Campbell 2003). Lamination is a contributor to the development of dough structure, it is an important processing step in the production of many bakery products (Qi et al. 2008). Lamination has an effect on the dough behavior and repeated lamination can build-up or break down the protein

network structure in the dough (Engmann et al. 2005). The objective of this study is to analyze the air bubble size and sphericity in dough as affected by mixing, lamination, yeast and DATEM treatments.

2. Materials and Methods

2.1 Materials

One commercial hard red winter flour with a protein content of 13.7% was used. It was obtained from Shawnee Milling Company (Shawnee, OK). Dough samples were prepared using 100 g of flour and other ingredients described in Table 1. Dough ingredients included sugar, dry instant yeast (Red Star yeast, Lesaffre Yeast Corporation, Milwaukee, WI) (0.4%), DATEM (DATEM 100, American Ingredients Company, Kansas City, MO) (0.5%), 2% sodium chloride (NaCl) and deionized water. Samples without DATEM were used as control.

2.2 Dough Preparation

The preparation of dough was made at three mixing levels, under-, over-, and optimum mixed as described in Table 2. Levels of mixing corresponded to under-mixed 7 minutes, optimum mixing 10 minutes, and over-mixed 12 minutes. After mixing (Swanson-Working pin-type mixer modified by Finney, National Manufacturing Co, Lincoln, NE), the dough was laminated with a sheet roller (National Manufacturing Company, Lincoln, NE), with roll gap positioned at the 1/8 inch in two directions (vertical and vertical + horizontal). After the lamination process, a 3 mm piece was cut and placed inside of a custom made mold (Fig. 1 and 2, Appendix I). The mold was used to assist in containing the air bubbles and align protein fibril in the direction the dough

was laminated. The mold assist in maintaining the alignment of the protein, it also helped in the transportation of dough to the microscopy lab. The dough and mold were place in a zip locked bag, labeled and stored at 0 to 4°C in a Styrofoam box with ice packs. Dough was examined with a light microscope (Leica SP2 Microscope, Leica Microsystem, Inc., Buffalo Grove, IL). Specimen samples were prepared from a thin piece (≈ 0.5 mm piece cut), spread on to the microscope slide (25.2 x 76.2 mm), and stained with Serva Blue solution. The solution contained 300 mg Serva Blue G (Serva Electrophesos, Generon Ltd., UK), 6 ml ethanol (96%) and 1 drop of acetic acid (30%). Digital images were obtained with a camera connected to the view piece of the microscope and two axes of the gas cells were measured from such images. Single bubbles were chosed in the field on vision, composites bubbles were not included. ImageJ software (National Institutes of Health (NIH), Bethesda, Maryland) was used to measure two axes lengths (L_1 and L_2) of 50 bubbles per sample preparation. For the conversion of pixels to micrometers we used 192 pixels which equal $300 \mu\text{m}^2$ (Ownby 2010). The two axes of the bubbles passed through the center of mass of each object. Analysis was performed in independent dough duplicates.

Table 1. Dough Ingredients and Levels of Yeast and DATEM

	Flour (g)	Salt (g)	Sugar (g)	Yeast (g)	DATEM (g)	D.I.Water (g)
No DATEM	100	1.5	0.45	0.4	0	60
No DATEM and yeast	100	1.5	0	0	0	60
No yeast and with DATEM	100	1.5	0	0	0.5	60
With yeast and DATEM	100	1.5	0.45	0.4	0.5	60

*De-ionized (D.I.) water was used.

Table 2. Mixing Levels of Dough

Levels of Mixing	Mixing Time (min)
Under-mixed	7
Optimum	10
Over-mixed	12

3. Statistical Analysis

A factorial arrangement in a completely randomized design with 2 replications of each treatment was used. The factors of interest were mixing time (optimum, under-mixed, and over-mixed), DATEM (0 and 0.5%), yeast (0 and 0.4%), and laminations (vertical and vertical + horizontal), which yielded a 3 x 2 x 2 x 2 factorial arrangement. Analysis of variance procedures were performed using PROC MIXED in PC SAS Version 9.2 (SAS Institute, Cary, NC). The responses were bubble mean area and sphericity. Interactions were examined, and main effects reported if no interactions were present. When interactions were significant, simple effects were calculated with a SLICE option in an LSMEANS statement. A significance level of 0.05 was used for all comparisons.

4. Results and Discussion

4.1 Effect of Lamination

A list of the abbreviation of the treatments and variables is reported in Table 1 (Appendix I). Comparison of air bubble area means in dough as a function of lamination treatments (vertical and vertical + horizontal) is reported in Table 2 (Appendix I) and Figure 1 and 2, for a given level of DATEM, yeast, and mixing times. Air bubble area

means of samples with no DATEM and given levels of yeast (0 and 0.4%), over-mixed and optimum mixing with vertical and vertical + horizontal laminations were significantly different ($p < 0.0001$ and 0.0006 , respectively). In the over-mixed and vertically laminated dough, the mean bubble area was 2.41 times larger than the vertical + horizontal lamination ($82,539$ versus $34,257 \mu\text{m}^2$, respectively). In the optimum mixed and vertically laminated dough, the mean bubble area was 1.76 times larger than in the dough receiving vertical + horizontal lamination ($86,921$ versus $49,523 \mu\text{m}^2$, respectively). The bubble area of dough samples with 0.5% DATEM was not significantly different (Fig. 2).

The results suggest that lamination affects the area mean of air bubbles in selected treatments and that the vertical lamination produced larger air bubbles in dough without DATEM or yeast. It also suggests that with the under-mixed treatment of dough, there was no effect of lamination in the air bubble area. To the best of our knowledge, there are no reports in the literature with studies of air bubble area mean and the effect of dough lamination, and more specifically on two lamination directions, i.e., vertical and vertical + horizontal. Leong et al (2008) measured the density of dough to indirectly evaluate the amount of gas incorporated into the dough before and after it was laminated. Another study conducted on laminated dough was focused on the elasticity of the dough after lamination (Chakrabarti-Bell et al. 2010). Although, some studies are found on the lamination of dough, to the best of our knowledge there are no studies that relate the effects of yeast and DATEM, with different mixing times, and the two lamination processes. Our study revealed the size of bubble mean area in dough without fermentation ranging from $12,425$ to $86,921 \mu\text{m}^2$, equivalent to 12.4 to 86.9 mm^2 (Table

2). The results suggest that within the time frame and the described experimental conditions, one would expect that the air bubbles mean area of samples containing DATEM (0.5%) and yeast (0.4%) from the two laminations (vertical or vertical + horizontal) would be for the most part similar.

4.2. Effect of Mixing

Table 3 (Fig. 3-4) describes the effect of the mixing time on mean area of air bubbles when compared to given levels of DATEM, yeast, and lamination treatments. In the set of comparisons with no DATEM, three treatments comparisons were statistically significant. The sets without DATEM and yeast and vertical lamination with contrasting mixing treatments of optimum, over-mixed, and under-mixed were significantly different (Table 3, Fig. 3) ($p < 0.0001$). Over-mixed dough had larger air bubbles mean area ($82,539 \mu\text{m}^2$) compared to optimum and under-mixed ($45,045$ and $34,412 \mu\text{m}^2$) (1.9 and 2.4 times larger, respectively). The next set of no DATEM and 0.4% yeast, and vertical and vertical + horizontal lamination, the comparison of optimum, over-mixed, and under-mixed treatments significantly influenced the air bubble area ($p < 0.0001$ and 0.0392 , respectively) (Table 3, Fig. 3). The bubble area mean of vertical lamination of the optimum mixing ($86,921 \mu\text{m}^2$) was about 2 times higher than over-mixed ($45,773 \mu\text{m}^2$) or under-mixed ($43,187 \mu\text{m}^2$). In the three mixing treatments with the vertical + horizontal lamination over-mixed ($59,592 \mu\text{m}^2$) mixing time, the bubble area mean was 1.8 times higher than under-mixed ($32,590 \mu\text{m}^2$) and 1.2 times higher than optimum ($49,523 \mu\text{m}^2$) mixing times. Treatments that contained 0.5% DATEM and/or (0 and 0.4%) yeast had similar air bubble areas, i.e., they were not significantly different

($p > 0.005$). Within these observations that were not significantly different, overall there is a slight trend of the over-mixed dough suggesting a modest large air bubble area mean compared to the optimum or under-mixed. It is possible that the number of bubbles per area mean was larger and thus more bubbles were represented in the over-mixed dough. After thorough research in the literature, no reports were found on the effects of mixing with comparison of lamination process with given levels of DATEM and yeast. A recent study on the structural changes of yeasted sweet dough and crumb grain with mixing (under-mixed, optimum, and over-mixed), fermentation times, and the dough pH was conducted with fermented and unfermented dough (Tlapale-Valdivia et al. 2010). These authors found that mixing did not affect luminosity of the crumb while fermentation decreased it. This was explained due to the increase in cell size in bread crumb causing a variation on the light reflection pattern. They also reported that mixing did not affect chromaticity parameters (a^* , b^*) except for the sample mixed at optimum time (25 min) without fermentation compared to the under-mixed dough (6 min). Tlapale-Valdivia et al. (2010) also reported that very small cells in sweet dough represent 84 to 76% of the total particles detected. The size distribution of the objects (area cells) revealed that two sizes 0.0072 (one pixel) and 0.014-0.072 mm² (2 to 10 pixels) represented 40% each (Tlapale-Valdivia et al. 2010). This study measures characteristic that may be of interest in future studies of this research, such as measuring the pH, fermentation, and baking of the bread to analyze the final product.

4.3 Effect of Yeast

Table 4 (Fig. 5-6) shows comparison of air bubbles area means of dough containing 0 and 0.4% yeast with lamination treatments (vertical and vertical + horizontal) and mixing times (optimum, over-mixed, and under-mixed). Comparing the block of samples without DATEM, air bubble area mean of optimum mixing with vertical lamination, 0% and 0.4% yeast were significantly different ($p=0.0002$). The samples containing yeast had air bubble 1.93 times larger than the sample with no yeast ($86,921$ and $45,045 \mu\text{m}^2$, respectively). The mean of samples, over-mixed with vertical and vertical + horizontal lamination containing 0% DATEM with given levels of yeast (0% and 0.4%) were significantly different (Table 4, Fig. 5) ($p=0.0008$ and 0.0182 , respectively). The vertical lamination that contained 0% yeast had a magnitude of change of bubble area mean of 1.8 times higher than sample with 0.4% yeast. The air bubble area in the vertical + horizontal lamination with yeast was 1.74 times larger than the no yeast sample. It is interesting to note that in the treatments without DATEM, over-mixed and vertical lamination, the bubble area mean was higher in the sample with no yeast compared to 0.4% yeast. One will expect a parallel with the other two comparisons with significant differences, in which the treatment with yeast had higher bubble area means compared to the no yeast. These observations suggest that the under-mixed dough, representing the least developed dough, has similar air bubble mean and yeast did not affect it.

Of the block of treatments with 0.5% DATEM, two comparisons were significantly different. Treatments with 0.5% DATEM (Table 4, Fig. 6), optimum and over-mixed with vertical + horizontal laminations were significantly different ($p=0.0294$

and 0.0328, respectively). Optimum and over-mixed mixing times with vertical + horizontal laminations and 0.4% yeast had larger bubble areas compared to the 0% yeast samples, with a higher magnitude of change (2.87 and 1.96 times larger, respectively). The overall trend suggests that samples with DATEM and yeast had a higher bubble area mean compared to samples without yeast.

4.4 Effect of DATEM

Table 5 (Fig. 7-8) illustrates the comparisons of air bubbles area mean of 0% and 0.5% of DATEM for given levels of yeast, mixing time and lamination treatments. Air bubble area of samples without yeast with optimum mixing time, vertical lamination treatments and (0 and 0.5%) DATEM were significantly different ($p=0.0051$). At optimum mixing times with vertical and vertical + horizontal lamination and 0% yeast, the samples with DATEM had lower area mean ($p<0.0001$). The magnitude of change (decrease area mean) compared to the control for optimum mixing with vertical and vertical + horizontal lamination was 3.05 and 4.6 times smaller, respectively. Observation of over-mixed with vertical lamination, comparing 0% and 0.5% DATEM with no yeast were significantly different ($p<0.0001$). The area mean of the samples containing DATEM was 2.7 times smaller than the sample with no DATEM (Table 5, Fig. 8). When yeast was present (0.4%), the optimum mixing time, with vertical lamination, and the sample with DATEM had lower area mean compared to no DATEM ($p<0.0001$). The mean area of the sample with DATEM was 2.63 smaller than with no DATEM present. Comparing the samples that were not significantly different, with yeast present there was an overall slight trend suggesting that samples with no DATEM tend to have a slightly

larger air bubble area mean versus 0.5% DATEM treatments. A possible explanation of the findings, only one sample with significant decrease in bubble area mean in the samples with yeast is that the physical state of dough with no fermentation has a more compact structure and the size of the air bubbles could not be homogeneously distributed. It is well reported in the literature that DATEM has an effect in improving loaf volume; crumb grain and uniformity of cells in the crumb (Bos and van Vliet 2001; Dickinson 2010; Gaupp and Adams 2007). These effects must be prominent during the fermentation and baking process, so in our samples that have not fermented, the effect of DATEM was not revealed.

4.5 Effect of Lamination on Air Bubble Sphericity (length ratio)

In a small cluster of bubbles defined as a central bubble of Volume V_c , surrounded by F bubbles, each of the same volume V , suggested that a foam structure in equilibrium minimizes its free energy (Jurine et al. 2005). These authors also propose that minimum free energy in bubbles is achieved by two important factors: a) average surface area and surface tension, and b) some function of shape. In the report of Jurine et al. (2005), the measurable variables were bubble area and ratio of a/c which are the two measure axes of spheroid bubbles. The measurable two axes in this study were labeled L_1 and L_2 . Theory states that when the mean ratio of the two axes (L_1/L_2) is close to one, the air bubbles are more spherical in shape, values smaller than one means the spheroid has more of an oblate shape, and the air bubbles appears to be “squashed” (Weisstein 2011). This is referred as the sphericity of the bubble, the closer to 1 the more sphere the bubble is.

Table 6 has air bubble ratio (sphericity) means in mixing time comparing lamination treatments with DATEM (0 and 0.5%) and yeast (0 and 0.4%). Only one significantly different treatment was observed in this study, over-mixed with vertical and vertical + horizontal lamination, without DATEM or yeast (0.799 and 0.875, respectively) ($p=0.0003$). Lower sphericity was observed in the vertical lamination compared to vertical + horizontal. This suggests that when the dough is over-mixed, the two laminations (vertical + horizontal) produced more spherical bubbles compared to one lamination (vertical). This could be explained by the rearrangement of the polymeric protein which is aligned into a more complex state when is laminated in one direction and then laminated again at 90° from the first direction, compared to only one direction. When DATEM was present, the sphericity of the air bubble was similar regardless of the direction of the lamination (Table 6, Fig. 10).

4.6 Effect of Mixing Time on Air Bubble Sphericity (length ratio)

Table 7 illustrates the effects of mixing time on air bubble mean sphericity when compared at given levels of DATEM, yeast, and lamination treatments. Treatment blocks with 0% DATEM and 0% yeast comparing the three sets of mixing times with vertical and vertical + horizontal laminations were significantly different ($p=0.0131$ and 0.0076 , respectively) (Fig. 11). When the vertical lamination was compared, the over-mixed treatment produced air bubbles deviating more from sphericity compared to optimum and under-mixed treatments compared to when the two lamination (vertical + horizontal) were applied. The over-mixed dough produced more spherical air bubbles compared to optimum and under-mixed dough (Table 7). This suggests that the two laminations

contribute to more stable bubbles, by making the environment favorable to aid bubble sphericity (lower energy system). Comparing the block of samples with DATEM and yeast, under-mixed dough has lower sphericity compared to the optimum mixed dough. We would expect that with DATEM and yeast, the bubbles would be more spherical. In Table 3 we proved that there was no effect of mixing in the area of the bubbles, however, the bubbles in the mentioned treatments were less spherical. The set of samples that contained 0.5% DATEM, the treatments with 0.4% yeast with a vertical + horizontal lamination, optimum mixing had more spherical bubbles compared to under-mixing (Fig. 12).

4.7 Effect of Yeast on Air Bubble Sphericity (length ratio)

Table 8 shows the comparison of yeast (0 and 0.4%) with mixing times, and lamination treatments that contain 0% or 0.5% DATEM. Samples with no DATEM at optimum mixing with vertical lamination were significantly different ($p=0.0495$). In this comparison with yeast bubbles were less spherical compared to the control with no yeast (0.815 vs. 0.855). No DATEM with over-mixed and vertical + horizontal lamination was significantly different (0.875 and 0.812, respectively; $p=0.0021$). Thus, in these two comparisons, the sample with yeast produced bubbles that were less spherical. When yeast was present, the bubble area was larger (Table 4, effect of yeast on area) but they were less spherical than samples with no yeast (Table 8, effect of yeast on sphericity). Comparing the bubbles area and sphericity of without DATEM and yeast, over-mixed with two laminations (vertical + horizontal), produced bubbles with larger area and lower sphericity compared to the treatments without yeast. The block of samples that contain

0.5% DATEM were not significantly different, but there was an overall trend within these samples and treatments with 0% yeast had a higher air bubble mean ratio than with yeast.

4.8 Effect of DATEM on Air Bubble Sphericity (length ratio)

Table 9 (Fig. 15-16) shows the comparisons of lamination and mixing times to given levels of DATEM (0% and 0.5%) with 0% or 0.4% yeast. Optimum with vertical and vertical + horizontal lamination with 0% yeast were significantly different ($p=0.0139$ and 0.0006 , respectively). Optimum with vertical and vertical + horizontal with DATEM was more spherical than no DATEM. Comparing the area and sphericity of the bubble, samples with DATEM had a smaller area (Table 5, effect of DATEM on area) and more sphericity (Table 9, effect of DATEM on sphericity). Over-mixed sample with vertical lamination and under-mixed mixing time with vertical + horizontal lamination were significantly different ($p<0.0001$ and 0.0086 , respectively). Over-mixed with vertical lamination when DATEM was present, was more spherical than no DATEM. Comparison of the area and sphericity of the bubble samples with DATEM had a smaller area (Table 5, effect of area) and were more spherical (Table 9, effect of DATEM on sphericity). This suggests that more air was incorporated during the over-mixed mixing time, but bubbles were stabilized with DATEM treatments. Treatments blocks containing DATEM (0 and 0.5%) and yeast with optimum mixing time and vertical lamination were significantly different ($p=0.00056$). Over-mixed with vertical and vertical + horizontal lamination with 0.4% yeast were significantly different ($p=0.0153$ and 0.0200 , respectively). Comparing the sample block with 0.4% yeast, the optimum

mixing time with vertical lamination and DATEM present was more spherical than no DATEM. Referring to Table 5, the effects of area on DATEM, bubbles had a smaller area with DATEM and in table 9 observations showed that bubbles had more sphericity.

When analysis were comparing the effects of laminations on air bubble sphericity (Table 6, Fig. 9-10), the significantly different treatments of over-mixed with vertical and vertical + horizontal lamination (0.799 and 0.875, respectively) sphericity mean values were less than one, therefore the air bubbles were more oblate shaped. The analysis comparing the effects of mixing (Table 7, Fig. 11-12), in the sample blocks with no DATEM and yeast with vertical lamination, showed that majority of optimum mixing times air bubble sphericity mean were close to one value, and over-mixed was second. So this study reveals that the average mixing times had the more spherical shape of air bubbles. However, observing the control significantly different observation, when the dough was laminated vertical to vertical + horizontal the optimum mixing times air bubble sphericity mean decreased in value (0.855 and 0.819, respectively) (Table 7, Fig 11-12). When treatments were compared using the effects of yeast, the treatments that had no DATEM and given levels of yeast in Table 8, optimum with vertical and over-mixed with vertical + horizontal laminations were the only treatments that were significantly different. Although, no studies have ever been conducted comparing DATEM, given levels of yeast with mixing times and lamination process, there have been somewhat similar observations conducted with analyzing the radii of the air bubbles. In Table 9, the majority of treatments were significantly different, and when DATEM and yeast were present air bubble ratio mean value were closest to one, presenting more spherical in shape.

Grenier et al. (2010) measured the radii of air bubbles during fermentation. In this study, zero time of fermentation of the air bubble radii mean was 0.15 mm and the maximum fermentation time was 45 minutes. The maximum air bubble radii mean which was observed at 40 minutes was 0.24 mm (Grenier et al. 2010). Our research observation were performed at zero fermentation with mixing time of optimum, under-, and over-mixed the values range from 0.996, 0.850, and 0.123 mm these bubble radii mean were smaller compared to the report of Grenier et al. (2010). Differences between the two studies maybe explained by different factor: Greiner et al. (2010) used a spiral kneader mixer; in our research we used a pin mixer and different flours were used in both studies. In our research we used three different mixing levels (optimum, under-mixed, and over-mixed) while in Greiner et al. (2010) used a complete baking process. We assume that the 17 minutes mixing time reported is the optimum even though it is not clearly specified by Greiner et al. (2010).

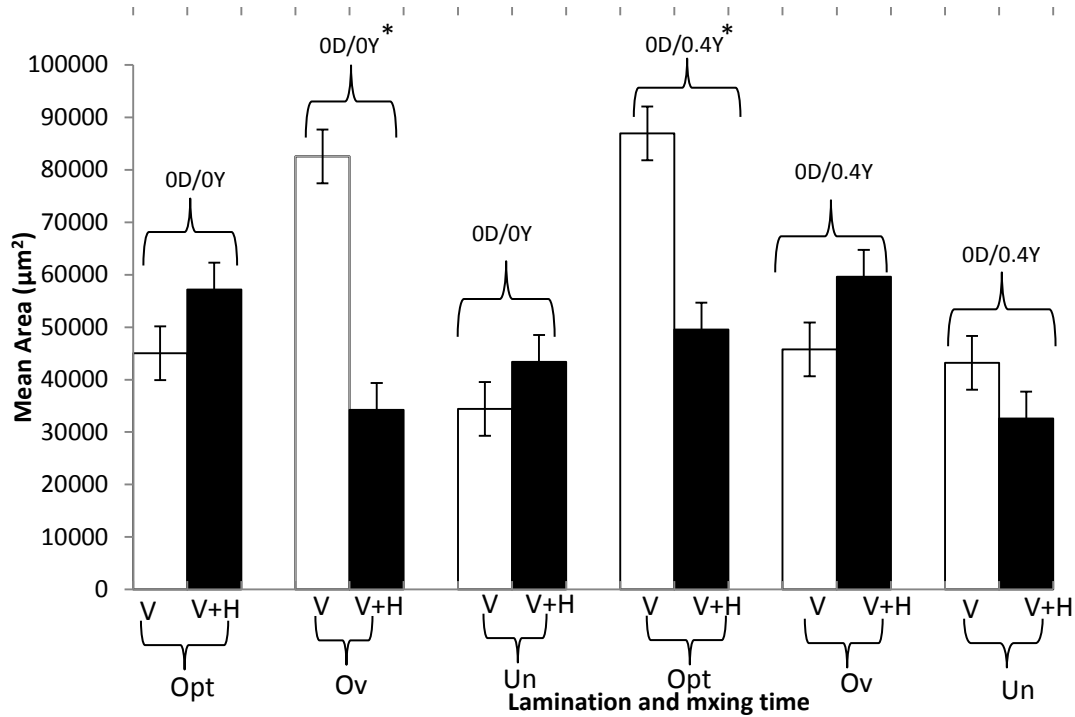


Figure 1. Mean area of air bubbles in dough comparing two lamination treatments (Vertical (V) and Vertical+ Horizontal (V+H)) for a given level of DATEM (0%) and yeast (0% and 0.4%) and mixing times (under-, optimum, over-mixed, 7, 10, and 12 min, respectively). Bars are standard error of mean. Mean based in two replicates each measured 50 bubbles. *Significantly different (p<0.05).

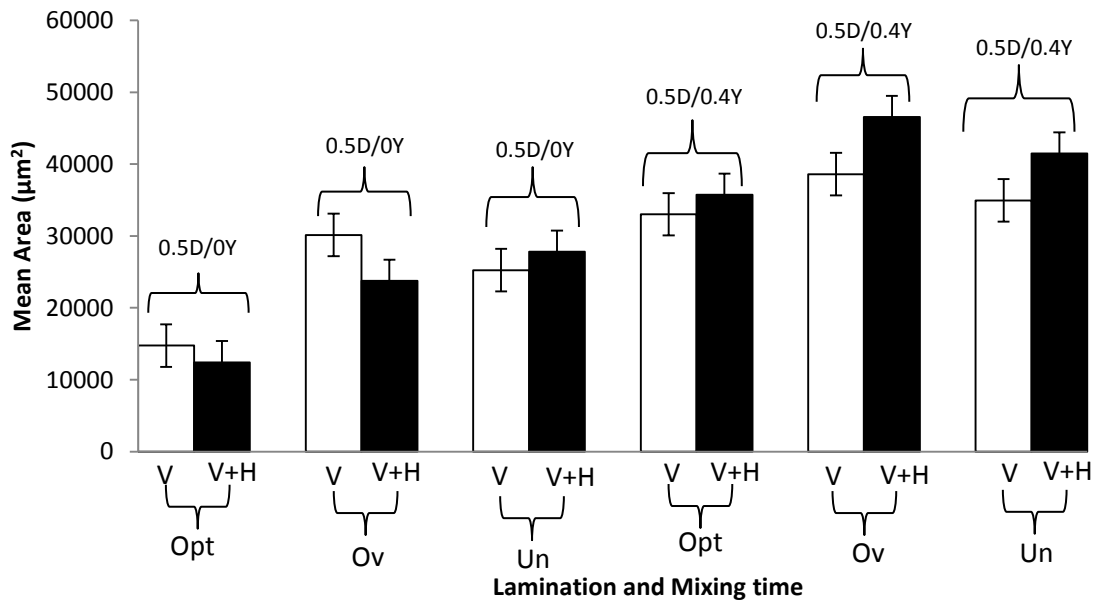


Figure 2. Mean area of air bubbles in dough comparing two lamination treatments (Vertical (V) and Vertical + Horizontal (V+H) direction) for a given level of DATEM (0.5%) yeast (0% and 0.4%) and mixing time (under-, optimum, and over-mixed 7, 10 and 12 min, respectively). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles.

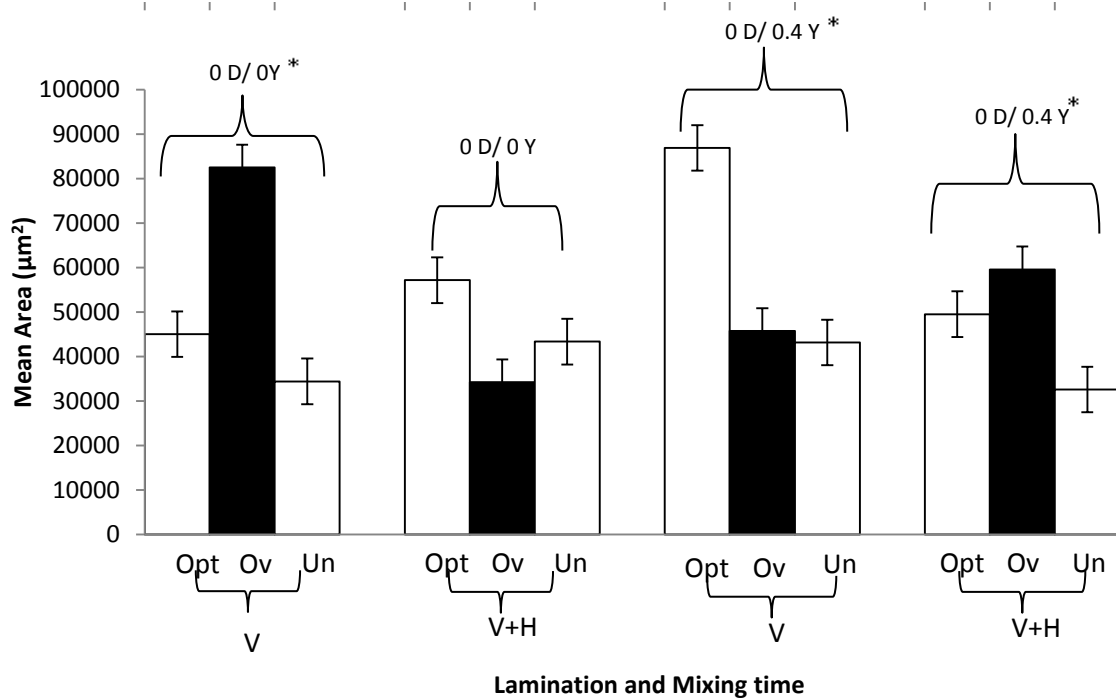


Figure 3. Mean areas of air bubbles in dough comparing mixing times (under, optimum, and over-mixed 7, 10, and 12 min) for a given level of DATEM (0%) and yeast (0% and 0.4%) and lamination treatments (Vertical (V) and Vertical + Horizontal (V+H)). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles. *Significantly different (p<0.05).

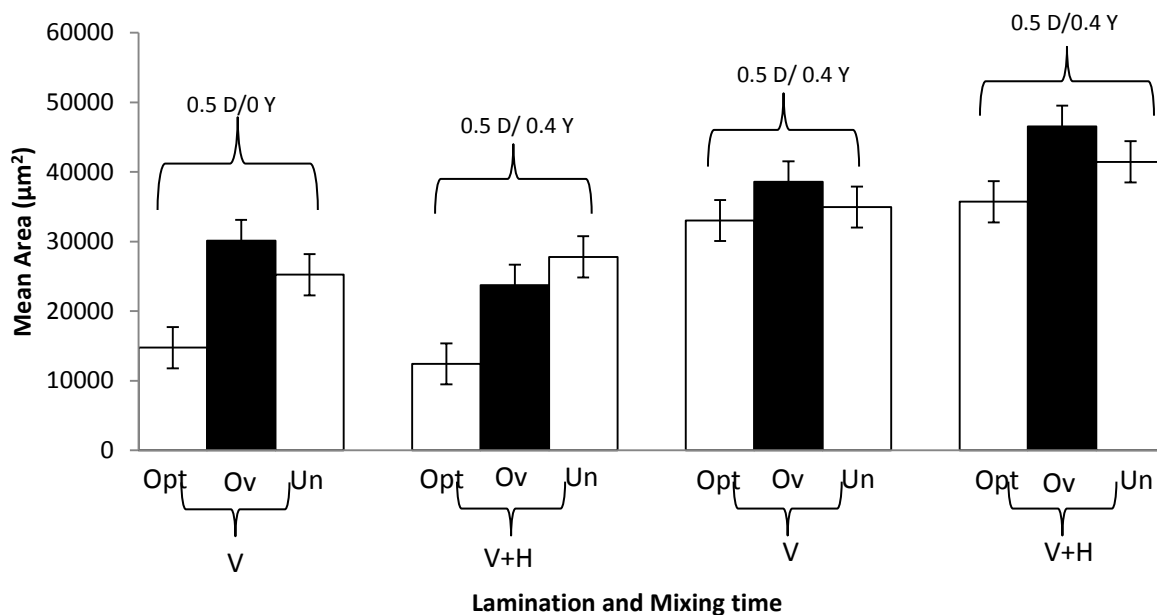


Figure 4. Mean areas of air bubbles in dough comparing mixing times (under, optimum, and over-mixed 7, 10, and 12 min) for a given level of DATEM (0.5%) and yeast (0% and 0.4%) and lamination treatments (Vertical (V) and Vertical + Horizontal (V+H)). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles. *Significantly different (p<0.05).

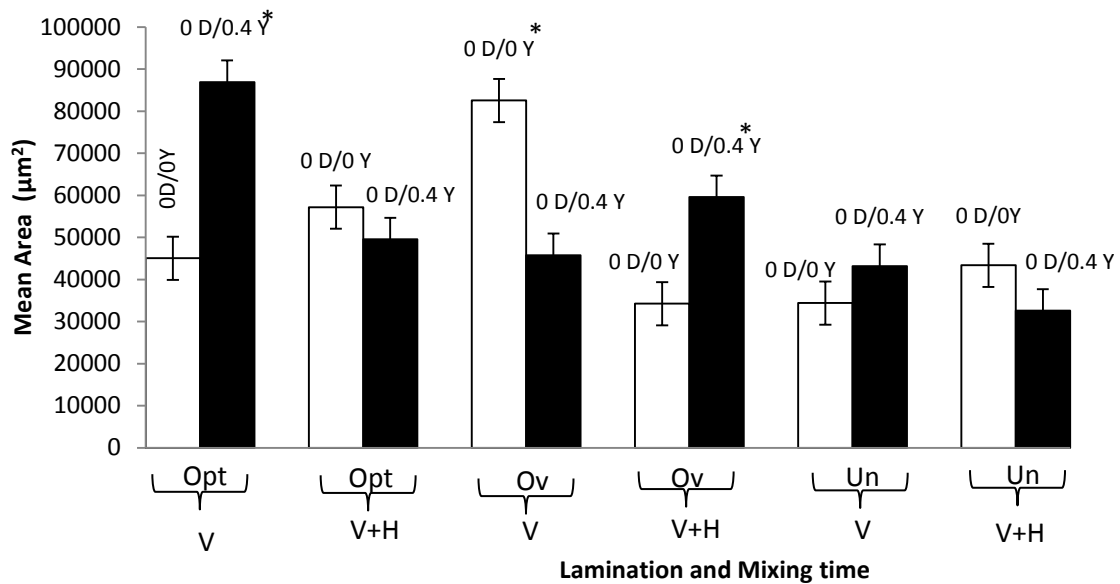


Figure 5. Mean Area of air bubbles in dough comparing two similar lamination treatments (Vertical (V) and Vertical+Horizontal (V+H) direction) and similar mixing time (under-, optimum, and over-mixed 7, 10, and 12 min, respectively) for given levels of DATEM (0%) and yeast (0% and 0.4%). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles. *Significantly different ($p < 0.05$).

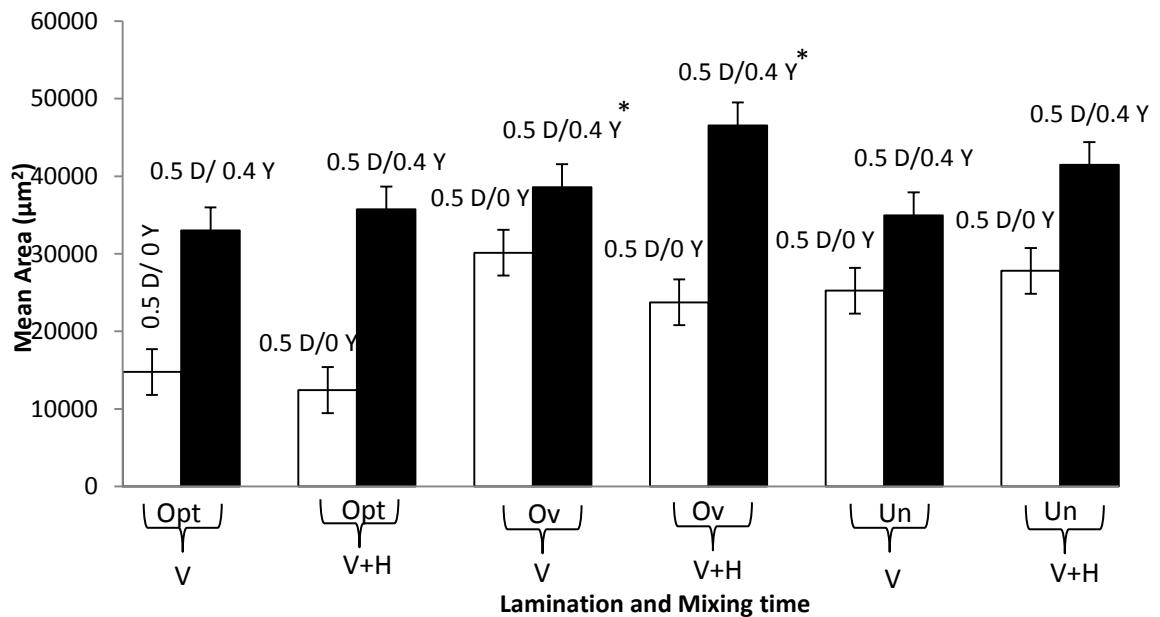


Figure 6. Mean Area of air bubbles in dough comparing two similar lamination treatments (Vertical (V) and Vertical + Horizontal (V+H) direction) and similar mixing time (under-, optimum, and over-mixed, 7, 10, and 12 min, respectively) for a given level of DATEM (0.5%) and yeast (0% and 0.4%). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles. *Significantly different ($p < 0.05$).

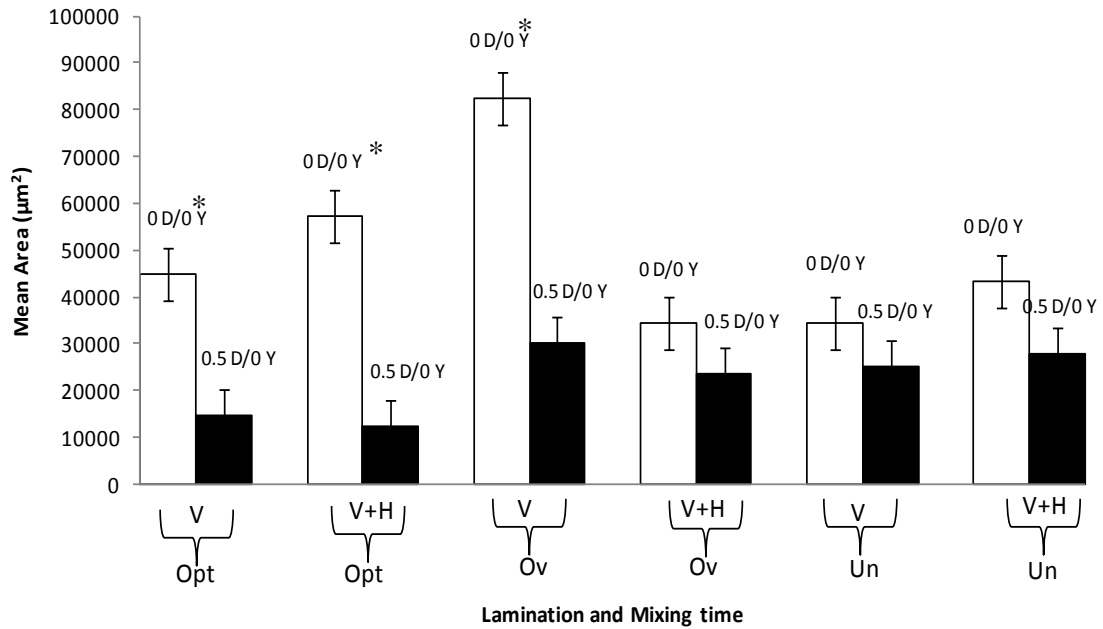


Figure 7. Mean area of air bubbles in dough with the effects of DATEM (0% and 0.5%) with a given level of yeast (0%) in comparing lamination treatment (Vertical (V) and Vertical + Horizontal (V+H) direction) and mixing times (under-, optimum, and over-mixed, 7, 10, and 12 min, respectively). Bars are standard error of mean. Mean area in two replicate each measuring 50 bubbles. *Significantly different ($p < 0.05$).

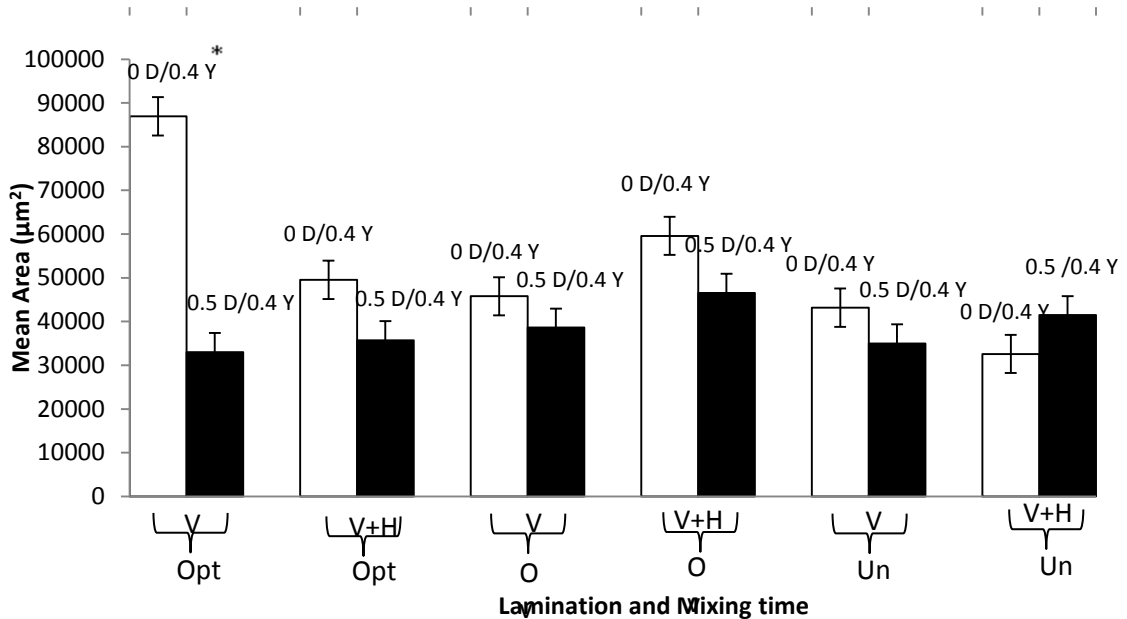


Figure 8. Mean area of air bubbles in dough with the effects of DATEM (0% and 0.5%) with a given level of yeast (0.4%) in comparing lamination treatment (Vertical (V) and Vertical + Horizontal (V+H) direction) and mixing times (under-, optimum, and over-mixed, 7, 10, and 12 min, respectively). Bars are standard error of mean. Mean based in two replicates each measured 50 bubbles. *Significantly different ($p < 0.05$).

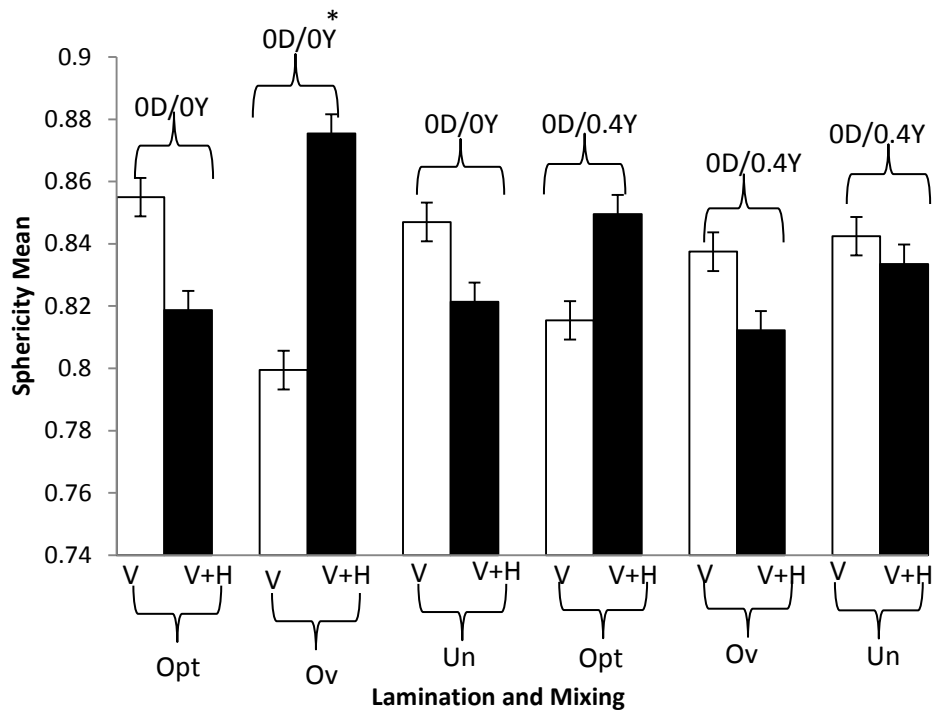


Figure 9. Sphericity mean of air bubbles in dough comparing the effects two lamination treatments (Vertical (V) and Vertical + Horizontal (V+H)) for a given level of DATEM (0% and 0.4%) and yeast (0% and 0.4%) and mixing times (under-, optimum, over-mixed, 7, 10, and 12 minutes, respectively). Bars are standard error of mean. Mean based in two replicates each measured 50 bubbles. *Significantly different ($p < 0.05$).

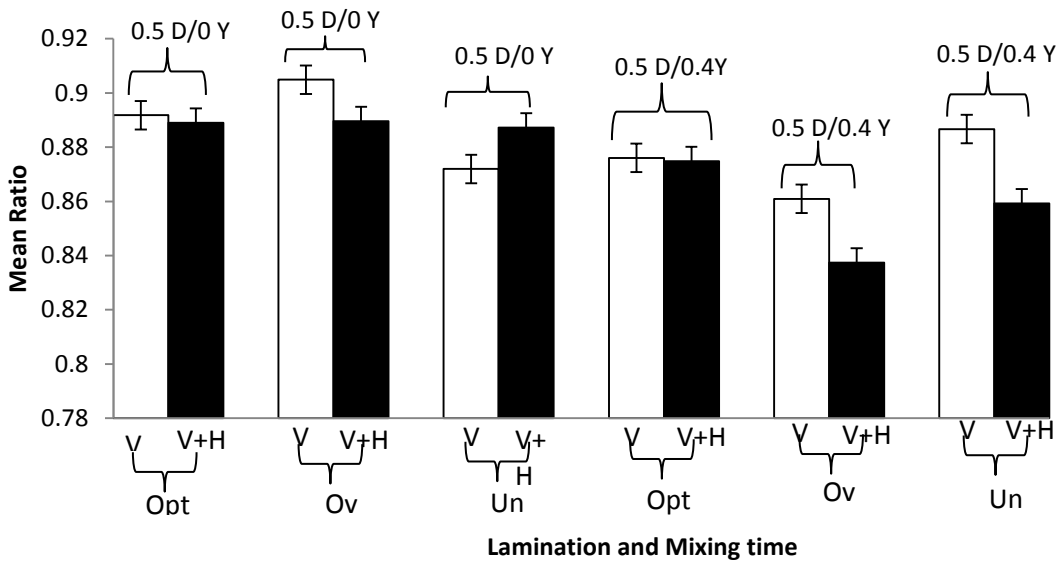


Figure 10. Sphericity mean of air bubbles in dough comparing the effects comparing two lamination treatments (Vertical (V) and Vertical + Horizontal (V+H)) for a given level of DATEM (0% and 0.4%) and yeast (0% and 0.4%) and mixing times (under-, optimum, over-mixed, 7, 10, and 12 minutes, respectively). Bars are standard error of mean. Mean based in two replicates each measured 50 bubbles.

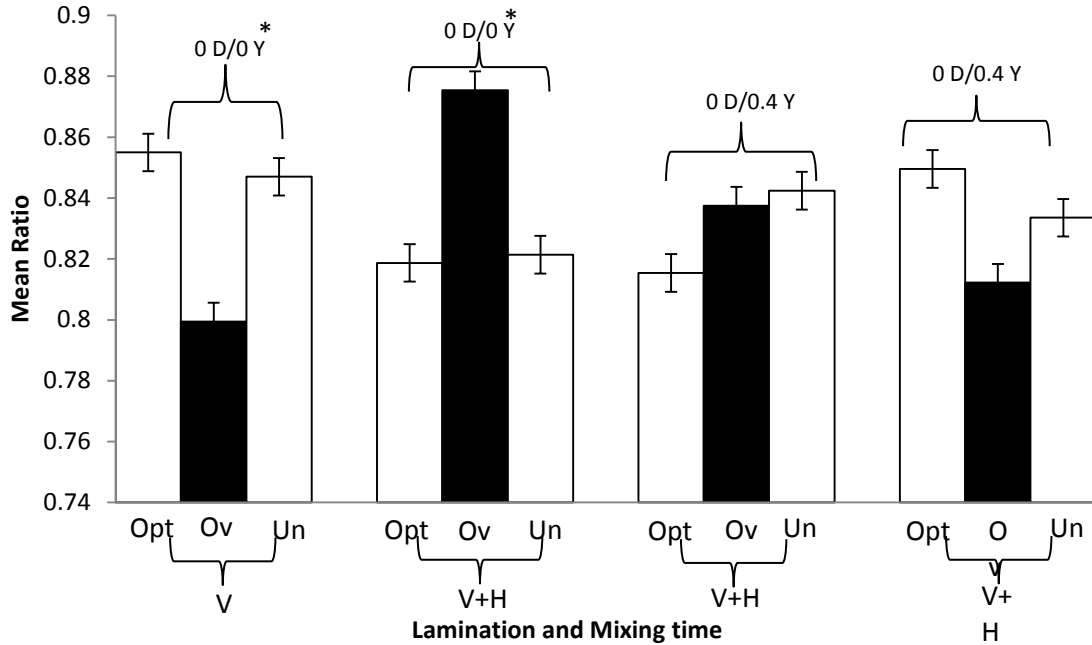


Figure 11. Sphericity mean of air bubbles in dough comparing the effects of mixing times (under-, optimum, and over-mixed, 7, 10, and 12 min, respectively) with given levels of DATEM (0%), yeast (0% and 0.4%), and lamination treatments (Vertical (V) and Vertical + Horizontal (V+H) direction). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles. *Significantly different (p<0.05).

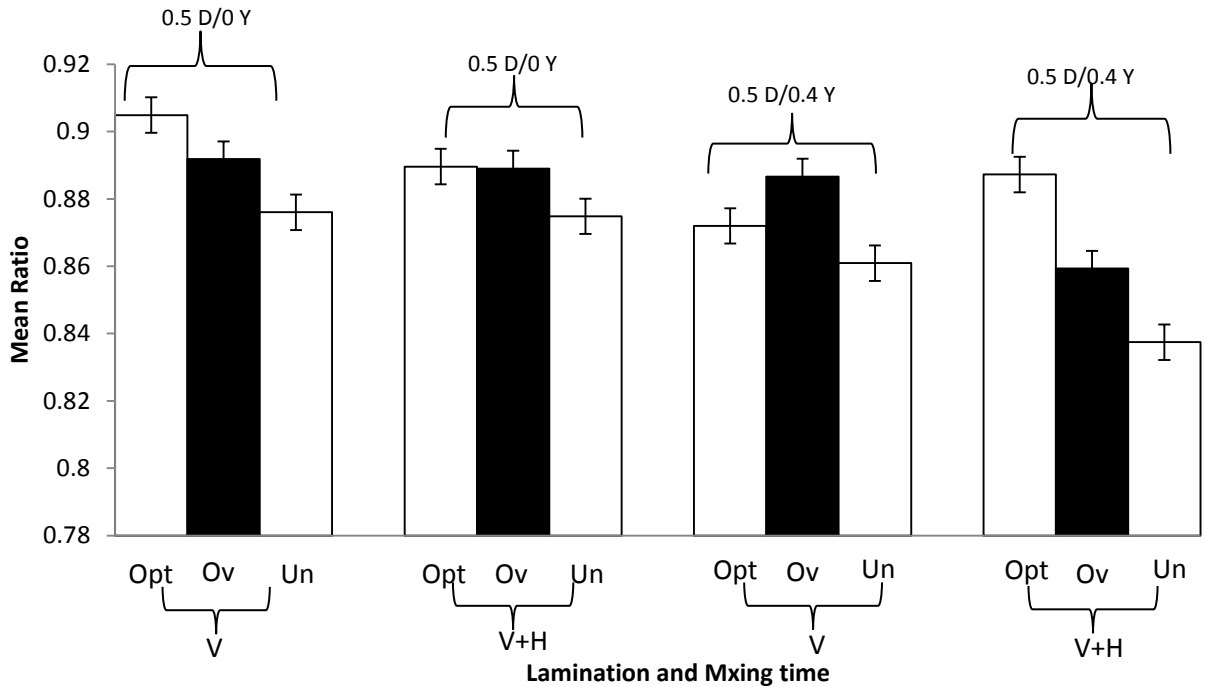
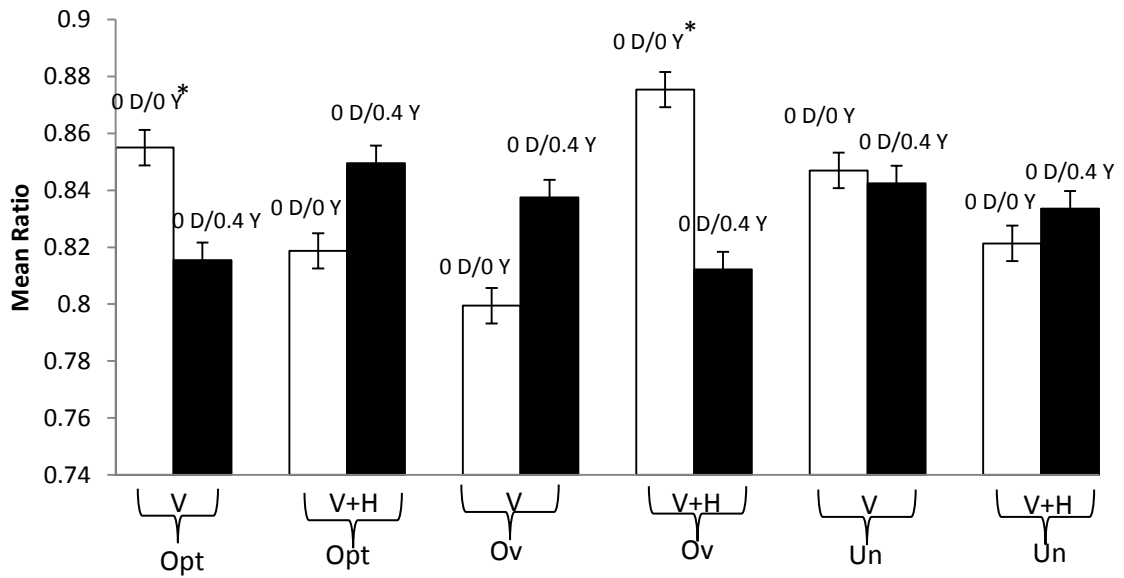
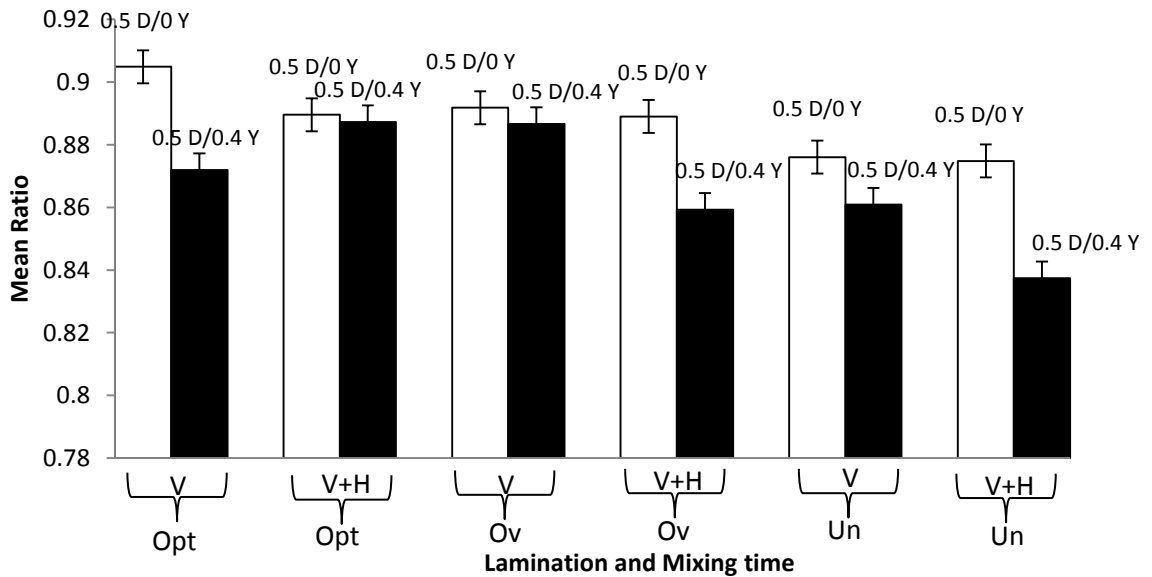


Figure 12. Sphericity mean of air bubbles in dough comparing the effects of mixing times (under-, optimum, and over-mixed, 7, 10, and 12 min, respectively) with given levels of DATEM (0.5%), yeast (0% and 0.4%), and lamination treatments (Vertical (V) and Vertical + Horizontal (V+H) direction). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles.



Lamination and Mixing time

Figure 13. Sphericity mean of air bubbles in bread dough comparing the effects of yeast (0% and 0.4%) and given levels of DATEM (0%) on lamination treatments (Vertical (V) and Vertical + Horizontal (V+H) direction) and mixing times (under-, optimum, and over-mixed, 7, 10, 12 min, respectively). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles. *Significantly different (p<0.05).



Lamination and Mixing time

Figure 14. Sphericity mean of air bubbles in bread dough comparing the effects of yeast (0% and 0.4%) and given levels of DATEM (0.5%) on lamination treatments (Vertical (V) and Vertical + Horizontal (V+H) direction) and mixing times (under-, optimum, and over-mixed, 7, 10, 12 min, respectively). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles.

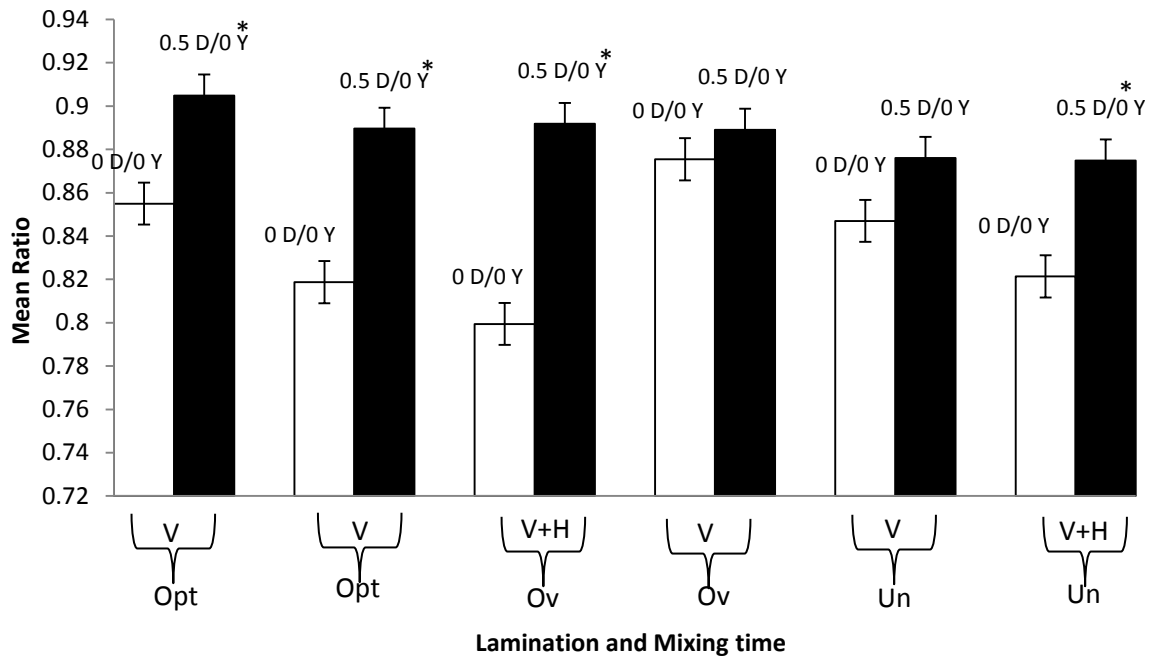


Figure 15. Sphericity mean of air bubbles in bread dough the effect of DATEM (0% and 0.5%) and a given level of yeast (0%) compared to similar lamination treatments (Vertical (V) and Vertical + Horizontal (V+H) direction) and mixing times (under-, optimum, and over-mixed, 7, 10, and 12 min, respectively). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles. *Significantly different ($p < 0.05$).

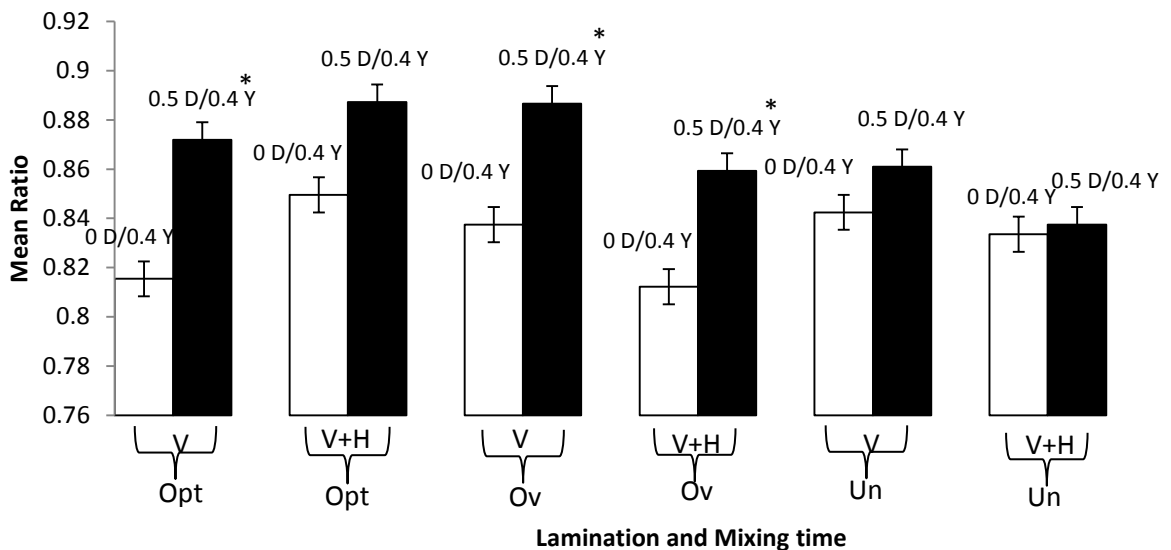


Figure 16. Sphericity mean of air bubbles in bread dough the effect of DATEM (0% and 0.5%) and a given level of yeast (0.4%) compared to similar lamination treatments (Vertical (V) and Vertical + Horizontal (V+H) direction) and mixing times (under-, optimum, and over-mixed, 7, 10, and 12 min, respectively). Bars are standard error of the mean. Mean based in two replicates each measured 50 bubbles. *Significantly different ($p < 0.05$).

CHAPTER IV

CONCLUSIONS

Overall, lamination affects the area mean of air bubbles in selected treatments and a trend suggested that vertical lamination produce large air bubbles in dough without DATEM or yeast. When dough is laminated in the vertical direction followed by horizontal direction, the process should align the protein fibril strands and assist in helping to control bubble diameter size. Although, effects of lamination were not significant in dough with DATEM and yeast, there was an overall trend suggesting a slightly smaller air bubble area mean when dough was laminated in the vertical + horizontal direction compared to a vertical lamination. When dough had DATEM (0.5%) and with or without yeast, the effect of mixing had no significant change in the air bubble area mean. Overall, yeast appears to affect the air bubble area mean of dough by increasing it in a number of treatments. This suggests that limited but detectable fermentation took place. Overall, a positive effect of DATEM was observed on the air bubble area by reducing it in the majority of the treatments. The sphericity of air bubbles was higher in the lamination vertical + horizontal direction applied to dough without DATEM and yeast, and over-mixed. The sphericity of air bubbles was limitedly affected by lamination and affected by mixing. Limited effect of lamination on sphericity suggests that lamination of vertical + horizontal direction promotes sphericity of air bubbles. Over-mixing with vertical + horizontal lamination of the dough produces more

spherical bubbles. Yeast produces less spherical air bubbles while DATEM promotes spherical bubbles. The study has shown that lamination, mixing time, and level of DATEM and yeast do have some effect on the bubble area in dough. The knowledge that we have obtained from this study can be utilized in the future studies to observe if the fermentation time will show changes in air bubble area mean and sphericity. Although some treatments showed no significant differences in a number of the treatments, many trends were observed within the analysis. For example, more spherical and smaller bubbles were observed when DATEM was present. When yeast was present the bubbles were less spherical and the area mean of the bubble was larger. This study also showed that when dough was laminated in the second direction (vertical + horizontal) that bubbles were more spherical than in the first vertical direction.

CHAPTER V

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CHAPTER VI

FUTURE RESEARCH

- Suggestion of further studies could be to compare the 1 hour fermentation analysis, not used because there was no significant interaction, to this study to determine if there are differences between the control (zero fermentation) and 1 hour fermentation time.
- Monitor the temperature and time of pre- and post-preparations of the dough, during the transportation of the dough from the two labs (mixing and microscopy labs). Although, precautions were taken to prevent fermentation by storing the samples at 0 to 4°C, a more strict control of time and temperature is suggested during dough preparation.

APPENDIXES

APPENDIX I

Table 1. List of abbreviations of treatments and variables measured

Abbr.	Unit	Treatment/Response variables
DATEM	%	Diacetyl (Tartaric (Acid) Ester of Mono and diglycerides
Yeast	%	Instant dry yeast (Baker's yeast)
LAM	-	Lamination
V+H	-	Vertical + Horizontal
V	-	Vertical
Opt	-	Optimum mixing, 10 min
Ov	-	Over-mixed, 12 min
Un	-	Under-mixed, 7 min
MNAREA	μm^2	Mean area of air bubbles in dough
MNSphericity	-	Mean ratio of air bubbles in dough
Std Error	μm^2	Standard error of area

-, no units.

Table 2. Comparison of area mean of air bubbles in dough for two lamination treatments (LAM) consisting in vertical (V) and vertical + horizontal (V+H) direction for a given level of DATEM, yeast or mixing time (Opt) optimum, (Ov) over-mixed, and (Un) under-mixed.

DATEM (%)	Yeast (%)	Mixing Time	LAM	MNAREA μm^2	Std Error	PVALUE
0.0	0.0	Opt	V	45045 a	4722	
0.0	0.0	Opt	V+H	57170 a	8806	0.2511
0.0	0.0	Ov	V	82539 a	8613	
0.0	0.0	Ov	V+H	34257 b	2972	<.0001
0.0	0.0	Un	V	34412 a	4388	
0.0	0.0	Un	V+H	43370 a	4086	0.3955
0.0	0.4	Opt	V	86921 a	9882	
0.0	0.4	Opt	V+H	49523 b	5025	0.0006
0.0	0.4	Ov	V	45773 a	3933	
0.0	0.4	Ov	V+H	59592 a	5512	0.1915
0.0	0.4	Un	V	43187 a	4512	
0.0	0.4	Un	V+H	32590 a	2795	0.3153
0.5	0.0	Opt	V	14757 a	1388	
0.5	0.0	Opt	V+H	12425 a	1816	0.8246
0.5	0.0	Ov	V	30143 a	2281	
0.5	0.0	Ov	V+H	23747 a	2216	0.5436
0.5	0.0	Un	V	25237 a	3085	
0.5	0.0	Un	V+H	27803 a	2785	0.8073
0.5	0.4	Opt	V	33019 a	3253	
0.5	0.4	Opt	V+H	35716 a	3065	0.7976
0.5	0.4	Ov	V	38598 a	4402	
0.5	0.4	Ov	V+H	46556 a	4038	0.4501
0.5	0.4	Un	V	34949 a	3654	
0.5	0.4	Un	V+H	41459 a	3695	0.5364

*Means within columns with different letter were significantly different (p<0.05).

Table 3. Comparisons of air bubbles area mean of mixing time (Opt) optimum, (Ov) over-mixed, and (Un) under-mixed given levels of DATEM, yeast and lamination treatments (LAM) consisting in vertical (V) and vertical + horizontal (V+H) direction. Significant comparisons are highlighted.

	DATEM (%)	Yeast (%)	LAM	Mixing Time	MNAREA μm^2	Std Error	PVALUE
	0.0	0.0	V	Opt	45045 b	4722	<.0001
	0.0	0.0	V	Ov	82539 a	8613	
	0.0	0.0	V	Un	34412 b	4388	
	0.0	0.0	V+H	Opt	57170 a	8806	0.0959
	0.0	0.0	V+H	Ov	34257 a	2972	
	0.0	0.0	V+H	Un	43370 a	4086	
	0.0	0.4	V	Opt	86921 a	9882	<.0001
	0.0	0.4	V	Ov	45773 b	3933	
	0.0	0.4	V	Un	43187 b	4512	
	0.0	0.4	V+H	Opt	49523 ab	5025	0.0392
	0.0	0.4	V+H	Ov	59592 a	5512	
	0.0	0.4	V+H	Un	32590 b	2795	
	0.5	0.0	V	Opt	14757 a	1388	0.3303
	0.5	0.0	V	Ov	30143 a	2281	
	0.5	0.0	V	Un	25237 a	3085	
	0.5	0.0	V+H	Opt	12425 a	1816	0.3203
	0.5	0.0	V+H	Ov	23747 a	2216	
	0.5	0.0	V+H	Un	27803 a	2785	
	0.5	0.4	V	Opt	33019 a	3253	0.8642
	0.5	0.4	V	Ov	38598 a	4402	
	0.5	0.4	V	Un	34949 a	3654	
	0.5	0.4	V+H	Opt	35716 a	3065	0.5876
	0.5	0.4	V+H	Ov	46556 a	4038	
	0.5	0.4	V+H	Un	41459 a	3695	

*Means within columns with different letter were significantly different ($p < 0.05$).

Table 4. Comparisons of air bubbles area mean of yeast (0 vs. 0.4%) for given levels of DATEM, mixing time (Opt) optimum, (Ov) over-mixed, and (Un) under-mixed, and lamination treatments (LAM) consisting in vertical (V) and vertical + horizontal direction (V+H). Significant comparisons are highlighted.

	DATEM (%)	Mixing Time	LAM	Yeast (%)	MNAREA μm^2	Std Error	PVALUE
	0.0	Opt	V	0.0	45045 b	4722	0.0002
	0.0	Opt	V	0.4	86921 a	9882	
	0.0	Opt	V+H	0.0	57170 a	8806	0.4680
	0.0	Opt	V+H	0.4	49523 a	5025	
	0.0	Ov	V	0.0	82539 a	8613	0.0008
	0.0	Ov	V	0.4	45773 b	3933	
	0.0	Ov	V+H	0.0	34257 b	2972	0.0182
	0.0	Ov	V+H	0.4	59592 a	5512	
	0.0	Un	V	0.0	34412 a	4388	0.4052
	0.0	Un	V	0.4	43187 a	4512	
	0.0	Un	V+H	0.0	43370 a	4086	0.3071
	0.0	Un	V+H	0.4	32590 a	2795	
	0.5	Opt	V	0.0	14757 a	1388	0.0857
	0.5	Opt	V	0.4	33019 a	3253	
	0.5	Opt	V+H	0.0	12425 b	1816	0.0294
	0.5	Opt	V+H	0.4	35716 a	3065	
	0.5	Ov	V	0.0	30143 a	2281	0.4224
	0.5	Ov	V	0.4	38598 a	4402	
	0.5	Ov	V+H	0.0	23747 b	2216	0.0328
	0.5	Ov	V+H	0.4	46556 a	4038	
	0.5	Un	V	0.0	25237 a	3085	0.3571
	0.5	Un	V	0.4	34949 a	3654	
	0.5	Un	V+H	0.0	27803 a	2785	0.1967
	0.5	Un	V+H	0.4	41459 a	3695	

*Means within columns with different letter were significantly different (p<0.05).

Table 5. Comparison of air bubbles area mean of levels of DATEM (0 vs. 0.5%) for a given level of yeast, mixing time (Opt) optimum, (Ov) over-mixed, and (Un) under-mixed and lamination treatment (LAM) consisting in vertical (V) and vertical + horizontal direction (V+H). Significant comparisons are highlighted.

	Yeast (%)	Mixing Time	LAM	DATEM (%)	MNAREA μm^2	Std Error	PVALUE
	0.0	Opt	V	0.0	45045 a	4722	0.0051
	0.0	Opt	V	0.5	14757 b	1388	
	0.0	Opt	V+H	0.0	57170 a	8806	<.0001
	0.0	Opt	V+H	0.5	12425 a	1816	
	0.0	Ov	V	0.0	82539 a	8613	<.0001
	0.0	Ov	V	0.5	30143 b	2281	
	0.0	Ov	V+H	0.0	34257 a	2972	0.3193
	0.0	Ov	V+H	0.5	23747 a	2216	
	0.0	Un	V	0.0	34412 a	4388	0.3842
	0.0	Un	V	0.5	25237 a	3085	
	0.0	Un	V+H	0.0	43370 a	4086	0.1418
	0.0	Un	V+H	0.5	27803 a	2785	
	0.4	Opt	V	0.0	86921 a	9882	<.0001
	0.4	Opt	V	0.5	33019 b	3253	
	0.4	Opt	V+H	0.0	49523 a	5025	0.1918
	0.4	Opt	V+H	0.5	35716 a	3065	
	0.4	Ov	V	0.0	45773 a	3933	0.4957
	0.4	Ov	V	0.5	38598 a	4402	
	0.4	Ov	V+H	0.0	59592 a	5512	0.2176
	0.4	Ov	V+H	0.5	46556 a	4038	
	0.4	Un	V	0.0	43187 a	4512	0.4344
	0.4	Un	V	0.5	34949 a	3654	
	0.4	Un	V+H	0.0	32590 a	2795	0.4002
	0.4	Un	V+H	0.5	41459 a	3695	

*Means within columns with different letter were significantly different (p<0.05).

Table 6. Comparison of air bubbles sphericity mean of lamination treatment (LAM) consisting in vertical (V) and vertical + horizontal direction (V+H), for given levels of DATEM, yeast and mixing time (Opt) optimum, (Ov) over-mixed, and (Un) under-mixed. Significant comparisons are highlighted.

DATEM (%)	Yeast (%)	Mixing Time	LAM	MNSphericity	Std Error	PVALUE
0.0	0.0	Opt	V	0.85499 a	0.008259	
0.0	0.0	Opt	V+H	0.81871 a	0.012553	0.0709
0.0	0.0	Ov	V	0.79944 b	0.010450	
0.0	0.0	Ov	V+H	0.87543 a	0.007164	0.0003
0.0	0.0	Un	V	0.84700 a	0.009553	
0.0	0.0	Un	V+H	0.82136 a	0.012266	0.1992
0.0	0.4	Opt	V	0.81544 a	0.010394	
0.0	0.4	Opt	V+H	0.84954 a	0.009098	0.0892
0.0	0.4	Ov	V	0.83747 a	0.009561	
0.0	0.4	Ov	V+H	0.81221 a	0.009339	0.2059
0.0	0.4	Un	V	0.84246 a	0.008746	
0.0	0.4	Un	V+H	0.83354 a	0.009573	0.6535
0.5	0.0	Opt	V	0.90489 a	0.005101	
0.5	0.0	Opt	V+H	0.88958 a	0.006134	0.4417
0.5	0.0	Ov	V	0.89180 a	0.006436	
0.5	0.0	Ov	V+H	0.88901 a	0.006328	0.8882
0.5	0.0	Un	V	0.87604 a	0.007152	
0.5	0.0	Un	V+H	0.87484 a	0.007621	0.9518
0.5	0.4	Opt	V	0.87198 a	0.007708	
0.5	0.4	Opt	V+H	0.88726 a	0.006840	0.4425
0.5	0.4	Ov	V	0.88666 a	0.006604	
0.5	0.4	Ov	V+H	0.85930 a	0.008825	0.1711
0.5	0.4	Un	V	0.86094 a	0.008623	
0.5	0.4	Un	V+H	0.83745 a	0.009093	0.2391

*Means within columns with different letter were significantly different (p<0.05).

Table 7. Comparisons of air bubbles sphericity mean of mixing time (Opt) optimum, (Ov) over-mixed, and (Un) under-mixed for a given level of DATEM, yeast and lamination treatment (LAM) consisting in vertical (V) and vertical + horizontal direction (V+H). Significant comparisons are highlighted.

DATEM (%)	Yeast (%)	LAM	Mixing Time	MNSphericity	Std Error	PVALUE
0.0	0.0	V	Opt	0.85499 a	0.008259	0.0131
0.0	0.0	V	Ov	0.79944 b	0.010450	
0.0	0.0	V	Un	0.84700 a	0.009553	
0.0	0.0	V+H	Opt	0.81871 b	0.012553	0.0076
0.0	0.0	V+H	Ov	0.87543 a	0.007164	
0.0	0.0	V+H	Un	0.82136 b	0.012266	
0.0	0.4	V	Opt	0.81544 a	0.010394	0.3533
0.0	0.4	V	Ov	0.83747 a	0.009561	
0.0	0.4	V	Un	0.84246 a	0.008746	
0.0	0.4	V+H	Opt	0.84954 a	0.009098	0.1742
0.0	0.4	V+H	Ov	0.81221 a	0.009339	
0.0	0.4	V+H	Un	0.83354 a	0.009573	
0.5	0.0	V	Opt	0.90489 a	0.005101	0.3498
0.5	0.0	V	Ov	0.89180 a	0.006436	
0.5	0.0	V	Un	0.87604 a	0.007152	
0.5	0.0	V+H	Opt	0.88958 a	0.006134	0.7016
0.5	0.0	V+H	Ov	0.88901 a	0.006328	
0.5	0.0	V+H	Un	0.87484 a	0.007621	
0.5	0.4	V	Opt	0.87198 a	0.007708	0.4312
0.5	0.4	V	Ov	0.88666 a	0.006604	
0.5	0.4	V	Un	0.86094 a	0.008623	
0.5	0.4	V+H	Opt	0.88726 a	0.006840	0.0473
0.5	0.4	V+H	Ov	0.85930 ab	0.008825	
0.5	0.4	V+H	Un	0.83745 b	0.009093	

*Means within columns with different letter were significantly different (p<0.05).

Table 8. Comparisons of air bubbles sphericity mean of yeast (0 and 0.4%) for given levels of DATEM, mixing time (Opt) optimum, (Ov) over-mixed, and (Un) under-mixed and lamination treatment (LAM) consisting in vertical (V) and vertical + horizontal direction (V+H). Significant comparisons are highlighted.

DATEM (%)	Mixing Time	LAM	Yeast (%)	MNSphericity	Std Error	PVALUE
0.0	Opt	V	0.0	0.85499 a	0.008259	0.0495
0.0	Opt	V	0.4	0.81544 b	0.010394	
0.0	Opt	V+H	0.0	0.81871 a	0.012553	0.1236
0.0	Opt	V+H	0.4	0.84954 a	0.009098	
0.0	Ov	V	0.0	0.79944 a	0.010450	0.0586
0.0	Ov	V	0.4	0.83747 a	0.009561	
0.0	Ov	V+H	0.0	0.87543 a	0.007164	0.0021
0.0	Ov	V+H	0.4	0.81221 b	0.009339	
0.0	Un	V	0.0	0.84700 a	0.009553	0.8191
0.0	Un	V	0.4	0.84246 a	0.008746	
0.0	Un	V+H	0.0	0.82136 a	0.012266	0.5401
0.0	Un	V+H	0.4	0.83354 a	0.009573	
0.5	Opt	V	0.0	0.90489 a	0.005101	0.1006
0.5	Opt	V	0.4	0.87198 a	0.007708	
0.5	Opt	V+H	0.0	0.88958 a	0.006134	0.9069
0.5	Opt	V+H	0.4	0.88726 a	0.006840	
0.5	Ov	V	0.0	0.89180 a	0.006436	0.7960
0.5	Ov	V	0.4	0.88666 a	0.006604	
0.5	Ov	V+H	0.0	0.88901 a	0.006328	0.1377
0.5	Ov	V+H	0.4	0.85930 a	0.008825	
0.5	Un	V	0.0	0.87604 a	0.007152	0.4477
0.5	Un	V	0.4	0.86094 a	0.008623	
0.5	Un	V+H	0.0	0.87484 a	0.007621	0.0628
0.5	Un	V+H	0.4	0.83745 a	0.009093	

*Means within columns with different letter were significantly different (p<0.05).

Table 9. Comparisons length of air bubbles sphericity mean of DATEM (0 and 0.5%) for given levels of yeast, mixing time (Opt) optimum, (Ov) over-mixed, (Un) Under-mixed and lamination treatments (LAM) consisting in vertical (V) and vertical + horizontal (V+H) direction. Significant comparisons are highlighted.

Yeast (%)	Mixing Time	LAM	DATEM (%)	MNSphericity	Std Error	PVALUE
0.0	Opt	V	0.0	0.85499 b	0.008259	0.0139
0.0	Opt	V	0.5	0.90489 a	0.005101	
0.0	Opt	V+H	0.0	0.81871 b	0.012553	0.0006
0.0	Opt	V+H	0.5	0.88958 a	0.006134	
0.0	Ov	V	0.0	0.79944 b	0.010450	<.0001
0.0	Ov	V	0.5	0.89180 a	0.006436	
0.0	Ov	V+H	0.0	0.87543 a	0.007164	0.4949
0.0	Ov	V+H	0.5	0.88901 a	0.006328	
0.0	Un	V	0.0	0.84700 a	0.009553	0.1465
0.0	Un	V	0.5	0.87604 a	0.007152	
0.0	Un	V+H	0.0	0.82136 b	0.012266	0.0086
0.0	Un	V+H	0.5	0.87484 a	0.007621	
0.4	Opt	V	0.0	0.81544 b	0.010394	0.0056
0.4	Opt	V	0.5	0.87198 a	0.007708	
0.4	Opt	V+H	0.0	0.84954 a	0.009098	0.0606
0.4	Opt	V+H	0.5	0.88726 a	0.006840	
0.4	Ov	V	0.0	0.83747 b	0.009561	0.0153
0.4	Ov	V	0.5	0.88666 a	0.006604	
0.4	Ov	V+H	0.0	0.81221 b	0.009339	0.0200
0.4	Ov	V+H	0.5	0.85930 a	0.008825	
0.4	Un	V	0.0	0.84246 a	0.008746	0.3535
0.4	Un	V	0.5	0.86094 a	0.008623	
0.4	Un	V+H	0.0	0.83354 a	0.009573	0.8440
0.4	Un	V+H	0.5	0.83745 a	0.009093	

*Means within columns with different letter were significantly different (p<0.05).

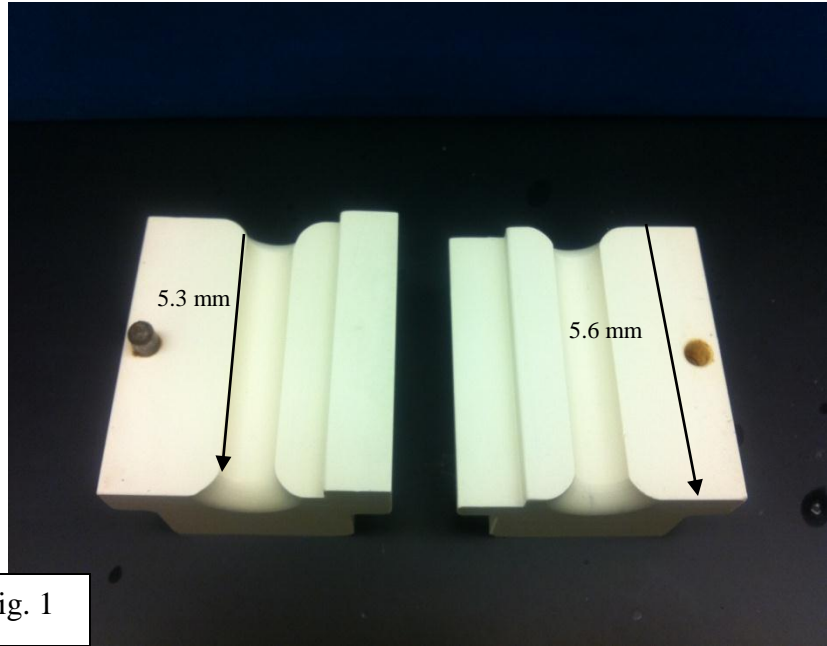


Fig. 1

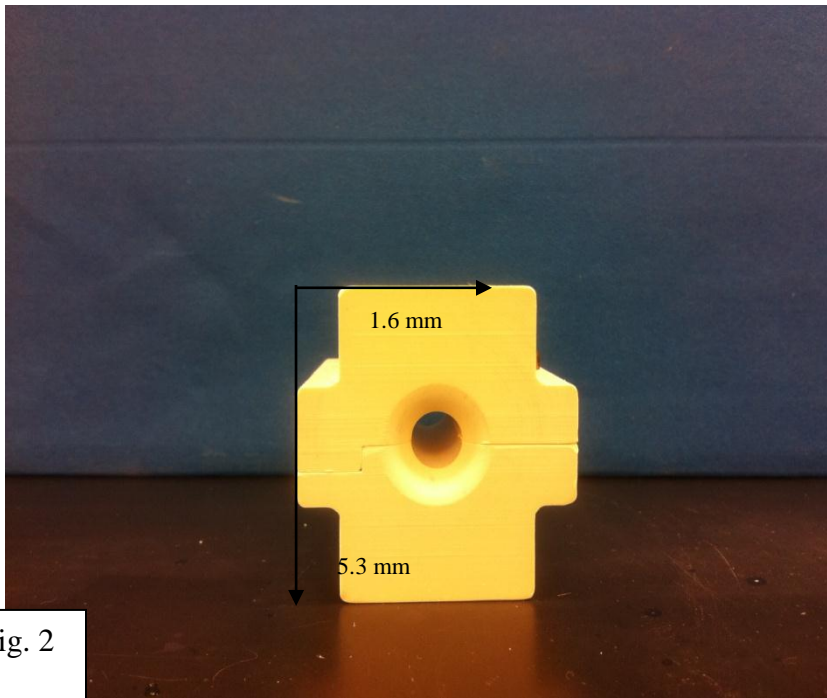


Fig. 2

Figure 1 and 2. Images of mold use to hold and form the dough after lamination. Diameter of hollow area on the center of mold is 1.6 mm.

APPENDIX II

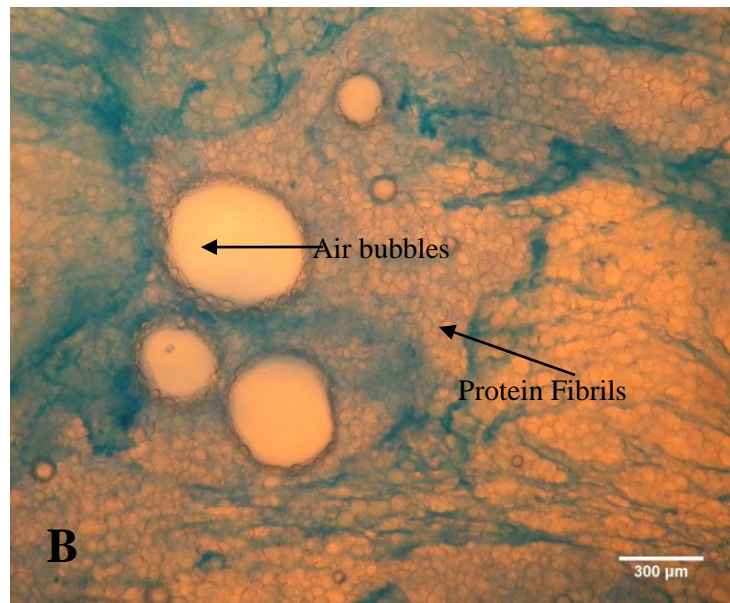
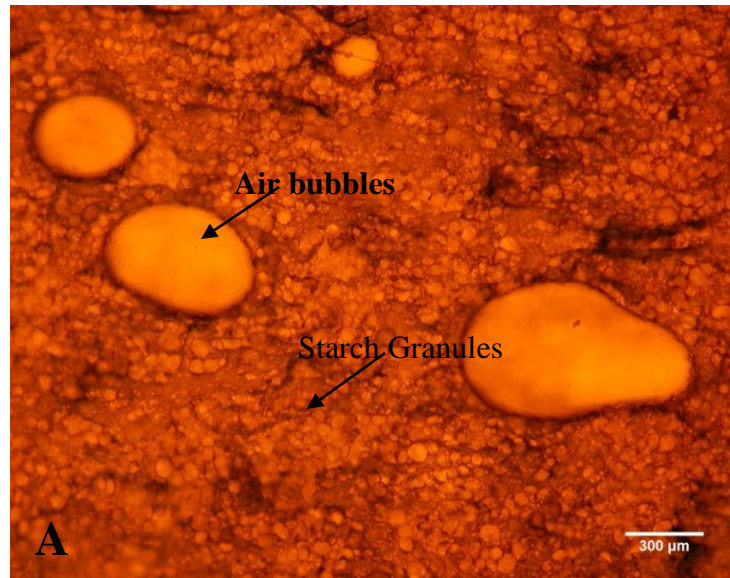


Figure A-B. Example of images of air bubbles, starch granules, and protein fibrils in dough at optimum mixing time with lamination treatments of vertical (V) (Fig.A) and vertical + horizontal (V+H) direction (Fig. B), with no DATEM 0% and yeast 0%.

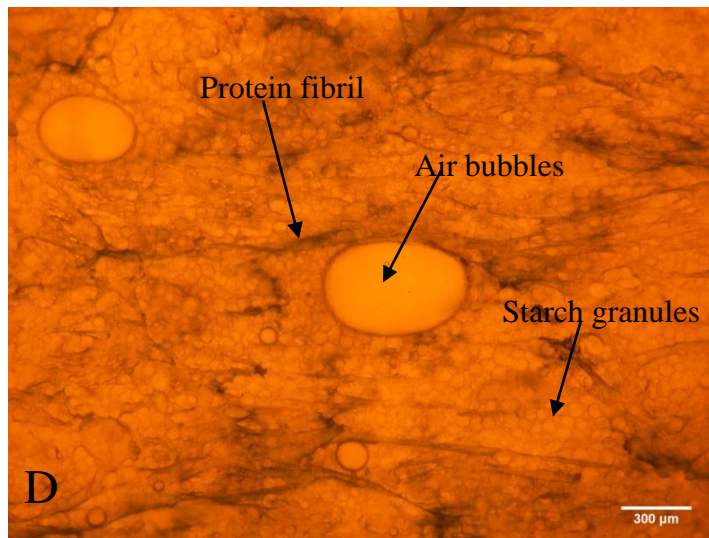
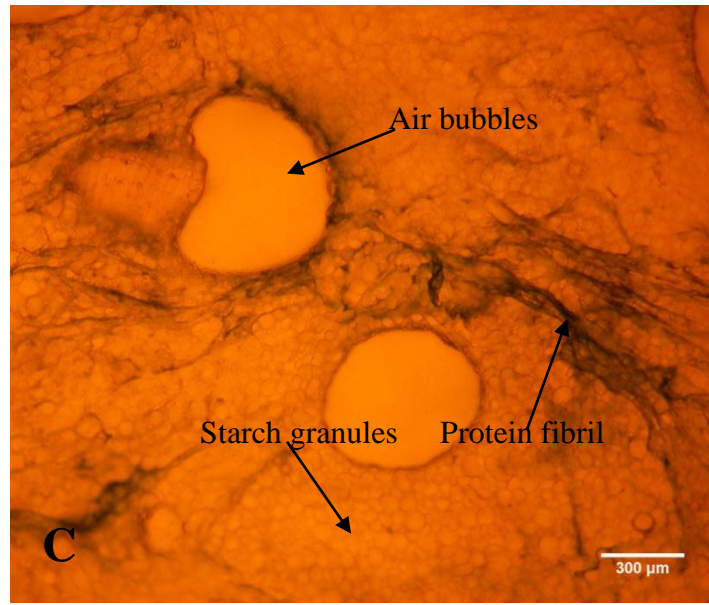


Figure C-D. Examples of images of air bubbles, starch granules, and protein fibrils in dough at optimum mixing time with lamination treatments of vertical (V) (Fig.C) and vertical + horizontal (V+H) (Fig. D), with a given level DATEM 0.5% and 0% yeast.

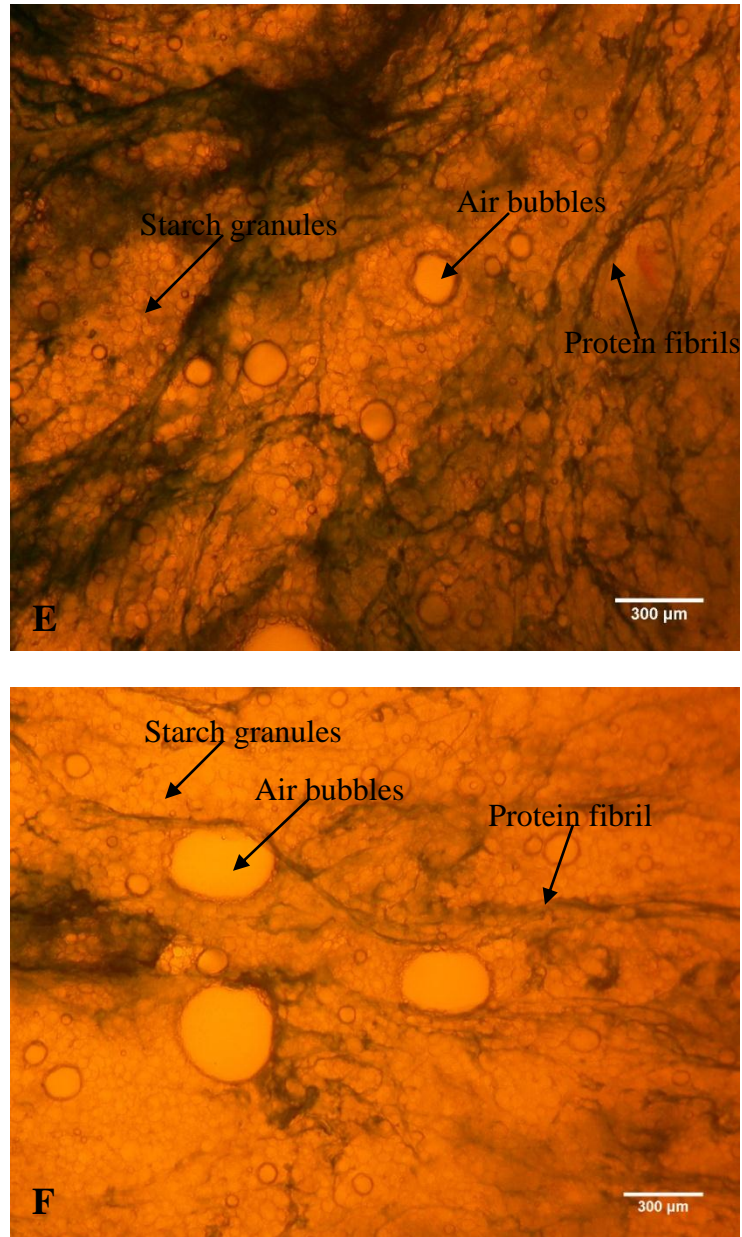


Figure E-F. Examples of images of air bubbles, starch granules, and protein fibrils in dough at optimum mixing time with lamination treatments of vertical (V) (Fig. E) and vertical + horizontal (V+H) (Fig. F), that has a given level DATEM 0% and 0.4% yeast.

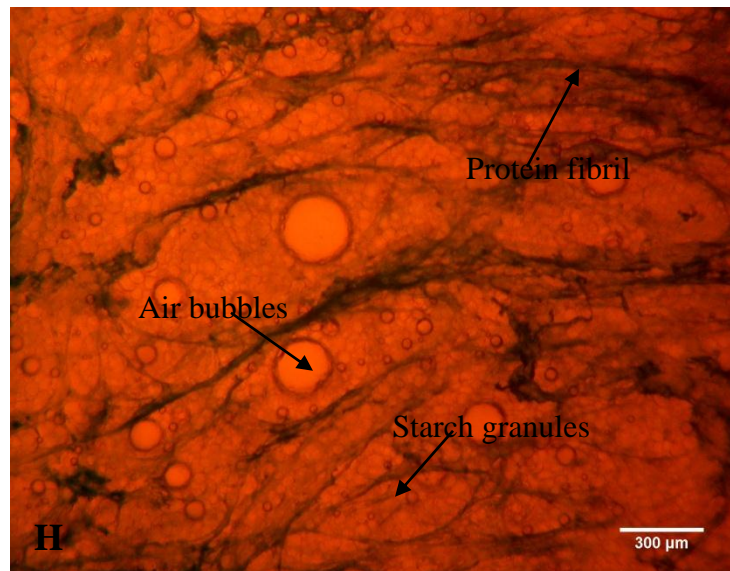
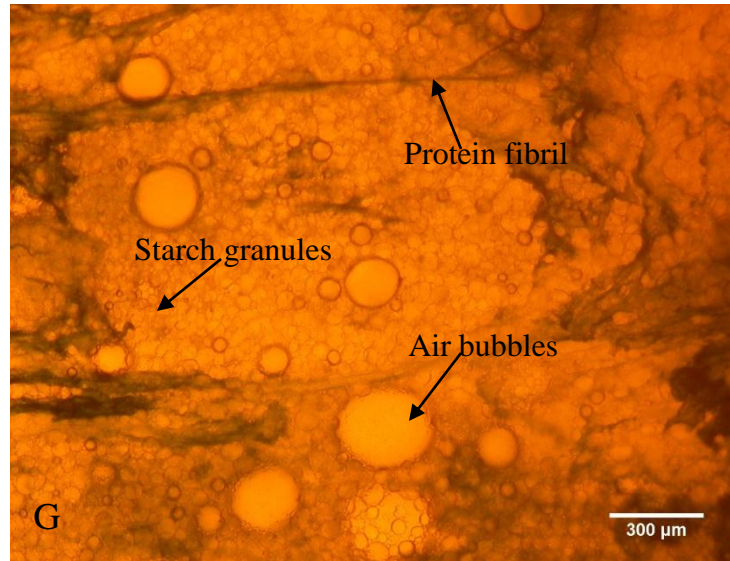


Figure G-H. Example of images of air bubbles, starch granules, and protein fibril in dough at optimum mixing time with lamination treatments of vertical (V) (Fig. G) and vertical + horizontal (V+H) (Fig. H), that has a given level DATEM 0.5% and 0.4% yeast.

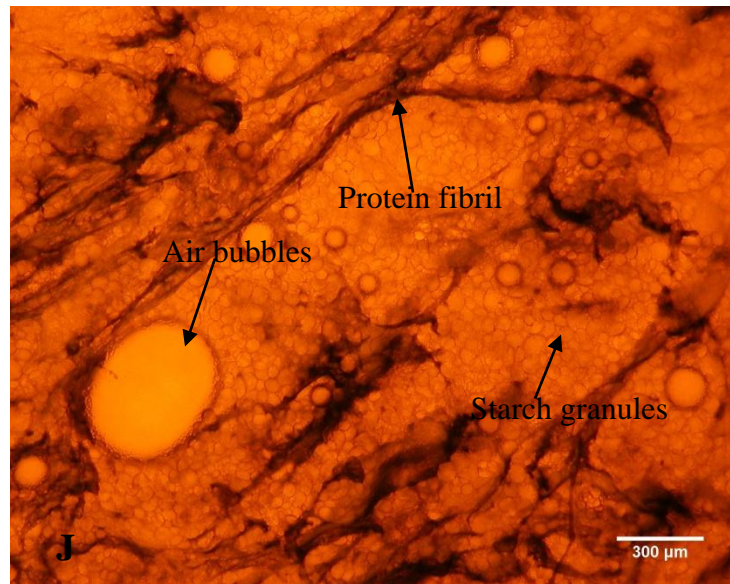
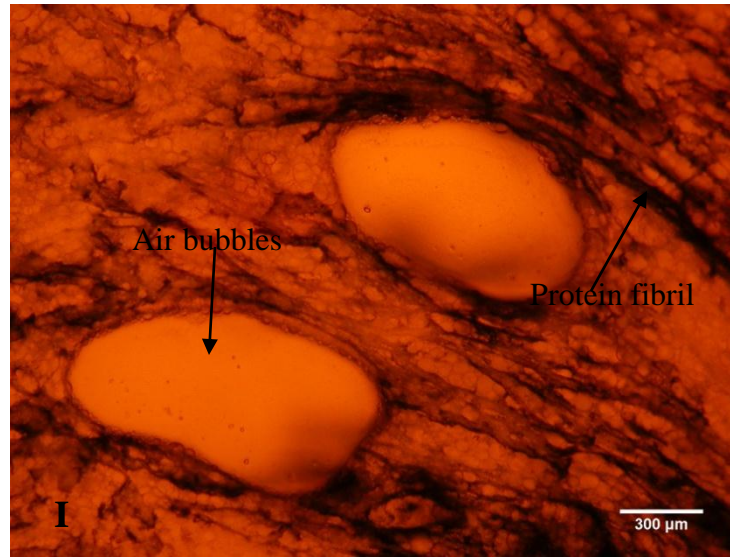


Figure I-J. Example of images of air bubbles, starch granules, and protein fibril in dough at over-mixed mixing time with lamination treatments of vertical (V) (Fig. I) and vertical + horizontal (V+H) (Fig. J), that has a given level DATEM 0% and 0% yeast.

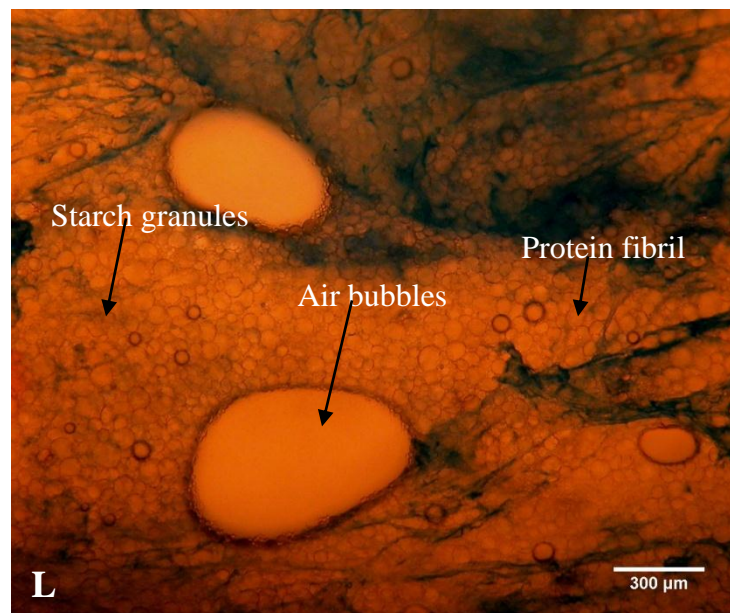
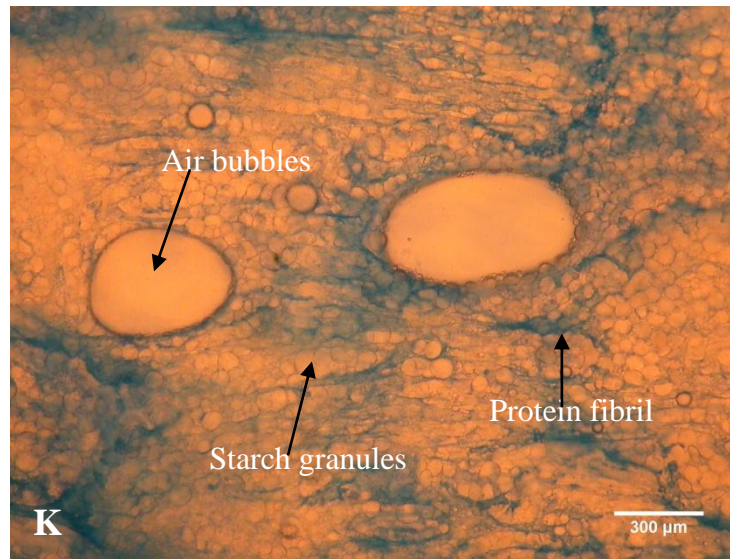


Figure K-L. Example of images of air bubbles, starch granules, and protein fibrils in dough at over-mixed mixing time with lamination treatments of vertical (V) (Fig. K) and vertical + horizontal (V+H) (Fig. L), that has a given level DATEM 0% and 0.4% yeast.

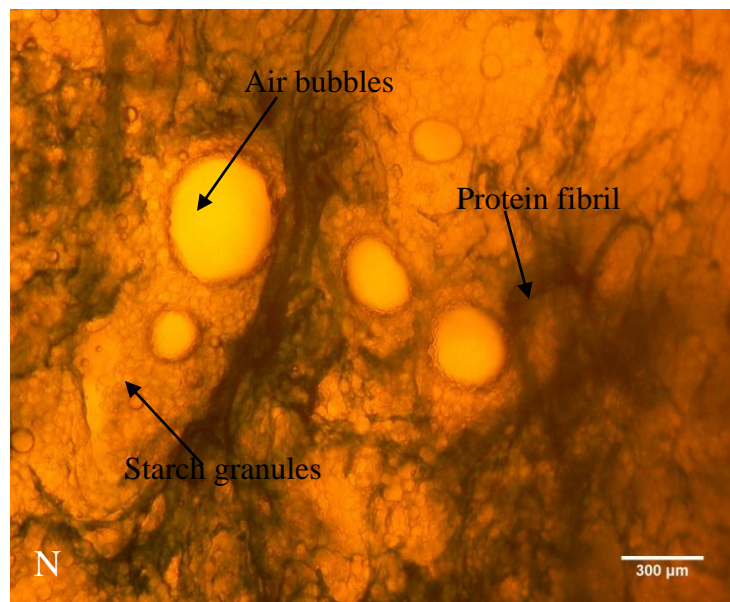
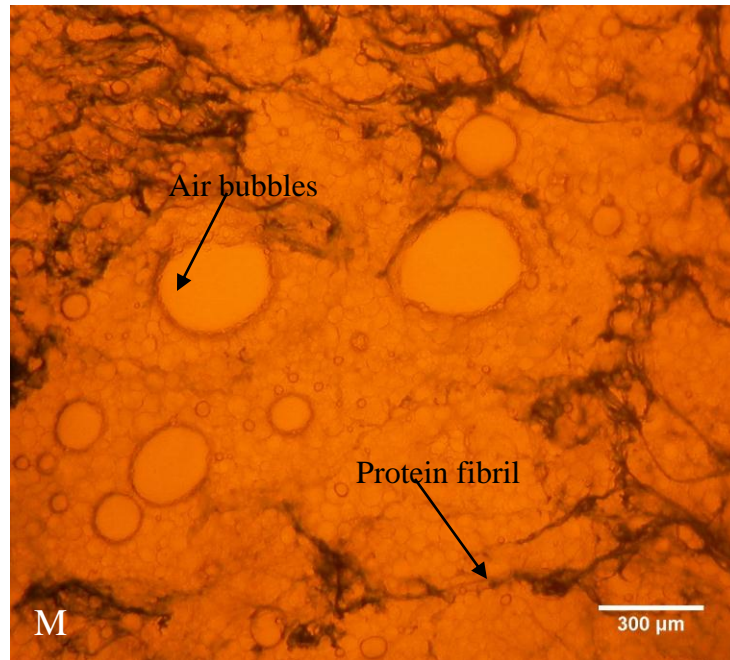


Figure M-N. Example of images of air bubbles, starch granules, and protein fibrils in dough at over-mixed mixing time with lamination treatments of vertical (V) (Fig. M) and vertical + horizontal (V+H) (Fig. N), that has a given level DATEM 0.5% and 0% yeast.

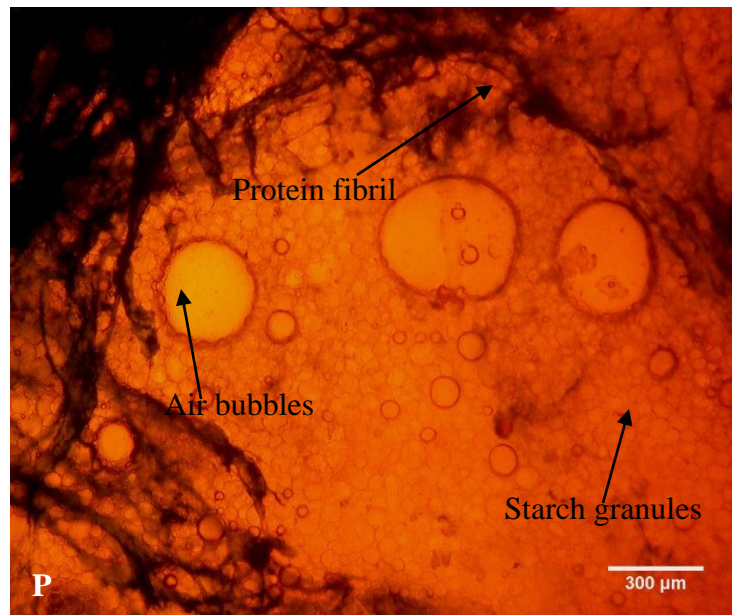
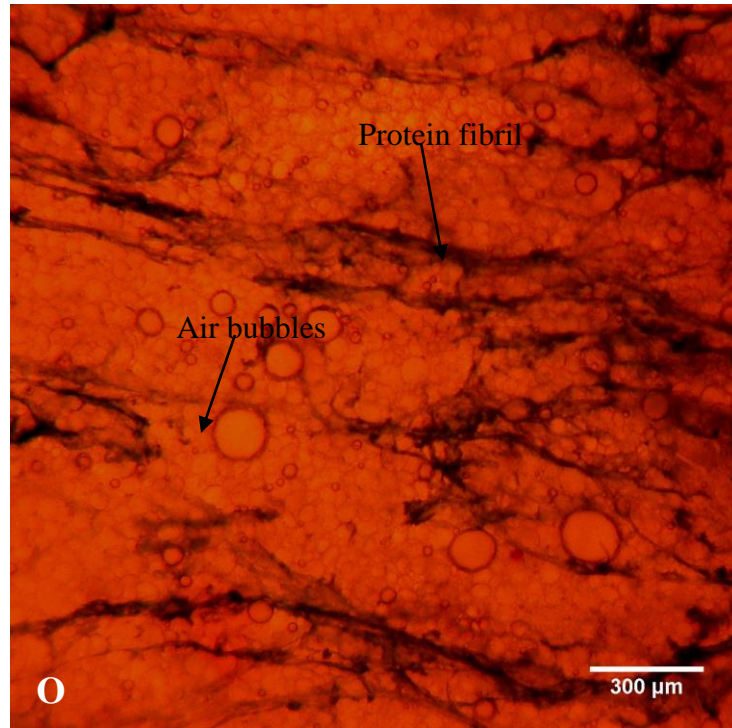


Figure O-P. Example of images of air bubbles, starch granules, and protein fibrils in dough at over-mixed mixing time with lamination treatments of vertical (V) (Fig. O) and vertical + horizontal (V+H) (Fig. P), that has a given level DATEM 0.5% and 0.4% yeast.

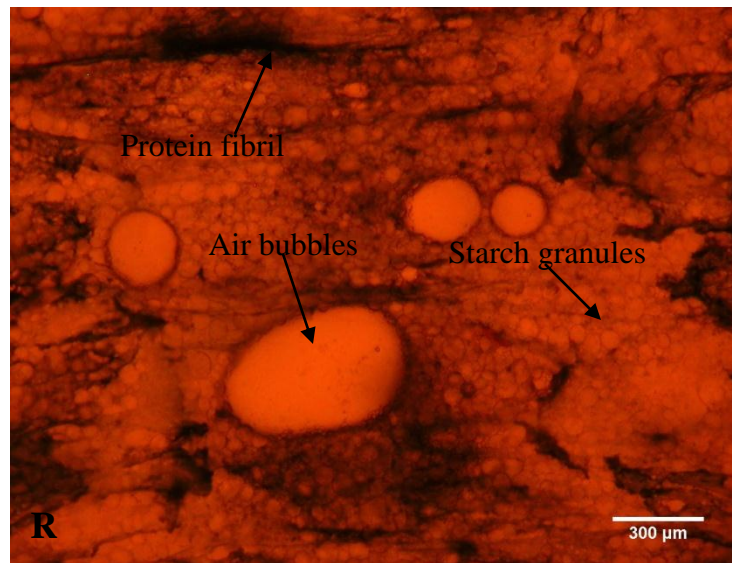
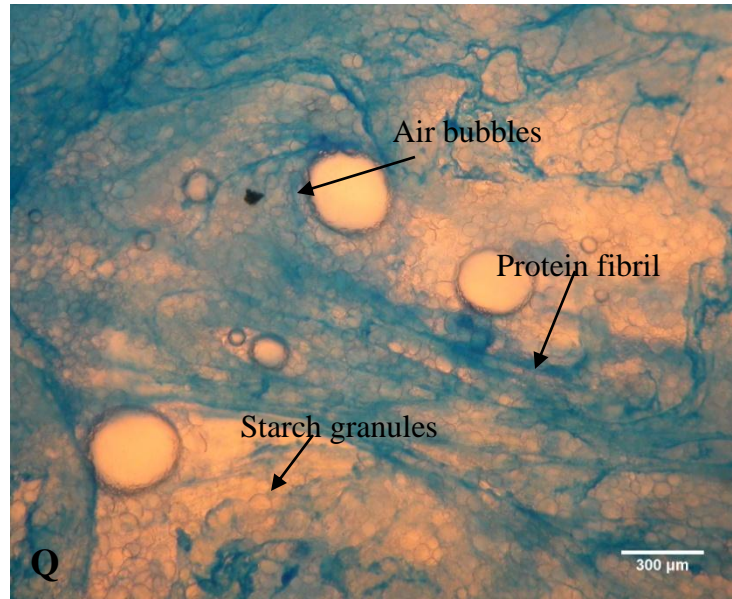


Figure Q-R. Example of images of air bubbles, starch granules, and protein fibrils in dough at under-mixed mixing time with lamination treatments of vertical (V) (Fig. Q) and vertical + horizontal (V+H) (Fig. R), that has a given level DATEM 0% and 0% yeast.

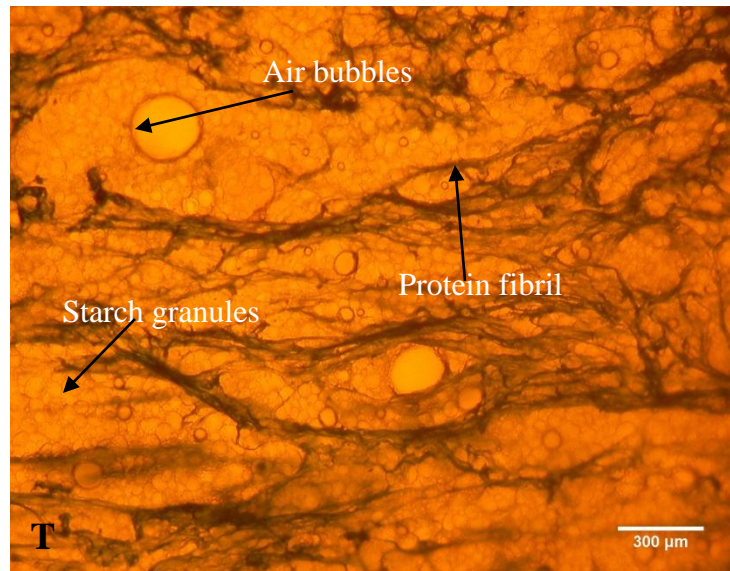
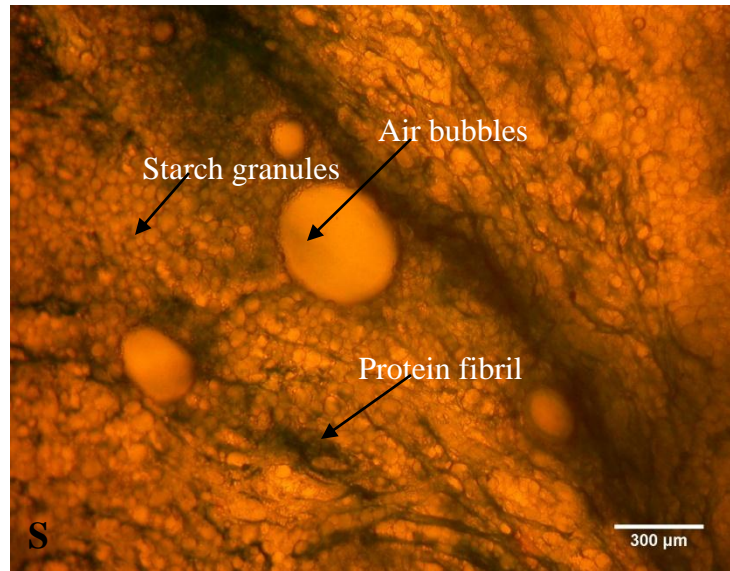


Figure S-T. Example of image of air bubbles, starch granules, and protein fibrils in dough at under-mixed mixing time with lamination treatments of vertical (V) (Fig. S) and vertical + horizontal (V+H) (Fig. T), that has a given level DATEM 0% and 0.4% yeast.

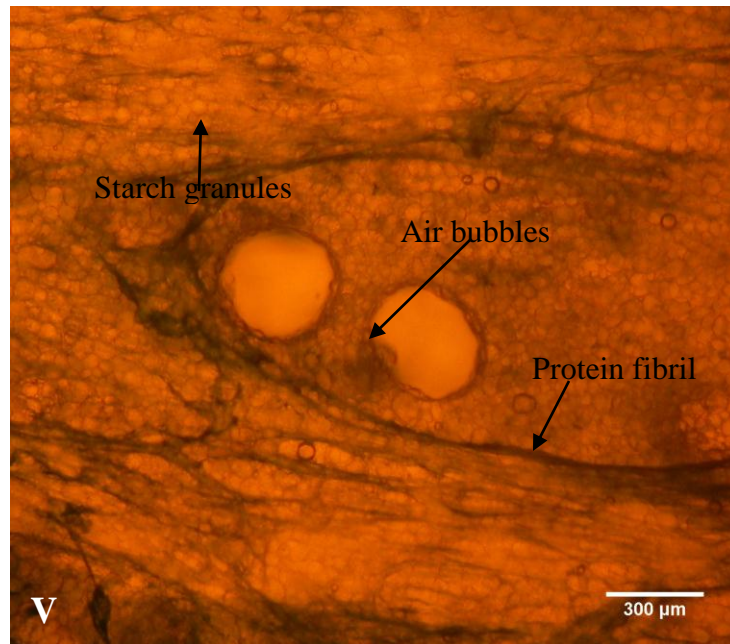
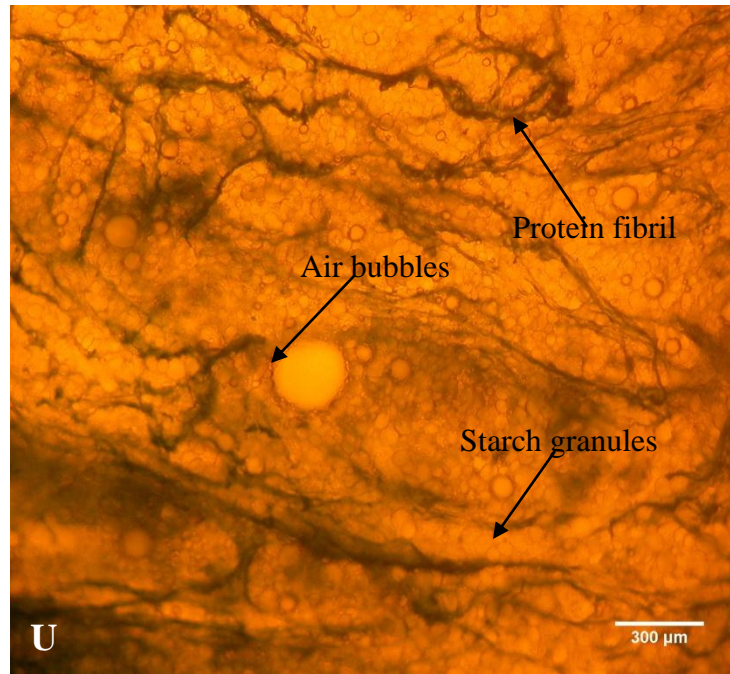


Figure U-V. Example of images of air bubbles, starch granules, and protein fibrils in dough at under-mixed mixing time with lamination treatments of vertical (V) (Fig. U) and vertical + horizontal (V+H) (Fig. V), that has a given level DATEM 0.5% and 0% yeast.

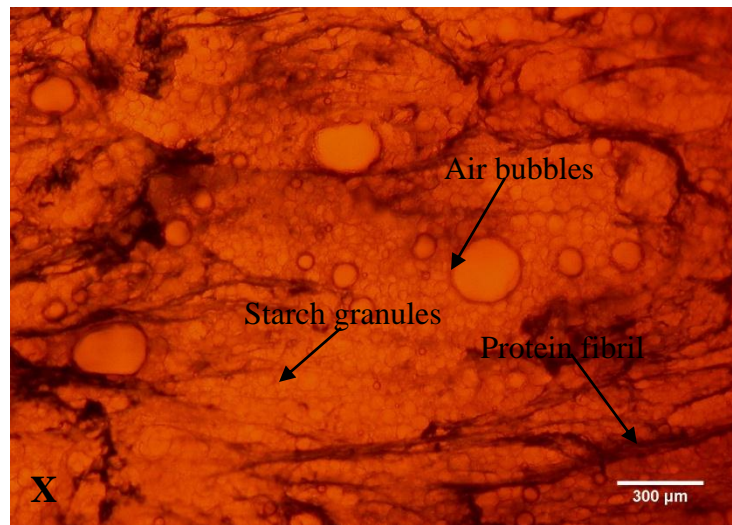
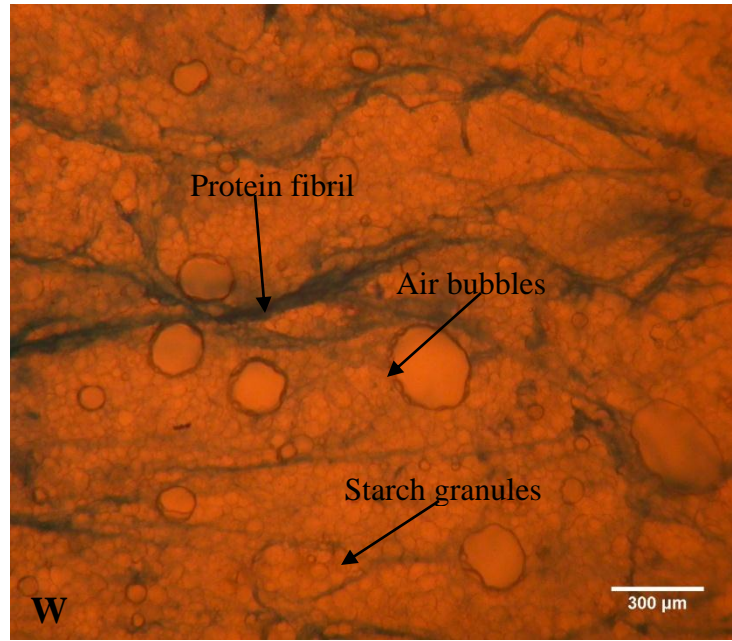


Figure W-X. Example of images of air bubbles, starch granules, and protein fibrils in dough at under-mixed mixing time with lamination treatments of vertical (V) (Fig. W) and vertical + horizontal (V+H) (Fig. X), that has a given level DATEM 0.5% and 0.4% yeast.

VITA

Shawna Rochelle Hughes

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF PROCESSING ON GAS CELL AREA AND SPHERICITY
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Date of Degree: July, 2011

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: EFFECT OF PROCESSING ON GAS CELL AREA AND SPHERICITY OF FOAM DOUGH: A MICROSCOPY STUDY

Pages in Study: 77

Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study: The formation of air bubbles in dough produce important physical changes that are translated into the aerated texture of different products including yeasted breads. Details of the physical characteristic of air bubbles in dough are not fully understood. The objective of this study was to investigate the effect of the factors; mixing (optimum, under-, and over-mixed), lamination (vertical and vertical + horizontal), DATEM and yeast on air bubbles area and sphericity.

Findings and Conclusions: Overall, lamination affects the area mean of air bubbles in selected treatments and a trend suggested that the vertical lamination produced large air bubbles in dough without DATEM or yeast. Although, effects of lamination on air bubble area were not significant in dough with DATEM and yeast, there was an overall trend suggesting a slightly smaller air bubble area mean when dough was laminated in the vertical + horizontal direction compared to a vertical lamination. Overall, yeast appears to affect the air bubble area mean of dough by increasing it in a number of treatments. A positive effect of DATEM was observed on the air bubble area by reducing it in the majority of the treatments. The sphericity of air bubbles was higher in the lamination vertical + horizontal direction applied to dough without DATEM and yeast, and over-mixed. The sphericity of air bubbles was limitedly affected by lamination and affected by mixing. Limited effect of lamination on sphericity suggests that lamination of vertical + horizontal direction promotes sphericity of air bubbles. Over-mixing with vertical + horizontal lamination of the dough produces more spherical bubbles. Yeast produces less spherical air bubbles while DATEM promotes more spherical bubbles. In summary, lamination, mixing time, and level of DATEM and yeast do have some effect on the bubble area in dough. For example, more spherical and smaller bubbles were observed when DATEM is present. Yeast produced less spherical and larger air bubbles. When dough was laminated in the second direction (vertical + horizontal) bubbles were more spherical than in the first direction (vertical).

ADVISER'S APPROVAL: Dr. Patricia Rayas-Duarte
