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**THE EFFECT OF EXTRUDING WHEAT AT LOWER TEMPERATURES ON  
THIAMIN LOSS AND PHYSICAL ATTRIBUTES WHEN USING CARBON  
DIOXIDE GAS AS A PUFFING AGENT**

**By**

**Abigail H. Schmid**

**A THESIS**

**Submitted to  
Michigan State University  
in partial fulfillment of the requirements  
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## **ABSTRACT**

### **THE EFFECT OF EXTRUDING WHEAT AT LOWER TEMPERATURES ON THIAMIN LOSS AND PHYSICAL ATTRIBUTES WHEN USING CARBON DIOXIDE GAS AS A PUFFING AGENT**

By

Abigail H. Schmid

Wheat flour with 0.3% (w/w) thiamin hydrochloride (vitamin B<sub>1</sub>) was extruded on a lab-scale extruder at lower temperatures and expanded using carbon dioxide (CO<sub>2</sub>) gas at 150 psi. Extrusion conditions based on preliminary work were barrel temperature profile of 40/40/50/70/80°C and screw speeds of 300, 350, and 400 rpm, at a flour feed rate of 3.6 kg/hr. These conditions were also repeated at 0 psi CO<sub>2</sub>. High-temperature control samples were extruded at 40/60/90/130/150°C and screw speeds of 200, 250, and 300 rpm at 5.2 kg/hr. Dough moisture content was 22% in the control samples, and was 22 and 25% in the low-temperature samples. Maximum expansion ratios were 2.4 for low-temperature samples and 2.9 for high-temperature samples. Without CO<sub>2</sub>, maximum expansion ratio was also 2.4. Expansion ratio increased with increasing screw speed, die product temperature, and energy input. Thiamin losses ranged from 10-16% in the control samples. With CO<sub>2</sub>, thiamin losses were between 3-11% at 22% moisture, compared to 24-34% at 25% moisture. Without CO<sub>2</sub>, thiamin losses were 0-1.5% at 22% moisture. Unlike typical high-temperature extrusion, thiamin loss in the low-temperature samples decreased with increasing screw speed. At 22% moisture using the extrusion set-up listed above, results indicate a potential for incorporating vitamins into raw flour prior to extruding at lower temperatures.

## **DEDICATION**

**To my wonderful family, Joe, Skip, and Sarah Schmid, for always being there and  
encouraging me to pursue my dreams.**

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## **Introduction**

Extrusion is a continuous process primarily used for shaping and/or heating foods and polymers. In extrusion of foods, granular material (containing carbohydrates and proteins) is mixed with water, and transformed into a viscoelastic melt when subjected to heating, shearing, and conveying, via rotating screw elements located inside the extruder barrel (Rizvi et al., 1995). A forming die is located at the end of the barrel where the doughy mass is pushed through and shaped. Extruded food products include pasta, confectionary products, texturized vegetable protein, pet foods, puffed snack foods and ready-to-eat cereals. Some of the advantages of extrusion, that also give the process longevity in the food industry, are versatility, high productivity, high product quality, and energy efficiency (Harper, 1981).

One unique aspect of extrusion is the ability to produce puffed products. Often the forming die is conical-shaped and the narrowest point is at the die exit. As the flow of dough is restricted at the die, high pressures are generated (600-900 psi) and at high barrel temperatures (140-180°C) the water inside the dough becomes superheated. When exiting the die into atmospheric pressure, the water converts to steam and expands and cools the extruded product as it moves through the dough and escapes to the atmosphere. Mercier and Feillet (1975) reported that the most influential variables on expansion ratio (diameter of extrudate/diameter of die) are extrusion temperature and feed material moisture content. They found that in a twin-screw extruder, maximum expansion ratio was obtained for cornstarch at 14% moisture content and temperatures between 180-200°C in the tested moisture content range of 10.5 to 28.5% (w/w). One disadvantage of extruding at high temperatures and low moisture contents is the loss of vitamins due to

the breaking of chemical bonds during mixing, or the loss of vitamin stability due to heat (Björck and Asp, 1983). For thiamin loss during extrusion, Beetner et al. (1974) reported 46% average loss and an increase in vitamin loss as temperature and screw speed increased. In extruded wheat flour, Guzman-Tello and Cheftel (1987) found a decrease in thiamin loss as the moisture content increased. Due to the significant loss of thiamin during extrusion, ready-to-eat cereals are fortified with thiamin and other vitamins post-extrusion, such as by spraying onto the surface of the extrudate (Burns et al., 2000).

There have been some investigations of lower-temperature (near 100°C) extrusion of cereals by injecting supercritical carbon dioxide (Mulvaney and Rizvi, 1993; Lee et al., 1999) or carbon dioxide gas (Ferdinand et al., 1990, 1992) as expanding agents. Vitamin degradation was not investigated. Other researchers have hypothesized that if the harsh conditions of the extrusion process are reduced, then vitamin loss will decrease (Lee et al., 1999; Sokhey et al., 1996). Killeit (1994) reported that vitamin degradation in extruded foods increased with increasing temperature, screw speed, and specific energy input, and with decreasing throughput, moisture, and die diameter.

Carbon dioxide injection is an inexpensive way of incorporating nucleated gas bubbles to expand the product at lower extrusion temperatures (Mulvaney and Rizvi, 1993). If vitamin loss is reduced at lower temperatures, then the post-extrusion addition of vitamins may become unnecessary. Thus, CO<sub>2</sub>-injection coupled with the addition of vitamins to feed material, has the potential of lowering processing costs, which would benefit breakfast cereal and snack food industries.

## **1. LITERATURE REVIEW**

### **1.1. Extruded Food Products**

The food industry began using extrusion approximately seventy years ago after borrowing the application from the plastics industry, which used extruders to shape products by forcing the plastic melt through an opening. Today, screw-type extruders are used to manufacture food and feed products. The screw-type design uses flighted screw elements on a rotating shaft to convey the material along the extruder barrel toward the exit die. Screw extruders can be categorized as single-screw, co-rotating twin-screw, or counter-rotating twin-screw. The co-rotating twin-screw extruder is the most efficient because it has the greatest capacity for mixing products uniformly (Harper, 1988).

In addition to acting as a former, an extruder can also be used to cook the shaped products. The cooking action takes place inside the extruder barrel through the combination of dissipated mechanical energy from the rotating screws plus optional added thermal energy by injected steam, or electrically heated rods or jackets. Maximum temperatures toward the exit die typically reach between 140°C and 190°C, but due to the movement of the material by the screws, the residence times at these high temperatures is much shorter than the total residence time inside the entire barrel. Because of this, extrusion cooking can be classified as a high-temperature/short-time (HTST) process. Similar to the HTST heat treatment of raw milk, this process is effective in the destruction of spoilage enzymes and microorganisms (Harper, 1981). In addition to improving product quality, some of the other advantages of using extrusion in food processing include the ability to produce a variety of products, continuous processing,

and energy efficiency (Hauck and Huber, 1989). These advantages are becoming even more important as the consumer demand for new food products increases.

#### **1.1.1. High-Temperature Expanded Products**

In the extrusion cooking process, molecular changes, such as the disruption of starch granules and protein denaturation, are likely to occur. These changes typically involve the conversion of a starchy and/or proteinaceous raw material into a viscoelastic melt. This transformation is important to the expansion of products since the viscoelastic properties of the melt allow for air cells to be retained, resulting in a rigid, porous, structure upon exiting the die (Kokini et al., 1992).

Starch gelatinization (often measured by loss of birefringence) results as the starch molecules are heated and mixed with water inside the extruder barrel. For wheat flour, Chiang and Johnson (1977) reported that increased extrusion temperatures increased starch gelatinization when the tested moisture content range was between 18 and 27% (w/w). For extruded cornstarch, Bhattacharya and Hanna (1987) found that expansion ratio increased and starch gelatinization was greater when barrel temperatures increased from 116°C to 166°C. According to their study, expansion ratio increased with decreasing moisture contents. They attributed this to higher moistures possibly having a decreased effect on product temperatures, thus causing expansion ratio to decrease. In summary, conventional puffing is typically done at high temperatures and low moisture contents to create ideal conditions for water vapor flash-off and starch gelatinization.

Expansion ratio, bulk density, water absorption index (WAI), and water solubility index (WSI) are physical characteristics of expanded products often used to represent extrudate quality. Expansion ratio measures how much the dough expands after exiting

the die. Bulk density determines the weight of product per unit volume and is related to expansion ratio, since the greater the expansion ratio the lower weight per volume the product will occupy. A low bulk density is desirable when trying to package expanded products in a cost effective manner. In the ready-to-eat breakfast cereal industry, WAI and WSI are directly related to “bowl life”, or the amount of time it will take for the product to become soggy in milk. The lower the WAI and WSI the longer the “bowl life” which will most likely result in a crispy and crunchy product throughout the breakfast eating experience (Sokhey et al., 1996).

#### **1.1.2. Carbon Dioxide-Expanded Products**

To date, limited research has been conducted on the use of injected carbon dioxide to expand extruded products. The idea of using carbon dioxide injection in food extrusion was also borrowed from the plastics industry, as gases were traditionally used to produce plastic foams (Ferdinand et al., 1990). The two types of carbon dioxide injection used by past researchers in the extrusion of cereal products have been supercritical fluid carbon dioxide and pressurized carbon dioxide gas (Lee et al., 1999; Sokhey et al., 1996; Rizvi et al., 1995; Mulvaney and Rizvi, 1993; Ferdinand et al., 1990, 1992). In both cases, at lower barrel temperatures, carbon dioxide injection of a fluid or gas was used to inject nucleated bubbles in the viscoelastic melt (Mulvaney and Rizvi, 1993). In the case of the supercritical fluid, the fluid changes to a gas as the pressure reduces to atmospheric conditions on exit from the die. In the supercritical CO<sub>2</sub> studies, process optimization was investigated by changing screw configuration, pressure profiles, barrel temperatures, screw speeds, moisture contents, and by adding texturizing agents (whey protein isolate and non-fat dry milk). To look at whether changing processing

variables had significant effects on physical attributes and chemical properties, extrudates were characterized for starch gelatinization, expansion ratio, breaking stress (amount of force required to break an extrudate), water absorption index, and water solubility index. In the CO<sub>2</sub> gas-injection studies, similar properties of extrudates were characterized, but for different extrusion conditions in which greater emphasis was placed on minimizing barrel temperatures (<100°C) in the cooking zone. The effect of extrusion variables on specific mechanical energy (SME) input was also investigated for supercritical and CO<sub>2</sub> gas injection (Mulvaney and Rizvi, 1993; Lee et al. 1999; Ferdinand et al. 1990, 1992). SME has been defined as the amount of mechanical energy required to process 1 kg of flour and water input, and is an important parameter in estimating manufacturing costs (Bhattacharya and Choudry, 1994).

In high-temperature expanded products, puffing is maximized under harsh extrusion conditions such as high temperature and low moisture contents (Mercier and Feillet, 1975). Because of this, some of the benefits that carbon dioxide-injected extrusion offers include reduced wear on the barrel, lower energy costs, and the ability to potentially add heat-sensitive ingredients like flavors, colors, and vitamins in with the raw material (Rizvi et al., 1995). In addition, carbon dioxide is a relatively inexpensive expanding agent. Oxygen is also a low-cost expanding agent, but it has not been investigated in food extrusion, due to its flammability and oxidizing capabilities.

## **1.2. Thiamin Degradation in Extrusion**

### **1.2.1. Introduction**

Thiamin, also called Vitamin B<sub>1</sub>, is an essential, water-soluble vitamin that is important in carbohydrate metabolism, energy production, proper function of the nervous system, and in the prevention of beriberi, an endemic disease in parts of Asia where polished white rice is a major dietary staple (Gubler, 1991). Beriberi is categorized into two main types, wet beriberi and dry beriberi. Wet beriberi symptoms include the accumulation of fluids in the feet, legs, and ankles, and is also referred to as edema. This edema may also cause the heart to enlarge and lead to congestive heart failure. Dry beriberi symptoms include muscle emaciation and nerve abnormalities. Other general symptoms include anorexia and difficulties in walking (Gubler, 1991).

The chemical structure of free thiamin is pyrimidine plus thiazole, attached by a methylene bridge. The coenzyme form is thiamin pyrophosphate, and is formed in the body when a pyrophosphate group is added to the structure of free thiamin (Rindi, 1996). The Daily Value (nutritional recommendation based on Reference Daily Intakes or the former U.S. Recommended Dietary Allowances) for thiamin is 1.5 mg/day (Food and Drug Administration, 1994). Dietary sources of thiamin include fortified ready-to-eat whole-grain cereals, enriched bread and flour, and pork, legumes, and dairy products (Rindi, 1996). Stability characteristics for thiamin have been reported as being readily destroyed at temperatures near 100°C and in solutions with a pH above 6.2 (Mulley et al., 1975a). Thiamin instabilities are due to the cleavage of the methylene bridge into pyrimidine and thiazole fragments when heated in alkaline solutions (Dwivedi and Arnold, 1973).



### **1.2.2. Thiamin Analysis in Cereal Products**

The categories of methods available for the analysis of thiamin include chemical, microbiological, animal, and physical. For food and feed products, thiamin content can be measured rapidly and economically using a chemical method (Association of Vitamin Chemists, 1966). The thiochrome method is the standard chemical method used by the Association of Official Analytical Chemists (AOAC, 1995) and the American Association of Cereal Chemists (AACC, 2000). When thiamin is oxidized, it forms thiochrome, which fluoresces under UV light. A fluorometer is then used to measure the amount of fluorescence, which is linearly proportional to the amount of thiamin under standard conditions and when other fluorescing compounds are not present (Mulley et al., 1975b). When thiamin loss was investigated in extruded wheat flour, Guzman-Tello and Cheftel (1987) used the thiochrome method for determining thiamin concentration. Pham and Del Rosario (1986) and Maga and Sizer (1978) also determined thiamin content in extruded legumes and extruded potato flakes, respectively, with the thiochrome method. Ilo and Berghofer (1998) and Beetner et al. (1974) used microbiological methods for measuring thiamin in extruded corn grits.

Although not listed as an official method by AOAC or AACC for thiamin analysis, Toma and Tabekhia (1979) and Kamman et al. (1980) have reported that high performance liquid chromatography (HPLC) is also a rapid and accurate method for measuring thiamin concentration in foods. In these two studies, HPLC and the thiochrome method were used to measure thiamin content in the same products. When thiamin concentrations of the two methods were compared, they found no difference in values. Since either method was acceptable and the thiochrome method has been

reported to be accurate within 5%, the thiochrome method was used in the present study to measure thiamin in raw flour and extruded products (Labuza and Riboh, 1982).

### 1.2.3. Effects of Extrusion Conditions on Thiamin Loss

Thiamin is naturally present in cereal grains, but thermally and mechanically degraded during processing, such as in milling and extrusion. Guzman-Tello and Cheftel (1987) extruded soft wheat flour mixed with thiamin at identical conditions for four different initial concentrations of thiamin. They plotted  $\log(\text{initial } B_1 \text{ concentration})$  vs.  $\log(\text{final } B_1 \text{ concentration})$ . The slope was 1.03, indicating that thiamin degradation in extruded wheat flour follows virtually a first-order kinetic reaction. As shown in Table 1, they found the destruction rate constant ( $k$ ) of thiamin to be a function of die product temperature and mean residence time (mean time the raw material spends inside extruder barrel):

**Table 1.1. Extent of Thiamin Loss in Wheat Flour (13% Moisture, w/w) as a Function of Temperature and Mean Residence Times<sup>a</sup>**

Treatment #	Product Temperature Just Before Die (C) <sup>o</sup>	Screw Speed (rpm)	Mean Residence Time in Heating Zone of Extruder (seconds)	% Thiamin Loss <sup>b</sup> (dry basis)	$k \times 10^3$ (per sec.)
1	131	100	42	11.5 ( $\pm 0.1$ )	3.23
2	145	100	41	21.5 ( $\pm 0.3$ )	6.86
3	160	100	42	30.0 ( $\pm 0.9$ )	9.34
4	176	100	40	42.5 ( $\pm 1.1$ )	14.4
5	159	100	43	27.0 ( $\pm 1.3$ )	7.92
6	159	125	40	41.1 ( $\pm 3.5$ )	13.7
7	160	150	34	47.2 ( $\pm 4.7$ )	19.5

<sup>a</sup> Adapted from Guzman-Tello and Cheftel, 1987

<sup>b</sup> Experiments conducted in duplicate. Standard deviations are given in parentheses.

In comparing treatments #3 to #7, it appears that at constant die temperatures, increasing screw speed from 100 to 150 rpm increased the percent thiamin loss by approximately

17%. With increasing screw speed, the amount of time the product spent inside the barrel decreased (34 vs. 42 seconds), indicating that shear effects may have contributed more to thiamin loss than thermal effects. Ilo and Berghofer (1998) reported thiamin losses in the extrusion of corn grits from 67-100%, when barrel temperatures ranged from 140-200°C, screw speeds from 65-81 rpm, and feed moistures from 11.8-14.2% . For non-enriched white flour, thiamin losses were 58% when the temperature at the die was 197°C, screw speed was 200 rpm, and feed moisture was 14.6% (Hankansson et al., 1987). Similar studies on thiamin degradation in extrusion indicated that thiamin loss decreased as energy input decreased or feed material moisture content increased (Asp and Björck, 1989; Pham and Del Rosario, 1986; Ilo and Berghofer, 1998; Killeit, 1994). These researchers suggested that an increase in moisture content reduces the dissipation of mechanical energy, resulting in a decrease in thiamin loss.

### **1.3. The Fortified Foods Trend**

Consumer demand for new, convenient products continues to grow rapidly (Sloan 2001). Based on consumer trends, a type of product worth exploring would be one that offers health benefits at a reasonable cost. Sloan (2000) reported that “nine out of ten shoppers now believe that healthy eating plays a role in disease prevention”. Sloan also reported that fortified foods are a \$50 billion dollar industry and two-thirds of those that grocery shop in the U.S. are buying more fortified foods. Ready-to-eat cereals, a type of extruded product, have traditionally been fortified, but with additional equipment to spray vitamins onto the product post-extrusion. The additives (vitamins, colors, flavors, and other nutrient additives) are added post-extrusion due to the harsh conditions inside the extruder barrel that degrade the chemical structure of some additives. Because of this, a

challenge remains to the extruded foods industry to modify the extrusion process where more nutrients are retained throughout processing, thus eliminating the need for an additional fortification step. One approach to addressing this challenge would be to add the nutrients to the raw material, thereby resulting in a more uniform distribution of the nutrients than that resulting from the spraying method.

#### **1.4 . Research Objectives**

Extruding foods at lower temperatures has potential for lowering processing energy costs and decreasing the loss of heat- and/or shear-labile nutrients. However, lower temperatures would decrease expansion normally caused by steam flashing off the extrudate, and would result in a less appealing product. Injection of CO<sub>2</sub> gas may decouple the dependence of expansion on high temperatures, and allow expansion at lower temperatures. A few studies investigated the potential of CO<sub>2</sub> gas extrusion but these studies did not report vitamin loss. Therefore, the research objectives are as follows:

##### **Objectives**

1. To find a screw configuration, moisture content range, screw speed range, die geometry, CO<sub>2</sub> pressure and barrel temperature profile near 100°C that will sufficiently melt and puff the extruded wheat dough.
2. To investigate the effects of moisture content, screw speed, and CO<sub>2</sub> pressure on the extruder specific mechanical energy input and mean residence time, and on the physical characteristics of expansion ratio, water absorption index, water solubility index, and bulk density.

3. To compare thiamin loss in products puffed at lower temperatures (near 100°C) to thiamin loss in products puffed at conventional temperatures (160°C).

## **Materials and Methods**

**“Images in this thesis are presented in color.”**

### **2.1. Raw Materials**

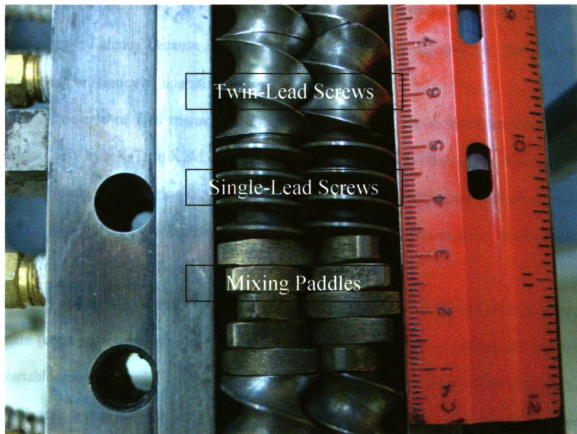
Michigan soft white wheat pastry flour (Star of the West Milling Co., Frankenmuth, MI) with a moisture content of approximately 13% was blended with 0.3% (w/w) food-grade thiamin hydrochloride (Spectrum Laboratory Products, Inc., Gardena, CA). Using a twin-shell dry blender (Patterson-Kelley, East Stroudsburg, PA), the flour and thiamin were mixed for 40 minutes, the minimum time to ensure adequate distribution of thiamin (coefficient of variance < 5%). The blender consisted of an inner mixing bar that held 4 cm long splines. The tip speed of one spline was measured with a manual tachometer as 5.9 m/s. According to Patterson-Kelley, high and medium speed blenders usually have tip speeds of 17 and 8.5 m/s, respectively. The mixing procedure and the chemical and physical properties of flour are outlined in Appendix 1, Table A.1.1.

### **2.2. Extrusion Conditions**

An APV (Grand Rapids, MI) MP19TC-25 co-rotating and intermeshing twin-screw extruder was used to extrude each sample in duplicate. The diameter of each barrel is 19 mm, thus making the length-to-diameter ratio of the extruder 25:1. The size of the extruder used in the present study was much smaller than an industrial-size extruder.

Along the length of the barrel and die, there are five zones in which the temperature is controlled via thermocouples, electrical heating elements, and water-cooling jackets. The direct current motor supply provides power to turn the shafts and screws up to a maximum screw speed of 500 rpm. A control display panel allows the

operator to monitor process variables such as the torque required to turn the screws (expressed as a percentage of the maximum torque), screw speed (rpm), temperature of the product and barrel ( $^{\circ}\text{C}$ ), and die pressure (psi). Die pressure was measured using a pressure transducer (Dynisco, Model # EPR3-3M-6) located 7 mm before the die entrance. The product temperature inside the die was measured by hand-inserting a T-type needle thermocouple (Cole-Parmer, Vernon-Hills, IL) into the die hole during extrusion. The exit dies both have circular openings of 3 mm and lengths of either 6 mm or 12 mm.



**Figure 2.1. Top View of Screw Elements Aligned Inside the Barrel of an APV MP19TC-25 Twin-Screw Extruder.**

Figure 2.1 shows how the screw elements appear inside the barrel. The helical metal rib around the screw is called a flight, and lead typically refers to the axial distance

between flights (Harper, 1981). In this case, a twin-lead screw has the geometry to fit two helices, whereas the single-lead fits one helix. The twin lead geometry is most ideal for conveying and the single lead geometry is better for compressing and heating the dough. When the screw elements are next to one another in Figure 2.1, twin-lead screws are perpendicular to each other, and the single-lead screws are fully intermeshed. They are aligned in this manner to ensure optimal mixing along the barrel.

The mixing paddles have a greater capacity for shearing and move along the barrel in either a clockwise (forward) or counterclockwise (reverse) motion. The paddle orientation can be 30, 60, or 90°. According to APV specifications, 30° has the greatest conveying efficiency because it operates at a low degree of fill, and 90° has a lower conveying efficiency by operating at a higher degree of fill.

The rate of feed material and deionized water into the extruder are controlled respectively by a K-Tron K2M twin-screw volumetric feeder (K-Tron Corp., Pitman, NJ) and E2 Metripump positive displacement metering pump (Bran & Luebbe, Northampton, UK). The twin-screw volumetric feeder is equipped with a safety feeder, variable speed drive, and digital control.

Prior to sample collection, the extruder was run for at least five minutes at steady state (constant torque, die pressure, and die temperature). After recording the process variables (screw speed, % torque, temperature profiles, die pressure, screw configuration, feed and water injection rates, and product temperature at the die), samples were collected for five minutes and held at room temperature in an area of subdued light prior to drying. Drying took place in a convection fan oven at 75°C for 16 hours, to a moisture content of 3.5 to 7%. After drying, the extrudates were stored in a light-resistant, sealed,



polyethylene bag at 1°C until further analysis. About 30 g of extruded material were ground using a Udy Cyclone Mill (Udy Corp., Fort Collins, CO) with a 0.5 mm screen. Moisture content was determined in duplicate on a 2.5-g ground sample, by heating at 130°C for 10 minutes using a Sartorius MA-30 moisture analyzer (Göttingen, Germany).

#### **2.2.1. Extrusion Set-up for High Temperature (Control)**

The screw configuration listed in Table 2.1 was one that had been used in preliminary experiments to expand extruded cereal products at high temperatures. Extrusion parameters included a constant temperature profile of 40/60/90/130/150°C (increasing temperature toward die), die geometry of 3 mm diameter x 12 mm length, feed rate of 5.2 kg/hr, and water injection rate of 0.69 kg/hr. The moisture content inside the barrel was 22% for the above feed rate and water injection rate. Three constant screw speeds of 200, 250, and 300 rpm were used. The above parameters had been tested in preliminary runs and identified as conditions that produced optimal puffing (expansion ratios > 2.0).

#### **2.2.2. Extrusion Set-Up for CO<sub>2</sub>-Injection**

The screw configuration for CO<sub>2</sub>-injection listed in Table 2.1 was based on preliminary experiments for producing a puffed product with CO<sub>2</sub> gas as the expanding agent. Placing mixing paddles at the point of CO<sub>2</sub>-injection in order to prevent the backflow of CO<sub>2</sub> gas further optimized this screw configuration. Ferdinand et al. (1990) used a similar mixing paddle set-up when the point of CO<sub>2</sub> injection was 165 mm away from the die entrance. In the present study, the point of CO<sub>2</sub>-injection was 110 mm away from the die entrance. The point of CO<sub>2</sub>-injection had been tested in previous studies

using the same APV extruder to produce expanded products. The optimal extrusion parameters for puffing were determined in a manner similar to the control samples. Each parameter (screw speed, moisture content, CO<sub>2</sub> pressure, and die geometry) was changed one at a time until a uniformly puffed product was obtained without barrel temperatures exceeding 100°C. These optimal parameters included a constant set temperature profile of 40/40/50/70/80°C, die geometry of 3 mm diameter x 6 mm length, CO<sub>2</sub> pressure of 150 psi, and feed rate of 3.6 kg/hr. The water injection rates were 0.36 kg/hr and 0.52 kg/hr resulting in feed material moistures of 22% and 25%, respectively. The screw speeds were 300, 350, and 400 rpm.

**Table 2.1. Screw Configurations for High-Temperature (Control) vs. CO<sub>2</sub>-Injection**

<b>High Temperature</b>	<b>CO<sub>2</sub>-Injection</b>
8D <sup>a</sup> Twin Lead	7D Twin Lead
7x30° Forward Mixing Paddle	7x30° Forward Mixing Paddle
8D Twin Lead	5D Twin Lead
3x60° Forward Mixing Paddle	1D Single Lead
3x30° Reverse Mixing Paddle	3x60° Forward Mixing Paddle
2D Single Lead	3x60° Reverse Mixing Paddle
4x60° Forward Mixing Paddle	2D Single Lead
3x30° Reverse Mixing Paddle	3x60° Forward Mixing Paddle
2D Single Lead	4x60° Reverse Mixing Paddle
	4D Twin Lead
	1D Single Lead

<sup>a</sup>D = 19 mm

### 2.3. Expansion Ratio and Bulk Density

After drying for 16 hours, calipers were used to measure the diameter of extruded products. Per sample, five strands were randomly selected and three measurements were taken along the product. To calculate expansion ratio, the average of 15 measurements was divided by the die diameter of 3 mm. Bulk density was determined by filling a 200 mL graduated cylinder with extruded pieces that were approximately 0.5 cm long. The graduated cylinder was tapped gently on a flat surface before weighing. The following equation was used to determine bulk density:

$$\text{Bulk Density} = \frac{\text{g of sample per 200 mL}}{200 \text{ mL}}$$

For each sample, bulk density was measured in triplicate.

### 2.4. Water Absorption Index (WAI) and Water Solubility Index (WSI)

The determinations of WAI and WSI were based on the method used by Anderson et al. (1969) where 2.0 g of ground extrudate is suspended in 20 mL of water at 30°C, shaken for 30 minutes and centrifuged at 3000 rpm (1075 x g) for 15 minutes. The supernatant was dried in a convection oven at 130°C for 2 hours. Taking two measurements per sample, WAI was calculated as the weight of sediment (or gel) obtained per gram of dry sample or:

$$\text{WAI} = \frac{(\text{weight of sediment} + \text{centrifuge tube}) - (\text{weight of centrifuge tube})}{\text{sample dry weight}}$$

WSI was calculated as a percentage of soluble material using the following equation:

$$\% \text{ WSI} = \frac{(\text{weight of dish} + \text{dried supernatant}) - (\text{weight of dish})}{\text{sample dry weight}} \times 100$$

## 2.5 . Specific Mechanical Energy Input

Specific mechanical energy (SME) is a measure of the viscous energy dissipation per unit mass of dough (Mason and Hosney, 1986). This energy originates from the direct current motor supply which provides the required torque to turn screws at a given screw speed. A portion of total power supplied to the shaft is used to push dough along the barrel against back-pressure generated at the die (Mohamed et al., 1990). The remaining power is transferred into the dough via fluid friction. Therefore, specific mechanical energy (SME) is calculated as the total power supplied to the shaft ( $P_w$ ), minus power to convey the dough ( $\Delta PQ$ ), divided by the mass flow rate:

$$\text{SME (kJ/kg)} = \frac{E_v}{\dot{m}} \frac{\left(\frac{\text{kJ}}{\text{s}}\right)}{\left(\frac{\text{kg}}{\text{s}}\right)}$$

$$\text{where } E_v = \frac{(P_w - \Delta PQ)}{1000}$$

$$P_w = (0.044)(\% \text{torque})(N), \text{ J/s}$$

↓

as given by the manufacturer

N = screw speed, rpm

$\Delta P$  = die pressure, Pa

$$Q = \text{volumetric flow rate, } \frac{\dot{m}}{\rho}, \text{ m}^3/\text{s}$$

$$\dot{m} = \text{mass flow rate of dough, } \dot{m}_{\text{feed flour}} + \dot{m}_{\text{water}}, \text{ kg/s}$$

$$\rho = \text{dough density, kg/m}^3$$

For the dough density measurement, a sample of dough was taken near the entrance of the die. The dough sample was wrapped tightly in plastic wrap and weighed. After recording the weight, the dough was placed into a 100 mL graduated cylinder filled with 30 mL deionized water. Dough volume was recorded as the volume of water displaced

by the dough. Dough density was calculated as the weight of dough (g) divided by the volume of dough (mL) and then units were mathematically converted from g/mL to kg/m<sup>3</sup>. Average SME was reported as the average between two replicate extrusion conditions.

## **2.6 . Mean Residence Time**

Due to axial mixing within the extruder barrel, there is a distribution of residence times for dough particles. Mean residence time is affected most strongly by screw configuration, screw speed, and feed rate (Altomare and Ghossi, 1986). Solid Red 40 dye in the amount of 0.3 g acted as a traceable color indicator and was poured by hand instantaneously where feed flour enters the extruder. Time at which red color first appeared in the extrudate exiting the die was measured. Extrudate strands were cut every five seconds until no obvious color appeared at the exit die. A strand was collected prior to color injection to represent the color value at time zero. A coffee grinder (Sunbeam Corporation, Maitland, FL) was used to reduce the particle size of the strands. Each sample in the amount of 1.6 g was then placed into a round disc with a 3.5-cm diameter and 0.5-cm height. A HunterLab D25 L color meter (Hunter Associates Laboratory, Reston, VA) measured the a\*-value (redness) of the ground samples. Standard number C2-30954 was the white plate used to standardize the color meter. Redness values were recorded and were used to represent the intensity (C(t)) of red color at exit time t. Mean residence time was calculated as a weighted average (Levenspiel, 1999):

$$\text{Mean residence time (seconds)} = \frac{\sum t C(t) \Delta t}{\sum C(t) \Delta t}$$

Microsoft Excel was used to construct a residence time distribution curve of E(t) (Normalized concentration) vs. t (seconds), where  $E(t) = \frac{C(t)}{\sum C(t)\Delta t}$ .

## 2.7. Thiamin Analysis

Thiamin concentration determination was based on the 1995 AOAC “Official Method 953.17 Thiamin (Vitamin B<sub>1</sub>) in Grain Products, Fluorometric (Rapid)”. In this method, potassium ferricyanide oxidizes thiamin to thiochrome, which fluoresces under UV light. The spectrofluorometer type was SF-330 from Varian (Palo Alto, CA) and methylacrylate sample cuvettes were used. The fluorometer was standardized with quinine sulfate at an excitation wavelength of 343 nm and emission wavelength of 459 nm. The wavelength range for measuring thiamin concentration was excitation wavelength of 373 nm and emission wavelength of 410 nm. The fluorometer settings used for analyzing all samples are listed in Table 2.2:

**Table 2.2. Fluorometer Settings for the Varian SF-330 Spectrofluorometer**

<b>Light Source</b>	Xenon Lamp
<b>Excitation slit</b>	5 nm
<b>Emission slit</b>	10 nm
<b>Sensitivity</b>	x 1/10, samples x 10, blanks
<b>Selector</b>	x 1

Modifications to the AOAC method were tested and implemented to improve the accuracy, precision, and efficiency of thiamin analysis. According to the AOAC method, sodium chloride should be added in the final oxidation step to act as a drying agent. However, Brubacher et al. (1985) reported that thiochrome yield may be reduced by 15-

19% if sodium chloride is present during oxidation and should therefore be added after oxidation is complete. Brubacher et al. (1985) also reported that when in excess, potassium ferricyanide can destroy thiamin. Based on this research, we did our own study and found results similar to Brubacher et al.'s (1985). The addition of NaCl and potassium ferricyanide was then adjusted in the oxidation step in order to optimize thiochrome yield (Appendix 2, Figures A.2.1 and A.2.2).

Using the exact quantities and volumes of reagents listed in the AOAC method, thiamin concentration is calculated using the following equation:

$$\mu\text{g thiamin in 5 ml assay solution} = \frac{(I - b)}{(S - d)}$$

In this equation,  $I$  is the fluorescence reading for the oxidized sample,  $b$  is the reading for the non-oxidized sample,  $S$  is the reading for the oxidized standard, and  $d$  is the reading for the non-oxidized standard. This method uses a standard concentration that covers the entire range of sample solutions and assumes that there is a linear relationship between thiamin concentration and fluorescence.

In the present study, a standard curve of fluorescence vs. thiamin concentration was constructed to confirm a linear relationship between the two (Appendix 3, Figure A.3.1). Since the day-to-day standard curve variability was very low when slopes were compared (<2% variability), a standard curve was not run on each day of analysis. Instead, one was constructed periodically, and averages of the y-intercept and slope values were used in the final calculation for percent thiamin. Percent thiamin was calculated in triplicate for each sample on a dry weight basis using the following equation:

$$\% \text{ thiamin} = \frac{(I - b) - z}{m} \times \frac{(F_1)(F_2)(10^{-6})}{W} \times 100\%$$

$I$  and  $b$  are the same as above and  $z$  and  $m$  are the average y-intercept and slope, respectively, from the standard curves.  $F_1$  is the dilution factor that occurs in the extraction step and  $F_2$  is the dilution factor that occurs in the oxidation step.  $W$  represents the dry weight of the sample (g). The equation for calculating percent thiamin loss was:

$$\% \text{ thiamin loss} = \left( 1 - \frac{P_i}{P_o} \right) \times 100$$

Where  $P_o$  is the average percent thiamin in feed flour (dry basis, based on four measurements) and  $P_i$  is the average percent thiamin in the extruded product (dry basis, based on three measurements). This modified method for thiamin analysis is presented in detail in Appendix 4.

## 2.8 Experimental Design

In the present study, the independent process variables included moisture content (%), die geometry (mm), screw speed (rpm), temperature profile (°C), carbon dioxide pressure (psi), screw configuration, feed rate (kg/hr), and initial thiamin concentration (0.3% w/w). The dependent variables were die pressure (psi), temperature of product at die (°C), specific mechanical energy (kJ/kg), expansion ratio, bulk density (g/mL), water absorption index, water solubility index, mean residence time (seconds), and % thiamin loss. Table 2.3 shows the experimental design for the control and CO<sub>2</sub> screw configurations. In addition to screw configuration, temperature profile, and moisture content, the constant independent variables for high temperature controls were feed rate (5.2 kg/hr), initial thiamin concentration (0.3% w/w), and die geometry (3 mm diameter x 12 mm length). A range of screw speeds was used to see if there was a significant



difference between dependent variables and changes in screw speed. In samples 7-30, screw configuration, temperature profile, feed rate (3.6 kg/hr), initial flour thiamin concentration (0.3%), and die geometry (3 mm diameter x 6 mm length) were the constant independent variables. A range of moisture contents, screw speeds, and CO<sub>2</sub> pressures were used to see if there was a significant difference between the dependent variables and different extrusion conditions. Samples 7-12 and 19-24 were also considered controls to show the effect of CO<sub>2</sub> pressure on the dependent variables when no CO<sub>2</sub> was injected. For each extrusion condition, replicate runs were conducted on different days. For example, samples 1, 3, and 5 were run on one day, and 2, 4, and 6 were run on a different day.

Listed in Table 2.4 is a set of additional experiments that were run after samples 1-30 were collected. The purpose of adding these two experiments was to investigate whether or not the CO<sub>2</sub> extrusion set-up (CO<sub>2</sub> screw configuration, 3 mm x 6 mm die, and 3.6 kg/hr feed rate) could produce quality expanded-products using the higher (control) temperature profile, 0 psi CO<sub>2</sub>, screw speed of 300 rpm, and 22% moisture content. Another reason was to see how these extrusion conditions affected mean residence time and thiamin loss, and also to compare these mean residence time and thiamin loss values to those measured in samples 5 and 6 (Table 2.4 compared to Table 2.3).

**Table 2.3. Experimental Design for Data Collection Using Control and Carbon-Dioxide Screw Configurations<sup>ab</sup>**

<u>Sample</u>	<u>Screw Configuration</u>	<u>Moisture Content</u>	<u>Temperature Profile (°C)</u>	<u>CO<sub>2</sub> Pressure (psi)</u>	<u>Screw Speed (rpm)</u>
1	Control	22%	40/60/90/130/150	N/A	200
2	Control	22%	40/60/90/130/150	N/A	200
3	Control	22%	40/60/90/130/150	N/A	250
4	Control	22%	40/60/90/130/150	N/A	250
5	Control	22%	40/60/90/130/150	N/A	300
6	Control	22%	40/60/90/130/150	N/A	300
7	CO <sub>2</sub>	22%	40/40/50/70/80	0	300
8	CO <sub>2</sub>	22%	40/40/50/70/80	0	300
9	CO <sub>2</sub>	22%	40/40/50/70/80	0	350
10	CO <sub>2</sub>	22%	40/40/50/70/80	0	350
11	CO <sub>2</sub>	22%	40/40/50/70/80	0	400
12	CO <sub>2</sub>	22%	40/40/50/70/80	0	400
13	CO <sub>2</sub>	22%	40/40/50/70/80	150	300
14	CO <sub>2</sub>	22%	40/40/50/70/80	150	300
15	CO <sub>2</sub>	22%	40/40/50/70/80	150	350
16	CO <sub>2</sub>	22%	40/40/50/70/80	150	350
17	CO <sub>2</sub>	22%	40/40/50/70/80	150	400
18	CO <sub>2</sub>	22%	40/40/50/70/80	150	400
19	CO <sub>2</sub>	25%	40/40/50/70/80	0	300
20	CO <sub>2</sub>	25%	40/40/50/70/80	0	300
21	CO <sub>2</sub>	25%	40/40/50/70/80	0	350
22	CO <sub>2</sub>	25%	40/40/50/70/80	0	350
23	CO <sub>2</sub>	25%	40/40/50/70/80	0	400
24	CO <sub>2</sub>	25%	40/40/50/70/80	0	400
25	CO <sub>2</sub>	25%	40/40/50/70/80	150	300
26	CO <sub>2</sub>	25%	40/40/50/70/80	150	300
27	CO <sub>2</sub>	25%	40/40/50/70/80	150	350
28	CO <sub>2</sub>	25%	40/40/50/70/80	150	350
29	CO <sub>2</sub>	25%	40/40/50/70/80	150	400
30	CO <sub>2</sub>	25%	40/40/50/70/80	150	400

<sup>a</sup> Die geometry = 3 mm diameter x 12 mm length, samples 1-6  
= 3 mm diameter x 6 mm length, samples 7-30

<sup>b</sup> Feed rate = 5.2 kg/hr, samples 1-6  
= 3.6 kg/hr, samples 7-30

**Table 2.4. Additional Experiments Using CO<sub>2</sub> Screw Configuration at Higher Temperatures <sup>ab</sup>**

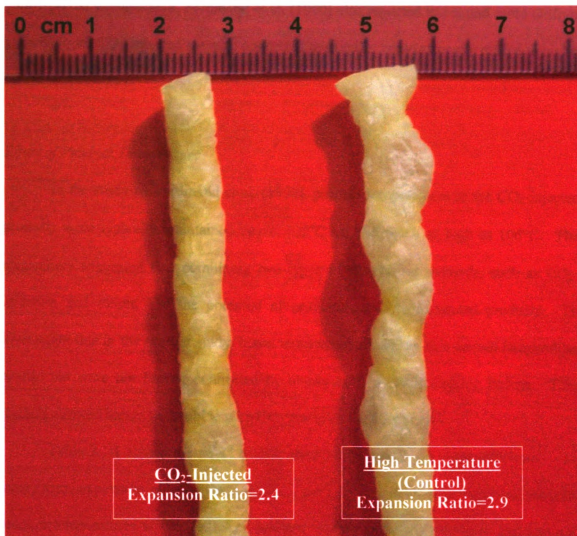
<u>Sample</u>	<u>Screw Configuration</u>	<u>Moisture Content</u>	<u>Temperature Profile (°C)</u>	<u>CO<sub>2</sub> Pressure (psi)</u>	<u>Screw Speed (rpm)</u>
31	CO <sub>2</sub>	22%	40/60/90/130/150	0	300
32	CO <sub>2</sub>	22%	40/60/90/130/150	0	300

<sup>a</sup> Die geometry = 3 mm diameter x 6 mm length

<sup>b</sup> Feed rate = 3.6 kg/hr

## Results and Discussion

### 3.1. Expansion Ratio



**Figure 3.1.1. Maximum Expansion Ratio for CO<sub>2</sub>-Injected and High- Temperature (Control) Extrudates.**

Figure 3.1.1 shows the maximum expansion ratio for the CO<sub>2</sub>-injected and control samples. For the CO<sub>2</sub>-injected extrudates, maximum expansion ratio was obtained at a screw speed of 400 rpm, 22% moisture, 150 psi CO<sub>2</sub> pressure, and die product temperature of 119°C. For the control samples, expansion ratio was maximized at a screw speed of 300 rpm, 22% moisture, and die product temperature of 161°C. Even

though the expansion was not as great for the CO<sub>2</sub>-injected samples, these samples typically had a more uniform expansion than the control samples (Figure 3.1.1). For CO<sub>2</sub>-injected wheat starch, Ferdinand et al. (1990) also reported lower and more uniform expansion for CO<sub>2</sub>-injected samples compared to samples conventionally puffed at higher barrel temperatures.

#### *Effect of Product Temperature*

In the study by Ferdinand et al. (1990), product temperatures in the CO<sub>2</sub>-injected products were typically maintained below 100°C but did reach as high as 106°C. The researchers suggested that combining two types of structuring methods, such as CO<sub>2</sub>-injection and steam, had the potential of producing quality expanded products. To investigate this in the present study, barrel temperatures were set to a certain temperature profile but were not further controlled by means such as water-cooling jackets. This method allowed steam to combine with CO<sub>2</sub> gas as expanding agents.

Table 3.1.1 shows the effect of die product temperature on expansion ratio. In most cases, expansion ratio between replicate samples increased with small increases in product temperatures at the die. For example, at 22% moisture, 300 rpm, and 150 psi CO<sub>2</sub> pressure, the expansion ratios were 1.8 and 1.4 for product temperatures of 112.7°C and 104°C, respectively. In addition, when moisture content inside the barrel decreased from 25 to 22%, product temperature and expansion ratio both increased (Table 3.1.1). Bhattacharya and Hanna (1987) also reported increases in product temperature and expansion ratio when cornstarch was extruded at lower moisture contents. In the present

study, when product temperatures were below 100° C, average expansion ratio did not exceed 1.4.

**Table 3.1.1. The Effect of Die Product Temperature on Expansion Ratios for Samples in the CO<sub>2</sub> (at Lower Temperatures) and Control (at Higher Temperatures) Screw Configurations**

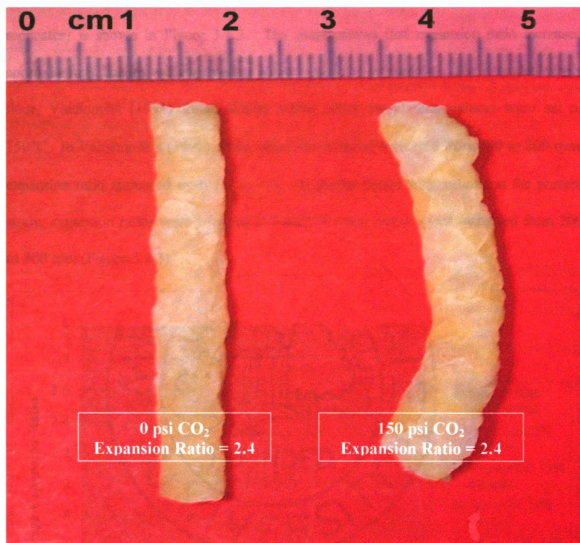
<b>Screw Config.</b>	<b>% Moisture</b>	<b>Screw Speed (rpm)</b>	<b>CO<sub>2</sub> Pressure (psi)</b>	<b>Product Temperature at Die (°C)</b>	<b>Expansion Ratio<sup>a</sup></b>
Control	22	200	N/A	161.3	2.3
Control	22	200	N/A	154.6	2.1
Control	22	250	N/A	160.2	2.6
Control	22	250	N/A	158.7	2.4
Control	22	300	N/A	161.3	2.9
Control	22	300	N/A	159.6	2.7
CO <sub>2</sub>	22	300	0	107.1	1.3
CO <sub>2</sub>	22	300	0	114.7	2.1
CO <sub>2</sub>	22	300	150	112.7	1.8
CO <sub>2</sub>	22	300	150	104	1.4
CO <sub>2</sub>	22	350	0	109.2	2.1
CO <sub>2</sub>	22	350	0	112.2	2.3
CO <sub>2</sub>	22	350	150	115.6	2.2
CO <sub>2</sub>	22	350	150	106.6	1.5
CO <sub>2</sub>	22	400	0	106.6	2.2
CO <sub>2</sub>	22	400	0	108.2	2.4
CO <sub>2</sub>	22	400	150	118.8	2.4
CO <sub>2</sub>	22	400	150	111.5	2.1
CO <sub>2</sub>	25	300	0	96.3	1.2
CO <sub>2</sub>	25	300	0	95.9	1.2
CO <sub>2</sub>	25	300	150	99.5	1.3
CO <sub>2</sub>	25	300	150	100.8	1.5
CO <sub>2</sub>	25	350	0	93.1	1.3
CO <sub>2</sub>	25	350	0	101.2	1.3
CO <sub>2</sub>	25	350	150	101.0	1.5
CO <sub>2</sub>	25	350	150	103.1	1.6
CO <sub>2</sub>	25	400	0	95.2	1.3
CO <sub>2</sub>	25	400	0	98.8	1.5
CO <sub>2</sub>	25	400	150	102.5	1.6
CO <sub>2</sub>	25	400	150	101.3	1.8

<sup>a</sup> Average based on fifteen measurements.

At 25% moisture without CO<sub>2</sub>-injection, die product temperatures did not exceed 100°C (Table 3.1.1). When CO<sub>2</sub> was injected, product temperatures at the die were slightly over 100°C, indicating that water vapor flash-off, along with CO<sub>2</sub>-injection, contributed to product expansion. At 22% moisture with and without CO<sub>2</sub>, product temperatures at the die also reached higher than 100°C. At this moisture content, expansion ratios were sometimes higher when no CO<sub>2</sub> was injected. Ferdinand et al. (1990) found similar results, in that when CO<sub>2</sub> pressure varied from 0, 175, 263, and 365 psi at 17% moisture and 200 rpm screw speed, maximum expansion ratio was obtained when CO<sub>2</sub> pressure was zero. In the present study, Figure 3.1.2 compares the expansion ratios between samples injected with 150 psi CO<sub>2</sub> and 0 psi CO<sub>2</sub>, at 400 rpm screw speed, and 22% moisture. Figure 3.1.2 shows similar expansion ratios between samples, in addition to uniform expansion. At screw speeds of only 300 rpm and 400 rpm, t-test results at  $\alpha=0.05$  showed that there was not a significant difference between mean expansion ratios at 0 psi or 150 psi CO<sub>2</sub> (Appendix 5, Table A.5.9).

The CO<sub>2</sub> screw configuration, die geometry, and feed rate were used at the higher (control) temperatures at 300 rpm, 22% moisture, and 0 psi CO<sub>2</sub> to investigate the effect of a large temperature increase on expansion ratio. Expansion ratios were 2.3 and 2.4 at die temperatures of 153.4 °C and 155.8 °C, respectively, compared to expansion ratios of 1.3 and 2.1 at die temperatures of 107.1°C and 114.7 °C in the lower temperature profile (Table 3.1.2 compared to Table 3.1.1). It appears that increasing temperature (die temperature increased from 80°C to 150°C) had only a modest effect on expansion ratio, and a maximum expansion ratio was reached. To achieve higher expansion ratios, variables other than temperature must be changed, such as screw configuration and feed

rate. For example, the expansion ratios for control samples at the higher feed rate of 5.2 kg/hr reached 2.9 (Table 3.1.1).



**Figure 3.1.2. Expansion Ratio Comparison for CO<sub>2</sub> samples at 22% Moisture and 400 rpm Screw Speed With or Without CO<sub>2</sub>-Injection.**

**Table 3.1.2. The Effect of Die Product Temperature on Expansion Ratios for Samples in the CO<sub>2</sub> Screw Configuration at Higher Temperatures**

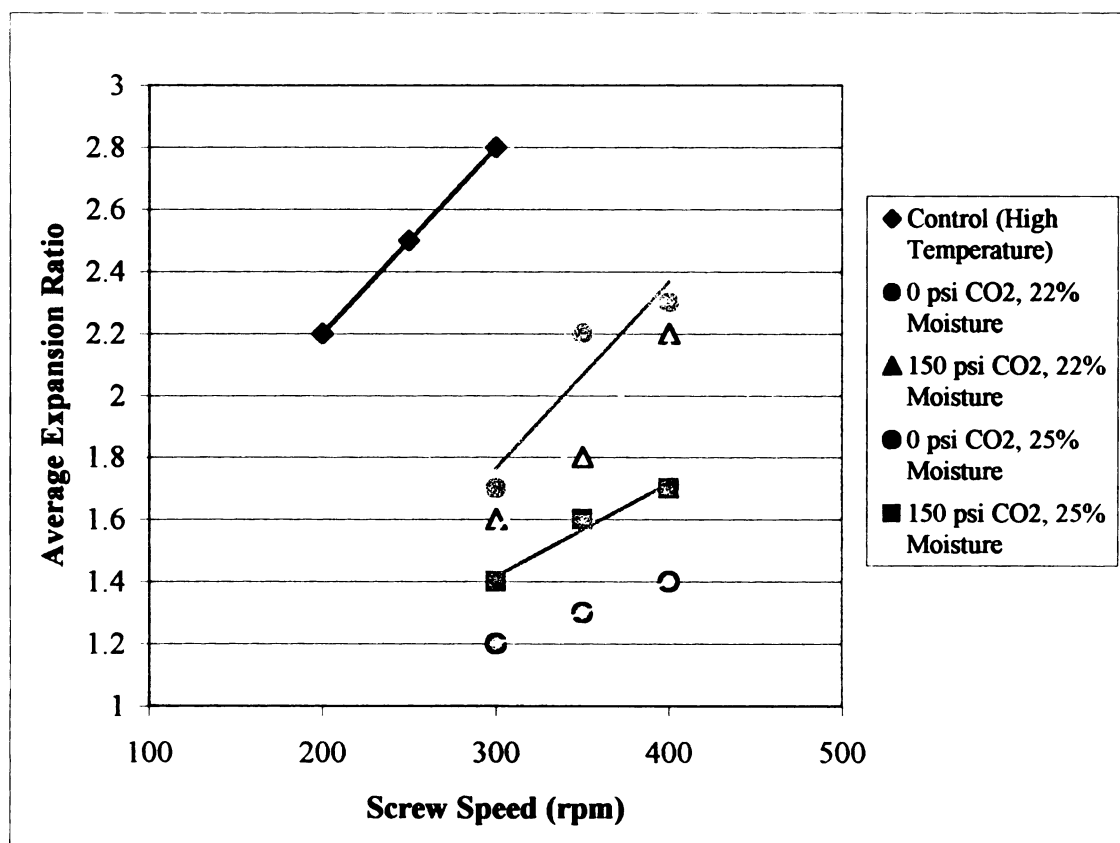
Screw Config.	% Moisture	Screw Speed (rpm)	CO <sub>2</sub> Pressure (psi)	Product Temperature at Die (°C)	Expansion Ratio <sup>a</sup>
CO <sub>2</sub>	22	300	0	153.4	2.3
CO <sub>2</sub>	22	300	0	155.8	2.4

<sup>a</sup> Average based on fifteen measurements.



### *Effect of Screw Speed*

The relationship between screw speed and average expansion ratio (between replicates) is shown in Figure 3.1.3. The graph shows that expansion ratio increased approximately linearly with increasing screw speed for all samples. For extruded wheat flour, Vainionpää (1991) found similar trends when barrel temperatures were set to 150°C. In Vainionpää's (1991) study, when screw speed increased from 100 to 200 rpm, expansion ratio increased from 1.4 to 1.6. At similar barrel temperatures in the present study, expansion ratios were between 2.2 and 2.8 when screw speed increased from 200 to 300 rpm (Figure 3.1.3).



**Figure 3.1.3. Average Expansion Ratio vs. Screw Speed Using the CO<sub>2</sub> (at Lower Temperatures) and Control (at Higher Temperatures) Screw Configurations.**

Lee et al. (1999) found a negative relationship between screw speed and expansion ratio when extruded cornstarch was injected with supercritical CO<sub>2</sub>. They attributed this relationship to mean residence time being reduced at higher screw speeds, which most likely caused a reduction in starch gelatinization and expansion. Although starch gelatinization was not measured directly in the present study, it appears that increasing shear rates with higher screw speeds may have had a greater effect on starch gelatinization than shorter residence times.

#### *Relationship Between Screw Speed and SME*

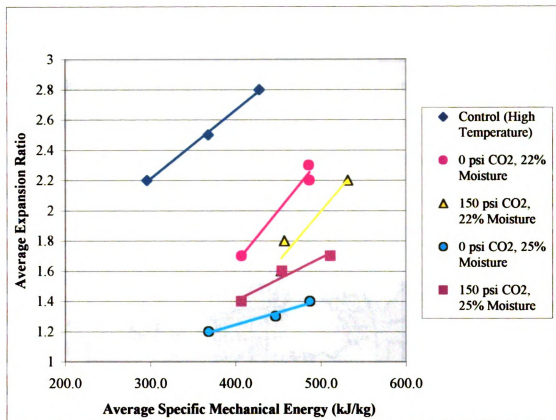
In addition to the heating elements along the barrel, the dissipation of viscous energy is also a source of heat (Harper, 1978). Figure 3.1.4 shows that increasing average SME<sup>a</sup> (average between replicate samples) had a positive effect on average expansion ratio. This trend is similar to the relationship between screw speed and average expansion ratio in Figure 3.1.3. This result suggests that the combination of increasing shear and viscous energy may have contributed to greater starch gelatinization in the dough, thus causing more expansion to occur. This result also indicates that in terms of processing energy costs for a given extrusion set-up, it will cost more to produce a highly expanded product.

At 150 psi CO<sub>2</sub> for both moisture contents, there was some decrease in expansion ratio at higher SMEs (Figure 3.1.4). Ferdinand et al. (1990) also reported that at higher CO<sub>2</sub> pressures, expansion ratio increased with increasing SME to a maximum, before gradually decreasing. In the present study, this decrease in expansion ratio with SME at higher CO<sub>2</sub> pressures may have been the result of inconsistencies in the flow of CO<sub>2</sub>.

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<sup>a</sup> The average value for dough density was calculated as 1250 kg/m<sup>3</sup>.

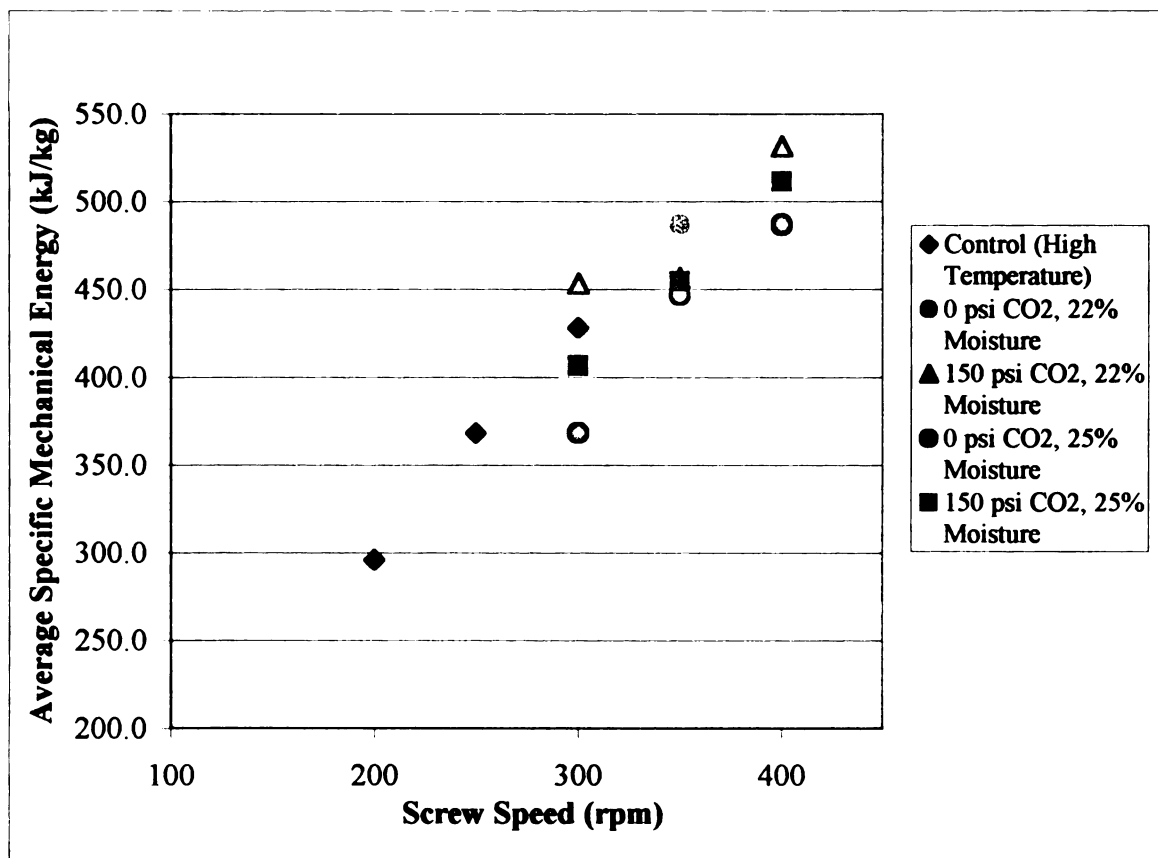
Carbon dioxide pressures were monitored with a pressure gauge and pressures stayed fairly constant. However, the actual flow of CO<sub>2</sub> into the extruder was not measured directly.



**Figure 3.1.4. Average Expansion Ratio vs. Average Specific Mechanical Energy Using the CO<sub>2</sub> (at Lower Temperatures) and Control (at Higher Temperatures) Screw Configurations.**

Even though SME is a function of torque, screw speed, and throughput, Altomare and Ghossi (1986) investigated the influence of changing only screw speed on SME in a twin-screw extruder. They reported that at moisture contents of 20 and 25%, SME input increased linearly when screw speed increased from 233 to 400 rpm. In the present study, a near linear relationship also existed between SME and screw speed (Figure 3.1.5).

The combination of factors influencing SME (increased torque, decreased screw speed, and increased feed rate) caused a net decrease in SME for the control samples compared to the CO<sub>2</sub> samples. It was reported in Section 1.1.2 that one of the potential advantages to extruding at lower temperatures using CO<sub>2</sub>-injection was reduced energy costs. However, in the present study the opposite result was found and more energy input was required to produce expanded products at lower temperatures than at higher temperatures (Figure 3.1.5). Also, approximately 70% more energy input was required to obtain similar expansion ratios of 2.2 at lower temperatures than at higher temperatures (Figure 3.1.4).



**Figure 3.1.5. Specific Mechanical Energy vs. Screw Speed Using the CO<sub>2</sub> (at Lower Temperatures) and Control (at Higher Temperatures) Screw Configurations.**

### **3.2. Bulk Density, Water Absorption Index, and Water Solubility Index**

#### *Bulk Density*

Average bulk densities for all products extruded at lower temperatures with or without CO<sub>2</sub> gas were higher than control products puffed at conventional temperatures (Table 3.2.1). Higher bulk densities were likely to occur since the expansion ratios were lower than that of the controls. Ferdinand et al. (1990) showed that bulk density was lower at a lower moisture content and decreased with increasing screw speed. In the present study, bulk densities in the CO<sub>2</sub> samples decreased when feed material moisture decreased from 25 to 22%. Also at 22% moisture, bulk density decreased with increasing screw speed (Table 3.2.1). These would be the expected trends for bulk density since expansion ratio increased with increasing screw speed and decreasing moisture content (Figure 3.1.3). No clear trend of bulk density with screw speed was shown at 25% moisture, which may have been due to the low expansion ratios found at this moisture content (Tables 3.1.1 and 3.2.1).

#### *WAI and % WSI*

The products extruded using the CO<sub>2</sub> screw configuration had lower average WAI than products puffed at higher temperatures (Table 3.2.1). Water absorption at room temperature is related to the extent of starch gelatinization in a extruded product that has been fully cooked. Native starches have zero water absorption capabilities at room temperature due to their compact granular structure, whereas gelatinized starch molecules absorb water at room temperature and swell. As the starch granules swell, WAI increases until a peak viscosity is reached, depending on the extent of starch damage. WAI then

decreases with the onset of dextrinization (Colonna et al. 1989). Since the amount of gelatinized starch molecules was not measured, further analytical tests would be required to determine the extent of starch gelatinization. One method of determining degree of gelatinization is the Rapid Viscoanalyzer.

**Table 3.2.1. Average Bulk Density, WAI, and % WSI for CO<sub>2</sub> (at Lower Temperatures) and Control (at Higher Temperatures) Screw Configurations**

Screw Config.	% Moist.	Screw Speed (rpm)	CO <sub>2</sub> Press. (psi)	Average Bulk Density <sup>a</sup> (g/ml)	Average WAI <sup>a</sup>	Average % WSI <sup>a</sup>
Control	22	200	N/A	0.206 (± 0.018)	8.95 (± 0.21)	11.5% (± 0.37)
Control	22	250	N/A	0.129 (± 0.013)	9.18 (± 0.24)	12.4% (± 1.02)
Control	22	300	N/A	0.099 (± 0.006)	9.13 (± 0.16)	13.6% (± 0.59)
CO <sub>2</sub>	22	300	0	0.516 (± 0.127)	7.00 (± 0.67)	12.0 (± 1.68)
CO <sub>2</sub>	22	300	150	0.501 (± 0.135)	6.66 (± 0.52)	11.7 (± 1.4)
CO <sub>2</sub>	22	350	0	0.329 (± 0.040)	7.88 (± 0.11)	14.0 (± 0.61)
CO <sub>2</sub>	22	350	150	0.434 (± 0.156)	7.26 (± 0.19)	11.4 (± 1.31)
CO <sub>2</sub>	22	400	0	0.291 (± 0.026)	7.36 (± 0.34)	13.2 (± 0.44)
CO <sub>2</sub>	22	400	150	0.284 (± 0.049)	7.86 (± 0.15)	16.6 (± 1.47)
CO <sub>2</sub>	25	300	0	0.532 (± 0.015)	7.92 (± 2.15)	6.71 (± 0.53)
CO <sub>2</sub>	25	300	150	0.605 (± 0.125)	5.31 (± 0.36)	7.39 (± 0.29)
CO <sub>2</sub>	25	350	0	0.718 (± 0.066)	7.85 (± 0.57)	7.54 (± 0.64)
CO <sub>2</sub>	25	350	150	0.683 (± 0.101)	5.62 (± 0.11)	8.09 (± 0.61)
CO <sub>2</sub>	25	400	0	0.689 (± 0.059)	7.42 (± 0.84)	8.68 (± 0.63)
CO <sub>2</sub>	25	400	150	0.616 (± 0.020)	5.89 (± 0.11)	9.51 (± 0.35)

<sup>a</sup> Average between replicate samples. Standard deviation given in parentheses.

Dextrinization is also referred to as starch fragmentation, and causes % WSI to increase. Dextrinization is related to the number of soluble molecules. At  $\alpha=0.05$ , t-tests comparing means between high and low-temperature samples at 300 rpm and 22% moisture showed that there was not a significant difference between mean % WSI values (Appendix 5, Table A.5.10). Another t-test comparison, also at  $\alpha=0.05$ , was conducted between mean % WSI at 22 and 25% moisture for samples extruded at lower

temperatures, 300 rpm screw speed, and 0 psi CO<sub>2</sub> (Appendix 5, Table A.5.11). As shown in Table A.5.11, % WSI is significantly lower at 25% moisture than compared to 22%. Colonna et al. (1984) reported for wheat starch that lower water solubilities occur under high moisture and low temperature extrusion conditions. In the present study, CO<sub>2</sub> samples at 25% moisture had lower SME values than CO<sub>2</sub> samples at 22% moisture (Figure 3.1.5). These results suggest that the extent of starch gelatinization decreased with decreasing temperature and SME, and increasing moisture content.

#### *WAI and % WSI: Effect of Screw Speed and CO<sub>2</sub>*

Within the CO<sub>2</sub> screw configuration at 25% moisture, % WSI increased with increasing screw speed (Table 3.2.1). When no CO<sub>2</sub> was injected at 25% moisture only, WAIs were higher and % WSIs were lower than the corresponding values for injection of CO<sub>2</sub> at 25% moisture. At these conditions, extrusion product temperatures were the lowest, indicating that the degree of starch gelatinization may have been reduced. At 22% moisture no apparent trends were found for increasing screw speed or CO<sub>2</sub> pressure on WAI and % WSI (Table 3.2.1).

#### *Bulk Density, WAI, and % WSI for Samples Extruded Using the CO<sub>2</sub> Screw Configuration at Higher Temperatures*

As stated in Section 3.1, there was a small increase in expansion ratio with a large increase in temperature for the CO<sub>2</sub> samples at 300 rpm, 22% moisture, and 0 psi CO<sub>2</sub>. The bulk density values in the CO<sub>2</sub> samples extruded at higher temperatures were lower than the CO<sub>2</sub> samples extruded at lower temperatures (Table 3.2.1 compared to Table 3.2.2). After drying, the moisture content in the CO<sub>2</sub> samples at higher temperatures was

4.8% compared to 6.7% at the lower temperature profile. This result suggests that for the CO<sub>2</sub> extrusion conditions, a reduction in bulk density at higher temperatures may have been the result of lower moistures in the final dried product.

Average WAI for the CO<sub>2</sub> samples extruded at higher temperatures was 7.62 (Table 3.2.2). This is higher than the average WAI of 7.00 for the low-temperature CO<sub>2</sub> samples at 300 rpm (Table 3.2.1). The average % WSI for the high-temperature CO<sub>2</sub> samples was 14.7 compared to 12.0 in the low-temperature CO<sub>2</sub> samples (Table 3.2.1 compared to Table 3.2.2). Higher WAI and % WSI values suggest that extent of starch gelatinization was greater with increasing temperature using the CO<sub>2</sub> extrusion set-up.

**Table 3.2.2. Average Bulk Density, WAI, and % WSI for the CO<sub>2</sub> Screw Configuration at Higher Temperatures**

<b>Screw Config.</b>	<b>% Moist.</b>	<b>Screw Speed (rpm)</b>	<b>CO<sub>2</sub> Press. (psi)</b>	<b>Average Bulk Density<sup>a</sup> (g/ml)</b>	<b>Average WAI<sup>a</sup></b>	<b>Average % WSI<sup>a</sup></b>
CO <sub>2</sub>	22	300	0	0.190 (±0.007)	7.62 (± 0.37)	14.7% (± 0.60)

<sup>a</sup> Average between replicate samples. Standard deviation given in parentheses.

### *Statistical Interpretation*

In Table 3.2.1, average values were the averages between replicate samples (repeated on different days) and ± standard deviation was given in parentheses. Standard deviation is a measure of how much the samples varied in physical attributes when extrusion conditions were repeated on a different day. Larger variations in bulk density, WAI, and % WSI appeared to occur only within the CO<sub>2</sub> screw configuration (Table 3.2.1). In the CO<sub>2</sub> screw configuration non-uniform mixing was visible in the extruded products only at 25% moisture and 0 psi CO<sub>2</sub>. In some of the extrudates, there were areas



along the strand where residual flour appeared. Therefore, the higher variability in physical characteristics within the CO<sub>2</sub> screw configuration at 25% moisture may have been due to this occurrence of non-uniform mixing.

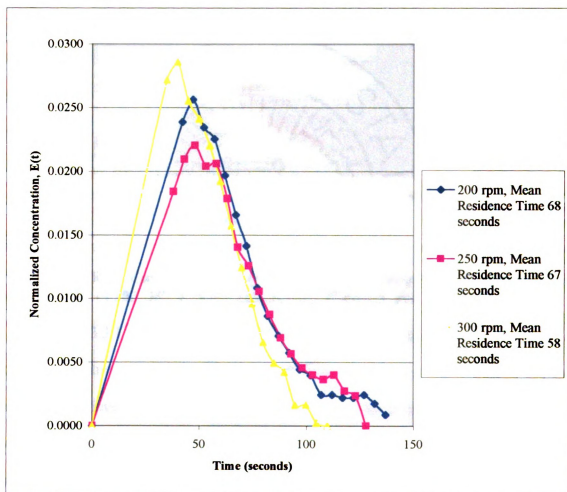
In Table 3.2.1, there was a higher variability in bulk densities (between replicates) at 22% moisture compared to 25% moisture. In addition, expansion ratios at 22% moisture, were less repeatable than 25% moisture, especially at the lower screw speeds (Table 3.1.1). These greater variations in bulk densities and expansion ratios were probably due to variations in die pressures and die temperatures when extrusion conditions were repeated on different days (Appendix 5, Table A.5.1).

### **3.3. Mean Residence Time**

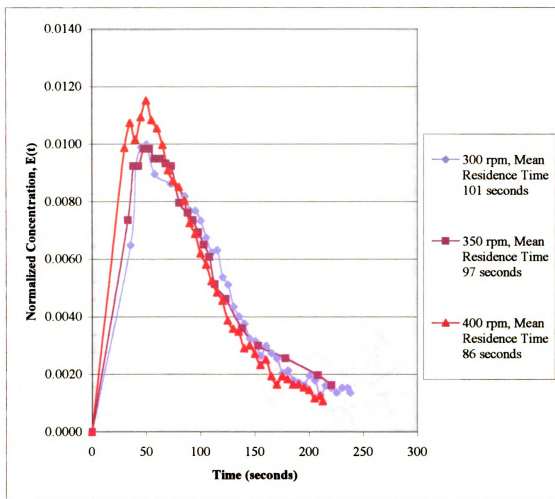
Figures 3.3.1 and 3.3.2 show examples of residence time distribution (RTD) curves, based on one replicate, for the CO<sub>2</sub> and control screw configurations. At each screw speed, mean residence time was longer in the CO<sub>2</sub> screw configuration than in the control screw configuration. As stated in Section 2.2, the conveying efficiency of the screw configuration is determined by the geometry of the screw elements. Almost twice as many mixing paddles at a 60° orientation are found in the CO<sub>2</sub> screw configuration (13 vs. 7, Table 2.1). Since the conveying efficiency of the CO<sub>2</sub> screw configuration is lowered, the CO<sub>2</sub> samples spend more time in the extruder barrel than compared to the controls.

The RTD curves in the control screw configuration show that as screw speed increased, mean residence time decreased. Altomare and Ghossi (1986) also reported that shorter mean residence times resulted when screw speed increased at a constant throughput. The researchers reported that when screw speeds were 150, 317, and 400

rpm, mean residence times were 28.4, 22.1, and 20.2 seconds, respectively. In the present study, where a smaller range of screw speeds was used compared to Altomare and Ghossi, there was only a one second difference in mean residence times between screw speeds of 200 and 250 rpm (Figure 3.3.1). When these extrusion conditions were repeated on a different day, the difference in residence times increased to 15 seconds between screw speeds of 200 and 250 rpm (Appendix 5, Table A.5.2), following the expected trend of shorter mean residence times at higher screw speeds.



**Figure 3.3.1. RTD Curves for Control (at Higher Temperatures) Screw Configuration at Screw Speeds of 200, 250, and 300 rpm at 22% Moisture.**



**Figure 3.3.2. RTD Curves for CO<sub>2</sub> (at Lower Temperatures) Screw Configuration at Screw Speeds of 300, 350, and 400 rpm at 22% Moisture, and 150 psi CO<sub>2</sub>.**

Table 3.3.1 lists the mean residence times (between replicates) for the CO<sub>2</sub> (at lower temperatures) and control (at higher temperatures) screw configurations. For these products, an inverse relationship typically existed between mean residence time and screw speed. At 22% moisture and 0 psi CO<sub>2</sub> this trend was not followed, which may have been due to the variability in residence times between replicate samples (Table 3.3.1). The higher standard deviations for some of the CO<sub>2</sub> samples show that it was more difficult to obtain highly repeatable results than for the control samples. However, the same trends were observed within any one run (Appendix 5, Table A.5.1).

**Table 3.3.1. Mean Residence Times for CO<sub>2</sub> (at Lower Temperatures) and Control (at Higher Temperatures) Screw Configurations**

Screw Configuration	% Moisture	CO <sub>2</sub> Pressure (psi)	Screw Speed (rpm)	Mean Residence Time <sup>a</sup> (seconds)
Control	22	N/A	200	77.08 (± 12.1)
Control	22	N/A	250	68.29 (± 1.3)
Control	22	N/A	300	57.52 (± 0.2)
CO <sub>2</sub>	22	0	300	129.24 (± 6.2)
CO <sub>2</sub>	22	0	350	136.42 (± 12.2)
CO <sub>2</sub>	22	0	400	116.96 (± 23.7)
CO <sub>2</sub>	22	150	300	124.42 (± 32.3)
CO <sub>2</sub>	22	150	350	114.18 (± 22.9)
CO <sub>2</sub>	22	150	400	100.51 (± 19.3)
CO <sub>2</sub>	25	0	300	129.73 (± 9.5)
CO <sub>2</sub>	25	0	350	126.98 (± 6.7)
CO <sub>2</sub>	25	0	400	118.0 (± 7.7)
CO <sub>2</sub>	25	150	300	135.10 (± 16.5)
CO <sub>2</sub>	25	150	350	128.94 (± 2.6)
CO <sub>2</sub>	25	150	400	103.89 (± 16.4)

<sup>a</sup> Average between replicate samples. Standard deviation given in parentheses.

The mean residence time for the CO<sub>2</sub> screw configuration at higher temperatures was  $112.9 \pm 16.2$  seconds, compared to the mean residence time of  $57.5 \pm 0.2$  seconds for control samples at 300 rpm (Table 3.3.2 compared to Table 3.3.1). Therefore, even at higher temperatures, the mean residence time for the CO<sub>2</sub> screw configuration continued to be about twice as long as that of the control screw configuration.

**Table 3.3.2. Mean Residence Times for the CO<sub>2</sub> Screw Configuration at Higher Temperatures**

Screw Configuration	% Moisture	CO <sub>2</sub> Pressure (psi)	Screw Speed (rpm)	Mean Residence Time (seconds) <sup>a</sup>
CO <sub>2</sub>	22	0	300	112.92 (± 16.2)

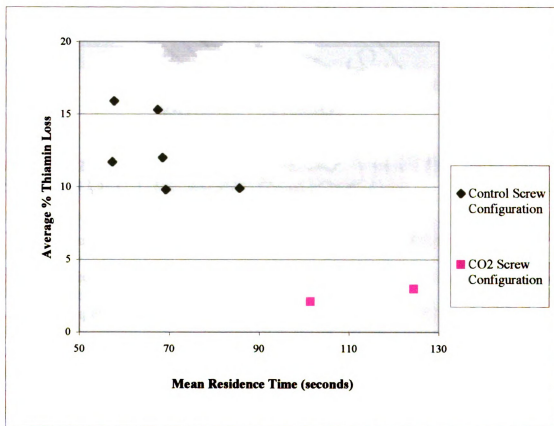
<sup>a</sup> Average between replicate samples. Standard deviation given in parentheses.

### 3.4. Thiamin Loss

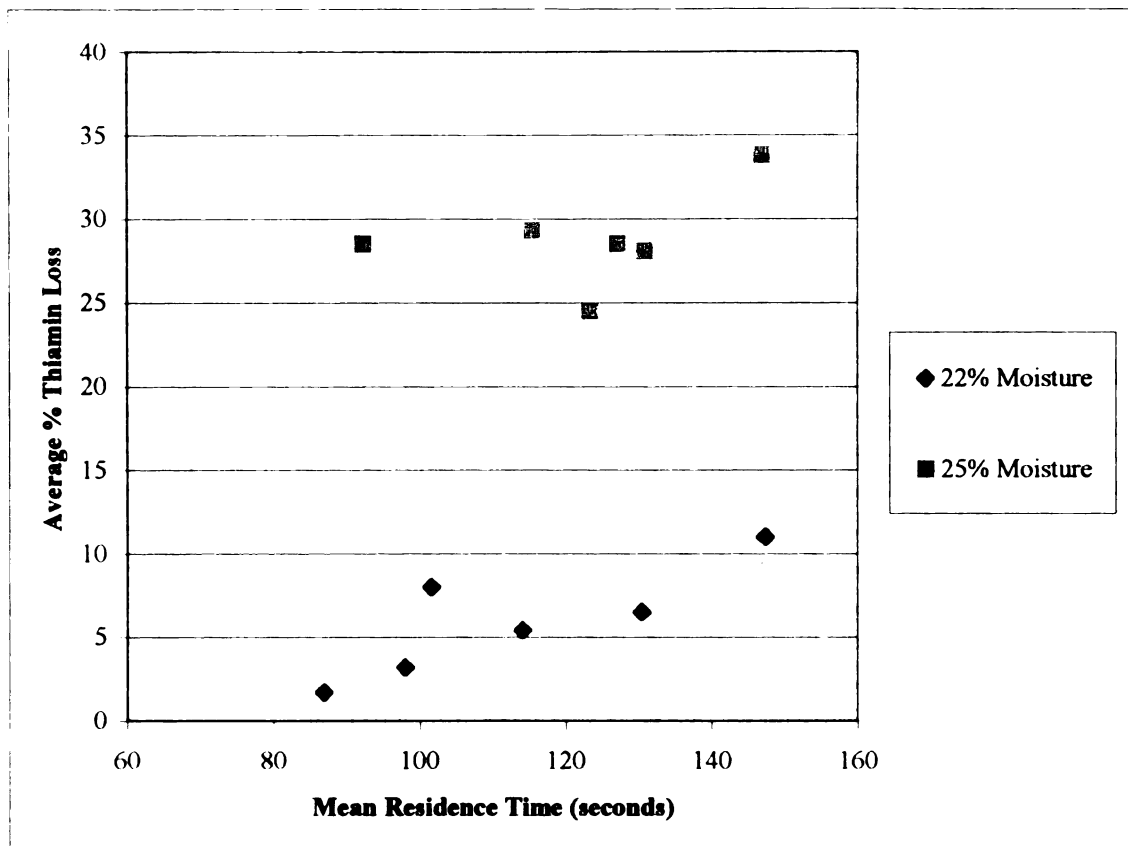
Average thiamin losses (based on three measurements) ranged from 10-16% in extrudates puffed using conventional methods (Figure 3.4.1). Guzman-Tello and Cheftel (1987) reported thiamin losses of 30% when the exit product temperature was 160°C for 14% moisture content wheat flour. At 160°C, they also reported that an increase in screw speed caused more thiamin destruction, even though residence time decreased. In the present study, the data also suggests a trend of decreasing thiamin loss with increasing mean residence time (decreasing screw speed) in the controls at higher temperatures (Figure 3.4.1). This trend did not change when samples were extruded using the CO<sub>2</sub> screw configuration at higher temperatures (Figure 3.4.1). Thiamin losses between replicates were 2.1 and 3.0% at the respective mean residence times of 124.4 and 101.4 seconds (Appendix 5, Tables A.5.3 and A.5.4). It appears that for high-temperature extrusion, thiamin loss increased with increasing screw speed, suggesting that shear effects predominated over thermal effects in thiamin degradation.

Compared to the control samples, lower thiamin losses were observed in the CO<sub>2</sub>-injected samples, but only when the moisture content was 22% (Figure 3.4.2). All CO<sub>2</sub>-injected samples at 25% moisture showed greater than 24.5% thiamin loss (Figure 3.4.2). Most of the literature has reported that as feed material moisture content increases, thiamin loss decreases (Maga and Sizer, 1978; Pham and Del Rosario, 1986; Guzman-Tello and Cheftel, 1987). Killeit (1994) suggested that lower thiamin losses at higher moistures were the result of decreased dough viscosities, which reduced the amount of shear and energy input on the product. In the present study, SME values were typically lower for most of the CO<sub>2</sub> samples at 25% moisture than the CO<sub>2</sub> samples at 22%

moisture (Figure 3.1.5). Unexpectedly, thiamin losses were about 15% greater at 25% moisture than the CO<sub>2</sub> samples at 22% moisture. Herrmann and Tunger (1966) suggested that in the dehydration of foods, changes in moisture contents may significantly affect the concentrations of the constituents and possibly the pH value of the products. Therefore, a higher thiamin loss at 25% vs. 22% moisture in the present work may have been the result of different chemical interactions occurring at the higher moisture content. For example, an increase in feed material moisture content may have resulted in an increase in dough pH, causing more thiamin degradation at 25% moisture.



**Figure 3.4.1. Average % Thiamin Loss vs. Mean Residence Time for High Temperature Samples Using the Control and CO<sub>2</sub> Screw Configurations.**



**Figure 3.4.2. Average % Thiamin Loss vs. Mean Residence Time for CO<sub>2</sub>-Injected Samples (at Lower Temperatures) at 22 and 25% Moisture.**

It can be concluded that unlike the high temperature extrudates, it appears that thermal effects had greater influence on thiamin loss than shear effects in the CO<sub>2</sub>-injected samples (Figure 3.4.2 compared to Figure 3.4.1). In Table 3.4.1, thiamin losses and mean residence times for low-temperature samples without CO<sub>2</sub> are shown. At 22% moisture, % thiamin losses were between 0 and 1.5%, which are lower than the samples at 150 psi CO<sub>2</sub> and 22% moisture (Table 3.4.1 compared to Figure 3.4.2). At 25% moisture and 0 psi CO<sub>2</sub>, thiamin losses were lower than at 25% moisture and 150 psi CO<sub>2</sub> (Table 3.4.1 compared to Figure 3.4.2). It appears that for both moisture contents, the addition of CO<sub>2</sub> gas caused an increase in thiamin loss (Table 3.4.1). In Section 3.1, an

increase in die product temperature was observed when CO<sub>2</sub> was injected, which may have contributed to greater thiamin losses (Table 3.1.1).

**Table 3.4.1. Average % Thiamin Losses and Mean Residence Times Without CO<sub>2</sub> at Lower Temperatures.**

<b>% Moisture</b>	<b>Screw Speed (rpm)</b>	<b>Average % Thiamin Loss<sup>a</sup></b>	<b>Mean residence time (seconds)</b>
22	300	0 (± 2.2)	124.8
22	300	1.5 (± 0.8)	133.6
22	350	0 (± 2.9)	127.8
22	350	0 (± 0.8)	145.0
22	400	0 (± 4.1)	100.2
22	400	0 (± 1.7)	133.7
25	300	26.4 (± 1.1)	136.4
25	300	0.5 (± 0.4)	123.0
25	350	16.1 (± 0.3)	131.7
25	350	1.1 (± 1.5)	122.3
25	400	11.6 (± 1.0)	112.6
25	400	0 (± 1.5)	123.4

<sup>a</sup> Average of three measurements. Standard deviation given in parentheses.

As shown in Table 3.4.1, thiamin loss was greater at 25% moisture than compared to 22% moisture. The variability in % thiamin loss among replicates at 25% moisture was also greater than the samples at 22% moisture (Table 3.4.1). Despite the variability, the trend of increasing thiamin loss with increasing mean residence time was typically followed at 25% moisture.

As stated above for the control screw configuration, thiamin loss decreased with longer residence times. However, a trend of higher thiamin losses with longer mean residence times for the CO<sub>2</sub> samples is shown in Figure 3.4.2. In Appendix 5 (Tables A.5.6 and A.5.7), the maximum % CV for % thiamin (w/w) in the feed flour was 6.0% for three 1-gram samples. Guzman-Tello and Cheftel (1987) reported the % CV of five



30-gram samples to be 3.6% when flour and thiamin were mixed in a high speed mixer for one hour. The % CV for % thiamin in extruded products did not exceed 5%, indicating that the analytical method for thiamin analysis was precise (Tables A.5.6 and A.5.7).

## **Conclusions and Recommendations**

### **4.1 Summary and Conclusions**

Physical characteristics were investigated in puffed-wheat flour extruded at lower temperatures (near 100°C) using carbon dioxide gas as the expanding agent. Specific mechanical energy inputs, mean residence times and thiamin losses were also investigated. These dependent variables in low-temperature extrusion were compared to puffed-wheat extruded at conventional puffing temperatures (160°C). The current work shows that using the screw configuration listed in Chapter 2 (“CO<sub>2</sub>-Injection”, Table 2.1) at a barrel temperature profile of 40/40/50/70/80°C, with CO<sub>2</sub> pressures of 0 and 150 psi, die geometry of 3 mm diameter x 6mm length, feed material moisture content of 22%, and using screw speeds and feed rates resulting in SMEs between 426 and 572 kJ/kg, and mean residence times in the range of 87 to 145 seconds, extruded wheat can be expanded (expansion ratios > 2.0) at lower temperatures. Even though the samples extruded at lower temperatures had more uniform expansion, the expansion ratios were still lower than those of the high-temperature control samples. The expansion ratios for CO<sub>2</sub>-injected samples at 22% moisture (1.4-2.4) were closer to the control than the CO<sub>2</sub>-injected samples at 25% moisture (1.2-1.8). When no CO<sub>2</sub> was injected, the expansion ratios were 1.3-2.4 and 1.2-1.5, for 22 and 25% moisture, respectively. Expansion ratio was higher with increasing product temperature, screw speed, and energy input. This trend of increasing expansion with increasing energy input indicates that it will cost more to process a more highly expanded product.

At 22% moisture, even though barrel temperatures were set below 100°C, product temperatures in the CO<sub>2</sub>-injected samples did reach up to 119° C, indicating that

expansion was due to the combination of water vapor flash-off and CO<sub>2</sub>-injection. When no CO<sub>2</sub> was injected, product temperatures reached as high as 115°C. At 22% moisture, for screw speeds of 300 and 400 rpm, t-Test ( $\alpha=0.05$ ) results showed that CO<sub>2</sub>-injection did not have a significant effect on expansion ratio.

Consistent with the expansion ratio results, bulk densities were higher in the samples extruded at lower temperatures compared to the high-temperature control samples. Bulk densities in the CO<sub>2</sub> samples decreased when feed material moisture decreased from 25 to 22%. At 22% moisture only, bulk density decreased with increasing screw speed.

In comparison to the control, all samples extruded at the lower temperature profile had lower WAIs. The % WSI values decreased with decreasing temperature and SME, and increasing moisture content. Lower % WSI values in the CO<sub>2</sub> samples at 25% moisture may be an indication that extent of starch gelatinization was lower than compared to the other samples. A Rapid Viscoanalyzer (RVA) is one way to measure the extent of starch gelatinization. A texture-measuring device could also be used to see how degree of starch gelatinization correlated with product texture. To obtain a better assessment of “bowl-life” in the low-temperature samples, additional texture measurements could be taken after soaking the extruded products in water.

Typically, for both screw configurations (CO<sub>2</sub> and control) mean residence time decreased as screw speed increased. The only condition that did not follow this trend was at 22% moisture and 0 psi CO<sub>2</sub>, and may have been due to the variability in residence times when extrusion conditions were repeated. The CO<sub>2</sub> screw configuration had mean

residence times that were approximately twice as long as the high-temperature screw configuration.

Percent thiamin loss for conventionally puffed products was 10-16% and thiamin loss values decreased with decreasing screw speed. The opposite trend was found for CO<sub>2</sub>-injected products, where thiamin loss increased with decreasing screw speed. These opposite trends suggest that for high temperature extrusion, mechanical effects predominate in thiamin degradation, and for lower temperature extrusion with CO<sub>2</sub>-injection, thermal effects predominate. In the CO<sub>2</sub>-injected products at 22% barrel moisture, thiamin loss was 3-11%. At 25% barrel moisture, thiamin loss was 24-34%. Processing variables, such as SME and product temperatures, were lower at this moisture content than at 22% moisture. Higher losses at 25% moisture may have been the result of different chemical interactions occurring at the higher moisture content, such as an increase in dough pH at 25% moisture. At 22 and 25% moisture, product temperatures at the die were lower (up to 10°C) when no CO<sub>2</sub> was injected compared to when CO<sub>2</sub> was injected. Thus, suggesting that higher temperatures caused increased thiamin loss. Thiamin losses at 22% moisture without CO<sub>2</sub> were between 0 and 1.5%. Thiamin losses ranged from 0 to 26.4% at 25% moisture without CO<sub>2</sub>, and more variability occurred between these replicate samples than compared to 22% moisture. Despite the variability, trends of increasing thiamin loss with increasing mean residence time were typically followed.

When samples were extruded using the CO<sub>2</sub> screw configuration at the higher temperature profile, feed rate of 3.6 kg/hr, 3 mm diameter x 6 mm length, 300 rpm screw speed, and 22% moisture content, expansion ratio was approximately 2.4. Between

replicates, average percent thiamin loss at these conditions was 2.5% when product temperatures reached as high as 156°C. The mean residence time using the CO<sub>2</sub> screw configuration at higher temperatures was also approximately twice as long as in the control screw configuration at 300 rpm screw speed. These results indicate that when a different extrusion set-up is used at higher temperatures, shear effects still predominate in thiamin degradation over thermal effects. Thus, suggesting that changing screw configuration, feed rate, and die geometry in higher temperature extrusion has the potential of decreasing thiamin loss in extruded puffed-wheat.

#### **4.2. Recommendations for Future Research**

It is important to note that the results summarized in Section 4.1 were found using a lab-scale extruder, and the range of extrusion conditions described in Section 2.2.1, 2.2.2, and 2.8. Because of these limitations, it is difficult to determine whether or not there is an advantage to using CO<sub>2</sub>-injection when extruding wheat at lower temperatures. Therefore, the following topics are recommended for future research:

1. Further optimize CO<sub>2</sub> injection set-up by changing screw configuration and point of CO<sub>2</sub> injection. Determine how these changes will impact residence time, retention of thiamin or other heat- and/or shear-sensitive additives (i.e. anthocyanins or beta-carotene), and physical attributes. Also add a flow meter at point of CO<sub>2</sub> injection to precisely measure amount of CO<sub>2</sub> gas entering extruder.
2. Investigate the effect of screw configuration, feed rate, and die geometry on the loss of heat- and/or shear-sensitive additives at higher temperatures.

Select a set of screw configurations with varying levels of conveying efficiency. At a constant initial concentration, investigate only how changes in those process variables affect the loss of the additives.

3. Repeat the experimental design in Table 2.3 and use equipment, such as RVA, to determine degree of starch gelatinization. Investigate the relationship between degree of starch gelatinization on expansion ratio, bulk density, WAI, and % WSI. Also use a texture-measuring device to correlate texture with degree of starch gelatinization. Compare all results in the low-temperature products to high-temperature controls.
4. Set-up an experimental design with a wide range of moisture contents and CO<sub>2</sub> pressures. Keeping initial concentration of heat- and/or shear-labile additives and other processing variables constant, investigate only the effects of CO<sub>2</sub> pressure and moisture content on degradation of the additives.

## **Appendices**

## Appendix 1



## Appendix 1. Mixing Method, Flour and 0.3% Thiamin

### Raw Materials:

4.5 g Thiamin Hydrochloride (5% Moisture)  
+ 1495.5 g Wheat Flour (13% Moisture)  
**Total Batch Weight:** 1500.0g

### Equipment:

- Hobart Corporation (Troy, Ohio) 5-quart all-purpose mixer with mixing bowl.
- Patterson-Kelley twin-shell dry blender.

1. Weigh out 4.5 g thiamin hydrochloride in weighing container.
2. Weigh out 500 g wheat flour in mixing bowl.
3. To 500 g flour add ½ of the thiamin. Place the flat mixing paddle on mixer head and mix on low for 1 minute. Add remaining thiamin and mix on low for 1 minute.
4. In a second mixing bowl weigh out 500 g of wheat flour. Do not tare scale. Add flour/thiamin mixture on top of 500 g of wheat flour, scale should read approximately 1004.5g. Add more wheat flour until scale reads 1500 g.
5. Take 1500 g of flour/thiamin mixture and spoon it into twin shell dry blender. Set mixing time for 40 minutes and begin mixing (inner bar speed measured by manual tachometer = 1400 rpm). Scrape down blender twice during mixing. During scrape down, also take about 200g out from bottom, add it back to the top of the mixer, and resume mixing.
6. Empty contents of mixer and put aside in a sealed container until needed.

**Table A.1. Physical and Chemical Properties of Soft White Wheat Flour (Star of the West Milling Co.)\***

Moisture	12.75-13.5%	AOAC 925.10
Protein	7.5-8.1%	AACC 96-12
Ash	0.42-0.47	AOAC 936.07
pH	5.9-6.1 (unbleached)	AOAC 943.02
Falling Number	250 seconds	AOAC 976.13

\* As stated in the manufacturer specification sheet.

## **Appendix 2**

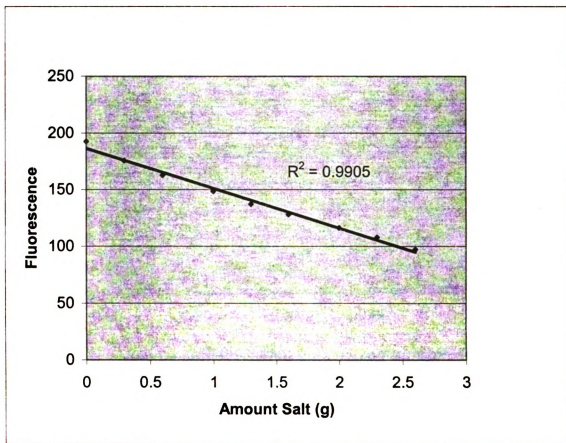
## Appendix 2. Modifications to AOAC Thiamin Analysis Method

### Study 1. Investigation of Salt Amount on Thiochrome Yield

#### **Methods:**

Varying amounts of salt were added to 7 different 2.5 ml standard aliquots (concentration 15.6 µg/ml) and the oxidation step then proceeded normally with the addition of oxidizing reagent and isobutanol (Appendix 4).

#### **Results:**



**Figure A.2.1. The Effect of Salt Mass on Thiochrome Yield.**

#### **Conclusion:**

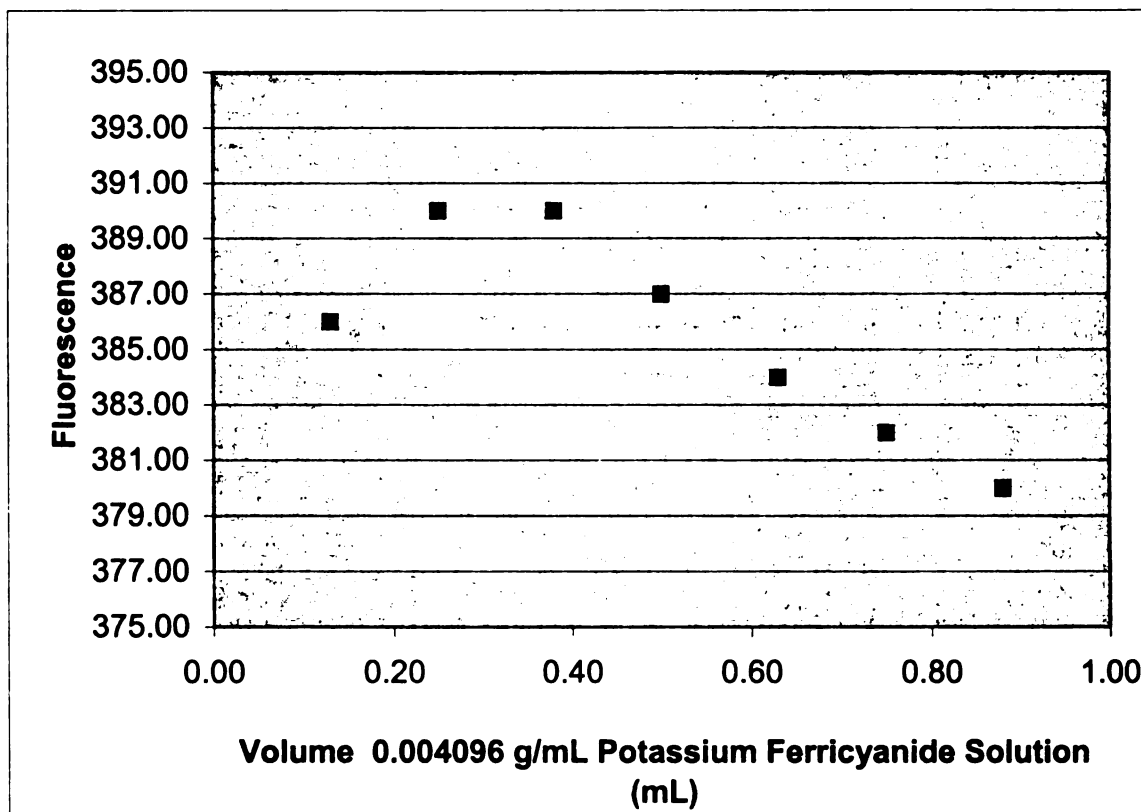
The amount of salt added in the oxidation step does affect thiochrome yield (directly proportional to fluorescence). It appears that when salt is present in the final separation of aqueous and isobutyl layers, it decreases thiochrome yield.

## **Study 2. Determination of the optimal amount of potassium ferricyanide to oxidize thiamin to thiochrome**

### **Method:**

Varying amounts of 0.4069% potassium ferricyanide solution were added to 7 different 2.5 ml aliquots of standard solution (concentration 15.6 µg/ml) and 15% NaOH. The reaction then proceeded as normal with the addition of isobutanol (Appendix 4).

### **Results:**



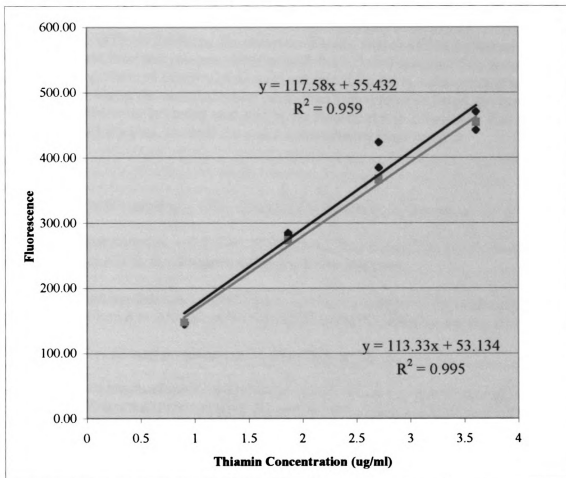
**Figure A.2.2. The Effect of Potassium Ferricyanide Amount on Thiochrome Yield.**

### **Conclusion:**

The optimal amount of 0.4069% potassium ferricyanide is between 0.25 and 0.38 ml's, for converting thiamin to thiochrome. This amount is 1/1000 of the amount used in the AOAC method. Therefore, for a 2.5 mL aliquot of standard solution at 100 µg/mL, approximately 0.001024 to 0.001556 grams of potassium ferricyanide are needed to optimally oxidize thiamin to thiochrome.

## **Appendix 3**

**Appendix 3. Standard Curves for Fluorescence  
vs. Thiamin Concentration ( $\mu\text{g/ml}$ )**



**Figure A.3. Standard Curves (Fluorescence vs. Thiamin Concentration)  
Constructed in Duplicate on Two Different Days.**

## Appendix 4

## Appendix 4. Thiamin Analysis Method

### Introduction:

This method was developed from the 1995 AOAC “Official Method 953.17 Thiamin (Vitamin B<sub>1</sub>) in Grain Products, Fluorometric (Rapid)” and modified for the analysis of extruded wheat flour that has been fortified with 0.3% (w/w) thiamin. The volumes and masses of reagents used in the method were optimized for the specific product under investigation. Using the extruded wheat product and the quantities listed, the maximum thiochrome concentration being analyzed by the fluorometer is 2.6 µg/mL. The standard is prepared at 3.6 µg/mL to cover the entire concentration range needed.

### Reagents:

**Sodium hydroxide solution** – 15%. Dissolve 15g NaOH in H<sub>2</sub>O to make 100 mL.

**Oxidizing Stock Solution.** – 0.2796% Dissolve 0.2796g K<sub>3</sub>Fe(CN)<sub>6</sub> in 15% NaOH Solution to make 100 mL. **Prepare solution on day it is used.**

**Oxidizing Working Solution** – 0.01398% Take a 10 mL aliquot of the oxidizing stock solution and dilute it to 200 mL with 15% NaOH. **Prepare solution on day it is used.**

**Isobutyl alcohol (2-methyl-1propanol)**– Use HPLC grade.

**Quinine sulfate stock solution** – Use quinine sulfate solution to govern reproducibility of fluorometer. Prepare stock solution by dissolving 10 mg quinine sulfate in 0.1N H<sub>2</sub>SO<sub>4</sub> to make 1 L. Store in light-resistant containers.

**Quinine sulfate standard solution** – Dilute 1 volume quinine sulfate stock solution with 39 volumes 0.1N H<sub>2</sub>SO<sub>4</sub>. (Solution fluoresces to ca same degree as does isobutanol extract of thiochrome obtained from 1µg thiamine-HCL.). **Store solution in light-resistant container.**

**Thiamin hydrochloride standard solutions** – (1) *Stock solution.* -- 100µg/mL. Accurately weight 50-60 mg USP Thiamin Hydrochloride Reference Standard that has been dried to constant weight over P<sub>2</sub>O<sub>5</sub> in desiccator (Reference standard is hygroscopic; avoid absorption of moisture.) Dissolve in 20% alcohol adjusted to pH 3.5 – 4.3 with HCl, and dilute to 500 mL with the acidified alcohol. Add enough additional acidified alcohol to make concentration exactly 100 µg thiamine-HCl / mL. Store at ca 10°C in glass-stoppered, light-resistant bottle.



### **Preparation of Standard Solution:**

Dilute 15.6 mL thiamin-HCl stock solution to 100 mL with ca 0.1N HCl (1 mL = 0.2 µg thiamin-HCl). *Note this concentration as (*Std<sub>conc</sub>*)*. Designate this as working standard solution. If NaCl is to be added to sample for extraction, add NaCl to working standard solution, before final dilution, to give final concentration of ca 5% [weight / volume]. (add about 5.0 g NaCl to give correct concentration)

### **Extraction:**

#### *95-100 °C Digestion*

Add ca 1 gram of sample and ca 10 grams salt in 250 mL centrifuge tube. (Addition of about 10 g NaCl to give final concentration of ca 5% [weight / volume] aids in subsequent separation of sample solution. *Note amount of sample used (*Smp<sub>mass</sub>*) and the amount of salt used (*Salt<sub>mass</sub>*)*. Thoroughly mix flour and salt with stirring rod before adding 0.1N HCl.) Add 200 mL 0.1 N HCl solution to flour and salt in 250 mL centrifuge tube, using part of acid to wash down sides of vessel. *Note amount of acid added (*mL<sub>HCl</sub>*)*. Shake vessel to thoroughly mix contents. Place vessel in H<sub>2</sub>O bath previously heated to 95-100°C. With caps on vessels, stir at frequent intervals to keep solids in suspension during thickening stage (5-8 min) for total heating time of 30 minutes. A shaking water bath is an acceptable way to heat and shake the tubes.

After hydrolysis has proceeded, place a drop of solution on a spot plate and test with thymol blue indicator. Solution should be distinctly red (pH 1.0-1.2). If not (indicating the presence of basic substances in sample), add ca 1N HCl in 1.0 mL portions until desired acidity is reached. Note volume of 1N acid required to supplement the 0.1N acid and REPEAT DIGESTION with new sample weight and necessary mixture of 1N and 0.1 N acids. Cool tubes to room temperature.

[Note: This pH test only needs to be done initially, and checked periodically thereafter.]

Centrifuge tubes at 5,000 rpm (4068 x g) for 10 minutes.

### **Oxidation:**

[Note: In the paragraphs that follow, the specific order in which reagents were added in the oxidation step is described. This order was tested in previous studies and was shown to produce the highest thiochrome yield when compared to adding the reagents in a different sequence.]

### **Standard Solutions:**

Using this method, standard curves do not have to be run on every day that a sample is analyzed. Standard curves should be checked periodically between days of analysis to

confirm low variability (<5%) between slopes. Once low variability is established, average y-intercept and slope values are to be used in the final calculation.

The standard curve is done at four concentrations in duplicate, making for 8 oxidized tubes and 8 un-oxidized tubes. The setup for one set of tubes is shown below.

**Table A.4. Reagent Amounts for the Construction of a Standard Curve (Fluorescence vs. Thiamin Concentration)**

<b>Tube #</b>	<b>Oxidized/ Non-oxidized</b>	<b>Volume Standard Added (mL)</b>	<b>Ending Thiamin Concentration (µg/mL)</b>	<b>Volume Oxidizing Solution (mL)</b>	<b>Volume 15% NaOH Solution (mL)</b>
1	Oxidized	3.00	3.60	12.00	
2	Oxidized	2.25	2.70	9.00	
3	Oxidized	1.55	1.86	6.20	
4	Oxidized	0.80	0.96	3.20	
5	Non-Oxidized	3.00	3.60		12.00
6	Non-Oxidized	2.25	2.70		9.00
7	Non-Oxidized	1.55	1.86		6.20
8	Non-Oxidized	0.80	0.96		3.20

#### *Oxidized Tubes*

To 1 ca 40 mL tubes (or reaction vessels) add necessary amount of working standard solution (amounts indicated in Table 4A.1). **UNIFORM TECHNIQUE MUST BE USED.** Protect solution from sunlight, which destroys thiochrome. Use pipet that delivers 10 mL in 1-2 sec for addition of oxidizing reagent. Place tip of pipet containing oxidizing reagent in neck of tube and hold it so that the stream of solution doesn't hit side of tube. Gently swirl tube to produce rotary motion in liquid and immediately add necessary amount of oxidizing reagent (amounts indicated in Table 4A.1). **IMMEDIATELY** add 13 mL isobutanol, stopper, and shake for at least 15 sec.

After isobutanol has been added to all tubes, shake again for ca 2 minutes (tubes may be placed in a shaker box for this additional shaking). Centrifuge tubes at ca 10,000 rpm (11,950 x g) for 5 minutes. Pipet ca 3.3 mL isobutanol extract (upper layer) from each tube into sample cuvette for thiochrome fluorescence measurement.

#### *Non-Oxidized Tubes*

To 1 ca 40 mL tubes (or reaction vessels) add necessary amount of working standard solution (amounts indicated in Table 4A.1). **UNIFORM TECHNIQUE MUST BE USED.** Protect solution from sunlight which destroys thiochrome. Use pipet that delivers 10 mL in 1-2 sec for addition of 15% NaOH solution. Place tip of pipet containing 15% NaOH solution in neck of tube and hold it so that the stream of solution

doesn't hit side of tube. Gently swirl tube to produce rotary motion in liquid and immediately add necessary amount of 15% NaOH solution (amounts indicated in Table 4.A.1). Remove pipet and swirl again to ensure adequate mixing. IMMEDIATELY add 13 mL isobutanol, stopper, and shake for at least 15 sec.

After isobutanol has been added to all tubes, shake again for ca 2 minutes (tubes may be placed in a shaker box for this additional shaking). Centrifuge tubes at ca 10,000 rpm (11,950 x g) for 5 minutes. Pipet ca 3.3 mL isobutanol extract (upper layer) from each tube in to cuvette for thiochrome fluorescence measurement.

### ***Sample Solutions***

#### ***Oxidized Tubes***

To 1 ca 40 mL tubes (or reaction vessels) add 2.5 mL sample solution from the 250 mL centrifuge tube. UNIFORM TECHNIQUE MUST BE USED. Protect solution from sunlight, which destroys thiochrome. Use pipet that delivers 10 mL in 1-2 sec for addition of oxidizing reagent. Place tip of pipet containing oxidizing reagent in neck of tube and hold it so that the stream of solution doesn't hit side of tube. Gently swirl tube to produce rotary motion in liquid and immediately add 10 mL oxidizing reagent. Remove pipet and swirl again to ensure adequate mixing. IMMEDIATELY add 13 mL isobutanol, stopper, and shake for at least 15 sec.

#### ***Non-Oxidized Tubes***

To 1 ca 40 mL tubes (or reaction vessels) add 2.5 mL sample solution from the 250 mL centrifuge tube. UNIFORM TECHNIQUE MUST BE USED. Protect solution from sunlight which destroys thiochrome. Use pipet that delivers 10 mL in 1-2 sec for addition of 15 % NaOH. Place tip of pipet containing 15 % NaOH in neck of tube and hold it so that the stream of solution doesn't hit side of tube. Gently swirl tube to produce rotary motion in liquid and immediately add 10 mL 15% NaOH. Remove pipet and swirl again to ensure adequate mixing. IMMEDIATELY add 13 mL isobutanol, stopper, and shake for at least 15 sec.

*Note amount of sample solution added to reaction vessel as **mL** smp aliquot*

*Note amount of isobutanol added to reaction vessel as **mL** smp isobutanol*

After isobutanol has been added to all tubes, shake again for ca 2 minutes (tubes may be placed in a shaker box for this additional shaking). Centrifuge tubes at ca 10,000 rpm (11,950 x g) for 5 minutes. Pipet ca 3.3 mL isobutanol extract (upper layer) from each tube in to cuvette for thiochrome fluorescence measurement.

## **Thiochrome Fluorescence Measurement:**

### ***Standardizing the Fluorometer***

To standardize the fluorometer, set excitation at 343 nm and emission at 459 nm, sensitivity at 10, and selector at 1. With nothing in fluorometer, use zeroing knob to zero machine. Put quinine standard in sample cell, and in fluorometer. Using variable knob, set machine to 10.00.

### ***Measuring Fluorescence***

#### ***Construction of Standard Curve***

After standardizing, set excitation at 373 nm and emission at 410 nm. Change sensitivity to 1/10 and using variable knob, set machine to 0.00. Measure fluorescence (S) of 3.3 mL of extract from oxidized assay standard solution. Change sensitivity to 10 and measure fluorescence (d) of 3.3 mL extract from assay standard solution which has been treated with 15% NaOH (standard blank). After fluorometric readings have been recorded, calculate (S-d) for each standard solution of known concentration. Using Microsoft Excel, plot fluorometric readings (S-d) vs. thiamin concentrations ( $\mu\text{g/mL}$ ) and derive a linear equation from the plotted data.

#### ***Measuring Sample Fluorescence***

After standardizing, set excitation at 373 nm and emission at 410 nm. Change sensitivity to 1/10 and using variable knob, set machine to 0.00. Measure fluorescence (I) of 3.3 mL of isobutanol extract from oxidized assay sample solution. Change sensitivity to 10 and measure fluorescence (d) of 3.3 mL extract from assay standard solution which has been treated with 15% NaOH (sample blank).

Calculation for % thiamin in sample ( $\mu\text{g}$  thiamin/g sample):

1. Use standard curve  
to solve for  
thiamin conc.

$$(\mu\text{g/mL}) : FC = \frac{y-z}{m}$$

Where, y = fluorometric reading (oxidized – blank)

z = average y-intercept

FC = thiamin concentration in fluorometric cell,  $\mu\text{g/mL}$

m = average slope

2. % thiamin calculation:

$$FC \times \left( \frac{SM}{SD} + \frac{FM}{FD} + \text{mL}_{\text{HCl}} \right) \times \frac{1}{\text{mL}_{\text{smp. aliquot}}} \times \frac{\text{mL}_{\text{smp. isobutanol}}}{1} \times \frac{10^{-6} \text{ g}}{\mu\text{g}} \times \frac{1}{\text{Smp}_{\text{mass}}} \times 100\%$$

Where, SM = salt mass (grams)

SD = salt density (g/ml)

FM = flour mass (grams)

FD = flour density (g/ml)

3. Percent thiamin calculation given the average slope is 119.4 and average y-intercept is 51.2, and the measured salt and flour densities are 2.17 g/mL and 0.48 g/mL respectively:

$$2.62 \frac{\mu\text{g}}{\text{mL}} \times \left( \frac{10.6 \text{ g}}{2.17 \frac{\text{g}}{\text{mL}}} + \frac{0.94 \text{ g}}{0.48 \frac{\text{g}}{\text{mL}}} + 200 \text{ mL} \right) \times \frac{1}{2.5} \times \frac{13 \text{ mL}}{1} \times \frac{10^{-6}}{\mu\text{g}} \times \frac{1}{0.94 \text{ g}} \times 100\% = 0.2997\%$$

$$\% \text{ thiamin (dry basis) for flour (13.5 \% moisture, w/w)} = \frac{0.2997\%}{(1-.135)} = 0.3465\%$$

## Appendix 5

## Appendix 5. Extrusion Data

**Table A.5.1. Processing Data and Expansion Ratio, and Bulk Density Values for CO<sub>2</sub> (Lower Temperature) and Control (Higher Temperature) Extrusion Conditions**

Screw Config.	% Moist.	Screw Speed (rpm)	CO <sub>2</sub> (psi)	Melt Temp. (°C)	Product Temp. at Die (°C)	% Torq.	Die Press. (psi)	Exp. Ratio <sup>c</sup>	Bulk Dens. (g/ml) <sup>b</sup>
Control	22	200	N/A	41/75/106/138/165	157.8	65	800	2.3 (±0.44)	0.222 (±0.006)
Control	22	200	N/A	44/79/102/136/164	154.6	47.5	620	2.1 (±0.59)	0.190 (±0.008)
Control	22	250	N/A	40/76/105/140/164	160.2	56.0	720	2.6 (±0.43)	0.141 (±0.007)
Control	22	250	N/A	41/77/104/138/164	158.7	55.7	720	2.4 (±0.53)	0.118 (±0.006)
Control	22	300	N/A	41/76/127/143/164	161.3	55.0	700	2.9 (±0.45)	0.093 (±0.002)
Control	22	300	N/A	41/77/104/142/163	159.6	53.0	680	2.7 (±0.42)	0.104 (±0.000)
CO <sub>2</sub>	22	300	0	41/61/71/89/96	107.1	32.5	600	1.3 (±0.02)	0.401 (±0.000)
CO <sub>2</sub>	22	300	0	41/62/71/91/97	114.7	35.9	820	2.1 (±0.23)	0.604 (±0.005)
CO <sub>2</sub>	22	300	150	41/64/72/85/97	112.7	44.0	940	1.8 (±0.28)	0.626 (±0.004)
CO <sub>2</sub>	22	300	150	41/59/69/86/93	104	32.3	600	1.4 (±0.31)	0.832 (±0.01)
CO <sub>2</sub>	22	350	0	41/60/71/92/97	109.2	34.5	690	2.1 (±0.19)	0.592 (±0.006)
CO <sub>2</sub>	22	350	0	41/60/70/91/96	112.2	35.6	790	2.3 (±0.10)	0.410 (±0.002)
CO <sub>2</sub>	22	350	150	41/63/72/86/97	115.6	34.0	700	2.2 (±0.23)	0.452 (±0.004)
CO <sub>2</sub>	22	350	150	41/60/69/85/93	106.6	31.8	620	1.5 (±0.14)	0.742 (±0.005)
CO <sub>2</sub>	22	400	0	41/56/65/82/89	106.6	30.0	610	2.2 (±0.17)	0.482 (±0.005)
CO <sub>2</sub>	22	400	0	41/58/67/84/91	108.2	31.2	760	2.4 (±0.11)	0.429 (±0.004)
CO <sub>2</sub>	22	400	150	41/65/75/88/98	118.8	36.0	700	2.4 (±0.19)	0.484 (±0.005)
CO <sub>2</sub>	22	400	150	41/59/69/87/95	111.5	30.9	600	2.1 (±0.20)	0.479 (±0.008)
CO <sub>2</sub>	25	300	0	42/63/76/84/85	96.3	32.5	335	1.2 (±0.10)	0.845 (±0.008)
CO <sub>2</sub>	25	300	0	41/61/71/83/89	95.9	31.5	400	1.2 (±0.02)	0.787 (±0.12)
CO <sub>2</sub>	25	300	150	41/63/74/87/91	99.5	34.4	530	1.3 (±0.14)	0.739 (±0.005)
CO <sub>2</sub>	25	300	150	41/69/85/94/91	100.8	36.2	300	1.5 (±0.15)	0.860 (±0.014)
CO <sub>2</sub>	25	350	0	41/62/75/82/84	93.1	32.0	340	1.3 (±0.12)	0.830 (±0.005)
CO <sub>2</sub>	25	350	0	41/61/71/86/92	101.2	34.5	540	1.3 (±0.03)	0.850 (±0.017)
CO <sub>2</sub>	25	350	150	41/61/74/87/92	101.0	33.1	560	1.5 (±0.13)	0.880 (±0.004)
CO <sub>2</sub>	25	350	150	41/70/86/95/92	103.1	34.5	330	1.6 (±0.39)	0.886 (±0.008)

**Table A.5.1. Processing Data and Expansion Ratio, and Bulk Density Values for CO<sub>2</sub> (Lower Temperature) and Control (Higher Temperature) Extrusion Conditions (cont'd)**

Screw Config.	% Moist.	Screw Speed (rpm)	CO <sub>2</sub> (psi)	Melt Temp. (°C)	Product Temp. at Die (°C)	% Torq.	Die Press. (psi)	Exp. Ratio <sup>c</sup>	Bulk Dens. (g/ml) <sup>b</sup>
CO <sub>2</sub>	25	400	0	41/57/68/77/85	95.2	29.3	290	1.3 (±0.13)	0.816 (±0.001)
CO <sub>2</sub>	25	400	0	44/59/69/84/89	98.8	34.1	560	1.5 (±0.11)	0.817 (±0.006)
CO <sub>2</sub>	25	400	150	44/59/69/87/92	102.5	34.0	600	1.6 (±0.30)	0.759 (±0.009)
CO <sub>2</sub>	25	400	150	41/60/84/89/91	101.3	32.5	300	1.8 (±0.50)	0.816 (±0.020)

<sup>a</sup> Average values based on two measurements. Standard deviation given in parentheses.

<sup>b</sup> Average value based on three measurements. Standard deviation given in parentheses.

<sup>c</sup> Average value based on fifteen measurements. Standard deviation given in parentheses.



**Table A.5.2. Processing Data and WAI, % WSI, SME and Mean Residence Time Values for CO<sub>2</sub> (Lower Temperature) and Control (Higher Temperature) Extrusion Conditions**

Screw Config.	% Moist.	Screw Speed (rpm)	CO <sub>2</sub> (psi)	Melt Temp. (°C)	Product Temp. at Die (°C)	% Torq.	Die Press. (psi)	WAI <sup>a</sup>	% WSI <sup>a</sup>	SME (kJ/kg)	Mean Res. Time (s)
Control	22	200	N/A	41/75/106/138/165	157.8	65	800	9.26 (±0.06)	11.2 (±0.06)	342.2	68.5
Control	22	200	N/A	44/79/102/136/164	154.6	47.5	620	9.00 (±0.28)	11.8 (±0.99)	249.6	85.6
Control	22	250	N/A	40/76/105/140/164	160.2	56.0	720	9.36 (±0.49)	13.2 (±0.99)	369.3	67.4
Control	22	250	N/A	41/77/104/138/164	158.7	55.7	720	9.00 (±0.18)	11.6 (±1.1)	367.0	69.2
Control	22	300	N/A	41/76/127/143/164	161.3	55.0	700	9.07 (±0.09)	13.9 (±0.57)	436.1	57.7
Control	22	300	N/A	41/77/104/142/163	159.6	53.0	680	8.82 (±1.1)	13.3 (±1.3)	419.9	57.3
CO <sub>2</sub>	22	300	0	41/61/71/89/96	107.1	32.5	600	6.41 (±0.03)	10.5 (±0.40)	386.9	124.8
CO <sub>2</sub>	22	300	0	41/62/71/91/97	114.7	35.9	820	7.58 (±0.30)	13.4 (±0.26)	426.2	133.6
CO <sub>2</sub>	22	300	150	41/64/72/85/97	112.7	44.0	940	7.12 (±0.11)	12.9 (±0.90)	522.4	101.6
CO <sub>2</sub>	22	300	150	41/59/69/86/93	104	32.3	600	6.21 (±0.12)	10.5 (±0.35)	383.9	147.3
CO <sub>2</sub>	22	350	0	41/60/71/92/97	109.2	34.5	690	7.97 (±0.08)	14.5 (±0.07)	479.4	127.8
CO <sub>2</sub>	22	350	0	41/60/70/91/96	112.2	35.6	790	7.79 (±0.48)	13.5 (±0.55)	494.0	145.0
CO <sub>2</sub>	22	350	150	41/63/72/86/97	115.6	34.0	700	7.42 (±0.03)	12.6 (±0.05)	471.9	98.0
CO <sub>2</sub>	22	350	150	41/60/69/85/93	106.6	31.8	620	7.10 (±0.24)	10.3 (±0.31)	441.4	130.4
CO <sub>2</sub>	22	400	0	41/56/65/82/89	106.6	30.0	610	7.65 (±0.25)	13.6 (±0.68)	476.9	100.2
CO <sub>2</sub>	22	400	0	41/58/67/84/91	108.2	31.2	760	7.07 (±0.14)	12.9 (±0.44)	495.0	133.7
CO <sub>2</sub>	22	400	150	41/65/75/88/98	118.8	36.0	700	7.73 (±0.04)	17.9 (±1.0)	571.9	86.9
CO <sub>2</sub>	22	400	150	41/59/69/87/95	111.5	30.9	600	8.00 (±0.22)	15.3 (±0.15)	490.8	114.1
CO <sub>2</sub>	25	300	0	42/63/76/84/85	96.3	32.5	335	9.78 (±0.43)	7.0 (±0.45)	374.3	136.4
CO <sub>2</sub>	25	300	0	41/61/71/83/89	95.9	31.5	400	6.07 (±0.06)	6.4 (±0.52)	362.4	123.0
CO <sub>2</sub>	25	300	150	41/63/74/87/91	99.5	34.4	530	5.00 (±0.24)	7.5 (±0.38)	396.0	123.4
CO <sub>2</sub>	25	300	150	41/69/85/94/91	100.8	36.2	300	5.62 (±0.09)	7.3 (±0.47)	417.4	146.8
CO <sub>2</sub>	25	350	0	41/62/75/82/84	93.1	32.0	340	8.31 (±0.55)	8.2 (±0.03)	430.3	131.7
CO <sub>2</sub>	25	350	0	41/61/71/86/92	101.2	34.5	540	7.40 (±0.07)	7.0 (±0.48)	463.0	122.3
CO <sub>2</sub>	25	350	150	41/61/74/87/92	101.0	33.1	560	5.52 (±0.03)	8.6 (±0.13)	444.7	127.1
CO <sub>2</sub>	25	350	150	41/70/86/95/92	103.1	34.5	330	5.72 (±0.02)	7.6 (±0.21)	464.1	130.8

**Table A.5.2. Processing Data and WAI, % WSI, SME and Mean Residence Time Values for CO<sub>2</sub> (Lower Temperature) and Control (Higher Temperature) Extrusion Conditions (cont'd)**

Screw Config.	% Moist.	Screw Speed (rpm)	CO <sub>2</sub> (psi)	Melt Temp. (°C)	Product Temp. at Die (°C)	% Torq.	Die Press. (psi)	WAI <sup>a</sup>	% WSI <sup>b</sup>	SME (kJ/kg)	Mean Res. Time (s)
CO <sub>2</sub>	25	400	0	41/57/68/77/85	95.2	29.3	290	6.72 (±0.05)	8.7 (±0.21)	450.6	112.6
CO <sub>2</sub>	25	400	0	44/59/69/84/89	98.8	34.1	560	8.12 (±0.08)	8.9 (±1.5)	523.2	123.4
CO <sub>2</sub>	25	400	150	44/59/69/87/92	102.5	34.0	600	5.98 (±0.01)	9.8 (±0.11)	522.3	92.3
CO <sub>2</sub>	25	400	150	41/60/84/89/91	101.3	32.5	300	5.80 (±0.01)	9.2 (±0.35)	500.0	115.5

<sup>a</sup> Average values based on two measurements. Standard deviation given in parentheses.

<sup>b</sup> Average value based on three measurements. Standard deviation given in parentheses.

<sup>c</sup> Average value based on fifteen measurements. Standard deviation given in parentheses.

**Table A.5.3. Processing Data and Expansion Ratios, Bulk Densities, WAI, % WSI, SME, and Mean Residence Time Values for CO<sub>2</sub> Extrusion at Higher Temperatures, 22% Moisture, Screw Speed of 300 rpm, and 0 psi CO<sub>2</sub>**

Melt Temp. (°C)	Product Temp. at Die (°C)	% Torque	Die Press. (psi)	Exp. Ratio <sup>c</sup>	Bulk Dens. (g/ml) <sup>b</sup>	WAI <sup>a</sup>	% WSI <sup>a</sup>	SME (kJ/kg)	Mean Res. Time (s)
44/75/102/ 132/162	153.4	24.9	300	2.3 (±0.15)	0.391 (±0.005)	7.81 (±0.27)	15.2 (±0.42)	297.4	124.4
46/78/103/ 135/164	155.8	26.6	350	2.4 (±0.16)	0.387 (±0.004)	7.42 (±1.07)	14.6 (±0.66)	317.5	101.4

<sup>a</sup> Average values based on two measurements. Standard deviation given in parentheses.

<sup>b</sup> Average value based on three measurements. Standard deviation given in parentheses.

<sup>c</sup> Average value based on fifteen measurements. Standard deviation given in parentheses.

**Table A.5.4. Processing Data and Average % Thiamin Losses for CO<sub>2</sub> (Lower Temperature) and Control (Higher Temperature) Extrusion Conditions**

Screw Config.	% Moist.	Screw Speed (rpm)	CO <sub>2</sub> Press. (psi)	Melt Temp. (°C)	Product Temp. at Die (°C)	% Torq.	Die Press. (psi)	Average % Thiamin Loss <sup>a</sup>
Control	22	200	N/A	41/75/106/138/165	161.3	55.0	700	12.0 (± 1.8)
Control	22	200	N/A	44/79/102/136/164	154.6	47.5	620	9.9 (± 1.2)
Control	22	250	N/A	40/76/105/140/164	160.2	56.0	720	15.3 (± 4.4)
Control	22	250	N/A	41/77/104/138/164	158.7	55.7	720	9.8 (± 0.5)
Control	22	300	N/A	41/76/127/143/165	161.3	55.0	700	15.9 (± 0.3)
Control	22	300	N/A	41/77/104/142/163	159.6	53.0	680	11.7 (± 0.7)
CO <sub>2</sub>	22	300	0	41/61/71/89/96	107.1	32.5	600	<0 (± 2.2)
CO <sub>2</sub>	22	300	0	41/62/71/91/97	114.7	35.9	820	1.5 (± 0.8)
CO <sub>2</sub>	22	300	150	41/64/72/85/97	112.7	44.0	940	<0 (± 2.9)
CO <sub>2</sub>	22	300	150	41/59/69/86/93	104	32.3	600	<0 (± 0.8)
CO <sub>2</sub>	22	350	0	41/60/71/92/97	109.2	34.5	690	<0 (± 4.1)
CO <sub>2</sub>	22	350	0	41/60/70/91/96	112.2	35.6	790	<0 (± 1.7)
CO <sub>2</sub>	22	350	150	41/63/72/86/97	115.6	34.0	700	8.0 (± 0.5)
CO <sub>2</sub>	22	350	150	41/60/69/85/93	106.6	31.8	620	11.0 (± 0.8)
CO <sub>2</sub>	22	400	0	41/56/65/82/89	106.6	30.0	610	3.2 (± 0.3)
CO <sub>2</sub>	22	400	0	41/58/67/84/91	108.2	31.2	760	6.5 (± 0.7)
CO <sub>2</sub>	22	400	150	41/65/75/88/98	118.8	36.0	700	1.7 (± 2.9)
CO <sub>2</sub>	22	400	150	41/59/69/87/95	111.5	30.9	600	5.4 (± 0.01)
CO <sub>2</sub>	25	300	0	42/63/76/84/85	96.3	32.5	335	26.4 (± 1.1)
CO <sub>2</sub>	25	300	0	41/61/71/83/89	95.9	31.5	400	0.5 (± 0.4)
CO <sub>2</sub>	25	300	150	41/63/74/87/91	99.5	34.4	530	16.1 (± 0.3)
CO <sub>2</sub>	25	300	150	41/69/85/94/91	100.8	36.2	335	1.1 (± 1.5)
CO <sub>2</sub>	25	350	0	41/62/75/82/84	93.1	32.0	340	11.6 (± 1.0)
CO <sub>2</sub>	25	350	0	41/61/71/86/92	101.2	34.5	540	<0 (± 1.5)
CO <sub>2</sub>	25	350	150	41/61/74/87/92	101.0	33.1	560	24.5 (± 1.3)
CO <sub>2</sub>	25	350	150	41/70/86/95/92	103.1	34.5	330	33.8 (± 1.1)
CO <sub>2</sub>	25	400	0	41/57/68/77/85	95.2	29.3	290	28.5 (± 0.7)
CO <sub>2</sub>	25	400	0	44/59/69/84/89	96.8	34.1	560	28.1 (± 0.6)
CO <sub>2</sub>	25	400	150	44/59/69/87/92	102.5	34.0	600	28.5 (± 1.4)
CO <sub>2</sub>	25	400	150	41/60/84/89/91	101.3	32.5	300	29.3 (± 1.5)

<sup>a</sup> Average based on three measurements. Standard deviation given in parentheses.

**Table A.5.5. Processing Data and Average % Thiamin Losses for CO<sub>2</sub> Extrusion at Higher Temperatures**

Screw Config.	% Moist.	Screw Speed (rpm)	CO <sub>2</sub> Press. (psi)	Melt Temp. (°C)	Product Temp. at Die (°C)	% Torque	Die Press. (psi)	Average % Thiamin Loss <sup>a</sup>
CO <sub>2</sub>	22	300	0	44/75/102/ 132/162	153.4	24.9	300	2.1 ± (1.2)
CO <sub>2</sub>	22	300	0	46/78/103/ 135/164	155.8	26.6	350	3.0 ± (3.4)

<sup>a</sup> Average based on three measurements. Standard deviation given in parentheses.

**Table A.5.6. Averages and % CVs for % Thiamin in Feed Flour and Extruded Products on a Dry Weight Basis**

Screw Configuration	% Moisture	Screw Speed (rpm)	CO <sub>2</sub> Pressure (psi)	Average % Thiamin Feed Flour <sup>a</sup> (Dry Wt.)	Average % Thiamin Extruded Products <sup>a</sup> (Dry Wt.)
Control	22	200	N/A	0.317 (3.7%)	0.279 (2.0%)
Control	22	200	N/A	0.331 (0.7%)	0.298 (1.1%)
Control	22	250	N/A	0.317 (3.7%)	0.268 (5.2%)
Control	22	250	N/A	0.331 (0.7%)	0.299 (1.6%)
Control	22	300	N/A	0.317 (3.7%)	0.266 (0.4%)
Control	22	300	N/A	0.331 (0.7%)	0.292 (0.8%)
CO <sub>2</sub>	22	300	0	0.316 (6.0%)	0.364 (1.9%)
CO <sub>2</sub>	22	300	0	0.349 (2.6%)	0.344 (0.8%)
CO <sub>2</sub>	22	350	0	0.316 (6.0%)	0.360 (2.5%)
CO <sub>2</sub>	22	350	0	0.349 (2.6%)	0.354 (0.8%)
CO <sub>2</sub>	22	400	0	0.316 (6.0%)	0.368 (3.5%)
CO <sub>2</sub>	22	400	0	0.349 (2.6%)	0.357 (1.7%)
CO <sub>2</sub>	22	300	150	0.323 (5.0%)	0.297 (0.6%)
CO <sub>2</sub>	22	300	150	0.316 (6.0%)	0.281 (0.9%)
CO <sub>2</sub>	22	350	150	0.323 (5.0%)	0.312 (0.3%)
CO <sub>2</sub>	22	350	150	0.316 (6.0%)	0.295 (0.8%)
CO <sub>2</sub>	22	400	150	0.323 (5.0%)	0.317 (2.9%)
CO <sub>2</sub>	22	400	150	0.351 (1.8%)	0.333 (0.0%)
CO <sub>2</sub>	25	300	0	0.320 (1.8%)	0.235 (1.4%)
CO <sub>2</sub>	25	300	0	0.349 (2.6%)	0.347 (0.4%)
CO <sub>2</sub>	25	350	0	0.320 (1.8%)	0.268 (0.4%)
CO <sub>2</sub>	25	350	0	0.349 (2.6%)	0.346 (1.5%)
CO <sub>2</sub>	25	400	0	0.320 (1.8%)	0.283 (1.1%)
CO <sub>2</sub>	25	400	0	0.349 (2.6%)	0.351 (1.5%)
CO <sub>2</sub>	25	300	150	0.327 (2.0%)	0.247 (1.7%)
CO <sub>2</sub>	25	300	150	0.320 (1.8%)	0.271 (1.7%)
CO <sub>2</sub>	25	350	150	0.327 (2.0%)	0.234 (3.7%)
CO <sub>2</sub>	25	350	150	0.320 (1.8%)	0.261 (0.8%)
CO <sub>2</sub>	25	400	150	0.327 (2.0%)	0.234 (1.7%)
CO <sub>2</sub>	25	400	150	0.320 (1.8%)	0.267 (2.2%)

<sup>a</sup> Average based on three measurements. % CV given in parentheses.

**Table A.5.7. Averages and % CVs for % Thiamin in Feed Flour and Extruded Products on a Dry Weight Basis, Using CO<sub>2</sub> Extrusion at Higher Temperatures**

<b>Screw Configuration</b>	<b>% Moisture</b>	<b>Screw Speed (rpm)</b>	<b>CO<sub>2</sub> Pressure (psi)</b>	<b>Average % Thiamin Feed Flour<sup>a</sup> (Dry Wt.)</b>	<b>Average % Thiamin Extruded Products<sup>a</sup> (Dry Wt.)</b>
CO <sub>2</sub>	22	300	0	0.349 (2.6%)	0.342 (1.2%)
CO <sub>2</sub>	22	300	0	0.349 (2.6%)	0.334 (3.5%)

<sup>a</sup> Average based on three measurements. % CV given in parentheses.

**Table A.5.8. Example: Mean Residence Time Calculation, High Temperature (Control) at a Screw Speed of 200 rpm**

<u>Time (s)</u>	<u>Rel. Conc.</u> <u>C (t)<sup>a</sup></u>	<u>Norm.</u> <u>Conc.</u> <u>E (t)</u>	<u>t</u>	<u>delta t</u>	<u>t {[C(t)] delta t}</u> <u>(Middle Time Weight)</u>	<u>[C(t)] delta t</u> <u>(Total Dye Amt.)</u>
0	0	0.0000	0	0	0	0
41.97	10.8	0.0236	44.47	5	2401.38	54
46.97	11.6	0.0253	49.47	5	2869.26	58
51.97	10.6	0.0231	54.47	5	2886.91	53
56.97	10.2	0.0223	59.47	5	3032.97	51
61.97	8.9	0.0194	64.47	5	2868.915	44.5
66.97	7.5	0.0164	69.47	5	2605.125	37.5
71.97	6.4	0.0140	74.47	5	2383.04	32
76.97	4.9	0.0107	79.47	5	1947.015	24.5
81.97	3.9	0.0085	84.47	5	1647.165	19.5
86.97	3.2	0.0070	89.47	5	1431.52	16
91.97	2.6	0.0057	94.47	5	1228.11	13
96.97	2	0.0044	99.47	5	994.7	10
101.97	1.8	0.0039	104.47	5	940.23	9
106.97	1.1	0.0024	109.47	5	602.085	5.5
111.97	1.1	0.0024	114.47	5	629.585	5.5
116.97	1.1	0.0024	119.47	5	657.085	5.5
121.97	1	0.0022	124.47	5	622.35	5
126.97	1	0.0022	129.47	5	647.35	5
131.97	1.1	0.0024	134.47	5	739.585	5.5
136.97	0.8	0.0017	139.47	5	557.88	4
141.97	0.4	0.0009			56.788	0
<b>Total</b>					31749.048	458
<b>Mean</b>						
<b>RTD</b>					<b>(Seconds)</b>	<b>69.32</b>

<sup>a</sup> C(t) adjusted to zero based on control sample with no dye.



**Table A.5.9. Results of MS Excel t-Test Comparing Mean Expansion Ratios at 0 and 150 psi CO<sub>2</sub>, for a Given Screw Speed and at 22% Moisture**

**t-Test: Paired Two Sample for Means at 300 rpm**  
(no significant difference at  $p < 0.05$ )

	150 psi CO <sub>2</sub>	0 psi CO <sub>2</sub>
<b>Mean</b>	<b>1.653</b>	<b>1.718</b>
Variance	0.126	0.178
Observations	30.000	30.000
Pearson Correlation	-0.015	
Hypothesized Mean Difference	0.000	
df	29.000	
<b>t Stat</b>	<b>-0.635</b>	
P(T<=t) one-tail	0.265	
<b>t Critical one-tail</b>	<b>1.699</b>	
P(T<=t) two-tail	0.530	
t Critical two-tail	2.045	

**t-Test: Paired Two Sample for Means at 350 rpm**  
(significantly different at  $p < 0.05$ )

	150 psi CO <sub>2</sub>	0 psi CO <sub>2</sub>
<b>Mean</b>	<b>1.897</b>	<b>2.287</b>
Variance	0.178	0.187
Observations	30.000	30.000
Pearson Correlation	-0.413	
Hypothesized Mean Difference	0.000	
df	29.000	
<b>t Stat</b>	<b>-2.971</b>	
P(T<=t) one-tail	0.003	
<b>t Critical one-tail</b>	<b>1.699</b>	
P(T<=t) two-tail	0.006	
t Critical two-tail	2.045	

**t-Test: Paired Two Sample for Means at 400 rpm**  
(no significant difference at  $p < 0.05$ )

	150 psi CO <sub>2</sub>	0 psi CO <sub>2</sub>
<b>Mean</b>	<b>2.213</b>	<b>2.277</b>
Variance	0.061	0.026
Observations	30.000	30.000
Pearson Correlation	-0.035	
Hypothesized Mean Difference	0.000	
df	29.000	
<b>t Stat</b>	<b>-1.156</b>	
P(T<=t) one-tail	0.128	
<b>t Critical one-tail</b>	<b>1.699</b>	
P(T<=t) two-tail	0.257	
t Critical two-tail	2.045	

**Table A.5.10. Results of MS Excel t-Test Comparing Mean % Water Solubility in the High- and Low-Temperature Samples at 22% Moisture, 0 psi CO<sub>2</sub>, and Screw Speed of 300 rpm**

**t-Test: Paired Two Sample for Means at 300 rpm  
(no significant difference at  $p < 0.05$ )**

	<b>High-Temperature (Control)</b>	<b>Low-Temperature</b>
<i>Mean</i>	<i>13.565</i>	<i>12</i>
Variance	0.336	2.82
Observations	4.000	4
Pearson Correlation	0.695	
Hypothesized Mean Difference	0.000	
df	3.000	
<i>t Stat</i>	<i>2.272</i>	
P(T<=t) one-tail	0.054	
<i>t Critical one-tail</i>	<i>2.353</i>	
P(T<=t) two-tail	0.108	
<i>t Critical two-tail</i>	<i>3.182</i>	

**Table A.5.11. Results of MS Excel t-Test Comparing Mean % Water Solubility in Low-Temperature Samples at 22% and 25% Moisture**

**t-Test: Paired Two Sample for Means at 300 rpm, 0 psi CO<sub>2</sub> at Lower Temperatures  
(significantly different at  $p < 0.05$ )**

	<b>22% Moisture</b>	<b>25% Moisture</b>
<i>Mean</i>	<i>12.000</i>	<i>6.675</i>
Variance	2.820	0.289
Observations	4.000	4.000
Pearson Correlation	-0.738	
Hypothesized Mean Difference	0.000	
df	3.000	
<i>t Stat</i>	<i>5.053</i>	
P(T<=t) one-tail	0.007	
<i>t Critical one-tail</i>	<i>2.353</i>	
P(T<=t) two-tail	0.015	
<i>t Critical two-tail</i>	<i>3.182</i>	

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