DEVELOPMENT OF A FUNCTIONAL MULTIVITAMIN MICROCAPSULE TO BE UTILIZED IN A READY-TO-EAT MEAT PRODUCT

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ABSTRACT

The objective of this study was to develop a multivitamin microcapsule to be utilized in a ready-to-eat (RTE) meat product. Commercial (COM) and laboratory (LAB) multivitamin microcapsules were implemented in a standard frankfurter formulation to produce a functional food. The control (CON) treatment consisted of the standard frankfurter formulation.Two trials (n = 20 / treatment / trial) were evaluated for sensory characteristics using a trained sensory panel and thiamine levels using high performance liquid chromatography (HPLC). While treatment did not have an effect on evaluated sensory characteristics ($P > 0.05$), an increase in display day (dd) aging increased cooking loss in Trial A. However, in Trial B, LAB and COM treatments had higher cooking loss ($P < 0.0001$) than CON; average initial juiciness scores increased depending on treatment ($P = 0.04$). Average initial and sustained tenderness in Trial B was dependent on dd with tenderness increasing throughout the aging intervals ($P < 0.0001$, 0.0002, respectively). Flavor intensity and off-flavor were not dependent on treatment or dd ($P > 0.05$) for both trials. In Trial A, overall acceptability was dependent on dd ($P = 0.0004$) with values ranging from a high in dd 1 (7.12 ± 0.10) to a low in dd 16 (6.47 ± 0.10). In Trial B, there were no differences in overall acceptability ($P > 0.05$). Thiamine levels were independent of trt, dd and trt x dd when analyzed by HPLC. Multivitamin microcapsule treatments did not have an effect on sensory characteristics when added to ready-to-eat meat products. Thus, multivitamin microcapsules may be added to frankfurter formulations to increase functional properties without adverse affects on sensorial properties.

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INTRODUCTION

Microencapsulation is the technology of encapsulating solids, liquids, or gaseous substances into miniature capsules that have the ability to release the encapsulated ingredients in a specific/controlled environment. Microencapsulation is beneficial for food processors to incorporate food ingredients that would otherwise be volatile and not functional in a food system (Desai and Park, 2005). According to Desai and Park (2005), the food industry commonly uses microencapsulation to entrap core material for means of protection from degradation by reducing the reactivity from the outside environment. This is important when core materials need to be released at a further point in the processing of a product to mask flavor undertones and/or to be conducive to the products solution and overall acceptability (Desai and Park, 2005). Some food ingredients are extremely complex and are expected to fulfill properties of a food for the consumer that would be unachievable without encapsulation of these ingredients. Microencapsulation is applied to many areas of the food industry and continues to grow due to the advances that have been accomplished with these processes. Because of this, microencapsulation can possibly broaden the application array of food ingredients (Gouin, 2004). The manipulation of the substances within the microencapsulation process can provide a value added product with unmatched characteristics (Gouin, 2004).

The simplest form of a microcapsule consists of two layers, a wall and a core interior with or without a consistent shaped capsule (Gouin, 2004). Some microcapsules have the ability to contain many wall layers depending on the desired release and the composition of the core material (Pothakamury and Barbosa-Canovas, 1995).

The general objective of this project is to develop a multivitamin microcapsule as a meat additive that can withstand the physical, chemical, and thermal process of a commonly consumed ready-to eat (RTE) meat product such as frankfurters. Meat emulsions, i.e. frankfurters, are an optimal food medium to apply the multivitamin microcapsule due to the large of amount of protein extraction that occurs in meat batter. This helps bind the food additive while also creating a uniform product. Some microencapsulation techniques that are utilized to develop microcapsules include spray drying, fluidized-bed coating, coacervation, liposome entrapment, and many others (Gibbs et al., 1999). . While many of these are used in the food industry, this literature review will solely focus on coacervation.

The method of release is also an important aspect of the microencapsulation process to be considered. Diffusing of the microcapsule into a fluid solution can cause quick release of the active ingredient. The release can be caused from biodegradation of the polymer in the food system into which it is placed. Another method of release is through osmotic pressure, this is achieved by allowing the wall material to be permeable to water resulting in the release of the core and by the swelling of the microcapsule from the application of heat that results in the release of the core ingredient (Pothakamury and Barbosa-Canovas, 1995). The above must be

considered when manipulating the controlled release of an ingredient (Pothakamury and Barbosa-Canovas, 1995).

The objectives of this research were to:

1. Create a multivitamin microcapsule that could withstand thermal, chemical, and mechanical agitation.
2. Evaluate the heat stability of the multivitamin microcapsule after processing.
3. Evaluate palatability of a RTE meat product which had an incorporated multivitamin microcapsule.

LITERATURE REVIEW

Coacervation

Coacervation microencapsulation has many applications in the food industry due to its capability of having a controlled release mechanism that can withstand high temperatures, mechanical stress, and have a sustained release. This process can encapsulate flavors, vitamins, nutrients, oils, preservatives, and enzymes. The process behind coacervation is the phase separation of hydrocolloids from the initial solution, and the new coacervate phase that forms around the desired ingredient that is emulsified or suspended in a solution as the hydrocolloid (Gouin, 2004). The formation of a liposome can be done simply by using this encapsulation technique. When forming a capsule/barrier around specific ingredients, a lipid bilayer has to be formed in order to allow hydrophobic interactions to maintain this stabile mechanism in a solution (Nii and Ishii, 2007). The formation of a phospholipid membrane is the key step in this phase separation technique because otherwise these polymers would disperse into the solution

(Nii and Ishii, 2007). Coacervation gives a high yield microcapsule with a high concentration of the core ingredient as well as abundant nucleation while still being relatively stabile (Sanchez et al., 2006).

Simple or complex coacervation methodology can be utilized. A microcapsule produced by simple coacervation is composed of only one colloid, whereas the complex method produces a microcapsule of multiple colloids (Huang et al., 2006). The simple method is composed of a highly polar substance and a substance that is much less polar, thus resulting in an encapsulation with only two layers (Huang et al., 2006). During a complex coacervation method, there is an interaction between a polyanion and a polycation that creates an interaction between the dispersed core material and coacervate, thus, coating the core material and solidifying to form the microcapsules (Huang et al., 2006).

The technique of complex coacervation can be done by first mixing the core material and a lipid bilayer (typically some form of oil). The coatings are typically oppositely charged amphoteric proteins that are mixed and heated in a solution before the core and oil mixture is introduced. Once one protein coating is mixed with the core/oil solution, the next coating is added and mixed thoroughly. This is then a three liquid phase system that contains the inner and outer layers of the microcapsule. To continue the coacervation process the pH of the mixture is altered and/or diluted (Green and Schleicher, 1957). The microcapsule solution is then cooled and the shell material hardens around the core. In numerous cases, the hardening step uses ingredients that are often undesirable in a food system due to the hazardous nature of these ingredients such as isopropynol alcohol. However, the step provides important cross-linking of

the wall material (Desai and Park, 2005). This step can also be accomplished by adjusting the pH after the cooling step, which works well in food (Green and Schleicher, 1957). The capsules can then be dried using chemicals, such as alcohols, or by filtering. In some cases, the capsules can be lyophilized to accomplish drying. This process can be done to create more than one wall layer which will help the microcapsule withstand different levels of stress to reach the desired time of release (Gouin, 2004).

Complex coacervation is the method of microencapsulation that was utilized in the presented research using the modified methods of Jizomoto et al. (1993), Dong and Rogers (1993), and Green and Schleicher (1957).

Gelatin and Gum Acacia

Complex coacervation using gelatin and gum arabic (acacia) is widely utilized in the food industry. Gum acacia is referred to as a heteropolysaccharide meaning that it is a polysaccharide that contains ~ 12% of a polypeptide (Sanchez et al., 2008). Gum acacia is useful in microencapsulation because the other 88% is the major molecule fraction that allows an acacia gum solution to self-assemble and interact with surrounding proteins (Sanchez et al., 2008). According to Sanchez et al. (2008), gum arabic is the oldest and most widely studied natural gum.

Coacervation is extremely dependent on pH. As the pH fluctuates, the amount of nucleation and growth of each microcapsule will vary (Liu et al., 2010). The entire process of forming a coacervate depends on non-covalent interactions that create strong electrostatic attractions to form the coating of the ingredient (Liu et al., 2010). The best time for coacervation

to occur is when the pH is between the pKa and the isoelectric point (pI) (Lui et al., 2010). Gelatin is utilized in the formation of polymers because of the strong ionic interactions that characterize this protein (Lii et al., 2002). Gelatin possesses a strong positive charge at a low pH, thus helping with the versatility of these interactions in the solutions (Lii et al., 2002). Gelatin is a polypeptide that contains a large amount of proline and glycine residues, which in the appropriate sequence help stabilize the protein (Fang and Bhandari, 2010). The utilization of gum acacia helps produce small microcapsules with a thick wall layer and a relatively stabile barrier from temperature fluctuations (Wilson and Shah, 2007). Gum acacia has a net negative charge, which works well with gelatin when forming microcapsules (Nii and Ishii, 2007).

Multivitamin

Vitamins are vital in maintaining proper health and are commonly found in a variety of foods as well as obtained from food supplements. Vitamins can be separated into the two categories of water and fat-soluble vitamins. Vitamins are considered micronutrients in that only small amounts are required by the body to maintain ideal health. However, in some cases when vitamins are not supplied correctly detrimental deficiencies can be noticed (Heudi et al., 2005). The food industry can assist with the vitamin intake of consumers by creating fortified food products (Heudi et al., 2005). Products that are enriched with vitamins are defined as "functional foods" due health benefit that the consumer receives by consumption of the product consumed (Jiminez-Colmenero et al., 2001). In order to be classified as a functional food it must meet the following criteria: can be consumed on a daily basis, produced from naturally occurring ingredients, and have positive health benefit (Jiminez-Colmenero et al., 2001). Some health

benefits include defense mechanisms treating and preventing diseases (Jiminez-Colmenero et al., 2001).

Although the addition of vitamins into certain food is not a difficult task, this additive is not conducive in many food systems including those, which are thermally processed, and can affect the quality (Hui, 2007). The meat processing environment is relatively antagonistic to the bioavailability of vitamins depending on the processing and storage of the food product (Klaui and F. Hoffman, 1979). Whether the vitamins are sensitive to heat, light, oxidation, reducing agents, humidity, or the pH, all of these factors would typically contribute some form of degradation when using encapsulation. Thiamine is highly sensitive to heat, light, moisture, and basic solutions, thus making the chances of thiamine withstanding the RTE meat product processing and cooking steps difficult.

Consumer Acceptance

Frankfurters are a highly consumed meat product in the United States. They are most commonly sold in retail stores, but are also highly consumed at ballparks and sporting events (AMI, 2010). Sausage, frankfurters, bacon, and ribs are all in the top 20 foods consumed by children ages 2 to 18 as a nutritional source (DGA, 2010). To be more specific, over 20 billion frankfurters are consumed each year by Americans (AMI, 2010). Not only is this a highly consumed food product, this food system has the ability to incorporate many different ingredients due to the amount of protein extraction that occurs when skeletal muscle is processed. In today's health conscious society, frankfurters are not always viewed in the most

positive light. However, increasing the nutritional value may allow consumers that have been skeptical in the past to enjoy this improved meat product.

HPLC

A common method for thiamine determination is the utilization of high performance liquid chromatography (HPLC). Thiamine and its phosphate esters are relatively heat sensitive and water-soluble. In meat, thiamine is at a relatively high level before the cooking process, but depending on how high of temperatures are reached, thiamine and other vitamins do not withstand this process (Tang et al., 2006). For example, the finding by Tang et al. (2006), showed that the thiamine levels before and after cooking were 0.90 ± 0.03 mg/ 100g and 0.32 ± 0.01 mg/ 100 g, respectively. Thiamine in the body helps in producing energy acting as a coenzyme in the decarboxylation of fats, carbohydrates, and alcohols (Lu and Frank, 2008). Some studies have reported difficulty separating thiamine from the phosphate esters using a C18 column, however the goal of this research is to measure the total thiamine thus making a C18 reverse phase column appropriate for the laboratory method utilized.

MATERIALS AND METHODS

Treatments

Two trials were completed at the Angelo State University Food Safety and Product Development Laboratory in San Angelo, TX. Two experimental treatments and a control were utilized to determine the effects of microcapsule addition to a RTE meat product. The control group (CON, n = 20/trial) consisted of a standard frankfurter formulation (Table 1). The

commercial treatment (COM, n = 20 / trial) consisted of a standard frankfurter formulation and a commercial multivitamin microcapsule (Table 2). The laboratory method (LAB, n = 20 / trial) consisted of a microcapsule developed at the Angelo State University Food Safety and Product Development Laboratory, which contained the same vitamin content as the COM microcapsule using the coacervation technique in the laboratory and a standard frankfurter formulation (Table 1).

The commercial microcapsule was donated from DSM Nutritional Products Inc. (Parsippany, NJ) and was utilized in the microcapsule treatments. The COM microcapsule is composed of the recommended daily intake of ascorbic acid, biotin, d-calcium pantothenate, niacinamide, pyridoxine hydrochloride, riboflavin, thiamine mononitrate, vitamin A palmitate, cyanocobalamin, cholecalciferol, tocopheryl acetate, phytoonadione, and maltodextrin as the carrier (Table 2). The COM microcapsule does not contain any minerals.

LAB Microcapsule Preparation

Modified procedures from Jizomoto et al. (1993), Dong and Rogers (1993), and Green and Schleicher (1957) were utilized to produce the laboratory developed microcapsule. LAB microcapsules were prepared by incorporating 100 g of the vitamin premix mixed with vegetable oil at 100% of solution (100 mL of vegetable oil). This mixture was homogenized into an aqueous solution (10 g of gelatin in 340 mL distilled water) that was previously warmed to 50°C. After these two solutions were emulsified, a gum acacia solution (10 g of gum acacia in 340 mL distilled water) that was previously heated to 50°C was added into the gelatin/oil solution. Once all of these mixtures were homogenized, the mixture went through a washing step by adding it to 1500 mL distilled deionized H_2O. The pH was also adjusted using a 10% acetic acid solution to

the pH of 4.0 then cooled using an ice bath until it reached 10°C or lower which accomplished initial capsule formation. After cooling, the pH was adjusted to 9 to 11 using 20% sodium hydroxide to facilitate further capsule hardening. The microcapsule solution was then placed in a separatory funnel for 1 h to separate the upper liquid from the supernatant. After the majority of the supernatant was removed, the microcapsule slurry was partially dehydrated using a Buchner funnel under a vacuum. The remaining microcapsules were then frozen overnight at 0°C to be lyophilized for 48 h. This resulted in a powder form of the microcapsules. To ensure the size of the capsules were more uniform, the powder was sifted and stored in a dry environment until the frankfurters were produced. Commercial (COM) and laboratory (LAB) treatment microcapsules were then incorporated into the frankfurter formulation at a rate of 270 mg / serving (44 g / treatment) (Table 2). All frankfurters (Control, Commercial, and Laboratory treatments) were produced at the Angelo State University Food Safety and Product Development Laboratory. An experimental unit (EU) was 680 g (8 frankfurters). Each treatment consisted of 20 EU or 13.6 kg of meat batter (n = 20 / trt / trial). The frankfurter batter was made, and the microcapsules were mixed after chopping.

Sample Preparation

The standard base frankfurter formulation utilized in all treatments is found in Table 1. Beef trimmings were 90% lean skeletal muscle (beef inside round) with 10% fat. Pork trimmings consisted of 50% lean skeletal muscle and 50% fat (jowl meat). All of the meat processing was conducted at the Food Safety and Product Development Laboratory at Angelo State University. Each treatment was prepared under refrigerated temperatures (7°C). The beef and pork trim was separately ground through a 0.79 cm grinder plate. The beef trim was placed in the bowl chopper

with the salt and half the water in the form of ice. This was mixed for approximately 3 min until the temperature reached 4 to 5°C or until the meat batter had a large amount of protein extraction. The pork trimmings, sodium nitrate, sodium erythorbate, seasonings were then added and chopped until the temperature reached 12 to 13°C. Each treatment had 13.6 kg of meat batter randomly partitioned out from the bulk batch of standard frankfurter formulation (Table 1) previously discussed. Once assigned, these treatments were mixed with the assigned microcapsule and vacuum stuffed. All of the meat batter was stuffed into 22-mm-diameter peelable cellulose casings (DeWeid International, San Antonio, Texas). The frankfurters were labeled for treatment identification and smoked in a climate-controlled smokehouse (Alkar, DEC International Inc., Lodi, WI). The frankfurters were cooked until internal temperature reached 72°C with a cold shower applied once this temperature was reached to reduce potential shrinkage (Table 3). The frankfurters were cooled to 7°C or below. The fully cooked not shelf stable frankfurter was then vacuum packaged with 1 EU per package. Each package was placed in a retail type setting for 16 days that had a light intensity of 1900 lux (lx). All packages within each treatment were randomly assigned to aging treatments. Packages or EU within microcapsule treatments and within aging treatments were subjected to sensory analysis and laboratory thiamine analysis.

In Trial B, the temperature of the meat batter was not regulated in the same manner as Trial A where ice water was used. Trial B used only water due to the batter in Trial A staying at a low temperature. The temperature reached with adding just the water was increased but not over desired levels. The CON treatment was stuffed immediately after being taken out of the

bowl chopper, while the COM and LAB microcapsules were mixed into the meat batter after the chopping before being stuffed in casing. The mixing increased the temperature of the meat batter, thus causing water loss in the cooking process (Alvarez et al., 2007).

Simulated Retail Display

Each package within a respective aging treatment was placed in a Tyler retail display case (Model NM8, Tyler Refrigeration Corporation, Niles, MI) at 4 to 7°C for 16 days on days and assigned to aging treatments of 1, 4, 8, 12, and 16 d. The retail display lighting (Promolux, Safe Spectrum t8 Platinum, Shawnigan Lake BC, Canada) was at a maintained intensity of ~1900 lx to simulate a retail setting. Sample ID was coded to maintain sample identification. Based on display day (dd) aging treatment, four EU from each treatment were removed from the retail coffin case at the appropriate assigned intervals. These packages were then each split into two different packages with each package having four frankfurters in them to be used for sensory and laboratory analysis. The separate packages were vacuum packaged and placed into a freezer at -20°C until subsequent analysis. The retail display research utilized the procedure according to Braden et al. (2007).

Sensory Evaluation Panel

Four EU from days 1, 4, 8, 12, and 16 were evaluated by a trained sensory panel. On the day of evaluation, the frankfurters were taken from the freezer to be defrosted in a cooler at 2 to 7°C and assigned a sample number to prevent panelist bias due to treatment. Frankfurters were placed in boiling water until the internal temperature reached 71°C. The samples were then cut into 1 cm slices using a grid cutting board. Uniform samples were placed in a labeled temperature maintained container and given to the trained panel to be analyzed. Trained panelists

evaluated the samples on juiciness, tenderness, color, flavor, off-flavor, and overall acceptability (Table 5). These procedures were modified from the procedures of Cross et al. (1978). Each panelist was given unsalted crackers, apple juice, and water to cleanse the pallet between each sample. There were at least six panelists on each sensory evaluation panel.

Laboratory Assay

To determine if the addition of the multivitamin microcapsule had an effect on the overall vitamin content of the frankfurter in the final product, thiamine levels were determined. Vitamin B_1 (thiamine) has been shown to be less stabile than Vitamin B_2, but relatively stabile when compared to other water-soluble vitamins (Batifoulier et al., 2005). Thiamine is highly stabile in high acid solutions but tends to decrease in stability as a solution becomes more alkaline (Batifoulier et al., 2005). The frankfurters were at a somewhat neutral pH making the thiamine potentially susceptible to degradation. Analysis using High Performance Liquid Chromatography (HPLC) was utilized to determine the amount of thiamine in the samples. The purpose of testing the thiamine levels was to determine the stability of the microcapsules as a whole if they are to be used to produce a functional food. Thiamine was utilized as an indicator vitamin for the remaining vitamins because of its heat sensitivity characteristic.

Chemicals and Reagent

All samples were HPLC analyzed at the Angelo State University Biochemistry Laboratory in San Angelo, TX. Thiamine hydrochloride, HPLC grade methanol, potassium phosphate buffer, and trichloroacetic acid (TCA) were purchased from VWR International (Texas) and donated by the Department of Chemistry at Angelo State University. The stock

solutions of thiamine HCl, trichloroacetic acid (TCA) (10%), and a potassium phosphate buffer (50 mM, pH 6) were prepared for the thiamine HPLC analysis.

Chromatographic Conditions

The HPLC system that was utilized was a Waters 2487 Dual λ Absorbance Detector .The 717 plus Autosampler (Waters, Milford, MA), and a 1525 Binary HPLC Pump (Waters, Milford, MA) was utilized with a reverse phase C18 (4.6 x 250 nm) column (Vydac).

Potassium phosphate buffer (50 mM, pH 6) in methanol (80/20 v/v) was the mobile phase that was prepared using dibasic and monobasic potassium phosphate and filtered to make the solvent HPLC grade. An isocratic method was utilized with an injection volume of 20 μL and a flow rate of 1 mL / min for 10 min.

Standards were made from a thiamine stock solution (1 mg thiamine HCl / 25 mL 10% TCA) to obtain a standard curve.

Thiamine Extraction

One gram of the frankfurter was added to 10 mL of the TCA (10%) solution. This mixture was homogenized using a Polytron homogenizer then placed on ice for 15 min. Each sample was centrifuged at 13000 rpm for 6 min at 10°C. The supernatant (1 mL) was placed in an epindorf tube and centrifuged at 12000 rpm for 5 min. All supernatant that could be removed was placed in a separate microcentrifuge tube to be centrifuged (12000 rpm for 5 min) again to obtain the most pure extracted vitamins possible. Each sample was diluted (1:5000) with the potassium phosphate buffer before the analysis.

Each treatment from the two trials had one sample from dd 1, 8, and 16 of each treatment from the two trials was sent to NP Analytical Laboratories (St. Louis, MI) commercial testing to be tested for thiamine levels utilizing an HPLC method (AOAC, 2000).

Statistical Analysis

Sensory scores and thiamine level data was analyzed as a completely randomized design using the general linear models procedures of SAS (SAS Inst. Inc., Cary, NC). Sensory scores and thiamine levels were included in the model as the dependent variables with treatment and display day as a fixed affects. Experimental units were 680 g of each treatment and significant ($P \leq 0.05$) treatment effect means were separated using Fisher's protected Least Significant Difference.

RESULTS AND DISCUSSION

Sensory Evaluation

In trial A, multivitamin microcapsule treatment and treatment by display day did not have any effect on cooking loss ($P = 0.7025$; 0.5194, respectively). Cooking loss was, however, dependent on dd. Display day four samples had a greater cooking loss of 2.17 ± 0.57 g compared to all other dd which range from 0.00 ± 0.57 to 0.33 ± 0.57 g ($P = 0.0417$). Treatment and treatment x dd did not affect the average initial juiciness ($P = 0.6036$; 0.4944, respectively). However, average initial juiciness was dependent on display day with scores lower on dd 4,8,12 and 16 when compared to dd1, thus decreasing as the aging interval increased ($P < 0.0019$; Table 7). There was no effect on the average sustained juiciness by treatment and treatment x dd

($P = 0.2098$; 0.3574, respectively). In Trial A, sustained juiciness was dependent on dd with dd 1 showing higher scores than all other display days ($P < 0.0001$; Table 7). Average initial tenderness was not dependent on treatment and treatment x dd ($P = 0.5855$; 0.3310, respectively). Average initial tenderness was affected by display day ($P < 0.0001$). The initial tenderness decreased as the aging increased with the exception of dd 4 that did not fit this trend (Table 7). Treatment and treatment x dd did not have an effect on average sustained tenderness ($P = 0.6561$; 0.4043, respectively). Sustained tenderness was dependent on dd with dd 1 showing higher mean scores than all other display day ($P < 0.0001$; Table 7). The average flavor intensity was dependent on dd ($P <0.0001$; Table 6) with the least square means ranging from 6.89 ± 0.11 on dd 1 and 6.24 ± 0.11 on dd 4. Display days 1, 8, 12, and 16 decreased in flavor intensity scores with the lowest score being on dd 4 ($P = 0.0002$; Table 7.). Treatment and treatment x dd did not have an effect on average flavor intensity ($P = 0.9248$; 0.7024, respectively; Table 7). Treatment, dd, and treatment x dd did not have an effect on off flavors ($P = 0.3952$; 0.2435; 0.8077, respectively). Average overall acceptability was not dependent on treatment or treatment x dd ($P = 0.1951$, 0.6899, respectively). However, overall acceptability was dependent on dd ($P = 0.0004$; Table 7). As the aging interval increased, the overall acceptability decreased with the mean scores ranging from 7.12 ± 0.11 on dd 1 and 6.47 ± 0.11 on dd 16 (Table 7).

Cooking loss in trial B was not dependent on dd or treatment x dd ($P = 0.6682$; 0.6777, respectively). However, microcapsule treatment did have an effect on cooking loss ($P < 0.0001$). The LAB and COM treatments had higher levels of cooking loss with values of 20.9 ± 1.79 and

20.6 ± 1.79 g when compared to the CON treatment at a level of 2.20 ±1.82 g (Table 6). The microcapsules were mixed after chopping in the LAB and COM treatments that could have played a role in the amount of cooking loss by raising the temperature of the meat batter. According to Alvarez et al. (2007), the temperature of the meat batter had to be controlled due an increase in temperature can cause the surface tension decreases on the fat particles thus creating more surface area for the protein to coat. If there is not enough protein to coat the fat particles, the cooking process allows the fat to "expand and melt" out of the product relating to the amount of cooking loss (Alvarez et al., 2007). While the average initial juiciness was affected by the treatment ($P = 0.0353$; Table 6), there was not an effect due to dd or treatment x dd ($P = 0.1314$; 0.2029, respectively). The COM and LAB treatments although having a higher amount of cooking loss had higher initial juiciness scores (7.20 ± 0.05, 7.17 ± 0.05; Table 6) than the CON treatment. Treatment, dd, and treatment x dd did not affect the average sustained juiciness ($P = 0.1458$; 0.1817; 0.1578, respectively; Table 6 and 8). The average initial tenderness was not dependent on treatment or treatment x dd ($P = 0.4234$; 0.0791, respectively). However, average initial juiciness was dependent on display day ($P < 0.0001$). As the aging interval increased, the initial tenderness increased with the mean scored on dd 1 being 6.99 ± 0.07 to dd 16 with mean scores of 7.15 ± 0.07 (Table 8). Treatment and treatment x dd did not have an effect ($P = 0.3739$; 0.0625, respectively), while the dd affected the average sustained tenderness ($P = 0.0002$; Table 8). The display day affect on average sustained juiciness was inconsistent with mean scores ranging from 7.46 ± 0.08 (dd 8) to 7.24 ± 0.08 (dd 1) ($P < 0.0001$). In Trial B, flavor intensity, off- flavor and overall acceptability were not dependent on treatment, dd, or treatment x dd ($P > 0.05$).

A study conducted by Kryitsi et al. (2011) showed that incorporating B complex vitamins in different cooked rice products, without any form of controlled release, had an effect on sensory characteristics. Flavor components such as metallic, bitter, and unpleasant characteristics were described with the addition of these vitamins (Kryitsi et al., 2011). This represents why the encapsulation technique can be useful when working with vitamins and other additives in food products. Microencapsulating multivitamins was shown to not have an effect on sensory characteristics in the standard frankfurter formulation, thus showing how encapsulation is important when applying vitamins to food products.

HPLC Data

The in-house laboratory analysis determined the amount of thiamine (mg/100g) in each EU. There was not an effect of trt, dd, or trt x dd in Trial A on the amount of thiamine present (P = 0.1714, 0.7273, 0.8660, respectively; Table 10). In Trial B, thiamine levels were not affected by the trt, dd, or trt x dd (P = 0.2742, 0.8593, 0.9440, respectively; Table 10).

The commercial laboratory (NP Analytical Laboratories) samples were not statistically analyzed due to the small samples size (n=1/1, 8, 16 dd / trt / trial). However, when these results were evaluated there was a distinct difference in magnitude of values reported of the control when comparing the COM and LAB treatment results. This reported thiamine levels in COM frankfurter and CON frankfurters points to the potential effect of the treatments. These CON frankfurter thiamine levels also relate to previous literature from Tang et al. (2006) showing that the amount of thiamine in the CON treatment is closer to the published levels in this literature and by the USDA Nutrient Database (2002). The NP Analytical Laboratories data from Trial A

and Trial B (Table 9) had differences between each treatment, which is contrary to the in-house HPLC method. Thus, data discrepancies could potentially be due to analysis methods used. The in-house HPLC thiamine extraction was not the AOAC (2000) method. The AOAC (2000) method includes an enzyme digestion step that was not utilized in the HPLC method (AOAC, 2000). Thiamine levels measured at the commercial laboratory were also measured by florescence method by converting the extracted thiamine to thiochrome to be measured with a fluorescence detector (AOAC, 2000). The levels of thiamine measured in the in-house HPLC laboratory were measured using absorbency, which demonstrated high amounts of thiamine that were not expected when looking at the USDA Nutrient Database (2002). Only using absorbance to measure the thiamine levels in the in-house HPLC method might not be specific enough, thus giving inflated thiamine readings. According to the USDA Nutrient Database (2002), pork and beef sausage contains 0.09 mg of thiamine / 26 g of meat. This is significantly smaller than that levels that were obtained using the in-house HPLC method in Trial A and B (Table 10 and 11). This can potentially be explained by the possibility that more vitamins were coming off the column at the same time as thiamine, which gave larger amount readings than were actually there. If the AOAC thiamine analysis method had been utilized, the thiamine data from HPLC method may have been a better representation of the actual amount of thiamine present. Samples (n= 1/ 1, 8, 16 dd / trt / trial) that were sent to NP Analytical Laboratories.

IMPLICATIONS

A multivitamin microcapsule may be added to a frankfurter formulation to increase functional properties of the RTE meat food product. The addition of a microcapsule that is able

to withstand chemical, thermal, and physical agitation will help broaden the use of the microencapsulation technique in the food industry. Although the production of a functional food is not a new idea, the implication of new food products could be widened and applied to the meat industry. Frankfurters are a largely consumed product by Americans and this alternative approach to a typical formulation could add another positive characteristic upon consumption. As the study did not accurately represent the in-house HPLC results to determine the different amounts of thiamine levels within each treatment, more studies can be conducted to change the method of thiamine extraction along with determining the remaining vitamins present in the cooked product. This study could also be applied to different RTE meat products not just specifically frankfurters.

Table 1. Frankfurter formulations for all treatments.

Ingredients	Amount (g)
Beef Trim (90/10)[a]	18144
Pork Trim (50/50)[b]	18144
Ice[cc]	3628
Non-Fat Dry Milk	1134
Salt	907
Dextrose	362
Ginger	48
Ground Mustard	51
Garlic Powder	24
Ground Nutmeg	48
Ground White Pepper	88
Ground Coriander	45
Ground Paprika	226
Onion Powder	24
Mace	45
Black Pepper	45
Sodium Erythorbate	16
Sodium Nitrite	88
LAB/COM Microcapsule[d]	48

[a] 90% lean skeletal muscle and 10% fat

[b] 50% lean skeletal muscle and 50% fat

[c] Water was used in Trial B

[d] Not used in CON treatment

Table 2. DSM Nutritional Products (Parsippany, NJ) Vitamin Premix XR05415000 Formulation Sheet.

Active Ingredients	Declared Ingredient Level (mg/serving)
Ascorbic Acid (Vitamin C)	78.00
Biotin (Vitamin H)	0.35
d-Calcium Pantothenate (Vitamin B5)	12.50
Niacinamide (Vitamin B3)	22.10
Pyridoxine Hydrochloride (Vitamin B6)	2.70
Riboflavin (Vitamin B2)	1.96
Thiamine Mononitrate (Vitamin B1)	1.80
Vitamin A Palmitate	24.00
Cyanocobalamin (Vitamin B12)	0.78
Cholecalciferol (Vitamin D3)	4.80
Tocopheryl Acetate (Vitamin E Acetate)	69.00
Phytonadione (Vitamin K1)	1.92
Maltodextrin (Carrier)	

* Use Rate: 270 mg/serving

Table 3. Frankfurter smokehouse cycle time, dry-bulb, wet-bulb, relative humidity, dampers, and smoke application for each trial (Alkar).

Step	Time (min)	Dry- Bulb (°C)	Wet- Bulb (°C)	Relative Humidity (%)	Dampers	Smoke
1	5	43.3	37.8	68	__	
2	5	43.3	0	__	Auto	
3	30	48.8	0	__	Closed	On
4	15	65.6	0	__	Auto	
5	15	73.9	54.4	36	Auto	
6	10	82.2	73.9	68	Auto	
7	12	__	__	__	__	

Cooking time ~ 90 minutes

Table 4. Scoring of sensory evaluation according to Cross et al. (1978).

Juiciness	Tenderness	Flavor Intensity	Off Flavor	Overall Acceptability
8- Extremely juicy	8- Extremely tender	8- Extremely intense	4- None	8- Like extremely
7- Very juicy	7- Very Tender	7- Very intense	3- Slight off flavor	7- Like very much
6- Moderately juicy	6- Moderately tender	6- Moderately intense	2- Moderate off flavor	6- Like moderately
5- Slightly juicy	5- Slightly tender	5- Slightly intense	1- Extreme off flavor	5- Like slightly
4- Slightly dry	4- Slightly tough	4- Slightly bland		4- Dislike slightly
3- Moderately dry	3- Moderately tough	3- Moderately bland		3- Dislike moderately
2- Very dry	2- Very tough	2- Very bland		2- Dislike very much
1- Extremely dry	1- Extremely tough	1- Extremely bland		1- Dislike extremely

Table 5. Least square means ± SEM of sensory evaluation attributes of the control (CON), commercial (COM), and laboratory (LAB) treatments of frankfurters for Trial A.

Attribute	Control	Commercial	Laboratory	*P* > F
Initial Juiciness[a]	6.12 ± 0.11	6.27 ± 0.11	6.15 ± 0.11	0.6036
Sustained Juiciness[b]	6.42 ± 0.09	6.65 ± 0.09	6.50 ± 0.09	0.2098
Initial Tenderness[c]	6.15 ± 0.08	6.23 ± 0.08	6.11 ± 0.08	0.5855
Sustained Tenderness[d]	6.51 ± 0.08	6.51 ± 0.08	6.41 ± 0.08	0.6561
Flavor Intensity[e]	6.50 ± 0.08	6.49 ± 0.08	6.46 ± 0.08	0.9248
Off Flavor[f]	3.99 ± 0.01	3.99 ± 0.01	4.00 ± 0.01	0.3952
Overall Acceptability[g]	6.63 ± 0.08	6.83 ± 0.08	6.67 ± 0.08	0.1951

[a] (Initial Juiciness) 1- Extremely Dry, 8- Extremely Juicy

[b] (Sustained Juiciness) 1-Extremely Dry, 8-Extremely Juicy

[c] (Initial Tenderness) 1-Extremely Tough, 8-Extremely Tough

[d] (Sustained Tenderness) 1-Extremely Tough, 8- Extremely Tough

[e] (Flavor Intensity) 1-Extremely Bland, 8- Extremely Intense

[f] (Off Flavor) 1- Extreme Off Flavor, 4- None

[g] (Overall Acceptability) 1- Dislike Extremely, 8- Like Extremely

Table 6. Least square means ± SEM of sensory evaluation attributes of the control (CON), commercial (COM), and laboratory (LAB) treatments of frankfurters for Trial B.

Attribute	Control	Commercial	Laboratory	*P* > F
Initial Juiciness[a]	7.00 ± 0.06[y]	7.20 ± 0.05[z]	7.16 ± 0.05[z]	0.0353
Sustained Juiciness[b]	7.29 ± 0.06	7.45 ± 0.06	7.33 ± 0.06	0.1458
Initial Tenderness[c]	6.97 ± 0.04	7.02 ± 0.04	6.94 ± 0.04	0.4234
Sustained Tenderness[d]	7.26 ± 0.05	7.20 ± 0.05	7.17 ± 0.05	0.3739
Flavor Intensity[e]	6.84 ± 0.05	6.90 ± 0.05	6.84 ± 0.05	0.5978
Off Flavor[f]	3.97 ± 0.02	3.95 ± 0.02	3.94 ± 0.02	0.5533
Overall Acceptability[g]	6.93 ± 0.06	6.99 ± 0.06	6.85 ± 0.06	0.2046

[yz] Means within a row lacking a common superscript differ ($P < 0.05$)

[a] (Initial Juiciness) 1- Extremely Dry, 8- Extremely Juicy

[b] (Sustained Juiciness) 1-Extremely Dry, 8-Extremely Juicy

[c] (Initial Tenderness) 1-Extremely Tough, 8-Extremely Tough

[d] (Sustained Tenderness) 1-Extremely Tough, 8- Extremely Tough

[e] (Flavor Intensity) 1-Extremely Bland, 8- Extremely Intense

[f] (Off Flavor) 1- Extreme Off Flavor, 4- None

[g] (Overall Acceptability) 1- Dislike Extremely, 8- Like Extremely

Table 7. Least square means ± SEM of sensory evaluation attributes of display day for frankfurters in Trial A.

Attribute	1	4	8	12	16	*P* > F
Initial Juiciness[a]	6.67 ± 0.14^{y}	5.93 ± 0.14^{z}	6.27 ± 0.14^{z}	6.14 ± 0.14^{z}	5.90 ± 0.14^{z}	0.0019
Sustained Juiciness[b]	7.05 ± 0.12^{x}	6.27 ± 0.12^{yz}	6.62 ± 0.12^{z}	6.41 ± 0.12^{z}	6.28 ± 0.12^{z}	< 0.0001
Initial Tenderness[c]	6.64 ± 0.10^{y}	5.98 ± 0.10^{z}	6.244 ± 0.10^{z}	6.01 ± 0.10^{z}	6.00 ± 0.10^{z}	< 0.0001
Sustained Tenderness[d]	7.09 ± 0.10^{x}	6.29 ± 0.10^{z}	6.55 ± 0.10^{y}	6.29 ± 0.10^{yz}	6.18 ± 0.10^{z}	< 0.0001
Flavor Intensity[e]	6.89 ± 0.10^{x}	6.24 ± 0.10^{xy}	6.62 ± 0.10^{yz}	6.44 ± 0.10^{yz}	6.23 ± 0.10^{z}	0.0002
Off Flavor[f]	4.00 ± 0.01	3.97 ± 0.01	4.0 ± 0.01	3.99 ± 0.01	4.00 ± 0.01	0.2435
Overall Acceptability[g]	7.11 ± 0.10^{x}	6.49 ± 0.10^{yz}	6.83 ± 0.10^{xy}	6.64 ± 0.10^{z}	6.47 ± 0.10^{z}	0.0004

[xyz] Means within a row lacking a common superscript differ ($P < 0.05$)

[a] (Initial Juiciness) 1- Extremely Dry, 8- Extremely Juicy

[b] (Sustained Juiciness) 1-Extremely Dry, 8-Extremely Juicy

[c] (Initial Tenderness) 1-Extremely Tough, 8-Extremely Tough

[d] (Sustained Tenderness) 1-Extremely Tough, 8- Extremely Tough

[e] (Flavor Intensity) 1-Extremely Bland, 8- Extremely Intense

[f] (Off Flavor) 1- Extreme Off Flavor, 4- None

[g] (Overall Acceptability) 1- Dislike Extremely, 8- Like Extremely

Table 8. Least square means ± SEM of sensory evaluation attributes of display day for frankfurters in Trial B.

Attribute	1	4	8	12	16	*P* > F
Initial Juiciness[a]	6.98 ± 0.07[z]	7.07 ± 0.07[y]	7.20 ± 0.07[y]	7.22 ± 0.07[y]	7.15 ± 0.07[y]	0.1314
Sustained Juiciness[b]	7.24 ± 0.07[z]	6.27 ± 0.07[y]	7.46 ± 0.07[y]	7.36 ± 0.07[y]	7.43 ± 0.07[y]	0.1817
Initial Tenderness[c]	6.69 ± 0.05[z]	6.88 ± 0.05[z]	7.05 ± 0.05[z]	6.01 ±0.10[z]	6.00 ± 0.10[z]	<0.0001
Sustained Tenderness[d]	6.99 ± 0.06[z]	7.11 ± 0.06[z]	7.27 ± 0.06[y]	7.37 ± 0.06[y]	7.33 ± 0.06[y]	0.0002
Flavor Intensity[e]	6.79 ± 0.06	6.89 ± 0.06	6.84 ± 0.06	6.82 ± 0.06	6.96 ± 0.06	0.2886
Off Flavor[f]	3.95 ± 0.02[y]	4.00 ± 0.02[y]	3.96 ± 0.02[y]	3.95 ± 0.02[y]	3.92 ± 0.02[z]	0.2323
Overall Acceptability[g]	6.85 ± 0.07	6.90 ± 0.07	6.93 ± 0.07	6.98 ± 0.07	6.95 ± 0.07	0.7525

[yz] Means within a row lacking a common superscript differ ($P < 0.05$)

[a] (Initial Juiciness) 1- Extremely Dry, 8- Extremely Juicy

[b] (Sustained Juiciness) 1-Extremely Dry, 8-Extremely Juicy

[c] (Initial Tenderness) 1-Extremely Tough, 8-Extremely Tough

[d] (Sustained Tenderness) 1-Extremely Tough, 8- Extremely Tough

[e] (Flavor Intensity) 1-Extremely Bland, 8- Extremely Intense

[f] (Off Flavor) 1- Extreme Off Flavor, 4- None

[g] (Overall Acceptability) 1- Dislike Extremely, 8- Like Extremely

Table 9. Thiamine levels results of control (CON), commercial (COM), and laboratory (LAB) treatments from NP Analytical Laboratories (St. Louis, MI) on dd 1, 8, and 16 in Trial A and Trial B (n = 1 / trt / dd).

Treatment	dd	Thiamine Levels (mg/100g) Trial A	Thiamine Levels (mg/100g) Trial B
CON	1	0.16	0.16
COM	1	3.94	3.77
LAB	1	0.47	1.17
CON	8	0.16	0.17
COM	8	3.88	3.22
LAB	8	0.43	1.24
CON	16	0.15	0.17
COM	16	4.27	2.94
LAB	16	0.47	1.19

Table 10. Least square means ± SEM of thiamine levels (mg/100g) based on control (CON), commercial (COM), and laboratory (LAB) treatments and by display day of Trial A and Trial B in the in-house HPLC laboratory method.

Effect		Trial A Thiamine (mg/100g)	Trial B Thiamine (mg/100g)	Trial A *P* > F	Trial B *P* > F
Treatment					
	Control	64.0 ± 0.03	63.0 ± 0.03	0.1714	0.2742
	Commercial	57.0 ± 0.03	57.0 ± 0.03	0.1714	0.2742
	Laboratory	62.0 ± 0.03	59.0 ± 0.03	0.1714	0.2742
Display Day					
	1	60.0 ± 0.03	57.0 ± 0.03	0.7273	0.8593
	4	58.0 ± 0.03	61.0 ± 0.03	0.7273	0.8593
	8	64.0 ± 0.03	59.0 ± 0.03	0.7273	0.8593
	12	63.0 ± 0.03	61.0 ± 0.03	0.7273	0.8593
	16	60.0 ± 0.03	58.0 ± 0.03	0.7273	0.8593

Table 11. Least square means ± SEM of thiamine levels mg/100g frankfurter) obtained by the in-house HPLC method (for control (CON), commercial (COM), and laboratory (LAB) frankfurter treatments.

Trial	Control	Commercial	Laboratory	Source	*P* > F
Trial A	64.0 ± 0.03	57.0 ± 0.03	62.0 ± 0.03	trt	0.1714
				dd	0.7273
				trt x dd	0.8660
Trial B	63.0 ± 0.03	57.0 ± 0.03	59.0 ± 0.03	trt	0.2742
				dd	0.8593
				trt x dd	0.9440

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APPENDICES

APPENDIX A. SENSORY EVALUATION

Sensory Evaluation

1. Frankfurters should have an internal temperature of 2 to 5°C before cooking. It is common to thaw food products before cooking at 2 to 5°C for 12 hours.

2. Take care and maintain sample identity throughout process by having labels with the product.

3. Pre-heat sample holding containers and pans by placing in the oven. Pans with separate suspended compartments can be utilized, with the addition of sand below to maintain temperature.

4. Boil a large pot of water to reheat the samples.

5. Internal temperature of each frankfurter should be taken in the geometric center and recorded. Temperatures should be in the range of 2 to 5°C.

6. Weigh each frankfurter in g before cooking and record.

7. Place each frankfurter in boiling water to reheat. The internal temperature of each frankfurter should be approximately 71°C.

8. Record weight and temperature of each steak recorded immediately after cooking utilizing same procedures as before cooking.

9. Slice each frankfurter into 1 cm pieces by using a premade cutting board to standardize the size of each sample.

10. Place all pieces of sample (excluding end pieces) in designated sample holding containers and maintain identity.

11. Panel room should be prepared before cooking to facilitate efficient panel time and minimize period after cooking until panel evaluations.

12. Panel set up and evaluations should be according to Cross et al., 1978.

13. Record all sensory data for analysis.

APPENDIX B. LABORATORY HPLC ANALYSIS

Laboratory Analysis

1. One gram of the frankfurter will be measured with a calibrated scale.
2. The 1 g sample will have 10 mL of trichloracetic acid (TCA) added to the tube.
3. The mixture will be homogenized using a polytron homogenizer.
4. This will then be placed on ice for 15 min.
5. The samples will be centrifuged at 13000 rpm for 6 min at 10°C.
6. The 1 mL of supernatant was transferred to an epidorf tube and centrifuged at 12000 rpm for 6 min two more times.
7. The samples were diluted to 1:5000
8. Place dilution in a 1 mL glass vial sample tube with polyethylene snap cap
9. The sample can then be put in the autosampler for analysis.

VITA

Kaci Lee Foote is the daughter of Jack and Tronda Foote. She was born on November 7, 1986 in Lubbock, Texas. Kaci grew up in Idalou, Texas and proceeding graduation enrolled Angelo State University in August 2005. She graduated with a B.S. degree in Animal Science in May 2009, and continued to study at Angelo State University in pursuance of a Master's of Science with an emphasis in Meat and Food Science. She is scheduled to graduate in May of 2011.