EFFECT OF PRODUCTION METHOD ON CHARACTERISTICS AND OXIDATIVE STABILITY OF MICROENCAPSULATED FISH OIL

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EFFECT OF PRODUCTION METHOD

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NOMENCLATURE

GC	Gas chromatography
HS-SPME	Head space solid phase microextraction
MS	Mass spectrometer
SEM	Scanning electron microscope
w/w	Weight to weight
w/v	Weight to volume

Units

%	Percent
^{o}C	Degree Centigrade
g	Gram
hr	Hour
kg	Kilogram
mL	Millliliter
µL	Microliter
min	Minute
sec	Second

CHAPTER I

INTRODUCTION

1.1 STATEMENT OF PROBLEM

It has been documented that traditional emulsion spray and freeze drying methods can produce microcapsules that improve the oxidative stability of oils high in polyunsaturated fatty acids, such as fish oil. However, emulsion processes require high energy homogenization steps that may initiate oxidation of fish oil. Conventional spray drying systems has employed pressure nozzles that utilize gas pressure to atomize microencapsulating materials. Pressure nozzles produce microcapsules that lack uniform size. Today, nozzles that mix oil and solutions containing encapsulating wall material at the point of atomization are available. Newer spray drying equipment introduces the option of eliminating the need for emulsion preparation prior to spray drying. The latest spray nozzles have introduced sonic energy as a means for atomization of solutions to be spray dried. Ultrasonic nozzles may present a means to produce more uniform microcapsules. Information on the physical and chemical characteristics of microcapsules produced by these new spray nozzles is not available.

1.2 HYPOTHESIS

Spray drying nozzles where oil is mixed with wall materials at the point of atomization will produce microcapsules with improved characteristics while increasing oxidative stability due to elimination of oil exposure to high energy homogenization process. Ultrasonic nozzles will produce more uniform microcapsules compared to pressure atomizing spray nozzles.

1.3 OBJECTIVES

The main objective of this thesis is to produce fish oil microcapsules by different microencapsulation techniques and compare the properties of microcapsules. The specific objectives include:

1) Physical and chemical characterization of produced microcapsules.
2) Evaluation of oxidative stability of microcapsules produced by various microencapsulation techniques.

CHAPTER II

REVIEW OF LITERATURE

2.1 OVERVIEW

Heart disease is the leading cause of death within the majority of developed countries (Heinzelmann and others 2000). Epidemiological studies have shown that long chain ω-3 polyunsaturated fatty acids (PUFA) have a positive effect on coronary health (Heinzelmann and others 2000). Specifically the ω-3 PUFA eicosapentaenoic acid [EPA (20:5n-3)] and docosahexaenoic acid [DHA (22:6n-3)] have been shown to reduce platelet aggregation, platelet vessel wall interactions, and blood plasma viscosity (Fantoni and others 1995). The health benefits of PUFA have received a lot of attention after the publication of a series of papers explaining the reduced incidence of heart disease among Greenland Eskimos whose diets are based on fish rich in PUFA (Whelan and Rust 2006). It has also been shown that DHA and EPA may prevent certain types of cancer, inflammations, allergies, and may improve development and function of the central nervous system (Connor 2000).

Fish oils contain the richest concentrations of DHA and EPA (Kolanowski and others 2004). It has been recommended to consume 0.2 g per day of DHA and EPA, which may be done by weekly consumption of fatty fish (Kolanowski, Ziolkowski and others 2006). However, in many western countries the amount of fish consumed is far below the recommended servings (Kolanowski, Ziolkowski and others 2006). Besides

changes in diets, increased intake of ω-3 PUFA may be accomplished through consumption of fish oil supplements or foods enriched with fish oil (Kolanowski and others 2004). Problems do arise when attempting to enrich foods with fish oil. Fish oil has a limited storage time because the PUFA are highly susceptible to oxidation (Cho and others 2003). Also fish oil has a strong, sometimes unpleasant, taste and smell that is unacceptable in most foods (Cho and others 2003). The focus of this thesis will be on preparation of fish oil microcapsules by spray and freeze drying methods to increase oxidative stability.

2.2 MICROENCAPSULATION

Microencapsulation provides a means to convert liquid fish oil into a more stable and easy to use dry powder (Kolanowski and others 2004). Basically, a microcapsule is made up of two parts: the core made up of fish oil and the outer wall which surrounds the entire surface of the inner oil core. The outer wall serves two basic purposes. One is to mask the undesirable “fishy” smell and taste. The second is to protect the easily oxidized fish oil from oxygen and light. The outer wall is usually made up of carbohydrates, proteins, and gums (Tan and others 2004). However, investigations are being conducted to evaluate new wall materials, such as sugar beet pectin (Drusch and others 2006). Some of the most common wall materials used for microencapsulation include gelatin, maltodextrin, sugars, starch, skimmed milk, milk and whey protein and plant gums. Combination of wall materials is often used to increase the efficiency of microencapsulation (Kolanowski and others 2004).

The most common way to produce microcapsules of fish oil is through spray drying of an emulsion (Augustin and others 2006; Drusch and others 2006; Hogan and others 2003; Rusli and others 2006; Tan and others 2004; Kolanowski and others 2004, Kolanowski, Ziolkowski and others 2006, Kolanowski, Jaworska and others 2006). Spray drying is the most common because it is a flexible, efficient, and an inexpensive process that produces good microcapsules (Ashady 1993). Emulsions are prepared by combining fish oil with the chosen wall material along with an emulsifier in water. The emulsion ingredients are stirred together creating a coarse emulsion. A high pressure homogenizer is then used to create a fine emulsion (Kolanowski, Ziolkowski and others 2006). Once the fine emulsion has been created it is spray dried. The emulsion is atomized through a nozzle into a chamber. Hot air (inlet temperatures usually close to 150ºC) circulating in the chamber quickly evaporates moisture from the atomized emulsion leaving the dried microcapsules (Hogan and others 2003).

There are, however, alternate methods to spray drying to create microcapsules. Heinzelmann and others (2000) were able to prepare microcapsules by using a freeze drying method. An emulsion was prepared and frozen. Then a freeze dryer was used to remove the frozen water from the emulsion by sublimation (Heinzelmann and others 2000). It was hypothesized that freeze drying may have advantages over spray drying. This is because freeze drying limits fish oil exposure to high temperatures that are required for spray drying. Furthermore, freeze drying is carried out under vacuum. Therefore, there is a smaller possibility to catalyze oxidation of fish oil at low temperatures and in the absence of oxygen during the microencapsulation process. Although Heinzelmann and others (2000) did not do a comparison of freeze dried fish oil

with spray dried fish oil, Kolanowski, Ziolkowski and others (2006) obtained experimental results confirming the Heinzelmann and others (2000) study. In the Kolanowski, Ziolkowski and others (2006) study the peroxide values (PV) of fish oil prior to microencapsulation was 1.05 meq and increased to 4.06 meq after spray drying indicating formation of oxidation products during the process.

Both spray and freeze drying require an emulsion to produce microcapsules as was stated previously. The production of emulsions requires fish oil to be exposed to some type of high energy homogenization. High energy homogenization is required to create emulsions with small oil droplet sizes usually around 1µm (Jafari and others 2006). Microfluidizing and ultrasonic homogenizers have been shown to be viable means for producing emulsions for spray and freeze drying (Jafari and others 2007). Microfluidization uses a pneumatic pump powered by pressurized air to force coarse emulsion fluid through a chamber of microchannels. The high pressure pump provides intense shearing action that can provide a fine emulsion (Jafari and others 2007). Ultrasonic devices employ cavitation as a means to create fine emulsions. The dispersed oil phase of the emulsion is disrupted and mixed as vapor cavities are formed and collapsed by ultrasonic waves (Jafari and others 2006).

High energy homogenizers have been shown to increase emulsion temperatures. Jafari and others (2007) showed that emulsion temperature at the exit of the microfluidizer chamber and in the area around the ultrasonic probe increased linearly with time and pressure. It was found that temperature of the sonicated emulsions increased up to 45 ºC after 100 s of homogenization. It has also been reported that high-pressure microfluidizing systems caused a temperature rise in emulsions up to 70-80 ºC

even though a cooling jacket was used (Floury and others 2004). Another aspect of emulsion production process that may lead to oxidation of fish oil is contact of fish oil with oxygen (Kolanowski, Ziolkowski and others 2006).

Most often purified fish oil with a PUFA content of 300 g per kg is used to produce microcapsules (Kolanowski and others 2004, Kolanowski, Ziolkowski and others 2006). Size and morphology of microcapsules depend on the wall materials and the process used to produce the capsules. Emulsion droplet size along with the method of drying the microcapsules can lead to a great amount of variation in the size of microcapsules (Ashady 1993). According to Kolanowski and others (2004) the diameter of microcapsules is less than 1000 μm. The standard method for assessment of microcapsule size is through the use of a particle size analyzer. Morphology is assessed through use of a scanning electron microscope (SEM) (Cho and others 2003; Drusch 2005). The desired morphology of microcapsules is to have a uniform spherical shape. While the size of microcapsules varies it is believed that the smaller the capsules, the better. This is due to the fact that smaller capsules may degrade more slowly leading to delayed oxidation of fish oil (Augustin and others 2006).

2.3 OXIDATIVE STABILITY

The oxidative stability of microencapsulated fish oil is the indicator of a successful or unsuccessful process. Measurements of the oxidative condition of encapsulated fish oil over a period of time under different storage conditions may be used to compare the protective strength of microcapsules produced by different methods (Drusch and others 2006). The most common measure of oxidation is PV which measures

primary oxidation products and p-anisidine values (AV) which is a measurement of secondary oxidation products (Hogan and others 2003). These byproducts of oxidized fatty acids are the indicators of oil degradation. The PV and AV are determined by extracting oil from the microcapsules and performing PV and AV tests created by the American Oil Chemists' Society (Hogan and others 2003).

Other parameters may also be monitored to further elucidate the oxidative condition of the microencapsulated fish oil. Oxidative changes in fish oil may be determined by measurements of conjugated dienes, propanal and other aldehydes (Drusch and others 2006). According to Drusch and others (2006) conjugated dienes reveal oxidative changes and may be easily measured by a photometrical method. Propanal is the major volatile aldehyde that results from degradation of PUFA (Faraji and others 2005). Propanal concentrations may be determined by use of a static headspace gas chromatograph with a flame ionization detector (Frankel 1993). Head space solid-phase microextraction (HS-SPME) has been shown to be a method for quick analysis and characterization of volatile oxidative compounds (Iglesias and others 2007).

Further judgments about the oxidative state of encapsulated fish oil can be assessed through detection of rancidity by sensory tests. Oils are considered rancid when a rancid odor is clearly recognized (Velasco and others 2006). Sensory panelists merely need to detect odor in this test. Other more complicated sensory evaluations were performed by Kolanowski, Jaworska and others (2006). Trained panelists were asked to compare odors of different samples with a reference sample while describing odor attributes and intensity (Kolanowski, Jaworska and others 2006). The test results showed

that microencapsulated fish oil oxidized rapidly in the presence of air while vacuum storage improved the shelf life of the encapsulated products.

2.4 SPRAY NOZZLE TECHNOLOGY

Conventional spray drying nozzles use pressure or centrifugal forces to atomize fluids (Klaypradit and Huang 2007). These nozzle types have been shown to have some disadvantages such as lack of control over droplet size consequently wide distributions of droplet size and clogging (Bittner and others 1999).

Ultrasonic atomizers employ ultrasonic vibrational energy as a means to atomize fluids (Klaypradit and Huang 2007). As their name implies, ultrasonic nozzles employ high frequency sound waves, those beyond the range of human hearing. Since wavelength is dependent upon operating frequency, nozzle dimensions are governed by frequency. In general, high frequency nozzles are smaller, create smaller drops, and consequently have smaller maximum flow capacity than nozzles that operate at lower frequencies. An important characteristic of ultrasonic nozzles is that they generate a soft spray which dramatically reduces overspray and minimizes clogging. These nozzles are recommended when extremely low flow rates are required. Another advantage of the ultrasonic nozzles is the ability to produce droplets with uniform size distribution (Topp and Eisenklam 1972). Bittner and others (1999) were able to produce microcapsules using an ultrasonic nozzle that had particle yields and encapsulation efficiencies that were within the range of conventional spray drying nozzles.

CHAPTER III

METHODOLOGY

3.1 FISH OIL ANALYSIS

3.1.1 Sample Characterization and Storage

Refined menhaden oil was obtained from OmegaPure (Houston, TX) containing 500 mg/kg mixed tocopherols and 200 mg/kg tert-butylhydroquinone. Fish oil was shipped frozen in 1 gallon jugs. Once received the fish oil was split into smaller glass bottles and the head space was filled with nitrogen. The fish oil was then stored in a -80 ºC freezer. Certificate of analysis provided by OmegaPure indicated typical and max values for free fatty acid, AV, and PV, as well as, percent values of long chain omega-3 fatty acids. In laboratory analysis of the fish oil was also performed for verification of these values. Monthly measurements of AV and PV were conducted to confirm the condition of the fish oil during the experimental period.

3.1.2 Free Fatty Acid

Free fatty acid (FFA) determination was performed using a colorimetric procedure. A 5 % (w/v) solution of copper acetate was prepared by dissolving 5 g of copper acetate in 100 mL of water. Pyridine was added to this solution 1 mL at a time until the pH was raised to a range of 6.0-6.2. A 100 mg/mL stock standard solution of oleic acid (National Formulary/Food Chemicals Codex grade, Fisher Chemical, Fairlawn, NJ) was prepared

by dissolving 100 mg of oleic acid in 1 mL of hexane. A standard curve was prepared by transferring 10, 20, 30, and 40 µL aliquots of stock standards to individual centrifuge tubes. To each tube 5 mL benzene and 1 mL copper acetate solution was added and vortexed for 2 min followed by centrifugation for 5 min. Approximately 2 g of fish oil was used to prepare samples in the same manner as the oleic acid standards. Absorbance of samples and standards was read at 715 nm on a spectrophotometer (DU 520, Beckman Coulter, Inc, Fullerton, CA). A standard curve was prepared and used to calculate FFA content in the samples. The results were reported as % (w/w) based on the initial oil weight used for the tests.

3.1.3 Peroxide Value

PV of the oil samples were determined by AOCS official method cd8-53 (2003). Approximately 5 g of the fish oil sample was weighed into a 250 mL flask. Then 30 mL of a 3:2 (v/v) glacial acetic acid/chloroform, both American Chemical Society (ACS) reagent grade (Fisher Chemical, Fairlawn, NJ), solution was added along with 0.5 mL of a saturated potassium iodide (ACS grade, Fisher Chemical, Fairlawn, NJ) solution. The solution was gently mixed and allowed to stand for 1 min before 30 mL of distilled water was added along with approximately 2 mL of a saturated starch solution. The solution was then titrated with a 0.01 N sodium thiosulfate (ACS grade, Fisher Chemical, Fairlawn, NJ) solution until the color changed from dark blue to colorless. The PV was calculated using the equation,

PV = [(mL of titrant)*(0.01)*1000]/(Sample mass).

3.1.4 p-Anisidine Value

p-Anisidine values for the oil samples were determined using AOCS official method Cd 18-90 (2003). Approximately 0.5 g of fish oil was weighed into 25 mL isooctane. The absorbance at 350 nm was measured using a spectrophotometer (DU 520, Beckman Coulter, Inc, Fullerton, CA). Five mL of the fish oil isooctane (ACS reagent grade, Fisher Chemical, Fairlawn, NJ) solution was placed in a test tube along with 1 mL of 0.25 g/100 mL p-anisidine (99 %, ACROS Organics, Morris Plain, NJ) solution in glacial acetic acid. After shaking and resting the mixture for 10 min the absorbance of the mixture was taken again at 350 nm. The AV was calculated using the following formula.

$$AV = [25 * (1.28 * As-Ab)]/m$$

Where; As = absorbance of the oil solution after reaction with the reagent, Ab = absorbance of the initial solution, and m = mass of the sample in g.

3.1.5 Fatty Acid Profile

The fatty acid profile of the fish oil was determined by using AOCS official method Ce 2-66 (2003). A HP 6890 Gas Chromatograph (GC) equipped with a flame ionization detector (FID) (HP Company, Wilmington, DE) was used to analyze the methylated fatty acids. A Supelco SP-2560 fused silica capillary column, 100 m x 0.25 mm x 0.20 µm film thickness (Bellefonte, PA) was used for analysis. Fatty acid standards were purchased from Supelco (Supelco 37 component FAME mix, Supelco, Bellefonte, PA). Helium (He) (Airgas, Tulsa, OK) was used as a carrier gas at a 20 cm/s flow rate. The injector temperature was held at 260 ºC. A temperature program was held at 140 ºC

for 5 min then increased at 4 ºC/min to 240 ºC and was held for 5 min. The detector conditions were maintained at 260 ºC, hydrogen gas flow 40 mL/min, air flow 450 mL/min and make-up gas (He) 45 mL/min. Fatty acid methyl ester samples (1 µL) were injected by an autosampler (HP 7683, HP Company, Wilmington, DE) with a 100:1 split ratio. Peak areas were calculated and data collection was managed using HP Chemstation (Revision. A.09.01, Agilent Technologies, Palo Alto, CA). Fatty acid peaks were identified using the standard FAME mixture. Undecanoic acid (99 % GC grade, Sigma-Aldrich, St Louis, MO) (11:0) was used as an internal standard for quantification.

3.2 MICROCAPSULE PREPARATION

3.2.1 Emulsion Preparation

BiPro whey protein isolate (WPI) containing 97.8 ± 2 % protein was purchased from Davisco Foods International (Eden Prairie, MN). The functional protein groups of the WPI were comprised of beta-lactoglobulin and alpha-lactalbumin. A 20 % (w/w) solution of WPI in de-ionized water was first created. The solution was created at a 20 % concentration due to the fact that higher concentrations were determined to be too viscous for spray drying. A 20 % WPI solution was also shown to be recommended among solutions of 10-30 % based on comparison of microcapsules by Rosenberg and Young (1993). Fish oil was added in a 1:2 ratio of fish oil to WPI by weight. Homogenization was carried out by first creating a course emulsion. A polytron electric homogenizer equipped with a small probe (PowerGen 700, Omni International, Marietta, GA) was used to create a course emulsion by homogenizing the mixture for three two min periods. The fine emulsion was created by using a Misonix Sonicator 3000 sonic probe

(Farmingdale, NY). The course emulsion was exposed to sonic energy for three two min periods, allowing the emulsion to cool in between periods. The emulsion was kept in an ice bath at all times during these processes and reached a maximum temperature of 22 ºC.

3.2.2 Freeze Drying

Following emulsion preparation the emulsions were frozen at -80°C in an ultra-freezer (Bio Freezer 8517, Forma Scientific, Waltham, MA). After 24 h the frozen emulsion was dried for 48 hours at -40 ºC and 100 millitorr (25 Liter Sentry Freezemobile, VirTis Company, Inc, Gardiner, NY). After the drying period the result was a dry matrix of microcapsules. The cross-links between microcapsules were broken by using a coffee grinder (SmartGrind, Black&Decker, Towson, MA) (5 sec grinding periods 5 times shaking) resulting in a free-flowing powder.

3.2.3 Spray Drying with 2-Fluid Pressure Nozzle

A Buchi-290 spray dryer (B-290, Flawil, Switzerland) equipped with a 2-fluid (liquid/gas) Buchi pressure nozzle and in conjunction with the B-296 de-humidifier (B-296, Flawil, Switzerland) equipped with a pre-heat exchanger was used for the microencapsulation experiments. A schematic diagram of the spray drier is shown in Figure 1 along with a picture of the system in Picture 1. The previously prepared emulsion was dried in a nitrogen environment where compressed nitrogen gas (Airgas, Tulsa, OK) is circulated through the spray dryer. The evaporated moisture was passed through the de-humidifier and condensed into a collection bottle. Figure 3 shows a schematic drawing of nitrogen flow through the spray drier, heat exchanger, and

dehumidifier. Inlet temperature of nitrogen gas was 180 °C. Outlet temperature, which is dependent on inlet temperature, was 90 ± 2 °C. The emulsion was delivered to the nozzle via a peristaltic pump at 10 % speed (2.75 ml/min).

3.2.4 Spray Drying with 3-Fluid Pressure Nozzle

The B-290 spray dryer was equipped with a 3-fluid (gas/liquid/liquid) pressure nozzle (Buchi 46555, Flawil, Switzerland). Figure 3 shows the design of the 3-fluid nozzle. Whey protein solution and the fish oil were pumped to the nozzle via a peristaltic and a syringe pump (12-05126, Sono-Tek, Milton, NY), respectively. The WPI solution was 20 % solids (w/w) in water. The pump rate of the peristaltic pump was 10 % (2.75ml/min). The ratio of fish oil to WPI was 1: 2 (w/w). Fish oil density was taken as 0.930 g/mL, according to the supplier, for conversion of fish oil volume to weight. As the previous experiments with 2-fluid nozzle the atomized microcapsules were dried in a nitrogen environment. During these experiments oil, wall material and gas flow in separate channels and did not mix until they met at the tip of the nozzle and atomized.

3.2.5 Spray Drying with 2-Channel Ultrasonic Nozzle

For these experiments the B-290 spray dryer was equipped with a 2-liquid channel 120 kilohertz ultrasonic atomizing nozzle (Sono-Tek, Milton, NY). A schematic diagram of the nozzle design is shown in Figure 4. The sonic nozzle was powered with a Broad Ultrasonic Generator (Sono-Tek 06-05108, Milton, NY) at a setting of 5.0 watts. The experimental conditions were the same as described in the previous paragraph. Similar to the experiments described in the previous section, oil and wall material flowed

in separate channels and did not mix until they met at the tip of the nozzle and were atomized. In these experiments atomization did not require gas pressure. The sonication was used for this purpose. Nitrogen gas was circulated through the nozzle to help keep the nozzle cool. Thermocouple readings indicated that the nozzle reached a maximum temperature of 50 ºC during the drying process.

3.3 MOISTURE CONTENT

A Karl Fischer titrator (758 KFD Titrino with 703Ti stand, Metrohm USA, Inc, Riverview, FL) was used to determine the moisture content of dried powder samples and the fish oil. The instrument was calibrated using water. For the fish oil approximately 3 g was used as the sample size. For dried powder samples 0.4 g was the sample size used. Samples were dispersed in Hydranal-Solvent CM (Sigma-Aldrich, St. Louis, MO) and titrated with Hydranal-Titrant 2 (Sigma-Aldrich, St. Louis, MO).

3.4 WATER ACTIVITY

Water activity of the samples was measured by using an AquaLab Water Activity Meter at 25 ºC (Series 3, Decagon Devices, Inc Pullman,WA).

3.5 TOTAL OIL

3.5.1 Soxtec Extraction

A Soxtec oil extraction unit (Tecator, Model 1043 Extraction Unit, Sweden) was used to extract the total oil from the microcapsules. Approximately 2 g of sample was weighed into extraction thimbles and mixed with Celite 545 (EMD Chemicals, Inc,

Gibbstown, NJ). The thimbles were loaded onto the instrument along with pre-weighed cups containing 40 mL of ACS reagent grade petroleum ether (Fisher Chemical, Fairlawn, NJ). The thimbles lowered into the boiling position for 10 minutes then raised into the rinse position for 20 minutes. The cups were removed and any residual petroleum ether was dried away. The difference in weight of the cups with and without oil was recorded as oil extracted. The thimbles were also dried. Once dried the remaining sample was ground in with a mortar and pestle and placed back into the extraction thimbles for a second extraction. The oil extracted from both extractions was added to equal the total oil.

3.5.2 Solvent Extraction

A second method for total oil was used to confirm the results obtained by Soxtec extraction. Solvent extraction of total oil was done based on the Rose-Gottllieb method (GEA Niro Method A 9a). Two grams of encapsulated oil sample was weighed out into a flask. Twenty ml of water was added to disperse the sample. Then the solution was placed in a water bath for 15 min at 60ºC, shaking occasionally. The mixture was then cooled and 25 ml of petroleum ether was added and mixed for 10 min. The mixture then was allowed to stand for at least 2 h until the ether phase was clear and a clear phase separation was observed between water and petroleum layers. The ether phase was then transferred into an Erlenmeyer flask and this extraction was performed two more times using the same flask of water phase. After the final transfer the ether was evaporated using a Rapid-Vap© vacuum system (Model 7900002, Labconco, Kansas City, MO). The flask was allowed to cool under vacuum and weighed.

$$\text{Total Oil (\%, w/w)} = (W1*100)/W2$$

W1 = weight in g of the evaporation residue.

W2 = weight in g of the power used.

3.6 SURFACE EXTRACTABLE OIL

Surface extractable oil fraction also known as the accessible or non-encapsulated oil was determined (Modified – GEA Niro Method A 10a). Two grams of encapsulated oil sample were placed into a 25 ml flask. Ten ml of petroleum-ether was added. The flask was closed and placed in a shaking device. The stirring speed was regulated so that the powder was moving but not splashing up on the upper sides of the flask. After 15 minutes shaking was stopped and the solution was filtered into a pre-weighed glass beaker and washed 2 more times with 10 ml petroleum-ether. Petroleum ether was completely evaporated from the filtrate under vacuum at 45ºC (Rapid-Vap© vacuum system 7900002, Labconco, Kansas City, MO).

Free oil (%, w/w) = (a*100)/(b)

a = weight of residue in the flask in grams.

b = grams of powder used.

The following equation was used to calculate microencapsulation efficiency (MEE) using the determined total oil and the surface extractable oil (Jimenez 2004).

MEE = [(total oil-extractable oil)*100]/total oil

3.7 PARTICLE SIZE ANALYSIS

A Malvern High Performance Particle Sizer (HPPS 5001, Malvern Instruments,

Ltd, Worcestershire, United Kingdom) was used to determine the average size of the microcapsules. Dried microcapsules were dispersed into inert silicone oil (silicone fluid 350 “100 % pure silicone,” Clearco Products Co, Bensalem,PA) for the analysis. The particle size analyzer performed 20 scans per sample and displayed an average diameter value.

3.8 BULK DENSITY

The bulk density of the dried powders was calculated by measuring the weight of 15 mL of non-compacted powder in a pre-weighed tube. The weight of the sample was divided by the volume to equal g/mL.

3.9 SURFACE MORPHOLOGY ANALYSIS

A scanning electron microscope (FEI Quanta 600, FEI Company, Hillsboro, OR) was used to analyze the surface morphology of the dried powders. Digital images where obtained at three magnifications, 1000, 5000, and 30,000.

3.10 OXIDATIVE STABILITY

3.10.1 Sample Storage

Approximately 0.5 g of dried microcapsules were weighed into 2 by 4 inch foil/poly bags (Sigma-Aldrich, Inc, St Louis, MO) and vacuum sealed (Ultravac 250, KOCH Supplies, Inc, Kansas City, MO). Picture 3 shows a foil/poly bag which were selected because of their ability to protect samples from light under vacuum. Then half of the vacuum sealed samples were stored in a refrigerator at 5 ºC and the other half in a

freezer at -18 ºC. In addition two 250 mL amber bottles of fish oil with nitrogen filled head space were wrapped in foil and were stored at the same two temperatures as the encapsulated samples.

3.10.2 Head Space Analysis

Two frozen and two refrigerated samples were removed from the storage every seven days and allowed to reach room temperature. Foil/poly bags were cut and 0.4 ± E-4 g of dried sample was weighed into 4 mL amber head space vials. Similarly 1.86 g of fish oil was weighed into 4 mL amber vials. Then 20 µL of 100 mg/L heptanoic acid ethyl ester (99 % GC grade, Sigma-Aldrich, Inc, St Louis, MO) was added into the sample vials as an internal standard. The head space vials were then placed on a 60 ºC heating block. The needle of a 75 µm Carboxen-Polydimethylsiloxane (CAR-PDMS) solid phase microextraction fiber assembly (Sigma-Aldrich, Inc, St Louis, MO) loaded in a manual holder was used to pierce the septum of the vial. The CAR-PDMS fiber was then exposed to the head space above the sample for 45 minutes. Then the fiber was retracted back into the needle. Figure 5 shows a drawing of the extraction process. Immediately following volatile extraction the assembly was manually inserted at the GC injection site set at 280 ºC. The GC oven method was started manually as the CAR-PDMS fiber was being exposed. The fiber was left in the injection site for 5 minutes before being retracted and removed. The samples were analyzed every 7 days in replicate over a 15 week period starting with the initial measurements immediately following microencapsulation.

Volatile compounds of the samples were analyzed by using a HP 6890 Series GC system equipped with an Agilent 5973N mass spectrometer (MS) (Palo Alto, CA). A

fused silica capillary Equity-5 column (30 m x 0.25 mm x 0.5 µm film thickness) from Supelco was used for the analysis. The oven temperature program started at 40 °C held for 5 minutes then increased at a rate of 3 °C/min to 9 °C, then 2°C/min to 110 °C, 10 °C/min to 200 °C, 20 °C/min to 240 °C and held for 3 minutes at this temperature. Helium was used as carrier gas at a flow rate of 1.0 mL/min. The inlet temperature was 280 °C. The samples were injected manually into the GC by a HS-SPME manual holder. The inlet was in splitless mode for the first minute before increasing to a split ratio of 100:1. The data collection and analysis were managed using an HP Chemstation (Rev. B.01.03 [204], Agilent Technologies, and Palo Alto, CA).

The peaks on the GC chromatograms were identified by using the MS spectral library (NIST/EPA/NIH Mass Spectral Library, Version 2, Gaithersburg, MD). Peak area ratio to internal standard was used to calculate semi-quantitative concentrations for comparison.

3.11 STATISTICAL ANALYSIS

All analytical tests were carried out at least in duplicate and in randomized order with the mean values being reported. Analysis of variance (ANOVA) of the results was performed using the Least Significant Differences (LSD) procedure of SAS for Windows (Software Version 9.1. SAS Institute Inc., Cary, NC).

CHAPTER IV

RESULSTS AND DISCUSSION

4.1 FISH OIL PROPERTIES

FFAs form as a result of the hydrolysis of triacylglycerides. FFAs often lead to undesirable flavor changes of oils (Barthet and others 2008). The fish oil used in this thesis was specified to have a FFA content of 0.06-0.10 % (w/w) by the supplier (Table 1). The actual FFA content of the oil determined in our laboratory was 0.062 % (w/w) which was within the limit declared by the supplier (Table 1).

PV measurements are conducted in order to determine the hydroperoxides or primary oxidation products in oils. The AOCS method used to measure the PV of the fish oil in this study expresses the amount of peroxides present in oil in meq/kg (Kulus and Ackman 2001). The acceptable range for PV set by the fish oil provider was 0-3 meq/kg. Our laboratory tests confirmed that PV of the fish oil, 0.43 meq/kg, was within the acceptable range (Table1).

AV represents the amount of secondary oxidation products in oil. Secondary oxidation products are formed by decomposition of primary oxidation products such as hydroperoxides. The AV range specified by the provider of the fish oil used in this study was in the range of 3-9.5. The actual AV was determined to be 7.52, which was close to the higher end of the range declared by the supplier (Table 1). Monthly PV and AV tests carried out on the fish oil did not show significant change indicating that quality of the

original product was maintained in the storage throughout the study.

PUFAs consisted of 25.8 % (w/w) of the total fatty acids in the fish oil (Table 1). This value was lower than the range specified by the provider of the fish oil (28-32%, w/w). EPA and DHA showed values of 13.6 % and 10.8 % of the total fatty acids, respectively. The EPA value was beyond the range designated (8-12%), while DHA was within the specified range. Fish oil was also rich in palmitic (18.3 %, w/w), palimitoleic (11.3%) and stearic acid (9.5%) (Table 2). The overall fatty acid composition was found to be in agreement with values published by Firestone (1999). As expected, moisture content of the original oil used for the encapsulation tests was low, 600 mg/kg (Table 2).

4.2 CHEMICAL AND PHYSICAL CHARACTERISTICS OF MICROCAPSULES

Fish oil was encapsulated in WPI because of the reported health benefits. WPI has been shown to be an ideal protein supplement for increasing lean muscle while at the same time helping to reduce fat in humans (Frestedt and others 2007). It is expected that fish oil encapsulated in WPI will deliver health benefits of both fish oil and WPI while delivering a product with extended shelf life. Furthermore, use of WPI eliminates the requirement for addition of a surface active ingredient to form an emulsion prior to generation of microcapsules because of the emulsifying properties of proteins. Moreau and Rosenberg (1993) stated that WPI exhibited effective microencapsulation properties.

4.2.1 Moisture Content

Moisture content of powders is an important parameter since high moisture may lead to caking/agglomeration of particles and promote microbial growth. Moisture

content of microcapsules and WPI used as wall material are shown in Table 3. The moisture content specified by the WPI supplier was 5% (w/w). However, our laboratory tests showed a higher moisture content, 7.7% (w/w), for this product. Higher moisture of the product might be due to moisture absorption during storage. The food industry has specified that dried powders should have moisture content between 3% and 4% (Masters 1991). The moisture content of microcapsules produced by spray drying with 2-fluid pressure nozzle was well below the specified maximum. Microcapsules produced by freeze drying and spray drying with the ultrasonic nozzle were near the 4% maximum while the 3-fluid pressure nozzle microcapsules were higher than the 4% maximum. Although there were statistically significant differences in moisture content of encapsulated products the variations were not large for practical purposes.

4.2.2 Water Activity

Water activity is the ratio of the vapor pressure of water in a material to the vapor pressure of pure water at the same temperature. Water activity (a_w) is one of the most critical factors in determining quality and safety of the foods. Water activity affects the shelf life, safety, texture, flavor, and smell of foods. Water activity was determined for the four microcapsule products along with WPI and fish oil (Table 4). As expected all the samples had low water activity, < 1. Our findings were in agreement with the literature (Klinkesorn and others 2005). Statistical analysis of the results indicated significant differences among samples. However, variations were not large to cause any concern for food applications.

4.2.3 Total Oil

Total oil describes the percent of oil that makes up the dried powder. Two oil extraction methods were used to determine total oil content in microcapsules (Table 5). The values determined by two different extraction methods were not significantly different. Among the 4 microcapsule products total oil content in microcapsules obtained by using the ultrasonic nozzle (about 28%, w/w) was significantly lower than that of other 3 microcapsule products. According to the literature oil content of microcapsules may vary between 20% and 50% (Drusch and Schwarz 2006). All the microencapsulated products examined in this study were within the range reported in the latter study.

4.2.4 Surface Extractable Oil

The surface extractable oil or “free oil” is often defined as the oil that may be extracted with organic solvents from the surface of unbroken microcapsules (Buma 1971, Sankarikutty and others 1988). The values in this study were determined to be significantly different among all 4 products (Table 6). The pressure nozzle with 2-fluid channels gave the best results with lowest surface oil, 2.6% while capsules prepared by using the sonication nozzle had the highest amount of surface oil, 6.8%. In general free oil contents of the microcapsules examined in this study were similar or lower than the values reported in the literature (Heinzelmann and others 2000).

4.2.5 Microencapsulation Efficiency

MEE is calculated to determine the amount of oil that was successfully encapsulated based on the values obtained for total oil and surface extractable oil.

Previous work has shown that the MEE can be directly affected by the materials and process used for production of microcapsules with efficiencies ranging from 0% to 95% (Baik and others 2004, Klinkesorn and others 2006, Hardas and others 2000, Heinzelmann and others 2000, Hogan and others 2001, Lin and others 1995, Velasco and others 2000). The values calculated for this study are displayed in Table 7. There were significant differences among MEE of all the products examined in this study. The 2-fluid nozzle had the highest (91.6%) while the ultrasonic nozzle had the lowest efficiency (76%). We believe that this was due to inconsistent function of the ultrasonic nozzle. It was observed that ultrasonic nozzle plugged frequently and atomization was not uniform throughout the drying process. Low encapsulation can be attributed improper nozzle function.

4.3 PARTICLE SIZE ANALYSIS

The average particle size diameter of the microcapsules was determined (Table 8). Freeze dried microcapsules had the largest average particle size at 56.2 µm. Microcapsules produced with the 2-fluid pressure nozzle were the smallest at 7.3 µm followed by the ultrasonic nozzle and 3-fluid pressure nozzle at 11.3 and 12.0 µm, respectively. A plot of % intensity versus diameter helps show the size distribution around the average (Figure 6). The graph indicates that the ultrasonic nozzle microcapsules had the most narrow size distribution, followed by the 2-fluid nozzle, 3-fluid nozzle, and freeze dried microcapsules. This finding supports the hypothesis that the ultrasonic nozzle produces more uniform particle size than the other nozzle types.

4.4 BULK DENSITY

Bulk density is a very important parameter to characterize food powders. It may vary with water content in product, and is dependent on the rate of shrinkage, which in turn is strongly affected by the drying method (Van Arsdel and Copley 1964). Spray dried products have to meet bulk density targets to provide consistent weight during packaging. Bulk density was determined for each of the dried products (Table 9). The bulk density determined for the powders was considered unpacked or aerated, because samples were not compacted. Bulk density of all the microcapsules was lower than that of the WPI. As expected freeze dried samples had the lowest bulk density followed by capsules produced with ultrasonic nozzle. There was no significant difference in bulk density of microcapsules produced by ultrasonic and 3-flluid nozzles.

4.5 SURFACE MORPHOLOGY

The surface morphology of the samples was observed by SEM. Pictures 4 through 15 display images captured for each dried microcapsule product at the same three magnifications (1000, 5000, and 30,000).

Freeze dried microcapsules were irregular in shape and lacked uniformity having a wide frequency of microcapsule sizes (Pictures 4-6). The surface of freeze dried capsules revealed fairly large surface cracks at higher magnifications. The microcapsules produced with the 2-fluid nozzle were more uniform in shape being round with some surface dents (Pictures 7-9) which may be associated with mid-air collisions during the spray drying process and improper atomization and drying rate (moisture rate). Moreau and Rosenberg (1993) have also observed that some surface dents are present on the

surface of whey derived spray dried powders that have been attributed to improper atomization and drying rate. Although it was less than the freeze dried samples, the 2-fluid nozzle microcapsules also showed wide particle size distribution lacking complete uniformity. The surface of the 2-fluid nozzle microcapsules did not reveal any observed surface cracks.

Microcapsules produced from the 3-fluid nozzle revealed round and some irregular shapes with surface dents (Pictures 10-12). Although the microcapsules were not completely uniform the frequency of larger to smaller capsules was observed to be less for 3-fluid nozzle in comparison to 2-fluid nozzle. The surface of the 3-fluid nozzle microcapsules did not reveal any observed surface cracks. There were, however, creases and blisters observed on the surface.

The ultrasonic nozzle produced round microcapsules with some irregular shapes (Pictures 13-15). It is plausible that irregularly shaped particles were formed when atomization was intermittent due to plugging of the nozzle during the drying process. However, as mentioned earlier the ultrasonic nozzle showed a significantly narrower particle size distribution than the other nozzles. Observations at higher magnitude revealed some small surface cracks on ultrasonic nozzle microcapsules (Picture 15).

4.6 OXIDATIVE STABILITY ANALYSIS

Oxidation of fish oil forms objectionable flavors and aromas and decreases the PUFA content. During oxidation primary and secondary oxidation products are formed. Secondary oxidation products include aldehydes, ketones, alcohols and hydrocarbons. Secondary oxidation products negatively influence the flavor and aroma of oxidized

lipids (Jacobsen 1999).

In this study relative quantity of volatile oxidation products in microcapsules and fish oil were compared by taking the ratio of the peak area of the compound of interest to the area of the internal standard (PAR). This method was similar to the method used by Jonsdottir and others (2005).

Propanal was selected for a direct comparison of a volatile oxidation product among the four microcapsules and fish oil as a control because it was the prominent peak present in each sample. In addition, propanal is a volatile compound associated with oxidation of EPA and DHA (Iglesias and others 2007). Tables 10 and 11 display the peak area ratio (PAR) for propanal over a 15 week period in the two storage conditions. The two storage conditions were common refrigerated (5 ºC) and freezing (-18 ºC) storage temperatures. These conditions were selected in order to simulate a consumer storage environment. Figures 6 and 7 plot the changes in propanal levels over the storage period at the two storage conditions.

Initial measurements (week 0) for propanal in microcapsules were indicators of oil degradation resulting from the production method. No propanal was detected in fish oil for two weeks when it was stored at 5^{o}C. However, some propanal was found in encapsulated products at week 0. These results were expected considering that fish oil samples did not go through the drying process and stored under nitrogen away from light at refrigerated conditions. Variations among initial PAR values for encapsulated products were not statistically significant.

The fish oil stored at 5 ºC began showing large increases in propanal levels between week 8 and 9. This would indicate that the fish oil began to lose stability at that

point. Propanal levels in all microencapsulated products were higher than the fish oil control up to 9th week (Table 10 and 11). Freeze dried microcapsules had an increase in propanal levels from week 8 to 9 similar to the fish oil control. An increase in propanal levels in sprayed dried products was observed a week later (week 9 to 10) than the freeze dried product. This increase in propanal levels may be due to oxidation of surface oil on encapsulated materials or release of volatiles that resulted from oxidation during the encapsulation process. Increase in propanal levels in fish oil and freeze dried samples continued for the rest of the stability tests. A slight decrease in PAR values was observed for the products obtained with 2 and 3-fluid nozzles. Propanal levels in products encapsulated with sonic nozzle remained steady during the rest of the study.

With regard to the -18 ºC stored samples no propanal was observed in fish oil until the third week. Slightly larger PAR values were observed towards the end of the study. In general PAR values for the samples stored at -18 ºC were lower than that for stored at 5 ^{o}C indicating the positive effect of lower temperatures on fish oil stability. The PAR data for all the samples indicate that the product quality was maintained fairly well throughout the study because of the very conservative storage conditions (low temperature, vacuum packaging or inert atmosphere for fish oil and no light exposure). Hence, no apparent differences in oxidative stability among samples were identified.

The other major compounds identified besides propanal were made up of aldehydes, alcohols, ketones, acids, and a large variety of hydrocarbons. Tables 12 through 21 display the most prevalent peaks for each sample over the storage period at each storage condition.

Fish oil was found to have prevalent peaks identified as 2-propenal, butanal,

formic acid, 2-ethyl-furan, and 1-penten-3-ol (Tables 20 and 21). These volatile compounds have been shown to be results of secondary oxidation of hydroperoxides (Lee and others 2003). 2-Propenal was the only volatile compound detected in fish oil durinSimilar to propanal levels of the 5 ºC stored sample these compounds remained at a steady level until a large increase from week 8 to 9.

CHAPTER VI

CONCLUSIONS

To the best of our knowledge this is the first study to compare microencapsulation technologies that utilize multiple fluid delivery and sonic energy with traditional spray and freeze dried emulsion microencapsulation methods. Comparison of chemical and physical characteristics revealed that ultrasonic did have one advantage. With regard to uniformity of size and shape, microcapsules produced by the 2-channel ultrasonic nozzle were observed to be more uniform in size and shape, determined by particle size distribution and SEM image comparisons. Disadvantages were also observed for ultrasonic nozzle microcapsules having lower oil encapsulating efficiency compared to pressure nozzles and freeze dried microcapsules at the same core to wall ratio. This may be due to frequent nozzle clogging during the microencapsulation process.

There was no observed initial advantage to spray methods that did not require the creation of an emulsion for microcapsule production, with regard to propanal as an indicator of oxidative stability. However, it was observed that microcapsules produced by multi-fluid nozzles propanal levels were lower throughout the course of a 14 week stability test. It should be stated that the 15 week sampling period may not have been long enough to adequately observe the induction of oxidation for all samples stored in the conditions chosen. Sample values fluctuated within a moderate range throughout the entire study. However, by observing propanal levels at the last data point of the stability

study some conclusions may be drawn. At the end of the 5 ºC test PAR levels for fish oil and freeze dried samples were observed to have a significant increase while spray dried samples values remained steady. This may indicate the beginning of oxidation among the fish oil and freeze dried samples while spray dried samples were remaining stable.

Among the microcapsule samples storage temperature did have an apparent affect on the PAR levels. With the low temperature samples having smaller PAR values. Again in many cases PAR values had increases relative to the corresponding propanal plot.

One noticeable difference between the fish oil control and the microcapsule samples was the presence of a variety of prevalent hydrocarbon peaks in the microcapsule samples. Hydrocarbons (alkanes and alkenes) result from free fatty acids and are precursors to the aldehydes, alcohols, and ketones associated with oxidation of fish oil.

FUTURE RESEARCH

Based on the observations and conclusions of this study there is still a need to study the potential for utilizing newer spray drying technologies for microencapsulation of fish oil. While the core to wall ratio was held constant in order to maintain comparative solids in the final products of this study. Optimization of core to wall solids needs to be investigated in order to increase microencapsulation efficiency for ultrasonic nozzles. Investigations of other wall materials and combinations of wall materials may reveal wall systems that are better suited for spray nozzles that encapsulate core materials at the point of atomization. A longer shelf life study on the microencapsulated fish oil samples produced by using different production techniques and nozzle designs is needed for better understanding of the effectiveness of these techniques to protect fish oil from oxidation.

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TABLES

Table 1: Chemical characteristics of the fish oil used for microencapsulation experiments

Parameter	Range Provided by Producer	Value Determined
FFA (%, w/w)	0.06-0.10	0.06 ± 0.01
PV (meq/kg)	0-3	0.4 ± 0.3
AV	3-9.5	7.5 ± 3E-4
EPA (%, w/w)	8-12	13.6 ± 0.2
DHA (%, w/w)	8-12	10.8 ± 0.2
EPA + DHA (%, w/w)	20-22	24.5
TOTAL Omega-3 (%, w/w)	28-32	25.8

Table 2: Fatty acid composition of fish oil used for encapsulation experiments

Fatty Acid	**(%, w/w)**
Myristic acid (C14:0)	8.05 ± 0.11
Palmitic acid (C16:0)	18.31 ± 0.26
Palmitoleic acid (C16:1)	11.30 ± 0.19
Stearic Acid (C18:0)	9.52 ± 0.28
Oleic acid (C18:1n9c)	3.37 ± 0.06
α-linolenic acid (C18:3n3)	**1.34 ± 0.02**
cis-13, 16 Docosadienoic acid (C22:2)	1.80 ± 0.03
Eicosapentaenoic acid (C20:5n3)	**13.64 ± 0.24**
Docosahexaenoic acid (C22:6n3)	**10.84 ± 0.19**

Table 3: Moisture content of the samples determined by Karl Fischer Titration

Sample	Moisture* (%, w/w)
Fish Oil	$0.06 \pm 4E\text{-}4^{a}$
Whey Protein Isolate	7.7 ± 0.2^{b}
Freeze Dried	4.5 ± 0.2^{c}
2-Fluid Pressure Nozzle	2.7 ± 0.03^{d}
3-Fluid Pressure Nozzle	5.3 ± 0.1^{e}
Ultrasonic Nozzle	4.2 ± 0.08^{f}

* Means with the same letter are not significantly different ($P > 0.05$).

Table 4: Water Activity of the samples

Sample	Aw*
Fish Oil	0.57 ± 0.01^{a}
Whey Protein Isolate	$0.25 \pm 6E\text{-}4^{b}$
Freeze Dried	$0.21 \pm 3E\text{-}3^{c}$
2-Fluid Pressure Nozzle	$0.15 \pm 3E\text{-}3^{d}$
3-Fluid Pressure Nozzle	$0.20 \pm 6E\text{-}4^{c}$
Ultrasonic Nozzle	$0.15 \pm 1E\text{-}3^{d}$

* Means with the same letter are not significantly different ($P > 0.05$).

Table 5: Total Oil content of microcapsules

Sample	Soxtec Extraction Oil* (%, w/w)	Rose-Gottlieb Solvent Extraction Oil (%, w/w)
Freeze Dried	31.9 ± 1.1^{a}	31.7 ± 0.5^{a}
2-Fluid Pressure Nozzle	31.3 ± 0.6^{a}	31.0 ± 0.6^{a}
3-Fluid Pressure Nozzle	31.3 ± 0.8^{a}	30.8 ± 0.4^{a}
Ultrasonic Nozzle	28.5 ± 0.9^{b}	28.0 ± 1^{b}

* Means with the same letter are not significantly different ($P > 0.05$).

Table 6: Surface oil content of microcapsules

Sample	Oil* (%, w/w)
Freeze Dried	5.3 ± 0.08^{a}
2-Fluid Pressure Nozzle	2.6 ± 0.03^{b}
3-Fluid Pressure Nozzle	4.4 ± 0.11^{c}
Ultrasonic Nozzle	6.8 ± 0.07^{d}

* Means with the same letter are not significantly different ($P > 0.05$).

Table 7: Microencapsulation efficiency (MEE) of different encapsulation techniques

Sample	MEE (%)*
Freeze Dried	83.3 ± 0.1^{a}
2-Fluid Pressure Nozzle	91.6 ± 0.1^{b}
3-Fluid Pressure Nozzle	85.8 ± 0.08^{c}
Ultrasonic Nozzle	76.1 ± 0.5^{d}

* Means with the same letter are not significantly different ($P > 0.05$).

Table 8: Average particle size of microcapsules analyzed by Malvern High Performance Particle Sizer

Sample	Average Diameter (µm)
Freeze Dried	56.2
2-Fluid Pressure Nozzle	7.3
3-Fluid Pressure Nozzle	12.0
Ultrasonic Nozzle	11.3

Table 9: Bulk Density of microcapsules

Sample	g/mL*
Freeze Dried	0.18 ± 0.01^{d}
2-Fluid Pressure Nozzle	$0.20 \pm 3.0E\text{-}03^{c}$
3-Fluid Pressure Nozzle	0.25 ± 0.01^{ab}
Ultrasonic Nozzle	0.24 ± 0.01^{b}
Whey Protein Isolate	0.26 ± 0.01^{a}

* Means with the same letter are not significantly different ($P > 0.05$).

Table 10: Samples stored at 5 ºC Propanal Peak Area Ratio (PAR) over 15 weeks.

Week	Freeze Dried	2-Fluid Nozzle	3-Fluid Nozzle	SONIC Nozzle	Fish Oil
0**	0.9 ± 0.4^{a}	1.6 ± 0.3^{a}	2.3 ± 1.5^{a}	3.0 ± 0.02^{a}	*
1**	6.4 ± 0.7^{a}	3.1 ± 0.7^{b}	2.1 ± 0.4^{b}	3.1 ± 0.1^{b}	*
2**	6.3 ± 0.8^{a}	7.5 ± 0.3^{a}	6.1 ± 1.1^{a}	2.3 ± 0.1^{b}	0.6 ± 0.4^{b}
3**	11.0 ± 0.6^{a}	7.7 ± 3.2^{b}	6.5 ± 1.1^{b}	5.5 ± 3.1^{c}	0.4 ± 0.1^{d}
4**	9.5 ± 0.2^{a}	7.3 ± 1.9^{ab}	6.8 ± 2.1^{ab}	3.7 ± 1.2^{bc}	0.9 ± 0.2^{c}
5**	6.9 ± 0.1^{a}	6.1 ± 0.8^{ab}	3.9 ± 0.7^{c}	5.6 ± 0.5^{bc}	1.2 ± 0.4^{d}
6**	4.9 ± 0.1^{ab}	6.0 ± 1.1^{a}	2.6 ± 1.3^{c}	3.1 ± 0.4^{bc}	0.4 ± 0.07^{d}
7**	7.8 ± 0.9^{a}	7.1 ± 0.8^{a}	2.1 ± 0.1^{bc}	3.3 ± 0.4^{b}	0.7 ± 0.3^{c}
8**	5.1 ± 1.3^{ab}	5.7 ± 2.2^{a}	1.9 ± 0.3^{bc}	2.8 ± 1.2^{abc}	1.2 ± 0.5^{c}
9**	13.0 ± 0.5^{a}	4.8 ± 2.1^{b}	1.6 ± 0.3^{c}	1.6 ± 0.2^{c}	6.4 ± 0.6^{b}
10**	13.4 ± 0.2^{ab}	14.8 ± 3.0^{a}	12.6 ± 0.9^{ab}	8.1 ± 2.3^{b}	8.7 ± 2.8^{b}
11**	13.3 ± 2.4^{ab}	14.1 ± 4.1^{a}	8.6 ± 1.3^{abc}	6.3 ± 0.02^{c}	7.6 ± 0.2^{bc}
12**	12.7 ± 0.1^{a}	6.8 ± 1.4^{b}	5.7 ± 0.8^{b}	7.7 ± 0.4^{b}	11.2 ± 2.6^{a}
13**	14.2 ± 0.4^{a}	12.6 ± 0.4^{b}	5.9 ± 0.1^{d}	7.6 ± 0.5^{c}	14.3 ± 0.5^{a}
14**	17.4 ± 0.2^{a}	7.0 ± 1.9^{b}	7.0 ± 0.5^{b}	8.4 ± 0.2^{b}	16.4 ± 2.3^{a}

* Compound not detected.

** Means with the same letter in the same row are not significantly different ($P > 0.05$).

Table 11: Samples stored at -18ºC Propanal Peak Area Ratio (PAR) over 15 weeks.

Week	Freeze Dried	2-Fluid Nozzle	3-Fluid Nozzle	SONIC Nozzle	Fish Oil
0**	0.9 ± 0.4^{a}	1.6 ± 0.3^{a}	2.3 ± 1.5^{a}	3.0 ± 0.02^{a}	*
1**	3.7 ± 0.1^{a}	1.8 ± 0.8^{b}	2.0 ± 0.3^{b}	2.9 ± 0.3^{ab}	*
2**	8.1 ± 0.5^{a}	2.1 ± 0.3^{b}	3.4 ± 0.9^{b}	1.9 ± 0.01^{b}	*
3**	12.7 ± 0.02^{a}	1.7 ± 0.1^{c}	1.9 ± 0.7^{c}	5.1 ± 2.6^{b}	3.9 ± 0.2^{ab}
4**	9.9 ± 0.3^{a}	6.5 ± 0.8^{ab}	4.4 ± 0.8^{bc}	6.9 ± 3.5^{ab}	2.1 ± 1.02^{c}
5**	6.3 ± 1.9^{a}	7.4 ± 0.7^{a}	4.9 ± 1.4^{ab}	4.6 ± 0.2^{ab}	2.3 ± 0.9^{b}
6**	6.3 ± 1.7^{a}	4.2 ± 1.3^{ab}	2.6 ± 0.8^{bc}	2.5 ± 0.7^{bc}	0.7 ± 0.3^{c}
7**	5.3 ± 0.16^{a}	3.7 ± 0.6^{ab}	1.2 ± 0.1^{b}	3.7 ± 2.5^{ab}	2.0 ± 0.9^{b}
8**	4.5 ± 1.1^{a}	4.1 ± 0.6^{a}	1.4 ± 0.5^{b}	1.7 ± 0.4^{b}	1.2 ± 0.7^{b}
9**	15.4 ± 1.3^{a}	4.2 ± 0.5^{b}	1.9 ± 0.02^{b}	1.9 ± 0.4^{b}	4.5 ± 2.2^{b}
10**	14.4 ± 0.9^{a}	16.1 ± 0.7^{a}	10.9 ± 0.02^{b}	8.6 ± 1.1^{c}	3.1 ± 0.1^{d}
11**	17.8 ± 0.1^{a}	10.5 ± 0.4^{b}	7.3 ± 0.4^{c}	6.2 ± 0.03^{c}	3.2 ± 2.1^{d}
12**	12.9 ± 0.1^{a}	8.5 ± 0.7^{b}	5.3 ± 0.8^{cd}	4.5 ± 1.0^{d}	6.4 ± 0.3^{c}
13**	12.5 ± 0.4^{a}	11.2 ± 1.7^{ab}	6.2 ± 1.3^{c}	7.5 ± 1.4^{bc}	11.1 ± 3.3^{ab}
14**	7.9 ± 0.2^{ab}	11.4 ± 1.7^{a}	5.8 ± 0.7^{b}	7.2 ± 0.4^{ab}	7.2 ± 3.5^{ab}

* Compound not detected.

** Means with the same letter in the same row are not significantly different ($P > 0.05$).

Table 12: PAR of volatile compounds detected for Freeze Dried Microcapsules stored at 5 ºC over 15 weeks.

	Freeze Dried 5 ºC							
Wk	Butanal	1-Penten-3-ol	Hexanal	2,5-dimethyl-Tridecane	Octane, 2,6-dimethyl-	Dodecane, 3-methyl-	Undecane, 3,6-dimethyl-	1,3-Cyclopentanedione, 4-(3-methyl butyl)-
0	*	*	*	*	*		*	*
1	*	*	*	4.4 ± 1.0	23.3 ± 3.3	27.8 ± 5.9	5.2 ± 0.8	3.9 ± 0.3
2	*	*	*	5.5 ± 0.2	34.5 ± 2.3	35.9 ± 2.8	7.5 ± 0.9	7.5 ± 0.9
3	5.6 ± 0.1	1.4 ± 0.3	4.0 ± 0.9	22.0 ± 0.6	101.0 ± 1.0	102.0 ± 4.9	17.0 ± 1.4	15.0 ± 1.4
4	4.2 ± 0.1	0.9 ± 0.03	2.4 ± 0.3	12.0 ± 0.04	69.5 ± 0.2	77.0 ± 0.7	14.0 ± 0.6	15.0 ± 1.3
5	1.9 ± 0.2	1.9 ± 0.2	2.2 ± 0.3	8.0 ± 1.6	46.4 ± 9.1	52.7 ± 9.5	12.0 ± 2.7	17.0 ± 5.2
6	0.9 ± 0.1	0.6 ± 0.3	2.2 ± 1.1	7.5 ± 3.1	31.0 ± 2.9	35.1 ± 1.8	8.9 ± 0.03	18.0 ± 3.0
7	1.7 ± 0.1	1.0 ± 0.2	2.7 ± 0.1	6.2 ± 0.2	40.4 ± 0.2	38.6 ± 1.1	10.0 ± 0.02	18.0 ± 0.1
8	2.3 ± 0.7	1.0 ± 0.4	2.8 ± 1.3	4.7 ± 0.9	28.1 ± 5.9	29.8 ± 6.0	8.3 ± 1.8	21.0 ± 6.8
9	6.6 ± 0.7	1.5 ± 0.7	3.2 ± 0.8	21.0 ± 0.9	117.0 ± 4.6	127.0 ± 0.2	23.0 ± 0.9	25.0 ± 5.9
10	5.2 ± 1.9	1.8 ± 0.6	3.4 ± 0.04	19.0 ± 0.2	120.0 ± 4.4	132 ± 9.3	25.0 ± 3.9	27.0 ± 6.7
11	5.8 ± 1.5	3.7 ± 1.5	3.9 ± 0.6	15.0 ± 3.0	108 ± 26	120 ± 29	28.0 ± 7.7	42.0 ± 13
12	5.8 ± 0.1	2.0 ± 0.3	4.9 ± 0.1	12.0 ± 0.8	84.8 ± 3.7	93.2 ± 3.0	22.0 ± 0.3	33.0 ± 1.0
13	7.5 ± 0.3	3.4 ± 0.4	4.7 ± 0.9	12.0 ± 0.03	87.5 ± 0.2	95.9 ± 0.1	24.0 ± 0.2	42.0 ± 0.1
14	11.0 ± 0.7	2.4 ± 0.1	11.0 ± 0.3	9.8 ± 2.6	17.9 ± 0.9	12.4 ± 1.5	5.8 ± 0.1	6.7 ± 0.8

* Compound not detected.

Table 13: PAR of volatile compounds detected for Freeze Dried Microcapsules stored at -18 ºC over 15 weeks.

	Freeze Dried -18 ºC							
Wk	Butanal	1-Penten-3-ol	Hexanal	Tridecane, 2,5-dimethyl-	Octane, 2,6-dimethyl-	Dodecane, 3-methyl-	Undecane, 3,6-dimethyl-	1,3-Cyclopentanedione, 4-(3-methyl butyl)-
0	*	*	*	*	*	*	*	*
1	*	*	*	*	11.0 ± 0.8	9.0 ± 1.4	*	*
2	*	*	*	*	27.4 ± 3.9	24.0 ± 5.1	*	*
3	*	1.7 ± 0.1	*	*	86.9 ± 6.8	66.0 ± 3.2	*	5.7 ± 0.3
4	*	1.3 ± 0.2	2.4 ± 0.1	*	60.0 ± 2.7	57.0 ± 0.9	*	7.4 ± 0.04
5	1.7± 0.8	1.5 ± 1.2	1.3 ± 0.9	5.4 ± 3.2	27.0 ± 15	28.0 ± 15	5.4 ± 2.8	7.3 ± 3.4
6	1.7 ± 0.6	1.3 ± 0.4	2.3 ± 0.9	6.4 ± 1.7	31.0 ± 8.0	34.0 ± 9.0	7.1 ± 1.8	11.0 ± 2.4
7	1.5 ± 0.03	1.2 ± 0.04	2.3 ± 1.2	5.7 ± 1.2	24.0 ± 0.6	26.0 ± 2.4	5.4 ± 1.2	7.4 ± 3.0
8	2.4 ± 0.04	0.8 ± 0.1	1.7 ± 0.4	4.3 ± 1.2	22.0 ± 4.5	24.0 ± 6.6	6.1 ± 1.7	11.0 ± 3.2
9	7.5 ± 0.2	5.2 ± 0.2	5.4 ± 0.5	26.7 ± 2.5	117.0 ± 3.8	157.0 ± 3.0	29.0 ± 0.6	28.0 ± 2.8
10	6.0 ± 1.9	1.0 ± 0.04	3.0 ± 0.4	24.5 ± 1.3	122.0 ± 4.1	116.0 ± 0.4	20.0 ± 0.1	20.0 ± 0.1
11	7.9 ± 0.7	3.4 ± 0.1	12.0 ± 0.4	23.5 ± 0.04	123.0 ± 2.2	126.0 ± 12.0	24.0 ± 4.5	29.0 ± 7.2
12	6.1 ± 3.0	1.9 ± 0.3	6.0 ± 2.6	12.5 ± 0.5	77.0 ± 11.0	84.0 ± 9.4	18.0 ± 3.1	26.0 ± 7.8
13	7.7 ± 1.8	1.9 ± 0.4	6.9 ± 2.8	12.3 ± 0.7	79.0 ± 16.0	87.0 ± 11	20.0 ± 2.4	30.0 ± 5.5
14	3.2 ± 0.3	0.9 ± 0.2	5.3 ± 0.4	5.0 ± 0.2	10.0 ± 1.5	4.2 ± 0.1	1.5 ± 0.1	2.9 ± 0.1

* Compound not detected.

Table 14: PAR of volatile compounds detected for 2-Fluid Nozzle Microcapsules stored at 5 ºC over 15 weeks.

	2-Fluid Nozzle 5 ºC									
Wk	Butanal	1-Penten-3-ol	Hexanal	2,5-dimethyl-Tridecane	2,5,6-trimethyl-Octane	2,6-dimethyl-Octane	3-methyl-Dodecane	3,6-dimethyl-Undecane	2,3,8-trimethyl-Decane	1,3-Cyclopentanedione, 4-(3-methyl butyl)-
0	*	*	1.16 ± 0.3	*	*	*	*	*	*	*
1	*	*	3.5 ± 0.02	*	25.0 ± 4.9	24.1 ± 7.1	18.3 ± 4.4	4.5 ± 0.9	*	*
2	3.0 ± 0.9	*	2.78 ± 0.19	*	41.2 ± 1.7	59.7 ± 3.2	11.9 ± 0.6	10.7 ± 2.8	36.0 ± 5.1	7.8 ± 0.6
3	5.6 ± 1.7	*	6.1 ±1.3	*	70.0 ± 6.4	108.7 ± 0.4	19.7 ± 2.9	16.9 ± 4.5	61.8 ± 8.7	13.0 ± 6.2
4	4.8 ± 0.5	1.4 ± 0.5	8.4 ± 0.5	*	87.1 ± 10	114.1 ± 15.4	121.7 ± 10.1	21.5 ± 0.3	84.9 ± 7.6	17.8 ± 0.8
5	3.9 ± 0.3	1.8 ± 0.2	5.3 ± 0.8	*	63.7 ± 8.6	86.8 ± 9.9	99.1± 8.8	19.6 ± 0.6	61.0 ± 5.4	21.2 ± 1.7
6	2.1 ± 0.1	1.3 ± 0.1	2.2 ± 0.2	*	52.3 ± 5.1	74.9 ± 5.6	83.5 ± 9.034	17.2 ± 3.1	51.0 ± 4.0	21.9 ± 3.1
7	2.4 ± 0.9	1.0 ± 0.3	5.6 ± 1.7	*	27.6 ± 3	54.2 ± 19.7	60.6 ± 22.1	16.4 ± 0.8	31.4 ± 4.5	20.5 ± 0.3
8	1.6 ± 0.4	0.9 ± 0.4	3.2 ± 1.6	6.0±2.7	19.4±7.6	31.1 ± 12.1	34.1 ± 12.8	13.9 ± 5.6	21.5 ± 8.0	16.8 ± 6.8
9	1.5 ± 0.6	1.3 ± 0.1	4.5 ± 0.9	7.9 ± 0.1	25.2 ± 4.7	43.1 ± 8.0	45.6 ± 8.8	17.2 ± 4.8	29.7 ± 4.8	26.6 ± 5.8
10	5.4 ± 1.3	9.5 ± 2	10.4 ± 1.7	33.4±7.2	121.1±30	160.3 ± 40.9	158.6 ± 36.3	30.9 ± 1.01	117.4 ± 6.6	24.4 ± 4.6
11	6.9 ± 0.1	17 ± 9.6	11.4 ± 6.4	24.1±5.7	96.8±17.3	125.6 ± 24.2	137 ± 23.3	35.0 ± 5.1	94.5 ± 21.5	27.1 ± 2.6
12	6.3 ± 1.2	3.8 ± 1.5	6.9 ± 0.3	16.5±1.6	71.5±10.3	106.1 ± 16.2	119.3 ± 19.6	27.5 ± 4.8	76.4 ± 12.2	40.2 ± 8.8
13	5.7 ± 1.7	13.1 ± 9.9	11.0 ± 0.9	15.2±0.3	57.3±2.5	90.4 ± 2.3	99.9 ± 2.7	36.9 ± 2.1	64.7 ± 1.3	42.4 ± 7.7
14	5.9 ± 1.5	6.5 ± 4.4	13.2 ± 4.8	12.3±2.3	48.7±4.6	74.4 ± 9.7	79.8 ± 11.4	21.1 ± 3.3	51.8 ± 6.8	36.8 ± 1.8

* Compound not detected.

Table 15: PAR of volatile compounds detected for 2-Fluid Nozzle Microcapsules stored at -18 ºC over 15 weeks.

	2-Fluid Nozzle -18 ºC									
Wk	Butanal	1-Penten-3-ol	Hexanal	2,5-dimethyl-Tridecane	2,5,6-trimethyl-Octane	2,6-dimethyl-Octane	3-methyl-Dodecane	3,6-dimethyl-Undecane	2,3,8-trimethyl-Decane	1,3-Cyclopentanedione, 4-(3-methyl butyl)-
0	*	*	*	*	*	*	*	*	*	*
1	*	*	*	*	*	10.8 ± 0.5	5.9 ± 1.1	*	*	*
2	*	*	*	*	*	11.7 ± 0.3	9.3 ± 0.9	*	*	*
3	*	*	*	*	*	10.9 ± 0.9	9.0 ± 1.6	1.56 ± 0.2	*	*
4	6.3 ± 1.4	*	4.4 ± 1.5	*	*	13.7 ± 1.7	67 ± 19	12.9 ± 0.5	*	*
5	5.4 ± 0.3	*	9.3 ± 1.0	*	*	14.9 ± 1.9	94 ± 5.5	15.4 ± 0.9	*	*
6	1.5 ± 0.7	*	4.8 ± 0.1	11 ± 2.8	*	22.1 ± 2.1	55 ± 5.6	10.3 0.6	*	*
7	2.2 ± 0.3	*	4.2 ± 0.9	10.4 ± 0.9	*	47.5 ± 5.3	52 ± 8.3	11.0 ± 2.7	4.2 ± 1.2	*
8	1.3 ± 0.1	*	3.5 ± 0.3	8.8 ± 0.7	28.3 ± 2.1	40.6 ± 3.1	44 ± 2.9	9.8 ± 0.1	28 ± 1.9	9.75 ± 0.5
9	1.4 ± 0.7	*	5.1 ± 1.5	9.9 ± 2	31.2 ± 0.1	52.8 ± 0.6	56 ± 0.1	12.7 ± 3	37 ± 1	33.1 ± 0.7
10	8.4 ± 2.5	2.7 ± 0.7	10 ± 2.8	49.4 ± 4	130 ± 4.1	210.7 ± 7.8	148 ± 5.4	23.8 ± 0.6	114 ± 5.8	15 ± 1.1
11	5.9 ± 2.3	2.9 ± 1.6	8.3 ± 2.5	29.5 ± 6.2	98 ± 2.5	118.1 ± 3.4	118 ± 16	22.1 ± 5.3	92 ± 2.2	14.6 ± 3.7
12	4.6 ± 2.2	3.6 ± 1.6	9.4 ± 0.2	18 ± 1.1	72.1 ± 0.2	104.4 ± 0.2	109 ± 7	22.1 ± 2.8	71 ± 3.8	22.1 ± 8.1
13	8.5 ± 2.4	14.0 ± 0.2	6.4 ± 0.5	22.1 ± 5.1	88.9 ± 18	110.7 ± 5.4	120 ± 3.2	27.9 ± 3.2	86 ± 15	31.3 ± 4.4
14	8.7 ± 0.5	8.0 ± 2.0	7.8 ± 0.7	19.2 ± 2	64 ± 11	99.01 ± 9.8	106 ± 8.2	27.4 ± 5.6	69 ± 5	36.9 ± 5.7

* Compound not detected.

Table 16: PAR of volatile compounds detected for 3-Fluid Nozzle Microcapsules stored at 5 ºC over 15 weeks.

	3-Fluid Nozzle 5 ºC							
Wk	2,5-dimethyl-Tridecane	2,5,6-trimethyl-Octane	2,6-dimethyl-Octane	3-methyl-Dodecane	3,6-dimethyl-Undecane	2,3,8-trimethyl-Decane	2,2,6-trimethyl-Decane	1,3-Cyclopentanedione, 4-(3-methyl butyl)-
0	*	*	*	*	*	*	*	*
1	4.4 ± 1.2	21.8 ± 6.2	33.5 ± 8.54	38.1 ± 9.6	7.5 ± 2.2	24.0 ± 5.9	13.0 ± 3.3	10.0 ± 2.6
2	7.7 ± 0.3	35.2 ± 0.5	52.5 ± 1.57	10.3 ± 0.6	11.0 ± 0.6	31.5 ± 4.9	15.0 ± 4.7	8.3 ± 0.4
3	12.0 ± 4.5	42.9 ± 3.1	64.4 ± 1.95	15.8 ± 4.4	14.0 ± 1.1	39.0 ± 6.6	34.0 ± 2.1	13.5 ± 0.1
4	15.0 ± 3.6	64.8 ± 14	82.9 ± 19	89.1 ± 19.0	15.0 ± 2.9	61.3 ± 13	28.0 ± 6.8	13.2 ± 2.2
5	6.3 ± 1.5	29.9 ± 7.5	40.6 ± 7.44	46.4 ± 8.6	9.5 ± 1.5	28.6 ± 5.0	15.0 ± 2.6	11.3 ±1.5
6	4.8 ± 0.8	22.5 ± 2.8	34.8 ± 1.59	36.9 ± 3.8	4.4 ± 5	22.1 ± 2.8	15.0 ± 1.9	14.9 ± 7.2
7	3.2 ± 0.04	15.8 ± 0.6	25.0 ± 1.01	27.5 ± 1.1	7.0 ± 0.4	17.1 ± 0.6	9.3 ± 0.4	13.4 ± 0.7
8	2.9 ± 0.5	14.4 ± 2.3	23.6 ± 3.2	25.4 ± 3.3	6.6 ± 0.7	15.9 ± 2.3	8.8 ± 1.3	12.7 ± 0.7
9	2.3 ± 0.7	10.7 ± 3.5	18.7 ± 6.3	19.2 ± 6.4	5.3 ± 1.7	12.7 ± 4.2	7.0 ± 2.4	13.3 ± 3.5
10	37.0 ± 0.1	143.0 ± 4.2	195.0 ± 7.3	185 ± 5.0	30.0 ± 1.0	135.0 ± 5.0	59.0 ± 1.7	26.3 ± 2.1
11	17.0 ± 1.2	83.6 ± 6.8	111.0 ± 9.6	123 ± 14.0	24.0 ± 2.9	105.0 ± 4.4	39.0 ± 4.8	26.3 ± 3.3
12	8.2 ± 2.2	41.2 ± 10	61.8 ± 15.4	69.3 ± 16.0	16.0 ± 3.9	44.1 ± 9.6	22.0 ± 5.6	24.2 ± 6.4
13	8.1 ± 0.04	43.1 ± 0.7	67.6 ± 3.06	74.6 ± 2.9	17.0 ± 1.7	47.8 ± 2.5	25.0 ± 1.2	27.3 ± 6.1
14	9.7 ± 0.7	48.7 ± 3.1	77.2 ± 3.37	83.4 ± 3.3	21.0 ± 0.6	54.0 ± 2.4	28.0 ± 1.2	37.0 ± 0.7

* Compound not detected.

Table 17: PAR of volatile compounds detected for 3-Fluid Nozzle Microcapsules stored at -18 ºC over 15 weeks.

	3-Fluid Nozzle -18 ºC							
Wk	2,5-dimethyl-Tridecane	2,5,6-trimethyl-Octane	2,6-dimethyl-Octane	3-methyl-Dodecane	3,6-dimethyl-Undecane	2,3,8-trimethyl-Decane	2,2,6-trimethyl-Decane	1,3-Cyclopentanedione, 4-(3-methyl butyl)-
0	*	*	*	*	*	*	*	*
1	4.9 ± 0.1	20.1 ± 2.2	36.8 ± 0.04	6.1 ± 1.7	32.9 ± 7.1	37.0 ± 4.0	*	*
2	3.5 ± 1.1	14.4 ± 3.9	23.1 ± 7.9	3.8 ± 0.8	21.5 ± 5.5	14.0 ± 0.6	3.0 ± 0.9	3.0 ± 0.9
3	4.5 ± 1.8	8.7 ± 1.6	13.9 ± 3.7	4.7 ± 1.3	11.5 ± 2.3	7.1 ± 1.2	3.5 ± 1.o	3.5 ± 1.0
4	7.2 ± 0.9	22.8 ± 0.8	39.9 ± 4.3	4.0 ± 0.04	26.9 ± 0.6	32 .0 ± 1.0	3.1 ± 0.1	3.1 ± 0.1
5	8.1 ± 1.5	31.2 ± 8.9	46.7 ± 12	6.9 ± 2.2	41.8 ± 13.0	37.0 ± 1.6	5.5 ± 1.9	5.5 ± 1.9
6	4.5 ± 1.0	18.6 ± 5.2	27.7 ± 8.4	5.1 ± 2.2	28.1 ± 11	18.0 ± 6.6	6.3 ± 3.5	6.3 ± 3.5
7	1.7 ± 0.1	6.93 ± 0.8	10.6 ± 1.1	2.2 ± 0.2	11.3 ± 1.2	7.2 ± 0.8	3.2 ± 0.3	3.2 ± 0.3
8	2.4 ± 1.1	10.7 ± 3.8	16.6 ± 5.6	4.5 ± 1.4	18.6 ± 6.4	12.0 ± 4.3	7.0 ± 1.0	7.0 ± 1.0
9	2.9 ± 0.1	14.1 ± 0.2	21.8 ± 0.5	6.1 ± 0.3	24.2 ± 0.2	15.0 ± 0.5	8.6 ± 0.6	8.6 ± 0.6
10	31.0 ± 0.3	112.0 ± 1.2	160.0 ± 0.1	22.0 ± 0.1	139.0 ± 0.03	102.0 ± 0.6	21.0 ± 0.04	21.0 ± 0.04
11	14.0 ± 0.4	71.0 ± 1.1	94.1 ± 0.1	20.0 ± 0.5	104.0 ± 1.4	88.0 ± 2.9	22.0 ± 0.6	22.0 ± 0.6
12	9.7 ± 2.0	45.6 ± 7	63.7 ± 10	14.0 ± 0.6	68.6 ± 8.1	44.0 ± 5.3	15.0 ± 3.6	15.0 ± 3.6
13	9.9 ± 2.0	46.3 ± 13	66.9 ± 18	15.0 ± 5.4	71.5 ± 2.2	46.0 ± 14	17.0 ± 9.4	17.0 ± 9.4
14	8.8 ± 0.4	40 ± 0.7	60.2 ± 1.4	12.0 ± 0.3	61.9 ± 1.3	41.0 ± 0.8	14.0 ± 2.9	14.0 ± 2.9

* Compound not detected.

Table 18: PAR of volatile compounds detected for Ultrasonic Microcapsules stored at 5 ºC over 15 weeks.

	Ultrasonic Nozzle 5 ºC							
Wk	HEPTANAL	Nonane, 3-methylene-	2,5-dimethyl-Tridecane	2,5,6-trimethyl-Octane	2,6-dimethyl-Octane	3-methyl-Dodecane	2,3,8-trimethyl-Decane	1,3-Cyclopentanedione, 4-(3-methyl butyl)-
0	*	*	*	*	*	*	*	*
1	*	*	*	7.8 ± 0.7	*	*	*	*
2	*	*	*	7.8 ± 1.1	13 ± 1	*	*	*
3	*	*	*	11.0 ± 0.8	12.5 ± 0.7	*	*	*
4	*	*	5.6 ± 0.9	28.0 ± 2.4	16.4 ± 0.4	5.0 ± 2.0	*	8.5 ± 0.7
5	5.2 ± 1.7	*	4.84 ± 1.7	11.4 ± 4.3	43.5 ± 0.6	7.1 ± 1.3	*	5.3 ± 0.4
6	5.1 ± 1.5	*	4.85 ± 0.9	14.3 ± 2.6	10.3 ± 3.5	7.1 ± 1.8	*	5.3 ± 0.4
7	7.4 ± 0.6	8.1 ± 1.1	6.8 ± 0.8	18.3 ± 0.5	12.6 ± 3.6	10.0 ± 1.49	1.6 ± 0.2	6.5 ± 2.9
8	5.3 ± 1.4	5.9 ± 3.3	4.8 ± 2.1	15.1 ± 4.4	15.0 ± 0.6	8.6 ± 2.9	1.2 ±0.2	8.4 ± 1.8
9	3.4 ± 1.8	2.2 ± 0.4	2.13 ± 0.4	6.9 ± 2.3	12.4 ± 4.1	3.7 ± 1.2	2.3 ± 1.3	15.0 ± 3.3
10	11.0 ± 4.1	5.2 ± 1.2	13.8 ± 3.4	40.9 ± 4.9	5.7 ± 2.4	23.0 ±1.1	15 ± 3.0	13.5 ± 5.4
11	13.0 ± 0.6	6.5 ± 1.9	10.7 ± 1.3	34.5 ± 5.3	42.5 ± 6	21.0 ± 2.8	12.0 ± 3.2	7.2 ± 2.9
12	10.0 ± 3.6	8.9 ± 0.2	11.7 ± 0.8	46.4 ± 5.3	33.4 ± 3.3	28.0 ± 1.4	19.0 ± 1.9	14.5 ±2.6
13	12.0 ± 2.5	6.6 ± 0.4	8.8 ± 0.7	34.3 ± 0.3	40.3 ± 1.9	20.0 ± 0.04	14 ± 0.2	16.1 ± 4.7
14	22.0 ± 4.6	10.2 ± 1.4	4.7 ± 0.2	1.5 ± 0.4	29.3 ± 0.8	1.2 ± 0.7	2.7 ± 1.6	28.5 ± 5

* Compound not detected.

Table 19: PAR of volatile compounds detected for Ultrasonic Microcapsules stored at -18 ºC over 15 weeks.

	Ultrasonic Nozzle -18 ºC							
Wk	HEPTANAL	Nonane, 3-methylene-	2,5-dimethyl-Tridecane	2,5,6-trimethyl-Octane	2,6-dimethyl-Octane	3-methyl-Dodecane	2,3,8-trimethyl-Decane	1,3-Cyclopentanedione, 4-(3-methyl butyl)-
0	*	*	*	*	*	*	*	*
1	*	*	3.2 ± 0.03	3.4 ± 0.03	1.8 ± 0.3	*	*	*
2	*	*	3.3 ± 0.1	3.3 ± 0.1	1.7 ± 0.02	*	*	2.3 ± 0.1
3	*	*	9.1 ± 0.6	9.5 ± 1.5	5.2 ± 1.6	*	*	2.6 ± 0.1
4	*	3.9 ± 0.03	6.5 ± 0.04	7.2 ± 1.4	2.6 ± 0.3	3.4 ± 1.4	*	6.5 ± 0.1
5	3.2 ± 0.1	5.6 ± 0.3	15.0 ± 1.2	30.0 ± 0.9	21.0 ± 1.0	6.67 ± 0.1	*	2.5 ± 0.1
6	2.6 ± 1.1	5.1 ± 1.4	15.0 ± 3.8	25.0 ± 6.4	22.0 ± 1.9	20.9 ± 5.4	*	3.3 ± 0.04
7	1.9 ± 0.1	3.1 ± 0.04	10.0 ± 1.4	15.0 ± 3.0	14.0 ± 5.3	3.5 ± 0.3	*	4.5 ± 2.2
8	1.8 ± 0.6	2.7 ± 0.7	8.9 ± 2.6	5.1 ± 1.4	16.0 ± 3.4	2.8 ± 0.6	3.4 ± 0.8	3.1 ± 0.8
9	1.7 ± 0.5	2.2 ± 0.3	5.9 ± 2.8	3.6 ± 1.4	10.0 ± 4.2	1.9 ± 0.7	4.8 ± 0.4	3.8 ± 0.2
10	4.8 ± 0.6	19.5 ± 1.7	65.0 ± 6.2	54.0 ± 4.9	73.0 ± 15	22.0 ± 4.8	16.0 ± 0.5	2.5 ± 1.1
11	3.7 ± 0.5	11.4 ± 0.4	34.0 ± 2.9	54.0 ± 1.1	40 ± 0.2	11.6 ± 0.01	13.0 ± 1.4	11.0 ± 0.3
12	3.9 ± 0.1	8.3 ± 1.6	29.0 ± 0.1	45.0 ± 0.7	41.0 ±2.3	7.9 ± 0.6	11.0 ± 2.5	6.5 ± 0.4
13	3.6 ± 0.1	10.1 ± 0.7	38.0 ± 5.6	59.0 ± 5.8	52.0 ± 9.3	9.3 ± 1.8	8.2 ± 2.5	9.7 ± 3.7
14	12.2 ± 2.1	4.2 ± 0.5	1.4 ±0.2	2.2 ± 0.3	2.0 ± 0.2	9.7 ± 1.2	5.4 ± 2.0	1.8 ± 0.1

* Compound not detected.

Table 20: PAR of volatile compounds detected for Fish Oil stored at 5 ºC over 15 weeks.

	Fish Oil 5 ºC				
Wk	2-Propenal	Formic acid	Butanal	2-ethyl-Furan	1-Penten-3-ol
0	0.8 ± 0.2	*	*	*	*
1	1 ± 0.5	*	0.2 ± 0	0.6 ± 0.2	2.7 ± 0.2
2	2.3 ± 0.9	*	1.1 ± 0.2	2.9 ± 0.1	2.3 ± 0.7
3	1.8 ± 0.6	3.4 ± 0.8	3.7 ± 1.3	4.3 ± 0.5	18 ± 5.4
4	1.9 ± 0.9	2.2 ± 0.9	6.8 ± 1.1	2.7 ± 0.3	12 ± 0.6
5	2.6 ± 0.04	1 ± 0.3	2.8 ± 0.7	3 ± 0.5	2.1 ± 0.3
6	1.1 ± 0.04	0.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.2	2.1 ± 0.3
7	1.1 ± 0.1	0.6 ± 0.4	1.7 ± 1.5	2.3 ± 0.2	1.2 ± 0.2
8	1.1 ± 0.1	0.9 ± 0.1	3.2 ± 0.8	2.6 ± 0.1	2.8 ± 0.2
9	8.6 ± 0.7	2.2 ± 1.8	11 ± 3.3	14.0 ± 0.4	18.0 ± 1.4
10	11 ± 5.1	6.1 ± 1.5	13 ± 3.7	14.0 ± 0.5	19.0 ± 1.4
11	19 ± 2.2	5.4 ± 2.0	9.3 ± 1.8	13.0 ± 0.2	13 ± 1.6
12	17 ± 3.8	15 ± 4.5	11.0 ± 2.9	19.0 ± 4.5	14 ± 3
13	6.3 ± 1.0	24.3 ± 0.2	21.0 ± 1.8	18.0 ± 0.2	14 ± 0.1
14	5.2 ± 3.8	47.0 ± 3.6	7.0 ± 5.5	13.0 ± 2.7	5.4 ± 2

* Compound not detected.

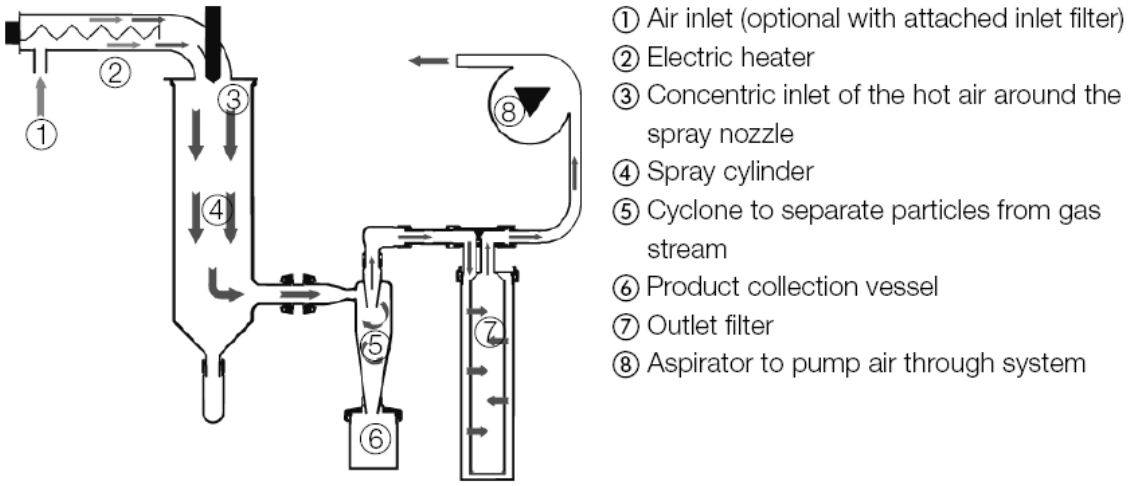
Table 21: PAR of volatile compounds detected for Fish Oil stored at -18 ºC over 15 weeks.

	Fish Oil -18 ºC				
Wk	2-Propenal	Formic acid	Butanal	2-ethyl-Furan	1-Penten-3-ol
0	0.8 ± 0.2	*	*	*	*
1	2.6 ± 0.2	*	*	1 ± 0.3	0.7 ± 0.1
2	2.2 ± 0.01	*	2.4 ± 0.6	2.3 ± 0.3	1.0 ± 0.2
3	1.8 ± 0.01	0.8 ± 0.3	1.2 ± 0.2	0.7 ± 0.2	1.0 ± 0.02
4	2.8 ± 1.6	0.8 ± 0.1	2.1± 0.2	2.8 ± 0.1	1.4 ± 0.1
5	7.6 ± 2.8	1.1 ± 0.8	2.3 ± 0.1	3.4 ± 0.2	2.1 ± 0.6
6	1.3 ± 0.5	0.6 ± 0.1	1.8 ± 0.8	1.5 ± 0.5	1.6 ± 0.4
7	0.9 ± 1.2	1.4 ± 0.03	4.3 ± 0.2	2.0 ± 0.3	2.0 ± 0.2
8	1.5 ± 2.1	1.1 ± 0.5	6.3 ± 2.9	1.9 ± 0.1	1.9 ± 0.5
9	11.0 ± 0.3	4.2 ± 1.4	15.0 ± 3.3	14.0 ± 2.2	7.6 ± 1.0
10	19.0 ± 11	5.5 ± 0.9	20 ± 2.8	10 ± 2.6	12.0 ± 3.8
11	8.2 ± 1.9	2.5 ± 0.7	23 ± 4.1	5.6 ± 0.4	5.0 ± 0.1
12	9.0 ± 2.0	9.0 ± 2.8	21.0 ± 0.03	8.6 ± 0.03	4.7± 0.2
13	9.2 ± 1.3	6.0 ± 0.9	18.0 ± 1.1	7.9 ± 1.6	10.0 ± 1.6
14	4.1 ± 1.9	7.7 ± 3.8	6.0 ± 2.0	2.8 ± 2.6	2.3 ± 0.5

* Compound not detected.

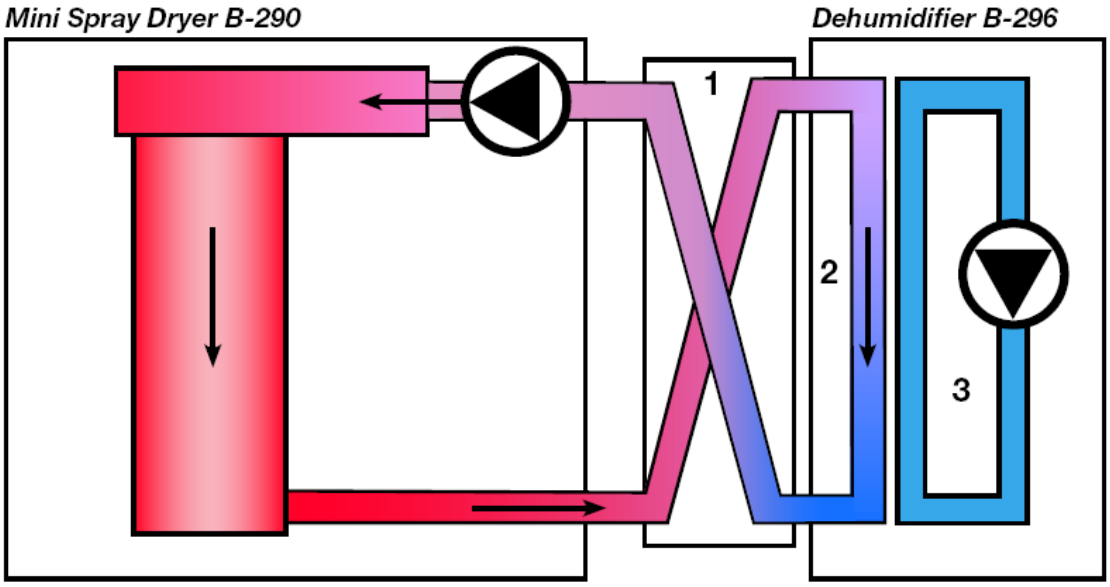
FIGURES

Figure 1: Schematic Drawing of Spray Drier used for encapsulation experiments



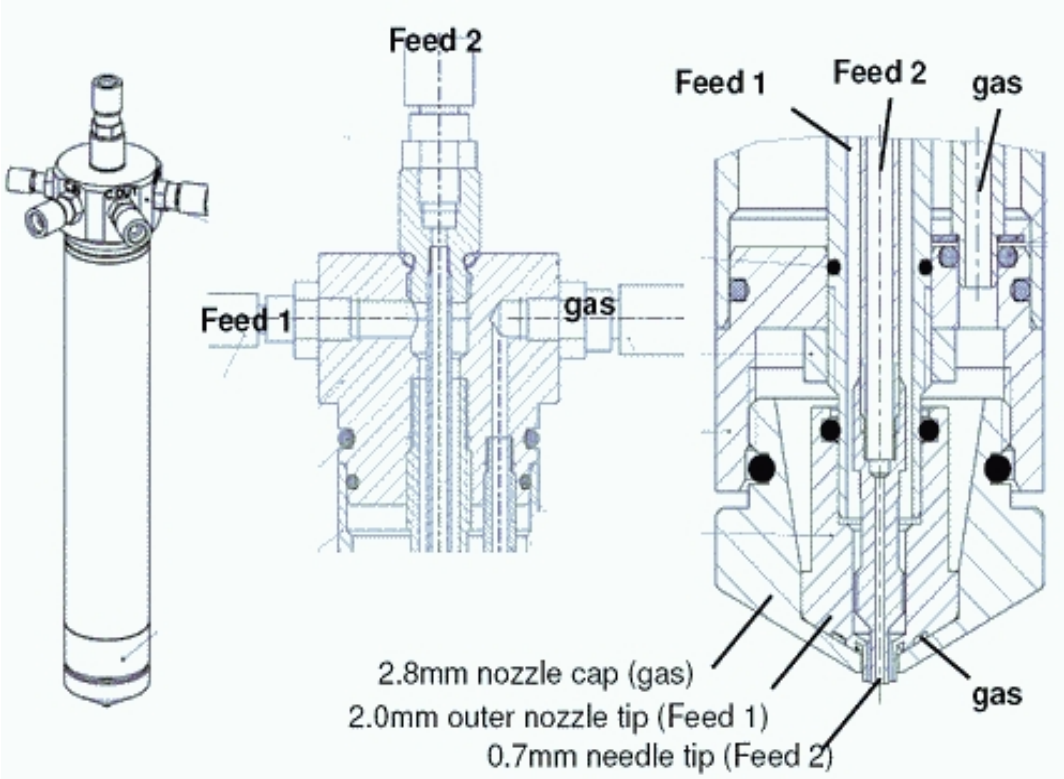
(B-290 operation manual, www.Buchi.com)

Figure 2: Schematic representation of drying gas cycle through the spray drying system.



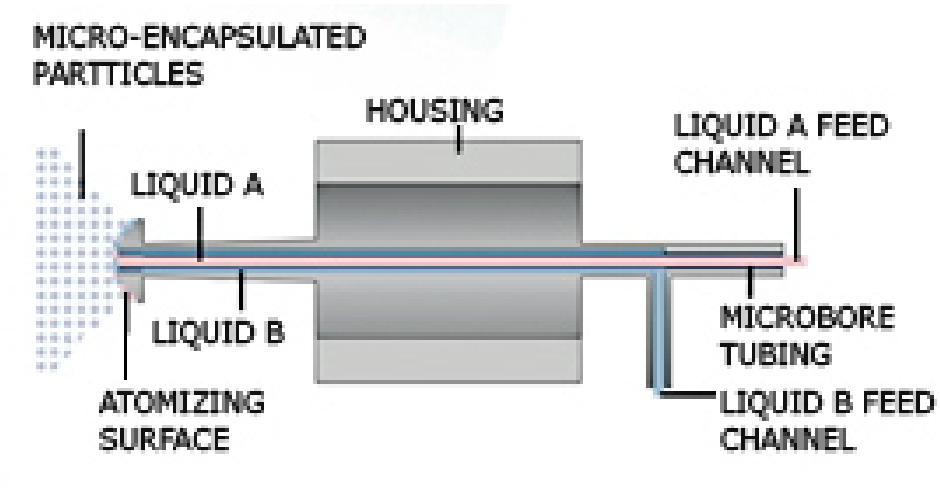
(www.Buchi.com)

Figure 3: A schematic diagram of 3-Fluid pressure nozzle used for microencapsulation.



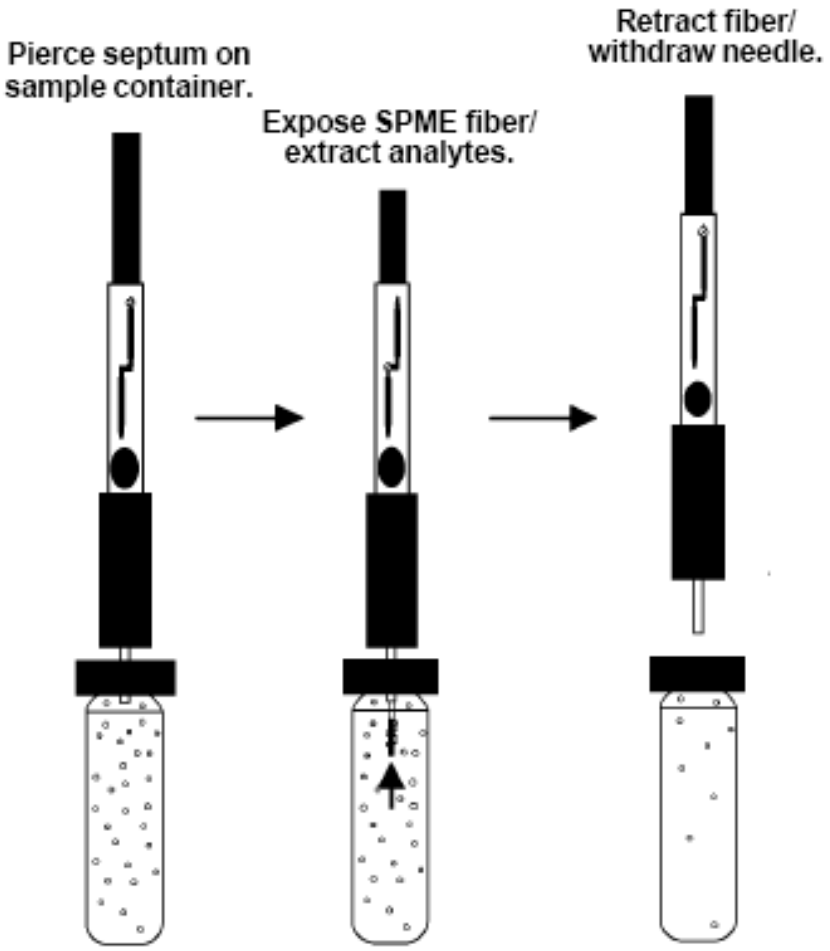
(www.Buchi.com)

Figure 4: Schematic drawing of Sono-Tek 2-Channel Ultrasonic Nozzle used for microencapsulation.



(www.Sono-Tek.com)

Figure 5: Solid Phase Microextraction schematic showing extraction method.



(www.Sigma-Aldrich.com)

Figure 6: Particle size analysis distribution plot of microcapsules.

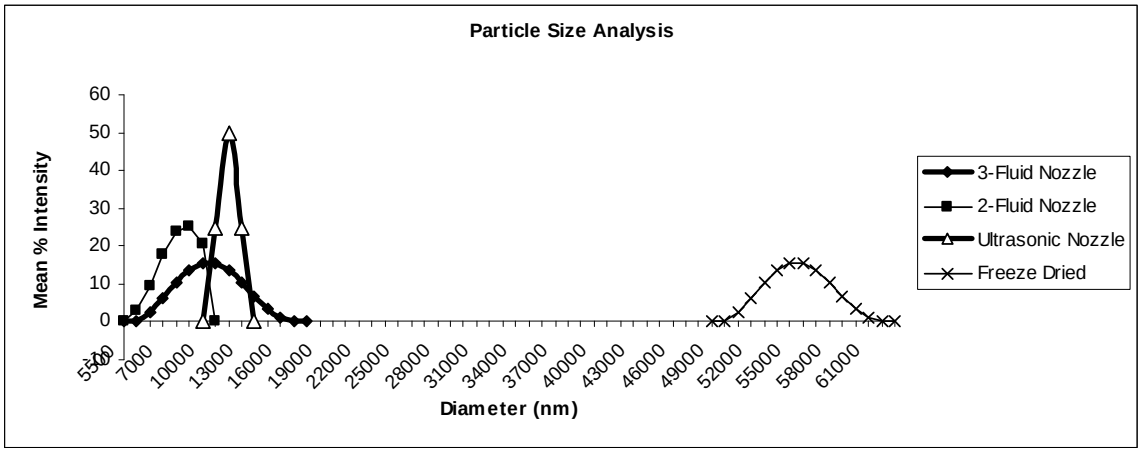


Figure 7: Propanal 5º C Peak Area Ratio (PAR) over Time

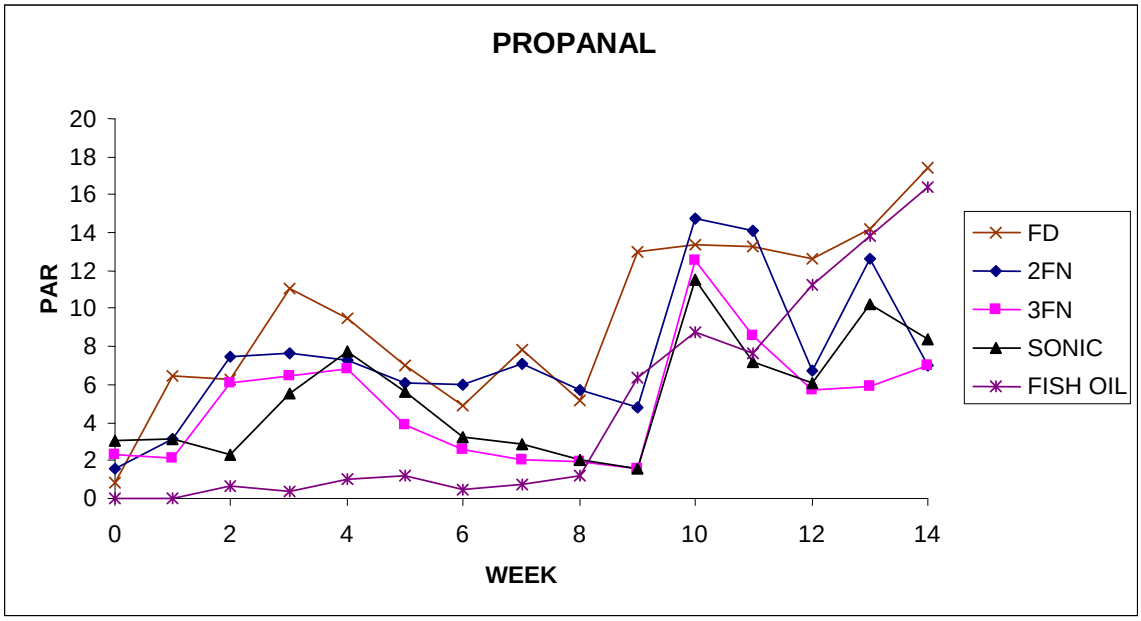
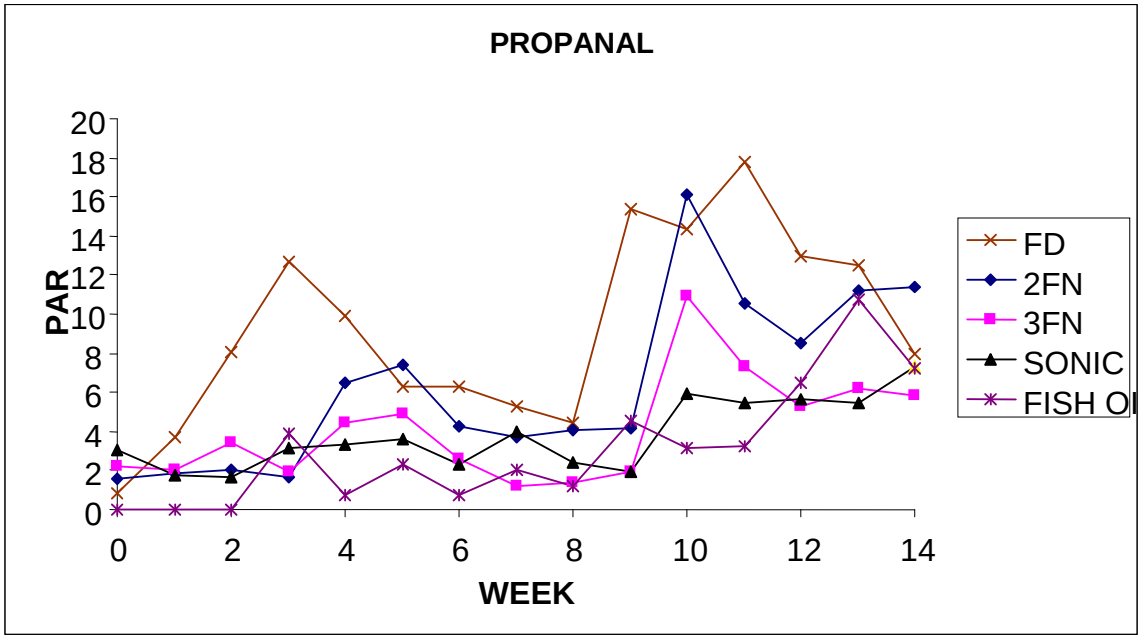
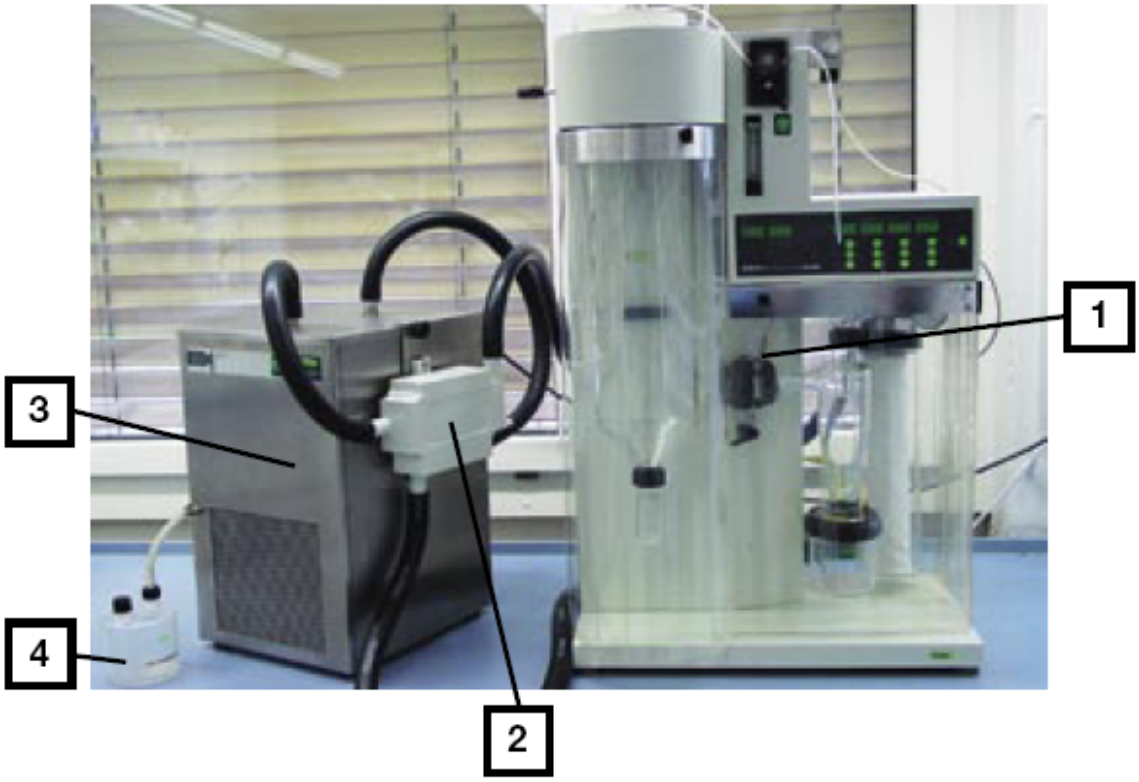


Figure 8: Propanal -18ºC Peak Area Ratio (PAR) over Time



PICTURES

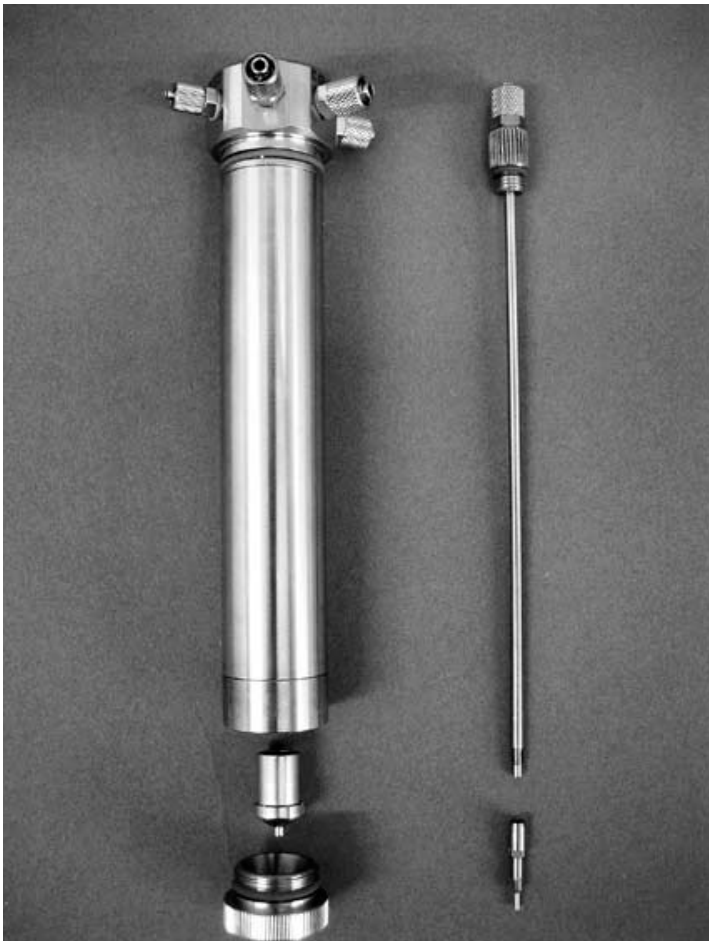
Picture 1: A picture of the spray drying system used for the experiments



1. B-290 Spray Drier
2. External heat exchanger
3. B-296 Dehumidifier
4. Water collection bottle

(www.Buchi.com)

Picture 2: A picture of 3-Fluid pressure nozzle

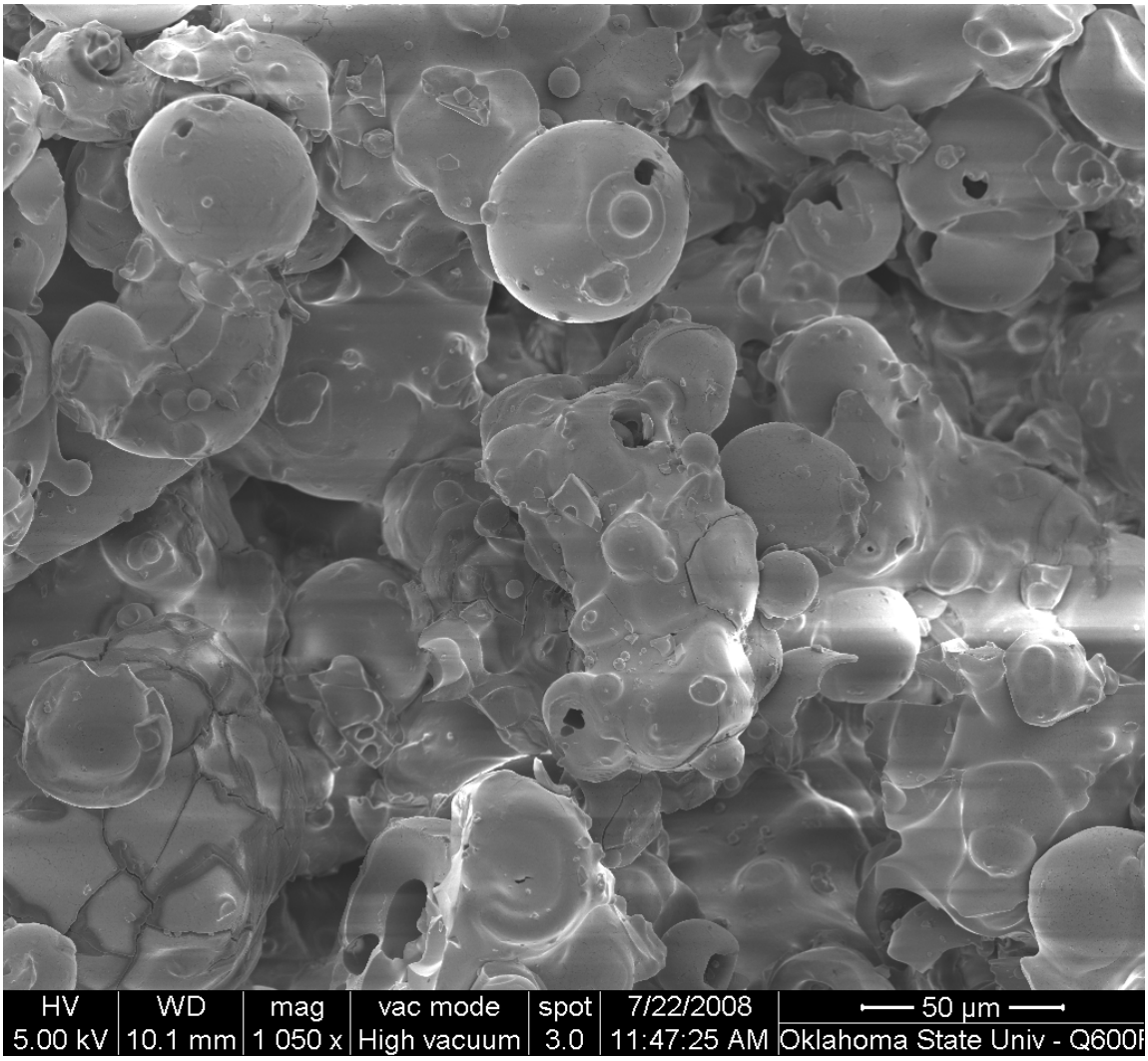


(www.Buchi.com)

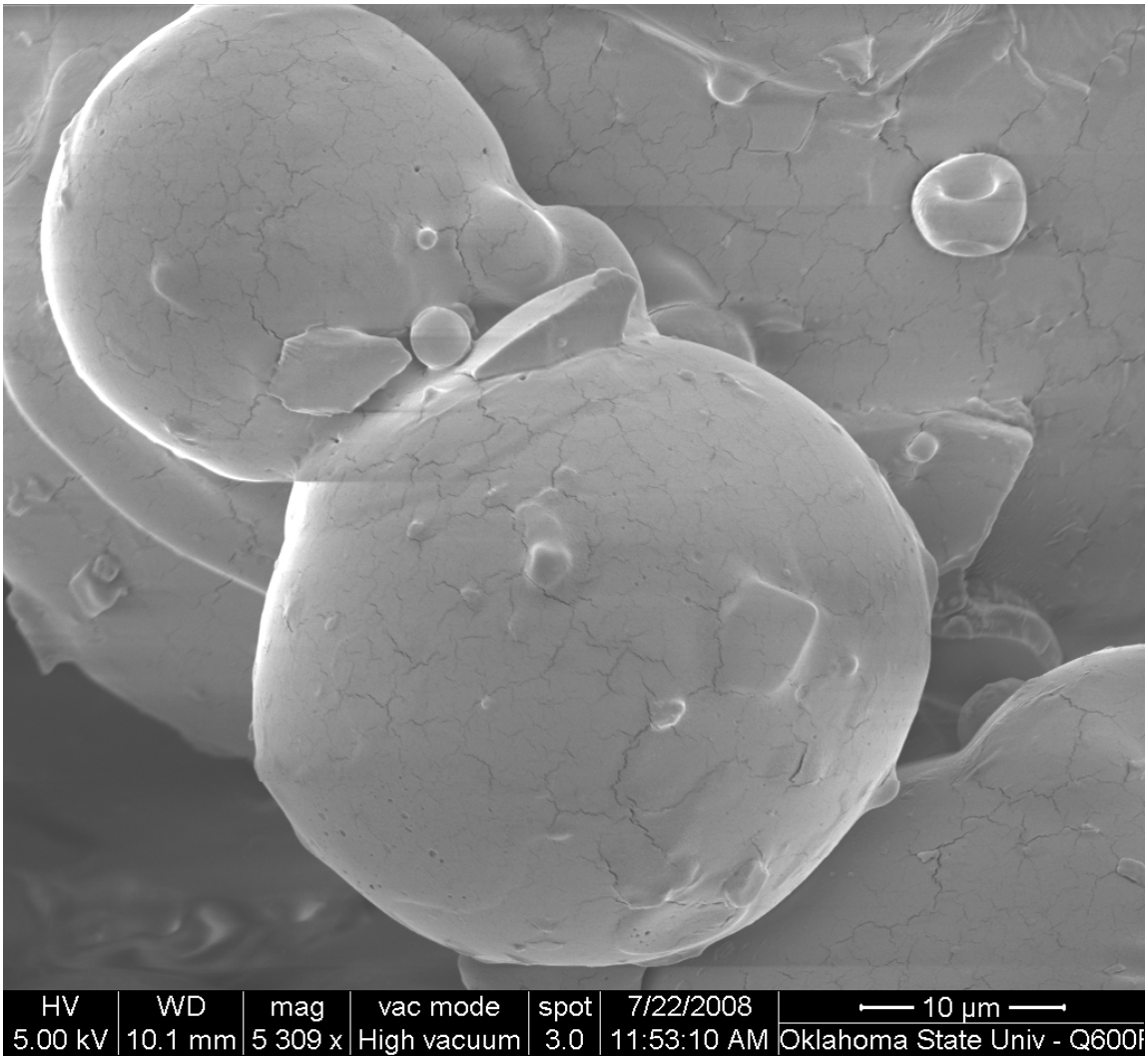
Picture 3: Foil/poly Bag

(www.Sigma-Aldrich.com)

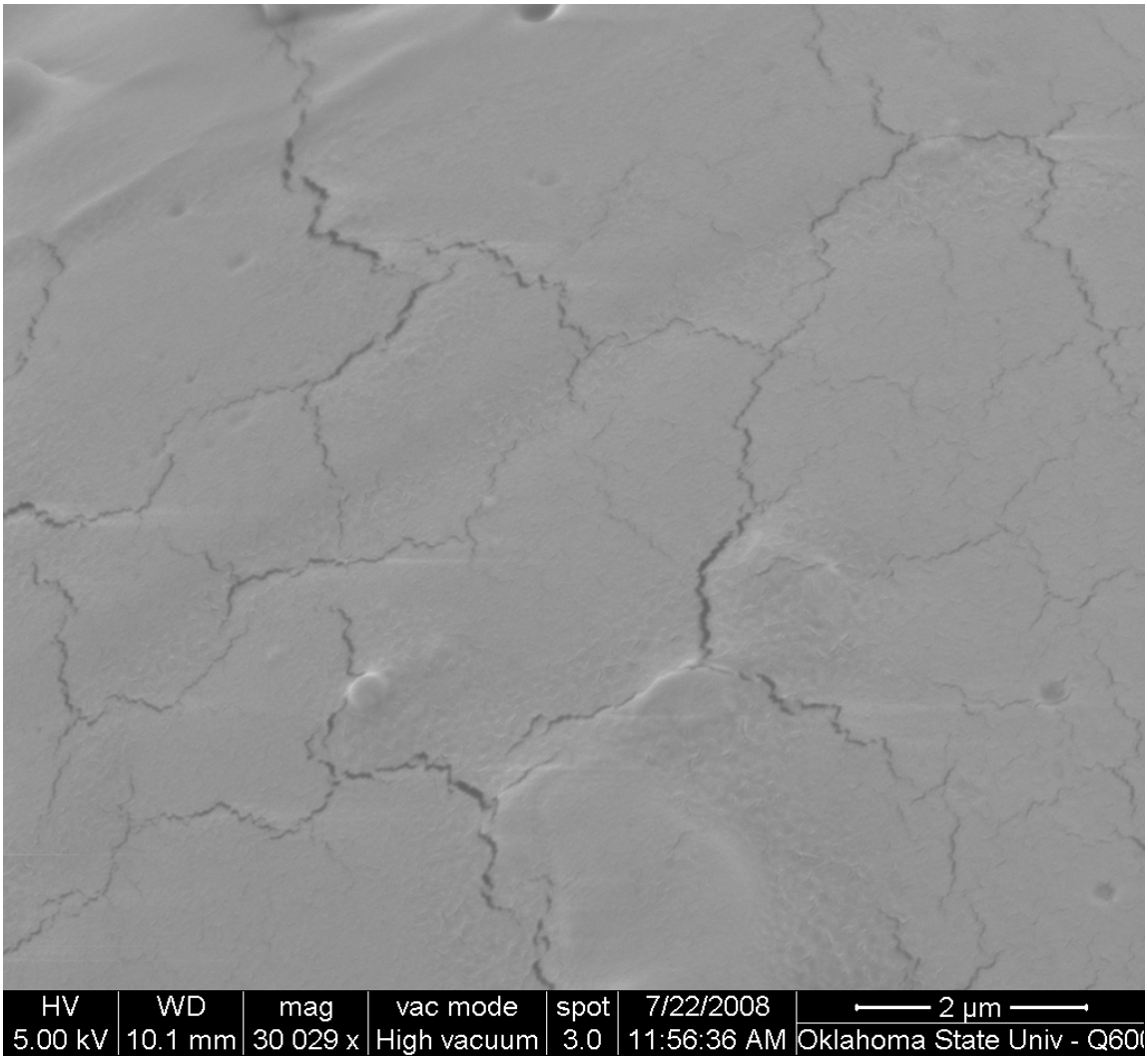
Picture 4: Freeze Dried microcapsules at 1000 times magnification.



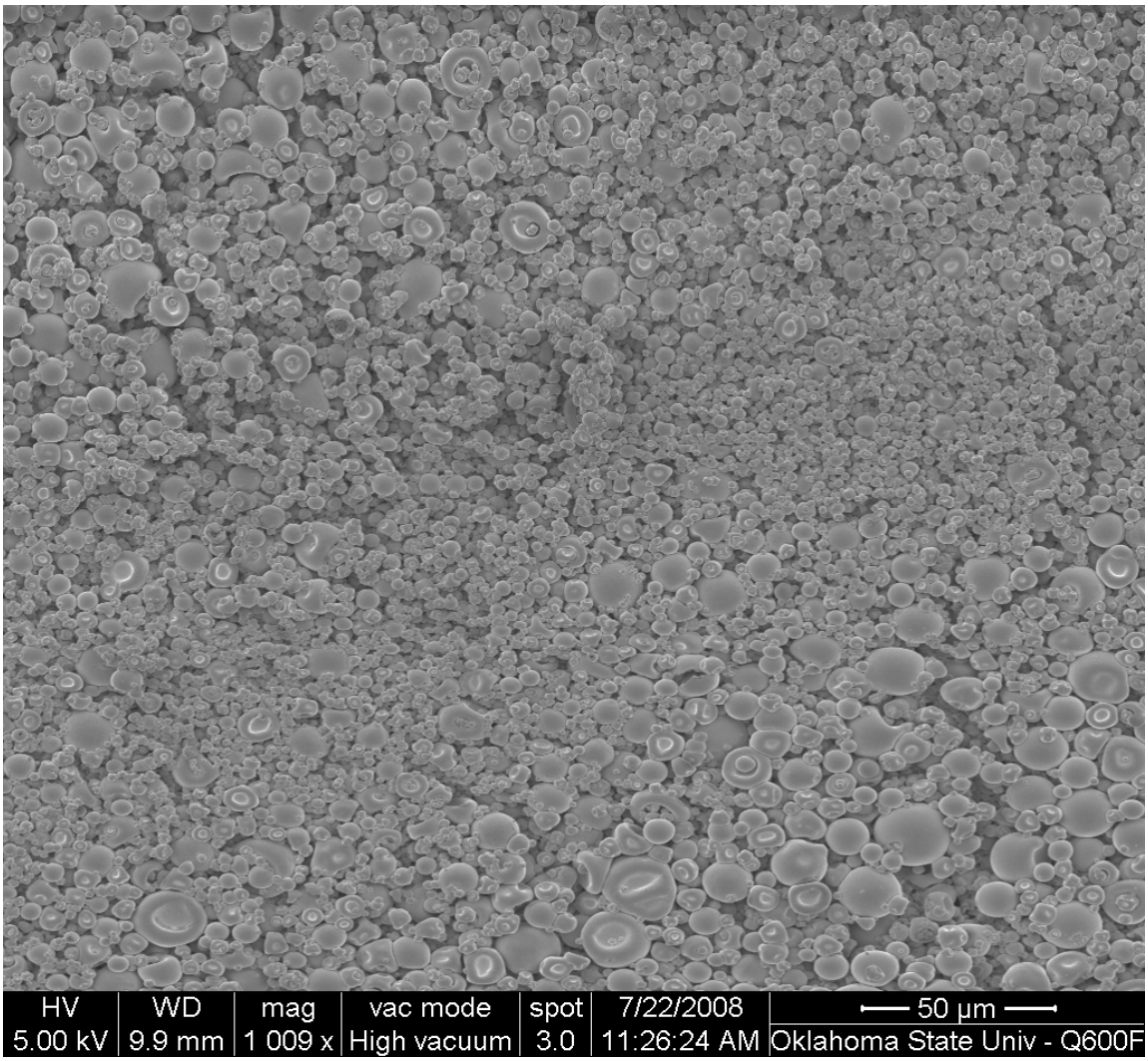
Picture 5: Freeze Dried microcapsules at 5000 times magnification.



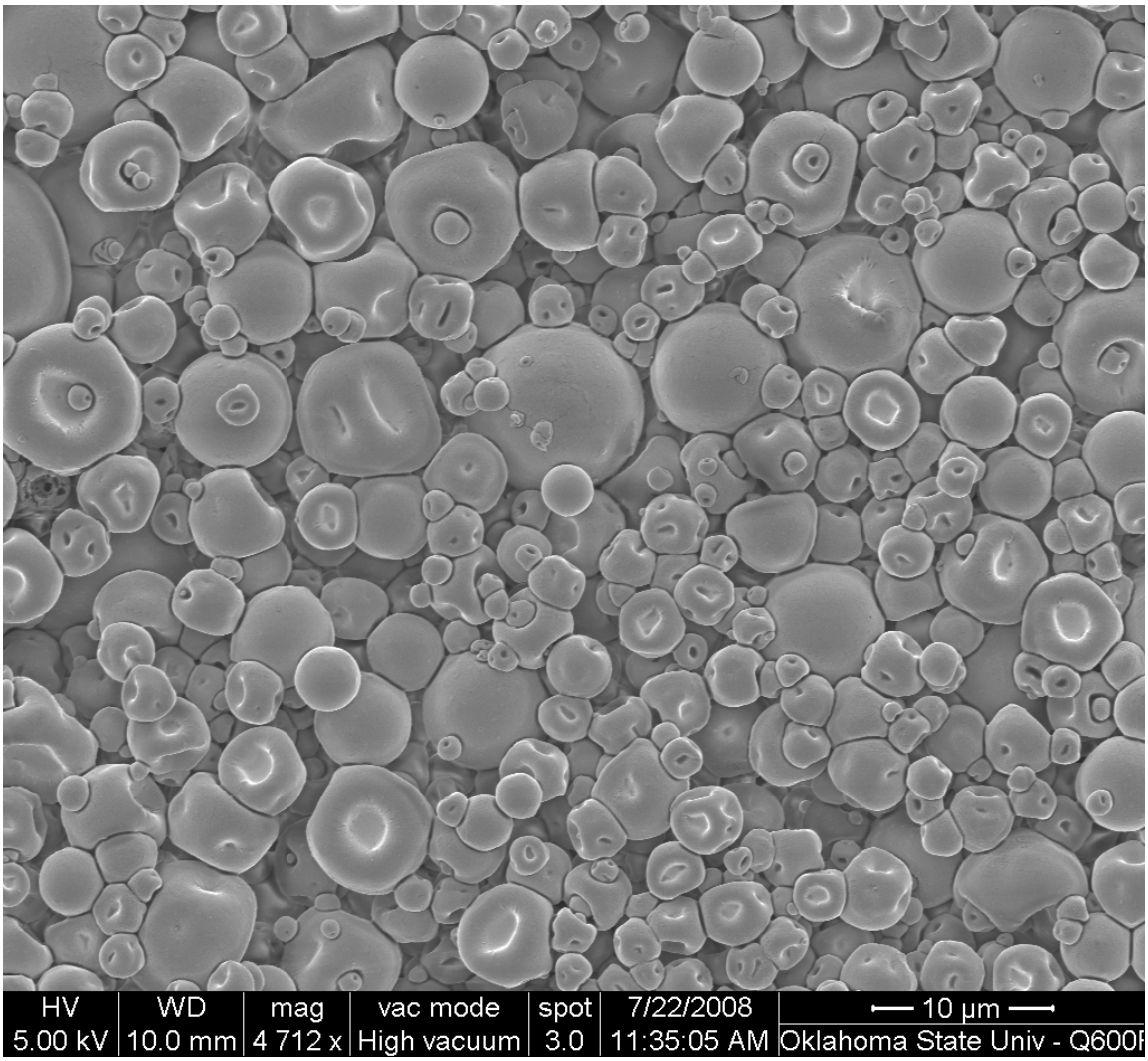
Picture 6: Freeze Dried microcapsules at 30,000 times magnification.



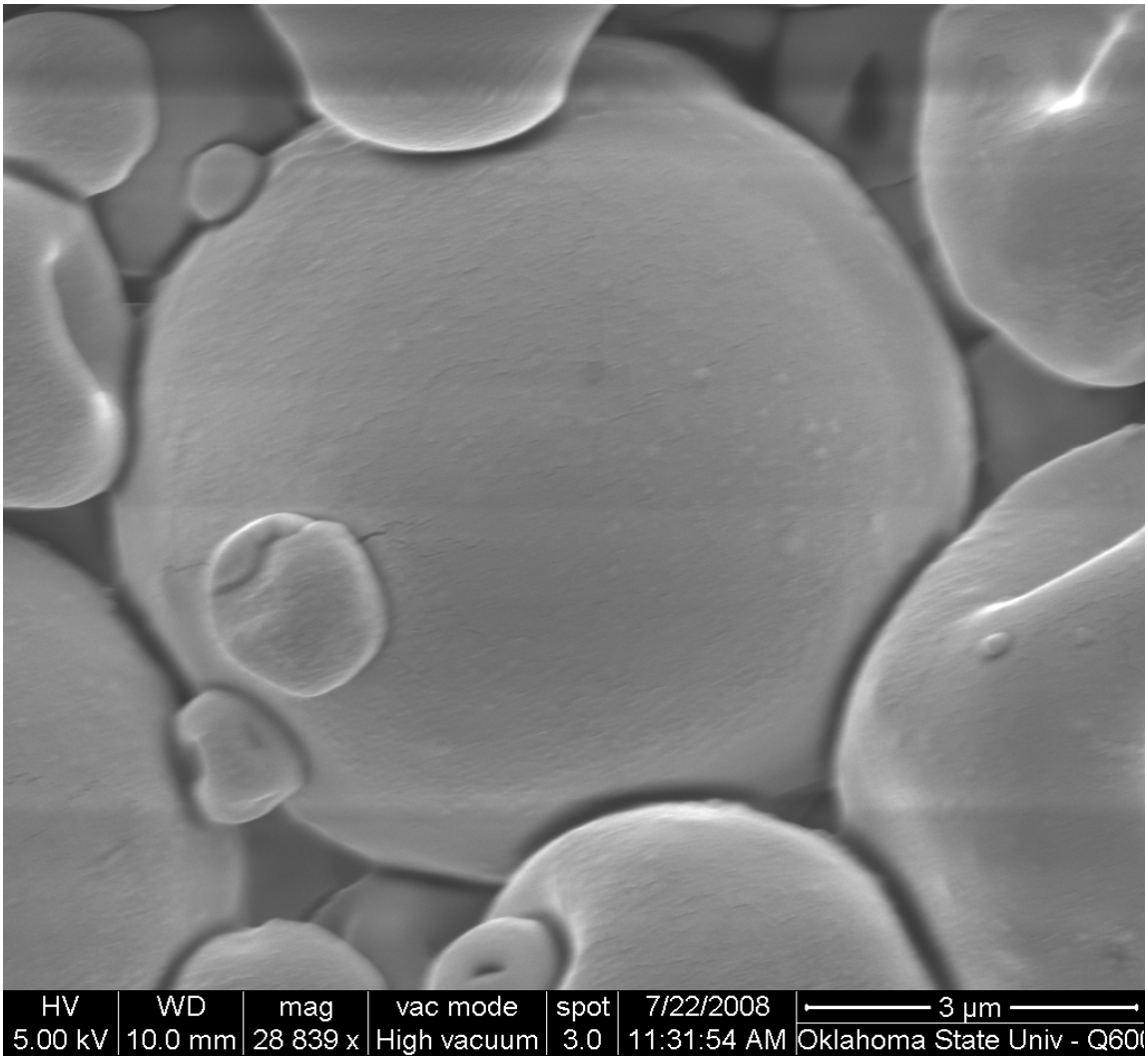
Picture 7: 2-Fluid Nozzle microcapsules at 1000 times magnification.



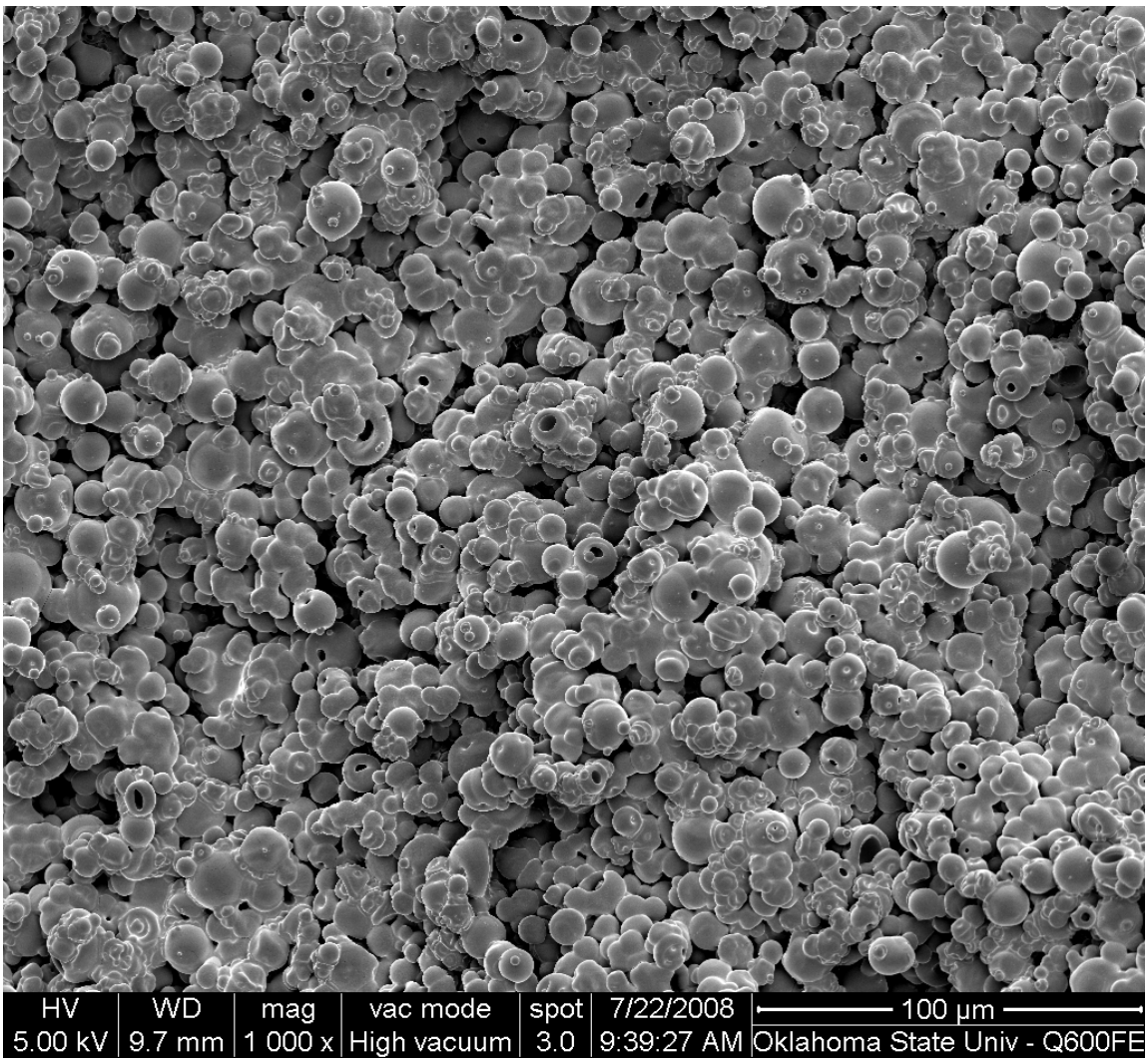
Picture 8: 2-Fluid Nozzle microcapsules at 5000 times magnification.



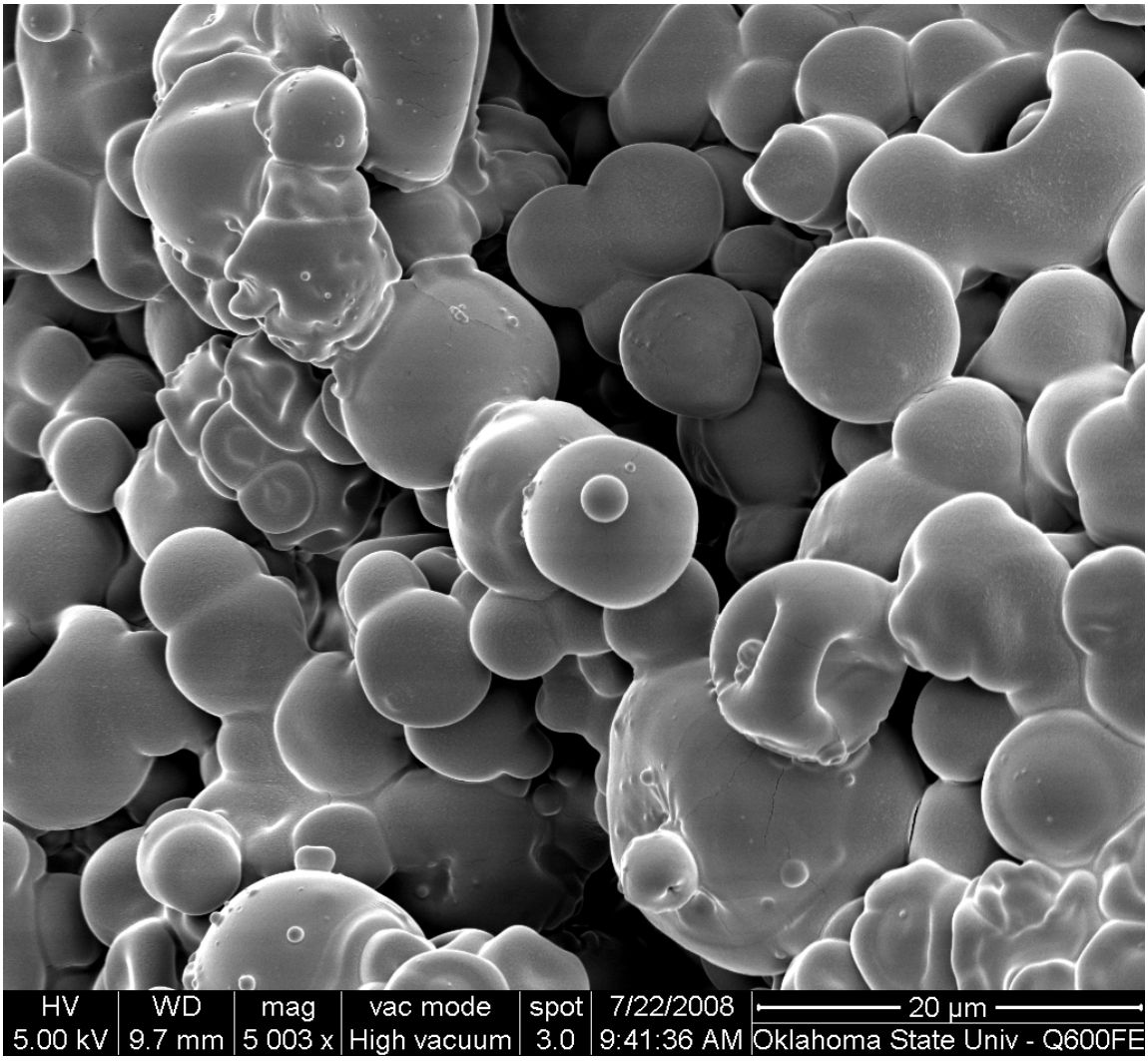
Picture 9: 2-Fluid Nozzle microcapsules at 30,000 times magnification.



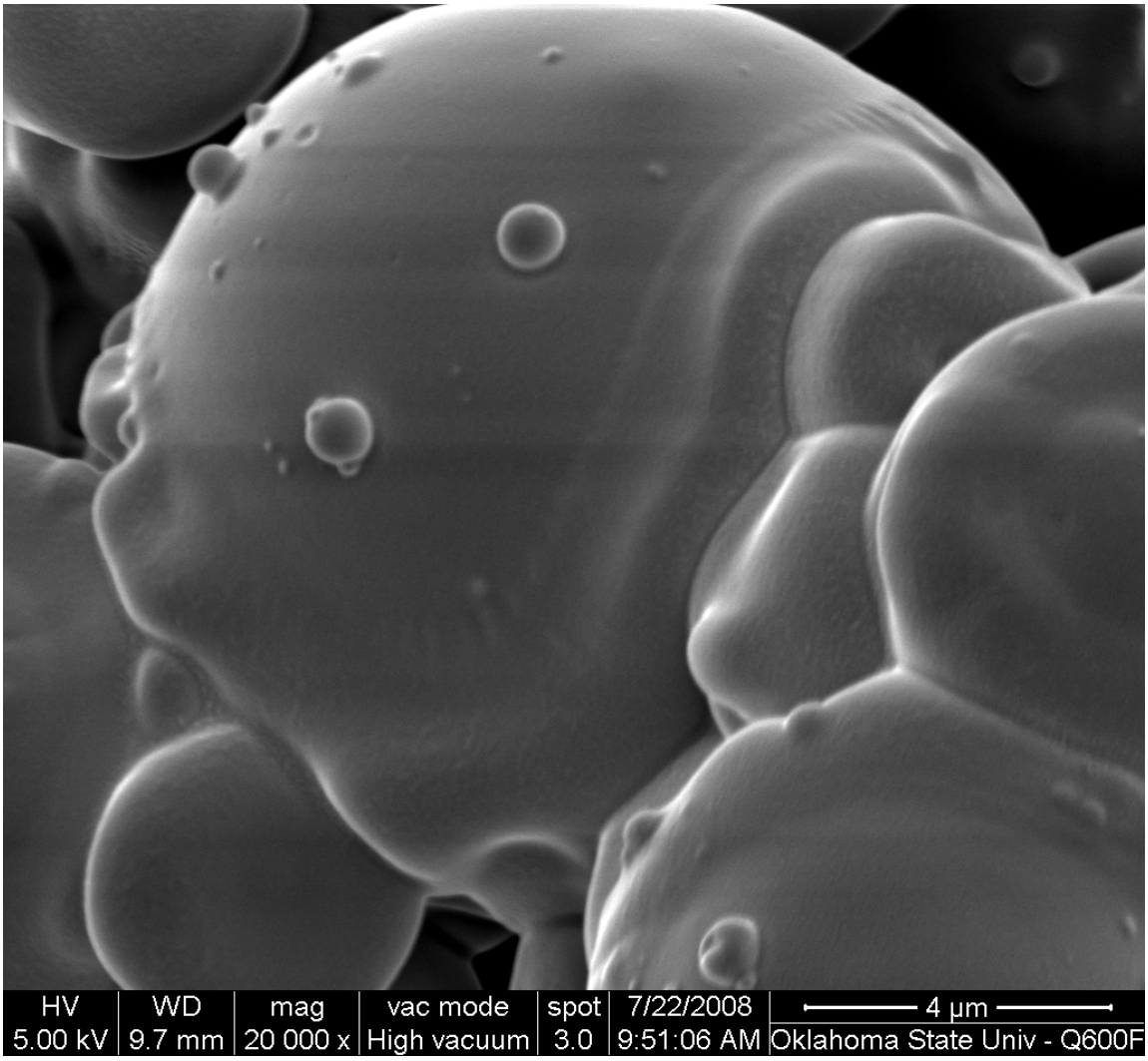
Picture 10: 3-Fluid Nozzle microcapsules at 1000 times magnification.



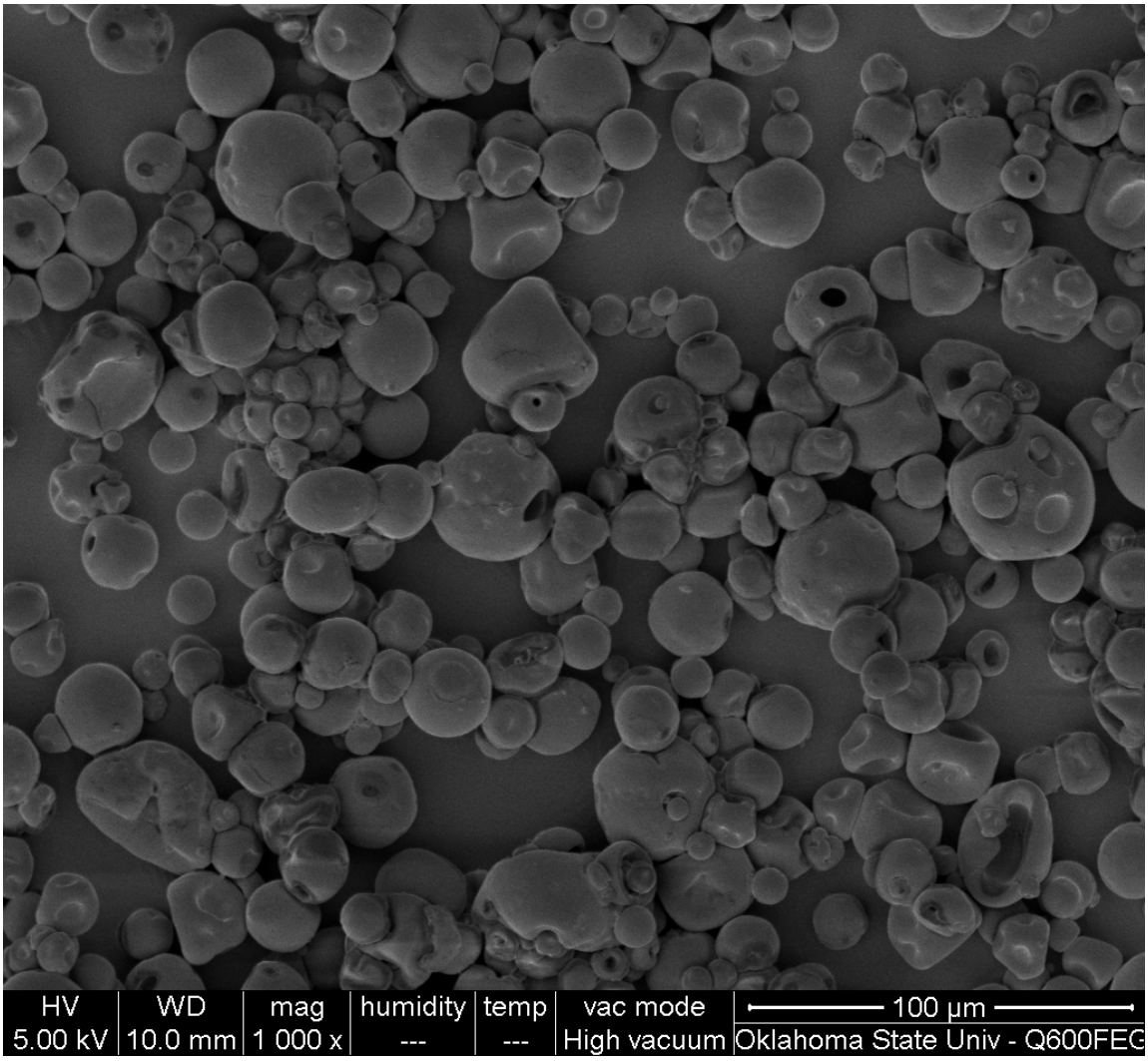
Picture 11: 3-Fluid Nozzle microcapsules at 5000 times magnification



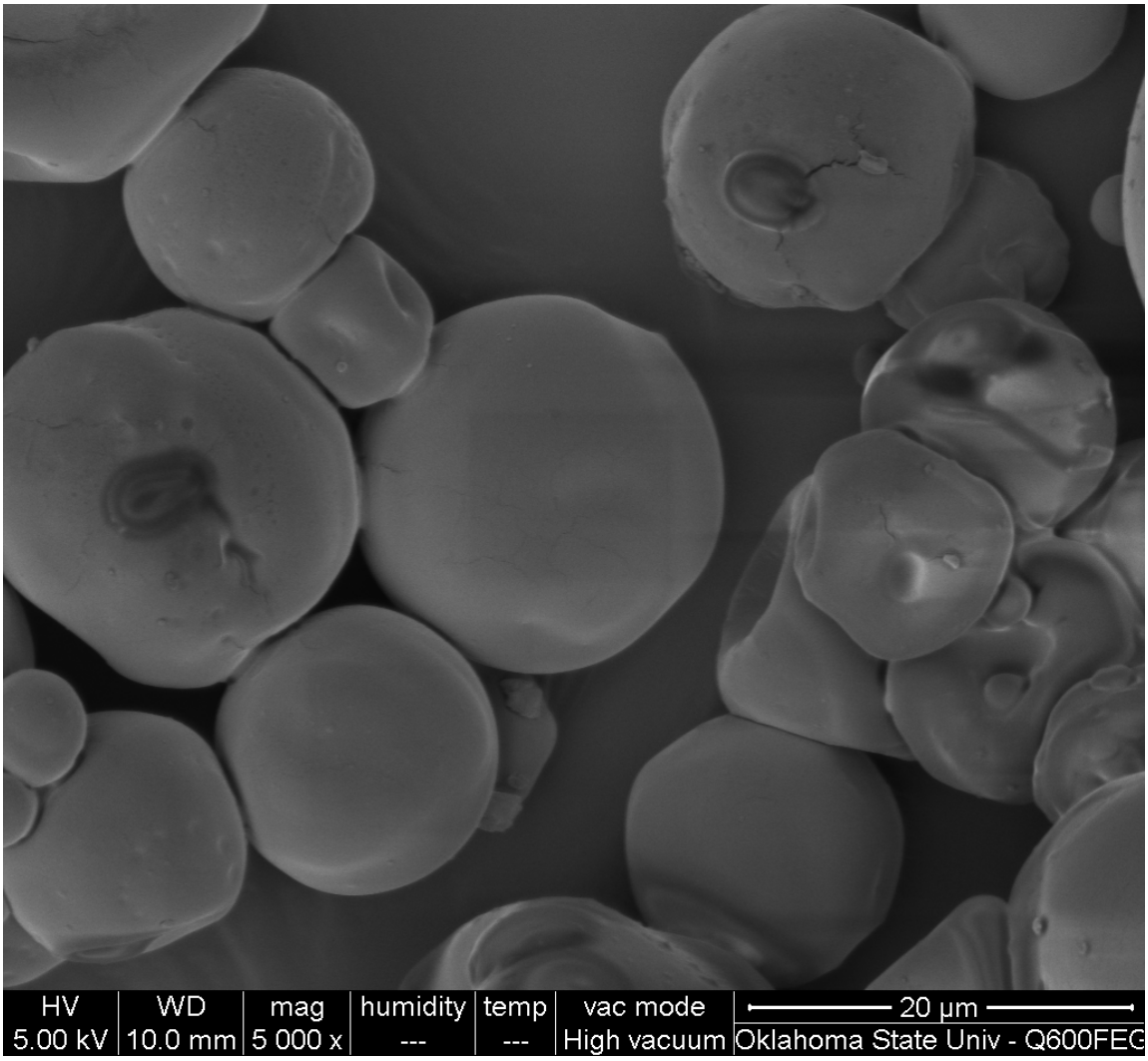
Picture 12: 3-Fluid Nozzle microcapsules at 30,000 times magnification.



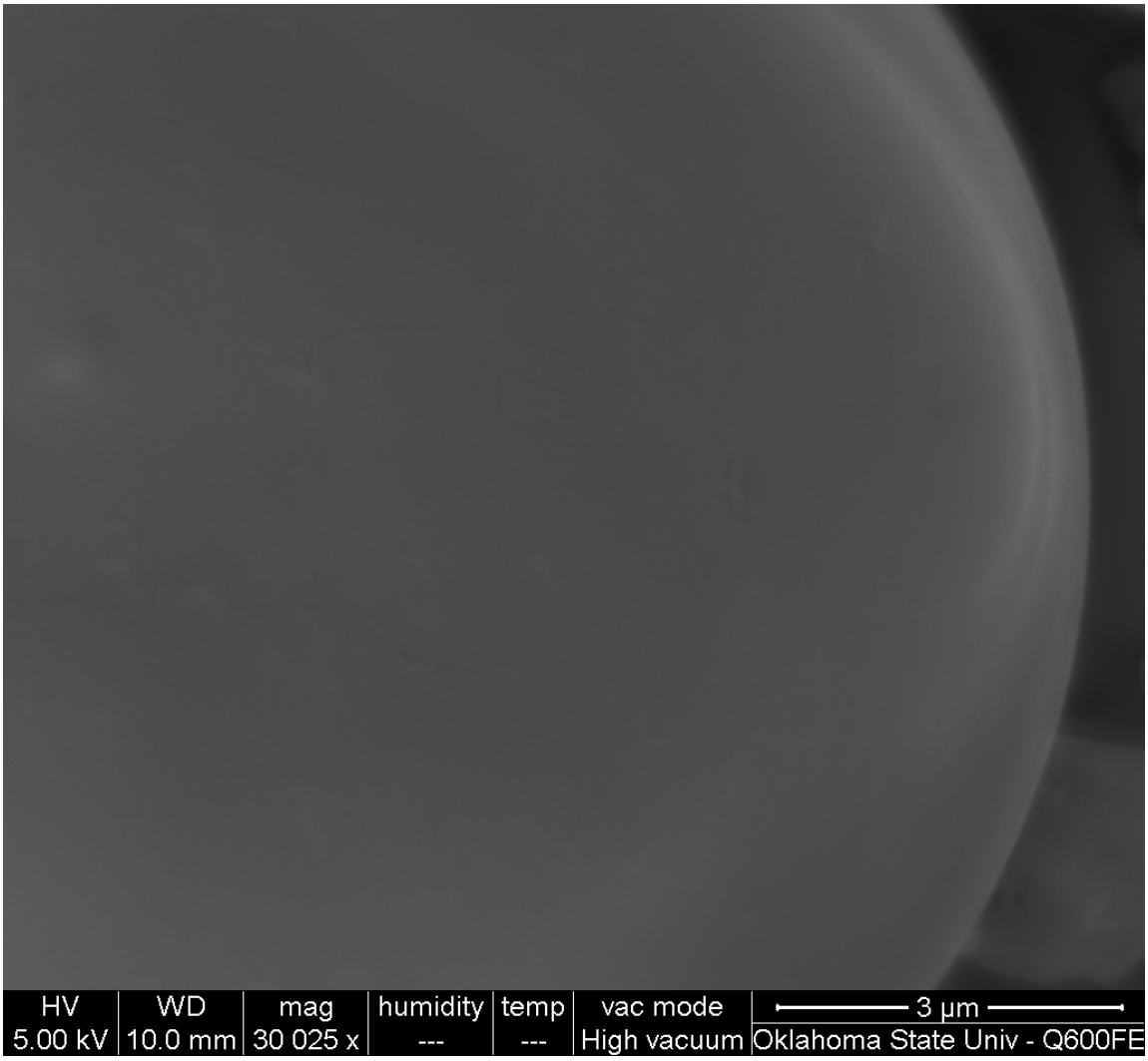
Picture 13: Ultrasonic Nozzle microcapsules at 1000 times magnification.



Picture 14: Ultrasonic Nozzle microcapsules at 5000 times magnification.



Picture 15: Ultrasonic Nozzle microcapsules at 30,000 times magnification.



VITA

JERRAD LEGAKO

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF PRODUCTION METHOD ON CHARACTERISTICS AND OXIDATIVE STABILITY OF MICROENCAPSULATED FISH OIL

Major Field: Food science

Biographical:

Personal Data: Born in Henryetta, Oklahoma, USA, on January 31, 1982, the son of Mr. and Mrs. Jospeh Legako

Education: Received Bachelor of Science degree in Biology from Texas Tech University, Lubbock, Texas in May 2005.
Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in December, 2008.

Experience: Employed by Oklahoma State University, Department of Biosystems and Agricultural Engineering as a graduate research assistant; Oklahoma State University, Department of Biosystems and Agricultural Engineering, 2006 to present.

Professional Memberships: Institute of Food Technologists, American Oil Chemists' Society

Name: Jerrad Legako Date of Degree: May, 2009

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: Effect of Production Method on Characteristics and Oxidative Stability of Microencapsulated Fish Oil

Pages in Study: 84 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study: The main objective of this study is to evaluate the effect of fish oil microencapsulation method on characteristics and oxidative stability. Four methods, freeze drying, spray drying by a 2 fluid pressure nozzle, spray drying by a 3 fluid pressure nozzle, and spray drying by a 2 channel ultrasonic nozzle were used in this research. Surface morphology of the microcapsules where determined by scanning electron microscopy. Total oil of the microcapsules was determined by soxtec and Rose-Gottlieb solvent extraction methods. Solvent extractable surface oil was also determined. Size of the microcapsules was determined by particle size analysis. Moisture content of the microcapsules was determined by Karl Fischer titration. Oxidative stability of the microcapules was determined over a 15 week period in which HS-SPME was used to analyze volatile oxidative compounds by GC-MS.

Findings and Conclusions: Comparison of chemical and physical characteristics revealed some differences. With regard to uniformity of size and shape, microcapsules produced by the 2-channel ultrasonic nozzle were observed to be more uniform in size and shape, determined by particle size distribution and SEM image comparisons. Disadvantages were also observed for ultrasonic nozzle microcapsules having lower oil encapsulating efficiency compared to pressure nozzles and freeze dried microcapsules. There was no observed initial advantage to spray methods that did not require the creation of an emulsion for microcapsule production. However, it was observed that microcapsules produced by multi-fluid nozzles propanal levels were lower throughout the course of a 14 week stability test. It should be stated that the 15 week sampling period may not have been long enough to adequately observe the induction of oxidation for all samples stored in the conditions chosen. Sample values fluctuated within a moderate range throughout the entire study. However, by observing propanal levels at the last data point of the stability study some conclusions may be drawn. At the end of the 5 ºC test PAR levels for fish oil and freeze dried samples were observed to have a significant increase while spray dried samples values remained steady. This may indicate the beginning of oxidation among the fish oil and freeze dried samples while spray dried samples were remaining stable.

ADVISER'S APPROVAL: Nurhan. T. Dunford