EFFECTS OF DRYING METHODS ON THE STABILITY OF 2,4-DECADIENAL ENCAPSULATED IN AN O/W NANOEMULSION

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ABSTRACT OF THE THESIS

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2,4-decadienal encapsulated in an o/w nanoemulsion

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The flavor industry has utilized many encapsulation methods in order to provide customers with stable flavors that maintain their integrity during various processing procedures. Savory flavors in particular have a unique hurdle to overcome, as they are subject to extreme temperature abuse, such as frying, baking, sautéing, and boiling. Highly sensitive compounds such as 2,4-Decadienal, that provide distinct characteristics to savory foods such as french fries and chicken, are particularly susceptible to change during these processes.

Using oil in water nanoemulstions of diameters between 20-800 nanometers as well as various drying methods to encapsulate volatile compounds have been an exciting avenue for flavor encapsulation. The present research will focus on the stability of 2,4-Decadienal using oil in water nanoparticles of medium chain triglycerides (Neobee) in addition to multilayer encapsulation; spray drying and freeze drying.

A slurry of 2,4-decadienal, maltodextrin, gum, Neobee and water were homogenized via high speed at 13,500 rpm and high pressure under 1500 bar to create a stable nanoemulsion. Half of the emulsion was then spray dried, while the remaining emulsion was freeze dried. Powdered finished samples were stored at 5°C (refrigeration), 25°C (ambient), 40°C (summer day), and 60°C (abuse/accelerated) for thirty days. Gas Chromatography with a flame ionization detector was used to measure ppm levels of 2,4-Decadienal in samples on appointed days. Mass Chromatography was then used to determine degradation compounds.

Freeze-dried samples yielded the best protection against oxidation, retro-aldol condensation, and over all degradation by as much as 50% amongst the widest range of shelf life temperatures. Higher temperature storage allowed twice as much development of degradation products, such as 2-octenal, hexanal, octanoic acid, hexanoic acid, and 2-nonenal compared to refrigerated storage. Freeze dried encapsulation faired the best at refrigeration temperatures due to its crystalline structure.

Medium chain triglycerides nanoparticles proved to be beneficial in retaining volatile compounds in conjunction with freeze drying. The higher heat, the longer the holding time, and the larger amount of oxygen present, accelerated 2,4-decadienal degradation in any encapsulation method. This study found that freeze drying a lipid nanoparticle with a volatile aldehyde, proved to be superior in retaining and preventing degradation compared to that of spray drying. Overall, the encapsulated samples retained 2,4-Decadienal significantly better than the unprotected reference. Future studies using various solid lipids should be examined.

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1 Introduction

The flavor industry has continually found innovative ways to provide their customers with high quality and long lasting flavors for various processed foods found in stores all over the world. Long lasting flavors is defined as a flavor that can maintain its intensity throughout its shelf life but also deliver continual flavor impact all the way through consumption. A high quality flavor is defined as a flavor that can withstand various processing techniques, such as heat, high shear and mixing without changing the delicate flavor profile or reacting with a customer's product formulation and still deliver to the end consumer. As food-processing techniques have advanced, so has the need for flavor development and stability throughout these processes. High temperature such as the case in frying and baking, high shearing, and a long shelf life are just a few of the obstacles that a flavor has to endure before being consumed.

Although there have been many advances in encapsulating flavor components, aldehydes, which are critical compounds in numerous flavor applications, have been a constant problem for flavor companies due to their volatility. Aldehydes, such as 2,4-decadienal, are very volatile, easily oxidized and have the ability to change a flavor profile from delicious to inedible.

2,4-Decadienal is known to contribute desirable aromas to many fried foods and meat products. However, it is also found in foods with stale and warmed over flavors. (Josephson et al., 1987) 2,4-Decadienal is also a major degradation product of linoleic acid, which is a main component of frying oils such as soybean oil and corn oil (Snyder et al., 1985; Patton et al., 1959). The off-notes associated with the decomposing of 2,4-decadienal can be attributed to reaction products of oxidation, Retro-Aldo, and thermal

degradation (Matthews, 1971; Josephson, 1987; Zhang, 1989). Hexanal, 2-octenal, hexanoic acid, heptanol, and octanoic acid have been identified as products and possible contributors of stale flavor deriving from the degradation of 2,4-decadienal (Matthews et al., 1971).

Preventing stale off-notes in foods due to flavor degradation has been widely researched and a proven technique is encapsulation. Encapsulation is the process of protecting specific compounds from the surrounding environment and other ingredients from foods (Rishch and Reineccius 1993). Flavors are bound in matrices made up of polysaccharides, gums or lipids, creating a barrier of protection from outside variables (Gharsallaoui et al., 2007). There are many methods and materials used in encapsulation technology in order to produce the desired protection needed for various products. 2,4-Decadienal specifically has been difficult to protect and will be studied in the following paper.

One-way of encapsulating flavor components is to spray dry, which is currently the predominant encapsulation process in the industry (Porzio et al., 2004). Spray drying takes a liquid product and atomizes it in a hot gas flow to quickly evaporate the liquid producing a stable powder (Gharsallaoui et al., 2007). Volatility and chemical reactivity of certain flavors containing 2,4-Decadienal will affect the final sensory character after spray drying, because of the high heat used (Porzio et al., 2004) This factor makes this method not ideal for aldehydes depending on the carrier, solvent, temperature, and emulsion type being used.

Freeze drying utilizes vacuum pressure vaporization to draw moisture out of a frozen emulsion there by leaving a powdered product. This process eliminates the

exposure to high temperatures commonly found in spray drying, eliminating reactivity of volatile compounds. Unfortunately, this process is currently expensive due to the amount of energy needed and is 30-50 times more time consuming than spray drying (Desobry et al., 1997).

Emulsions are utilized in both of the above drying methods as a prepatory step in which aroma compounds are partitioned between water and fat (Dumont and INRA 2006). Typically the lipids used in these emulsions are liquid at room temperature and emulsifiers are used in addition to homogenization in order to retain consistent water in oil or oil in water mixtures. 2,4-Decadienal is ideal in oil in water emulsions due to its high solubility.

Solid lipid nanoparticles (SLN's) are a new technique in encapsulating volatile aromas as an alternative to colloidal systems (Manjunath et al., 2005). Instead of using lipids such as medium chain triglycerides, fats such as palm kernel oil and coconut fat, which are solid at room temperature, provide an innovative protection barrier. In addition, SLN's reduce coalescence at room temperature during storage or prior to further processing, retaining constant nanoparticle size (Muller 2005). SLN's have been widely used in pharmaceutical and neutricitical products for controlled drug delivery (Muhlen et al., 1998). The following research will focus more on medium chain triglycerides to encapsulated 2,4-decadienal but will touch upon the use of SLN's for further studies.

Nanoparticles are in the submicron size range of 50-1000nm (Muhlen et al., 1998). This particle size can be achieved using high-pressure homogenization where particles of an emulsion are put under high pressure, shear and turbulence. Nanoparticles have the

potential to improve controlled release of flavor and stability compared to microparticles delivery systems with a particle size range of 10-1000μm (Mozafari et al., 2006).

Developing ways of encapsulating aldehydes such as 2,4-decadienal will assist in better protecting other volatile compounds in flavors from harsh processing conditions in order to deliver a high quality products to the consumer and help the bottom line of flavor companies. The goal of this research is to find the best drying method using lipid nanoparticles to provide stability of 2,4-decadienal against oxidation and to identify its degradation compounds. 2,4-Decadienal was analyzed after various encapsulation methods and storage temperatures using gas chromatography with a flame ionization detector. In addition, particle size analysis was conducted to correlate particle size with drying efficiency and stability.

2 Literature Review

2.1 2,4-Decadienal

Aldehydes are a class of compounds important in flavors such as bread, french fries, beer and various proteins such as beef, lamb and chicken (Feneroli, 1963; Brunton, 2002, Moyano, 2005). 2,4-Decadienal is a major contributing aldehyde to those flavors. 2,4-Decadienal is used universally in sweet, beverage, and savory flavors. At low levels of 1-2ppm, this aldehyde can provide citrus grapefruit notes and at levels of 10ppm desirable fatty fried aromas are attributed. 2,4-Decadienal is formed from the autoxidation of polyunsaturated fatty acids. Linoleic acid is the most abundant polyunsaturated fatty acid in fresh corn and soybean oil for example (Parliament et al. 1988). However, the cost of this raw material is expensive but essential in many flavor creations. In addition, aldehydes tend to chemically react with protein amino groups and result in irreversible binding as Schiff bases (Hansen and Heinis 1991, 1992). When this occurs, flavor perception decreases and off-notes can develop.

Much research has been done on the degradation compounds of 2,4-decadienal in various mechanisms such as autoxidation, retro-aldo condensation, and thermal reaction. Matthews et al. (1971) identified hexanal, 2-octenal, hexanoic acid, 2-octenoic acid, and other trace compounds as the autoxidative degradation products of 2,4-decadienal. It was found that oxygen affects primarily at the olefinic centers and that autoxidative cleavage between carbons two and three would produce 2-octenal. In addition, cleavage at carbons four and five would produce hexanal which is a key identifier of degraded 2,4-decadienal (Matthews et al. 1971). The presence of oxygen needs to be limited in order to preserve the flavor and aroma benefits of 2,4-decadienal in a flavored product.

Retro-Aldo condensation is yet another degradative pathway that yields distinctive chemical compounds. In the case of Josephson et al. (1987), 2,4-decadienal was degraded via retro-aldo condensation of water mediated alpha/beta double bond hydration to 2-octenal, which further formed hexanal and ethanal. This poses a problem with isolating procedures such as steam distillation or food applications where water is present since 2,4-decadienal is susceptible to nonoxidative water mediated degradation (Josephson et al., 1987). This study found the rate of retro-aldol degradations of 2,4-decadienal in an aqueous system was independent of oxygen but particularly accelerated with heat. Food systems that contain water and 2,4-decadienal are more susceptible to retro-aldo condensation.

Thermal degradation is a crucial factor in the degradation of 2,4-decadienal. Heat processing is in virtually every food product on the market and causes 2,4-decadienal to be susceptible to multiple pathways of degradation and in some cases accelerates them. Zhang et al., (1989) studied the volatile compounds formed from thermal exposure of 2,4-decadienal reacted with cysteine and glutathione, which are components commonly found in natural food materials. Up to forty-five volatile compounds were identified in this reaction and more specifically the cysteine interaction produced four times as many volatiles as glutathione (Zhange et al. 1988). The majority of the carbonyls were derived from thermal degradation of 2,4-decadienal, which further reacted via autoxidation (Farmer et al., 1943; Matthews et al., 1971; Michalski and Hammond, 1972; Schieberle and Grosch, 1981; Josephson and Lindsay, 1987). Spray drying, frying, baking, sautéing, and boiling, all of which have the potential of being high heat applications can affect the stability of this volatile compound.

2.2 Spray Dry Encapsulation

Spray drying is the most common and cheapest technique to encapsulate a food material. An encapsulation entraps a sensitive ingredient, such as 2,4-decadienal, in a coating "wall" to isolate and protect from the environmental (Desobry et al., 1997). Spray drying is a process by which a liquid product is atomized in a hot gas to instantaneously yield a powder (Gharsallaoui et al., 2007). Generally, an emulsified stable liquid is fed into a stream of hot air or an inert gas such as nitrogen, and is sprayed into a dry chamber immediately evaporating any liquid solvent.

There has been copious research done on the variables of spray drying, such as the inlet and outlet temperatures, flow rate of the drying air, and more importantly the carrier composition of the protective wall. The carrier should dissolve easily, be water-soluble, tasteless, odorless, inexpensive, and have excellent oil emulsifying properties (Porzio et al., 2007). Martinez et al., (2004) studied the optimal spray drier encapsulation process of orange oil, while Soottitantawat et al., (2004) researched the influence of powder size on the stability of encapsulated D-limonene by spray drying. The majority of the encapsulating research done has focused on micro encapsulation (0.2-5000μ) rather than nano-encapsulation (< 0.2μ or 2000Å) (King 1995).

Materials ranging from the pharmaceutical industry to the food industry to the cosmetics industry have unique compositions that need to be addressed and customized when spray drying in order to properly protect. In addition, surface space in ratio to particle size of the spray dry particles play a significant role in the stability of the product encapsulated. This research will study the spray drying stability of 2,4-decadienal using a nano-encapuslated approach.

2.3 Freeze Dry Encapsulation

Freeze drying was introduced to the food industry in the mid 1960's and utilized as a way to dry coffee while retaining it's delicate aroma (Petersen and Lorentzen 1973). Freeze drying, as a method of encapsulation has not been widely accepted in the flavor industry due to a high processing price. Desobry et al., (1997) found that freeze-drying is 30-50 times more expensive than spray drying. The theory of freeze-drying is similar to spray drying in that an unstable material is protected from the environment in a coating or wall with little moisture present. However, the major difference between the two is that freeze-drying has no heat associated with its method. This not only eliminates a key-contributing factor of degradation of volatile compounds it requires less processing over all. Freeze drying occurs at low temperatures from a frozen solid state, this avoids any water phase reactions and because of the vacuum most oxidation is evaded (Desobry et al., 1997). The process uses a stable emulsion solution identical to the solution prior to spray drying and is frozen anywhere from -10°C to -35°C where it is then placed under a vacuum to extract any water and solvent. This yields a solid mass, which needs to be further processed and broken down into a powder.

The typical polysaccharide used in both spray drying and freeze-drying is maltodextrin. Maltodextrin has many average molecular weights otherwise known as DE or dextrose equivalent values. Cheman et al., (1999) concluded that the use of low DE maltodextrins in a freeze-dried product provided a better encapsulation of the oriental fruit durian aroma than the use of high DE maltodextrins. In contrast, the use of high DE maltodextrins exhibited greater hygroscopicity leading to matrix instability and aroma release. Voilley and Rifai (1982) measured the loss of aroma from freeze-dried

maltodetrins under various relative humidity's and found a break down of the matrix occurred between 30 and 75% of relative humidity. The current research, however, will utilize one of the lower molecular weight maltodextrins with a dextrose equivalent (DE) of 10 for encapsulating 2,4-decadienal under various temperatures rather than humidity's. This particular maltodextrin carrier was chosen for this study as it is a widely used carrier for spray drying in the flavor industry, does not react with most flavor compounds and is relatively inexpensive.

2.4 Emulsions

Prior to spray drying or freeze drying, a stable emulsion needs to be produced using carriers that have strong emulsification properties and an effective homogenization technique (Vilstrup 2001). A typical food emulsion is an oil-in-water emulsion similar to mayonnaise or hollandaise sauce. However when encapsulating, an emulsion is where the active compound, such as 2,4-decadienal, is entrapped in a hydrocolloid solution, added to a large volume of water and homogenized (Risch 1995). 2,4-Decadienal is a perfect chemical to encapsulate using this technique due to its oil solubility and need for protection.

Various materials have been encapsulated using emulsions. One such study examined the stability of lycopene emulsions in skimmed milk, orange juice, and water (Ribeiro, et al., 2003). It was found that dissolving lycopene in an oil and water emulsion was a promising method to incorporate lycopene into water dispersible systems. In addition, little to no coalescence and aggregation of the lycopene-loaded emulsion were observed in the food (Ribeiro, et al., 2003). Lycopene encapsulation is similar to 2,4-

decadienal because of their volatile nature and need in various food products to either deliver health benefits or flavor perception respectively.

A unique class of emulsions is the nanoemulsions, which are a class of extremely small droplets that provide stability due to high interfacial tension and require lower amounts of stabilizers for their formation (Huang, 2008). This is beneficial in reducing material costs while increasing the protection of the encapsulate from water, air and other environmental conditions. The emulsifier used in a food nanoemulsion should be nontoxic, compatible with other materials, and capable of maintaining a stable emulsion with a minimum amount used (Manjunath, et al., 2005). It is this class of emulsions that will be utilized in the current research to encapsulated 2,4-decadienal in a nano-oil-in-water emulsion.

2.5 Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNP's) are an emerging technology in the food industry. It is already successfully used in the pharmaceutical and cosmetic industry to deliver bioactive compounds to specific sites in the body. Nanoparticles for flavor application have a particle diameter size of 20-800ημ. Nanoencapsulation has the potential of improved controlled release and improve solubility (Mozafari et al., 2005). A solid lipid nanoparticle is a lipid that is solid at room temperature such as coconut fat, palm kernel oil, rice wax, or hydrogenated oils, with a melting point ranging from 75°F to above 100°F. In addition, other lipids, phospholipids, triacylglycerols, waxes, fatty acids, or their mixtures can be used to form solid lipid nanoparticles (Reithmeier et al., 2001). These lipids are mixed with the material to be encapsulated in addition to a solvent, emulsifier, and polysaccharide carrier, forming an emulsion. In order to achieve the

nanoparticle size, the emulsion is typically high pressure homogenized under pressures ranging from, 1000-1500 bar (Muller-Goymann 2002).

Lipid particles have been an alternative colloidal delivery system allowing flexibility to the size of the particle in conjunction with high-pressure homogenization (Chambi, et al., 2007). Very little has been done in the flavor industry to utilize SLNP's to protect volatile flavor chemicals that undergo various harsh processing conditions. Manjunath, et al., 2005 examined various SLNP's prepared by high-pressure homogenization, for drug delivery systems and found that by using solid lipids, it dramatically reduced coalescence after reaching room temperature, had better physical stability and provided good mobility of the drug molecule.

Lipid based nanoencapsulation of antioxidants has also been studied in regards to their roles in food products. Mozafari, et al., 2006, employed techniques to enhance delivery and retention of antioxidants in cells and tissues. Mozafari found that reactive or sensitive micronutrients, similar to volatile flavor compounds, could be turned into stable ingredients through a nanoencapsulated carrier system. 2,4-Decadienal could be an ideal compound to encapsulate in this research study, due to its volatile nature and necessity in many flavor profiles. However the main goal of this research is to focus solely on medium chain triglycerides as the lipid encapsulate for 2,4-decadienal as one way of protecting this volatile aldehyde from temperature abuse in addition to either, spray drying or freeze drying. The following study can then possibly parlay the use of solid lipid nanoparticles in future studies.

3 Materials and Methods

3.1 Materials

2,4-Decadienal Natural (95% pure), heptanol (99.5% pure), octenal (94% pure), hexanoic acid (99% pure), nonenal (93% pure), octanoic acid (98% pure) and maltodextrin (10 DE) were provided by Firmenich Inc. (Princeton, NJ); Medium chain triglyceride (Neobee ^{TM} 1053 Stepan Co., Northfield, IL); Purity gum 2000 (National Starch, Bridgewater, NJ).

3.2 Preparation of Emulsion

Deionized water at a proportion of 58.0% was heated via a hot plate to 90 °C with agitation, while the emulsifier (purity gum 2000) at a load of 7.5% and maltodextrin at 29.0% were slowly added until completely dissolved. The mixture was then chilled for 12 hours/overnight under refrigeration temperature (5°C) to ensure a uniform and stable mixture. A 0.50% load of 2,4-Decadienal and 5.0% of the corresponding medium chain triglyceride (Neobee) were mixed together. Under high-speed homogenization (Ultra-Turrax T-25 basic, IKA Works, Inc., Wilmington, USA) at 13,500 rpm the chilled gum and starch mixture were placed in an ice water bath to maintain a temperature below 45°C, while the 2,4-decadienal and lipid mixture were added. High-speed homogenization was maintained for 15 minutes while the temperature was carefully monitored.

The homogenized mixture was then transferred to the Emulusiflex C-3 nitrogen induced high-pressure homogenizer (Avestin Inc., Ottawa, Canada) under 1500 bar or 20000 PSI, and homogenized for five cycles until finally being passed through an ice chilled copper coil in order to solidify the lipid. This processing step produces

nanoparticles for medium chain triglycerides (Neobee). The thermal circulator attached to the high-pressure homogenizer was set to a constant 45°C. The homogenized slurry was then split into two parts ready for spray or freeze-drying.

3.3 Spray Drier

The 2,4-decadienal encapsulated in the Neobee oil slurry was placed in the model Pulvis GB22 fluid bed spray dryer (Yamato, Santa Clara, Ca). The drying air was set to 0.43-0.45 m^3/min, air inlet was set to 110°C and the outlet temperature was set to 70°C. The powdered product was then collected from the filter chamber and a small portion was taken in order to measure particle size. The remaining samples were split into appropriate plastic jars in preparation for shelf life studies.

3.4 Freeze Drier

The slurry was placed in multiple plastic 50ml sterile centrifuge tubes and frozen overnight (-20°C). The following day the samples were removed from the freezer, which completely solidified the emulsified slurry and placed in the freeze dryer (Freezone 4.5, Labconco, Kansas City, MO), open to the system and under vacuum until all moisture was removed and a dry solid mass remained. All samples were collected and ground to a granular form using a bench top mortal and pestle and sieved through a #18 mesh to ensure uniformity. A small portion was taken in order to measure particle size and the remaining samples were then placed in plastic jars in preparation for shelf life study.

3.5 Particle Size Measurements

The average diameter and size distribution of the emulsified samples after high speed homogenization for Neobee were observed under dynamic light scattering particle size analyzer (Model 90 Plus, Brookhaven Instrument Corp., Holtsville, NY) at a fixed

angle of 90° at 25°C and then again after high pressure homogenization. Powdered samples after spray drying and freeze-drying were re-hydrated with deionized water and also measured for particle size under the above method. All samples were weighed at 0.5g and diluted with 10g of deionized water and measured in triplicate to ensure accuracy.

3.6 Shelf Life Studies

Approximately 40 grams of each powdered sample were placed in four separate opaque plastic jars, in order to eliminate light affects, with tight fitting lids and labeled. Both spray dried or freeze dried variables containing 2,4-decadienal encapsulated with Neobee were placed in refrigeration temperature of 5°C, room temperature of 25°C, warm conditions of 40°C and hot conditions of 60°C. The warm and hot conditions were maintained using a hot box set at the respective temperatures in a closed system. Small amounts of 1.5 grams were sampled from each jar at each temperature point at day zero, one, three, seven, ten, fourteen, twenty, and thirty for gas chromatograph testing.

3.7 Gas Chromatography Assay

An Agilent 6890 gas chromatographer with a flame ionization detector (FID) capillary column (Restek 10123 RTX-1) was used to quantify levels of 2,4-decadienal as well its degradation components in various samples. The capillary column measured 30.0 meters in length, 250.0 micrometers in diameter, and had a film thickness of 0.25 micrometers. The FID was set to 250°C with a hydrogen flow of 40.0 ml/min, an airflow of 450.0 ml/min, and used Helium as the gas type. Finally the split flow ratio was set 10:1 with a flow rate of 13.6 ml/min and an initial temperature of 240°C. A Hewlett

Packard automated sample injector was attached to the GC and injected 1.0 microliters of sample.

First steps of measuring a reference curve using only Decadienal and Dodecane were quantified in order to mark retention times as well as peak areas for future sample measurements. Chloroform was chosen as the solvent to extract volatile compounds from both Neobee and palm kernel oil in each sample. 1.5 grams of powdered sample was dissolved with 5ml of deionized water in a 50ml centrifuge tube for 1 minute using a vortex mixer. Subsequently, 10ml of chloroform and 750ppm of the internal standard Dodecane was added and mixed for another minute until there was a homogenous white cloudy mixture. The mixture was then centrifuged for 10 minutes to get good separation of particulates as well as water and chloroform. The clear chloroform layer was then extracted from the centrifuge tube and placed in a standard auto injector vial and sealed.

A single GC mass spectrometer of neat 2,4-decadienal was run on an Agilent 6890N with an Agilent 5973 mass selective detector (MSD), to identify chemical compounds. After the compounds were identified, neat reference samples of each identifying chemical were run on the FID GC under the same conditions as the testing samples in order to accurately classify the retention times and peak areas. All samples were run in triplicate to ensure accuracy. Data was collected for 2,4-decadienal and all major chemical peaks.

4 Results and Discussion

4.1 Particle Size Significance

4.1.1 Neobee High Speed & High Pressure Homogenization

There is a significant difference when comparing the lognormal mean diameter of Neobee after high speed homogenization and after both high speed and high pressure homogenization, as seen in Table 4.1 below. The sample after being high speed homogenized had a mean diameter of 572.4 nm while the sample after both processes had a mean diameter of 380.7 nm. It is apparent that during high pressure homogenization, particle size dramatically reduces due to the force of 20000 psi being placed on the emulsion in order to achieve the nanoparticle size. High speed homogenization, although effective as an intermediate step to create a stable emulsion for high pressure homogenization, clearly does not provide enough force to create nanoparticle droplets.

In Figure 4.1 it is important to point out that the distribution of particles, or the bell shape curves are slightly different for both high speed and high pressure homogenization. High speed homogenization leads to a slightly narrower curve than that of high pressure homogenization. This could be due to the fact that the rate of processing for high speed homogenization is greater than that of high pressure homogenization. It could be stated that by processing under high pressure homogenization for a longer period of time, could yield an even closer particle distribution, narrower curve, to the high speed homogenization curve.

Given the larger particle size of high speed homogenization, there is less surface area exposed over all for oxidation compared to the smaller particles of high pressure homogenization. In addition a larger portion of 2,4-decadienal is encapsulated in each

particle, which can possibly degrade at a faster rate than that of the smaller particle size of high pressure homogenization. Due to the fact that this study further encapsulates 2,4-decadienal using spray dry or freeze dry, degradation will be quantified only after the drying methods are implemented. The role of particle size in determining flavor retention is controversial. Reineccius & Coulter could find no effect of particle size on retention in their research, however it is often desirable to produce large particulates to facilitate rehydration, as small particles disperse poorly especially in cold water (Vilstrup 2001). The following research will analyze the benefits or pitfalls of a larger particle size in regards to degradation and flavor retention using 2,4-decadienal as the encapsulate.

Sample Type	**LOGNORMAL SUMMARY (mean diameter) nm**
NEOBEE HIGH SPEED HOMOGENIZED	572.4
NEOBEE HIGHT SPEED & PRESSURE HOMOGENIZED	380.7
NEOBEE SPRAY DRIED REHYDRATED	324.7
NEOBEE FREEZE DRIED REHYDRATED	519.5

Table 4.1 Lognormal mean diameter (nm) of each variable of processing using medium chain triglycerides (Neobee).

4.1.2 Spray Dried Versus Freeze Dried Particle Size Distribution

Particle size distribution is the relative amounts of particles present, sorted according to size (Jillavenkatesa et al., 2001). It is apparent in Figure 4.2 that the freeze-drying process had a larger lognormal mean diameter than that of the spray dried sample. The freeze dried sample had a tendency to coalesce either during the freezing process or when slightly thawed from a frozen state. The lipids have strong aggregate properties that even after grinding to a powdered form make for large particle sizes. Perhaps

because of the inefficiency of the freeze dry system being used, some temperature abuse occurred allowing the samples to thaw while being freeze-dried. The lipid structure of medium chain triglycerides such as that of Neobee are not as tightly packed as they are with solid fats such as palm kernel oil and therefore tend to come together easier when dispersed in a liquid.

Spray drying on the other hand, had an almost forty percent reduction in lognormal particle size mean at 324.7nm compared to freeze dried samples at 519.5nm. This could be due to the fact that the spray dried samples were immediately processed after high pressure homogenization and spray drying inherently forces the liquid product through a small nozzle causing smaller particle sizes.

Even more significant when comparing the two powdered forms, is when both are compared to that of the emulsion system after high pressure homogenization, as seen in Figure 4.3. All three samples have a similar bell shaped curve distribution, where no one curve is narrower than the other. However, the freeze dried sample has the largest particle size average compared to that of even high pressure homogenization. In fact the spray dried sample at a mean of 324.7nm is relatively close in size to that of the high pressure sample with a mean of 380.7nm. It can be stated that spray drying further reduces particle size, in contrast to freeze drying which increases particle size due to the nature of each process.

Spray drying yields similar particle size distribution as well as mean diameter compared to that of high pressure homogenization. Whereas, the freeze dried sample produced larger particle sizes over all but had a similar distribution curve compared to

that of the other two samples in Figure 4.3. 2,4-Decadienal retention and its correlation with particle size and distribution will be discussed further in this research.

4.2 Stability Storage Results

4.2.1 Stability of 2,4-Decadienal Encapsulated in Neobee

Freeze dried samples of the encapsulate Neobee had the best overall retention of 2,4-decadienal across the four temperatures over the thirty day shelf life study. In Figure 4.5 all four temperature studies show trends of degradation relatively close to each other. When looking at each trend line, 5°C had the most 2,4-decadienal retention, following 25°C, 40°C, and 60°C. Looking at the 30-day shelf life mark, there is only about a 100ppm difference of 2,4-decadienal retention from 5°C compared to that of the hotbox temperature of 60°C.

In comparison, Figure 4.4 shows samples of spray dried Neobee encapsulate with a much wider but even spread amongst each shelf life temperature. However, the trend remains the same in regards to, the lower the temperature, the higher the retention of 2,4-decadienal. Again, when looking at the 30-day shelf life point of 5°C compared to that of the 60°C in Figure 4.6, there is almost a 200ppm difference. This is twice the reduction compared to that of the freeze-dried Neobee retention discussed above. Freeze-dried maltodextrin particles tend to be more regular, thinner, and smoother (Desobry et al., 1997). This reasoning could be why the freeze-dried samples showed better than that of the spray dried samples that have smaller particle sizes. Clearly for a medium chain triglyceride, freeze-drying provides better protection for its encapsulated material and therefore yields more retention of volatile compounds in all temperature storage conditions.

4.2.2 Shelf Life Storage at 5°C and 25°C

Typically 2,4-decadienal neat is stored in refrigeration temperature in many flavor companies in order to preserve its shelf life and prevent off notes from developing. In Figure 4.6, the freeze-dried sample fared better at retaining 2,4-decdienal at the end of the 30 day shelf life study by 50ppm compared to that of the spray dry. This could be due to the fact that the crystalline matrix of the freeze dry had better protection qualities in cooler temperatures than that of spray drying. What is more significant is the rate, or curve contrast of the two samples. Freeze dried lost 2,4-decadienal at reduced rate with an almost flat curve over the 30 day period, while the spray dried sample had a steeper curve which symbolizes a more dramatic rate or reduction over time. The reference curve, however, showed a higher overall retention of 2,4-decadienal compared to both samples due to the fact that this was the ideal storage temperature for this aldehyde. Nevertheless, this study focuses on the elevated temperatures of food processing in order to protect volatile compounds and not cold temperature food products, which would not need encapsulated forms of 2,4-decadienal. Cold storage comparisons still play a considerable role when comparing various drying methods and as a baseline to elevated temperature studies.

In contrast, Figure 4.7 shows lower overall concentrations of 2,4-decadienal in all samples, including the reference sample. The same trend can be seen here in regards to the rate of reduction, where the freeze dried sample has an almost flat curve with an ending concentration of about 275ppm. The spray dried curve again has a steeper drop, indicative of a faster rate of 2,4-decadienal reduction with an ending concentration of about 200ppm. Even at room temperature, the reference sample of neat 2,4-decadienal

loses a considerable amount over the 30 day period, almost matching the final concentration of the freeze dried sample. It can be inferred that unencapsulated 2,4-decadienal stored at 25°C over a longer period of time, over 30 days, does not retain as much as freeze dried encapsulated samples. It is apparent that with the slight twenty degree elevation in storage temperature the overall concentration of 2,4-decadeianl also proportionately dropped compared to that of samples stored at 5°C. In both cases, the freeze dried samples performed and protected better than that of the spray dried samples in retaining 2,4-decadienal.

4.2.3 Shelf Life Storage at 40°C and 60°C

It is important to focus on the elevated temperatures in order to examine what type of retention is maintained at slightly higher than room temperature, similar to that of summer at 40°C and an abuse temperature of 60°C. As discussed above, it is apparent to note the trends at extreme storage temperatures of 5°C and 60°C. However, when looking at Figure 4.8, it is clear that freeze dried encapsulation retains 2,4-decadienal better than that of spray dried samples. This could be due to the fact that the particle sizes of freeze-dried samples tend to be larger and therefore have less over all surface area then the smaller particles of spray-dried samples. Also, the minimal processing of freeze-dried samples could help retain 2,4-decadienal better than the harsher spray dry techniques used. The crystalline structure of freeze-dried samples allow for less amorphous regions for aroma compounds to migrate and reach the surface, where they can be degraded via oxygen, heat, light, and moisture (Voilley et al,. 2006).

In Figure 4.9 a similar distribution is observed of 2,4-decadienal retention, however, the curve positioning was shifted down compared to Figure 4.8. This

represents a less overall retention at 60°C compared to that of 40°C, even though the rate and proportion of degradation between spray dried and freeze dried were similar. Neobee freeze-dried seemed to have maintained its integrity as a matrix barrier for 2,4-decadienal compared to its spray-dried counterpart. In contrast, all encapsulated samples, Neobee, spray-dried or freeze-dried proved to have better retention and protection against loss then un-encapsulated 2,4-decadienal under both 40°C and 60°C.

4.3 Degradation Products and Possible Pathways

4.3.1 Autoxidation of 2,4-Decadienal

The main products of 2,4-decadienal autoxidation are hexanal and 2-octenal as found by Sulzbacher et al., (1960). The same was found in this study. As seen in Figure 4.10, a mass spectrum was run on neat 2,4-decadienal in order to identify degradation compounds. After which, reference samples of the same compounds were run on a GC with an FID in order to classify retention times and compare to test samples, this can be seen in Table 4.2.

When looking at Figure 4.11, hexanal development was more significant in the spray dried sample versus the freeze dried encapsulate at 60°C for 30 days. This is most likely the result of 2,4-decadienal degradation as being exposed to atmospheric oxygen while in storage. Furthermore, in Figure 4.18 as oxidation of hexanal occurred, hexanoic acid developed in only the spray dried sample, proving the degradation of the spray dry sample was more severe. This trend can be seen clearly in Figure 4.19 where the samples were stored at a harsher 60°C. Only after day 14 at such a high temperature did the freeze dried sample develop hexanoic acid, where none was developed at 40°C. Again, the freeze dried sample held up better in such high temperatures and had less barrier

breakdown than that of the spray dried sample. In addition, 2,4-decadienal retention was better in the freeze dried sample as seen in Figure 4.12. Both isomers of 2,4-decadienal were detetcted at more than double the concentration in the freeze dried sample compared to that of the spray dried. This is indicative of a better encapsulation method, provided by freeze drying.

Reference Name	Retention Time	Peak Area	Retention Time	Peak Area
Hexanal	4.049	130.2	4.05	132.9
1-Heptanol	6.183	239.1	6.185	237.6
Hexanoic Acid	6.348	161.9	6.461	160.7
Limonene (Levo)	7.181	291.2	7.187	286.5
2-Octenal	7.338	243.6	7.338	244
Nonanal Nat	8.038	201.1	8.038	201.1
Methyloctanoate	8.349	203.1	8.3448	204.2
Trans-2-Nonenal	8.79	313.3	8.789	315.9
Octanoic Acid	9.051	248.7	9.178	250
Cis-4-Decenal	10.207	344.6	10.207	338.5

Table 4.2 Retention identification times of degradation compounds.

Secondary proof of spray drying not holding up as a protective encapsulation can be seen in Figure 4.15. Clearly Octanoic Acid development was highest in the spray dry system. In Figure 4.19 the largest levels of hexanal can be found in the spray dried sample at 60°C at day 3, then followed by the spray dried variable at 40°C on day 7 in

Figure 4.18. Surprisingly, in both Figures 4.18 and 4.19, both samples had a slight increase in hexanal early in the study, which then consistently decreased by day 30. Hexanoic acid did not start developing until day 14 at the 40°C temperature. This could be contributed to the maximum degradation occurring of what small amount of 2,4-decadienal was unencapsulated at day 7 and 3 respectively, then flashing off or further converting to hexanoic acid slowly as hexanal is a volatile aldehyde.

When comparing Figure 4.18 with Figure 4.19 the significant observation is the development of hexanoic acid towards the end of each shelf life. At 40°C hexanoic acid only developed in the spray dried sample but in Figure 4.19 hexanoic acid developed, although a small amount, in both the freeze dried and spray dried sample. This temperature point could be the threshold limit of the freeze dried matrix in maintaining its integrity as a protective barrier. It is important to state that 60°C is not a typical industry condition in which to store any flavors, liquid, paste, or powders under. This temperature was chosen in order to accelerate the shelf life study and examine each samples behavior. This can be clearly seen in Figure 4.13, where it is apparent that the sample stored at 60°C for 30 days has significantly larger peaks of hexanoic acid, octanoic acid, methyloctanoate and hexanal compared to the same spray dry sample stored at 5°C for 14 days. The warmer and longer the storage temperatures are, the higher rate of degradation compounds are present as well as a decrease in 2,4-decdienal retention is observed as seen in a close up view, in Figure 4.14.

The autoxidation of 2,4-decadienal has been found to cleave the carbon atoms four and five producing hexanal and when cleaving the double bond between carbon atoms two and three, yields 2-octenal (Matthews et al., 1971). The current research has

found that both samples using Neobee as an encapsulate to protect 2.4-decdienal shield against autoxidation better than if unencapsulated. Also, the larger particle size of the freeze dried sample seem to show a better resistance to autoxidation and retain 2,4-decadienal more efficiently. Further reactions of hexanal will yield 2-octenal, which is another important marker in determining 2,4-decadienal degredation.

2-Octenal, another product of autoxidation of 2,4-decadienal was abundantly found in both samples being tested at 25°C and 40°C. However, the freeze dried sample again proved to be a slightly better barrier for 2,4-decadienal against oxygen compared to spray dried at 25°C as seen in Figure 4.16 but the opposite was found at 40°C at day 4 as seen in Figure 4.17. The spike in 2-octenal in the freeze dried sample at day 4 could be caused by a breakdown in a pocket of the freeze dried matrix which allowed the 2,4-decdienal encapsulate to interact with heat and oxygen or perhaps an inferior batch, creating an outlier in the data. Overall at both storage temperatures the common trend was a larger development in Octenal in the spray dried sample. In Figure 4.16, the lowest levels of 2-octenal development came from the Neobee freeze-dried sample. In this case, particle size or the encapsulation method were not as of a significant factor in 2,4-decdienal protection, due to the fact that the concentration between the two were a difference of only 1-2ppm.

4.3.2 Retro-Aldo Condensation

In relation to autoxidation, retro-aldol condensation yields similar products when degrading 2,4-decadienal. Josephson et al., (1987) found that 2,4-decdienal was degraded to 2-octenal and ethanal by a water mediated alpha/beta double bond hydration, retro-aldol condensation reaction series. In addition, the similar degredation of 2-octenal

resulted in the formations of hexanal and ethanol. These retro-aldol related degradations of 2,4-decadienal in an aqueous system was independent of oxygen but greatly accelerated with heat (Josephson et al., 1987). In the current research, heat was the defining variable to whether 2,4-decadienal would degrade. Again when looking at Figure 4.16 2-octenal levels are clearly higher in the spray dried sample when stored at room temperature. Interestingly enough, another indicator of 2,4-decadienal degradation is the presence of 2-nonenal. In the case of Figure 4.16, 2-nonenal levels in both samples matched the concentration level of the freeze dried sample. Although 2-nonenal can be used as a good indicator of degradation, in this experiment it did not show significant differences in the samples to quantify its validity. Although relative humidity was not controlled in this experiment, the containers the samples were stored in were not air tight and therefore could be susceptible to the changes in humidity.

The combination of retro-aldol condensation with autoxidation could explain the increase of hexanal on day 7 of Figure 4.18 and then a slow decrease over the 30 day shelf life. As the unencapsulated 2,4-decadienal degrades via heat, humidity, and oxygen, a variety of pathways can yield hexanal and 2-octenal. Specifically, 2-Octenal should be formed non-oxidatively only through alpha/beta double-bond hydration but continued degradation would result in the formation of hexanal and ethanol.

4.3.3 Neobee Degradation

Neobee as a medium chain triglyceride (MCT) is derived by esterifying glycerol with a mixture of caprylic (C:8) and capric (C:10) fatty acids which are fractionated from coconut or palm kernel oils and hold superior oxidative stability (Stepan Co. 2008). It is then composed of 68% octanoic acid, 30% decanoic acid and traces of other various acids.

By looking at Figure 4.15 and 4.17 it is clear that octanoic acid is a compound found in Neobee based samples. However, when looking at Figure 4.10 which is a mass spectrum of degraded neat 2,4-decadienal, octanoic acid is clearly a compound found in its degradation, whereas hexanoic acid is not normally found. Neobee degradation could be caused by oxidation which is accelerated with the addition of heat. Elevated temperatures can break down the fatty acid structure leading to rancidity. In Figure 4.15 it is clear that octanoic acid does not start to develop in any of the samples until day 7 at a shelf life temperature of 60°C. By the end of the 30 day shelf life it is clear that Neobee spray dried yields the largest amount of octanoic acid with Neobee freeze dried coming in second with 50% less.

In regards to protection against oxidation, the Neobee spray dried sample does not seem to protect as well as its freeze-dried counterpart. It is apparent that Neobee being composed of 68% octanoic acid yields more of this compound that can lead to rancidity and degradation, which makes it a good indicator for 2,4-decadienal.

5 Conclusions

In this research, medium chain triglycerides were used to encapsulate 2,4-decadienal in lipid nanoparticles followed by either spray drying or freeze drying. A shelf life study was conducted to determine the quantity of 2,4-decadienal retained in each sample after 30 days under 5°C (refrigeration), 25°C (ambient), 40°C (typical summer), and 60°C (accelertated test), in order to identify which combination of processes and material would be optimal in addition to quantifying specific degradation products.

Neobee freeze-dried samples yielded the best protection against oxidation, retro-aldol condensation, and over all degradation amongst the widest range of shelf life temperatures. In regards to particle size, freeze-dried samples with their larger particle size proved to be better protection versus the smaller particles of spray-dried samples. It was apparent that the higher temperature storage allowed severe development of degradation products, such as 2-octenal, hexanal, octanoic acid, hexanoic acid, and 2-nonenal. This particular aspect of the research might not be true to industry standards as the majority of flavors are stored at ambient or cooler temperatures. Nevertheless, freeze dried encapsulation technology faired the best at refrigeration temperatures due to its crystalline structure and solid physical state. However, for this research, accelerated testing was needed for dramatic results.

Medium chain trigylcerides being used as a lipid nanoparticle proved to be beneficial in retaining volatile compounds such as 2,4-decadienal in conjunction with freeze drying. Although the particle size of the freeze dried sample were larger than that of spray dry, they were still in the nanometer range and does not necessarily prove that a larger particle size equates to better protection properties. Rather, nanoparticles assist in providing a high quality preliminary encapsulation in order to process further using a drying method as the secondary encapsulation. The higher heat, the longer the holding time, and the larger amount of oxygen present, all accelerate 2,4-decadienal degradation in any encapsulation method. This study found that freeze drying a lipid nanoparticle with a volatile aldehyde, such as 2,4-decadienal proved to be superior in retaining and preventing degredation compared to that of spray drying. Although spray drying is practiced industry wide as an inexpensive and efficient way of drying and encapsulating a

flavor, freeze drying technologies should be looked at if monetary capital was of no concern and an even higher quality product was desired.

Flavor loss associated with high temperature processing and cooling is a typical problem in the industry. Utilizing the successful techniques of solid lipid nanoparticles employed by the pharmaceutical and nutrition industry to flavors is a promising avenue to research. Most flavorings are encapsulated in a water-soluble polymer and are then released easily with the addition of water. Encapsulating a flavor in a solid lipid nanoparticle would slow its release and offer some protection and prevent changes in the delicate flavor profile. Nanoencapsulation to protect 2,4-decadienal could be a way to prevent off flavors, increase shelf life, and provide an alternative to typical microencapsulation methods.

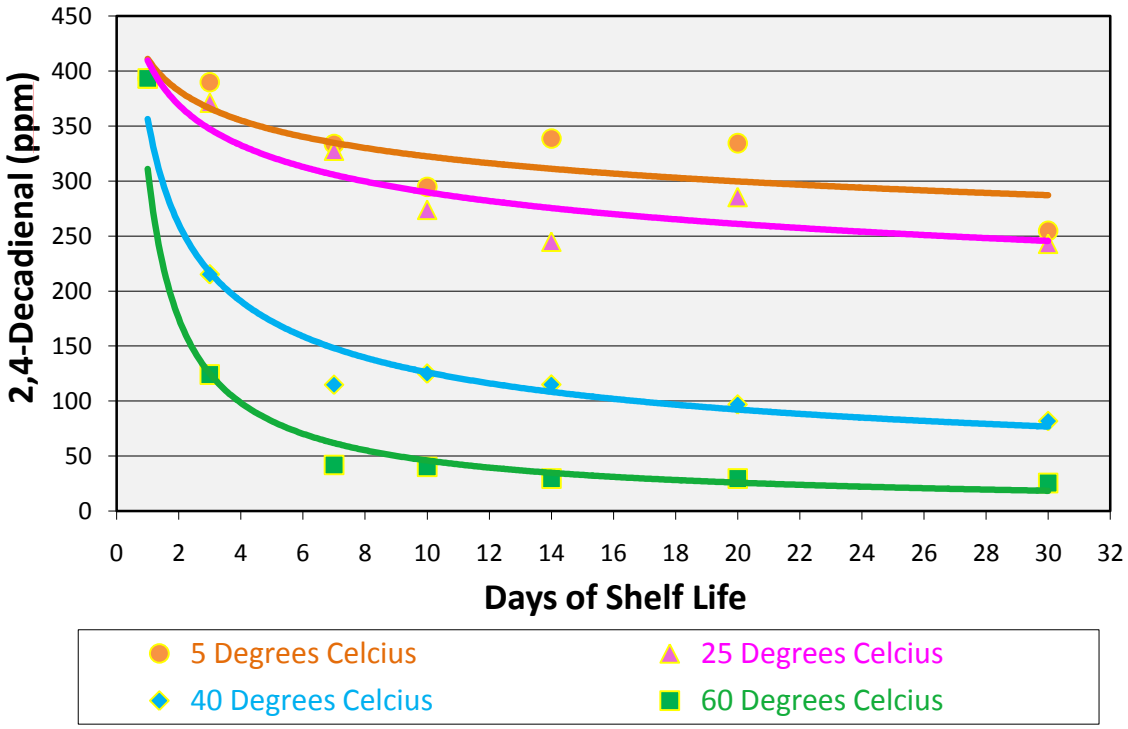
6 Future Studies

6.1 Preliminary Results of Palm Kernel Oil

6.1.1 Stability of 2,4-Decadienal Encapsulated in Palm Kernel Oil

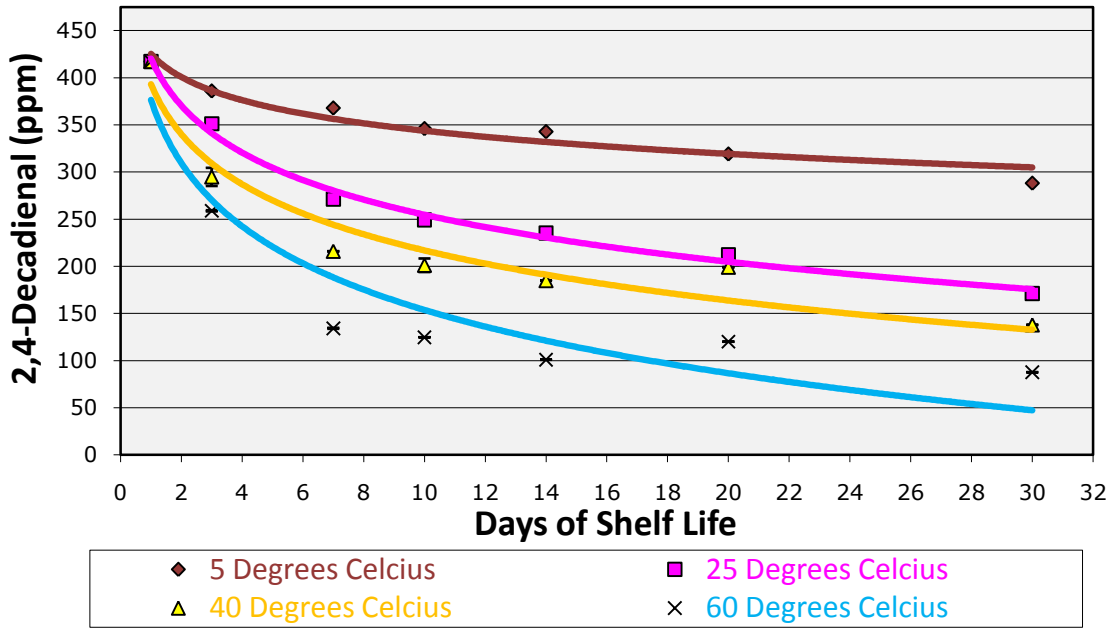
The same pattern that Neobee showed was not the case with palm kernel oil. In Graph 6.1 there is a clear trend that the cooler storage temperatures of 5°C and 25°C maintained the majority of 2,4-decadienal. However, when looking at the curves of 40°C and 60°C there is a large drop of retention, with 60°C loosing almost all traces of 2,4-decadienal. Palm kernel oil although solid at room temperature has a melting point of 40°C. The loss in 2,4-decadienal at this shelf life temperature and warmer confirms that freeze dried palm kernel oil in its powdered state melts at these temperatures and exposes the encapsulated material to both oxidation and degradation. It is important to point out that at the 5°C storage, both freeze dried palm kernel oil and Neobee retained equal

amounts of 2,4-decadienal. Only at higher temperatures, was there a significant difference in how each lipid performed. Although these storage temperatures do not reflective industry practices, it is safe to assume that a volatile compound encapsulated using SLN of palm kernel oil is beneficial for protection but cannot be temperature abused and stored in a cool facility.



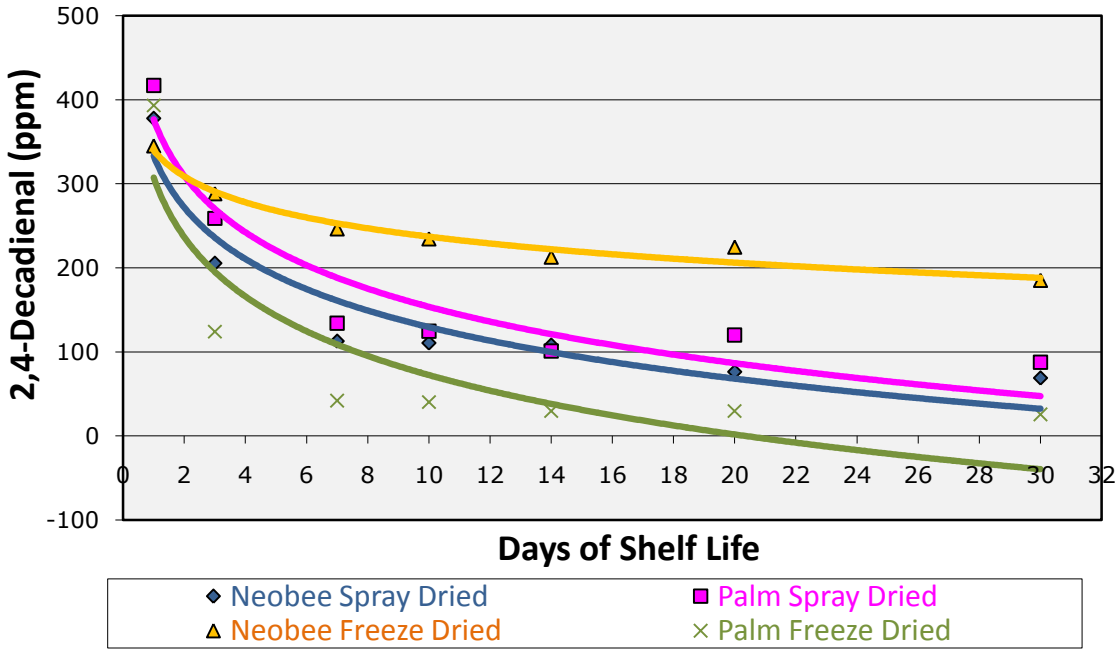
Graph 6.1 Freeze dried 2, 4-decadienal encapsulated in palm kernel fat; Shelf life study.

Spray dried palm kernel showed a typical ratio lose of 2,4-decadienal as the storage temperature increased, as seen in Graph 6.2. At the two warmer storage temperatures, spray dried palm kernel oil retained double the amount of 2,4-decadienal at the 30-day mark. For spray dried 40°C there was 150ppm retention while at the same temperature for freeze dried it was 80ppm.



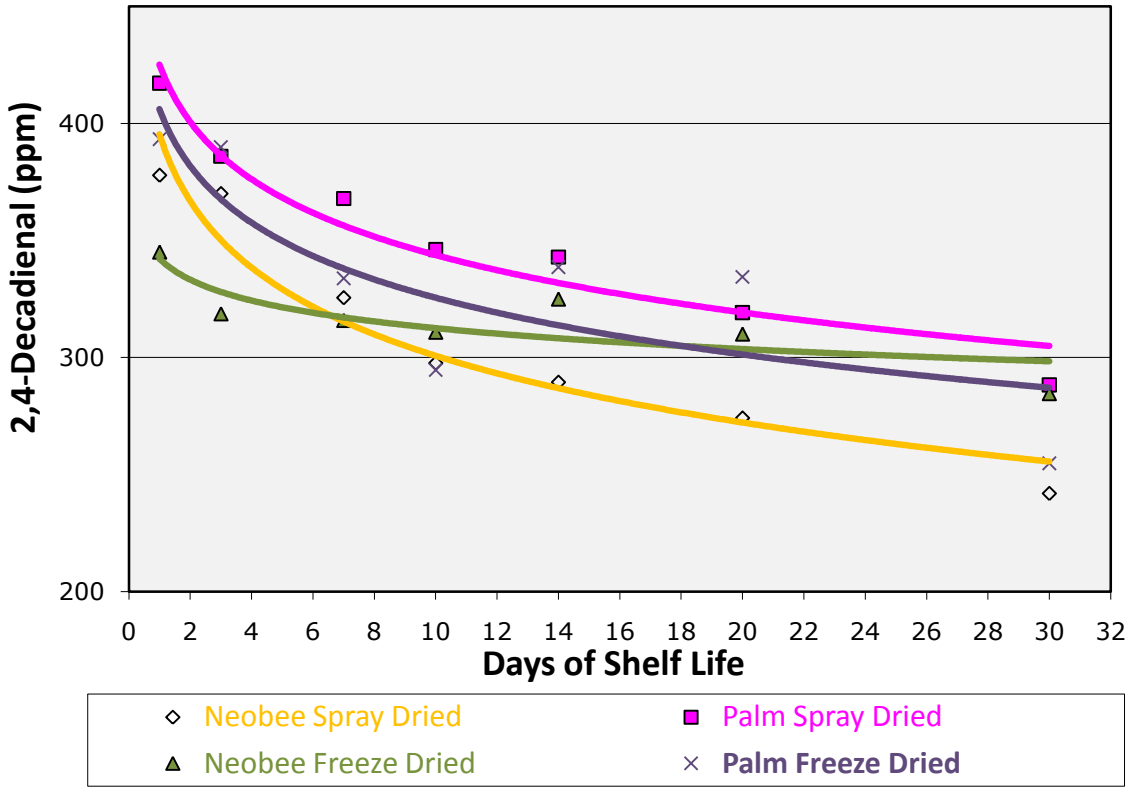
Graph 6.2 Spray dried 2,4-decadienal encapsulated in palm kernel fat; Shelf life study.

Even a more drastic example would be at the 60°C temperature at the 30-day mark, spray dried palm kernel retained 90ppm while freeze dried palm kernel oil was close to zero parts per million as seen in Graph 6.3.



Graph 6.3 Shelf life study at 60°C over a 30 day period.

Interestingly enough, spray dried palm kernel oil at a storage temperature of 5°C retained 25% more 2,4-decadienal at the end of the 30-day shelf life study than that of spray dried Neobee. This contrast can be seen in Graph 6.4. It is clear that palm kernel oil maintains better protective properties as a SLN coating in cooler storage conditions due to its glass transition temperature and physical structure. The glass transition state is critical since it can initiate other phenomena such as crystallization of amorphous zones or breakdown of the matrix leading to flavor loss and degradation (Voilley et al., 2006).



Graph 6.4 Shelf life study at 5°C over a 30 day period.

6.1.2 Autoxidation of Linoleic Acid

Linoleic acid is found in many vegetable oils such as soybean, corn, and palm oils. Palm kernel specifically has 10.0% linoleic acid and is also a good source of medium chain triglycerides. Ishii et al., (1992) found that a major degradation compound of the autoxidation of linoleic acid is 2,4-decadienal. When looking at Graph 6.4 where we would expect Neobee spray dried to surpass that of the palm kernel samples, palm kernel spray dried retains more 2,4-decadienal. It is possible that spray-dried palm kernel oil retained more 2,4-decadienal in addition to actually developing slightly more through the autoxidation process of linoleic acid. Whether palm kernel in this case retains more of the volatile 2,4-decadienal encapsulated in its matrix or if in fact it forms 2,4-decadienal through the autoxidation of the linoleic acid in the palm kernel is basis for future studies.

6.2 Additional Variables

In future studies, relative humidity could be controlled to specifically determine the effects of retro-aldo condensation. Also various solid lipids with higher melting points could be tested as encapsulation matrixes, as well as various starches and processing methods. This research demonstrates the potential of solid lipid nanoparticles as an encapsulation material for volatile aldehydes such as 2,4-decadienal.

7 References

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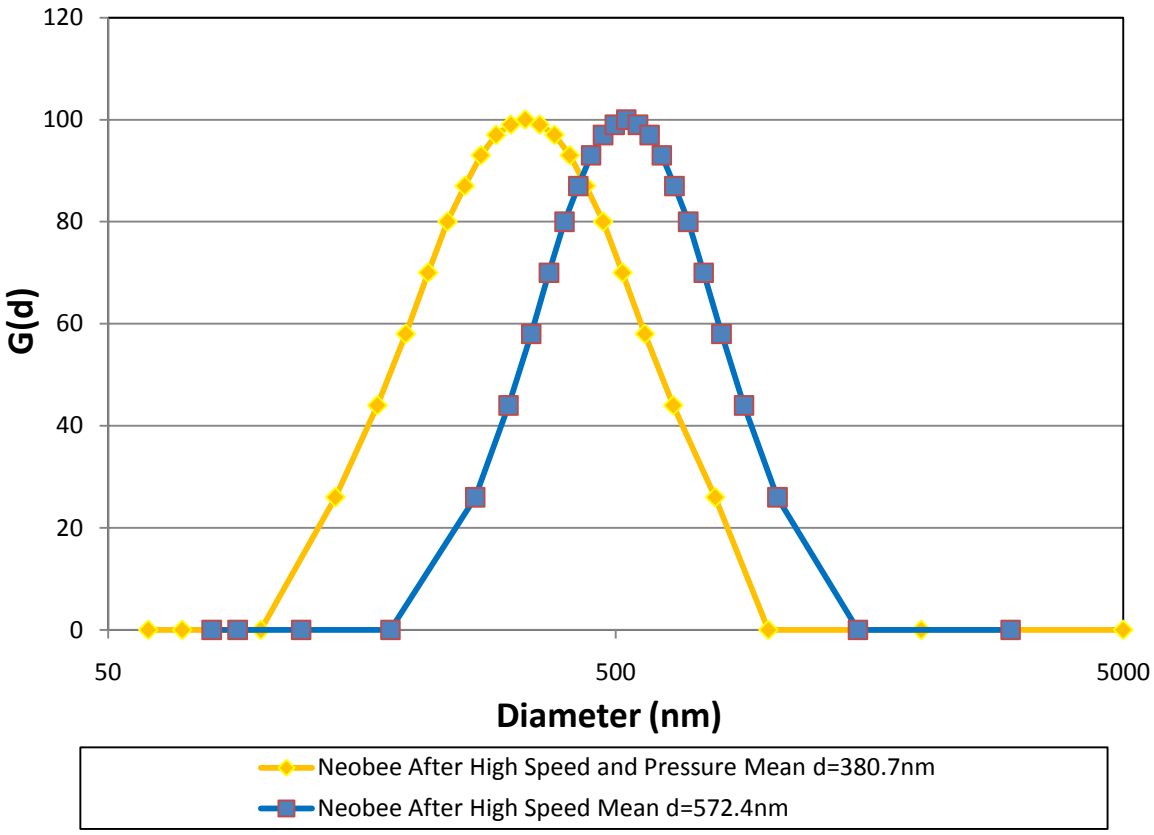


Figure 4.1 Particle size distribution of Neobee after high speed homogenization versus a combination of high speed and high pressure homogenization with 2,4-decadienal encapsulated.

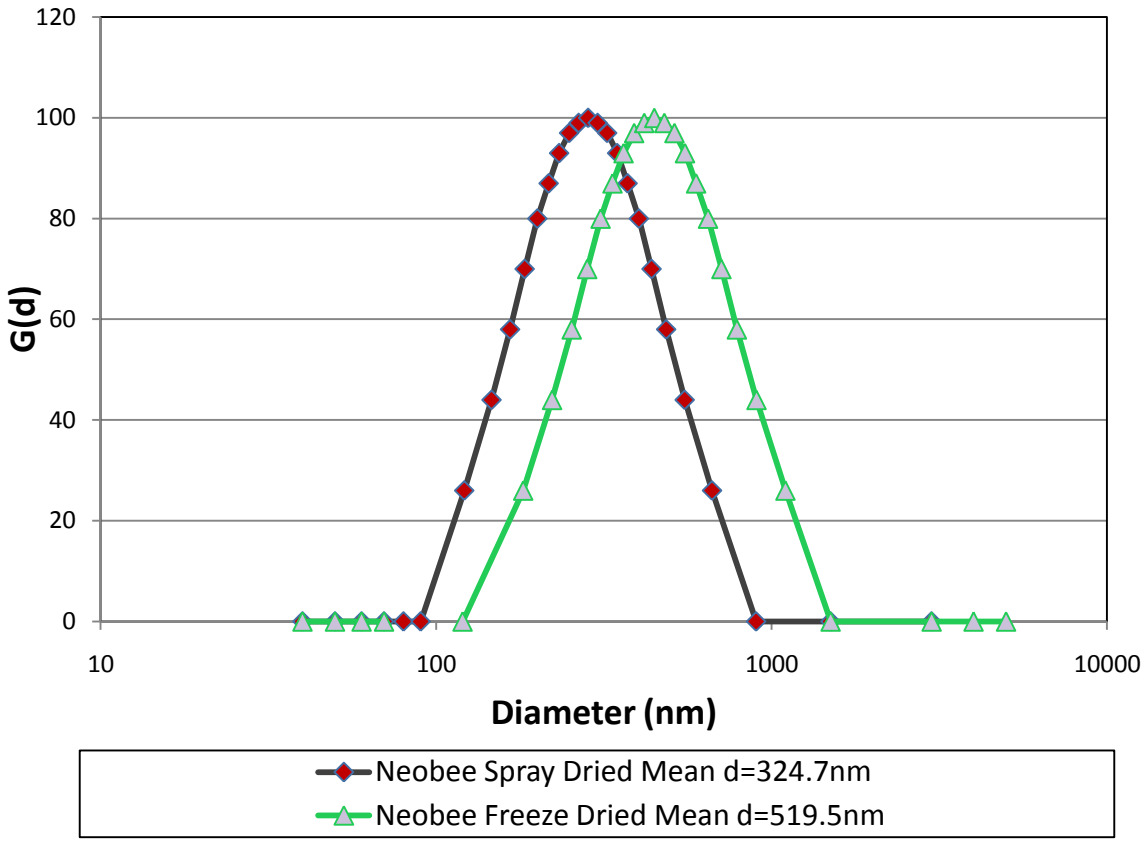


Figure 4.2 Particle size comparison of Neobee spray dried versus Neobee freeze dried with 2,4-decadienal encapsulated.

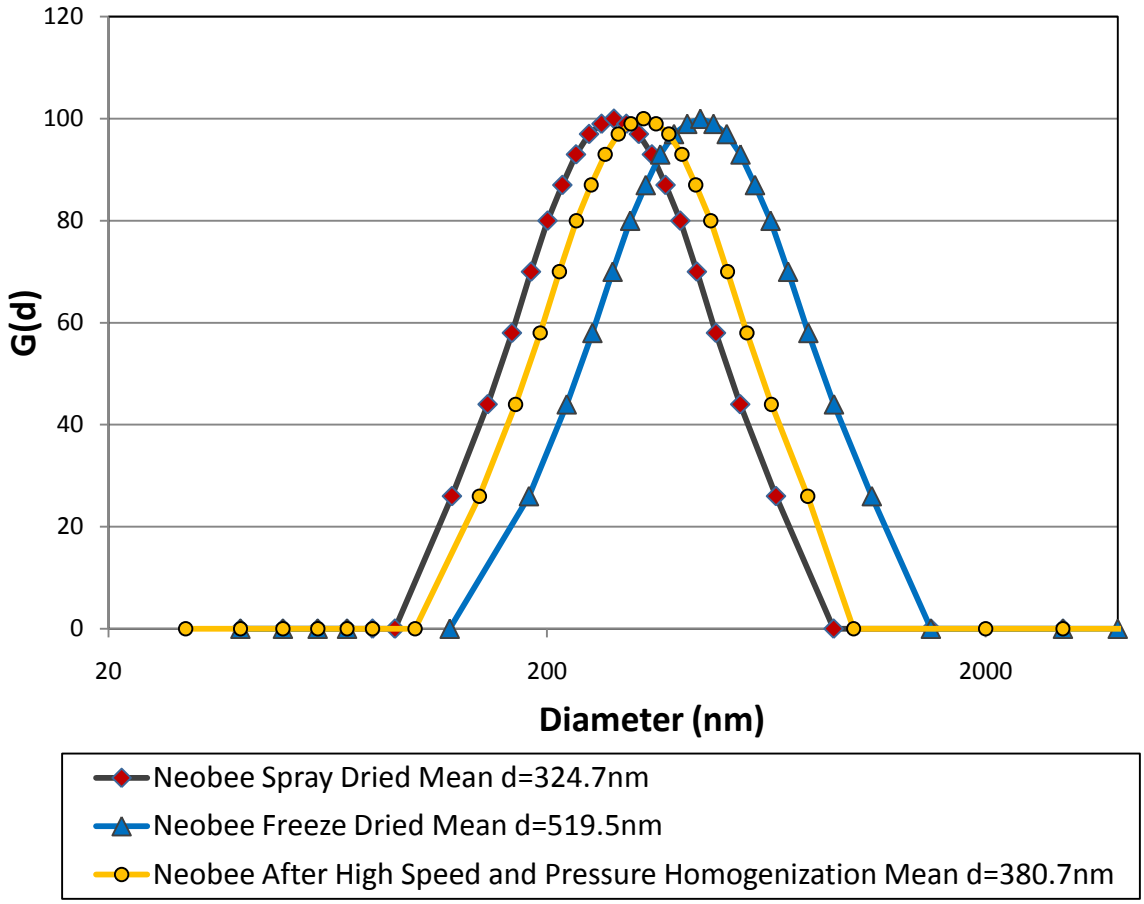


Figure 4.3 Particle size comparison of Neobee after both high speed and high pressure homogenization versus Neobee spray dried and Neobee freeze dried with 2,4-decadienal encapsulated.

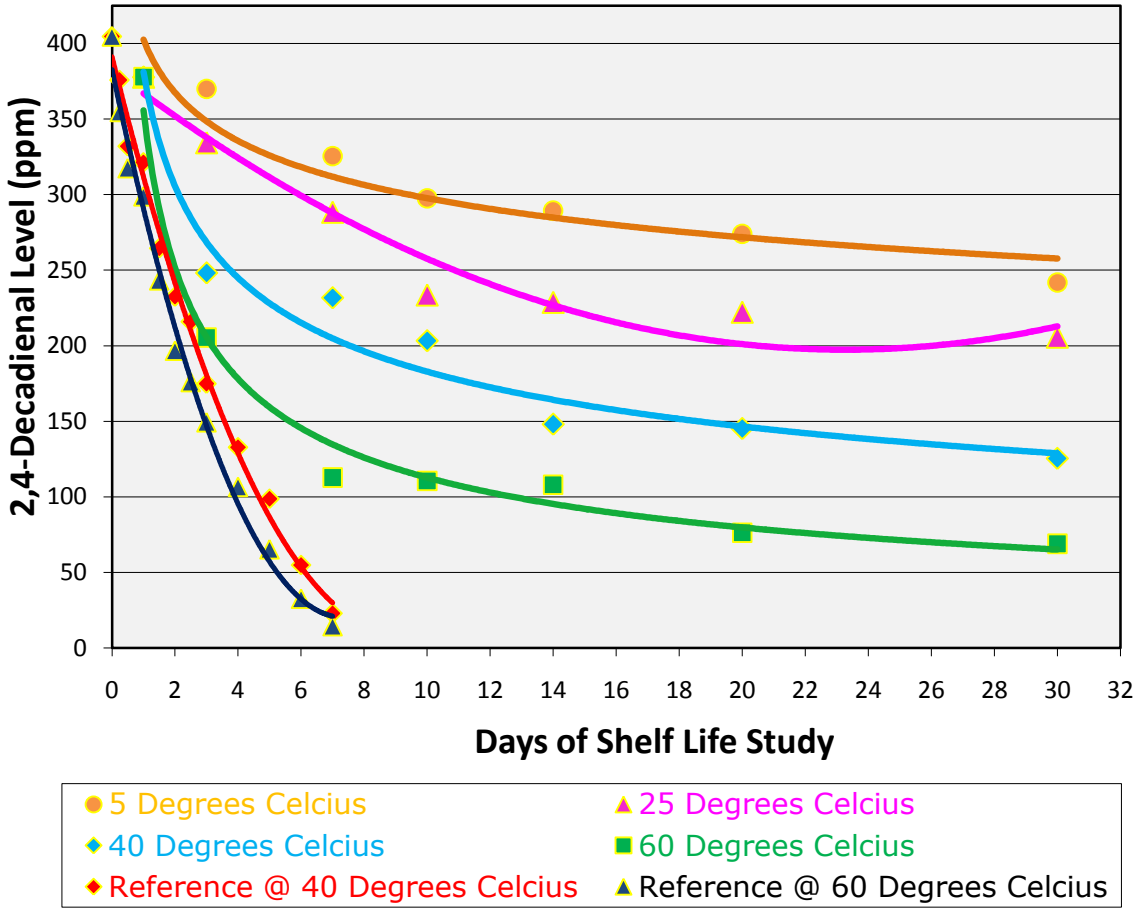


Figure 4.4 Spray dried 2,4-decadienal encapsulated in Neobee; Shelf life study.

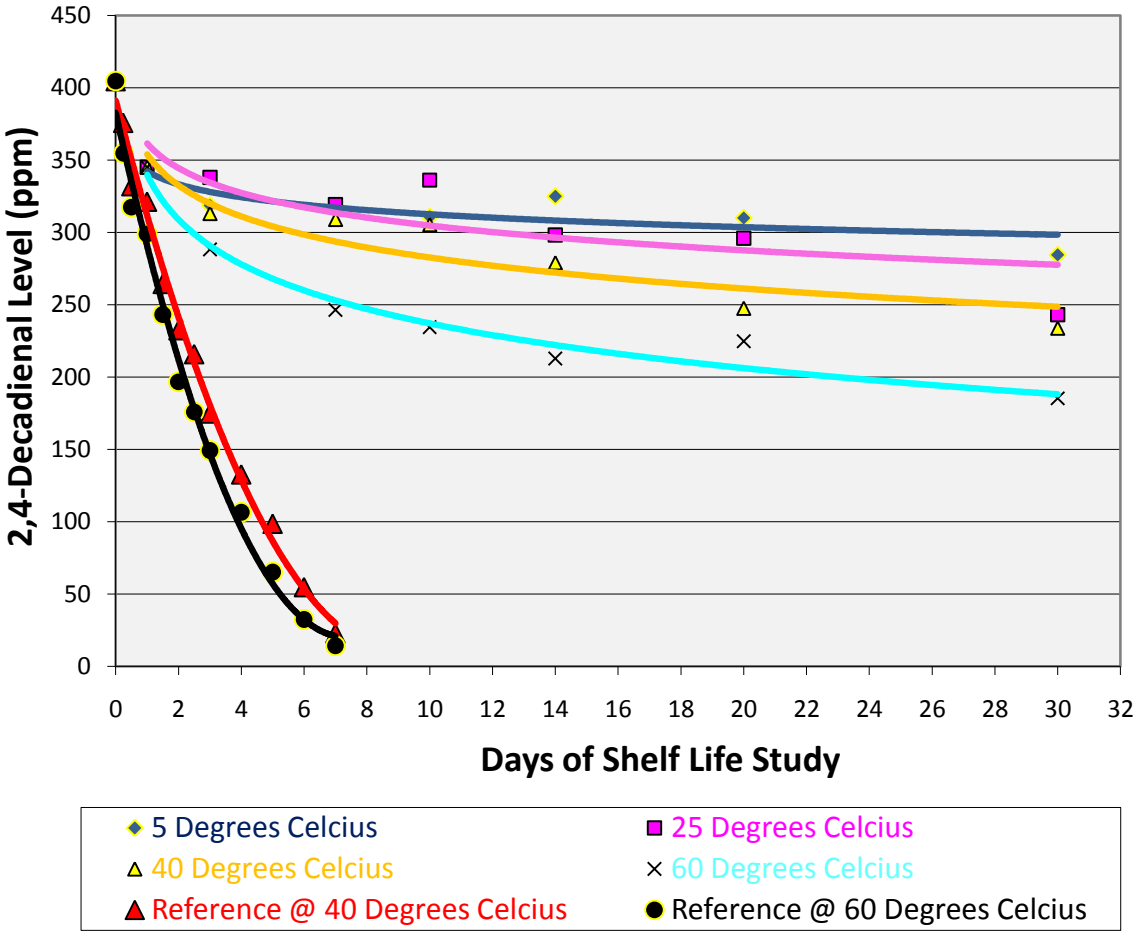


Figure 4.5 Freeze dried 2,4-decadienal encapsulated in Neobee; Shelf life study.

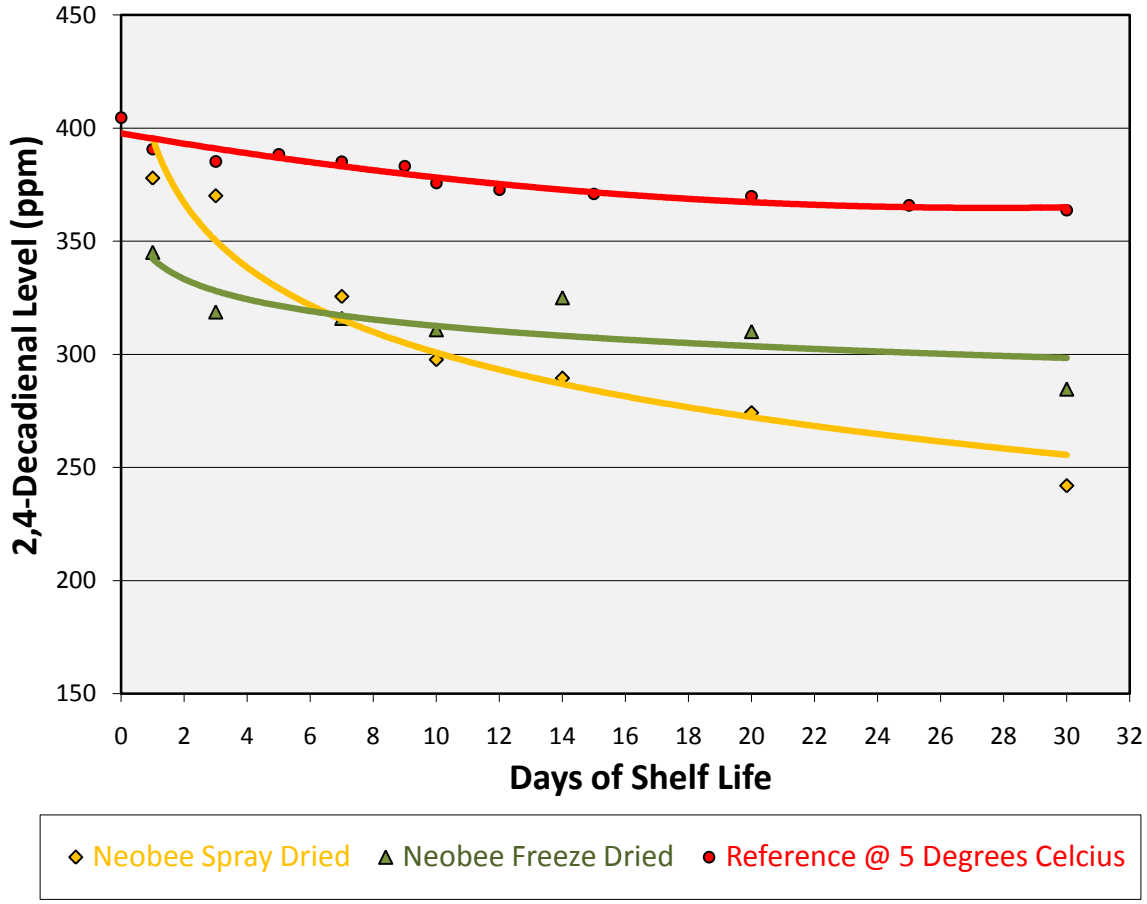


Figure 4.6 Shelf life study of Neobee freeze dried versus spray dried at 5°C over a 30 day period.

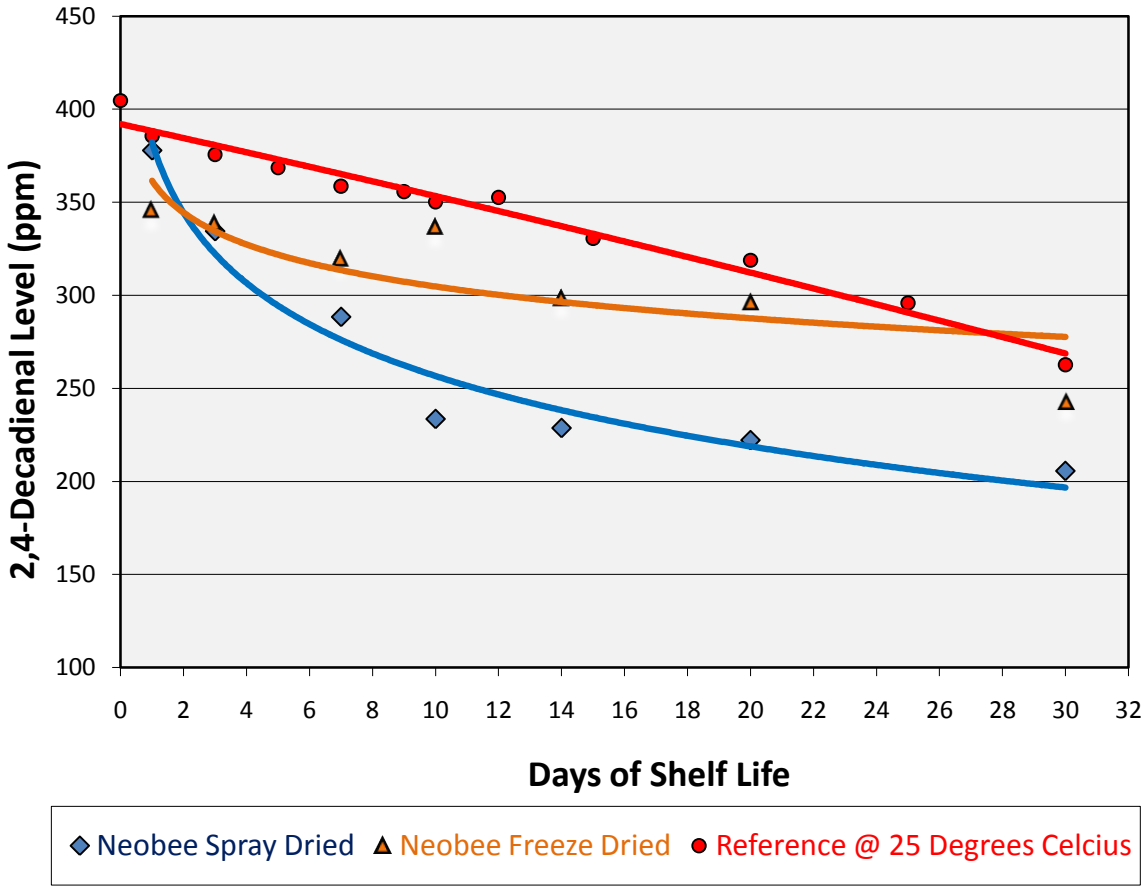


Figure 4.7 Shelf life study of Neobee freeze dried versus spray dried at 25°C over a 30 day period.

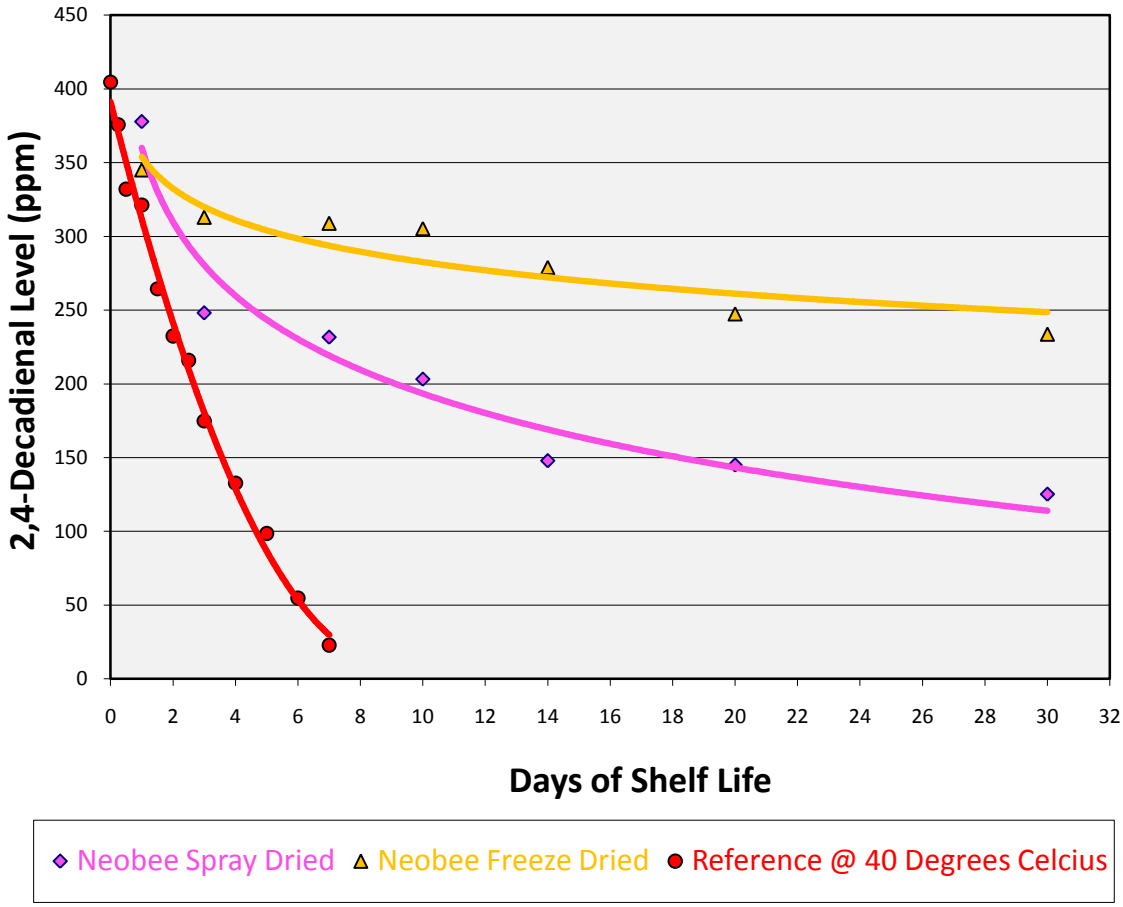


Figure 4.8 Shelf life study of Neobee freeze dried versus spray dried at 40°C over a 30 day period.

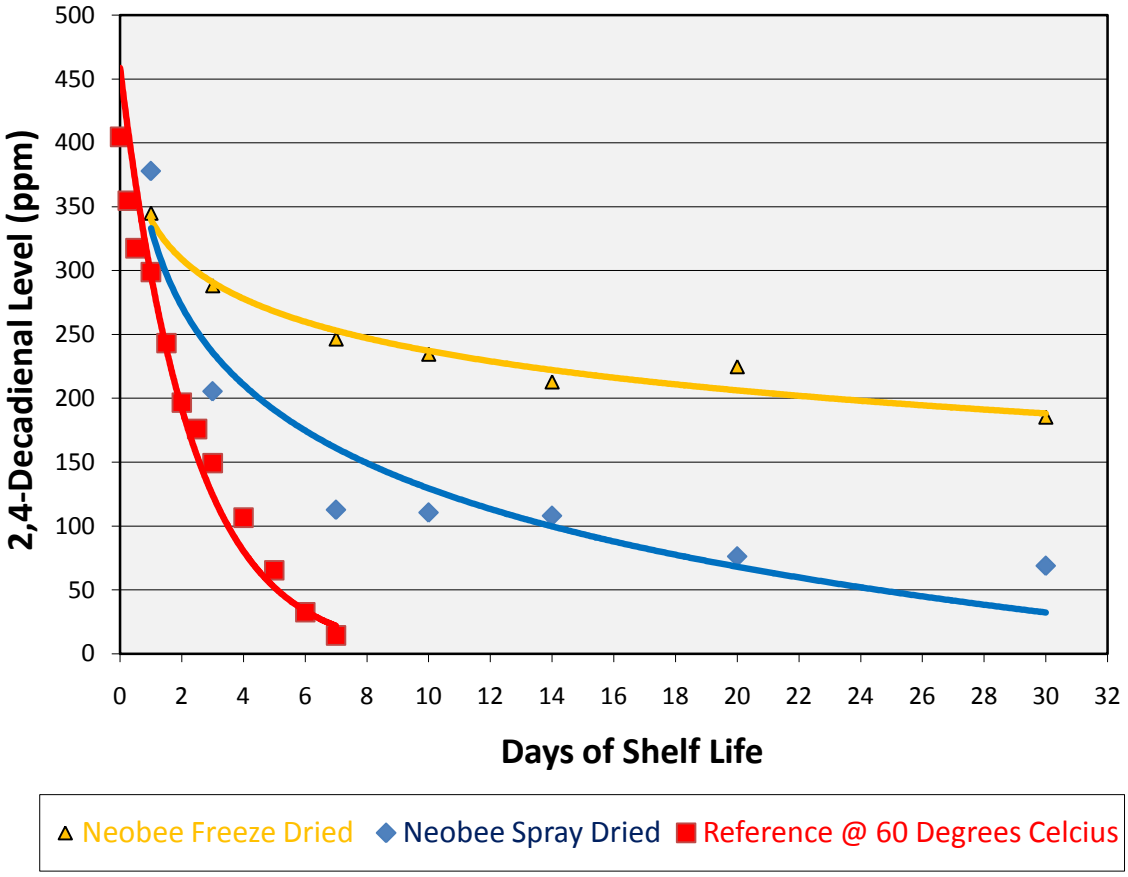


Figure 4.9 Shelf life study of Neobee freeze dried versus spray dried at 60°C over a 30 day period.

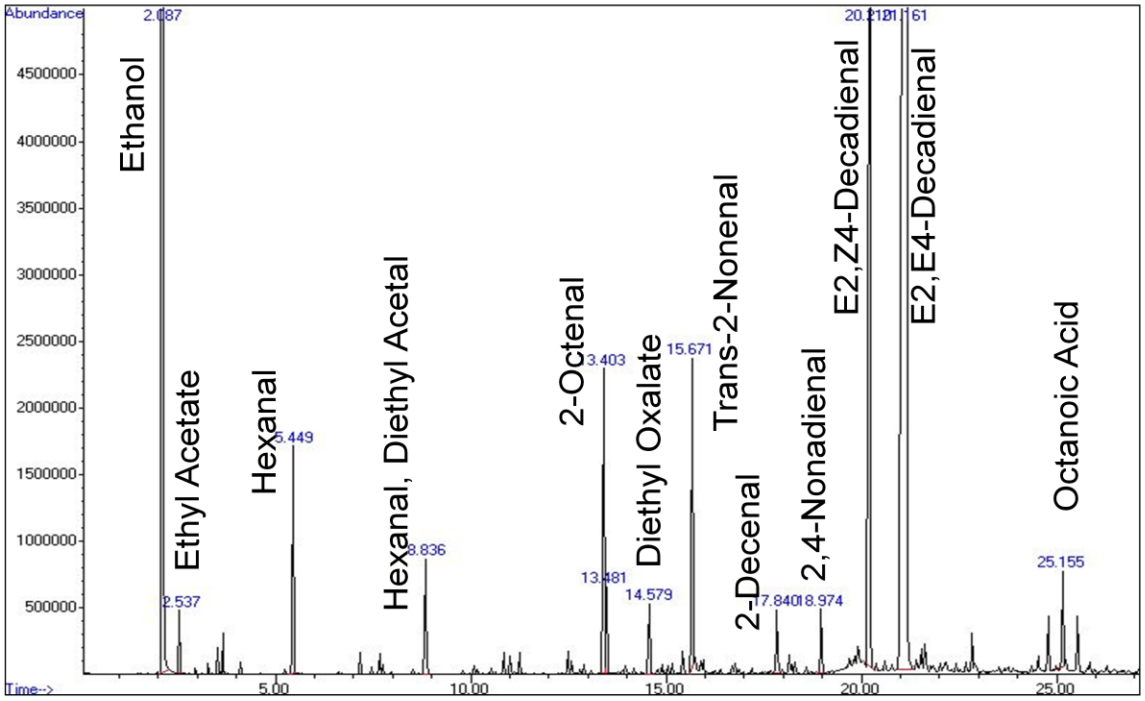


Figure 4.10 Mass spectrum of degraded neat 2,4-decadienal run on a polar column

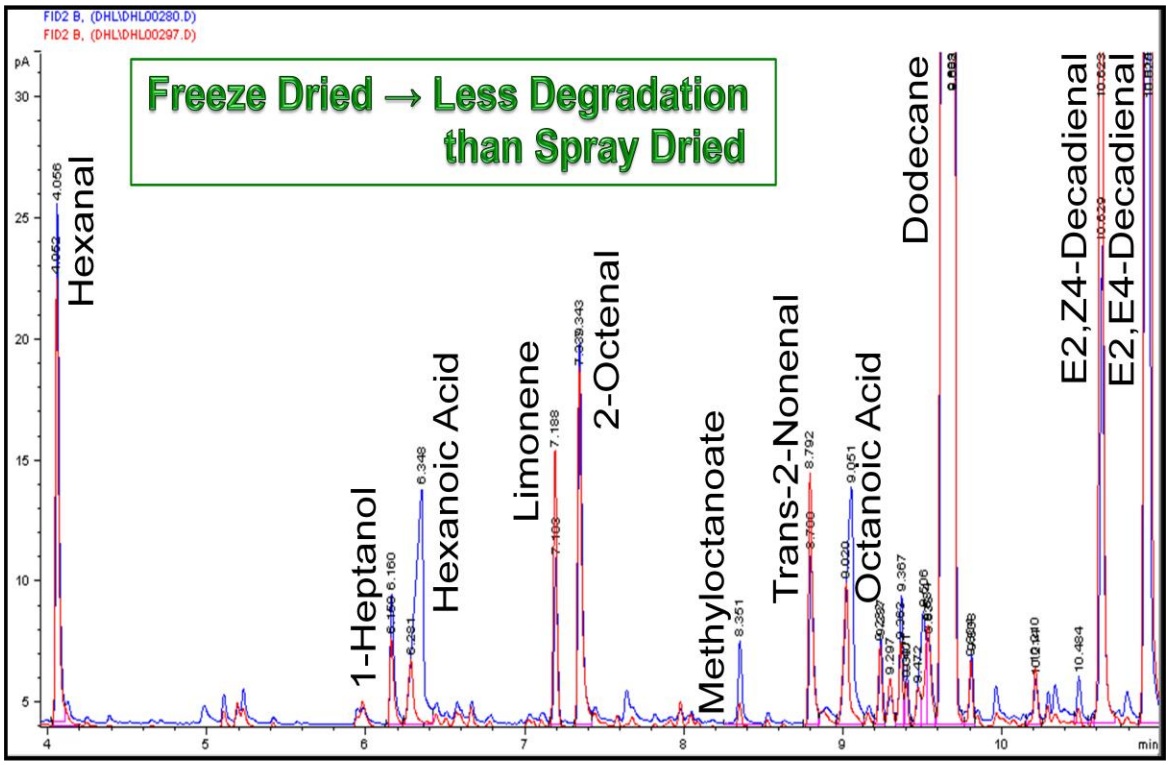
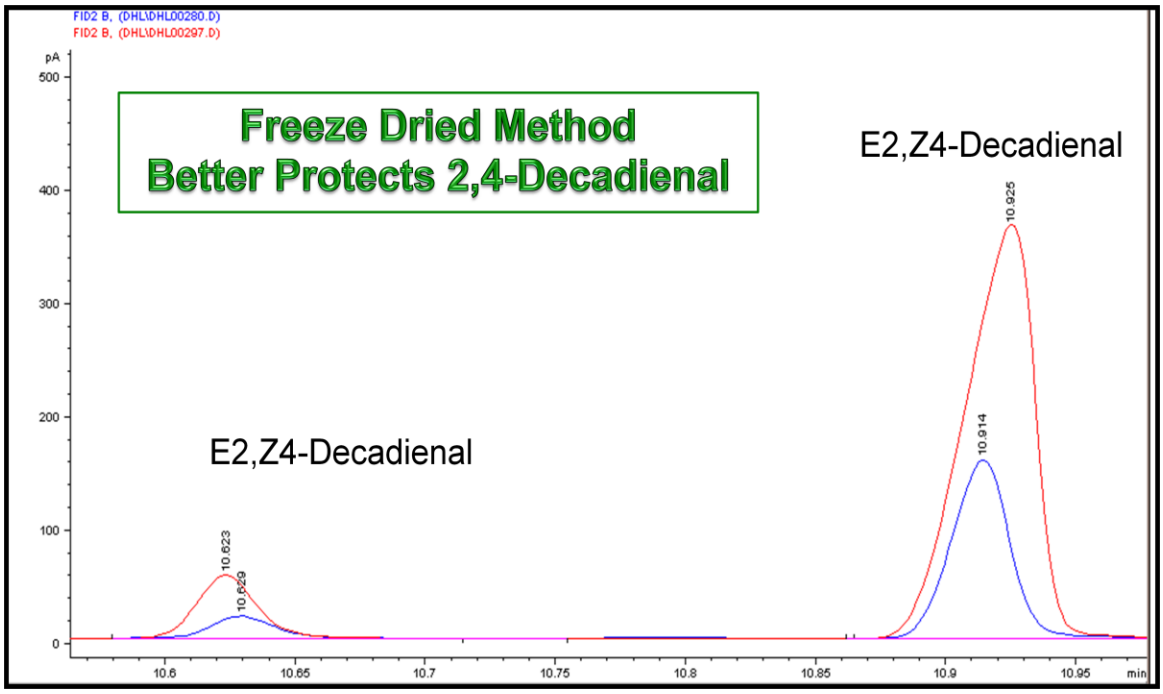


Figure 4.11 Gas chromatogram of spray dried Neobee with 2,4-decadienal encapsulated, stored for 30 days at 60°C transposed over the gas chromatogram of freeze dried Neobee with 2,4-decadienal encapsulated and stored for 30 days at 60°C.



Blue Peaks: Neo SD 60°C 30 Days Red Peaks: Neo FD 60°C 30 Days

Figure 4.12 Detailed view of 2,4-decadienal peaks of spray dried Neobee with 2, 4-decadienal encapsulated, transposed over freeze dried Neobee with 2,4-decadienal encapsulated and stored at 60°C for 30 days.

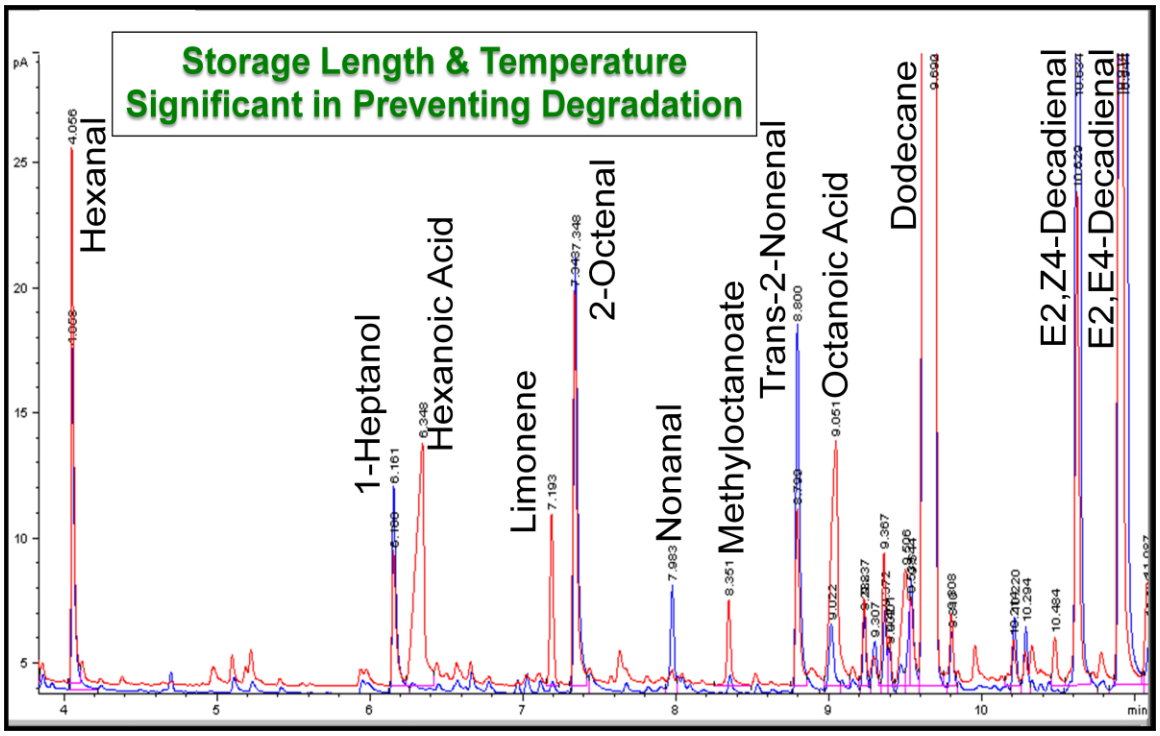
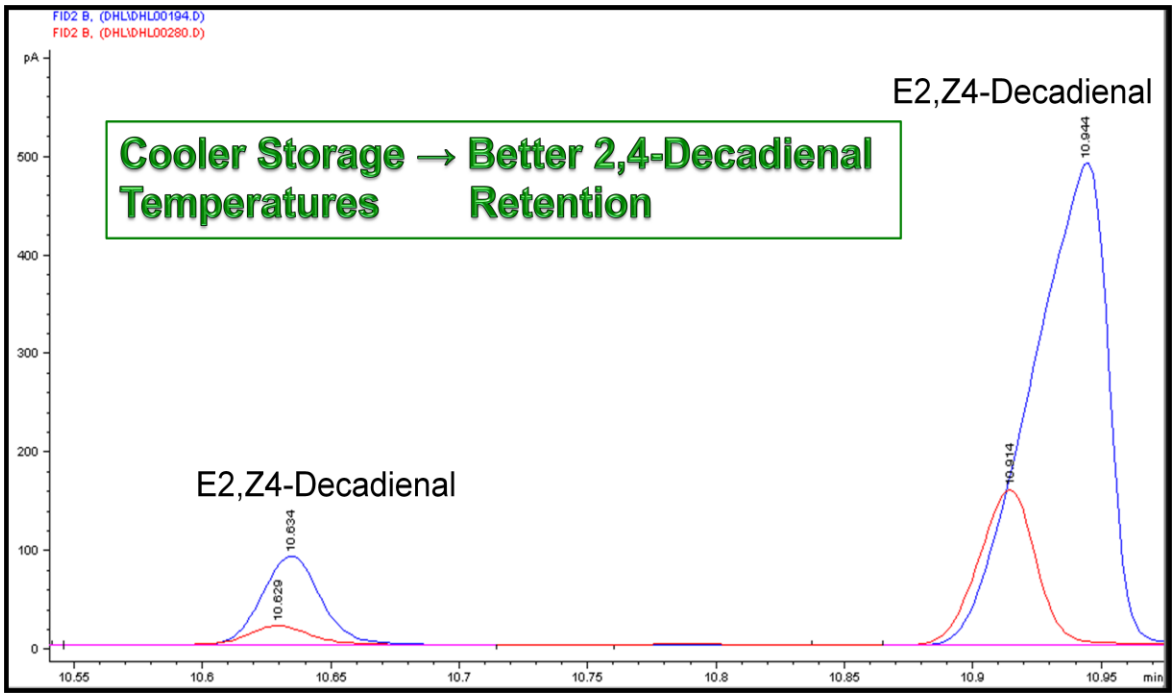


Figure 4.13 Gas chromatogram of spray dried Neobee with 2,4-decadienal encapsulated, stored for 14 days at 5°C and transposed over the gas chromatogram of spray dried Neobee with 2,4-decadienal encapsulated and stored for 30 days at 60°C.



Blue Peaks: Neo SD 5°C 14 Days Red Peaks: Neo SD 60°C 30 Days

Figure 4.14 Detailed view of 2,4-decadienal peaks of spray dried Neobee with 2,4-decadienal encapsulated, stored at 5°C for 14 days transposed over freeze dried Neobee with 2,4-decadienal encapsulated and stored at 60°C for 30 days.

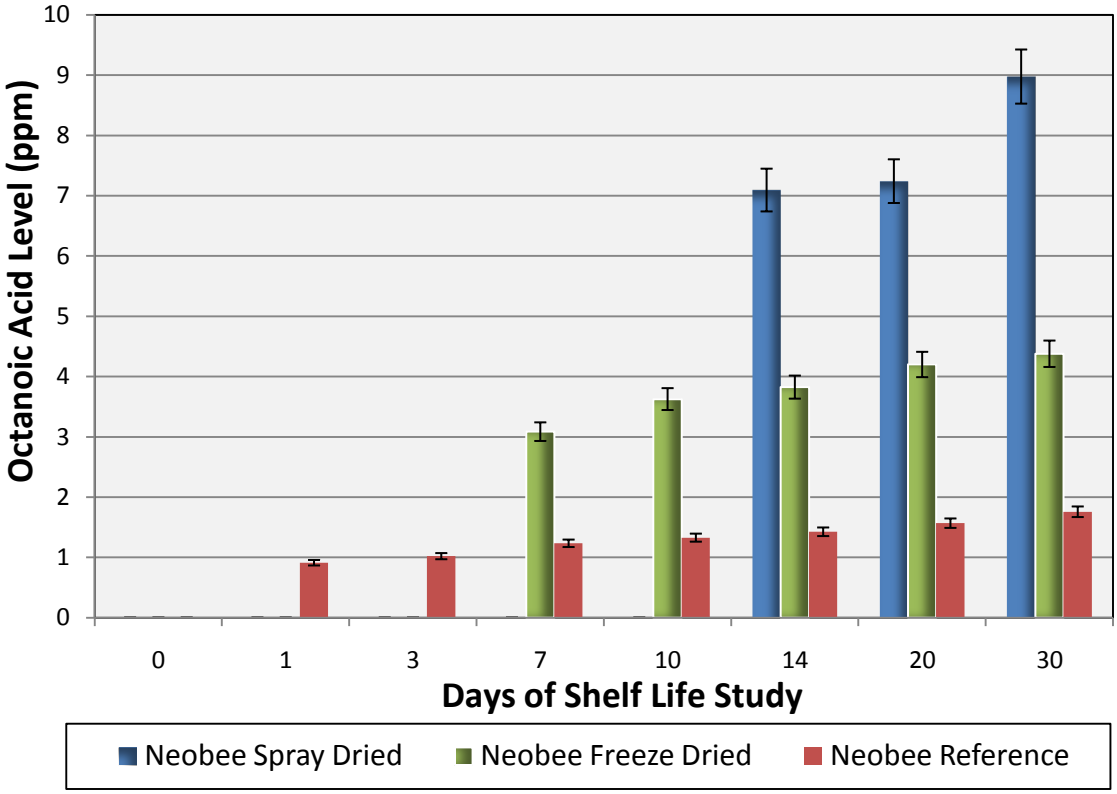


Figure 4.15 Octanoic Acid development of encapsulated 2,4-decadienal over 30 day shelf life study at 60°C.

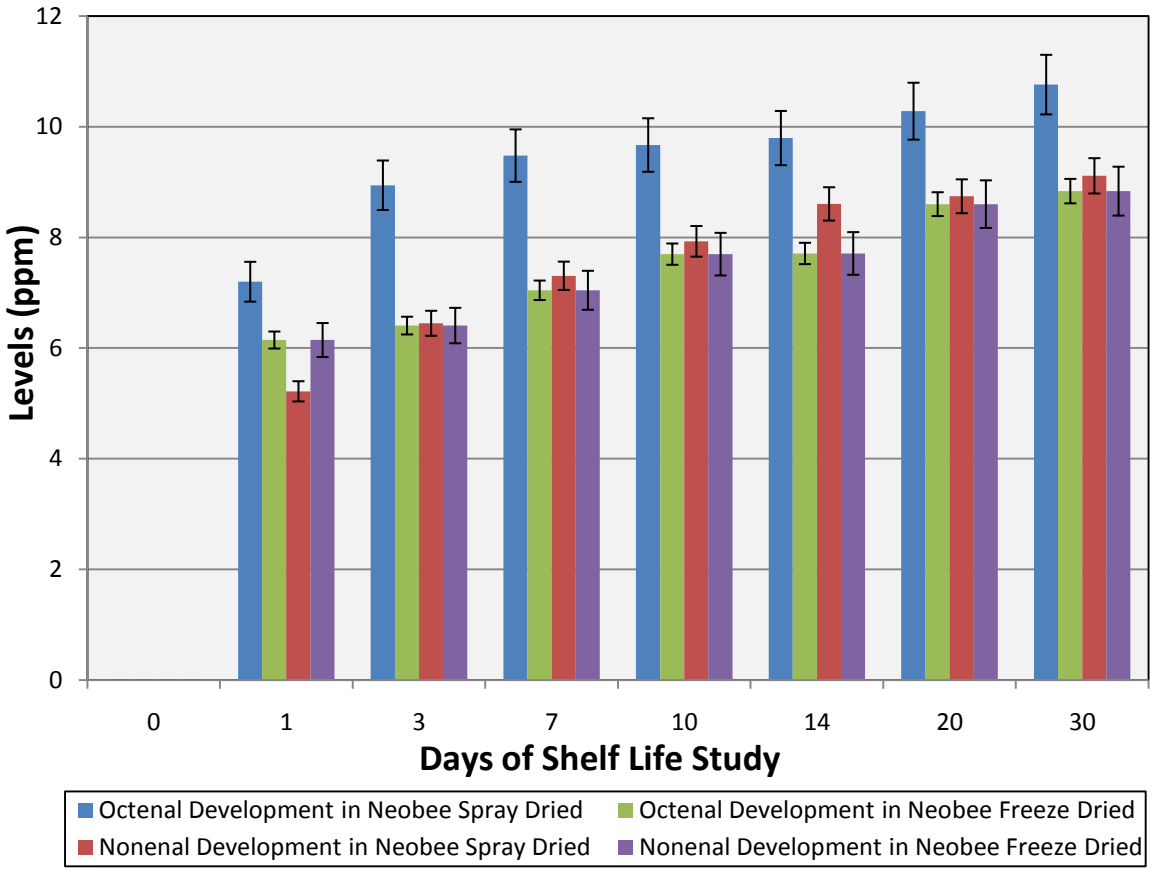


Figure 4.16 2-Octenal development versus Nonenal development of encapsulated 2,4-decadienal over 30 day shelf life study at 25°C.

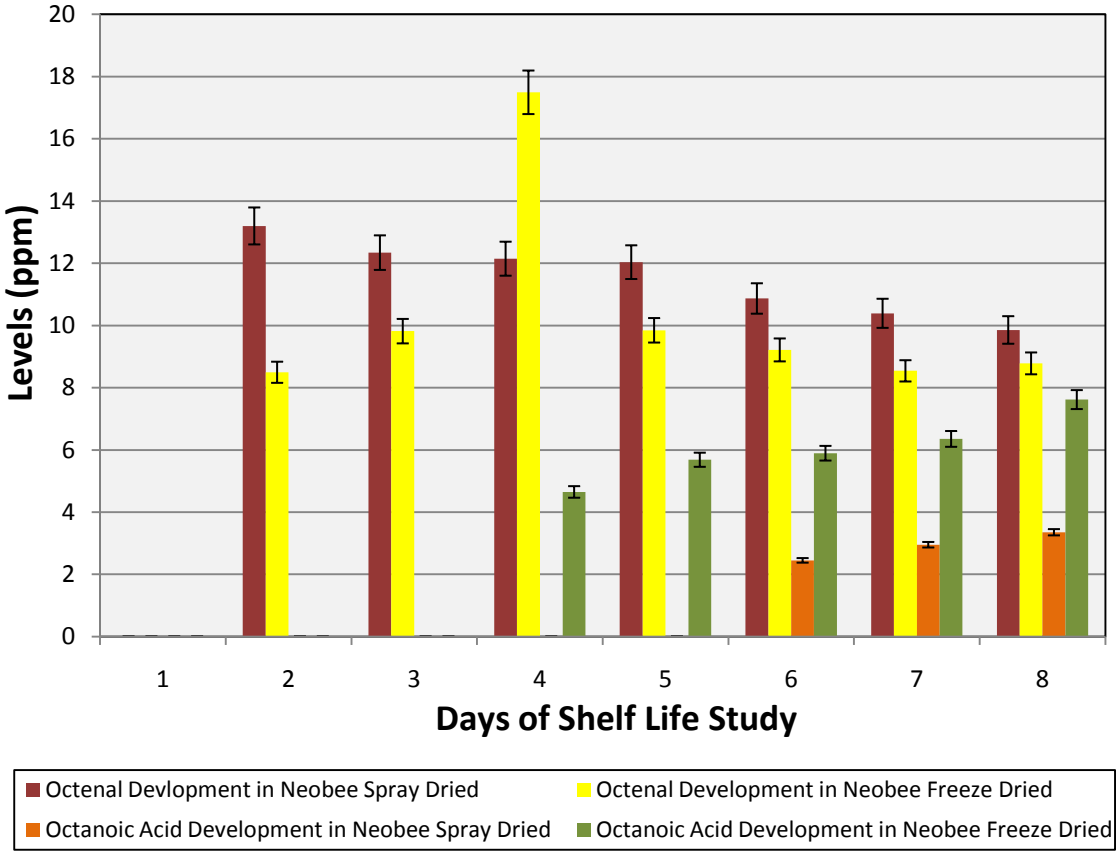


Figure 4.17 2-Octenal development versus Octanoic Acid development of encapsulated 2,4-decadienal over 30 day shelf life study at 40°C.

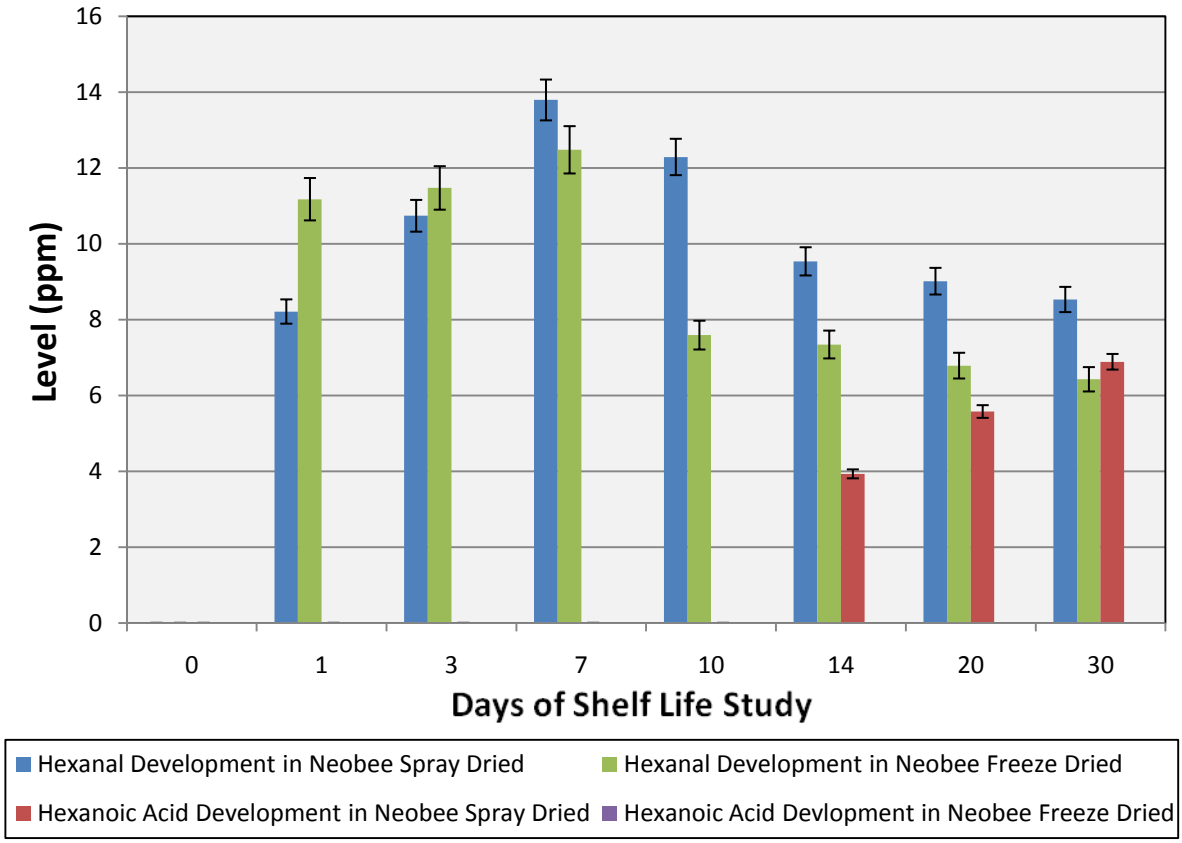


Figure 4.18 Hexanoic Acid development versus Hexanal development of encapsulated 2,4-decadienal over 30 day shelf life study at 40°C.

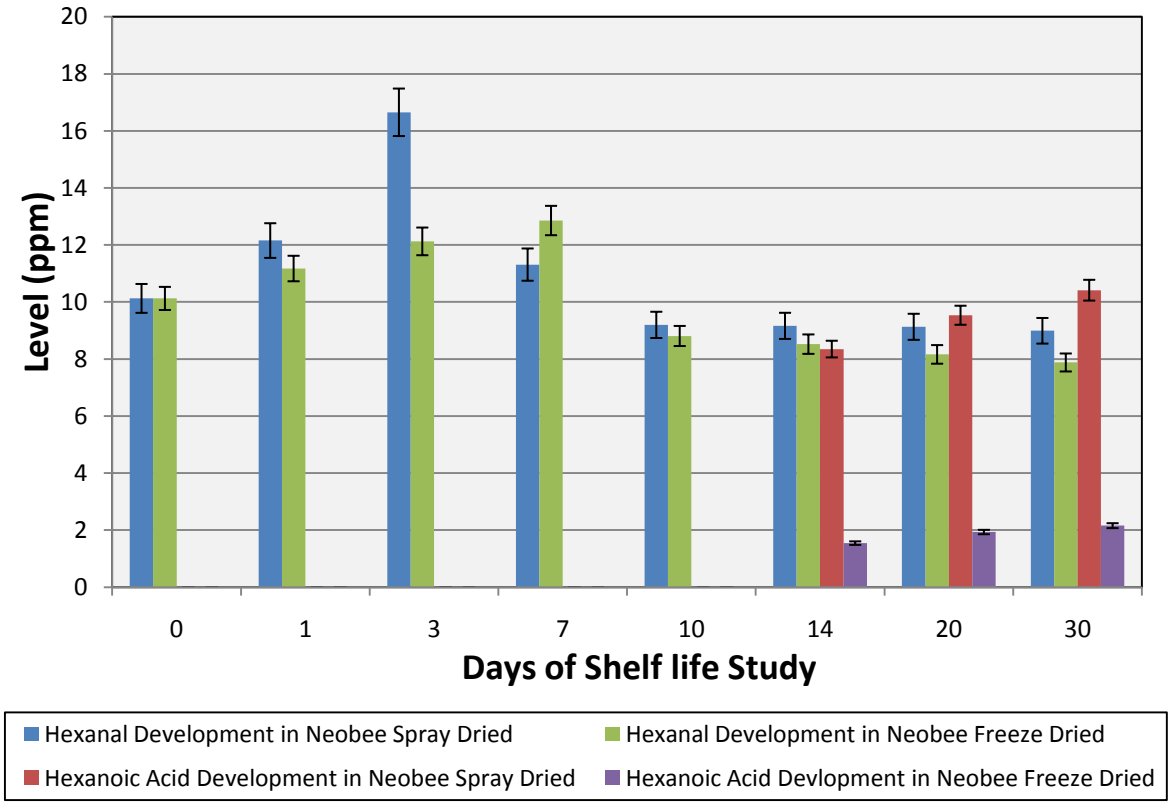
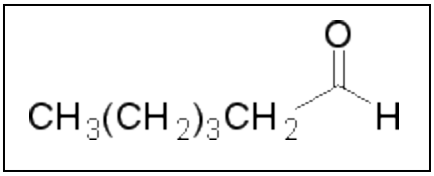
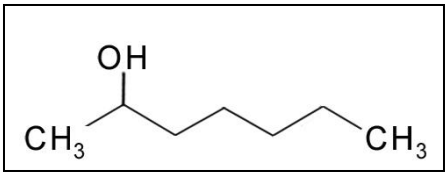


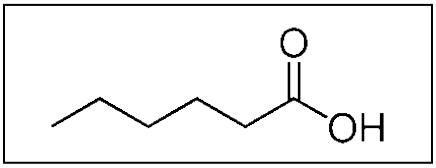
Figure 4.19 Hexanoic Acid development versus Hexanal development of encapsulated 2,4-decadienal over 30 day shelf life study at 60°C.



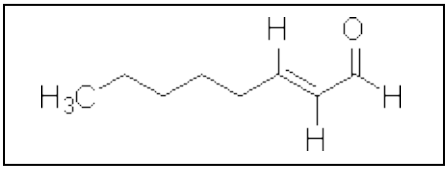
Hexanal



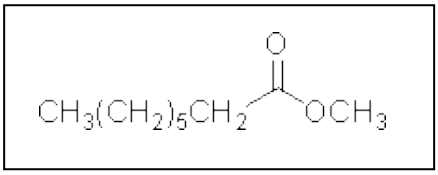
1-Heptanol



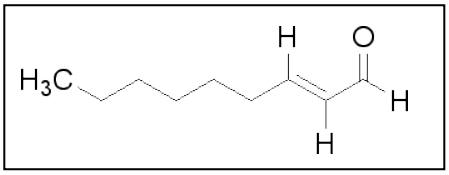
Hexanoic Acid



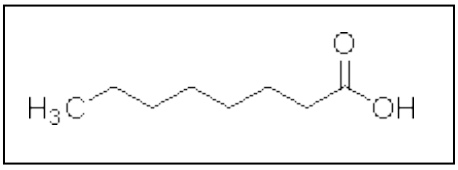
2-Octenal



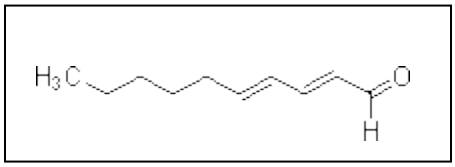
Methyloctanoate



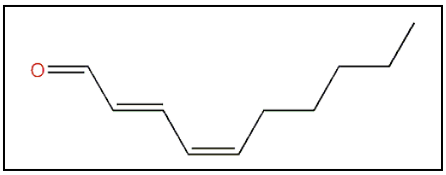
Trans-2-Nonenal



Octanoic Acid



(E,E)-2,4-Decadienal



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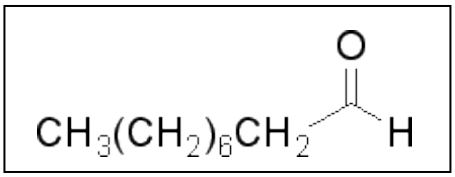
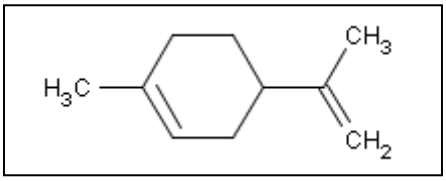
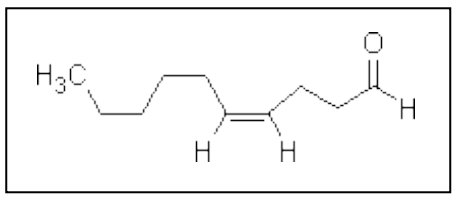


Nonanal



Limonene



Cis-4-Decenal

Table 4.3 Chemical Structures of Degradation Products