MICROENCAPSULATION OF BITTER PHYTONUTRIENTS FROM CITRUS BY-PRODUCTS AND APPLICATION THEREOF TO FRUIT JUICES

By

TEODORO RIVERA

A Dissertation submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Food Science

Written under the direction of

Dr. Chi-Tang Ho and Dr. Henryk Daun

And approved by

_____

_____

_____

_____

New Brunswick, New Jersey

JANUARY, 2015

ABSTRACT OF DISSERTATION

Microencapsulation of Bitter Phytonutrients from Citrus By-products and Application Thereof to Fruit Juices

By: TEODORO RIVERA

Dissertation Directors: Dr. Chi-Tang Ho and Dr. Henryk Daun

Every year, there are over 30 million metric tons of by-products generated by the citrus juice industry. It is well known that these types of by-products contain components which have been clinically proven to fight disease. Among them are the phytonutrients; of high interest are specifically the limonoids and flavonoids.

It is unfortunate that many of these by-products go to “waste” due to several reasons; one of them being off taste! In fact, limonin is a component found in citrus fruit that in high levels directly lowers the taste quality of the juice due to its bitter notes. The scope of this project is to capture the nutritious by-products and incorporate them back into juices in a way where the off flavors are concealed to consumers. For this project, two by-products were collected; orange seeds and orange rag. These by-products were stabilized via dehydration followed by milling. Then, microencapsulation trials were conducted to identify a suitable water-proof coating material that would be effective in concealing the off

flavors these ingredients impair. At the completion of experimental trials, it was determined that hot melt fluidized bed coating using food grade carnauba wax was the most effective path for application work. Although shelf life studies did not meet the 10 week requirement for typical chilled juices, there is potential to apply these microcapsules to products which have a shelf life requirement of 6 weeks or lower. Application potential also exists for products which have a twist cap or sachets for used at time of consumption. Recommended next steps include microencapsulation work where a multi-layer coating can be applied to prolong shelf life of the microcapsules.

Research done on citrus phytonutrients indicates many diverse benefits including the potential to fight cancer and cardiovascular disease. The ideal outcome of this field of work is to re-incorporate by-products back into juices to make them more wholesome and significantly increase the health and wellness potential.

DEDICATION

To my wonderful wife Maggie and son Sergio, daughters Karina & Alondra for all the support and for being patient with me working late nights, weekends and traveling to obtain this degree. To my parents and brothers for all their support throughout my life. To Mrs. Suzanne Wallace for all the support on editing my paper.

ACKNOWLEDGMENTS

Great Friends, Mentors, Co-workers and Supporters for making my idea a reality!

Ph.D. Committee

Dr. Chi-Tang Ho (Advisor)

Dr. Henryk Daun (Advisor)

Dr. Kit Yam

Dr. Peter Given

PepsiCo Teams

F&V Apps / Tropicana Management

Long Term Research Department

Analytical and Sensory Departments

External Support

Balchem Corporation

Freund-Vector Corporation

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

I. INTRODUCTION

The onset of baby boomers getting older coupled with a rise in chronic diseases, has increased consumer demand for foods and beverages fortified with functional ingredients. Functional ingredients added to everyday food give it an edge to go beyond inherent benefits. In recent years, numerous scientific papers have been published on the many health benefits of these functional ingredients including, but not limited to phytonutrients. Phytonutrients are those components found in plants which provide nutrition and or function above and beyond the common micro and macronutrients. This paper will focus on phytonutrients which derive from the orange citrus fruit; two of the important groups include limonoids and flavonoids. Although many of these phytonutrients such as the limonoids have been associated with many health benefits, there are some negatives, one of them being the off taste some of these compounds impart at high levels. Conventional juice producers actively work to remove certain flavonoids and limonoids from the juice in order to avoid bitter notes. Therefore, there is great opportunity to develop a process to incorporate these phytonutrients back into juices and other beverages without the negative taste.

1. Citrus Fruit

Citrus fruits are juicy, aromatic, flavorful, and highly nutritious. Citrus fruits are in the evergreen tree of the Rutaceae family. There are many kinds of citrus fruits but the oranges appear to be its main representative. Oranges have many kinds of derivatives hybridized from other species and varieties such as tangerines, mandarins, clementines, satsumas, as there are other citrus types too which are different in texture, appearance and taste such as grapefruit, lime, calamondin, lemon, pomelo and others. Some documents indicate citrus fruits date back from 2,400 to 4,000 years ago indicating to Southeast Asian as the point of origin. Nowadays, citrus fruit can be grown anywhere in the world with the top producers being China, United States, Brazil, Mexico, Spain, India, Italy, and Egypt (Ladaniya 2008).

Each layer or part of the citrus fruit has a particular use. The peel, skin or rind also called epicarp or flavedo, which serves as covering or protection of the pulp, is not eaten normally because of its highly bitter taste but bits of it are included in cooking as zest or to add spice or flavor to food. More importantly, the rind or peel contains the essential oils that emit the fragrance and aromatic characteristic of the fruit. The white, spongy part called mesocarp or albedo is the part of the peel along with the epicarp that contains many phytonutrients and enzymes with particular health functions. The internal part of the fruit, the pulp, is

the most important being the edible portion where the juice is contained. It is subdivided into sacs separated by a radial film called endocarp or segments (UNCTAD 2011). The juice can be very sweet, tart or sour, or bland depending on the varietal and the stage of maturity. Whichever taste it has, the flavorful liquid is filled with vitamins and nutrients such as soluble sugars, vitamins such as C and other vitamin types, pectin, fibers, different organic acids, potassium salt and some others as the important phytonutrients. All these give the citrus fruit its citrine flavor and other unique flavors (Citrus Pages 2011).

The following figure illustrates the various parts of the orange fruit. Note the yellow flags, which highlight the parts of the fruit where this project focuses: the rag and the seeds.

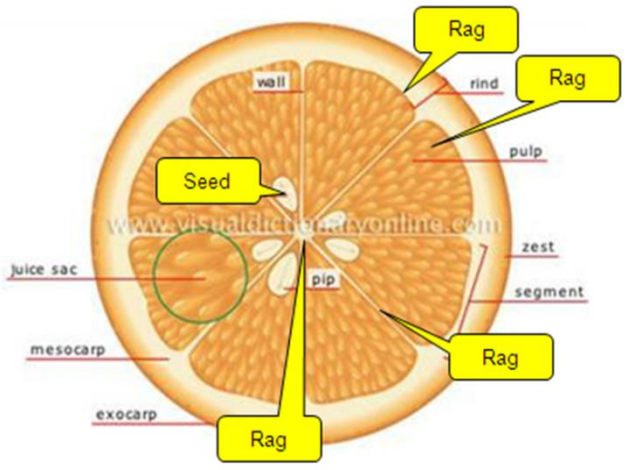


Figure 1. The Parts of the Orange Fruit

Citrus fruits are generally consumed fresh, typically as a dessert or as a snack especially with the sweet-tasting varieties. The juice is ingested straight from the pulp, scooped out, pressed or “juiced”. Sometimes the juice is diluted with water and sugar to make refreshing beverages, or further processed to be made into puree and in some cases, even for the making of wine. Other citrus types with astringent, sharper flavor as lemon, lime and calamondin are used with other ingredients for culinary purposes such as flavor for cooking food, baking, marinating, tenderizing meat, steaming, garnishing, or in some cases, to eliminate off odor, aside from being a beverage (New World Encyclopedia 2012). Although the focus of this project is not to utilize orange ingredients for use in commercial cleaning, it is fair to mention that at times, citrus fruit components have industrial uses for cleansing purposes due to their acidic content. The juices and rind are also used for flavoring in marmalade and sauces. Other parts such as the seeds and pulp, to a small extent, are made into topical antibacterial and antifungal agents. The leaves, incorporated with other plant parts, are claimed to be used medicinally for mouth sores, internal ailments and fractures. The bark can either be boiled or chopped finely to treat postpartum sickness, the flu, and some internal injuries (Manner and others 2011).

Furthermore, in terms of additional nutrition to note, orange fruit apart from being rich in vitamin C, it also contains; folic acid, potassium, folate, thiamin,

niacin, and contains a good source of dietary fiber, at least 10% of the daily value or 2.5 grams of fiber per serving. In the case of a juice beverage, where the by-products will be re-applied for this project, the serving size is defined as 8 fluid ounces; this is equivalent to approximately 250 ml. In addition to fiber and all of the nutrients listed above, there are the phytonutrients. They are generally fat-free, cholesterol-free and sodium-free, making them a primary choice for dieters and those suffering from chronic diseases. An abundance of literature over the last few decades indicates there is good evidence of these phytonutrients having potential to fight cancer, hypertension and other heart-related diseases (Murray 2007).

This project focuses on the re-incorporation of some key phytonutrients (also known as phytochemicals) back into fruit juices. The benefit of this re-incorporation is that these phytonutrients are captured from citrus by-products which unfortunately, are typically turned in to cattle feed or discarded directly to landfills. Citrus phytonutrients are non-nutritive chemicals produced by plants for their own protection. They are neither vitamins nor minerals but in nature, are compounds that shield plants against bacteria, viruses, fungi, insects, and even environmental challenges such as droughts and the sun. They provide the aroma, texture, color and flavor that give food its unique appeal. More than two thousand phytonutrients are pigments that put color in the plants (ADA 2012).

For humans, phytonutrients are not biologically necessary for survival but they are indispensable when a person aims for optimum health. Literature indicates a strong correlation of phytonutrients with well-being and disease-fighting compounds which can help prevent strings of diseases by keeping the body balanced. For example, as antioxidants, they promote the healthy function of the immune system and indicate to prevent cancer. In addition, they protect against problematic bacteria and viruses, reduce inflammation and are associated with the treatment or prevention of cardiovascular disease among other ailments (Murray 2007).

It is believed that a large number of the population in highly industrialized nations lack phytonutrients in their diet as shown by the rising figures of cardiovascular diseases and similar ailments. Indulgence of over processed foods coupled with preservatives and other additives is seen as one of the major causes of disease. Some over-processed and chemically preserved foods are harmful to human health because they can destroy the naturally-occurring phytonutrients in raw food specifically fruits and vegetables during the manufacturing process (Watson and Smith 2007). It is important to note that not all processing of food is bad. There are some processing practices which actually increase the bioavailability of nutrients by exposing the nutrients to enhanced absorption. Plant-based natural foods such as fruits, vegetables, nuts, seeds,

grains and beans, have specific phytonutrients of interest. These have a direct effect on the foods' color, flavor and function in their supportive and protective action in the body cells and tissues (Watson and Smith 2007). The vibrant colors of fruits and vegetables such as orange, red, and yellow pigments found in peppers, pumpkins, cantaloupes, cherries, papaya, mango, cabbage, carrots and others are due to phytonutrients. Some good examples include: lutein which is responsible for the bright yellow color of corn, lycopene which makes the tomato red, carotene which makes the carrot orange, and anthocyanin makes the blueberry blue (Watson and Smith 2007). It is interesting to note that when carrot juice is excessively consumed by people, the high beta-carotene content found in carrots can give the skin an orange hue pigment. More importantly, the ingested beta-carotene converts to vitamin A in the body and is responsible for making good eye sight, promotes good skin health and a good immune system (Watson and Smith 2007).

It has been noted that phytonutrients help the body in many ways. One of the most important benefits is they serve as antioxidants. As antioxidants, they offer protection from free radicals by enhancing immunity and communication or synapsis between body cells, detoxifying carcinogens, repairing damage in DNA caused by smoking and other toxins, and also in killing established cancer cells (Watson and Smith 2007). Free radicals are the reactive, self-damaging

molecules that accumulate in the body. It is well established that a large presence of free radicals can lead to cancer. It has been reported that Indole, a phytonutrient found in cabbage, stimulates enzymes that render estrogen less effective which reduces the risk of breast cancer. The saponins found in beans interfere with the replication of certain cellular DNA which prevents the reproduction of cancer cells. Capsaicin found in hot peppers helps protect DNA from carcinogens. Allicin from garlic has antibacterial properties (Watson and Smith 2007).

Phytonutrients also reduce inflammation. This health concern is generally associated with the swelling, redness and pain of organs, muscles and joints. These conditions are typically deemed as superficial and ordinary, yet they should be attended to. Normal inflammation is a good reaction to disease because the swelling effect means the body is naturally resisting the bacteria, virus or toxin. But, constant and too much inflammation should not always happen and is therefore cause for concern. The kind of inflammation referred to here as negative occurs right in the internal organ of the body system many of times, without the person being aware of it, without feeling anything different or strange from within. The onset of a rapid and prolonged inflammation can disrupt the immune system and lead to chronic problems or disease. Researches have proved that there is a link between inflammation, weight gain and obesity.

Prolonged inflammation is considered to be responsible for the occurrence of degenerative diseases such as heart disease, Alzheimer's, cancer, autoimmune disease, diabetes. It is also associated with an accelerated aging process by creating an imbalance in the body which stimulates negative effects to the person's health and the ability to lose weight (Watson and Smith 2007). With the consumption of phytonutrients, the degenerative aging process will slow down as it will also reduce the risks of diseases such as cancers, heart disease, stroke, high blood pressure, cataracts, osteoporosis, urinary tract infection and other chronic health conditions (ADA 2012).

2. Phytonutrients in citrus fruit

It has been known since ancient times that citrus fruits contain bioactive compounds that benefit human health. In addition to large amounts of vitamin C and folic acid, which traditionally has been the main characteristics of citrus fruits, other components such as bioactives or phytonutrients are found in citrus including but not limited to; flavonoids, limonoids, furocoumarins, and some which also provide pigments such as chlorophylls, carotenoids and anthocyanins (Terry 2011). The chlorophylls are responsible for the green colors found in the peel during the growth and maturation of the citrus fruit. This is high during the fruit's immaturity stage but later fades when the fruit has grown fully and has been exposed to cool temperature. The important consideration given to high

chlorophyll content of the peel should depend on the type of citrus. At times, the very green peel gives the notion that the fruit is still raw, covering the presence of carotenoids, which give the fruit its characteristic attractive orange and yellow color in the pulp. Practically speaking, when a consumer looks for a ready-to-eat citrus fruit, particularly oranges, he or she avoids those with green peel thinking the fruit is unripe and is therefore not ready for consumption when in fact for some varietals, the citrus can be overly ripe inside. For lime, the green color is an important quality indicator because lime is supposed to be green (Terry 2011).

The carotenoids are pigments found in the peel, fruit flesh, flavedo and juice of the citrus. It makes the pulp deep orange, red, or in some cases, light pale orange. Citrus carotenoids are considered one of the most complex carotenoid groups because aside from the common vitamin A carotenoids such as Alpha carotene, Beta carotene, and Beta-cryptoxanthin, it also contains lesser known provitamin A carotenoids and unique carotenoids such as C_{30} apocarotenoid, which is found exclusively in citrus fruits (Terry 2011). The concentration and distribution of carotenoids in citrus are related to the variety, maturity, tissue type, climate and season. Most carotenoids are concentrated in the fruit peel while the distribution of carotenoid in the juice and in flavedo is of equal amount. The region where the citrus is grown also plays a factor in the carotenoid content. It is said that citrus fruits grown in the Mediterranean climate

have more carotenoid content than those cultivated in the tropical areas owing it to the farms' unique stress condition. In addition, carotenoid content and distribution in citrus is highly complex and can be explained based on genes, mutation and even changes in plant hormones (Terry 2011). Some studies, as revealed by Ladaniya (2008) indicate that carotenoids increase in the peel and pulp as the fruit ripens; likewise with the presence of lycopene in pink and red grapefruit and some mutants of sweet orange, commonly known as "blood oranges" because of their red juice. It is clear how the fusion of vitamins, minerals and disease-fighting phytonutrient compounds in citrus fruits already make it a power food indispensable in every one's diet. Note that the helpful compounds are also found in the skin or rind of the fruit, the uneaten parts. On the other hand, desirable taste is the fundamental factor in food selection. The conflict here is on how to reconcile consumer's aversion to the bitter tasting, phytonutrient packed fruit parts in exchange of having a healthier body. Some scientists, in their search for solutions to disease-free human health even suggested to increase the bitterness of some plant foods such as brussels sprouts because the more bitter it is, the larger concentration of glucosinolates which fight diseases. This suggestion is opposite to the practices most commonly found in the food industry, which is to eliminate the bitter taste in foods for palatability and "improving food quality". In commercial practices, this means that the useful compounds cited earlier, the phenols, flavonoids, isoflavones,

limonoids and terpenes are removed through de-bittering processes. An interesting field of work being done to do this naturally is via the cultivation stage and not in the factories. More work is also being done through the field of genetic (Drewnowski and Gomez 2011).

a) Limonoids – Limonin

Seeds - For years, people have had the notion that oranges are beneficial for humans from the fruit pulp, peel, leaves, flowers and the seeds. In terms of seeds, it has been stated that they offer various practical and medicinal uses. The most common substances extracted from seeds are the oils. In the olden times, it is known that civilizations had used orange and lemon oils in making perfumes, medicine and cosmetics. Nowadays, orange seeds are considered as a new supply of edible oil and also serve as a good source for essential fatty acids. There are more than 60 fatty acids found in various citrus seed oils. >65% unsaturated fatty acids are present, which is a very high amount. Linoleic is >30%, oleic is >18%, and 2–12% linolenic acids are the largest unsaturated fatty acids present (Shahidi and Zhong 1993, 2005). It was also reported by the same source as noted that citrus seeds contain about 36% oil, which can be recovered from seeds by crushing, drying and solvent extraction.

According to the World Journal of Dairy & Food Sciences (2012) an orange seed has about 22% protein, 40% lipids, 6% fiber and 32% carbohydrate. It is important to note that oranges differ greatly, depending on fruit varietal, soil conditions, growing region and even the level of maturity when they are harvested. Minerals such as calcium, magnesium, potassium, manganese, sodium, copper, iron, zinc and phosphorous are also contained in the seed as well as some amount of amino acids. Crude citrus seed oils are said to have eight classes of chemical constituents that are obtained by using a thin layer chromatography method. These are the triacylglycerols, free fatty acids, diacylglycerols, monoacylglycerols, sterols, phospholipids, alcohols and hydrocarbons. Triacylglycerol's, being the major oil class in all citrus seed oils, can give off partial acylglycerols and free fatty acids during its partial enzymatic hydrolysis in the period of seed storage. Shahidi (1993) reported that other edible oils have lesser amount of volatile fatty acids compared to citrus seed oil. For instance, mandarin seed oil has a high content of triacylglycerol while citron oil has a large amount of free fatty acids. Orange oil is in some ways similar to cotton seed oil in its degree of saturation. It has been proven that orange seed present numerous health benefits. People have done work which indicates that orange seeds are good antioxidants which can defend our bodies from the harmful effects of free radicals. These free radicals, as stated earlier, are responsible for premature aging, tissue damage and may trigger diseases.

Literature indicates that limonoids found in orange seeds are therefore believed to be effective against breast, mouth, skin and lung cancers (Berhow and others 2000).

Limonoids, are a family of multi-ring structures unique to citrus fruits. A large portion of the health benefits of citrus fruits are attributed to limonoids (Polouse and others 2006). The therapeutic efficacy of citrus seed extracts also depends on the presence of polyphenolic flavonoids (Drozdowicz 2011). When taken orally, substances high in limonoids are used to treat bacterial, viral and fungal infections. The vaporized citrus seed extract is also used to treat lung infections. Citrus seeds provide the naturally high repository of citrus limonoid. There have been tests in animals incorporating citrus seeds in the diet and results have shown that seeds can detoxify other chemicals and suppress the growth of tumor cells. These claims have yet to be fully tested in humans (Codoner 2010).

A good example of these limonoid compounds are the limonoid glycosides as well as the limonoid aglycones. Note that limonoids are phytochemicals which are derived from other plants too beyond citrus. According to an article in the Journal of Medwell (2011), limonoids are found mainly in the Rutaceae and Meliaceae families of fruits and they differ from flavonoids in chemical structure. Limonoids are very concentrated in the seed portion of the citrus fruit. Limonoid

aglycones (do not contain any glucose units) are essentially water-insoluble compounds that are responsible for the very bitter taste in citrus fruit while the limonoid glucosides (glucose containing) are water-soluble and essentially tasteless. Limonoids are described as modified triterpenes having a 4,4,8 trimethyl-17 furanyl steroid skeleton and this characteristic is assumed to be one of the reasons why limonoid indicate to have anti-carcinogenic, insecticidal, insect growth regulation, antibacterial, antiviral and antifungal properties. Researchers have discovered and characterized more than fifty limonoids and the latest studies show that the ingestion and treatment of limonoids have been proven to be chemo-preventive (Berhow and others 2000). Common citrus fruit is associated with very high antiproliferative properties as well as with increased anticarcinogenic properties in different cancer cells (Turner and Burri 2013). An important general health benefit of citrus fruit consumption is the improvement in the overall cardiovascular health as well as insulin productivity. In a study involving obese children, it was shown that citrus juice (mandarin) consumption significantly reduced the oxidative stress biomarkers, helped improve insulin homeostasis and lowered overall insulin intake (Cvetnic and Kenezevic 2009). Citrus juice intake for an average of 250 to 500 ml per week for four weeks also helped lower blood pressure in adults who have long-standing history of high cholesterol and blood pressure. There was also a significant improvement in the overall plasma lipid reduction of hypercholesterolemic individuals. Cholesterol

concentrations have also improved in those individuals who incorporated orange juice consumption into their daily habitual diet for a period of four weeks (Cvetnic and Kenezevic 2009). There have also been similar effects on animals. Weight gain, fat and cholesterol build up has been inhibited in mice who were given orange juice three times a day for four weeks as part of their regular diet. Other interesting findings in animals show that citrus consumption improved insulin homeostasis and insulin sensitivity, decreased blood cholesterol levels and LDLs, reduced liver stress and improved blood pressure level (Poulose and others 2009). These anti-obesity effects of citrus fruits on animals have been very remarkable; therefore, further tests are being conducted to show its proliferative effects in human subjects as well. In additional to fruit juice, other studies point specifically to the extracted compounds from the seeds and peels, generally contain limonoids, flavonoids and pectins which have the specific health benefits (Cvetnic and Kenezevic 2009).

Citrus limonoids, along with certain flavonoids, are responsible for the bitter taste in citrus fruits as explained in the introduction. Limonin and Nomilin are considered as the most prevalent types of limonoids in citrus fruits (Turner and Burri 2013). As compounds, limonoids are known to contain substituted furan moieties, and based on certain animal studies; these derivatives have significant contribution to overall health, because of their chemotherapeutic

effects. Limonoids are also considered as a detoxifying enzymes, in which the main action is to catalyze the conjugation of glutathione S-transferase (also known as GST), which is commonly associated with carcinogenic activation (Poulose and others 2009).

The importance induction of GST inhibits carcinogenic activation, because they help facilitate excretion of these compounds through a water soluble process. If there is an increase in the GST activity, following intake of limonoids rich in substituted furan moiety, there will be an increase in the mechanistic property to protect the body against the negative effects such as carcinogens. This means that GST can help forestall carcinogen activation through inhibition process, where chemicals enhances GST intake to further inhibit chemically-ingested compounds containing carcinogens (Cvetnic and Kenezevic 2009). The substituted furan moiety, or furanoids, induces GST activity through a breakdown process where the 2-alkyl substituted compound, the sulfur analogue 2-n-butyl thiopen, and 2-n-heptyl furan form into more complex compounds that have significant roles during the entire enzyme induction phase. This GST-inducing ability of nominilins and limonins play an important role in the induction of enzyme and cataclysmic properties of these compounds. Several studies have already been conducted and indicate that GST induction properties of citrus

limonoids, where forestomach tumors have been inhibited following the induction of GST activity through nomilin and limonin intake (Kuttan and others 2011).

The activation of the properties of citrus limonoids has shown profound effects in the protection against forestomach tumor activation. Similarly, they have also shown significant effects on the inhibition of lung carcinogenesis through effective activation of GST compounds. There are some animal studies that indicate limonoids can also provide benefits against skin tumor activation. But, these studies focus on animal subjects alone, so it is necessary to test human cancer cells to identify their overall effectiveness and to address possible challenges during the chemotherapeutic activation phase. In the past, the problems found associated with human subject testing involve the consumption of extremely bitter citrus limonoids and the capability of the chemicals to be catalyzed in organic solvents (Turner and Burri 2013). In general these problems would post a consumption limit on the capability of and the type of limonoids to be included in specific citrus products including supplements and food additives (Poulose and others 2009). The solution for these ingestion challenges is to either create or utilize limonoid compounds with high molecular properties and highly water-soluble (limonin glycosides), so that they are tasteless and more soluble. Or, by microencapsulating the compounds, by doing so, products

containing higher concentrations of citrus limonoids can be consumed, hence, the premise of this project.

The following are additional noteworthy examples cited in literature on the benefits of citrus limonoids (limonin).

Pancreatic cancer cell inhibitor: A specific anti-cancer property of citrus limonoids is that it provides compounds that help inhibit the formation of pancreatic cancer cells. According to Kim and others (2012), limonoids possess anti-pancreatic cancer properties by suppressing cancer cell proliferation and apoptosis induction. This process results in inhibition of enzyme activities and obstruction of signal transduction pathways, all of which are key characteristics of pancreatic cell activation. The researchers, however, emphasized the need for extended work to identify the specific cellular process involved in the enzyme activation of pancreatic cancer cells. By doing this, a concise idea about the specific cellular targets of citrus limonoids and whether increasing the intake of limonoid-containing citrus products could have beneficial effect to fight against pancreatic cancer cell formation (Kim and others 2012). Once the work is fully completed, limonoids can then be used specifically for their anticancer properties with emphasis on pancreatic cancer cell enzyme inhibition. Another study that

supported the anti-cancer property of limonoid in inhibiting human pancreatic cancer cells was conducted by Murthy and others (2013). In this study, the researchers examined the capability of limonin and nomilin – derived from citrus by-products to inhibit cancer cell proliferation. The researchers also found that when limonoids are combined with curcumin (another compound that contains anticancer properties), the cytotoxicity, or cell death, can be increased and proliferated more effectiverly. In this experiment, cellular proliferation was assessed by combining limonoid compound in varying doses on pancreatic cancer cell samples. Limonoids were added in varying ratios, where increased potency is expected to result in more profound results. This is one of the studies that provided compelling outcomes for exploring the anticancer properties of limonoids by analyzing its pharmacodynamic effects for inhibiting pancreatic cancer cells (Murthy and others 2013). Evidently, the cancer cell samples that had the higher amounts of limonoids show increased cytotoxicity to cancer cells. The researchers suggested that there is indeed a strong correlation between limonoid consumption and increased protection against colon cancer cells (Murthy and others 2013). Note that this is the early stages of looking at the current status of citrus limonoids and their important anti-cancer roles, which means that future researches should be conducted to expand the current outcome of this study as recommended by Murthy and others (2013). Also, the sample size used in this study is relatively small so in order to examine the

potential benefits of citrus limonoids, there should be larger sample pool to properly evaluate the potential.

Apoptotic effects against oral cancer: Nagoor and others (2011) conducted a study that aimed to investigate the apoptotic and cytotoxic effects of limonoids on oral squamous carcinoma of human cell samples. The limonoid extract was derived from an orange seed powder, which is known for its anti-proliferative and cell inhibitory characteristics. Nagoor and others (2011) explained that the main target cells of their research were the oral HSC-4 squamous cancer cell lines. By using these sample cells and analyzing the chemical composition and characterization of the citrus limonoid, researchers were able to find that HSC-4 oral cancer cells lose its viability through a cytotoxic and apoptotic process, where inhibition of growth and replication are the primary reactive outcomes of the citrus limonoids. Under these conditions, the HSC-4 cancer cells could be killed, further cementing the anti-tumor properties of citrus limonoids. At this time, however, oral cancer cells were investigated and the results are quite similar to other studies focused on anti-carcinogenic characteristics of limonoids (Nagoor and others 2011). But, because there is limited work on citrus limonoid tested for human oral cancer cell studies, the authors emphasized that there be other possible effects of citrus limonoids if larger cell samples or actual human clinical trials are conducted. This means that

the study of Nagoor and others (2011) is the first stage of the larger picture which involve more trials containing citrus limonoids for oral cancer treatment research.

Breast cancer cells: Because of its growing range in the application for cancer treatments, researchers have expanded the use of citrus limonoids to specific cancer diseases. Vikram and others (2011) investigated the cell-cell signaling that citrus limonoids do against breast cancer cells. The citrus limonoids used in this study were purified and extracted to get its respective active compounds, including limonin, deacetylnomilinic acid glucoside, isolimonic acid and ichangin. These purified limonoids, according to the results, the cell-cell signaling process helped induce the expression of cancer cell death and inhibit cancer growth (Vikram and others 2011). The researchers further explained that the main target action of the citrus limonoid compounds was the activation of the Harvey auto-inducer (also known as HAI). However, as in other cases above, Vikram and others (2011) as well as Somasundaram and others (2012), stressed that this is the first time a study found significant results and that future and more expansive work should be conducted to either support or refute their findings.

Anti-obesity properties: One of the first studies to show the anti-obesity properties of citrus limonoids which can lead to preventing further cardiovascular issues, was performed in mice by Ono and others (2011). It has been previously discussed that citrus limonoids have the capability to activate GST that help in

the inhibition of cancer cells. This is the same process that has been identified in the anti-obesity properties of limonoids, because they also allow for the activation of TGR5 protein receptors. These receptors play an important role in the development of metabolic conditions such as obesity (Ono and others 2011). In the study, the specific limonoid extracts were limonin and nomilin from lemon seeds, which are capable of inhibiting TGR5 activation. The mice were put on a diet involving daily intake of citrus seeds rich in limonoids for nine weeks. Before the start of the experiment, the mice were already obese so that the researchers could identify whether or not inclusion of limonoid in the diet would have potential benefits. After the nine weeks trial period, the mice did not gain additional weight and even lose some weight in the process. Insulin sensitivity was also improved, which led to enhanced tolerance to glucose and above-average serum glucose level, all of which are attributed to the inclusion of citrus seeds in the diet (Ono and others 2011). This finding supported the researchers' hypothesis that citrus limonoids indeed have anti-obesity properties. Once again, it was suggested that further research should be conducted to investigate its applicability directly in humans.

Weight Management - Another promising health benefit of citrus limonoids is that its daily consumption could lead to better nutrition and weight management and decreases the incidence of obesity (O'Neil and others 2012).

Unlike the previous anti-cancer studies, which focused on experimental aspect to determine the mechanism of action of citrus limonoid, the study of O'Neil and others (2012) relied on statistical survey to analyze whether or not consumption of whole foods containing citrus limonoids have beneficial effects in terms of weight management and decreasing obesity. This study used the National Health and Nutrition Examination Survey of 2004-2008 as their basis of the evaluation. More than 9,000 adult participants have been included in the study, most of which were either obese or overweight during the start of the survey. They were all encourage to include an orange juice powder in their daily diet for a period of 4 years, so that researchers could identify if consumption of citrus orange powder as a whole food was attributed with lower cholesterol levels and improvement in body weight. In order to see if there are significant changes in the body weight and proportion of the participants, the researchers divided the participants into two groups: the first group had included orange juice powder in their diet and the second group is a control group, where no treatment intervention was given. No additional food intake in their daily food consumption was given (O'Neil and others 2012). This will help the researchers properly analyze whether or not there were significant changes that happened during the course of the study. In terms of results, first, males who consume more orange juice powder were less likely to become obese and become at risk for metabolic syndrome than the female counterparts. Second, at the end of the study, the total cholesterol level of the

participants who consumed orange juice powder was lower in consumers than non-consumers, regardless of gender. More specifically, the total low-density-lipoprotein counts of those who have consumed orange juice powder were lower than the non-consumers. Third, upon conducting a follow-up survey six months after the study, the authors found that there was no overall increase in weight or BMI on those people who have consumed orange juice powder (O'Neil and others 2012). The researchers also stated that higher intake of fruit juice powder and its habitual inclusion in an individual's diet could likely decrease the risk of obesity as well as other co-morbid conditions associated with obesity. This large scale survey study was one of the first hand studies that shed light to the health benefits of citrus juice consumption. It also gave a deeper understanding of the compounds found in orange fruit – including limonin and hesperidin, which are two key components in the fruit powder produced for this project. Therefore, consumption of citrus by-products containing large amounts of citrus limonoids and flavonoids should be encouraged and possible included in an individual's daily dietary intake to achieve good health results.

Limonin – Limonin is a type of limonoid. It is a white, crystalline and highly bitter substance found in high concentration within orange and lemon seeds. It is also found in orange juice especially in the un-mature or green fruit. Limonin is also known as limonoate D-ring-lactone and as limonoic acid di-delta-

lactone. It is a member of the class of compounds known as furanolactones. Limonin just as limonoids stated above, the bitter type, also known as the aglycone or a-glycone, do not contain any glucose. On the other hand, limonin glucoside or glycoside contain a glucose unit and are not bitter. Concentrations of limonin glucoside can reach very high levels in some orange juices since they are not bitter as the aglycones. The limonin or limonin aglycones are responsible for the development of bitterness in citrus during fruit maturing. The structure is highly oxygenated; the furan is a cyclic compound of a five-member aromatic ring with four carbon atoms and one oxygen. A Lactone (cyclic ester) is composed of a carbonyl next to an ether. Limonin as well as other limonoids are highly oxygenated triterpenoids with anticarcinogenic activity against human breast cancer cells (Shahidi and others 2000). There are two enzymes which complete the inter-conversion of free carbohydrate to carbohydrate-bearing moieties; they are limonoid glycosyltransferase and limonoid D-ring lactone hydrolase. There is also the “delayed bitterness reaction” which takes place within the fruit or when the fruit is juiced in many cases. It starts with the non-bitter compound, Limonoate A-ring Lactone, then, under presence of acidic conditions with the enzyme Limonoid D-ring lactone hydrolase, it produces the bitter compound of limonin a-glycone (Berhow and others 2000). Note that limonin glycosides are abundant in the fruit and also of importance in health but are not bitter so they will not be the focus of this project. Limonin or limonin aglycones are very bitter,

hence needing the encapsulation protection to conceal the off taste which is the goal of this project. The following figure is the chemical structure of limonin.

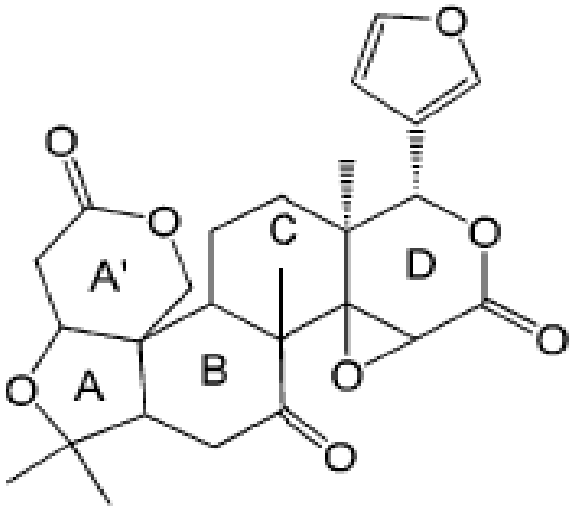


Figure 2. Structure of Limonin

Limonin can be easily detected in citrus fruit as the bitter flavor when one bites a citrus fruit. Although it does not taste good at all, this specific phytonutrient offers a variety of health benefits. The most important benefit associated with limonin as well as for some other limonoids is its anti-carcinogenic properties. It has been stated that in cancers such as neuroblastomas which are often found in the neck, chest, spinal cord or adrenal glands, limonoids have been able to stop the progression of these neuroblastomas (Bayazit and others 2010). Researchers state that limonoids

also produce no side effects when compared to some cancer drugs which can be toxic. According to Bayazit and others (2010), limonin may be capable of “impeding, slowing or even killing cancer cells so it could be used as food additives in preventative measures”. In that study conducted by Bayazit and others (2010) effects were observed where D-limonene from citrus, orange oil and lemon oil on stimulated neoplasia of the lungs and forestomach of female mice subdued pulmonary adenoma formation and the occurrence of forestomach tumors, therefore showing that non-nutrient constituents of the diet may inhibit carcinogen-induced neoplasia. A large number of other studies were also conducted over the past few years about the cancer-fighting properties of limonoids. In the issue of Food and Chemical Toxicology (2010), it was written that limonoids from the White Marsh grapefruit seed effectively hindered the growth as well as killed colon cancer cells. In the December 2010 issue of Anti-Cancer Agents in Medicinal Chemistry a study associated with limonoids exhibited benefits for breast cancer, melanoma and lung cancer as well.

Toxicology - There are a few indications of negative effects of consuming citrus seeds, although research is much more limited than the many known studies which indicate positive effects.

A. Allergies

Despite the promising benefits of eating citrus seeds, whether fresh, dried, or extracted, one should still be careful. Allergic reactions to fruits, especially to citrus have been documented. In a case study conducted by Turner and others (2011), children who were able to tolerate fruit pulp showed adverse allergic reactions to orange seed extracts. This case study suggests that citrus seeds can be a potential cause of allergic reactions including oral allergy syndrome or even systemic allergic reactions.

B. Counter-reactions

Excessive consumption of grapefruit citrus fruit is also observed to increase breast cancer risks because it inhibits an enzyme that metabolizes estrogen. More evidence is needed to prove this (Therapeutic Research Faculty 2012). Moreover, due to lack of studies that seek to know the counter-reactions of citrus seeds in humans, it is possible that more counter-reactions may be observed especially when the consumption is excessive. When trying to incorporate citrus fruits in diets, especially with the added citrus seeds, there is a need for moderation until further research is completed in this field of work.

b) Flavonoids – Hesperidin

Flavonoids or bioflavonoids are a class of secondary metabolites. In general, flavonoids are plant pigments from phenylalanine comprising of a large group of compounds present in fruits and vegetables. Flavonoid comes from the Latin word “flavus” which means yellow color. During the 1930s, flavonoids were discovered by the American-Hungarian scientist Albert Szent-Gyorgi. Flavonoids were originally referred as *vitamin **P***, this was because of the effect they had on the **P**ermeability of vascular capillaries. Since then, that name has not been used frequently. In terms of synthesis, bioflavonoids as other phytonutrients cannot be produced within the body of an individual. As such, many experts recommend that flavonoids be implemented or acquired within ones diet. These compounds are vital so individuals may have optimum well-being. Flavonoids have become prominent with human health researchers because of their health function activities in clearing the body of free radicals. In addition, flavonoids have been associated with having anti-inflammatory, anti-allergy as well as cancer-preventive capability (Buslig and others 2002; American Chemical Society 2012). In citrus, quercetin, lutein and hesperidin are among the most commonly known bioflavonoids. In citrus fruit, flavonoids can be found and are extracted from the white pulpy parts (albedo) that surrounds the fruit (Murray 2007). Other kinds of flavonoids in citrus are as follows: Proanthocyanids – a natural flavonoid compound found in the tree bark that was made into tea by the Quebec Indians.

It was said that the antioxidant activity of this compound is 20 times greater than vitamin C and 50 times greater than vitamin E. The compound is highly curative and can treat various illnesses. Naringen and rutin are bioflavonoids associated to work closely with vitamin C. They have allergenic properties and blocking abilities in inhibiting the release of histamine. Nobiletin – is associated with blocking tumor growth and helping an enzyme that can detoxify elements leading to cancer (Gibbs 2011). Tangeretin is associated with inhibiting the growth of tumors. Terpenoids are associated with helping prevent cancer, even if the cancer has already started. Monoterpenes are components found in the essential oils of citrus fruits and have been associated with anti-tumor activity. Monoterpenes have been identified to promote chemo preventive activity against skin, liver, lung and stomach cancers (Economos 2011; FAOUN 2011). Quercetin helps reduce the risk of coronary disease by dilating and relaxing blood vessels and has a protective effect against certain types of arrhythmias. Quercetin also helps protect the body against viral and bacterial invasion. Xanthones, found in the rind and skin, indicate to be good for asthma and are antiviral, antibacterial and exhibit immune boosting properties (Gibbs 2011).

Hesperidin is a flavonoid found largely in the white spongy part of the peel and membrane of oranges and tangerines. Citrus Hesperidin has other

common names including but not limited to; bioflavonoid, bioflavonoid complex, bioflavonoid concentrate, bioflavonoid extract, citrus bioflavonoid, citrus bioflavonoid extract, citrus bioflavones, citrus flavones, citrus flavonoids, hesperidin methyl chalcone, hesperidina, hespéridine (eMedicineHealth 2011). As opposed to limonin, hesperidin does not contribute much to the bitter flavor of juices. It is insoluble in neutral aqueous solutions and slightly soluble in acidic solutions such as orange juices. Therefore, once the juice is extracted from the fruit, the hesperidin can form crystals. Under the microscope, these crystals are long and needlelike. In processing equipment, they appear as a white scale or film that can build up until it breaks off as white flakes. Therefore, hesperidin also contributes to the cloud in juice. These crystals can clog finishing screens thereby decreasing the juice yield of juice making. During evaporation, concentration of the juice accelerates hesperidin crystallization, causing white flakes to appear in the concentrate or reconstituted juice. The appearance of hesperidin flakes in citrus juices increases with fruit maturity and may become acute in the late season especially in valencia juice. This is due to lower acid levels later in the seasons which reduce the solubility of the hesperidin (Kimball 1999).

Hesperidin has good scientific support when it comes to the benefits that one can utilize. Hesperidin with diosmin is being used in Europe for the healing

process of hemorrhoids (eMedicineHealth 2011). The reason to this is because hesperidin has the ability to scale back the capillary permeability as well as anti-inflammatory action. There have been studies which indicate that hesperidin has antioxidant activity as well. Although most of the work has been done in-vitro, the possible anti-inflammatory action of hesperidin is perhaps because of the action of the aglycone *hesperetin* in the hesperidin. There is also evidence that hesperidin prevents histamine from being unleashed from mast cells. This is perhaps the reason why hesperidin has the potential to act as an anti-allergic medication (Chiba 2012). Hesperidin has positive effects on the immune system, hesperidin alone can be bought as a supplement but it can also be combined with other products which produce benefits to the blood vessels. It has been reported that the combination of hesperidin and diosmin can ease the symptoms of venous leg ulcers, arthritis, capillary fragility and it may even help lower the blood pressure of people with Type 2 diabetes (eMedicineHealth; 2011 Kimball 1999).

There is evidence that hesperidin also works as an antihistamine, works as a stimulant for energy metabolism, ease diabetic complications, ease gastrointestinal disorders and aid in heart conditions. It can prevent heart diseases and high blood pressure. It also promotes weight loss and wound healing (Kimball 1999). The list goes on and on. Although there is a long list of

the many benefits of hesperidin, further reseach on humans is necessary. The following figure illustrates the chemical structure of hesperidin; note the glucoses on the left side of the structure.

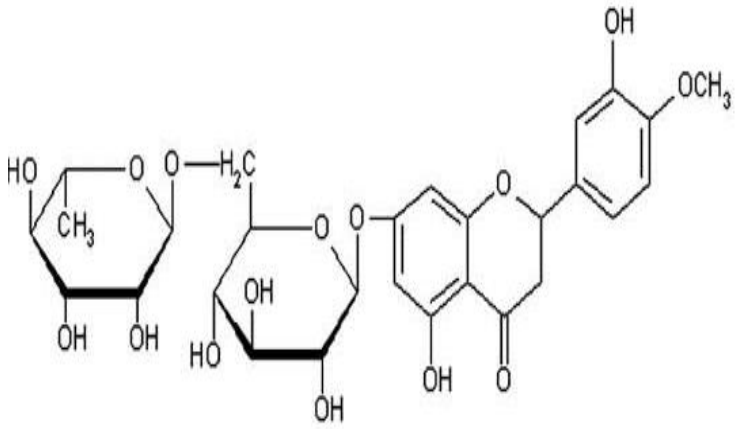


Figure 3. Structure of Hesperidin

Further to the above, the following are additional examples and details to the known benefits of citrus flavonoids and other phytonutrients found in citrus fruit.

A. Anti-carcinogenic activity

Flavonoids also have the potential to become anti-carcinogenic agents. Due to antioxidants, they also have anti-mutagenic effects because they protect

DNA from oxidative damage and neutralize free radicals that commonly promote mutation leading to different forms of cancer (Codoner 2010).

B. Antioxidants

Ample research indicates that orange extracts are good antioxidants. In a study conducted by Moulehi and others (2012), twenty-two phenolic compounds were identified in mandarin orange and bitter orange seeds. These include but not limited to; hydroxybenzoic acids, hydroxynnamic acids, flavones, flavonols, simple phenol and coumarin. In a different study, the antioxidant properties of citrus fruit seeds were also observed in the glyceric extract of which grapefruit seeds contain limonoids and flavonoid glycosides. However in this study, the researchers used aqueous solutions in the seed extraction while the solutions of grapefruit seeds available in the market today contain ethanol (Giamperi 2004).

C. Anti-viral , Anti-parasitic and Anti-bacterial

In a study by Drozdowicz (2011) it was observed properties of anti-bacterial, anti-viral and anti-parasitic activity of grapefruit seeds. It was also learned that the presence of polyphenolic flavonoids in grapefruit and many other citrus fruits inhibit platelet aggregation. This decreases the risk of developing coronary thrombosis, myocardial infarction and other heart diseases. Polyphenolic flavonoids have antibacterial properties that can also be used as

protection against gastrointestinal infections. It has been confirmed that citrus seed extracts have antimicrobial properties against a wide range of gram-positive and gram-negative organisms. It was found that the seed extract disrupts the bacterial membrane and releases its cytoplastic contents within fifteen minutes after contact. These properties make citrus seed extract an effective aid to help prevent urinary tract infection (Oyelami 2005). The anti-viral activities of citrus seed flavonoids can also be associated with non-glycosidic compounds. The anti-viral activities of flavonoids found in citrus seeds are also observed in cases of influenza virus and hepatitis C virus (Codoner 2010).

D. Anti-inflammatory

Flavonoids in citrus can act on the immune system and inflammatory responses that are associated with inhibition of key enzymes. These have properties that control, or inhibit the biosynthesis of pro-inflammatory agents. These are also able to inhibit the kinases and phospodiesterases that are essential for cellular signal reduction and activation. In the same way, they also inhibit the cells responsible for inflammation and immune response (Codoner 2010).

E. Anti-atherosclerotic activity

Flavonoids in citrus are also involved in cardiovascular protection. They inhibit the pathogenesis of atheroma that is responsible for many cardiovascular problems. The free radicals facilitate rapid inactivation of the nitric oxide that maintains normal vascular homeostatics. This inhibits the platelet aggregation and clot formation. It's known that clot formation is a common cause of heart attacks. Moreover, the reduction of plasma cholesterol is associated with effects on specific liver functions that are related to lipid processing (Codoner 2010).

3. Microencapsulation overview

Food microencapsulation plays an essential role in the food industry. Consumers want their food to be delicious, healthy, affordable and ready-to-eat. As such, food microencapsulation tries to respond to these consumer demands. Microencapsulation of foods has been generating interest from food consumers because of its relationship with increased acceptability and the increased quality of the foods. Many vitamins and minerals and several phytonutrients are considered unpleasant because of the off taste or aroma they produce. Microencapsulation is a suitable solution to these challenges. In addition, some microencapsulation techniques done ahead of food processing can mitigate the loss of nutrient content. It was noted that over the last couple of decades, microencapsulation technologies have been well accepted in the food industry; many items have been microencapsulated including, aroma

compounds, minerals, vitamins, enzymes, colorants and functional ingredients. At the advent of microencapsulation and for a long time after, it had been considered as too specific and costly for the food industry. In recent decades, cost-efficient preparations, advances in equipment manufacturing and increased volume of production added to the affordability of microencapsulated ingredients. Microencapsulation is aimed to protect, stabilize and even slow the release of food ingredients. It also comes useful as some nutrients are delicate and volatile so protecting them has been a major concern of food manufacturers. Most of the available aroma compounds are created through extraction or chemical synthesis. Foods that contain synthetic flavors are usually avoided, because food consumers see them to be unhealthy and harmful to their health (Shahidi and Han 1993).

In more detailed terms microencapsulation is a technique where tiny droplets or particles are enclosed in a coating in order to provide the microcapsules with useful and helpful properties. A microcapsule is a very small sphere that has a uniform wall built around it. The material that is inside a microcapsule is called the core, the fill, or the internal phase. The wall, on the other hand, is often referred to as the coating, membrane, or the shell (Zeller and Salleb 1996). Most of these microcapsules have diameters that measure somewhere between a few millimeters all the way down to a few microns. There

are a growing number of opportunities in the food industry where great efforts are being done to ensure the integrity of the microcapsules. The incorporation of very small amounts of food flavors into different foods not only has a great effect on the cost and quality but also in consumer satisfaction of the finished product. Over the last decade, the food industry has increased demand for products that are microencapsulated. The food industry is also making a continuous effort to develop processing methods, quality ingredients and useful and practical packaging materials so that they can produce more improved food preservation and better transport and delivery options (Zeller and Salleb 1996).

Coating of particulate materials is a basic practice in many chemical industries including food, pharmaceuticals, cosmetics and biomedical. Furthermore, the coated particles can also be pelletized or serve as a final product enclosed in soluble coating materials depending on the application needs. The coating process involves the covering of particulate materials including agglomerates, pellets and powders with a surrounding layer of coating material. The coating process can also be applied to a variety of particles ranging from sub-micron particles to very large objects in the millimeter range or lager. The coating thickness might vary from a few nanometers to several micrometers and even millimeters. The active component can be contained in core particles or in some cases, as part of the coating material. There are several methods to

introduce the coating agent into the system; through dispersing or dissolving in an evaporable solvent, molten or applied in the form of a very dry powder (Saleh and Guigon 2007). The typical coating process is performed to achieve many benefits such as the following:

- To protect the powder from incompatible elements such as oxygen, acidity, humidity, and light
- To delay and control the release of core particles or active agents
- To reduce the affinity of powders to liquid or organic solvents
- To avoid caking phenomena during transport and storage
- To improve appearance, taste and odor of products
- To conserve nutrients

Many options are available to encapsulate food ingredients. The following are just three examples of microencapsulation techniques often used by the food industry with the last one being a fluidized bed dryer; which is the technique adapted for this project.

a) Spray drying

This mechanical procedure is a widely used commercial process. Spray drying is generally used in the production of a large amount of food encapsulation. This technique is also favored in the microencapsulation of

volatiles (Deis 1997). The numerous merits of this process have created its dominance over the years. Spray drying of food is favorable, because the equipment is readily low in cost and low to operate as compared to other microencapsulation options. There is also a very wide selection of carrier options for this technique. The retention of volatiles is enhanced, because the finished products can have good stability properties. In addition to these benefits, a large-scale production mode can be used continuously as opposed to a batch process, which can also contribute to efficiency. Spray drying is conveniently applied to materials that are heat-labile or have a low boiling point. This is because the core material reaches very low temperatures. This process involves the dispersion of the material to enter the microencapsulation process to be placed in a carrier material. Then, it is submitted to an atomization process. The mixture is then sprayed into a hot chamber. The microcapsules that are formed are eventually transferred to a cyclone separator for a recovery process (Shiga and others 2001).

b) Coacervation

This method is a chemical phenomenon that occurs in colloidal solutions. Some regard this technique to be the first used for encapsulation. This method was one of the original encapsulation processes used to create pressure sensitive dye microcapsules that could be used in the manufacture of carbonless

copy paper. Coacervation consists of the separation from the solution of the particles of a colloid. It will then agglomerate to clusters and then into a liquid and the separate phase is referred to as the coacervate. The core material that is used in the coacervation method should be compatible with the receiver polymer. It also should be insoluble in the medium to be used in coacervation. Simple coacervation techniques enable food flavor to have a prolonged release. The complex coacervation technology however, produces a prolonged release or diffusion and a started release that includes dehydration, pH, mechanical effect, enzymatic effect or dissolution (Desobry and others 1997).

c) Fluidized bed dryer

Another way to microencapsulate ingredients is by use of fluidized bed dryers. This is the technique selected for this project. The process of spray coating fluid bed entails three general steps. First, the particles to be coated must be *fluidized* in the control environment of the chamber intended for the coating session. Second, the coating material has to be sprayed by the use of a nozzle into the particles. Finally, the film formation needs to be a succession of wetting and then drying steps. The tiny drops of the sprayed liquid will spread into the surface of the powder particle and will be joined together. The mixtures or the solvent will then be evaporated by the hot air (Lee and Krohta 2002). The size of the end product range is rather large; it can be from a few microns to several

millimeters. The fluid bed process has been widely employed in the cosmetic and pharmaceutical industries for a long time. Both sectors have a large enough budget that can be used in day to day processing. This technique is becoming more common with the food industry as cost for equipment and operation has reduced over the years. The fluid bed process is one of the most appropriate methods that could be used for encapsulation of flavors. It's evident by the fact that the wall or coating materials that are used in the food systems become fully dissolved. The coating in turn will form robust inter-particle bridges during the re-drying procedure. Fluidized bed technology also allows definite distribution of particle size to ensure uniformity. The fluidized bed process has high rates of drying capacity because it applies good gas particle contact. The flow area entailed by the fluid bed process is relatively small. Another positive characteristic of this technology is high thermal efficiency. This process can be used for gums, starches, and maltodextrins, because shells can be formed as soon as the hot air disperses the solvent (Lee and Krohta 2002).

A fluidized bed is created when a solid particulate is placed under appropriate conditions to cause the solid to behave as a fluid. The achieved phenomenon is referred to as *fluidization*. For a free-standing bed there will be an ideal point, known as the incipient fluidization point whereby the bed's mass is

suspended by the flow of the fluid stream. Its corresponding fluid velocity is referred as the minimum fluidization velocity (Grace and others 2008).

There are many benefits of utilizing fluidized bed dryers such as:

- A high surface area contact between fluid and solid per unit bed volume
- High relative velocities between the fluid and the dispersed solid phase
- High levels of intermixing
- Frequent particle-particle and particle-wall collisions

For the fluidized bed dryer approach, the spray nozzle can be positioned in one of three ways. If at bottom, it is referred as the *Wurster* process. A side spray is referred to as a Tangential process. The third option is a Top spray (Holdich 2002).

Fluidized bed dryers can also employ *the hot-melt coatings* where waxes and fats are utilized as the coating materials. The solidification step, also known as congealing, is achieved by the use of cool air, ambient air, or slightly warm air for some types of hot coatings. This is the conversion of the melted coating material i.e. wax, going from a liquid phase to a solid. The fluidized bed dryer process coupled with the hot melt system was the selected approach for this project. In part, there is a need to spray coating materials which will serve as

good moisture barriers and they are geneally fat based. After microencapsulation trials were completed, the critical next step was to perform water binding capacity to evaluate the effectives of the selected coating material for moisture barriers.

It is important to remember that the droplets of the hot-melt coating must be very small so that solid bridges and bubbles will not form during the entire process. However, the quality of the coating used should be dependent on the actual coating zone so that proper coating provisions could be done. A well-utilized hot-melt fluidized bed coating allows the creation of film quality that is evaluated based on the thickness and uniformity of the film coating. This is very important to achieve in the fruit powder, because they serve to improve the overall storage time and handling of the products as well as the key reason for coating the fruit powder; for concealing the off taste of the citrus fruit phytonutrients. The procedure for hot-melt coating involves application of molten wax or other hard fat materials onto the fruit particles. The quality of the finished coating material will depend on the amount and rate of the solidification of the coating droplets, and this was achieved by maintaining a constant liquid temperature of around 40°C to 60°C above the average melting point of the carnauba wax or other coating materials. Proper temperature control and monitoring is very critical when applying hot-melt fluidized bed coating on fruit powder to achieve the desired coating quality (Aulton, 2010). Another advantage

of the hot-melt process is that it only requires short processing time, because the carnauba wax or other coating material to be used has no evaporative properties, such as water or other organic solvents. Therefore, the coating can be applied in a relatively short period of time. A documented disadvantage of hot-melt coating is that it can be time-consuming for first-time operators, because it requires extensive equipment set-up, including nozzle insulation and air atomization, melting of the coating material and clean-up (Aulton 2010). More detailed information about the coating parameters of the fluidized bed dryer and the physical and chemical mechanisms will be discussed in section IV of this paper.

II. HYPOTHESIS AND OBJECTIVES

The hypothesis

1. Identifying an adequate water impermeable coating for a developed fruit powder will prevent leaching of the bitter phytonutrients found in citrus by-products and conceal the off taste over the 10 week shelf life of a chilled orange juice beverage

The objectives

1. Identify coating ingredients to develop a suitable water proof barrier to conceal the bitter notes found in orange fruit by-products, when applied to a juice beverage

2. Incorporate the coated fruit by-product to an orange juice beverage to increase the limonin levels by 5 to 8 times higher than the amounts typically found in commercial orange juice

III. BY-PRODUCT SOURCING AND PREPARATION

Every year, there are over 30 million metric tons of citrus by-products generated by the juice industry (FAOUN 2011). For the most part, close to half of the orange fruit is unutilized and go to “waste”. In some cases as cattle feed but in other cases to landfills. This is unfortunate since these citrus by-products contain many nutrients which are not consumed by humans. Most commercially produced fruit juices lack the dietary fiber found in whole fresh fruit. In addition, a good portion of other nutrients such as vitamins, minerals and specifically phytonutrients found in the by-products go unused. Literature outlines that good levels of phytonutrients and health benefits found in the albedo, rag, and even in the seeds and peel of many fruits as mentioned in the introduction of this paper. For this project, the opportunity is about capturing and re-incorporating the phytonutrients found in the rag and seed of the orange fruit.

In terms of processing, in a simplistic way, the orange fruit is collected from the field otherwise known as the groves, and it is then sized to facilitate the cleaning and extracting of the oils from the peel. The oils are removed by making many perforations on the skin of the peel with needle-like probes on rollers. Oil leaches out and then collected as a liquid. Next, the whole fruit goes to extraction which is where the peel is removed. Many types of equipment exist that can remove the peel. Note that peel in oranges is a by-product which is not typically

consumed by humans. Then, the peeled liquidly fruit (fruit puree) goes through some metal channels called throughs directly to fruit finishing. Fruit finishing is the step where the “juicing” takes place. Juicing is where the juice is separated from the rest of the fruit solids and collected to be further processed i.e. pasteurized, packaged and sold to market. After finishing the fruit or “juicing”, the leftover by-products are the rag and seeds. Just as is done in every home’s kitchen, in terms of juicing, the rag is separated from the fruit juice. But, when people eat a whole orange, the rag is a component which is consumed. The so-called rag is composed of; the white spongy parts of the peel, the core in the middle of the orange, the segment walls dividing the sections of the fruit and the sack walls of the pulp where the juice comes from. The rag is essential where much of the fiber is concentrated as well as flavonoids. To bring further clarity to the processing of the orange fruit, the following figure (4) with the flow diagram illustrates the key steps in orange fruit processing.

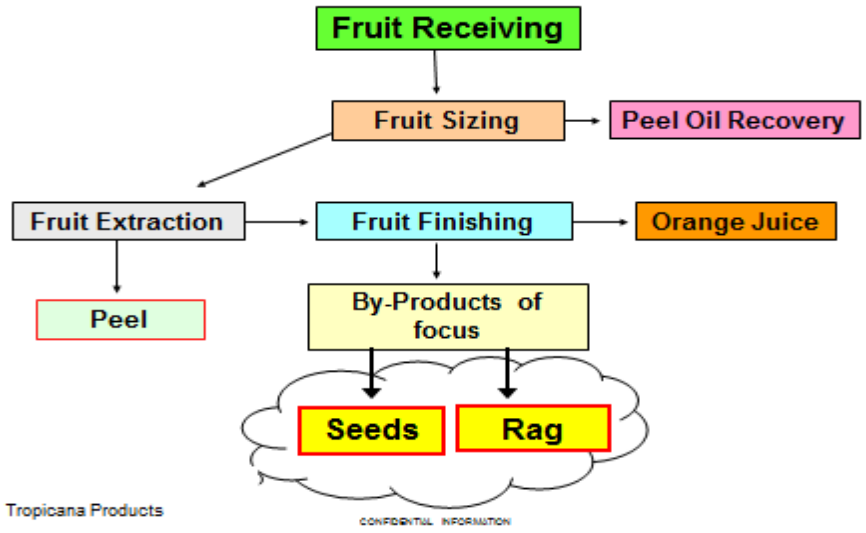


Figure 4. Flow Chart of Fruit Processing

1. Dehydration

The first step of the sourcing and preparation section was to obtain by-product materials to conduct the hands-on part of this project. The two by-products; rag and seeds were collected in separate containers. After collecting twelve (12) five-gallon pails of rag and six (6) five-gallon pails of seeds from a fruit processing facility located in Bradenton Florida, the pails containing the by-products were immediately stored frozen at a temperature of -20ºC to avoid spoilage. Products were then shipped to a location in Barrington, Illinois to

perform sample preparation. Once in Barrington, samples were quickly thawed in a warm water bath of approximately 35ºC. Note, there was no direct contact between the by-product samples and the water. The samples were maintained in a sealed plastic bag.

To elaborate on the nomenclature of the two by-products of focus, Table 1 has definitions along with actual photographs of the fresh by-product ingredients. It is rather obvious what seeds are, but most people are not as familiar with the term “rag” of the citrus fruit.

Table 1. By-Product Nomenclature

Seeds	The seeds are imbedded within the segments of the orange, they are separated from the rag. Contain high levels of **limonin**	
Rag	The portion of the orange which is leftover after the juice is expressed and seeds are removed. Contain high levels of **hesperidin**	

The next step of the preparation process was to remove moisture since the materials are wet and raw. This step is very critical to stabilize the by-product

ingredients and to avoid spoilage and to facilitate further handling and processing steps. Initial moisture of the seeds was approximately 50% and initial moisture of the rag was approximately 80%. The two by-products were dehydrated individually utilizing an electric forced air Aeroglide oven (Figure 5). The oven has an air delivery system capable of generating a volume of 150 feet per minute (FPM). For this project, the velocity was set to 100 FPM utilizing perforated metal pans with a surface area of 9 square feet. This then translates to 900 cubic feet per minute (CFM) of hot air. (100 FPM times the cross sectional area of the drying trays used which is 3 feet x 3 feet equates to 900 CFM). The oven is equipped with two heaters and are used singly or jointly depending on temperature or drying (energy) needs. One heater is rated at 30 KW to deliver 102,400 BTU/hr and the other heater is rated at 70 KW which can deliver 239,000 BTU/hr. To dehydrate the by-products, first, an 8 kilogram sample (one pail full of material) of the citrus rag was placed on a perforated stainless steel pan at a bed depth of 3 inches. For the citrus seeds, a 10 kilogram sample was placed on a separate perforated stainless steel pan with a bed depth of 2 inches. The dehydration approach for both the rag and seeds was the similar but done separately. There were a total of five drying cycles. Air speed was set to a constant speed of 900 CFM. For the first three cycles, the oven temperature was set at 120^{o}C and timing was four minutes with air flow in the up direction and four minutes with air flow in the down direction. For the subsequent three cycles, the

oven temperature was reduced and set to a temperature of 100^{o}C and timing was four minutes with air flow in the up direction and four minutes with air flow in the down direction. Note that these times, temperatures, and air flows were the final conditions but were derived after several trials of drying the product as effectively as possible. It is obvious that the goal of drying was to remove the moisture as efficiently as possible but with caution not to damage the material by heating too quickly. Therefore, these conditions were specifically selected to minimize the degradation of the by-product ingredients, the nutrition and the sensory characteristics. Note that especial attention was given to the rag. i.e. avoid excessive heat and drying to minimize or eliminate burning, scorching or caramelizing due to its high sugar content. Values for physical, chemical and nutritional characteristics of the by-products will be disclosed and discussed later in this section.

Figure 5. Dehydration via Electric Aeroglide Oven

The finished product target moisture for each of the two by-products was 5% independently. Selecting a 5% moisture range was ideal for grinding the product in the next phase of the process and ultimately creating a good powder to facilitate the microencapsulation portion of the project. After samples were dehydrated, a small portion of each by-product ingredient was retained to conduct analysis of the phytonutrients and other macronutrients including dietary fiber, sugars, protein and moisture content. At this point, the data in Table 2 below showcases the initial counts for the phytonutrients of interest. The data is based on analysis of the dried by-products. Note the extremely high levels of limonin in the seed powder. It is in the range of four-thousand parts per million

(ppm). To set some context, this level is very high since a typical commercial orange juice beverage may be in the range of 3 to 5 ppm of limonin. Further details for characterization equipment utilize to analyze the ingredients and procedures are found in the next section of this paper under micronization.

Table 2. Characterization of Rag and Seed Powders

Phytonutrient Profile (average values from 6 lots)		Rag (dry basis)	Seeds (dry basis)
Limonoids (ppm)	**Limonin**	164	**4,207**
	Nomilin	36	430
Flavanones (ppm)	Narirutin	2,961	1,144
	Hesperidin	**16,085**	5,993
	Didymin	1,212	397

2. Micronization

The next step of the sample preparation was to micronize the dried by-product materials by grinding them through a mill. For this project, a FitsMill was

utilized. FitsMill equipment is from the Fitzpatrick Co. Chicago, IL. Model DR00700, 1700 RPM (figure 6). First, approximately 10 kilograms of the dried rag material was slowly fed through the inlet of the mill. The targeted particle size was 100 to 200 microns. This range is optimal for generating a good fluidized bed for microencapsulation based on literature review, equipment potential, personal experience, application parameters and equipment availability. Therefore, a screen size of US 70 (equivalent ~200 microns) was placed at the outlet of the FitsMill to serve as the top end of the allowed particle size range. Placing this screen on the outlet is designed to only allow particles smaller than ~200 microns to exit the mill. Particles which are larger than ~200 microns are re-circulated through the mill and spinning cutters at 1700 revolutions per minute (RPM) continue to micronize or cut down the by-product material until they become small enough to exit the FitsMill outlet screen. Figure 7 below shows the cutting head of the FitsMill. Since the mill utilized for this work is a pilot scale unit, this step was conducted for approximately two hours of slow feeding and micronizing of the dehydrated rag. Next, approximately 5 kilograms of the dried seed material was set to be fed slowly through the inlet of the FitsMill. Screen size and all other running conditions were kept constant as utilized for milling the rag material. After feeding a small portion of the dried seed material, very little passed through the exit screen. A second attempt was done but again no powder was generated. The FitsMill was taken apart, inspected and noted that the

housing of the blades was completely coated with seed material and the screen at the bottom of the outlet was completely plugged with a liquidly, oily, paste-like consistency material. After further inspection, it was determined that the high fat/oil content (approximately 35-40%) of the seeds was the cause for this issue. The unit had to be thoroughly dismantled and cleaned. It was then determined to combine the rag with the seed material at a ratio of 2:1 to “thin-out” the seed material and to facilitate micronizing and further handling. This approach proved out to be successful since the combined (combo) ingredients were able to pass through the cutters and exit screen without causing further binding or clogging of the unit. After the combo (blend of rag and seed) sample was completely micronized, a small portion of this mixture was retained to conduct sieve analysis and particle size distribution. The rest of the material was stored in a bag-in-box in a cool, dry, warehouse at 5ºC and low relative humidity <50%.

Figure 6. Micronization via FitsMill

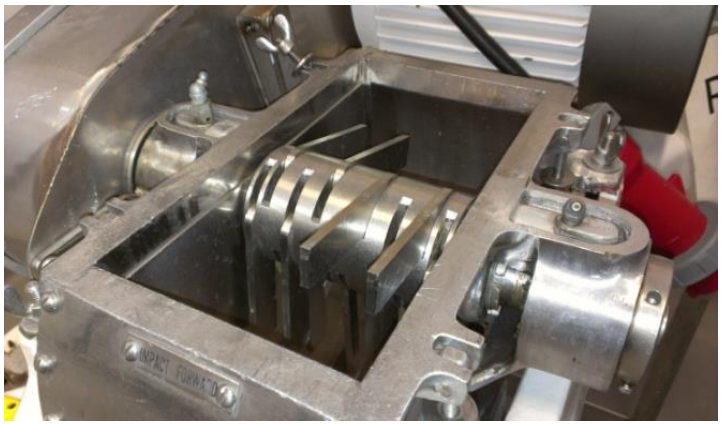


Figure 7. FitsMill Cutting Head

3. Material characterization

Following the completion of the micronization step, the combo powder was analyzed for particle size via a traditional Alpine sieve analysis. This is essentially

a series of perforated sieves (screens) where particles are fed through the screen by gravity with assistance by a brush moving the particles around and through the screens. The process starts with a highly porous screen on the top (large perforation/hole size) followed by smaller and smaller screen openings. The selected screen sizes are listed in Table 3. The selection of these screens offered a broad range of particle size screening from 300 microns down to 20 microns. Based on this set of results, since a smaller amount of product was collected on the 150 and on the 300 micron screens, this indicates that the majority of the particles were between 150 and 300 microns. It was noted that if the screen size on the FitsMill had a 200 micron screen, the particles on the Alpine sieve analysis should have all passed the 300 screen. It was determined at a later date that agglomeration of the fruit powder mixture was an issue but was eventually successfully addressed before conducting the last set of microencapsulation trials.

To better understand the particle size distribution of the combo powder ingredient, a more sophisticated analysis was conducted. The ingredient was analyzed through a Malvern Analyzer (special thanks to the Barrington Analytical team for conducting this analysis). This method is essentially done via laser diffraction where a light is scattered to different angles as particles pass by the laser. The particles size is then calculated in relation to the diffracted angles.

Results are then reported as particle surface weighted mean and volume weighted mean. Figure 6 illustrates the *volume* weighted mean (VWM) of 233 microns. The *surface* weighted mean (SWM) was at 105 microns. Note that the VWM distribution has a large range based on the bell curve. Some particles registered at 500+ microns. The reason for this large value is that some agglomeration was taking place and clustered particles were being measured as a single particle or sphere. As part of the next phase of the project, high resolution microscopic images were taken of the fruit combo material before and after microencapsulation to find more concrete answers. Details to follow in the Applications section (V) portion of this project.

Table 3. Particle size Distribution via Sieve Analysis

Alpine Sieve Analysis		
ON #635 (20 microns)	%	99.92
ON #325 (45 microns)	%	96.64
ON #200 (75 micron)	%	78.72
ON #100 (150 micron)	%	47.52
ON #50 (300 micron)	%	10.88

Figure 8. Particle Size Distribution via Malvern Analyzer

Analysis for phytonutrients counts was done internally. Special thanks to the Valhalla, NY Analytical group for conducting the assay work. Methodology is essentially an acid digestion and methanol extraction via HPLC. There is also a method utilizing a LC/MS. Note that no details will be shared regarding characterization in terms of sample preparation, extraction and other aspects of the analysis as information is proprietary to PepsiCo.

In terms of phytonutrients, to obtain the baseline on some reference materials, the profile of fresh oranges and commercial orange juice was

established. Subsequently, the fruit combo ingredient (mixture of rag and seeds at 2:1 ratio) was characterized to understand the phytonutrient profile of the fruit powder. Note that all data is reported “As Is” basis. i.e. powders as dry basis and juices and whole fruit as wet basis. The microencapsulated powders were then analyzed for phytonutrient levels after microencapsulation trials were completed to ensure targeted levels were achieved as designed. The profile of the coated powder will be showcased in the Application (V) portion of this project. For Table 4 below, the first column lists the key phytonutrients of interest, including two limonoids and three flavonoids. It is obvious that several other dozen limonoids and flavonoids exist in the citrus fruit, but only this small subset was displayed for ease of illustrating the key points. The second column has the levels of a typical whole orange fruit accounting for only the “edible portions of the fruit”. Hence, the non-edible portions of the fruit were removed, i.e., the peel and the seeds. This value is a composite of twelve oranges collected during a period of one month (three oranges per week) consisting of four different lots. The third column has the profile of a typical commercially available Not from Concentrate (NFC) 100% orange juice. Note the whole fruit has a significantly higher level of limonin and of hesperidin than the commercial orange juice. For the fresh orange fruit, the limonin analyzed at 8 ppm which is two to three times higher than the juice. Since limonin (aglycone) is not soluble in aqueous solution, some of these compounds would be entrapped in the solid portions of the fresh fruit including the cell wall of

the pulp sacks, in the membrane of the segment wall including the albedo portion of the fruit. For hesperidin, the orange fruit analyzed at 402 ppm and orange juice at 288 ppm. Objective no. 2 of this project is to significantly increase the limonin level of the “NEW beverage product” by about 5-8 times by means of using the encapsulated fruit powder.

Table 4. Characterization of an Orange and Commercial Juice

Phytonutrient Profile parts per million (ppm)	Whole Orange Fruit (Edible Portions) Wet Basis	100% Orange Juice Wet Basis		
Limonin	8	3	Utilize by-products	Create Enhanced Beverage w/ significantly higher limonin levels!
Nomilin	1	1		
Narirutin	73	103		
Hesperidin	402	288		
Didymin	33	24		

The fruit powder combo ingredient was also analyzed for additional standard physical and nutritional parameters typical ingredient suppliers would provide to buyers. Table 5 below outlines some of the key elements of the developed fruit powder being utilized for this project. Note that the Bulk Density

as report is 247.37 grams per pint. Converting this value into common International units is approximately 450 kilograms/cubic meter, which is a rather light product as compared to other powders in the field of grain flours.

Table 5. Fruit Powder Ingredient Specification

Fruit Powder Combo Specification		
Bulk Density	g/ pint	247.37
Fat	%	13.52
Protein	%	7.60
Sugar	%	22.23
Dietary Fiber	%	49.96
Moisture	%	4.30

IV. MICROENCAPSULATION OF THE FRUIT POWDER

1. Coating materials overview

Barriers to moisture transfer - Since the ultimate goal of this project is to add back the recaptured citrus by-products to fruit juices in the form of a stable powder ingredient, the initial screening of materials had to provide a barrier to moisture transfer (the first objective of this project) due to the fact that orange juice contains ~88% water. The following categories were selected based on literature review, consulting with colleagues and past work experience.

a. **Proteins** – Whey proteins provide a clear manifestation of the functional properties that are desirable as coating materials. Applications that utilize whey proteins for microencapsulation purposes have been done for international markets. Proteins are available as whey protein concentrate (WPC) powders; typically WPC 50 and WPC 70 and as whey powder isolates (WPI); typically WPI 95 and WPI 96. Numbers indicate the % protein concentration. WPI have been known to provide good barriers in opposition to oxidation for the microencapsulation of orange oil. WPI are able to provide an efficient foundation for microencapsulating of volatiles through spray drying. WPC 70 has been used to provide the surface properties that are required to stabilize emulsions.

b. **Shellac Resin** – This ingredient is secreted by an insect from India. It is

well-suited with waxes and will give the coated products a glossy appearance. This compound has been used as a food additive for candies and fresh fruits. Shellac and resins have low permeability to gases and have a low permeability to water as well.

c. **Waxes** – There are many options here, some of the most comon waxes include andelilla, paraffin, carnauba and beeswax. Carnauba wax is commonly used as a moisture barrier for food products. Synthetic paraffin wax is used as a coating for vegetables, candies, chewing gums, fruits and chesses.

d. **Corn zein** – Zein is a prolamine that is derived from corn gluten. It is insoluble in water except during a very low or very high pH. This is because zein has a high content of non-polaric amino acids. It is soluble in alcohol. After drying, it has a glossy surface that is grease and moisture resistant. The film is brittle. Plasticizers are necessary to add plasticity. It is frequently used as a good substitute for shellac because it dries faster. Corn zein has a high gloss appearance and possesses an increased stability during its shelf life.

e. **Beeswax**- Bees are known to be one of the most favored insects in the world. Aside from being active in collecting pollens from flowers and making honey, bees are also busy producing natural beeswax in their hives. The glands in the abdomens of young bees produce wax that are removed, masticated and

blended with the bees' enzymes and saliva. This processed wax is then attached to the honeycomb which carefully stores honey and protects the hive from foreign contaminants. Beeswax is a mixture of various compounds. Chemistrydaily.com (2012) enumerated the following compositions for Beeswax: 14% hydrocarbons, 35% monoesters, 14% diesters, 3% triesters, 4% hydroxyl monoesters, 8% hydroxyl polyesters, 1% acid esters, 2% acid polyesters, 12% free acids, 1% free alcohols and 6% of the composition is still unidentified. Beeswax has fatty acid esters of straight-chain alcohols. The temperature in the hive has to be 33° to 36°C for the wax to be secreted by the bees. Such wax is solid at room temperature. It doesn't boil, but it melts if it reaches the temperature 62° to 64°C (Cracolice and others 2007). For centuries, beeswax has been used in countless ways. Aside from using it for making candles, it was also used in the embalming process in ancient Egypt to cover parts of the corpse such as the eyes, mouth and the nose according to eHoneybees.com (2010). In ancient Chinese medicine, it was used to heal skin problems such as wounds and eczema. Ear disease, coughs, tonsillitis, nose inflammation, asthma and periodontitis conditions can also be treated with beeswax. Literature states that if beeswax is mixed with other substances such as butter, it can help address other issues associated with skin diseases (Beeswax 2007). When mixed with honey, it can treat damaged corneas. It has also been confirmed in a study performed at Dubai Specialized Medical Center (2005) in the United Arab Emirates that beeswax

may have mild antibacterial properties. A mixture of honey, olive oil and beeswax has been used and applied to laboratory plates where *Staphylococcus aureus* bacteria and the fungus *Candida albicans* are proliferating. The mixture with the added beeswax inhibited the growth of bacteria and fungus. Thus, it can be used to treat diaper rash and other bacterial skin conditions. Oral administration of beeswax can help cure some diseases. However, it can cause gastrointestinal blockage if extreme quantities are consumed. A person may feel other symptoms of beeswax poisoning if consumed in large quantities; namely, abdominal pain, nausea, vomiting, abdominal swelling, lack of bowel movements and tender abdomen. Treatment may be in the form of decontamination by use of laxative to move the wax through the GI tract and prevent bowel blockage. Medical advice from a qualified physician should always be sought before treating any severe conditions (Beeswax 2007; Beeswax benefits 2012).

f. **Hydrogenated soy bean oil (HSBO)** - Vegetable oils such as soybean oil and sunflower oil are liquid at room temperature. These oils contain unsaturated fats that are likely to turn rancid when exposed to air. Oils will stale, therefore, have an unpleasant odor and flavor. Hydrogenation is a chemical process where the liquid oil is heated to high temperatures after adding a catalyst and hydrogen is forcefully passed through the liquid. Filtration at the end helps remove the catalyst and the result is hydrogenated oil. Hydrogenation produces a uniform

product with required texture as the fatty acids from the oil acquire some of the hydrogen and turn dense. Hydrogenation helps change the liquid oil into solid form which has a longer shelf life than the liquid. Stopping the procedure of hydrogenation before is fully completed will provide partially hydrogenated oils. It has a consistency like butter; it forms a semi-solid form type of product. It offers good flavor and texture and it is much more cost effective than butter. It is commonly used as “butter substitute” in the food industry.

g. Carnauba wax – Carnauba wax has been widely used in the wax industry for many years. Floor waxes and car polishers contain a significant amount of carnauba wax to give it a hard, shiny surface. Nowadays, carnauba wax is also used in cosmetics, glamour products, ointments, tablet coatings, pharmaceuticals, candles, gums, confections, auto polish, floor polish, shoe polish, carbon paper, inks, paper coatings, cheese coating and fruit coatings. Carnauba wax is compatible with most animal, vegetable and mineral fats and other waxes and also a large variety of natural synthetic resins. To date, carnauba wax is not proven to be toxic in humans yet there are some studies that show minute toxicity in rats (Ekelman and others 2012). Further research needs to be conducted to ensure there are no toxicity concerns for use in the coating of food ingredients. Especially for the fruit powder at the levels anticipated to be consumed in the finished juice beverage. There is also a need to find out

consumption limits as it may cause gastrointestinal issues as other waxes as noted above for the beeswax if consumed in excess amounts.

2. Screening of coating materials

The first hands-on step of this phase of the project was to screen the overall performance of the coating ingredients from the various groups listed above. Ingredients were dissolved in a solvent, water in some cases, depending on their compatibility and dissolution needs. In some cases the solvent was an alcohol (ethanol or methanol). Then, the coating material along with solvent was placed in a glass beaker utilizing a stirring rod on a magnetic stirring plate under a hood to completely dissolve the coating material i.e. protein, etc. Then, the by-product powders (powder rag and powder seed) where added to the beaker mixtures, stirring continued under a hood overnight to completely evaporate the solvent. The solidified material in the beaker was then manually removed with a metal spatula. Big pieces of this “coated material” were turned into a powder. This was done by hand, utilizing a cup and mortar. See Figure 9 for visual representation of these steps.

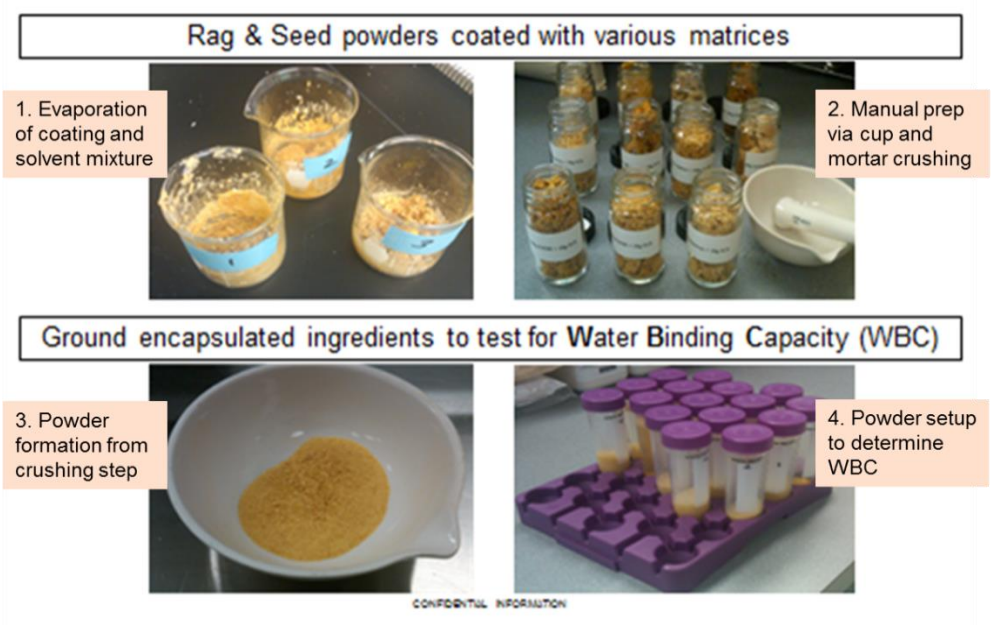


Figure 9. Steps to Screen for Coating Material

To test effectiveness of the coating materials against moisture, a water binding capacity (WBC) testing was conducted. This test determines how effective the water barrier or coating was on the fruit powder to guard against water transfer. Many WBC procedures exist, this particular one was chosen to more aggressively test the microcapsule coating and obtain an initial read within hours of mixing of the microcapsule in a water, sugar and acid solution. Note that the water solution was adjusted with the added sugar and acid to more precisely mimic the conditions of orange juice. The following table showcases the formulation of the simulated juice solution and respective chemistry parameters.

Table 6. Liquid Solution Mixture to Determine WBC

Liquid solution to determine WBC			
		% wt.	Grams
Water	Beverage grade filter water	87.9450	879.4500
Sugar	Granular white table sugar	12.0000	120.0000
C6H8O7	Citrix acid (anhydrous)	0.0550	0.5500
		100.0000	1000.0000
	Solution parameters:		
	pH	3.5	
	Brix	12.1	

Water Binding Capacity (WBC) Procedure:

1) Add 3 grams of encapsulated fruit powder into 50 ml centrifuge tube. Record weight in grams as (W1)
2) Add 40 grams of special water, sugar, acid solution which simulates an OJ beverage to the 50 ml centrifuge tube. Record weight as (W2)
3) Stir tubes by hand vigorously for 2 minutes. Let tubes set to "bind water" for a period of 60 minutes
4) Centrifuge tubes for a period of 5 minutes at 2,000 revolutions per minute (RPM)
5) Decant supernate and weight contents in grams. Record weight as (W3)

6) The WBC is calculated using the following equation: WBC = (W2-W3)/(W1)
7) WBC units are expressed as the number of times water was absorbed per unit of fruit powder

As an example, a factor of 2 indicates two grams of water were absorbed by one gram of fruit powder.

Equipment utilized for centrifugation was a Beckman Coulter Allegra X-12 bench top model. The analysis was conducted at the Barrington, IL R&D facility. Conditions for the centrifuge were 5 minutes at 2000 RPM. After a series of WBC tests were conducted, in duplicates, it was quickly determined that the most effective options to protect against moisture transfer were the waxes and hydrogenated soy bean oil (HSBO). It is important to re-establish that this initial screening was done by coating the powder “by-hand”, in the lab using the stir plate, evaporation hood, cup and morter to grind to a powder to obtain preliminary effectiveness of the coating material group options. Although some of the WBC results for waxes were close to 0.50 units, in reality, these numbers are higher than expected. Ideally a value of zero units would be expected to ensure there is no water binding. Hence, no water transfer and no leaching of the phytonutrients from the microcapsule to the fruit juice. However, there is reason to believe that the manual grinding by cup and mortar ruptured some of the

coating and water made its way to the fruit powders. Table 7 provides the results of the sample screening work. Samples were done in triplicates, results were consistent and only the average is reported for each of the sample types. Values are more clearly depicted in Figure 10, this graphs shows that the most effective protection against moisture transfer were the waxes and the HSBO. Note that the HSBO used in this project was obtained from Balchem Corporation and details about this ingredient are proprietary. In general terms the ingredient is claimed to be very effective for coating purposes vs traditional HSBO sold in market.

Table 7. WBC Summary of Results for Screening Phase

Sample	Description	W1	W2	W3	**WBC**
A1	zein solution in ethanol w/10 grams of rag	3.0	40.0	26.4	**4.53**
A2	zein solution in ethanol w/10 grams of seed	3.0	40.0	31.2	**2.93**
A4	cellulose solution in ethanol w/10 g of rag	3.0	40.0	27.3	**4.23**
A5	cellulose solution in ethanol w/10 g of seed	3.0	40.0	31.1	**2.97**
A6	zein solution in ethanol w/10 grams of rag	3.0	40.0	32.5	**2.50**
A7	20 grams of bees wax w/10 grams of rag	3.0	40.0	38.8	**0.40**
A8	20 grams of bees wax w/10 grams of seed	3.0	40.0	38.7	**0.43**
A9	zein solution in ethanol w/10 grams of seed	3.0	40.0	33.4	**2.20**
A10	20 grams of carnauba wax w/10 grams of seed	3.0	40.0	38.6	**0.47**
A11	20 grams of carnauba wax w/10 grams of rag	3.0	40.0	38.2	**0.60**
A12	Seed control (unencapsulated)	3.0	40.0	27.1	**4.30**
A13	Pomace control (unencapsulated)	3.0	40.0	21.0	**6.33**
A14	20 grams of HSBO w/10 grams of seed	3.0	40.0	38.4	**0.53**
A15	20 grams of HSBO w/10 grams of rag	3.0	40.0	38.2	**0.60**
B1	12.5% shellac sol. in water, 5 min combo sample	3.0	40.0	23.2	**5.60**
B2	12.5% shellac sol. in water, 20 min combo sample	3.0	40.0	25.6	**4.80**
B3	12.5% shellac sol. in water, Final 30 min combo sample	3.0	40.0	27.7	**4.10**
B4	12.5% shellac sol. in methanol, 20 min combo sample	3.0	40.0	23.4	**5.53**
B5	12.5% shellac sol. in methanol, 40 min combo sample	3.0	40.0	23.8	**5.40**
B6	12.5% shellac sol. in methanol, 60 min combo sample	3.0	40.0	24.3	**5.23**
B7	12.5% shellac sol. in methanol, 70 min combo sample	3.0	40.0	24.3	**5.23**
B8	PVP solution in water w. 10 grams of fruit combo. 20 min	3.0	40.0	27.7	**4.10**
B9	PVP solution in water w. 10 grams of fruit combo. 60 min.	3.0	40.0	28.0	**4.00**

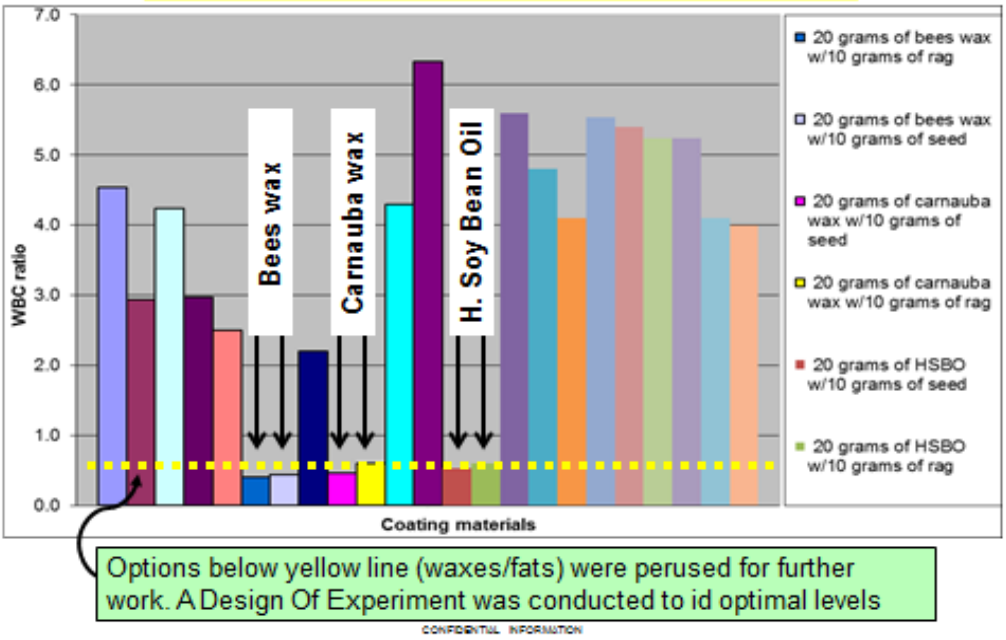


Figure 10. WBC Summary of Results from Screening Phase

After the screening work was completed, noting that the carnauba wax and the HSBO were the most effective, the following step was focused on understanding how these coatings perform under controlled conditions. Microencapsulation work was conducted mechanically via a hot melt, fluidized bed dryer unit at a large scale. But, before delving into the manufacturing work, a Design of Experiment (DOE) was developed and the work was conducted as designed to understand if there were synergies by using the two coating materials as a mixture. At this point, it is important to further discuss the two

coating materials of interest to set a better understanding of these two finalist coating options.

Coating Materials of interest - The suitability of the coating material is important to achieve the desired quality of the finished microcapsules. For the coating layer, if the material is suitable to the core particles, it will provide good moisture and oxygen protective effects. For this project, these two materials were considered not only because of the WBC results but also because they are readily available in the food industry. Although, each has its own advantages and disadvantages that need to be considered.

Hydrogenated soy bean oil (HSBO) - Oils have been hydrogenated for many decades to prolong their shelf life and to make the oils more stable. Hydrogenated oil is oil in which essentially fatty acids have been converted to a different form chemically, which has several functional effects. Hydrogenated oil is far more shelf stable and will not go rancid as quickly as untreated oil. It also has a higher melting point and is often used in frying and for pastry-baking reasons. During hydrogenation, the chemical structure of the oil is changed. Scientists in the 1990s began to realize this could result in negative health effects due to several issues, including solidification and plaque formation. Hydrogenated oil is essentially made by forcing hydrogen gas into liquid oil at high pressure. Both animal and vegetable fats are hydrogenated. In general, the

more solid the oil is, the more hydrogenated it is. Two common examples of hydrogenated oil are Crisco and Margarine. HSBO is a viable option for coating of the orange fruit powder. However, there is the need for chemical modification of soy bean oil so that it can possess the needed functionality to become a solid. Processing of the HSBO may be needed in order to enhance biocompatibility and antibacterial properties of the soy bean oil as coating material (Bakshi 2013). For biodegradable feed-stock, the preparation of castor oil and soybean oil to become biocompatible requires incorporation of silver, zinc, cadmium, or boron particles (Bakshi 2013). However, this would not be safe to be used when coating substrates for human consumption. In some studies, hydrogenated vegetable oil such as soy bean and castor oil are used as binding agents in the coating process (Rahman and others 2011). Vegetable oils consist of triglycerides, fatty acids, phospholipids and other compounds. They are often used as coating materials because of favorable physiochemical properties including melting point, hydrophilic lipophilic balance and digestibility. However, the lipid must not melt too early for the coated material to be stable (Jannin and Cuppok 2013). Another major quality consideration for soybean oil is its oxidative stability. This refers to resistance of soy bean oil to oxidative reactions during processing and storage. Often, the outcome of these oxidative reactions leads to off-flavor development. Soy bean oil lacks flavor stability especially during processing, application and storage. But, this can be improved in two ways;

processing and genetic breeding. Some of the processes that may be implemented include interesterification, fractionation and winterization (Singh 2010). The advantages of HSBO are summarized as follows; they are viable to be purchased in commercial food grade, favorable physiological properties (melting point hydrophilic and digestibility). As noted earlier, the HSBO utilized for this project was a “high grade ingredient” developed by Balchem Corporation. This ingredient has some unique melting characteristics making it a good candidate for coating the fruit powder material.

Carnauba Wax – Many plants have the ability of protecting their leaves very well from drying up. Some plants excrete waxes that shield them from the heat of the sun. These waxes serve as a coating for plants for various types of protection. Waxes slow down the evaporation of water and also prevent microbes from invading the plant. One of the toughest waxes found in plants is the carnauba wax. Commercial carnauba wax is obtained from the Brazilian Palm tree, *Copernica carifera*. The tree exudes a wax through the petioles of its fan-shaped leaves, preventing dehydration from the hot equatorial climate. The leaves are harvested, dried, boiled in water and the wax that floats to the top is skimmed and filtered. Carnauba wax has a high melting point of 82° to 85°C, the highest melting point of waxes (Cracolice 2007). According to a study conducted by Melo (1998) La Universidad Federal do Rio Grande do Norte - Centro de

Tecnologia. Carnauba wax contains mainly wax esters at ~85%, accompanied by small amounts of free acids and alcohols, hydrocarbons and resins. Carnauba wax also contains carboxylic acid components with hydroxyl groups. The hydroxyl groups tie together next to carbon chains by hydrogen bonding. These cross-links are responsible for the tough, relatively high melting point.

Waxes are very reliable when used as coating material because of their capability to control the release of the core particles, which in this case is the fruit powder. Melting carnauba wax does not require solvents and does not constitute to toxicity of organic material residues of the fruit powder, which would be the case when using solvents for dissolving and applying powder coatings. In addition, the use of heated wax is completely insoluble with other forms of liquids, which could further increase its sustained release functions. Another positive of the carnauba wax is that it contains no alcohol unlike some other wax products used for coating processes (Srivastava and Mishra 2012). Carnauba wax as the main coating material does not have any known chemical interactions with the fruit powder and in most cases, with other pharmaceutical elements of the citrus fruit. According to Srivastava and Mishra (2012), the importance of carnauba wax as a coating material should be weighed based on its chemical compatibility with the coated powder, where it could provide substantial controlled release properties through proper melting procedures. But by the time

that the coated fruit powder comes in contact with the digesting liquid (bile) in the upper small intestine, to breakdown its components. The purpose of the coating is sometimes targeted for the powder contents to be consumed easier; it could have a more pleasant outlook than ingesting the powder contents alone which are essentially bitter due to the citrus limonin components. Wax coatings can resist gastric acids in the stomach, because the enteric coating are made of tough chemical adhesion and bonds that can only be broken and dissolved in the intestines by bile as the case with other fats. This way, the contents of the powder can be absorbed slowly, depending on its controlled release property characteristics such as type of wax, crystallinity level, transit time and thickness of the coating. In this case, we know the coated powder will be placed in an acidic beverage, i.e. orange juice. The fatty coated substance will generally remain intact and stable. Care must be exercised to apply enough coating layers to the fruit powder to achieve the application, processing and shelf life requirements but it needs to be balanced with ensuring the bioactive (phytonutrients) will be released and become bioavailable for consumers to take advantage of the benefits. Note that the scope of this project does not include testing the bioavailability and absorbability of the phytonutrients.

Using Carnauba Wax - waxes are considered as one of the materials that can be used to coat powders in order to control the release of the fruit powder.

This can be achieved by liquifying the wax in a hot melt unit. Hot melt coating techniques refers to the application of fine layer of coating material in molten state over the main core substrate. Hot melt wax coating has some advantages over solvent coating. For one, toxicity as captured above, of organic solvent residues and influence of environment are some major problems that are associated with solvent coating (Padsalgi and others 2008). Moreover, the use of wax in melt coating is also advantageous, because wax is inert to most pharmaceutical active compounds and also has controlled release characteristics. As waxes are melted, similar to other substances such as ethylene glycol, hydrogenated vegetable oils, phospholipids and cocoa butter are completely immiscible with other liquids such as water. Waxes are esters of fatty acids and long chain alcohols. They are hydrophobic so they cannot be combined with water and other solvents. Their melting temperatures are typically higher than 60ºC; hence, they are used for prolonged release of the core particles. Carnauba wax has a high melt point in the range of 78º to 85ºC. In the food industry, it is used often with candelilla wax, rice wax, hydrogenated jojoba oil and paraffin wax between 10 to 30% wt. for taste-masked formulations and sustained release (Jannin and Cuppok 2013). Carnauba wax is inert or non-reactive to core particulates and most pharmaceutical materials. Generally, waxes have a high melting point compared to other solid coating options. They have a low cooling point therefore are easy to solidify.

3. Wurster fluidized bed dryer process

In 1959, Dr. Dale Wurster from the University of Wisconsin introduced an air suspension coating technology that would be known as the Wurster process. Today, it enjoys wide use in the pharmaceutical industry for layering and coating the powdered particles of pellets and also controlled-release of bioactives. The product container or load vessel usually ranges in size from 3.5 inches up to 46 inches in diameter or from 500 grams batch size to as high as 800 kilograms. Commercially, the Wurster process is used for coating substrates from less than 100 microns for tablets and for layering in order to produce core materials (Jones 2009). The Wurster process, also called bottom spray processing, refers to the coating process where the coating liquid is sprayed from the bottom of the vessel. This format was developed to increase the collision probability of the particles and coating droplets. This significantly improves coating material efficiency and also reduces the time of spray drying (Onwulata 2005). The basic design of the apparatus is composed of a coating chamber that is slightly conical and houses a cylindrical partition that is open at both ends of the chamber. The bottom end of the chamber is about half the diameter of the top part of the coating chamber. An orifice plate is divided into two regions and placed at the base of the chamber. The first region is an open area of the plate that is under the partition. This region is very permeable, because it permits a high volume

and velocity of air to transport the particles vertically upward. As the particles accelerate upward, they pass through the nozzle mounted at the center of the plate (Jones 2009). The Wurster coater is a specialized variant of the conical spout-fluid bed apparatus used with a spray nozzle placed at the porous base with an axial draft tube. This set-up allows simultaneous fluidization and circulating of particles inside the vessel of the apparatus (Wittal 2013). The nozzle then sprays the coating agent. As the particles move upward and pass the area within spray nozzle's reach, they become encapsulated by the coating agent. The coating material adheres to the particle surface by evaporation of the solvent; the coating agent then coats the core particles. This process is repeated until the desired thickens of the coating wall is obtained. This process can be time-consuming but the multi-layer coating is effective in reducing defects (Baldwin and others 1994). The fluidized beds are also used for granulation but agglomeration of particles can be high, although, the Wurster approach minimized this problem (Wittal 2013). Initial spray drying process was very efficient when spraying larger particles but have high agglomeration risks for small particles due to the high concentration of wet particles at the bottom of the container. This is ultimately the reason why, Dr. Wurster invented the insert bottom sprayer. The circulation of particles hence increased the drying rate and reduced risks of agglomeration. Overall, the circulation leads to great

homogeneity in the quality of coating, making it smooth and uniform (Onwulata 2005).

Many researchers have concentrated on the evaluation and improvement of the apparatus and of product quality. To achieve better performance of the apparatus, some of the identified requirements include; changing bed velocity, rate of circulation and elimination of dead zone through gas. To prevent plugging of the nozzles, special shields are placed near the outlet head. Some nozzles also alter the gas distributor in such a way that it would create an additional airflow that will blow particles away (Wittal 2013). The Wurster fluid-bed process is acknowledged by the pharmaceutical industry as the primary technology for precision application of film coating for various materials including powders, crystals and granules. Film coating using the Wurster process is also effective in masking the taste, color and odor of the drug or supplement with a top layer to make it more palatable. This process can also be used either to improve capsule barrier or its release properties. The Wurster apparatus is one of the most suitable instruments for film coating of small particles. Through this design, the particles are able to follow a circulating flow trajectory as desired by the operator based on air speed, exhaust speed and spray patterns (Hampel and others 2013).

Pellets and small substrates are coated extensively through the Wurster process when using water, organic solvents, and even through spraying molten coating materials as fats or waxes. All fluidized-bed techniques are known for high rates of mass and heat transfer. Highly water-soluble coating materials can be coated using water-based applications without problems with core penetration. Droplets applied through the surface spread when forming continuous layer and then quickly eliminate moisture to the warm and dry air. After the thin film layer has been applied, the spray rates can be increased to isolate the soluble core. Coatings like volatile solvents can also be applied with high quality, because the formed droplets impinge on the substrates quickly. This also minimizes need for spray drying. However, there are some limitations about its application when using molten coating materials, specialized equipment to melt, transport and spray high temperature liquids is required. In this process, the coating is applied through spraying a molten substance that congeals on the surface of the substrate. The Wurster process is adapted to molten coating because of congealing and its impact on the fluidization behavior (Jones 2009). In this regard, the Wurster system is the most suitable coating process that can be implemented in coating the developed powders. Special thanks to the team in Hawthorne, NY for supporting this project and allowing me the use of their fluidized bed dryer system and other lab equipment. Figure 11 below showcases the actual unit utilized for phase two of this project.

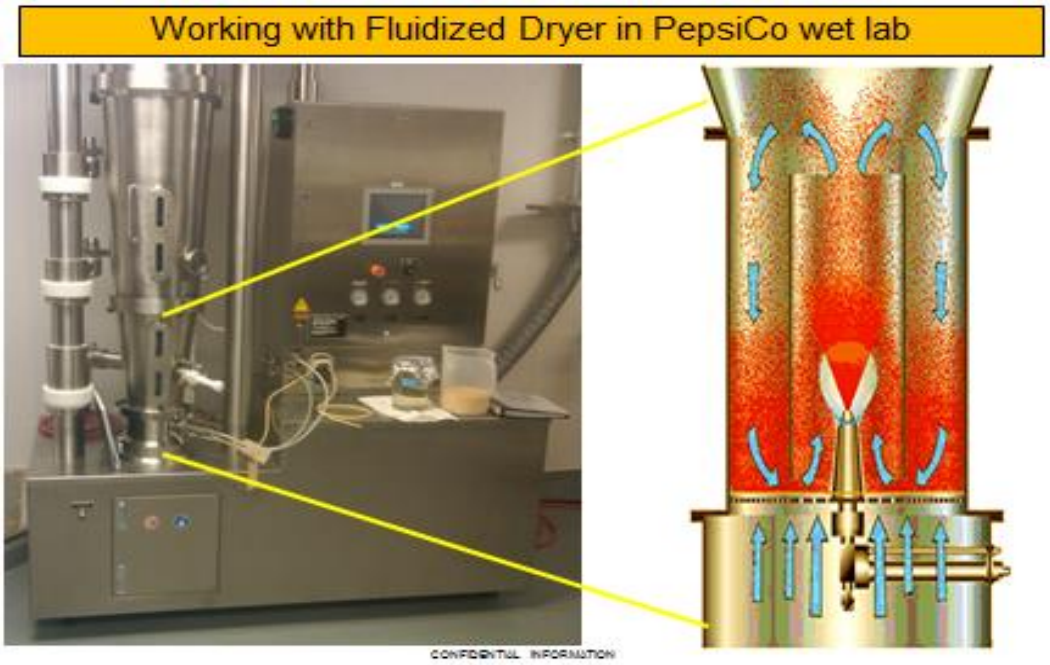


Figure 11. Fluidized Bed Dryer and Loading Vessel

Contrary to the wet spray process which uses hot air for liquid removal and solidification of the coating matrix, hot melt coating uses cool air, ambient air, or slightly warm air to solidify or congeal the coating material. For this project, the carnauba wax was heated to 120ºC which is considerably higher than the specified melting point of approximately 85ºC. The higher temperature at the melt tank and the hold tank is required to account for the transport of the liquified wax through the various lines, valves, pump and meters all the way through the spray nozzle. This results in the coating material being liquified for ease of pumping

and spraying over the fruit powder. Higher temperature of the liquid also reduces the viscosity making the wetting process more effective. Once at the desired location in the chamber, the blowing of typically ambient air quickly solidifies the deposited wax or fat layer. Hot melt coating is often used to provide moisture resistant, gastric resistance, acid resistance and maintained intact until release in the small intestine. Hot melt coating has also been adopted by some industries, because the technique is faster and cheaper compared to the traditional alternatives which require expensive, tedious and time consuming processes to evaporate and recover solvents (Dhuppe 2012). However, the hot melt process also requires high energy input, because a high temperature is necessary to melt the waxes or fats and also the need of specialized equipment to maintain the wax as a liquid and the transportation of the equipment all the way through the spray nozzle. The following table summarizes the advantages and disadvantages of utilizing the hot melt process.

Table 8. Advantages and Disadvantages of Hot Melt Process

Advantages	Disadvantages
No solvent or water in the process	Requires high energy input
Drying steps are eliminated so processing steps are shortened	High melting temperatures may affect the core particles
Simple, continuous, and efficient process	Needs close attention to maintain high temperatures and caution to operator not to burn
Uniform dispersion of fine particles	
Good stability of the final product at varying pH and moisture conditions	

a. DOE layout

The pre-work to determine the optimal levels for utilizing in the stability study was determined by conducting a Design of Experiment (DOE) using *Design Expert*® software. The HSBO and the carnauba wax where selected as part of the DOE since they were two of the most promising options off the screening exercise as id by the initial WBC results. The following layout, Figure 12, showcases the DOE distribution of the HSBO and the carnauba wax. Note the various points of each independent coating material plus certain mixtures of the two coating materials.

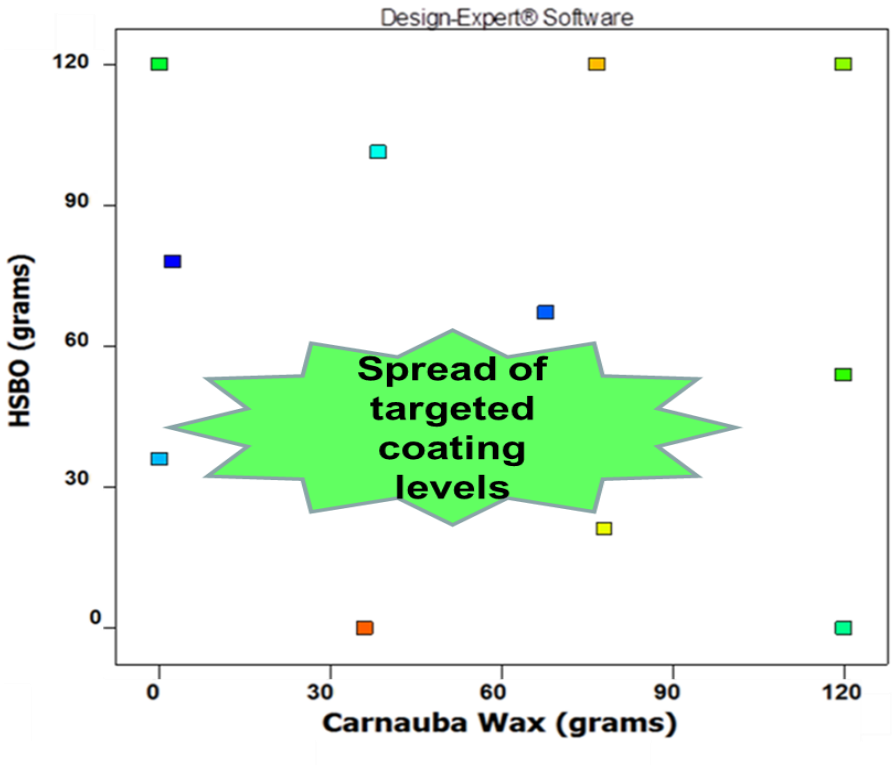


Figure 12. DOE Layout of the Carnauba wax and HSBO

In a more direct approach, the numerical DOE values are showcased below in Table 9. It specifically outlines the experimental design including the number of runs, the factors (HSBO, carnauba wax) and also the response (WBC) results of the experiment. In total, there were eleven runs conducted utilizing a consistent initial load of the fruit powder at 100 grams per run. The level of HSBO and the carnauba wax varied as listed in the table. This DOE was carried out in the lab, bench-top setting working with the traditional “under the vent hood” to

melt the wax and HSBO in a glass beaker on top of hot plates, adding the fruit powder combo, solidifying over nigh and grinding by hand with cup and mortar process, followed by implementing the WBC process and analysis. The WBC analysis was done in triplicates. Results listed are the average of the three measurements.

Table 9. DOE Values for the Carnauba Wax and HSBO Study

Run #	Carnauba Wax (grams)	HSBO (grams)	Water Binding Capacity (units)
1	2.4	78.0	**4.25**
2	67.8	67.2	**3.55**
3	0	36.0	**6.00**
4	38.4	101.4	**3.30**
5	120.0	0	**2.80**
6	0	120.0	**3.50**
7	120.0	54.0	**2.75**
8	120.0	120.0	**1.55**
9	78.0	21.1	**4.00**
10	76.8	120.0	**2.60**
11	36.0	0	**5.50**

Analysing the DOE results (Figure 13) in a plot format for a more visual perspective indicate, as expected, the more coating applied, the more protection against water moisture transfer. Since the colored lines on the plot are slightly curbed, this indicates there is a synergistic effect between the HSBO and the carnauba wax when combined. Although, the level of synergy is not high.

Therefore, the feasibility of mixing two diverse ingredients, which have distinct melting and solidification points, makes it difficult to control in a commercial setting. Furthermore, having two ingredients in the *Ingredient List* of a package does not keep the side panel as simple and clean as possible, especially with the *hydrogenated* wording required for the soy bean oil. As the results are analysed and the HSBO and carnauba wax are looked at independently, the carnauba wax is directionally better than the HSBO. Furthermore, in practical consideration, the combination of the two ingredients were not implemented for the plant trials as there is tremendous cost and time consumption for using the large scale system at a manufacturing level which went outside the funding limitations of this project.

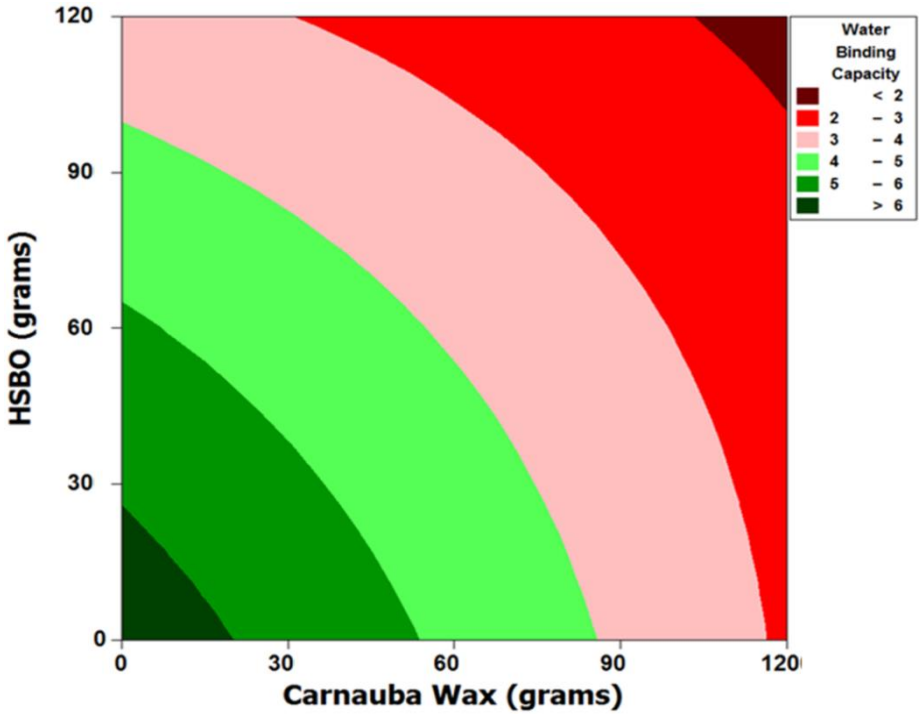


Figure 13. WBC from DOE Experiment

Leveraging from the DOE results, a larger trial was conducted in a manufacturing plant using the full scale hot-melt fluidized bed dryer equipment. Samples were produced for application work and for conducting the shelf life study. The focus of this particular trial included only the use of HSBO and carnauba wax, separately at select coating levels.

b. Physiochemical Interactions

It is important to further discuss chemical, physical and biological interactions that can be expected or at least be considered between the coating materials and the fruit powder. Interactions are of high importance since the fruit powder has to be coated precisely to conceal the bitter taste of the limonoids, maintain in place during the shelf life of the beverage, and bioavailable during consumption of the beverage. Coating properties can be enhanced by the use of the molten HSBO or carnauba wax as binding agents in order to improve the consistency of the coating material and the adhesiveness to the core fruit particles. Although technologies such as the cooling process will be utilized to ensure binding of the hot coatings to the core material, an oxidative process may still take place due to the nature of the coating materials, specifically HSBO. According to Agnihotri and others (2012), the coating agent should possess the capability of developing into a film that is cohesive with the core substrate. It must also be chemically compatible to the core. In general to be able to produce a viable encapsulated product, the coating material should possess the following attributes:

- Able to stabilize the core material
- Inert toward any active ingredients
- Able to control release of substrate under specific conditions

- Tasteless, odorless and stable
- Non-hygroscopic, without high viscosity, semi-flexible, yet durable
- Economical for its intended application

In line with these base attributes, it is essential to identify the chemical characteristics of the coating material to be used, as well as the qualities of the core material. Ascertaining the chemical properties of the lipid coating agent and the fruit powder will determine the subsequent chemical, physical and biological interactions.

HSBO and carnauba wax are both lipids. Lipids are macromolecules that include fatty acids, glycerides, phospholipids, sphingolipids, waxes and sterols (Sudke and Sakarkar 2013). The majority of these lipids are insoluble in water and are amphiphilic. They are identified by their fatty acid composition, melting point, Hydrophilic-Lipophilic Balance (HLB) and solubility in organic solvents (Sudke and Sakarkar 2013). Lipids as coating agents have a wide variety of application from the pharmaceutical to the food industry. Generally, lipids with medium to high melting temperature are in the range of 55ºC to 80°C and are used for both taste masking and prolonged release applications. This is to avoid the premature release of the core material or active ingredient due to the lipid melting. Carnauba wax, which is one of the coating materials used in this project, has a melting range of 81ºC to 85°C, depending on its source and purity (Sudke

and Sakarkar 2013). Moreover, carnauba wax is hard, brittle, non-toxic and highly hydrophobic. These qualities make carnauba wax a suitable coating agent for the desired application of the fruit powder in a juice beverage.

The same could be said for the properties of HSBO. This coating agent is also nontoxic and is digested by lipases located in the body's stomach and in the upper intestine by bile generated by the liver. Hydrogenated oils such as HSBO consists of mixtures of triglycerides and there are two types namely, Type I, melts in the range of 57ºC to 70°C and has an iodine value of 0 to 5, typically more saturated fats. Type II, has a melting range of 20ºC to 50°C and an iodine value of 55 to 80, typically higher levels of unsaturation (Sudke and Sakarkar 2013). Partially hydrogenated oils belong to the latter type. Additionally, HSBO, for the most part, has neutral flavors, similar to carnauba wax which is a critical and positive attribute since the goal of this project is to conceal the bitter and other off notes of the limonoids found in the fruit powder and become "tasteless" or transparent once added to the fruit juices.

Since the core material; the orange fruit powder has a significant amount of fat due to the seed content and the coating materials are essentially lipid based, this indicates that both lipid coating agents are partially adhesive to the surface of the fruit powder mixture. Since there are both polar and nonpolar qualities in the fruit powder, dispersive adhesion can be administered between

the shell and the core material. Van der Waals forces and hydrophobic interactions are both present, as well as London dispersion forces, because they are useful for adhesive action due to temporary polarities. To measure adhesiveness of the coating material, the contact angle needs to be understood. Although, aside from contact angles, there are many parameters that affect the properties of the coating liquid (after melting) and how it binds to the core material. Factors such as the amount of the core material, the diameter of the core material, surface tension, hygroscopicity, viscosity of the coating material, temperature as well as fluidizing air flow and drying temperature need to be understood and controlled to obtain an optimal microcapsule. The morphology of the HSBO or carnauba wax coating (crystalline or amorphous) and surface roughness of the core can influence the adhesion properties of the microcapsule formation. Controlling the quality of the deposited layer of coating will be dependent upon the wetting parameters which are responsible for how well the coating spreads over the surface of the core fruit powder. These parameters are not intrinsic characteristics of the coating material but result from localized interactions between the coating and the core (Saleh and Guigon 2007). Generally, the wetting of the solid core by the liquified coating is a function of three parameters: the contact angle, the surface tension of the liquid and its viscosity. According to Saleh and Guigon (2007), the contact angle and the surface tension control the wetting maximum and equilibrium, whereas the

viscosity determines the wetting kinetics. It was also stated that the surface tension of the liquid regulates the droplet size distribution as well as the liquid distribution on the surface of the particles (Saleh and Guigon 2007). On the other hand, an increase in the extent of agglomeration is the result of an increase in the viscosity of the coating material. Thus, the more viscous the coating material is, the poorer the wettability which can translate to the formation of non-uniform coating layers. Figure 14 below showcases illustrations of several contact angles and effect on coating effectiveness.

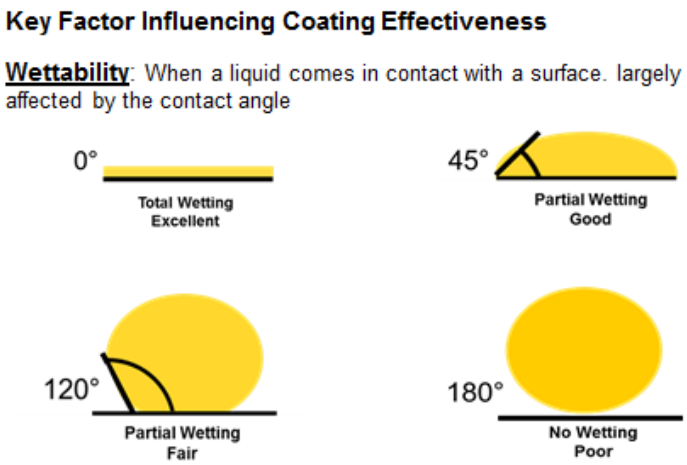


Figure 14. Contact Angles and Wetting Effectiveness

It was learned during the plant trial experiments to increase the hold temperature of the carnauba wax to ensure low viscosity of coating material through the spray nozzle to minimize agglomeration of particles.

Although agglomeration is considered a negative side-effect for most coating processes, it can be the desired outcome in some cases, namely, for the granulation process. Granules are produced by the drying of the liquid and the bridges between small adhering powder particles which eventually form fixed agglomerates. With the aid of intermolecular attractive forces, Van der Waals forces, and Electrostatic forces, aggregation of particles is initiated. Planinsek and others (2000) further stated that the main parameters affecting optimum granulation conditions include the following: spreading of the coating over the core, adhesion between the coating and the core as well as coating cohesion. Hence, wetting parameters have the effect on the agglomeration of particles in the fluidized bed process. For this project, agglomeration was not targeted or a desired outcome since the microcapsule production was aimed at producing very small particles to conceal them from the drinking experience. Agglomerates larger than about 150 microns are perceived as gritty or sandy to the human palate. Therefore, application to fruit juices, fruit nectars or fruit drinks are not as ideal. But, larger particles or microcapsule agglomerates would work properly for

smoothie type of fruit beverages or those that by design include particulates, such as pulp, fruit bits, fruit chunks or similar.

Since the carnauba wax is said to have relatively high crystallinity, (Endlein and Peleikis 2011), it produces a very hard and brittle shell when dried, which is a good property for a coating agent in most food applications to prevent moisture transfer. Carnauba wax also has very good emulsification properties and has good oil binding capacity for ester oils as well as for mineral oil (Endlein and Peleikis 2011). Therefore, it is compatible with the fruit powder properties and will be good in binding to form uniform microcapsules. The same also can be said for HSBO, although, its crystalline structure is much weaker than carnauba wax. Generally, both bonding and adhesive forces, which control the growth mechanism and the coating efficiency respectively, depend on the liquid surface tension and liquid–solid contact angle (Saleh and Guigon 2007). It is also explained by Saleh and Guigon (2007) that for wetting to occur, coating molecules situated in the three phase interface must break off with their surrounding semi-liquid molecules, push away the gas molecules adsorbed at the powder surface and adhere to the core by forming bonds with its molecules. Spontaneous wetting occurs if the coating-core adhesive forces are stronger than both coating cohesive and core/surrounding gas adhesive forces. The forces involved in this interaction can be classified according to their relative strength as

primary, donor–acceptor and secondary bonds. The primary bonds entail chemical bonds, either ionic or covalent, while the secondary bonds pertain to hydrogen and Van der Waals bonds (Saleh and Guigon 2007). Usually, the most common bonds are the primary donor/acceptor bonds. Low contact angles suggest that the coating material wets the surface well and will spread readily across it evenly, while high contact angles suggest that the coating does not wet the surface as effectively (as shown in figure 14) and has a tendency to form beads. Based on all the underlying mechanisms regarding wetting and layering, it can be concluded that for a given set of operating conditions, the coating efficiency depends on physicochemical properties which condition the coating spreading and adhesion on the core particles' surface (Saleh and Guigon 2007). For this project, the fact that the fruit particles have a low contact angle due to the particle size of ~150 microns, the roughness of the core material surface, and the viscosity of the coating material being initially low, positive modifications were implemented and ultimately increasing the hold temperature of the carnauba wax, the adequate surface tension of the core-shell bonding forces, the cohesion was relatively effective.

Biologically, there are essentially no interactions that can be expected between the fruit powder and the coating materials. The fruit powder is relatively inert and does not have any biological reactions with the lipid coatings. The

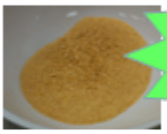
flavonoids and limonoids contained in the powder which provide the bitter taste are poorly miscible in lipids and as such cannot be dissolved in the coating material during the wetting process. Therefore, the flavonoids and limonoids which are rich in health benefits are contained within the capsule until the coating is digested in the body, digestion is a process achieved by lipase in the stomach and bile produced by the liver and delivered to the upper intestine by the gallbladder.

V. APPLICATION WORK

The application work followed the microencapsulation trials conducted at the manufacturing plant. For the application phase, there was an anticipated level of phytonutrient load with the microcapsules to be added to the fruit juice beverage. Projected levels within the microcapsule assuming a load of 50% (meaning half core material and half coating material by wt.) are illustrated in Table 10. Based on the mixture combination of 2:1 rag to seeds powder, the actual level of the fruit powder would contain limonin at 1,140 ppm. The level of the hesperidin would be 5,606 ppm. Application work consisted of using the most successful microcapsules from the design of experiment (DOE) which then were mass produced at a manufacturing plant scale using fluidized bed dryer equipped with the hot melt system. The coated fruit powder will ultimately be added to a Not from Concentrate (NFC) orange Juice beverage. But, for this project a *simulated* OJ beverage was created and utilized to more precisely monitor the shelf stability of the microcapsules. In terms of limonin targets, when using the microcapsules that are 50% active, at a usage level of 2% by weight of the juice beverage, the “NEW” juice beverage would contain about **8 times** the amount of limonin found in commercial orange juice as illustrated in table 10 below. Certain studies as written in the introduction of this paper indicate anti-carcinogenic and cardiovascular benefits via consumption of limonoids and flavonoids. It is

essential to clarify again that this project's scope is to increase the availability of these limonoids by several fold, 5 to 8 times higher than those found in commercial orange juice. As it is well known in the food industry, foods cannot have any claims on them which list anything to do with: Prevent, Mitigate or Cure diseases. The following table illustrates the complete list of values. For reference, the values for a whole orange fruit are also included.

Table 10. Phytonutrients Targets for a New Juice

Phytonutrient Profile parts per million (ppm)	Whole Orange Fruit (Edible Portions) Wet Basis 	100% Orange Juice Wet Basis 	Microencap-sulated Fruit Power (MFP) (50% Load or 50% Active) 	NFC Orange juice w/ MFP Powder 2% wt.) 
Limonin	**8**	**3**	**1,140**	**24**
Nomilin	1	1	101	3
Narirutin	73	103	920	120
Hesperidin	402	288	5,606	400
Didymin	33	24	268	30

1. Perspective on coated fruit powder in fruit juices

When it comes to the stability of the microcapsules, the erosion of the wax or fat coating layer due to flow of fluid pressures by pumping and the acidic properties of orange juice may contribute to the difficulty of maintaining the coated fruit powder stable in the juice. That is why some applications still combine the wax melt coating process with the traditional polymer film coating in order to ensure that the coated core material remain stable for longer under diverse applications and environments. Nevertheless, the fact that no water or any other solvent is used in the hot melt wax coating process simplifies the procedure. The coating material which is carnauba wax or HSBO are expected to make the finish microcapsules relatively stable in fruit juices, as the materials are durable, especially the carnauba wax. It is hydrophobic; it is completely immiscible in liquids like water. It was therefore hypothesized that the coating will do a good job at protecting the citrus core particles for the term of an NFC chilled juice shelf life, typically 10 weeks. Furthermore, the process of spraying the coating through the Wurster system also ensures uniform and high quality coating to the fruit powder when compare to other coating practices. To ensure that the coating remains stable, there is the need to implement a relatively high thickness of the coating material, specifically for longer shelf life needs in acidic conditions. Therefore, for the final phase of the microencapsulation section,

which was producing microcapsules for the stability study, multiple microcapsules with varying coating thickness were produced and ranged from 30% coating by weight all the way up to 75% coating by weight, conversely, from 25% active/core up to 70% active/core.

The table below (Table 11) displays the samples which were selected to conduct the stability study in a simulated orange juice beverage. Note that variables include a large array of coating levels. This was purposely done to ensure the study had an expansive offering of fruit powder active/core and coating levels. Note that a "*positive control"* was also added in the mix which is identified a 100% active (code C) which is only fruit powder with no coating as a benchmark for the work.

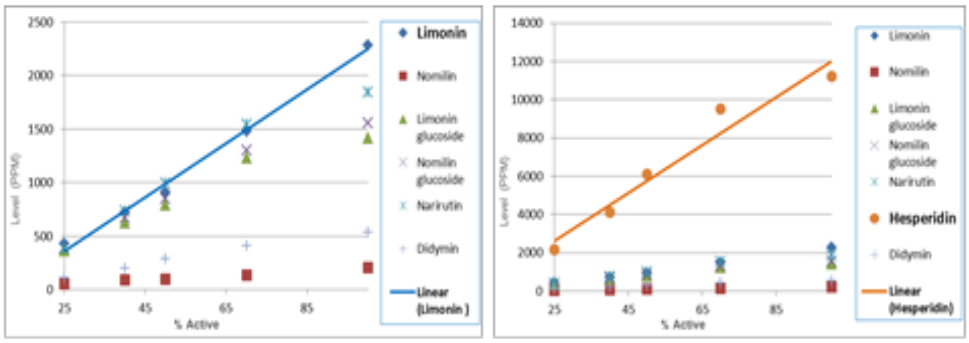
Table 11. Variables Selected for Stability Study

Selected Samples for Stability Study					
Code	Description	W1	W2	W3	WBC
C	100% Active	3.0	40.0	13.5	8.83
8	70% Active	3.0	40.0	17.0	7.67
5	50% Active	3.0	40.0	28.7	3.77
2	40% Active	3.0	40.0	32.5	2.50
4	25% Active	3.0	40.0	36.2	1.27

After producing the microcapsules outlined above in a manufacturing facility, it was determined to conduct content analysis of these microcapsules to obtain confirmation that the bioactives such as the phytonutrients of interest in the fruit powder (limonin, etc.) were at adequate levels as well as coating levels turned out as designed. Results in Table 12 below showcase the produced microcapsules and their associated phytonutrient counts. Overall, this data confirms that targeted coating levels as well as bioactive levels were close to the designed targets. As illustrated in the graphs, the variation off the line for some phytonutrients was approximately +/- 20%.

Table 12. Confirmation of Coating Levels to Fruit Powder

	Values in parts per million						
Samples	**Limonin**	Nomilin	Limonin glucoside	Nomilin glucoside	Narirutin	**Hesperidin**	Didymin
100 % Active	**2279.5**	203.6	1416.2	1551.7	1840.0	**11212.1**	537.3
70 % Active	**1475.5**	135.5	1229.9	1297.4	1540.1	**9494.8**	411.1
50 % Active	**914.4**	98.7	788.4	846.4	996.6	**6089.5**	286.0
40 % Active	**723.7**	87.6	624.0	671.3	731.8	**4117.3**	197.4
25 % Active	**428.1**	50.4	366.1	387.7	384.7	**2152.5**	107.3



CONFIDENTIAL INFORMATION

2. Incorporation of microcapsules to fruit juice

The incorporation of the microcapsules to the orange juice beverage is ultimately the goal of this project. As Table 10 illustrates, the 50% Active microcapsules are added at a level of approximately 2% by weight. At this usage level, the microcapsules will increase the limonin level by about 8 times higher than levels found in commercial orange juice. Note that as the microcapsules were added to the “juice” (simulated orange juice beverage), the usage level had to be adjusted with the various microcapsules since the amount of active/core varied per microcapsule depending on the coating %. i.e., need to add more capsules with the thicker coating and less amount of capsules with the thinner coating to deliver the same net amount of fruit power (active).

Figure 15 below illustrates the actual photographs of the uncoated fruit powder (100% active) and a series of coated fruit powder which range from 70% active down to 25% active. Photographs were taken with a *Digital WILD* Stereomicroscope - light microscope at 16X magnification. It is important to note that particle size is reported as Surface Mean Diameter (SMD). Based on the photographs and particle size distribution readings, the coated particles turned out much larger than anticipated. As an example, if the core, uncoated fruit particles had a SMD of 110 microns, then, a coating of 30% by weight should of yield particles in the range of 150 micron for SMD. But, the actual size was at

343 micron SMD. The subsequent capsules also exhibit the same larger outcome than expected to as large as 613 microns SMD for the 25% active microcapsules. Note that these large capsules present a sensory challenge since any particle at or above the 150 to 200 micron range will be perceived by people consuming the product. Larger than 150 to 200 micron SMD microcaps exhibit a granular, sandy, grainy mouth feel. This can be fine for products which have the consistency of a fruit smoothie or for products that have added particulates such as bits of pulp, bits of fruit, etc. but for ordinary 100% juice with no pulp or for nectars or juice drinks, the mouth feel and the visual experience will certainly be evident.

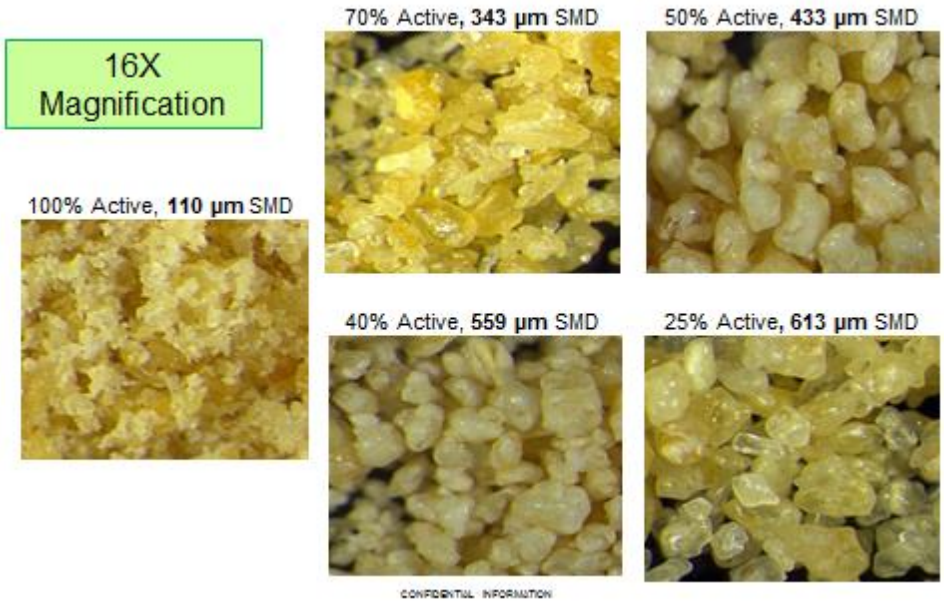


Figure 15. Photographs of Uncoated and Coated Fruit Powder

To further investigate the unexpected large capsule size, another photograph was taken of the 25% active microcapsules at **40X** magnification. It was evident as shown in Figure 16 with the red arrow; the capsules had imbedded fruit powder in clusters. The clustering of the fruit powder, in part, is an effect of significant amount of fat in the seed powder, coupled with high temperature in the fluidized bed dryer vessel making the granules “sticky” and therefore bind with each other as explained in the milling section when the screens plugged up. Furthermore, it is speculated that the highly viscous liquid due to initial challenges with keeping a high and controlled temperature of the

coating material, contributed to the wetting characteristics of the coating and the fruit clusters affected the wetting angle which translated in to irregular coating layers. The clustering of the fruit particles formed larger cluster structures; perhaps in the range of 300 to 400 micron SMD, hence, once the coating was applied, the formed microcapsules were in the range of 500 to 600 microns SMD.

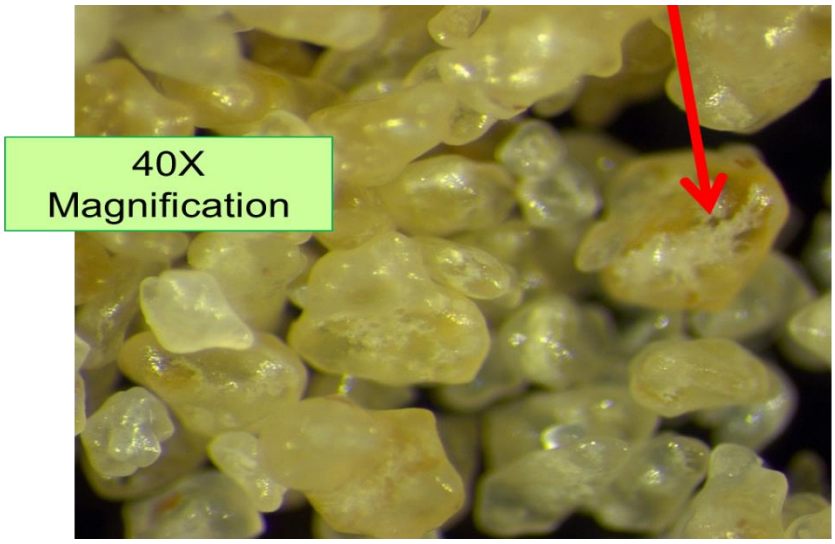


Figure 16. Photograph of Microcapsule at 40X Magnification

To address these irregularities, during the subsequent and final encapsulation plant trial, three adjustments were implemented to the process to enhance the coating effectiveness between the melted coating and the fruit powder. First, to improve the flowability of the fruit powder and also to reduce the clustering or clumping effects, an “anti-caking” agent was mixed-in with the fruit

powder. The flow agent selected was calcium silicate. The ingredient was obtained from *Huber Engineer Materials* of Atlanta, Georgia (one of many suppliers of these types of ingredients). Benefits for calcium silicate are listed as follow:

- Reduce or eliminate caking
- Improve material flow
- Increase packaging rates
- Reduce or eliminate build up in spray drying operations
- Reduce or eliminate lumping
- Efficiently absorb liquids, fats, and oils for powders
- Decrease clogging and bridging during
- Decrease or eliminate dusting

There are many types of these anti-caking agents which work in a similar fashion, other options include; silicon dioxides, calcium silicates and sodium aluminosilicates. For this project, a 1% wt. addition of calcium silicate was incorporated to the fruit powder. This translates into 0.5% weight for the 50% active microcapsules once the coating is account for. The net usage level of calcium silicate in the finished juice beverage is much lower since capsules were added at a level of approximately 2% weight. After adding and tumble mixing the

calcium silicate with the fruit powder, it was immediately noted that the flow of the fruit powder significantly improved. Viewing the fruit powder under the light microscope also showed significantly less clustering. The second modification implemented was to reduce the fruit powder temperate in the fluidize bed dryer product hold vessel. Lowering the temperature also reduced the clumping of the powder since high heat “melted” the fats in the fruit powder derived from the seeds. Hence, lower temperature reduced the “sticky” effects. Third, for the last plant trial, the carnauba wax was used instead of the HSBO. This decision was made since in the DOE, the carnauba wax performed directionally better than the HSBO but had not been used before due to not being able to maintain the hot melt unit at the required temperature (high enough to melt the carnauba wax and be pumpable all the way through the spray nozzle). Using the carnauba wax and precisely controlling the delivery temperate improved the coating parameters and the adjustments of the other two modifications to the powder (flow agent and lower product temperature) enhance the wetting conditions, so the coating was more effective. This particular trial was the final one conducted and wad done in collaboration with Freund-Vector, which is the supplier where the fluidized bed dryer and hot melt equipment was purchased. The trial was conducted at the Freund-Vector facility located in Marion, Iowa for a period of two days. Table 15 below outlines the operating conditions of this final phase of the coating work.

Table 13. Fluidized Bed Dryer Run Conditions for Carnauba Wax

Element	Parameters and units	Amount
Fruit Powder	Per batch (KG)	1
Carnauba wax	Per batch for 50 active (KG)	1
Hold tank amount	Molten wax amount (KG)	2
Dryer vessel	Airflow (CFM)	75
Coating amount	Target (%)	50
Inlet air	Temp (°C)	65
Product - powder	Temp (°C)	53
Exhaust air	Temp (°C)	46
Solution tank	Heater temp (°C)	120
Solution line	Heater temp (°C)	120
Spray pump speed	RPM	20
Spray pump rate	Grams/min	30
Nozzle air	Pressure (PSI)	30
Nozzle air	Rate (Liter/min)	145
Nozzle air	Heater temp (°C)	120
Nozzle tip	Orifice size (mm)	1
Exhaust filter	Mesh size (us mesh)	60
Base screen	Mesh size (us mesh)	60

Running with the adjusted equipment and product conditions listed above and using the anti-caking agent produced enhanced microcapsules. Note that due to the time limitation and cost associated with using a full scale testing facility; only one sample was produced using carnauba wax, the 50% active and 50% coating variable. After examination of these microcapsules under the light microscope at both the 16X magnification and at 40X magnification, the particle surface mean diameter (SMD), the particle size distribution and the coating consistency was significantly improved. Figure 17 below showcases the

uncoated fruit powder as well as the microcapsule utilizing the carnauba wax. Initial SMD for the fruit powder was 102 microns, the microcaps with the 50% wt. added carnauba wax yield a particle size of 184 microns which is more in-line to what is expected by a simple mass balance calculation.

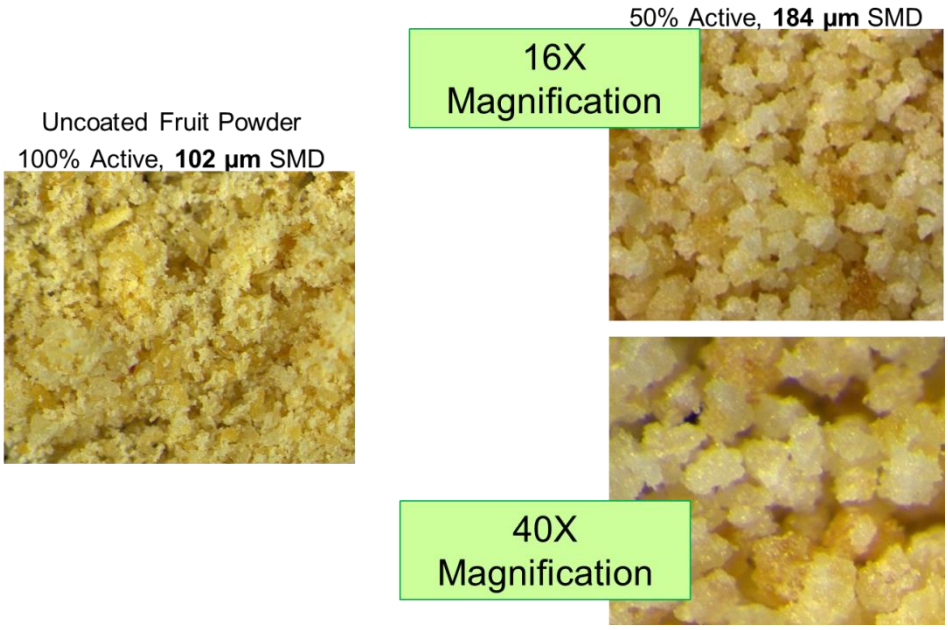


Figure 17. Photographs of Carnauba Wax Coated Microcapsules

3. Stability study design to determine shelf life

In order to determine the performance of the microcapsules (coated fruit powder with HSBO or carnauba wax) in an orange juice beverage and conduct

the shelf life assessment with the aim to achieve a 10 week shelf life under refrigerated conditions, a unique approach was developed for two reasons. First, to limit the amount of fruit powder ingested by the panelists drinking the samples. This was done since this fruit powder is not yet a GRAS certified ingredient. GRAS or Generally Recognized as Safe is a term the food industry uses to label an ingredient safe for human consumption under specified conditions and at specified levels. Food companies have strict procedures and protocols in place to ensure all ingredients are safe for human consumption. Conditions Include stages during scoping, research, testing and development, inclusive of conducting shelf life studies. Second, the simulated beverage was utilized to better monitor the effectiveness of the microcaps and measure the amount of phytonutrient leaching. Therefore, a clear (color-free) beverage was developed to conduct this work. Leaching of color is common with fruit powders which contain compounds as annatto, anthocyanins, beta-carotene, caramel, carmine, chlorophyll, curcumin, lutein, turmeric, etc. As the microcapsule's coating dissolves or ruptures, there will be color leaching into the juice beverage. Having created a simulated orange juice (OJ) beverage without color was instrumental to visually measure the level of color leaching.

To set up the shelf life study, it was determine to first set up the threshold of bitter levels which would come from the fruit powder. A simulated OJ beverage

was created and portions of *uncoated* fruit powder were added in increments of 0.1% wt. A control was also prepared with common levels of limonin, which is in the range of ~ 3 parts per million (ppm) as illustrated in Table 14.

Table 14. levels for Determination of Bitterness Thresholds

Sample	% wt. uncoated powder added	Limonin PPM
Control	0.12	2.9
T1	0.20	4.8
T2	0.30	7.2
T3	0.40	9.6
T4	0.50	12.0
T5	0.60	14.4
T6	0.70	16.8

The uncoated fruit powder was added to the simulated OJ beverages which contained the ingredients as illustrated in Table 15. In this case, it included beta-carotene as a coloring agent and cloud for turbidity to ensure, visually, the samples looked the same to the panelists. Essentially, the mouth feel, the appearance and aroma of the control plus the five test samples was relatively the same to the expert panel tasting the sample series. The only changing variable was the amount of uncoated powder in each sample. Figure 18 illustrates the actual simulated OJ beverage with the uncoated fruit powder dissolved into the beverage but “invisible” to the naked eye as the cloud and beta-carotene concealed the added fruit powder.

Table 15. Simulated OJ Beverage to Establish Taste Thresholds

Simulated OJ to establish taste thresholds			
		% wt.	Grams
Water	Beverage grade filter water	87.8400	878.4000
Sugar	Granular white table sugar	12.0000	120.0000
C6H8O7	Citrix acid (anhydrous)	0.0550	0.5500
Cloud	Natural cloud emulsion Robertet NV8182	0.0450	0.4500
Flavor	Natural Orange Flavor key	0.0100	0.1000
Powder	Uncoated Fruit powder combo at diverse levels (0.12% to 0.70%)		
Colorant	Beta-carotene powder - lucarotin BASF 1CWD/Y	0.0500	0.5000
		100.0000	1000.0000
	Solution parameters:		
	pH	3.5	
	Brix	12.1	

Figure 18. Photograph of Samples Containing Uncoated Fruit Powder

4. Sensory panel to establish bitter thresholds

A group of 15 expert judges, skilled in the art and *heavy-users* of orange juice were recruited. Eleven judges from within the company and four external judges. Control was labeled and identified as such (control). All test samples were presented “blind”, each having a 3 digit code, including a blind control within the set of test samples. Sequence of test samples was randomized with each judge. A rating sheet was provided to assess each test sample vs the control. The methodology utilized was Degree of Difference (DOD) with a 5 point scale. The 5 point scale descriptors are as follow:

1. **No difference** = Sample test tastes and feels exactly like the Control
2. **Slight difference** = Similar overall profile, small magnitude difference (some panelists able to describe the nature of the difference)
3. **Moderate difference** = Similar overall profile, with noticeable differences of moderate magnitude (all panelists able to describe the nature of the difference)
4. **Large difference** = Similar overall profile, with noticeable differences of large magnitude (all panelists able to describe the nature of the difference)

5. **Very large difference** = Similar overall profile, with noticeable differences of very large magnitude (all panelists able to describe the nature of the difference)

Only ***no*** difference and ***slight*** difference were allowed as parity or as ***"pass".*** These passing samples were then identified as only T1 and T2 samples based on the expert panel results. Figure 18 established that test sample T1 contained ~ 5 ppm limonin and test sample T2 contained ~7 ppm limonin. All other results were deemed as ***fail*** due to the high level of bitterness. Once the bitter thresholds were identified, these values were used to determine the limits of color leaching of the fruit powder. This determination was done with a similar set of test samples without the cloud and beta-carotene. Figure 19 has the range of the color-free simulated OJ beverage showcasing the effect of natural coloration deriving directly from the uncoated fruit powder. The goal of the shelf life study was to use this correlation of bitterness vs. color leaching of the microcapsules in the uncolored OJ simulated beverage.

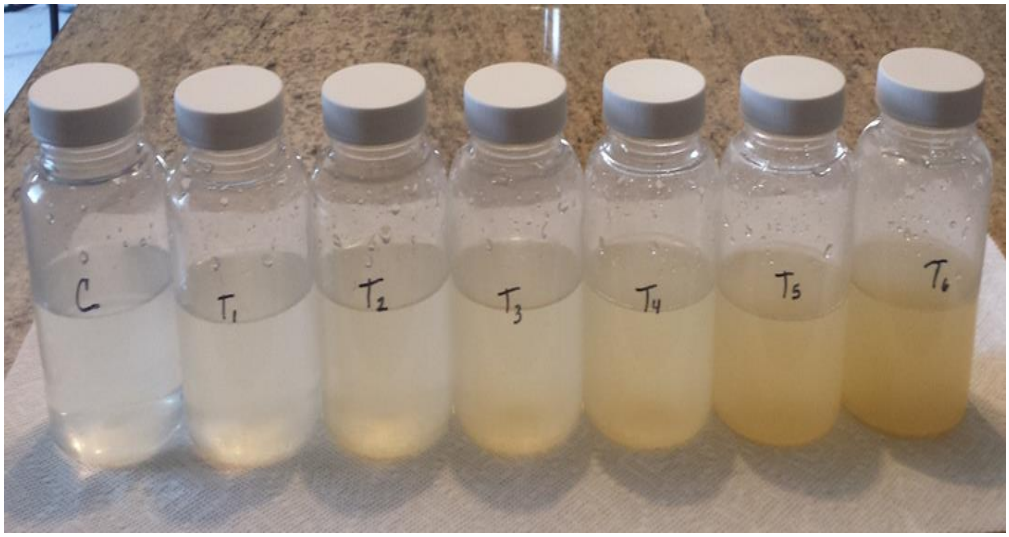


Figure 19. Color Leaching Effects

5. Shelf life study set-up

To establish a shelf life ***pass or fail*** determination, a color correlation skid was developed. Note that the color skid as identified in Table 16 was correlated with the samples in Figure 18 which included the uncoated powder.

Table 16. Color Skid Utilized for Shelf Life Determination

COLOR SKID	
Control	Pass
T1	Pass
T2	Pass
T3	Fail
T4	Fail
T5	Fail
T6	Fail

For the shelf life study, all samples were stored under refrigerated conditions at a temperature of 2ºC. The targeted shelf life per the hypothesis of this project was 10 weeks. The microcapsules used for the stability study included several samples from the HSBO trial and one microcapsule from the carnauba wax trial which was the 50% Active & 50% coating sample. Table 17 illustrates the complete list of microcapsules used in the stability study followed by Table 18 which includes the simulated orange juice beverage without the colorants to establish when the color leaching would fail based on established color skid limits.

Table 17. List of Microcaps Utilized for Shelf Life Study

Coating type	None	None	HSBO	Carnauba	HSBO	HSBO	HSBO
Amount of Coating	Base Sol. (No MFP / Active)	100% Active	70% Active	50% Active	50% Active	40% Active	25% Active

The simulated OJ beverage utilized for the shelf life study did not contain any added color or cloud. The microencapsulated powders were added, either coated with HSBO or carnauba wax.

Table 18. Simulated OJ Beverage for Establishing Shelf Life

Simulated OJ to establish shelf life			
		% wt.	Grams
Water	Beverage grade filter water	85.9350	859.3500
Sugar	Granular white table sugar	12.0000	120.0000
C6H8O7	Citrix acid (anhydrous)	0.0550	0.5500
Capsules	Coated Fruit powder (50% active, 50% coating)	2.0000	20.0000
Flavor	Natural Orange Flavor key	0.0100	0.1000
		100.0000	1000.0000
	Solution parameters:		
	pH	3.5	
	Brix	12.1	

On a weekly basis, samples were pulled from the refrigerator to determine amount of liquid coloration (color leaching) by visual examination. Coloration/leaching level was compare vs color skid with pre-established *pass or fail* thresholds. Results were then recorder in the shelf life tracker as illustrated in Table 19 below.

VI. RESULTS AND CONCLUSION

The results for the stability study indicate that the tested microcapsules did not reach the targeted 10 weeks shelf life. Table 19 contains the list of tested microcapsules and respective results. In the stability study, a blank variable was added as a negative control (Variable A) to ensure there was no issues with the base beverage as formulated. Note that a positive control (Variable B) containing the fruit powder with no coating was added to ensure the coloration or leaching would be immediate. As expected, the positive control sample failed the stability study within minutes of adding the uncoated powder to the beverage solution. Next, a series of coated fruit powder with the HSBO coating was added to the study. A sequence starting with the higher % Active (fruit powder) followed by lowering % Active was incorporated in the study. Note that in the mix, there was the 50% active fruit powder coated with carnauba wax (Variable E). Based on the results, the carnauba wax coating held up one week better than its counter 50% active fruit powder coated with the HSBO. Note that a 70% Active sample was also added to the study. This was strategically done monitor failure at various levels. As expected, this variable only lasted two weeks. Overall, the microcapsules with the 25% Active; 75% coating lasted the longest. Based on table 19 results, the 25% Active microcapsules (Variable G) were stable up to 6 weeks but then failed at week 7.

Table 19. Stability Study Results

Visual assessment based on liquid coloration							
Variable	A	B	C	D	E	F	G
Coating type	None	None	HSBO	HSBO	Carnauba wax	HSBO	HSBO
Fruit Powder (Active) wt. amt.	Bev. Soln. (No Active)	100% Active	70% Active	50% Active	50% Active	40% Active	25% Active
week 1							
week 2							
week 3							
week 4							
week 5							
week 6							
week 7							
week 8							
week 9							
week 10							

Key	
Pass	
Fail	

During the term of the shelf life study, samples were also monitored under a *Stereo Light Microscope* using direct top light and a *PLM Microscope* using polarized light at 100X magnification to track coating effectiveness. It was determined that water concentration gradient is the driving force for particle hydration through diffusion which then leads to expansion of the particles and rupturing of the coating wall which then leads into leaching of the phytonutrients. Failure modes can be targeted to thin spots of the coating wall where it facilitates water to diffuse into the microcapsule, it was established earlier through the WBC testing that the fruit powder is hydrophilic therefore, it can absorb up to 3x its

weight in water, this can further accelerate the mode of microcap expansion and rupture of the coating wall.

Figure 20 below are photographs of the progression of the shelf life and associated microcap engulfing with the final stage being the rupturing of the coating wall. The first two photographs represent the uncoated fruit powder. The second set of photographs are an example of the coated fruit powder with the carnauba wax at a 50% wt. coating level. The third and final set has two photographs, the first one on the left was taken with the stereo light microscope at 100X and the second one on the right is the same image but done with the PLM LeicaDMLB microscope to better illustrate the via polarized light as pointed out by the yellow color arrow. Eventually the coating, being somewhat crystalline in nature will rupture when too much water enters the microcapsule as illustrated by the red arrow followed by the leaching of the fruit powder into the liquid.

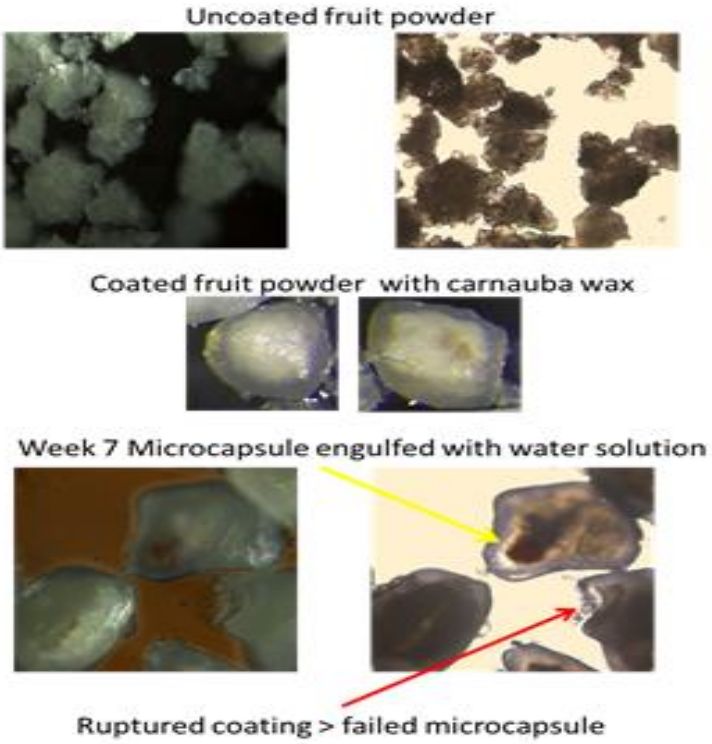


Figure 20. Photographs of Microcaps through Coating Rupture

The thicker the coating wall, the more it will inhibit or retard diffusion of water into the fruit particles. The coating mechanism of the wax on the fruit powder is essentially via the ability of the molten wax to coat the particle (wetting) then, it's simply solidification of the melted coating on the particle. Temperature of the cooling or tempering air has a direct effect on the extent of particle aggregation. The rate of cooling and congealing can also dictate the crystallinity morphology of the microcapsules. As part of future work, it is recommended in point # 3 to conduct studies to determine the rate of solidification and effects on

coating strength. It is also suggested for the recommended next steps on item #4 to develop a quantitative method for evaluating the point of failure of the microcaps as the leaching of the micronutrients colored the liquid solution. An initial start was carried-out at the end of this project to at obtain some understanding by looking at levels of a *L,a,b* color profile via a UV Reflection conducted on a *Greta MacBeth X-Rite Colorimeter*. Table below outlines the levels associated with Pass and Fail for color value *(a)* and color value *(b)*. As the red circle below indicates, values lower than a -0.80 for *(a)* and higher than a 1.78 for *(b)* will be the cut-off for Pass from a sensory perspective as it was established with the expert panel.

Table 20. Profile of L,a,b color spectrum for quantifying failure cut-off

Sample Code	% wt. uncoated powder	Limonin PPM	Sensory Association	Color (L)	Color (a)	Color (b)
Control	0.12	2.90	Pass	30.52	-0.62	0.91
T1	0.20	4.80	Pass	30.39	-0.71	1.55
T2	0.30	7.20	Pass	29.65	-0.80	1.78
T3	0.40	9.60	Fail	27.59	**-0.90**	**1.85**
T4	0.50	12.00	Fail	28.63	**-1.08**	**1.97**
T5	0.60	14.40	Fail	28.11	**-1.12**	**2.04**
T6	0.70	16.80	Fail	30.03	**-1.34**	**2.63**

The opportunity is great, citrus by-products such as citrus rag and citrus seeds generated by the orange juice industry are immense in volume. It was established that more that 30 million metric tons of by-products are produced annually. It has also been well established that these types of by-products have many health promoting components; including dietary fiber, vitamin C, carotenoids, flavonoids and limonoids. Most of the health benefits associated with citrus seeds and citrus rag such as; anti-oxidant, antiviral, anti-bacterial, anti-inflammatory and anti-carcinogenic are associated with the inherent limonoids and flavonoids in these by-products. It is important to cover or to coat these two components especially in the case of limonoids, because they are very bitter and

cannot be consume directly in high quantities. Therefore, the work conducted for this project gets closer at identifying formats to utilize these vastly unused materials which have good health and wellness potential. Coating aims at conserving, then consuming the phytonutrients while concealing the bitter taste. Based on the work conducted, identified advantages and disadvantages of using coating materials for this application, it was determined that carnauba wax was the best coating material. It is inert and non-reactive to the core fruit powder and it is non-soluble in water and highly hydrophobic. In addition, no solvent is needed to apply the coating material when the hot melt unit is utilized.

In conclusion, the data results based on experiments conducted did not fully support the hypothesis to achieve a 10 week shelf life under refrigerated conditions for an orange juice beverage

1. Microencapsulating fruit powder by fluidized bed dryer showed good potential when utilizing waxes and fats as coating materials for developing a water proof barrier
2. Although the microcaps did not fulfill the targeted shelf life of 10 weeks, there is application potential for fruit beverages that require a much shorter shelf life such as some fruit smoothies, freshly squeezed juices and cold press juices

3. The addition of the flow agent or anti-caking agent - calcium silicate to the fruit powder greatly improved its flowability and reduced the clumping leading to smaller, more adequate and uniform microcapsules
4. Although microcapsules larger than ~200 um, have a gritty mouth feel, they are suitable for products such as fruit smoothies where the consistency is thicker and fruit bits and other food particulates are commonly added by design
5. The thin coated microcapsules, i.e., 70% active, 30% coating do not appear to be conducive for long shelf life but can be a suitable option for "*direct add*" at time of consumption. i.e. added through *sachets* or *in twist caps*
6. Microcapsules with thin coating should also be considered for opportunities in non-beverage applications where moisture is not as high, i.e. bakery toppings, granola bars, trail nut mixes, etc.

Incorporation of largely unutilized citrus by-products containing bitter phytonutrients via the use of microencapsulation can be a good tool to increase the health and wellness potential of juice beverages. Although, one must always be aware of not only the positives but also the potential negative effects of the foods that we eat and drink, prescribed drugs that we take, cosmetics that we

use. We often hear in the news of people accidentally getting harmed or even killed by combining two kinds of drugs, or in some cases, getting very sick by consuming too much of a certain type of food. These incidents could be avoided just by knowing and understanding that not everything we eat and drink is safe, even the natural food. It is important that we are conscious of the contents of the food we ingest and potential interactions within them and with medications we take. Because, as the famous quote from Paracelsus said, “All substances are poisons - the difference is in the dose”.

VII. FUTURE WORK

There are several recommended next steps to be considered for future work along this line of research.

1. Conduct microencapsulation research applying multi-layer coating materials to expand the versatility of the microcapsules and extend shelf life of finished orange juice beverage beyond 6 weeks
2. Conduct work of the developed microcapsules using carnauba as and apply to non-citrus type of fruit or vegetable juices, more neutral pH and lower acidity to understand if shelf life can be greater than 6 weeks
3. Conduct a microencapsulation DOE utilizing wax coating materials to derive an understanding of coating mechanisms at it relates to rate of wetting and crystallinity for finding the optimal morphology layer
4. Continue work to optimize the develop a quantitative, analytical method for color assessment to identify all the components which contribute to color leaching which are associated with bitterness

REFERENCES

Agnihotri Nitika, Mishra Ravinesh, Goda Chirag, Arora Manu. 2012. Microencapsulation. A Novel Approach in Drug Delivery: A Review. Indo Global Journal of Pharmaceutical Sciences 2.1: 1-20.

American Dietetic Association (ADA) Complete Food and Nutrition Guide. 2006. Duyff Roberta, Larson Duyff. Third ed. New Jersey: John Wiley & Sons, Inc.

Auton, E. 2010. Pharmaceutics: The science of dosage form design. Edinburgh: Churchill Livingston

Bakhshi Hadi. 2013. Synthesis and characterization of antibacterial polyurethane coatings from quaternary ammonium salts functionalized soybean oil polyols. Materials Science and Engineering C. vol.33, no.1: 153-164.

Baldwin Elizabeth, Hagenmaier Robert, Bai Jinhe, Krochta John. 1994. Edible Coating and Films to Improve Food Quality: New York, NY: Taylor Francis & Group.

Bayazit R. 2010. Biochemical and physiological evaluations of Limonoids - A potential cancer destroyer. Medwell journal. Web November 1 <http://www.medwelljournals. com/fulltext/?doi=javaa.2010.1099.1107>

Beeswax. 2007. Web. October 28, 2012 <http://chemistrydaily.com/chemistry/Beeswax>

Beeswax benefits. 2012. Web. October, 2012 <http://ehoneybees.com.>

Berhow A. Mark, Hasegwa Shin, Manners Gary D. 2000. Citrus Limonoids, Functional Chemicals in Agriculture and Foods. American Chemical Society, Volume 758, US. AOAC Press.

Buslig Bela and John F. Manthey. 2002. Flavonoids in Cell Function: Advances in Experimental Medicine and Biology. New York: Plenum Publishers.

Chiba Andrew. 2012. Hesperidin, a Citrus Flavonoid, Inhibits Bone Loss and Decreases Serum and Hepatic Lipids in Ovariectomized Mice. Journal of Nutrition. Web. 2 Nov, 2 < http://jn.nutrition.org/content/133/6/1892.full.pdf+html.>

Citrus Pages. 2011. “Lemon.” October 30, 2011. <http://users.kymp.net/citruspages/ lemons.html.>

Codoner Frank. 2010. Citrus as Functional Foods. Current Topics in Nutraceuticals Research. Vol.8, no.4: 173-183.

Cracolice Mark S., Peters Edward I. 2007. Introductory Chemistry: An Active Learning Approach. United States of America: David Harris Print.

Crowell P. L. 2011. Prevention and Therapy of Cancer by Dietary Monoterpenes. November 3. <www.ncbi.nlm.nih.gov/pubmed/10082788>

Cvetnic, Z. and Kenezevic, S. 2009. “Antimicrobial activity of grapefruit seed and pulp ethanolic extract.” Acta Pharm. 13: 243 – 250.

Deis C. Ronald. 1997. Spray-drying-innovative. Use of an old process. Food Product Design, 7: 97–113.

Desobry, S., Netto, F.M., Labuza, T.P. 1997. Comparison of spray-drying, drum-drying and freeze-drying for β-carotene encapsulation and preservation. Journal of Food Science, 62: 1158–1162.

Dhuppe Shital. 2012. Recent techniques of pharmaceutical solvent-less coating: a review. International Journal of Pharmaceutical Sciences and Research. Vol.37, no.7: 1976-1986.

Dictionary of Food Science and Technology. 2009. International Food Information Service. UK: IFIS Publishing.

Drewnowski Adam, Carmen Gomez-Carneros. 2011. Bitter Taste, Phytonutrients: A Consumer Review. November 3 <http://www.ajcn.org/content/72/6/1424.full>

Drozdowicz D. 2011. Gastro protective activity of grapefruit (Citrus paridisi) seed extract against acute gastric lesions. Nuts and Seeds in Health and Disease Prevention. Vol. 5, no. 5: 553-560.

Economos Christine, Clay William D. 2011. Nutritional and Health Benefits of Citrus Fruits. FAO Corporate Document Repository. November 5. <http://www.fao.org/docrep/x2650T/ x2650t03.htm#TopOfPage>

Ekelman B. Karen, Ckhang Hui-Chen. 2012. Carnauba wax. Web. 2 Nov, <http://www.inchem.org/documents/ jecfa/jecmono/v30je13.htm>

eMedicineHealth. 2011. Citrus Hesperidin. 31 October. <http://www.emedicine health.com/hesperidin/vitamins-supplements.htm.>

Endlein E., Peleikis K. 2011. Natural Waxes Properties, Compositions and Applications. International Journal for Applied Science 4: 1-8.

FAOUN - Food and Agricultural Organization of the United Nations. 2011. Economic and Social Department, the Statistical Division. Citrus fruits.

Giamperi L. 2004. Antioxidant activity of Citrus paradise sees glyceric extract. Fitoterapia. Vol. 75, no. 2: 221-224.

Gibbs Tracy. 2011. Phytonutrients, the Natural Drugs of the Future. November 3. <http://www.worldscibooks.com/etextbook/6358/6358_chap01.pdf.>

Grace John R., Leckner Bo, Zhu Jesse, Cheng Yi. 2008. Fluidized Beds, Clayton T. Crow, Multiphase Flow Handbook, CRC Press, p. 5:71, DOI 10.1201/9781420040470. Ch 5. ISBN 978-1-4200-4047-0

Hampel N, Buck A, Peglow M, Tsotsas E. 2013. Continuous pellet coating in a Wurster fluidized bed process. Chemical Engineering Science, vol. 86, no. 1: 87-98.

Hesperidin Health. 2012. October 31. <http://www.health24.com/natural/Herbs/17-666-676,65571.asp.>

Holdich Richard Graham. 2002. Fluidization, Chapter 7. Fundamentals of Particle Technology, Midland Information Technology and Publishing. ISBN 978-0954388102

Introduction to Toxicology. 2012. Web. October 29, <http://service004.hpc.ncsu.edu/toxicology/websites/courses/tox401/bookchapters/Hodgson_Ch1.pdf.>

Jannin V., Cuppok Y. 2013. Hot-melt coating with lipid excipients. International Journal of Pharmaceutics. Vol.457, no.1: 480-487.

Jones D. 2009. Development, Optimization and Scale-up of Process Parameters: urster Coating. Developing Solid Oral Dosage Forms. p. 807-825.

Kim, J., Jayaprakasha G, Vikram, A. Patil, B. 2012. “Cancer Chemopreventive Properties of Citrus Limonoids.” American Chem Soc. 3: p. 37 – 50.

Kimball Dan. 1999. Citrus Processing: A Complete Guide, 1999. Maryland: Aspen Publishers, Inc.

Kotamballi, N., Murthy, C., Jayaprakasha, G. & Patil, S. 2013 “Citrus limonoids and curcumin additively inhibit human colon cancer cells.” TX: Texas A&M University: College Station, TX.

Kuttan, G., Manu, P. & Kuttan, R. 2011. “Inhibition of tumor progression by naturally occurring terpenoids.” Pharm Biol. 49(10): p. 995 – 1007.

Ladaniya Milind. 2008. Citrus fruit: Biology, technology and evaluation. California: Academic Press.

Lee S.Y., Krochta J.M. 2002. Accelerated shelf life testing of whey protein coated peanuts analyzed by static gas chromatography. Journal of Agricultural and Food Chemistry, 50: 2022–2028.

Melo J. 1998. Carnauba wax used as a hydrophobic agent for expanded vermiculite. Brazilian Journal of Chemical Engineering. Web. 2 Nov,<http://www.scielo. br/scielo.php?pid=S0104-66321998000100010&script=sci_arttext>

Moulehi Ikram, Bourgou Soumaya, Ourghemmi Ines, Tounsi S. Moufida. 2012. Variety and ripening impact on phenolic composition and antioxidant activity of

mandarin (Citrus retucalate Blanco) and bitter orange (Citrus aurantium L.) seed extracts. Industrial Crops and Products. Vol.39, no.1: 74-80.

Murray Frank. 2007. Health Benefits Derived From Sweet Orange: Diosmin Supplements from Fruits. California: Basic Health Publications, Inc.

Nagoor, N., Muttiah, K., Lim, T. & Awang, K. 2011. “Regulation of Apoptotic Effects by Erythrocarpine E, a Cytotoxic Limonoid from Chisocheton erythrocarpus in HSC-4 Human Oral Cancer Cells.” PLOS One. 6(9): 112 – 120.

O’Neil, C., Nicklas, T., Rampersaud, G. & Fulgoni, V. 2012. “100% Orange juice consumption is associated with better diet quality, improved nutrient adequacy, decreased risk for obesity, and improved biomarkers of health in adults: National Health and Nutrition Examination Survey, 2003-2006.” Nutri Journ. 11: p. 107 – 113.

Ono, E., Inoue, J., Hashidume, T. & Shimizu, M. 2011. “Anti-obesity and anti-hyperglycemic effects of the dietary citrus limonoid nomilin in mice fed a high-fat diet.” Biochem BioPhys Res Comm. 410(3): p. 677 – 681.

Onwulata Charles. 2005. Encapsulated and Powdered Foods. New York: Taylor & Francis Group. p 435

Oyelami OA. 2005. The effectiveness of grapefruit (Citrus paradise) seeds in treating urinary tract infections. The Journal of Alternative and Complementary Medicine. Vol. 11, no.2: 369-371.

Padsalgi Amol, Bidkar Sanjay, Jadhav Vijay, Sheladiya Deepak. 2008. Sustained release tablet of theophylline by hot melt wax coating. Asian Journal of Pharmaceutics. Vol.2, no.1: 26-29.

Planinsek O., Pisek R., Trojak A., Srcic S. 2000. “The utilization of surface free-energy parameters for the selection of a suitable binder in fluidized bed granulation.” International Journal of Pharmaceutics 207: 77-88.

Plus Herbal Extracts. 2011. Hesperidin October 31. <http://www.herbalextracts plus.com/hesperidin.cfm>.

Poulos, S., Harris, E. & Patil, B. 2009. “Citrus Limonoids Induce Apoptosis in Human Neuroblastoma Cells and Have Radical Scavenging Activity.” Journ Nutr. 135 (4): p. 870-877.

Pouluse M. Shibu, Harris D. Edward, Patil S. Bhimanagouda. 2006. Anti-proliferative effects of citrus limonoids against human neuroblastoma and colonic adenocarcinoma cells. Nutrition and Cancer. Vol.56, no.1: 103-112

Rahman A. Md, Harwansh R., Biswas R., Mirza Mohd. 2010. Hot melt extrusion and freeze pelletization: better alternative for drugs having stability problem due to presence of water. International Journal of Pharmaceutical Sciences and Research. Vol.1, no.11: 22-31.

Saleh Khashayar, Guigon Pierre. 2007. Granulation. Compiegne University, France: Elsever B.V. p 235.

Samia El-Safy F., Salem Rabab H., Abd El-Ghany, 2012. M.E. Chemical and Nutritional Evaluation of Different Seed Flours as Novel Sources of Protein. World Journal of Dairy and Food Sciences 7: 59-65.

Shahidi Fereidoon, Chi-Tang Ho. 2000. Phytochemicals and Phytopharmaceuticals. US: AOCS Press ISBN 1 893997 05 7

Shahidi Fereidoon and Han X.Q. 1993. Encapsulation of food ingredients. Critical Reviews in Food Science and Nutrition, 33: 501–547.

Shiga Hirokazu, Yoshii Hidefumi, Nishiyama Rumiko. 2001. Flavor encapsulation and release characteristics of spray-dried powder by the blended encapsulant of cyclodextrin and gum arabic. Drying Technology, 19: 1385–1395.

Singh Guriqbal. 2010. The Soybean: Botany, Production and Uses. UK: CAB International North America. SBN-13: 9781845936440

Somasundaram, S., Price, J., Pearce, K. & Shuck, R. 2012. “Citrus Limonin Lacks the Antichemotherapeutic Effect in Human Models of Breast Cancer.” Journ Nutrigenet Nutrigenomics. 5: p. 106–114.

Sudke Suresh G, Sakarkar Dinesh M. 2013. An Extensive Insight on Physico-Chemical Characterization of Hot-Melt Coating Excipients. International Journal of PharmTech Research 5: 879-893.

Terry A. Leon. 2011. Health Promoting Properties of Fruits and Vegetables. UK: CAB International Inc.

The New World Encyclopedia – Citrus. 2011. <http://www.newworldencyclopedia.org/ entry/Citrus>

Therapeutic Research Faculty. 2012. Grapefruit. Natural Medicines Comprehensive Database.

Turner, K. & Burri, S. 2013. “Potential Nutritional Benefits of Current Citrus Consumption.” Agriculture. 3(1): p. 170 – 186.

Turner Paul, Gray Paul, Wong Melanie, Campbell Dianne. 2011. Allergy to fruit seeds presenting with anaphylaxis. Clinical and Translational Allergy. Vol.1, no.1: 58.

UNCTAD Citrus Fruit. 2011: Characteristics. November 3. <http://unctad.org/ infocomm/anglais/orange/characteristics.htm>

Vikram, A., Jesudhasan, P., Jayaprakasha, R. & Pillai, S. 2011. “Citrus limonoids interfere with Vibrio harveyi cell–cell signalling and biofilm formation by modulating the response regulator LuxO.” Microbio. 157: p. 99 – 110.

Watson Brenda, Leonard Smith. 2007. The Fiber 35 Diet: Nature’s Weight Loss Secret, Definition of Phytonutrients. New York: Free Press.

Wittal Vikas. 2013. Encapsulation Nanotechnologies. New Jersey: Scrivener Publishing LLC p. 147

Zeller B.L. and Salleb F.Z. 1996. Production of micro porous sugars for adsorption of volatile flavors. Journal of Food Science, 61: 749–759