MICRONIZATION, MICROENCAPSULATION AND IN-VITRO DIGESTION OF TART CHERRY PURÉE FOR IMPROVING POLYPHENOL EXTRACTABILITY AND BIOACCESSIBILITY

by

NISHTHA LUKHMANA

(Under the Direction of Rakesh K. Singh and Fanbin Kong)

ABSTRACT

There has been a significant amount of interest in tart cherries due to their health-promoting properties. However, full understanding about the limited bioavailability of polyphenols upon consumption is a critical challenge for the cherry industry. Also, much of the previous research catering to this problem has focused on sweet cherries and that leads to the knowledge gap in the area of tart cherry processing and nutrient bioaccessibility. The long-term goal of the study is to develop value-added tart cherry products with enhanced health benefits through particle size reduction (micronization) and microencapsulation to improve the extractability and bioaccessibility of polyphenol compounds. In our study, we conducted a comparative analysis of micronization methods for tart cherry purée and selected megatron and high-pressure homogenization (HPH), based on the desired particle size reduction obtained. With micronization, there was a significant increase in the polyphenol (including flavonoid and anthocyanin) extractability, perhaps due to the disruption of plant cell wall, which resulted in the release of metabolites into the extracellular environment. Furthermore, tart cherry purée was microencapsulated by spray

drying with five combinations of two wall materials, maltodextrin (MD) and gum arabic (GA). The results showed a significantly higher release of polyphenols and flavonoids for microcapsules coated with MD: GA at 15%:15%. Upon storage at different temperatures (4°C, 25°C and 42°C) and relative humidity conditions (11%, 22.5%, 32%, 57%, 75%) for 30 days, it was observed that an equal blend of MD and GA (15%: 15%) was the best wall material combination in improving the stability and the chemical properties of spray-dried tart cherry microcapsules. Also, upon sequential digestion in the mouth, stomach, and intestine, it was found that there was an increase in the release of polyphenols up to 2 h (stomach) and then a sharp decline during the 4 h (intestine), possibly due to reduced stability of the flavylium cation at higher pH values. Our studies have thus demonstrated the importance of tart cherry purée processed with micronization and microencapsulation, to generate microparticles and spray dried microcapsules respectively, in improving the polyphenol extractability and bioaccessibility.

INDEX WORDS: Tart Cherry, Micronization, Microencapsulation, Spray Drying, *In-vitro* Digestion, Bioaccessibility.

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A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2019

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August 2019

DEDICATION

In the fond memory of my father, Late Mr. Raman Kumar Lukhmana, who left untimely for his heavenly abode on the 17th day of March 2009. His moral values and teachings serve as an unrelenting source of my courage and optimism, and I shall forever continue in my constant quest towards making him proud.

ACKNOWLEDGEMENTS

Journey through this dissertation would not have been possible without his valuable, vigilant, compassionate tutelage, whole hearted involvement and unwavering patience. I express sincere and heartfelt gratitude for my revered major Professor, Dr. Rakesh K. Singh, who painstakingly fostered and steered this dissertation to its completion while allowing me the room to work in my own way. I will always be grateful to him for his unrelenting optimism and a strong vision about the study that kept me going.

I humbly express my deepest sense of gratitude to my co-major Professor, Dr. Fanbin Kong, for providing me the opportunity to indulge in sincere research. I am indebted to all my committee members Dr. Joan G. Fischer, Dr. Jose Reyes De Corcuera and Dr. William L. Kerr for their proficient guidance and a thoroughly enriching experience during my graduate school years. I am thankful to the staff and faculty at the Department of Food Science and Technology for their direction and advice, and to Dr. Kristin Navara, Department of Poultry, for helping me hone my scientific writing skills.

I am grateful to Carl Ruiz for his untiring help, intellectual support, suggestions and useful insight during the micronization phase of the study. I would also like to acknowledge the technical and administrative staff for their kind words and amicable smiles that reassured me in exhaustive times during the execution of the study. I consider myself fortunate to have colleagues like Anuj, Ebenezer, Pragati, George and best friends like Georgina, Nabaneeta, Akanksha, Anubhav, Shruti, Rohit, Prathamesh, Joshita, Nehal,

Anuja, Farah, Rajani, Urvashi, Ayush, Dnyanada, Rakesh and Mayuri, and would like to thank them for their friendship, support and for being my family away from family.

My family is literally the wind beneath my wings. Many thanks to my sister, Dr. Shveta Lukhmana and to my brother-in-law, Dr. Indrish Bhatia, for always cheering me up with their motivational pep talks from half-way across the world. My sister paved the path for me to dream big and brightened my journey with the sunshine of her inspirational demeanor. To my mother, Mrs. Veena Lukhmana, I owe my existence. Her sense of accomplishment in my efforts is the reason I thrive to be better. I will forever be indebted to her for all that I am and for all that I'll ever be. Her annual gifts to me in the form of her visits to the US, wrapped in positivity and moral support, throughout the duration of my program, played a pivotal role in the successful completion of my research work and dissertation.

Words cannot do justice to just how grateful I am to my best friend of thirteen years and now husband, Sahil Tahiliani, for his unrelenting faith in my abilities and strengths, for being my anchor through all the highs and lows of my Ph.D. journey, and for his scientific help with data analysis and data visualization for my dissertation. I'm also thankful to my father-in-law, Dr. Suresh K. Tahiliani and my mother-in-law, Mrs. Kamini Tahiliani for their love, words of encouragement and reassurance that there is light at the end of the tunnel.

Last but not the least, with folded hands, I thank God for giving me such a warm mix of mentors, family and friends, and for blessing me with this gift of life and for the wisdom to make the best use of it.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

1.1 Introduction

Over the last decades, epidemiological studies have proved that the consumption of fruits and vegetables that are rich in antioxidants results in improved health and reduced risk of chronic diseases in consumers (Tomás-Barberan, Ferreres, & Gil, 2000). These health benefits have been attributed to the presence of phenolic compounds that impart health promoting properties due to their antioxidant activity (Mellor & Naumovski, 2016). Flavonoids and anthocyanins are the most studied class among these phenolic compounds, and much interest is focused on the structure, stability and bio-accessibility of the flavonoids (Heim, Tagliaferro, & Bobilya, 2002; Rice-Evans, Miller, & Paganga, 1996). Sour cherries (Prunus cerasus L.) are the richest in phenolic compounds, among all the members of drupe family of fruits, and exhibit a broad spectrum of health benefits contributing toward the prevention of diseases (King & Youez, 2003; Stoner, Wang, & Casto, 2008; Yao et al., 2004). Studies have found that sour cherries exhibit robust antioxidant and anti-inflammatory properties due to inherently high amount of anthocyanins present (Seeram, Momin, Nair, & Bourquin, 2001). Consumption of sour cherry adds a good amount of anthocyanins in the diet and they have been found to enhance the level of superoxide dismutase and conversely reduce the level of antioxidant activity in serum and in rats, they reduced arthritis (He et al., 2006). Recent research shows that there

is an increased interest in using sour cherry in consumer food products since their health benefits have become widely known (Llanes, Bolling, Kirakosyan, Kaufman, & Seymour, 2008). It has been known that anthocyanins (ACNs) are easily degradable when subjected to neutral pH, thermal stress and the presence of metal ions, oxygen and enzymes (Frank et al., 2012). To protect and prevent the ACNs from degenerating, they need to be stabilized and that can be done by microencapsulation while also specifically timing their release in the digestion (Betz & Kulozik, 2011). It is mainly used to protect bioactive compounds or functional foods from light, temperature, oxidation. By encapsulating the dried micron-sized powders using the spray-drying method, we can protect the functional foods from extreme external factors that can degrade the ACNs (Murugesan & Orsat, 2012).

Studying the in vivo feeding and digestion in animals and humans provides the most approximate way to get nutritional data for food sciences (Hur, Lim, Decker, & McClements, 2011). However, the problem with human trials is that they are expensive, have long timelines and resource exhaustive, and have ethical issues (Minekus et al., 2014). To overcome this problem, researchers have come up with innovative strategies to develop an affordable, practical and basic in vitro models that simulate gastrointestinal (GI) conditions (Liang et al., 2012). Among all types of flavonoids, the anthocyanins have lowest bioavailability due to their limited release within the matrix and that leads to lower absorption by the body (Yang, I. Koo, O. Song, & K. Chun, 2011). The total concentration of a nutrient or non-nutrient in a food does not provide information about its bioaccessibility or bioavailability. Only a proportion (sometimes highly variable depending upon the food matrix, processing and storage) of these food components is absorbed and utilized (Cabañero, Madrid, & Cámara, 2004). Therefore, in order to accurately make

conclusions about health benefits of a food, the bioaccessibility of nutrients should be investigated.

While much is known about the content, composition, and antioxidant activity of bioactive compounds of tart cherry, there is little knowledge about aspects related to their micronization, bio-accessibility of micronized purée, the effect of microencapsulation on the release properties of polyphenols and their storage stability. These knowledge gaps give way to the need for this study, which is to enhance the understanding towards the bioaccessibility of bioactive compounds in cherry purée and implementing novel improvement strategy through particle size reduction and further encapsulating it with varying combinations of coating material. The information obtained through this research can be used to expand purée applications and develop value added products for nutraceutical/dietary supplement industry.

1.2 Hypotheses

We hypothesized that micronization of tart cherry purée can enhance the extractability of cherry polyphenols and improve the antioxidant capacity. This is based on the rationale that the particle size reduction in cherry purée will generate microparticles with bigger surface area that promotes better digestion by enzymes and higher release of polyphenols from the matrix. Another hypothesis of the study was that microencapsulation of tart cherry purée can generate microcapsules that will possess improved storage characteristics and display a higher release of polyphenols in the gastric and intestinal phases of digestion. This is based on the rationale that microencapsulation is known to

protect the anthocyanins against degradation in the gastric environment and hence increase their bioaccessibility in the intestinal digestion.

1.3 Objectives

The long-term goal of the study was to develop value-added tart cherry products with enhanced health benefits through particle size reduction (micronization) to improve extractability and bioaccessibility of polyphenol compounds.

The specific objectives include:

I. Conduct a comparative study for different micronization methods to obtain the desired particle size and highest extraction yield.

II. Determine effect of micronization and wall material combination on release properties of spray dried tart cherry purée to obtain the conditions for enhanced polyphenol extractability.

III. Investigate the storage stability of tart cherry microcapsules and determine the range of temperature and relative humidity conditions that result in the lowest polyphenolic degradation.

IV. Assess the release of polyphenols in tart cherry purée during gastric and intestinal digestion as affected by micronization and microencapsulation to determine the method with the highest polyphenol bioaccessibility.

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CHAPTER 2

REVIEW OF LITERATURE

2.1. Tart Cherry

Tart cherry (Prunus cerasus L.), a member of family Rosaceae, subfamily Prunoideae, is a rich source of vitamins, polyphenols, alkaloids, melatonin and fibers (Gao, Wang, Feng, & Zhu, 2012), all contributing to its great nutritional value. It has been used in the juice, wine and jam industries for processing purposes (Petersen & Poll, 1999). While sweet cherries (Prunus avium L.) are mainly consumed fresh, with only 20-25% being processed, i.e. canned, dried, frozen or juiced. Almost 97% of sour cherries, on the other hand (Prunus cerasus L.) are processed into juice and jam (Chaovanalikit & Wrolstad, 2004b). Juice produced from sour cherries has a high acidic value. Reported acidity values range from 13.5-29.2g/L (Ekşi, Reicheneder, & Kieninger, 1980), while other researchers have reported a narrower range of 17.5-25.5 g/L (L-malic acid, main acid in sour cherry juice, contributes to acidity values of 14.3 – 29.2 g/L) (Ekşi et al., 1980). Sour cherry juice also has high levels of anthocyanins, which give its characteristic purplish red color.

2.1.1. Cherry production and distribution

Cherry production is prevalent in the areas with a temperate climate. Turkey is the leading producer of cherries in the world, producing almost 481,000 metric tons (Mt) annually and contributing to approximately 24% of the total cherries produced the world

over (2,033,300 metric tons) (FAO, 2015). Turkey is also the world leader in production of sour cherries (187900 Mt), accounting for almost 17% of the total world production of 1131500 Mt (FAO, 2015). The Russian Federation, Poland, Ukraine and Iran immediately follow Turkey as other major producers of sour cherries (FAO, 2015). Other geographical areas where production of both sweet and sour cherries is prevalent include Mediterranean and Central Europe, North Africa, Southern Australia and New Zealand, and temperate zones of the Americas (USA, Canada, Argentina and Chile) (Basanta, De Escalada Plá, Raffo, Stortz, & Rojas, 2014; Mariette et al., 2010).

2.1.2. Chemical composition of cherry

Cherry is low in calories and high in nutrient value. It is rich in fiber, carotenoids, polyphenols, vitamin C and potassium (McCune, Kubota, Stendell-Hollis, & Thomson, 2011). It also has good amounts of tryptophan, melatonin and serotonin (Barriga et al., 2009; María Garrido et al., 2013). The nutrient composition of cherries is affected by many factors, including the cultivar, the stage of fruit ripening, the part of the fruit, the duration of storage, the analytical method used etc. (Bianconi et al., 2017). All cultivars of cherries are grouped into two major categories – the sweet cherry (Prunus avium L.) and the tart cherry (Prunus cerasus L.) (Ferretti, Bacchetti, Belleggia, & Neri, 2010). The most common sweet cherry cultivar in the USA is Bing, the most common tart being Montmorency. Polyphenols are an important nutrient in both sweet and tart cherries (Bianconi et al., 2017; Ferretti et al., 2010; McCune et al., 2011).

Tart cherries have a high phenolic content, ranging from 2704 to 4998 mg gallic acid equivalent (GAE) per kg (Bonerz, Würth, Dietrich, & Will, 2007). Predominant among phenolics are colored-phenolics, i.e. anthocyanins, major ones being Cyanidin-3-

glucosylrutinoside (361–515 mg/kg), cyanidin-3-rutinoside (125–213 mg/kg), cyanidin-3-sophoroside (37–185 mg/kg) and peonidin-3-rutinoside (5–15 mg/kg) (Bonerz et al., 2007). Neochlorogenic acid (67–278 mg/kg) is the main non-coloured phenolic. Chlorogenic acid (6–58 mg/kg), p-coumaric acid derivatives (6–41 mg/ kg), quercetin-3-rutinoside (10–44 mg/kg), isorhamnetin-3-rutinoside (3–26 mg/kg), quercetin-3-glucoside (2–4 mg/kg) and kaempferol-3-rutinoside (0–13 mg/kg) have all been identified in varying quantities (Bonerz et al., 2007).

While tart cherries have higher concentration of total phenolic compounds, sweet cherries have more anthocyanins (McCune et al., 2011). Bing cherries have 134, 92 and 333 mg of total phenolics/100 g fresh weight in the flesh, pits and skins respectively. For Montmorency cherries, these values are 301, 157, and 558 mg/100 g, respectively (Chaovanalikit & Wrolstad, 2004b; McCune et al., 2011). Anthocyanin concentrations in the flesh, pits and skins of Bing cherries are reported to be 26.0, 10.4, and 60.6 mg/100 g fresh weight, and those in Montmorency cherries are 0.2, 0.8, and 36.5 mg/100 g respectively (Chaovanalikit & Wrolstad, 2004a; McCune et al., 2011). Other cultivars of red sweet cherries (Benton, Black Gold, Hongdeng) had anthocyanin concentrations ranging from 82-297 mg/100 g fresh weight. Yellow sweet cherries (Gold and Rainer) had 2–41 mg/100 g of anthocyanins, and red sour cherry cultivars (Montmorency and Balton) had 27–76 mg/100 g (Mulabagal, Lang, Dewitt, Dalavoy, & Nair, 2009). Six other cultivars of cherries (Delta Marca, Celste, Bigarreau, Durone Nero, Lapins and Moretta) had anthocyanin concentrations ranging from 2.1–344.9 mg/100 g fresh weight (approximately 0.6–22% of the total phenolics (Martini, Conte, & Tagliazucchi, 2017). The major

anthocyanins in both Bing and Montmorency cherries are cyanidin-3-glucoside and cyanidin-3-rutinoside.

Cherries are also rich in other phenolic compounds like hydroxycinnamates and Flavan-3-ols. These two compounds make up about 25% and 40% of the total phenolics in Montmorency cherries and 50% and 5% in Bing cherries (Darshan S Kelley, Rasooly, Jacob, Kader, & Mackey, 2006; Wojdyło, Nowicka, Laskowski, & Oszmiański, 2014). Melatonin, linked to the sleep regulation pathway, is another antioxidant found in both sweet and tart cherries, at concentrations ranging from 10–20 ng/g fresh weight in both Hongdeng and Rainier ripe sweet cherries (Li et al., 2013) and 2.1 ng/g and 13.5 ng/g, in Balton and Montmorency tart cherries (Burkhardt, Dun Xian Tan, Manchester, Hardeland, & Reiter, 2001).

2.2. Health benefits of tart cherry

Cherry consumption has been shown to reduce the risk of chronic inflammatory diseases like diabetes, cardiovascular disease and cancer in animal and human studies. Sleep and cognitive function have also been shown to improve, as has post exercise pain recovery. This is not surprising, considering the high concentration of polyphenols and vitamin C in cherries, both of which have anti-oxidant and anti-inflammatory properties (Bell, Mchugh, Stevenson, & Howatson, 2014; Coelho, De Lima, De Oliveira Assumpção, Prestes, & Denadai, 2015; McCune et al., 2011)

2.2.1. Effect of cherry consumption on oxidative stress

To date, 10 studies have reported the effects of cherry products on oxidative stress markers in humans. 8 of those reported reduced oxidative stress (Bell et al., 2014; Bowtell,

Sumners, Dyer, Fox, & Mileva, 2011; María Garrido et al., 2009; Howatson et al., 2010; Jacob et al., 2003; Levers et al., 2016; Lynn et al., 2014; Traustadó et al., 2009), while 2 reported no change (Levers et al., 2016; McCormick, Peeling, Binnie, Dawson, & Sim, 2016). Among antioxidant capacity markers, Plasma ORAC (Jacob et al., 2003), FRAP (Lynn et al., 2014) and serum TAS (Bowtell et al., 2011; Howatson et al., 2010; Levers et al., 2016) levels were reported to be increased, whereas plasma F2-isoprostane (Traustadó et al., 2009) and LOOH (Bell et al., 2014) levels were reduced. All these findings point to a role of cherries and cherry products in reducing oxidative stress in humans. Corroborating these findings are the results from animal and cell culture studies, which showed cherry extracts to increase the activity of hepatic antioxidant enzymes and decrease in vitro lipid peroxidation caused by iron or copper (Alba C., Daya, & Franck, 2017; Ferretti et al., 2010).

2.2.2. Effect of cherry consumption on inflammation

Out of sixteen reported human studies, eleven have showed decrease in inflammatory biomarkers with consumption of cherries or cherry products (Bell et al., 2014; Bowtell et al., 2011; Dimitriou et al., 2015; Howatson et al., 2010; Jacob et al., 2003;; Kelley et al., 2013; Darshan S Kelley et al., 2006; Levers et al., 2016). Four studies reported no change (McCormick et al., 2016; Vargas, Ashbeck, Thomson, Gerner, & Thompson, 2014), while one actually showed an increase (Garrido, Gonzalez-Gomez, et al., 2013). ESR (Martin, Bopp, Burrell, & Hook, 2011), C-reactive protein levels (Howatson et al., 2010; D S Kelley et al., 2013), Tumor Necrosis Factor (Levers et al., 2016), IL-6 (Howatson et al., 2010; Levers et al., 2016), IL-8 (Bell et al., 2014), NO (Darshan S Kelley et al., 2006), and upper respiratory tract symptoms (Dimitriou et al., 2015) are

inflammatory markers that have been shown to be reduced in different studies. In one study, plasma C-reactive protein levels reduced by almost a quarter within 5 hours of consumption of 45 fresh Bing cherries when compared to baseline values, although the change did not attain statistical significance (Jacob et al., 2003). Two other studies (Levers et al., 2016) reported significant reduction in post exercise plasma levels of inflammatory cytokines (IL-6, IL-8, and TNF) in the tart cherry group compared to the placebo group, when pre-exercise levels in the two groups were comparable. Kelley et al. (2013) reported decrease in IL-18 and ferritin levels and increase in IL-1R antagonist after cherry consumption. The above studies establish the anti-inflammatory nature of cherries in humans. Supporting these findings are in vitro studies showing inhibition of COX 1 and 2 enzymes by cherry extracts (Mulabagal et al., 2009; Seeram, Momin, Nair, & Bourquin, 2001) and inhibition of NF-κB by anthocyanins in cultured human blood monocytes (Carrillo-Vico, Guerrero, Lardone, & Reiter, 2005).

2.2.3. Effect of cherry consumption on exercise induced muscle damage and recovery

Eight out of nine studies showed reduction in muscle pain, soreness and strength loss post exercise with consumption of cherries (Bell et al., 2014; Bowtell et al., 2011; Connolly, McHugh, & Padilla-Zakour, 2006; Howatson et al., 2010; Kuehl, Perrier, Elliot, & Chesnutt, 2010; Levers et al., 2016). One study on water polo athletes showed no difference from placebo (McCormick et al., 2016). Another study showed reduction in muscle damage after exercise, as ascertained by plasma concentrations of creatine kinase (CK) and lactate dehydrogenase (LDH), with the use of cherry products as compared to placebo (Howatson et al., 2010). However, some studies did not replicate these findings

(Bell et al., 2014; Bowtell et al., 2011). The antioxidant and anti-inflammatory properties of anthocyanins and phenolic compounds, as discussed above, contribute to the reduction in exercise induced muscle damage. (Coelho et al., 2015).

2.2.4. Effect of cherry consumption on diabetes

Human studies have shown no effect of cherry products on glucose and fasting insulin levels in healthy non-diabetic subjects (Kelley et al., 2006; Garrido, González-Gómez, et al., 2013). Consumption of concentrated tart cherry juice in diabetic women, on the other hand, showed significant reduction in HbA1c levels after 6 weeks of regular use (40 ml/day of cherry juice providing 720 mg/day of anthocyanins) (Kuehl et al., 2010). Studies have shown prevention of alloxan-induced diabetes with cherry extract consumption in rats and in mice (Saleh, El-Darra, & Raafat, 2017). Cherry extracts or anthocyanins, when added to high fat diets of mice and rats, reduced blood glucose, insulin and triglyceride levels, when compared with rodents being fed the high fat diets alone. (Jayaprakasam, Olson, Schutzki, Tai, & Nair, 2006; Seymour et al., 2008; Snyder et al., 2016). Inhibition of glucosidase and dipeptidyl peptidase-4, both enzymes of glucose metabolism and known to increase blood glucose, has been shown with tart cherry juice and chlorogenic acid (Cásedas, Les, Gómez-Serranillos, Smith, & López, 2016; Crepaldi et al., 2007). Similarly, tart cherry extract and its component anthocyanins have been shown to reduce the activity of the human enzyme amylase in an in-vitro study (Homoki et al., 2016). In vitro cell culture studies showed increase in glucose stimulated insulin secretion from cultured beta cells from rodent pancreas. (Jayaprakasam, Vareed, Olson, & Nair, 2005). Other cell culture studies have suggested that anthocyanins cause favorable

changes in glucose metabolism at many levels to induce an overall reduction in blood glucose levels. (Crepaldi et al., 2007). All aforementioned studies, and more, provide overwhelming evidence to support the role of cherry consumption in healthy glucose regulation.

2.2.5. Effect of cherry consumption on blood lipids

As seen with glucose metabolism above, both sweet and tart cherry products consumed by healthy subjects, showed no change in their lipid profile (triglycerides, low density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), total cholesterol, and number of different lipoprotein particles and their sizes (Kelley et al., 2006; Lynn et al., 2014). Obese subjects with elevated lipid profile, on the other hand, showed favorable changes following 4 weeks of tart cherry juice consumption resulted in a reduction in VLDL and triglycerides/high-density lipoprotein (TG/HDL) ratio (Martin et al., 2011). Changes in triglyceride levels with consumption of cherry extracts and anthocyanins in rodents have been discussed earlier (Jayaprakasam et al., 2006; Seymour et al., 2008; Snyder et al., 2016).

2.2.6. Effect of cherry consumption on blood pressure

Two studies monitoring the immediate impact of Montmorency cherry concentrate consumption on systolic blood pressure (SBP) showed significant reduction at 1 and 2 hours post consumption, but not at 4 and 5 hours (Keane et al., 2016b; Keane, 2016a). The acute effects were believed to be due to increased serum vanillic and protocatechuic acids, both metabolites of cyanindin-3-glucoside- an anthocyanin found in high concentrations in

cherries (Keane et al., 2016a). The 6-week tart cherry concentrate consumption study on diabetic women, as mentioned above, showed significant reduction in both systolic and diastolic blood pressure (Ataie-Jafari, Hosseini, Karimi, & Pajouhi, 2008). These long-term beneficial effects of cherries on blood pressure may have been due to reduction in endothelin-1 (ET-1), a powerful vasoconstrictor (Kelley et al., 2013). Cell culture studies on human and animal endothelial cells showed increased expression of endothelial nitric oxide synthase (eNOS) and consequent increased production of the vasodilator nitric oxide (NO) upon addition of the anthocyanin cyanidin-3-glucosdie (Edwards, Czank, Woodward, Cassidy, & Kay, 2015). Anthocyanins have also been shown in in-vitro studies to inhibit expression of NF-κB and other mediators involved in pathogenesis of cardiovascular diseases (Wallace, 2012). Studies in rabbits have shown decreased plaque formation and improved cardiac functions on addition of tart cherry extract to an atherogenic diet (Szabo et al., 2013). All these studies suggest that cherry consumption may improve glucose tolerance and reduce the risk of cardiovascular diseases.

2.2.7. Effect of cherry consumption on gout-related arthritis

In the earliest study of its kind, consumption of cherries, fresh or canned, was shown to prevent attacks of arthritis and reduce plasma uric acid (UA) levels in 12 patients with gout. Some patients in this study also reported greater freedom of joint movements. Another recent study on 633 gout patients showed almost one-third lower risk of gout attacks with consumption of cherries or cherry extract over a 2-day period (Zhang et al., 2012). These and other studies suggest that cherry consumption reduces the chances of arthritic attacks.

2.2.8. Effect of cherry consumption on sleep, mood and cognitive functions

Improvements in both sleep quality and quantity have been reported with the use of sweet (Garrido et al., 2013; Garrido et al., 2009) and tart cherries (Pigeon, Carr, Gorman, & Perlis, 2010), as early as within 3 days with sweet cherries (141 g or 25 cherries/day) and 5 days with tart cherries (240 mL of tart cherry juice; approximately 100 cherries/day). A study on early changes in mood and cognitive functions with consumption of tart cherry concentrate, however, showed no alteration within the first 5 hours (Keane, Haskell-Ramsay, Veasey, & Howatson, 2016). Improvements in memory functions and autophagy have been reported in ageing rats with tart cherry powder supplementation. (Thangthaeng et al., 2016)

2.3 Micronization

Micronization is a cost-effective and successful technique for synthesizing smaller sized particles ranging from 1 mm to 100 mm (Zhao et al., 2009a). In the last decade, various micronization treatments such as microfluidization (Chen et al., 2013; Wang et al., 2012), ultrafine grinding (Tao et al., 2014; Zhao et al., 2009b; Zhu et al., 2010), and high pressure micronization (Chau et al., 2007) have been efficaciously utilized to micronize foods and beverages. The reduction of particle sizes modifies their structures and surface characteristics, which in turn changes the functional properties of foods (Wang et al., 2012). Megatron is one of the particle size reduction methods that works by superfine grinding due to particle-particle and particle-surface impact that reduces particle size by generating friction (Ramachandriah and Chin, 2016); the time and rotational speed being

key factors for obtaining microparticles (Chau et al., 2007). This process has been demonstrated to increase the extractability and antioxidant properties of bioactive compounds in agricultural wastes and food matrix such as cereals, fruits, vegetables and mushrooms (Ma et al., 2016; Wang et al., 2016; Drakos et al., 2017) but have not been applied to tart cherry purée yet. Micronization has been shown to release compounds that are strongly bound to the food matrix (Zhu et al., 2014), therefore enhancing the amount of antioxidant activity shown by extractable polyphenols and modifying the dietary fiber fraction (Ma et al., 2016; Zhu et al., 2014).

In a micronization study involving olive pomace (Speroni et al., 2019), researchers observed that particle size reduction was effective in achieving granulometric fractions, around 10 times smaller after micronization (31.1 ± 0.6 μm). In another study, by employing 16 h of micronization, the particle size of dietary fiber extracted from carrot was reduced to the range of 10–302 μm (Ma et al., 2016). Celery stalk (Apium graveolens) generated particle sizes in the low μm ranges when it was micronized for 24 h (Ramachandraiah and Chin, 2017). In our study with tart cherries, we have chosen to explore short micronization times to avoid thermal degradation of polyphenols due to heating during prolonged friction in the megatron. Moreover, reduced grinding times save energy and reduces production costs. In another study on the effect of micronization on polyphenol bioaccessibility of rice bran, the researchers studied the effect of strong mechanical force in grinding process and agglomerated microstructures in the food matrix under a scanning electron microscope (Zhao et al., 2018).

Post-micronization, higher values of total polyphenolic content have been reported for fruits known for being high antioxidant sources, such as açai pulp and apple (Pérez-

Jiménez and Saura-Calixto, 2015; Rufino et al., 2011). These increase in values is related to superfine grinding that agitates the structure of sample thereby releasing the polyphenols linked to the fiber matrix (Zhu et al., 2014). This release is a major indication of potential increase in the bioaccessibility of these compounds, as observed by Rosa et al. (2013). A few studies have shown the positive effects of micronization in improving the antioxidant capacity, which is in line with the increase in the extractable polyphenol content. Micronization has also been shown to improve the DPPH radical scavenging in green tea, mushroom and isolated compounds such as trans-resveratrol (Hu et al., 2012; Wang et al., 2016; Aguiar et al., 2018). Micronization has been shown to increase the ABTS radical scavenging activity of dietary fiber from grape pomace (Zhu et al., 2014) by altering the fiber structure and releasing matrix-bound polyphenols.

Another area of focus in our study is micronization by high pressure homogenization. Homogenizers used in the industry have pressures that normally are between 20 and 50 MPa, but current crop of homogenizers can reach much higher pressures. The principle of high-pressure homogenization is that high pressure forces the increases its velocity, leading to high shear stress in the valve gap and depressurization. Immediately after the gap outlet, the flow kinetic energy is converted into turbulence and cavitation, as the jet breaks down and impacts in internal solid surfaces of the homogenizer (Dumay et al., 2013; Floury et al., 2004; Innings and Trägårdh, 2007). Thus, the macromolecules and suspended particles in the fluid are subjected to great mechanical stress that causes disruption of the cell wall (Floury et al., 2004). Homogenization has an effect on the particle size distribution, colour, cloudiness, flavonoid content of vegetable-based beverages. Betoret et al. (2009) found that homogenization pressure of 20 MPa or

more, improves the quality of fresh fruit juice. The homogenization decreases the particle size with no negative impact on the color of juice, as well as the total flavonoid content. Apart from decreasing the particle size, high-pressure homogenization (HPH) has been known to influence the water holding capacity and rheological properties of orange pulp due to the inverse relation between the particle size and water holding capacity. Silva et al. (2010) observed that at higher pressure, the formation of smaller aggregates due to inter-particle attraction could be observed. In another work, HPH treatment caused the change of color of tomato juice because of the leakage of lycopene from the disrupted cells, (Kubo et al., 2013). Moreover, HPH increased the absolute zeta potential, lightness and total suspended solids of the homogenized sample. In a study done on taro pulp, the effect of HPH on the microstructure showed that the constituent suspended particles were strongly broken down during HPH processing, increasing the consistency and stability of taro pulp (Yu et al., 2016).

2.4. Microencapsulation

Microencapsulation is a process of coating individual solid particles or droplets with a continuous film (polysaccharide, gums, resins, proteins, and lipids) to produce microcapsules. The capsules thus produced as in the range of micrometer or millimeter and can vary in shape, depending on the several process related parameters (heat, pressure etc.) and on the physical and chemical properties of the ingredient and coating material (Tyagi, Kaushik, Tyagi, & Akiyama, 2011). Microencapsulated food materials are essentially composed of two components: a core and a polymer/inorganic shell as the outer covering to the core. The core material and the process of microencapsulation also dictate the shape

of the microcapsules produced. While the shape of microcapsules can range from regular shapes such as spherical, tubular, oval to irregular, the morphology of microcapsules varies from mononuclear, polynuclear to matrix and multiwall particles (Ghosh, 2006).

2.4.1. Methods of microencapsulation

There are numerous methods of microencapsulation used for the production of microcapsules. The most common methods are divided in three categories viz. physical methods (such as pan coating, air-suspension coating, centrifugal extrusion, vibrational nozzle, spray drying, solvent evaporation), physic-chemical methods (ionic gelation, coacervation, sol-gel) and chemical methods (such as interfacial polymerization, suspension polymerization and emulsion polymerization) (Tyagi et al., 2011; Zhao & Zhang, 2011). In our study, we have focused on the spray drying technique for microencapsulating tart cherry purée and producing microcapsules coated with polysaccharides/complex carbohydrates such as maltodextrin, gum arabic and combinations of the two. The process of spray drying was selected because it offers various advantages such ease to scale-up, versatility, wide availability of the equipment know-how and uniform coating of the particles.

2.4.2. Need for microencapsulation

Encapsulation is the process of coating one substance, the active agent, with another, the wall material. The encapsulated substance forms the core, fill, or internal or payload phase. The coating substance can be called the membrane, capsule, shell, external phrase or matrix (Fang & Bhandari, 2010; Zuidam & Nedović, 2010). There can be number

of reasons for which encapsulation may be required in the food industry. The major reasons are to mask the flavor, improve flowability and storage ability, improve delivery of the active core agent, either bioactive molecules (e.g. antioxidants, minerals, vitamins, phytosterols, lutein, fatty acids, lycopene) or living cells (e.g. probiotics) into foods (de Vos, Faas, Spasojevic, & Sikkema, 2010; Zuidam & Nedović, 2010). In most cases, the bioactive components are completely encased and protected by a physical barrier (de Vos et al., 2010). Encapsulation has also been used for packaging materials in small capsules for controlled release at steady rates under specific conditions (Desai & Park, 2005). The particles thus produced typically vary from a few nm to mm in diameter (Zuidam & Nedović, 2010).

In recent years, addition of functional compounds to food products has become increasingly popular. These compounds may be added to control flavor, color, texture, provide health benefits, or for preservation purposes. These compounds are usually susceptible to damage from food processing techniques, environmental conditions or stomach enzymes, and therefore, need to be encapsulated. Encapsulation preserves stability of these compounds during processing and storage and prevents undesirable interactions with food matrix. This allows the product to be delivered in undegraded condition to the desired site (Lesmes & McClements, 2009). By protecting sensitive bioactive materials from the environment, this technique allows taste and aroma differentiation, masking of bad taste or smell, stabilization of food ingredients and increase in bioavailability.

Encapsulation can also be used for modification of physical characteristics of the original material to (a) allow easier handling, (b) keep reactive components of the food

mixture separate (c) to provide adequate concentration and uniform dispersion of an active agent (Desai & Park, 2005).

2.4.3. Applications of microencapsulation in the food industry

Probably the most important application of microencapsulation is to provide improved stability of active core ingredient during processing and in the final product. Probiotics, for example, are highly sensitive to a lot of external and gastric environmental factors (de Vos, Faas, Spasojevic, & Sikkema, 2010). Encapsulation helps to increase their bioavailability and functionality. The encapsulating material should be efficient and not alter the taste or texture of the food. Sometimes, as with the case of active agents like lactobacilli and bifidobacteria, the encapsulation matrix actually benefits the microbes during dehydration and lyophilization techniques. There are various techniques for encapsulation. Among the gentler approaches is extrusion. It is advisable for delicate active agents like probiotics, using matrix molecules that promote the functionality of the active agent. Other encapsulation techniques are spray-, freeze- or vacuum-drying. Carrier materials typically include carbohydrate – protein mixtures, gum Arabic, skim milk powders, soy, modified starch, maltodextrin and sugars (de Vos et al., 2010; Zuidam & Nedović, 2010).

Another application of encapsulation is to prevent evaporation and degradation of volatile aromatic agents added to the food (Milanovic et al., 2010). Encapsulation protects the aromatic compound against evaporation and chemical reactions (light or oxidation induced, or with other flavor adding agents) (De Roos, 2003; Madene, Jacquot, Scher, & Desobry, 2006). Spray-drying, spray-chilling or –cooling and spray bed drying, among

others, are the common methods used for encapsulation of such agents. Spray-chilling produces aromatic lipid particles (Zuidam & Heinrich, 2010). Encapsulation is also used to mask unpleasant taste and astringency of food components like polyphenols. Using encapsulated polyphenols, instead of the free agents, preserves their bioactivity and ensures bioavailability, in addition to alleviating the unpleasant effects (Fang & Bhandari, 2010, Bell, 2010). Also, most phenolic compounds are unstable under conditions encountered during food processing and in the gut (acidic environment, enzymes etc.) (Bell, 2010). Encapsulation helps maintain the active ingredients until they reach their physiological target in the organism (Chen, Remondetto, & Subirade, 2006).

Encapsulation is also employed to prevent reaction of the core agent with other food components like water or air, as in case of essential oils. These oils dissolve in water, imparting to it their taste and smell. Essential oils have antimicrobial properties and are encapsulated into nanospheres as controlled release devices which release the product at the desired site, under specific conditions (Parris, Cooke, Moreau, & Hicks, 2008). Other food ingredients and fortifying agents, like vitamins, peptides, etc. are also subjected to encapsulation to meet one of the many aforementioned objectives (Zuidam & Heinrich, 2010).

2.5. Spray Drying

Spray drying is one of the most popular microencapsulation techniques on account of its simplicity and speed. It involves coating a hydrophobic (water repellant) core agent with a water solubilized shell, thus creating an oil-in-water type emulsion. A dry capsule powder is produced from this emulsion by the spray drying technique, using the following

steps (Moreno et al., 2012). First, the emulsion is sprayed into a heated chamber by means of an atomizer, which ensures a steady stream of tiny droplets. This step is called 'Atomization'. These droplets next come in contact with a heated stream of gas, which ensures evaporation of water from the outer shell, thereby producing tiny dry capsules. The temperature of this gas stream is preset, based on the core and coating material properties. In the last step, the dry capsule powder is separated from the gas stream using a cyclone separator. Being a rapid process, the major limitation of this technique is the agglomeration of the particles (Ghosh, 2006). On the plus side, though, scaling up of the process is easy and production of particles of desired size just needs the proper atomizer (Borreguero et al., 2011).

2.5.1 Steps of spray drying

The entire process of spray-drying involves the following 5 steps: 1) Feed preparation 2) atomization 3) droplet-hot air contact 4) drying of atomized droplets, 5) separation of dried particles from humid air (Verma & Singh, 2015).

2.5.1.1. Preparation of feed

Feed preparation involves concentration of the emulsion that is to be spray dried. Concentration of the emulsion reduces its water contact, thereby ensuring requirement of lesser energy to dry the atomized particles in the subsequent steps. Some carrier materials are also added to improve the properties of the final product. Some of the common carrier materials include, but are not limited to, maltodextrin, gum Arabic and milk proteins (de Vos et al., 2010; Zuidam & Nedović, 2010).

2.5.1.2 Atomization

Atomization converts the prepared emulsion into a mist or spray of tiny droplets, which are then fed into the drying chamber. The aim is to ensure effective drying of the particles by providing an increased surface area. Rotary atomizers and different kinds of nozzles employing hydraulic, pneumatic or ultrasonic mechanisms can be employed to achieve atomization (Cal & Sollohub, 2010).

2.5.1.3. Droplet-air contact

In this step, the atomized feed comes in contact with a current of hot air in the drying chamber. Heat of the air evaporates water from the droplets, producing tiny particles. At the same time, the air cools down and this cooled air pneumatically transports the dried particles downstream (Universiti Putra Malaysia. Fakulti Sains dan Teknologi Makanan., 2008). Based on the direction of the hot air current and the feed, drying can be of 3 models. The commonest is the co-current model, where direction of the feed and hot air current is the same. In this model, the feed is exposed upfront to the hottest air. In the counter current model, the directions of the two streams are opposite. Here, the atomized droplets meet the coldest air first, with steadily increasing temperature thereafter. However, this model is not suitable for sensitive feeds to the temperature. The third, combined model, employs both co-current and counter-current drying. (Cal & Sollohub, 2010; Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007; Murugesan & Orsat, 2011; Zuidam & Heinrich, 2010).

2.5.1.4. Drying of droplets

There are three phases of drying of a particle. In the first phase, the droplet temperature increases to dry bulb temperature, just after coming in contact with hot air. Evaporation of water from the droplets then ensues and continues at constant temperature and vapor pressure. In the final phase, drying rate decreases as crust forms on the particle surface. This increases the particle temperature further. Drying is complete when temperature of the drying particles matches that of the hot air (Gharsallaoui et al., 2007).

2.5.1.5. Separation of dried particles

This is achieved using cyclones, bag filters, wet scrubbers, and electrostatic precipitators (Verma & Singh, 2015).

2.5.2. Types of carrier materials

The choice of carrier material is determined by many criteria, important ones being the functionality that it adds to the final product, the desired stability, cost issues, the type of release, rheological characteristics at high concentration, and material restrictions. In general, the material chosen should be edible, biodegradable, and able to form an effective barrier. It should protect the active core material from environmental conditions and during processing and storage and should not react with the same. Also, it should be easy to use for encapsulation and have stability under extreme conditions. The most commonly used molecules for encapsulation in the food sector are polysaccharides (Zuidam & Nedović, 2010).

2.5.2.1. Hydrolyzed starch

Products of starch hydrolysis produced using enzymes or acids make good carrier materials, due to their low cost and odorlessness. Hydrolyzed starch is described by a parameter called dextrose equivalent (DE), which denotes the level of hydrolysis. At less than 20 DE, it is called Maltodextrin, and if more, it is called dried glucose syrup. The higher the DE, lesser the oxygen permeability and greater the protective effect, but greater the likelihood of caking during storage (Shahidi & Han, 1993).

2.5.2.2. Gum Arabic

This is another carrier material used in the food industry, though its use is limited due to the high cost and inherent impurities. It is a naturally occurring gum produced from Acacia tree exudates. Chemical composition of gum Arabic is largely complex highly ramified heteropolysaccharide with proteins contributing only about 2% of the total structure. The functional properties of gum Arabic are largely due to the proteins in its structure (McNamee, O'Riordan, & O'Sullivan, 1998; Turchiuli et al., 2005), whereas the arabinogalactan fraction provides film forming properties (McNamee et al., 1998). Gum Arabic is highly soluble in both cold and hot water.

2.5.2.3. Proteins

Proteins serve as excellent carrier materials for encapsulation of sugar rich products by spray drying (Fang & Bhandari, 2010; Jafari, Assadpoor, He, & Bhandari, 2008; Shahidi & Han, 1993) due to their unique properties like high solubility, low viscosity, good emulsifiability and film forming capacities. These properties of proteins are due to their chemical structure with flexible molecular chains, their high molecular weight and ability to associate with other molecules.

2.5.3. Types of atomizers/nozzles

Atomization can be achieved by rotary atomizers, hydraulic nozzles, pneumatic nozzles, and ultrasonic nozzles (Cal & Sollohub, 2010).

2.5.3.1. Rotary atomizers

These make use of centrifugal force to achieve droplets of desired size. The design consists of a rotating disc with grooves at the edges. Feed is supplied at the center of the rotating disc. It gets pushed to the edges by centrifugal force, where by coming in contact with the grooves, it is broken into particles of desired size (Cal & Sollohub, 2010). However, the yield with this method of atomization is low, and so it cannot be used for expensive feeds (Huang, Kumar, & Mujumdar, 2006).

2.5.3.2. Hydraulic nozzles (one-fluid nozzles)

In these types of nozzles, the feed is squeezed through a nozzle of decreasing diameter. It utilizes the kinetic energy of the feed to break it into tiny droplets. As can be understood, feed of high viscosity is not effectively atomized by this type of nozzle. The feeding rate is the only controllable parameter in this process, and it determines the size of the droplets. (Cal & Sollohub, 2010).

2.5.3.3. Pneumatic nozzles (multi-fluid nozzles)

These types of nozzles use pneumatic (gas) pressure over the feed to achieve atomization. Compressed carrier gas creates high frictional forces over the feed, which causes it to disintegrate into tiny droplets. As expected, droplet size is dependent on both - the feed characteristics like surface tension, density and viscosity, and the compressed gas characteristics, especially velocity (Cal & Sollohub, 2010).

2.5.3.4. Ultrasonic nozzles

These use ultrasonic vibrations to atomize the feed. Droplet size depends on the ultrasonic frequency. The advantage with these types of atomizers is that the droplets move at a slow speed, and so the product yield is higher. Also, the particle size distribution is narrower with ultrasonic nozzles (Cal & Sollohub, 2010).

2.6. Moisture Isotherm

The sorption properties of foods indicating its hygroscopicity and dehydration kinetics are important parameters that help in estimating the shelf life of dried foods. To assess factors like dehydration in food, we employ equation for modelling water sorption isotherms that enable us to better understand the thermodynamic properties of water in food at various storage conditions. There have been many proposed mathematical isotherm models to describe water sorption but none of them can give accurate results and cover the entire range of water activity for various food products (Labuza, 2011; Labuza, 2014). The reason one single model is able to cover the entire range of water activity is because of different mechanisms of water attachment with the food matrix at different water activities (Labuza, 2014). Of all the models seen in literature, these are the most commonly used (C. van den Berg & Bruin, 2013) are discussed below.

2.6.1 The Brunauer-Emmett-Teller (BET) Equation

The Brunauer, Emmett and Teller (BET) sorption equation, formulated in 1938, gives us an estimation of moisture adsorbed on the monolayer surface. The monolayer moisture content of many foods has been previously seen to be directly related with the

physical and chemical stability of dehydrated foods (Hill & Rizvi, 1982; Karel, 1973). However, it has been seen in a lot of cases that BET plots are only linear over the lower relative pressure region (a_w) of the sorbate. However, the equation has been useful in outlining an optimum moisture content for drying and storage stability of foods, and in the estimation of the surface area of a food (Cornelis van den Berg, 1991).

The BET equation is generally expressed in the form:

$$\frac{M}{M_0} = \frac{Ca_w}{(1-a_w)(1-a_w+Ca_w)} \quad (2.1)$$

where M is the moisture content (kg=kg dry solid), M_0 is monolayer moisture content (kg/kg dry solid), a_w is the water activity, and C is a constant related to the net heat of sorption. The estimation of the constants is based on linearization of equation.

2.6.2 Halsey Equation

The isotherm equation developed by Halsey (Halsey, 2005), details an expression for condensation of multilayers that may be present at a relatively great distance from the surface.

$$a_w = \exp(-A/RT\theta^r) \quad (2.2)$$

where A and r are constants, $\theta = M/M_0$, R is universal gas constant (8.314 kJ mol^{-1} K^{-1}), and T is the absolute temperature (K). Halsey based this equation on the theory that potential energy of a molecule varies as the inverse *r*th power of its distance from the surface. The equation also expressed the fact that the magnitude of the parameter 'r' characterizes the type of interaction between the vapors and the solid. (C. van den Berg & Bruin, 2013)

2.6.2 Henderson Equation

Henderson equation is one of commonly used isotherm models relating water activity to the amount of water sorbed (García-Pérez, Cárcel, Clemente, & Mulet, 2008). This can be written as:

$$M = \left[\frac{\ln(1-a_w)}{-A}\right]^{1/B} \quad (2.3)$$

where M is the moisture content (kg/kg dry solid), A and B are constants. A linearized plot of ln $[-\ln(1-a_w)]$ versus moisture content has been previously used to to give rise to three 'localized isotherms' which may or may not directly give any accurate information on the physical state of water. (García-Pérez et al., 2008)

2.6.3 Oswin Equation

Another model used was described by Oswin (Oswin, 2007) that uses an empirical model which is a series expansion for sigmoid shaped curves, and can be written as:

$$M = A\left[\frac{a_w}{1-a_w}\right]^B \quad (2.4)$$

where M is the moisture content (kg/kg dry solid), A and B are constants. Some research groups have found Oswin equation model to be the best one for describing the isotherms of starchy food, and a reasonably good fit for meat and vegetables. (Boquet, Chirife, & Iglesias, 1978)

2.6.4 Guggenheim-Anderson-de Boer (GAB) Equation

The three parameters GAB equation derived independently by Guggenheim (Guggenheim, 1966), Anderson (Anderson, 1946), and de Boer (Deboer, 1953) is a multi-molecular, localized, semi-theoretical and homogeneous adsorption model.

$$M = \frac{M_0 C K a_w}{(1-Ka_w)(1-Ka_w+CKa_w)} \quad (2.5)$$

where M is the moisture content (kg/kg dry solid), M_0 is the monolayer moisture content; C and K are constants related to the energies of interaction between the first and further molecules at the individual sorption sites.

The GAB model represents a refined extension of the BET theory, postulating that the state of the sorbate molecules in the second and higher layers is equal, but different from that in the liquid-like state. This assumption introduces an additional degree of freedom (an additional constant, K). Lower sorption than that demanded by the BET model is predicted by the GAB isotherm, making it more versatile and successful up to high water activities (i.e. aw^o 0.9).

2.6.5 Moisture Isotherm studies on food

Moisture sorption isotherms describe the amount of water vapor that can be absorbed by a product depending on its chemical composition, physical-chemical state, and physical structure. Food manufacturers need to know how long it will be before their product molds, gets soggy, goes stale, becomes rancid, cakes, clumps, crystallizes, and becomes unacceptable to the consumer by examining isotherm shape, using different isotherm model equations discussed before to predict drying times though to date no

equation give accurate results throughout the food matrices as we have seen before. (Al-Muhtaseb, McMinn, & Magee, 2002)

The Oswin model has been found very suitable for a considerable number of nuts, spices, and coffee. (Al-Muhtaseb et al., 2002; Lomauro, Bakshi, & Labuza, 1985) The Hasley model is generally found to be best for study of the sorption isotherms of meats, milk products, and vegetables (Boquet et al., 1978). Some researchers have (Iglesias, Chirife, & Lombardi, 1975) applied the Hasley model to describe reasonably the sorption of dried figs, apricots, and raisins.

As for the Henderson model, this model is one of the most widely used, relating water activity to the amount of water. The model was applied to study the sorption isotherm of potatoes (Wang & Brennan, 1991), lentils (Menkov, 2000), onion (Adam, Mühlbaucr, Esper, Wolf, & Spieβ, 2000), pineapple (Hossain, Bala, Hossain, & Mondol, 2001), and chestnut (Vázquez, Chenlo, & Moreira, 2001).

The GAB and the BET models are the most accepted models for foods or edible materials due to the value of the monolayer moisture content in each model that indicates the amount of adsorbed water to specific sites at the food surface and used as a tool to consideration the optimum value to assure food stability. (Rachtanapun, Kumsuk, Thipo, & Lorwatcharasupaporn, 2010)

2.7 In-vitro digestion models

There are two major types of in vitro digestion models i.e. static and dynamic models. (Venema et al. 2009). In the static model of in vitro digestion, the sample to be tested is subjected to step-wise chemical and enzyme solutions with variable pH for certain

time durations simulating digestion (Venema et al. 2009). Alternatively, in the dynamic models, the test sample is subjected to a sequential flow of samples with real time variation in pH (Venema et al. 2009).

2.7.1. Static Model

When dealing with static model of digestion, one can find models from simple to complex models. The simplest ones may just deal with very low pH solutions or solutions with just pepsin enzyme in a stirring beaker mimicking the peristalsis action. The simplistic intestinal models include pancreatin and bile salt mixtures at neutral pH. The more complex models include organic molecules and gastric juice containing enzymes, buffers, electrolytes and salts. (McClements and Li, 2011; Shim et al. 2010; Oomen et al. 2003; Miller et al. 1981). In-vitro digestion models are of two kinds based on the number of digestion steps i.e. single or multi step (mouth, stomach, small or large intestine) being simulated in the model (McClements and Li, 2011).

2.7.2. One-Step model

In an animal/human digestive tract, the mouth plays two major roles, one being mastication and the other is enzymatic. The chewing step breaks down food at forces approaching 1000 N and chemically, it facilitates the saliva which contains α-amylase, a starch digestion enzyme, and mucin for lubrication (Aura, 2005). However, the food stays in the mouth for about 50 - 60 seconds on an average and for even shorter amounts of time for the liquid foods, thus limiting the scope of any major changes in the chemical components (McClements and Li, 2011). This is the basis of the one-step model, which

essentially eliminates the need to mimic the salivary phase, as seen in some of the previous studies excluding the mouth step (Minekus et al. 1995; Gil-Izquierdo et al. 2002; Miller et al. 1981; Tarko et al. 2009; Tsai et al. 2008). Furthermore, the mastication effect could be mimicked by homogenizing the sample prior to the in-vitro digestion (Vallejo et al., 2004). However, despite the aforementioned reasons, there has been equal popularity of studies that choose to include the mouth step during in-vitro digestion (Lan-Pidhainy et al. 2007; Wood et al. 2002; Shim et al. 2010; Beer et al. 1997).

2.7.3. Two-Step Model

One of the most commonly used in vitro digestion model was designed by Miller et al (1981). It is based on simulating the digestion in the stomach (by adding HCl to lower the pH to 2) and the intestine (using dialysis bag) and is divided into two steps. The contents are retained in the stomach and then passed into the second section of the model, containing the dialysate (serum mixed with the retentate from the stomach treated food). The small intestinal functionality is replicated for enzyme action and absorption and bioaccessibility of the food is effectively measured by this model (Miller et al, 1981). Toor et al (2009) measured bioaccessibility of tomatoes using this process with slight modifications.

2.7.4. Dynamic Model

A dynamic model system, called TNO gastrointestinal system, of the gastrointestinal tract, has been tested by Minekus et al (1995). This model mimics the human GI tract by including churning functions like squeezing and peristalsis, followed by nutrient absorption by the small intestine (Yoo and Chen, 2006). This model takes into

account the gastric and intestinal transit times to be 70 and 160 minutes, respectively. The pH of the model, with stomach close to 2 and the small intestinal portion of the model is approximated to 6.5, based on the duodenal pH. Actual gastric, pancreatic and bile secretions are used in the model to mimic the internal GI environment precisely (Yoo and Chen, 2006). To make in vitro digestion models resemble the in vivo environment closely, it is very important to consider the factors like pH, temperature, the concentration of the enzyme, enzyme buffers/inhibitors and well-regulated incubation time. Any changes in these factors could potentially affect the enzyme activity and inaccuracy of the model (Hur et al. 2011).

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CHAPTER 3

RHEOLOGICAL AND STRUCTURAL PROPERTIES OF TART CHERRY PURÉE AS AFFECTED BY PARTICLE SIZE REDUCTION[1]

[1]Lukhmana, N., Kong, F., Kerr, W. L., & Singh, R. K. (2018). LWT, 90, 650-657. Reprinted here with permission of the publisher. Copyright 2018, with permission from ELSEVIER.

Abstract

The objective of this study was to determine the effect of micronization techniques for particle size reduction from D (v, 0.9) ≥ 800 μm to D (v, 0.9) ≤ 100 μm on the rheological and structural characteristics of tart cherry purée. Commercially frozen cherry purée was thawed and micronized using Megatron MT5000 at 15000 rpm for three cycles (M1, M2, M3; 10 min each) followed by high-pressure homogenization at 200 MPa. The particle size distribution was measured using Malvern laser particle size analyzer and total soluble and insoluble solid contents by using the AOAC official method 20.5 or 30.2. All samples were further characterized for rheology, total solids content and particle morphology. Results showed a significant reduction in apparent viscosities (2.59 Pa.s to 0.0144 Pa.s) with a reduction in particle size (800 μm to 100 μm) and this trend was consistent in different shear rate ranges. The change in serum viscosity followed the similar trend as observed in purée viscosity with micronization. Comparative analysis of the effect of shear rate (0 to 1000 s^{-1}) on viscosity showed an inverse relationship, suggesting a shear thinning behavior of the fluid. This effect on serum viscosity (0.0119 Pa.s to 0.0047 Pa.s) was found to be higher in the shear rate range of 0 to 10 s^{-1}, as opposed to a relatively less reduction from 0.0047 Pa.s to 0.0022 Pa.s and 0.0015 Pa.s at higher shear rates in ranges of 20 to 100 s^{-1} and 20 to 1000 s^{-1} respectively. The viscosity–shear rate changes were approximated by Ostwald-de Waele model (power law relation) for serum and Herschel Bulkley model for purée. The total solid content (14.20 ±1.10 °Brix) was not significantly affected by particle size reduction. Results of this study provide useful information about the rheological properties of fruit juices/purées, which are influenced by the interactions

between the dispersed phase and the serum, and are critical indicators of textural properties, mouth-feel, commercial acceptability, requirements of mixing and transport equipment.

Keywords: micronization, rheology, viscosity, non-Newtonian fluids, shear thinning, cherry purée.

3.1 Introduction

In recent years, many researchers have undertaken a number of studies into the health benefits of cherries (Prunus spp.). The quality of cherry fruit is primarily influenced by color, sweetness, sourness, and firmness. The presence of glucose and fructose contribute to the sweetness in the cherry fruit, while sourness is primarily due to the presence of organic acid (malic acid) (Bernalte, Sabio, Hernández, & Gervasini, 2003; Esti, Cinquanta, Sinesio, Moneta, & Di Matteo, 2002). The consumer acceptance appears to be reliant upon the ratio between acid concentrations and sugar (Crisosto, Crisosto, & Metheney, 2003). There is a higher content of sugar (13 g/100 g) in sweet cherries as opposed to sour cherries (8 g/100 g). Cherries are characterized by the presence of some carotenoids such as beta-carotene, lutein and zeaxatine and liposoluble (A, E and K) and hydrosoluble (B, C) vitamins (Ferretti, Bacchetti, Belleggia, & Neri, 2010). As common antioxidants, anthocyanins can demonstrate a broad range of biomedical functions, like preventing cardiovascular disorders, age-induced oxidative stress, inflammatory responses, diverse degenerative diseases and several cancers (Wang et al., 1999). Anthocyanins (ACNs) are not stable which leads to color disappearance if used alone. Isolated ACNs can degrade easily under thermal stress, neutral pH and the presence of oxygen, metal ions and enzymes (Frank et al., 2012). To prevent ACNs from degrading, microencapsulation is a good choice to stabilize ACNs and control their release at a specific time in the digestion (Betz & Kulozik, 2011). Microencapsulation is mainly used to protect bioactive compounds or functional foods from light, temperature, oxidation (Murugesan & Orsat, 2012).

Rheological properties can provide analytical tools to yield meaningful insight on the structural organization of food (Ahmed & Ramaswamy, 2007). Fruit purées behave as non-Newtonian fluids: in general, purées of fruits and vegetables are shear-thinning fluids (Rao, 1992) presenting a yield stress. The properties of different plant-based food dispersions depend on the raw material and are influenced not only by the concentration but also by the particle size, stiffness, composition, and elastic properties (Day, Xu, Øiseth, Lundin, & Hemar, 2010; F. W. C. Den Ouden, Van Vliet, Sciences, & Box, 2002; H Kunzek, Kabbert, & Gloyna, 1999; Herbert Kunzek, Opel, & Senge, 1997; Maceiras, Álvarez, & Cancela, 2007; Nindo, Tang, Powers, & Takhar, 2007)

The rheological parameters include apparent viscosity, yield stress and consistency index of the product. Moreover, they serve as a useful tool in understanding food structure and quality control of the product during processing. Solids content, particle size distribution of solids and serum viscosity are equally significant in understanding the rheological behavior of plant-based food dispersions (Rao, 1992). To study the rheological properties of fruit purées, we need to analyze the variation of apparent viscosity with changing shear rate. If this variation is non-linear, it is indicative of non-Newtonian nature of fluids. Since fruit purée is made up of serum which is mostly a Newtonian fluid and particles of various sizes and shapes dispersed within it, it is possible that the fruit pulp with its associated pectin may be the constituent contributing to the non-Newtonian behavior (Nindo, Tang, Powers, & Takhar, 2007).

Furthermore, many researchers have reported that fruit purées behave as non-Newtonian fluid due to the complex interactions among soluble sugars, pectic substances and suspended solids (Ahmed & Ramaswamy, 2007). Plant-based food suspensions mostly

constitute plant tissue-based particles in a continuous serum phase with pectin, sugars and organic acids solubilized in it and commonly present in vegetable juices, purées and soups (Anthon, Diaz, & Barrett, 2008; Tanglertpaibul & Rao, 1987). The rheological properties of plant-based materials are determined by two factors, namely the particle properties of the dispersed phase and the properties of the serum phase particularly of the solubilized pectin (Anthon, Diaz, & Barrett, 2008; Beresovsky, Kopelman, & Mizrahi, 1995; Ouden and Vliet, 1997; Servais, Jones, & Roberts, 2002; Tanglertpaibul and Rao, 1987; Yoo and Rao, 1994). The particle properties that form the rheology of the material include the concentration, size distribution, shape, surface properties, inter-particle forces and defects (Fischer, Pollard, Erni, Marti, & Padar, 2009; Gallegos & Franco, 1999; Genovese, Lozano, & Rao, 2007).

The choice of an appropriate model depends on the application and model parameters and is very crucial in determining the relation between the product viscosity and shear. In order to obtain accurate measurements, laminar flow conditions are maintained during experiments. However, stability may be challenging to accomplish at very low or high shear rates for fruit purées containing non-uniform particles. At high shear rates, the dispersed particles that are induced by turbulent flow may cause structural breakdown of the fruit purée. Despite these variations, most purées commonly show non-Newtonian flow patterns. Therefore, the non-Newtonian model in equation (3.1) was applied for explaining the flow of tart cherry purées. The Ostwald de Waelle model, or the Power Law model [Eq. (3.1)], is commonly used in studies regarding the flow behavior of foods. This model provides a good description of fluid flow behavior in the shear range,

which is easily measured by many rheological instruments. However, it does not accurately fit data obtained at a wide range of shear rates. This model is in the form:

$$\sigma = (K \times \gamma)^n \quad (3.1)$$

where, σ (Pa) is shear stress, K (Pa.s) is consistency index, γ is shear rate (s^{-1}) and n is the dimensionless flow behavior index. Most fruit purées show shear-thinning behavior ($0<n<1$), which can indicate a breakdown of structural units in a food caused during shear by the hydrodynamic forces that are generated (Rao, 1992). A potential method of studying these changes in product structure could include quantification of flow parameters within some specific shear rate ranges. Herschel-Buckley (HB) model can be used by including the yield term of a foodstuff with a finite yield stress in the Power Law Model.

In this study, the effect of particle size reduction on the rheology and particle structure of tart cherry purée was investigated systematically. The change in serum and purée viscosity with micronization was also studied. Comparative analysis of the effect of shear rate on viscosity and their relationship with the behavior of the fluid was analyzed.

3.2 Materials and Methods

3.2.1 Sample preparation

Nineteen litres of frozen tart cherry purée was obtained from Cherry Central, Inc, Michigan, USA. The frozen tart cherry purée was thawed for 6 h at room temperature, followed by 6 h in warm water (30˚C). The sample was then divided into 36 batches of 500 mL each in plastic (polyethelene) freezer safe bags and stored at -20˚C for further analysis. Various size reduction equipment such as megatron and hammer mill, that can generate particle size of ~300 μm and a high-pressure throttling system that can create particle size

<100 μm were used. The cherry dispersions thus obtained were compared with the cherry purée obtained from the commercial supplier, Cherry Central, Inc. with particle size 800-1000 μm. Separate batches of 500 ml of sample were passed through each method: Super Mass Colloider (stone MKE6-46, Model MKCA6-3, Masuko Sangyo Co. Ltd., Saitama, Japan), Hammer Mill (0.1270 and 0.0508 cm screens, Model JT, Fitzpatrick Co., Elmhurst, Ill., U.S.A.), or Megatron MT5000 (Model MTK 5000Q, Kinematica, Inc., Cincinnati, Ohio, U.S.A.) at 15000 rpm for three cycles (M1, M2, M3; 10 min each) followed by High Pressure Homogenizer (Model nG7900, Stansted Fluid Power Ltd., Essex, UK) at 200 MPa. The temperature was monitored using a digital thermometer during micronization and was not allowed to exceed 45˚C to prevent any chemical alteration of the sample. The desired goal was to reduce the particle size to about 300 μm or less. To determine the reduction in particle size produced by the Megatron, the sample was analyzed with Malvern Mastersizer (Malvern Instruments, Southborough, Mass.). Since the particle size distribution was found to be above 300 μm, the sample was cooled to 20˚C and the process was repeated twice. Therefore, three cycles of 10 min each, with a constant initial temperature of 20˚C and a final temperature of 45˚C (or below) were carried out to reach the desired range of particle size distribution.

3.2.2 Particle size distribution

The particle-size distribution was measured using Malvern Laser Particle Size Analyzer, Mastersizer S with 300 mm lens (Malvern Instruments, Southborough, Mass.). Tart cherry purée samples were dispersed in deionized water until an obscuration point (10–20%) was obtained in the diffractometer cell at a pump speed of 2020 rpm. An optical

model based on the Mie theory of light scattering by spherical particles was applied to calculate the predicted scattering pattern with the refractive indices of the cherry purée and water as follows: real refractive index, 1.47; refractive index of water, 1.33. The instrument's software calculated the average volume-weighted diameter, D(4,3)= Σnidi4/ Σnidi3 (where n is the number of particles in a size class of diameter d_i), surface-weighted mean diameter, D(3,2) = Σnidi3/ Σnidi2, the diameter below which 99% the volume of particles are found, D(v,0.99), the diameter below which 90% of the volume of particles are found, D(v,0.9), the diameter below which 80% of the volume of particles are found, D(v,0.8), the diameter below which 60% of the volume of particles are found, D(v,0.6), the diameter below which 50% of the volume of particles are found, D(v,0.5), the diameter below which 40% of the volume of particles are found, D(v,0.4), the diameter below which 20% of the volume of particles are found, D(v,0.2), the diameter below which 10% of the volume of particles are found, D(v,0.1), from the size distribution were calculated by the instrument software. The measurement of particle size distribution of tart cherry purée as affected by different methods of particle size reduction was done. Five measurements from three replications were used for the analyses and the analyses were done on the diluted samples in distilled water.

The relative width of the PSD, referred to as the spread, was calculated as:

$$Spread = \frac{\text{D (v,0.9)} - \text{D (v,0.1)}}{\text{D (3,2)}} \quad (3.2)$$

3.2.3 Color measurements

Color properties (L*, a*, b*) of tart cherry purée were determined using MiniScanEZ 4500L portable spectrophotometer (HunterLab), referring to color space CIE

Lab. The samples were measured against a white ceramic reference plate (L*= 93.92; a*= 1.03; b* = 0.52). The data were mean of three measurements. Total change in the color, hue and Chroma were calculated according to Wojdyło, Figiel, Lech, Nowicka, & Oszmiański, 2014.

3.2.4 Total solid content

The total solid content of each suspension was determined using the AOAC official method 20.5 (Horwitz, Kamps, & Boyer, 1980) by drying 3.0 ± 0.1 g of each sample in a vacuum oven at 70 °C for 4 – 5 h. All analyses were conducted in triplicate.

3.2.5 Rheological properties

Rheological flow behavior was measured using a stress-controlled rheometer (TA Instruments). The geometry of the rheometer was designed in a way to avoid the wall slip and involved a six-blade vane with a diameter of 10 mm and a height of 15 mm. Approximately 50 mL of tart cherry purée was loaded into the instrument and the shear rate distributions measured were representative shear rates. Since, the duration of each measurement was relatively short (i.e. 20 – 30 min), the extent of evaporation during the experiments was considered negligible. The vane geometry blade has an advantage over the cone and plate setup, especially when the food samples involve vegetable and fruit purées. This is because the pulp in the purée has a tendency to disperse easily when used for rheological measurements with the cone and plate set up. Viscosity of tart cherry purée processed using different methods of particle size reduction was measured at varying shear rates. Three experimental replications were conducted for each method of particle size

reduction. The software used for measurements was allowed to equilibrate to a pre-determined temperature before the data collection was started. During pumping and mixing of liquid foods, the shear rates in the range of 10–1000 s^{-1} are often experienced. Therefore, rheological experiments were carried out within the shear rate ranges that cover most applications (Steffe, 1996).

3.2.6 Bright Field Microscopy

Light micrographs were taken using a Nikon Eclipse E400 light microscope and were used to analyze the microstructure of the suspensions. The samples were studied using an objective of ×10 or ×40 magnification. The images were captured using OMAX A35100U3 camera and viewed using the TopViewX software.

3.2.7 Statistical analysis

Statistical significance of treatment factors and its interactions were determined according to two-way factorial design with three replications of each treatment using SAS version 9.1 (SAS Inst., Inc., Cary, N.C.). The factors were method of micronization and shear rates; each at three levels. Analysis of variance was used to study the effects of treatment factors on response variables (particle size diameter and viscosity) quantifying tart cherry properties.

Similarly, three-way factorial design with three replications was used to study the statistical significance of treatment factors and the interactions on the rheological properties. Student's t-test was used further for pair wise comparison between the means of each variable. Non-micronized tart cherry was used as a control for statistical

comparison with the experiment samples for the response variables of total solids and particle size distribution.

3.3 Results and Discussion

3.3.1 Particle size analysis

Particle size distributions (PSD) for micronized samples were different for different methods of micronization as shown in Table 3.1. Measurements showed a monomodal size distribution, both for the separated fractions and the purées. The cumulative volumetric PSD of all samples of micronized and non-micronized tart cherry purée were performed. Based on the PSDs, various characteristic diameters and their spread were calculated (Table 3.1). It is important to mention that values were calculated assuming spherical particles. Particle size was also measured as the area-based (D[3,2]) or the volume-based diameter (D[4,3]). D[4,3] is highly influenced by the presence of large particles, whereas the D[3,2] values are contributed by the presence of smaller particles. Although values of D[4,3] were larger than those of D[3,2], however the trends in both parameters were similar. As expected, it can be seen from Table 3.1 that particle sizes of the sample prior to micronization were significantly higher than the particle sizes obtained by different methods of micronization. Particle sizes were also larger for particles micronized using the megatron (Kinematica) in first cycle and were reduced in subsequent cycles. Megatron was chosen as the best method for micronization based on reduction in particle size, yield and ease of use of the respective equipment. Table 3.1 summarizes the observations for each cycle and gives a comparison of it with that of control (before micronization). Temperature was monitored during each cycle at an interval of 1 min and controlled below 45˚C to avoid

degradation of anthocyanins and other bioactives. In addition to these characteristic diameters, also the relative width of the PSD, expressed as the spread, was calculated using Eq. 3.2. D [3,2] was used for the calculation of spread because D [4,3] and D [v,0.5] are highly influenced by larger particles that can distort the spread. As expected, the spread of the control turned out to be larger than the spread of the micronized tart cherry purée.

3.3.2 Color measurements

Values of lightness (L*), redness (a*) and yellowness (b*) followed a similar trend, in terms of increase, with particle size reduction. Significant ($P < 0.001$) and linear effects on L* were recorded with decreasing particles from 520 to 291 μm, with consequential increase in L*, noticeable but dependent on particle size distribution as seen in Figure 3.1. Similar increase in values were noted in a*and b* with decreasing PSD. Thus, processed tart cherry purée became lighter as D90 decreased from 520 to 291 μm. Hutchings (1994) stated that L*, a* and b*, represent food diffuse reflectance of light, which are dependent on particulate distribution and scattering factors or coefficients. With increase in micronization over subsequent cycles of particle size reduction using the megatron, L* value increased from 16.37 to 22.62, a* value increased from 18.95 to 20.56, b* value increased from 11.27 to 13.61. These increments in L*, a* and b* values can be explained on the basis of light scattering, which increases with a reduction in particle size. Hence, a sharp increase in the L* values (that denotes the lightness of the sample) was observed in Figure 3.1. Graf & Saguy, (1991) showed that in food suspensions, scattering factor is inversely related to particle diameter. Tart cherry purées with varying particle sizes have

different structural shapes and arrangements influencing light scattering coefficients and thus appearance.

3.3.3 Analysis of sample and serum viscosity

It has been reported that the flow behavior of most fruit products deviates from the simple Newtonian behavior (Steffe, 1996). Similarly, all samples of tart cherry purées listed in Table 3.1 were found to be non-Newtonian liquids showing a yield stress. This non-Newtonian character of the purée sample is a result of the structural modifications produced while shearing (Gallegos & Franco, 1999; Steffe, 1996).

Size reduction is expected to increase the viscosity as the reduction in particle size increases the resistive forces of the fluid. However, in our study there was a noticeable decrease in viscosity with decrease in particle sizes, which was also in coherence with the results obtained by Yoo & Rao (1994) in tomato purée and by Ahmed, Shivhare, & Raghavan (2000) in green chilli purée. This can be further explained based on photomicrographs obtained (Fig. 3.8 (a)-(c)]. An increase in shear rate caused a non-linear decrease in the viscosity, which meant that the sample had a shear thinning behavior.

The flow curves were fitted to different models to investigate the influence of the particle size on the model parameters. Both the Bingham and the Herschel–Bulkley models were used. However, the Bingham model simulates linear flow behaviour, the Herschel–Bulkley model involves parameters to describe shear thinning or thickening. The Herschel–Bulkley model better described the experimental flow curve, indicating that the suspensions are not simple Bingham liquids. Indeed, the Herschel–Bulkley model is more often used to describe the non-Newtonian flow behavior of food systems presenting a yield

stress like several fruit or vegetable products (purées and juices) (Duran & Costell, 1982; Steffe, 1996).

The flow index (n) and the consistency coefficient (K) in the Herschel–Bulkley model were estimated for the experimental flow curves and good fits of the experimental data points were obtained for all cases ($R_{adj}^2 > 0.95$). For the micronized samples, n increased with particle size and concentration, whereas no effect of particle size on the parameter K was noticeable (Figure. 3.7).

The flow curves of the tart cherry purée samples were highly dominated by the yield stress. Although fitting of the Herschel–Bulkley model to the flow curves was good within lower shear rate ranges, for the flow behavior at higher values ($> 100\ s^{-1}$), i.e. shear thinning cannot be unambiguously predicted. The contribution of the yield stress to the measured viscosity was large.

The duration and magnitude (both shear rate and shear stress) of flow determine the viscosity of non-Newtonian fluids. Rheology cannot be adequately explained by a single number due to the varying range of shear rates that prevails in fluid processing and application. In addition, continuous deformation of the fluid may cause changes in viscosity over time. Therefore, the flow behavior of non-Newtonian fluids cannot be described by viscosity measurements at a constant shear rate. The non-Newtonian flow phenomena originate from different kinds of solids particles that are dispersed in the liquid phase in food systems or are polymeric macromolecules that are solubilized in it. The main effect of this is the momentum transfer as it flows through the bulk fluid. This momentum transfer change is caused by the translocation and rotation of particles under the influence of the superimposed flow. Thus, shear stresses may increase or reduce depending on (a)

the interactions between the disperse and dispersing phase, (b), degree of particle aggregation, as determined by particle-particle interactions and (c) mechanical interactions of the particles that are dispersed due to hydrodynamic effects.

Bulk rheology can also be affected by the state of particles' flocculation and aggregation. These particle associations occur due to particle-particle interactions which generate formation of internal structural network in suspensions. Before shearing begins, the potential energy barrier determines how well the liquid flow and dispersion can occur. At lower shear rates, the suspension structure keeps its form and flow doesn't take place until internal network damage is completed. At higher shear rates, the frequency of collisions increases which causes the internal structure to break. In the above curves (Figure 3.2 to 3.6), the samples exhibit non-Newtonian flow phenomenon. Viscosity decreases with shear rate as a result of primary and secondary bonds breaking isothermally and reversibly. Structure breaking due to hydrodynamic effects cause clusters and aggregates to be destroyed. This is due to a higher rate of particle dissociation compared to the rate of their association. Another factor includes favorable orientation of dispersed particles in the flow field. Rheological properties of fluids can also influence transfer equipment requirements. In Figure 3.2 to 3.6, heterogeneity of the non-micronized sample was observed at low shear rates. This could be explained by the settling of solid particles due to gravitational forces which cause the particles to settle to the bottom, since the forces always act perpendicularly to the direction of flow. As a result of this, the solids concentration gradient in the sheared volume creates a non-uniform and unknown distribution of rheological properties. Problems involving inhomogeneity are usually seen when viscosity of the liquid phase is low, and solids volumetric concentration is high. The

variation of flow behavior index over varying shear rates was analyzed for different methods of micronization (Figure. 3.7) and it was found that the flow behavior index (n) increases with increasing shear rates showing non-Newtonian behavior. However, with the particle size reduction, the values of the flow behavior index (for a certain shear rate) decrease, indicating that for values of n >1, the increase in shear thinning observed for high shear rates is much more as opposed to that observed at low shear rates for values of n <1.

3.3.4 Structural Analysis

All tart cherry purée samples were observed under a light microscope at 10X. Figure 3.8(a) depicts a cherry purée sample that has not undergone any micronization. This means that the particles are relatively large and tend to form a purée composed mostly of large cell clusters that could be seen in Figure 3.8(a). Samples that are not ground, as this one is, tend to have a higher viscosity than samples that undergo micronization. This may be due to the uneven surfaces of the cell clusters, which make it easy for cell clusters to agglomerate together in larger clusters that increase viscosity. The tart cherry purée sample shown in Figure 3.8(b) contains a mix of cell clusters and smaller micronized particles. This sample should have an intermediate viscosity that is somewhat lower than that seen in the sample from Fig 3.8(a), but higher than the viscosity of the sample in Figure 3.8(c). This decrease in viscosity occurs because there are less cell clusters in this sample that can aggregate together and thicken the purée. The smaller particles in this sample likely have smaller and smoother surfaces that are less conducive to clustering. The purée shown in Figure 3.8(c) has received the highest degree of micronization. This sample does not appear to contain many cell clusters. The particles in this sample are likely small and relatively

smooth. Since this sample contains the smallest, smoothest particles, the purée should have the lowest viscosity of all three samples studied. This decrease in viscosity is likely due to the absence of cell clusters that can thicken the purée by agglomeration. The viscosity trend observed here is contrary to that observed in commercial fruit juices, in which viscosity increases with decreasing particle size. The trend of viscosity decreasing with decreasing particle size concurs with the trend discussed by (Leverrier, Almeida, Espinosa-Mu noz, & Cuvelier, 2016) in which the same trend was observed in apple purées that had been ground to different degrees. It can be concluded that for apple and tart cherry purées, the viscosity of the sample is largely dependent on the size of the particles in the sample, which is determined by the degree to which the sample is ground. Samples that receive less micronization tend to have higher viscosities.

3.4 Conclusions

Based on the results, tart cherry purée showed a significant reduction in apparent viscosities with a reduction in particle size and this trend was consistent in different shear rate ranges. The changes in serum viscosity followed the similar trend as observed in purée viscosity with micronization. Comparative analysis of the effect of shear rate on viscosity showed an inverse relationship, suggesting a shear thinning behavior of the fluid. The viscosity–shear rate changes were approximated by Ostwald-de Waele model (power law relation) for serum and Herschel Bulkley model for purée. The total solid content was not significantly affected by particle size reduction. The structural analysis reveals that the samples that are not ground, tend to have a higher viscosity than the samples that undergo micronization. The reduction in viscosity occurs because there are less cell clusters in the

micronized sample that can aggregate together and thicken the purée. The smaller particles in the micronized sample are likely to have smaller and smoother surfaces that are less conducive to clustering and agglomeration. Results of this study provide useful information about the rheological properties of fruit juices/purées, which are influenced by the interactions between the dispersed phase and the serum and are critical indicators of textural properties and commercial acceptability.

3.5 References

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Table 3.1: Particle size distribution (PSD) of tart cherry purée micronized using different methods of particle size reduction[1].

Methods of Micronization	D [4,3]	D [3,2]	D [v,0.1]	D [v,0.5]	D [v,0.9]	Spread
Before Micronization (control)	480.34 (7.02)	87.71 (5.35)	78.25 (6.23)	518.90 (10.21)	789.09 (12.31)	8.10
Super Mass Colloider	405.01 (8.97)	45.46 (4.65)	71.92 (8.34)	408.10 (9.76)	718.17 (11.21)	14.21
Hammer mill	270.38 (6.79)	38.42 (3.27)	32.83 (4.73)	248.44 (6.56)	530.35 (8.99)	12.94
Megatron (Cycle 1)	250.54 (4.54)	38.87 (5.43)	33.08 (5.76)	213.11 (6.65)	519.42 (7.34)	12.51
Megatron (Cycle 2)	221.34 (5.32)	36.81 (4.98)	31.88 (6.48)	171.28 (4.77)	451.91 (6.38)	11.41
Megatron (Cycle 3)	143.67 (4.39)	25.63 (2.76)	20.52 (2.54)	124.21 (3.27)	291.26 (4.65)	10.56
High Pressure throttling system	37.96 (3.65)	10.22 (2.33)	4.48 (1.21)	30.52 (2.45)	83.64 (3.42)	7.74

[1](Standard deviations are indicated within parentheses for experiments done in triplicate). It can be observed that every method of micronization leads to particle size reduction. Although, High Pressure throttling system gave the highest particle size reduction in the purée; the yield was low and therefore, Megatron was chosen as the preferred method of micronization as it gave a good yield and an effective particle size reduction.

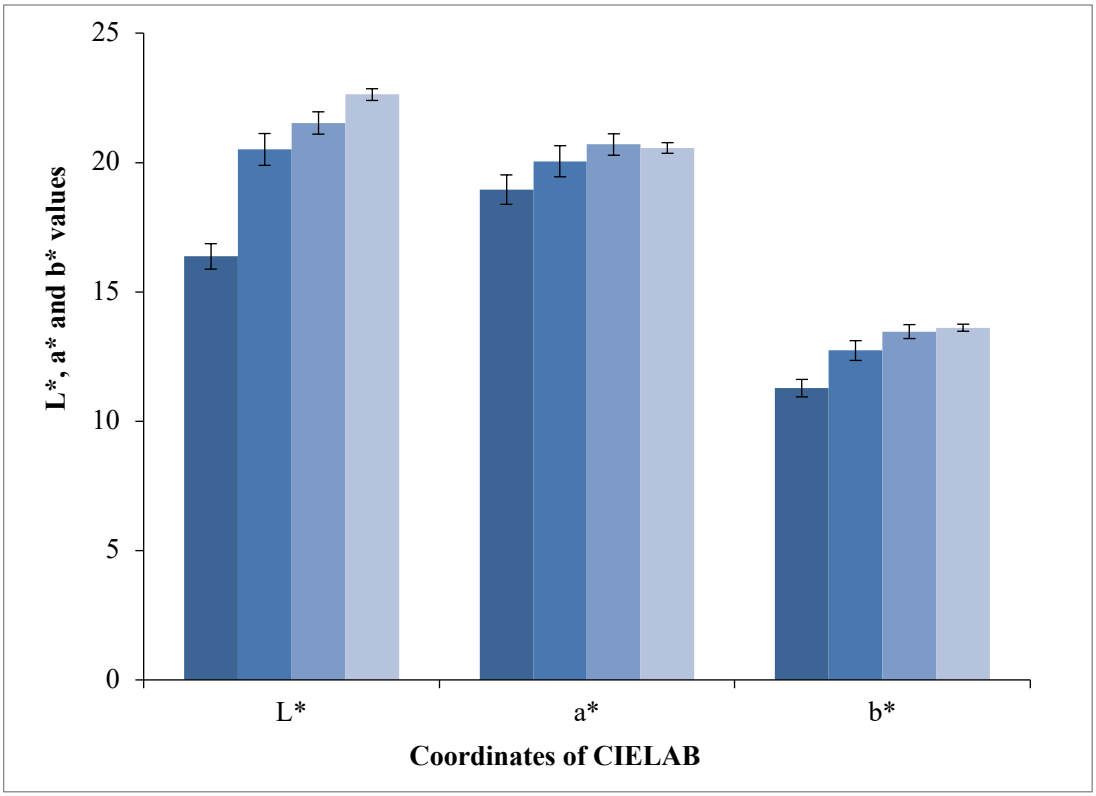

Figure 3.1: Effect of micronization on color of tart cherry purée during different cycles of the sample micronized using the Megatron (■: Before micronization, ■: 1st cycle, ■:2nd cycle, ■: 3rd cycle)

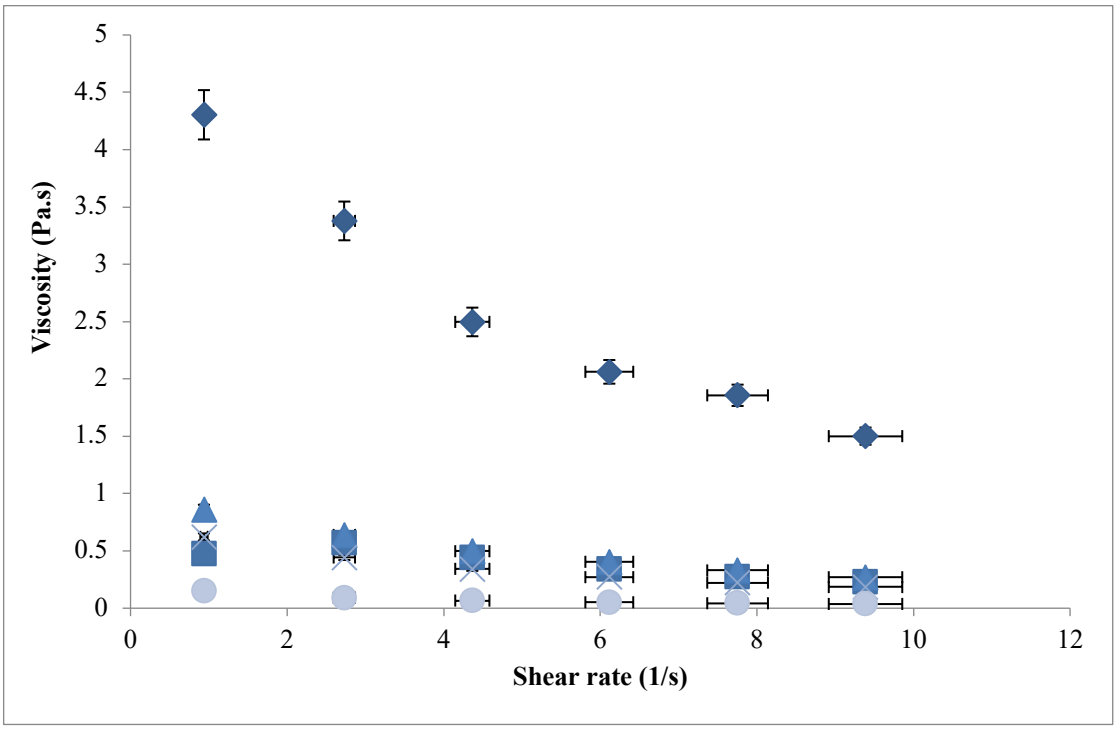

Figure 3.2: Comparative analysis of the effect of varying shear rate (0-10 /s) on the viscosity of micronized and non-micronized sample.

(◆Non-micronized, ■: Megatron (cycle 1), Δ: Megatron (cycle 2), ×: Megatron (cycle 3), ●: High pressure throttling system). With the reduction in particle sizes, there was decrease in viscosity over varying shear rate of range 0-10/s.

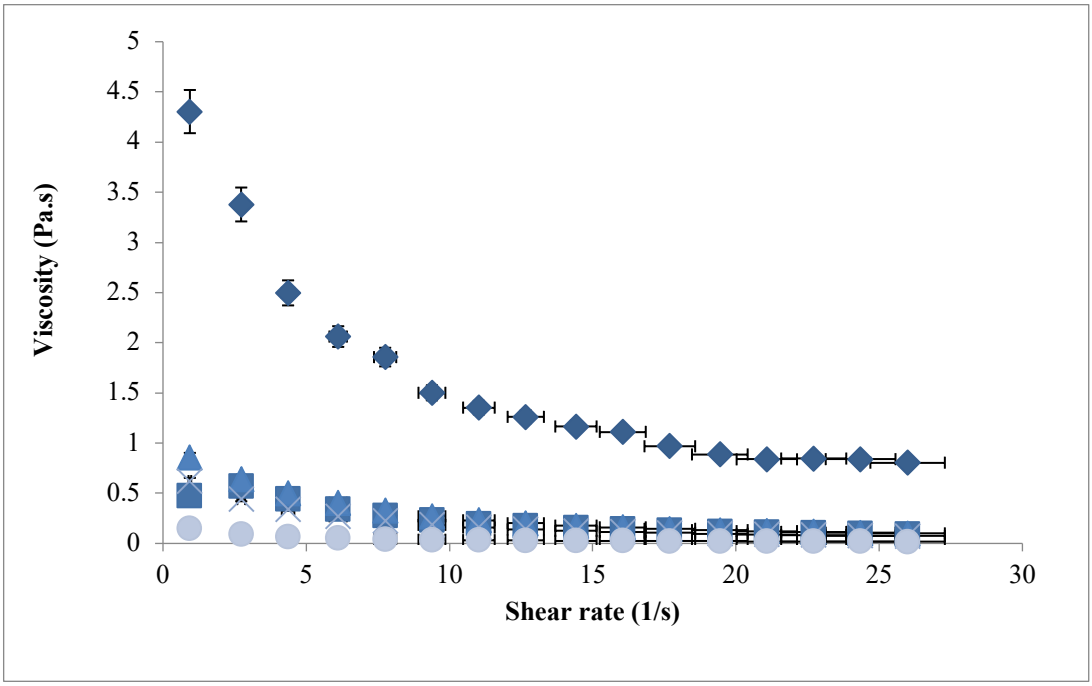

Figure 3.3: Comparative analysis of the effect of varying shear rate (0-25 /s) on the viscosity of micronized and non-micronized sample. (◆Non-micronized, ■: Megatron (cycle 1), Δ: Megatron (cycle 2), ×: Megatron (cycle 3), ●: High pressure throttling system). With the reduction in particle sizes, there was decrease in viscosity over varying shear rate of range 0-25/s.

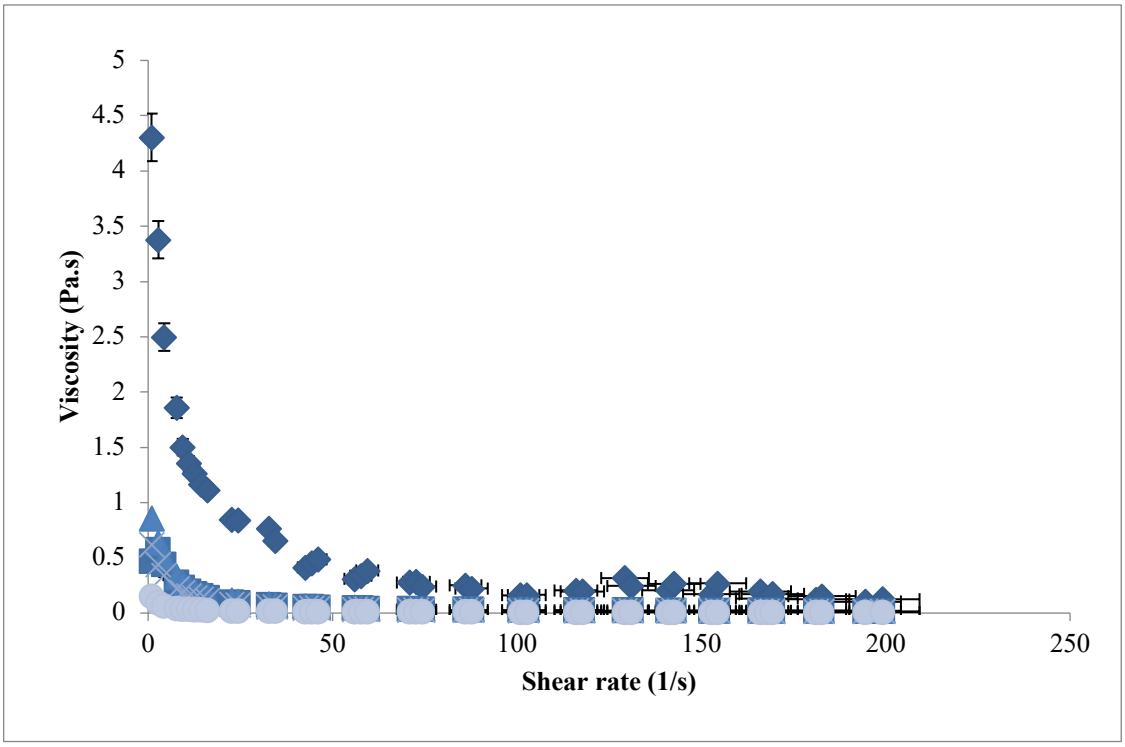

Figure 3.4: Comparative analysis of the effect of varying shear rate (0-200 /s) on the apparent viscosity of micronized and non-micronized (◆Non-micronized, ■: Megatron (cycle 1), Δ: Megatron (cycle 2), ×: Megatron (cycle 3), ●: High pressure throttling system). With the reduction in particle sizes, there was decrease in viscosity over varying shear rate of range 0-200/s.

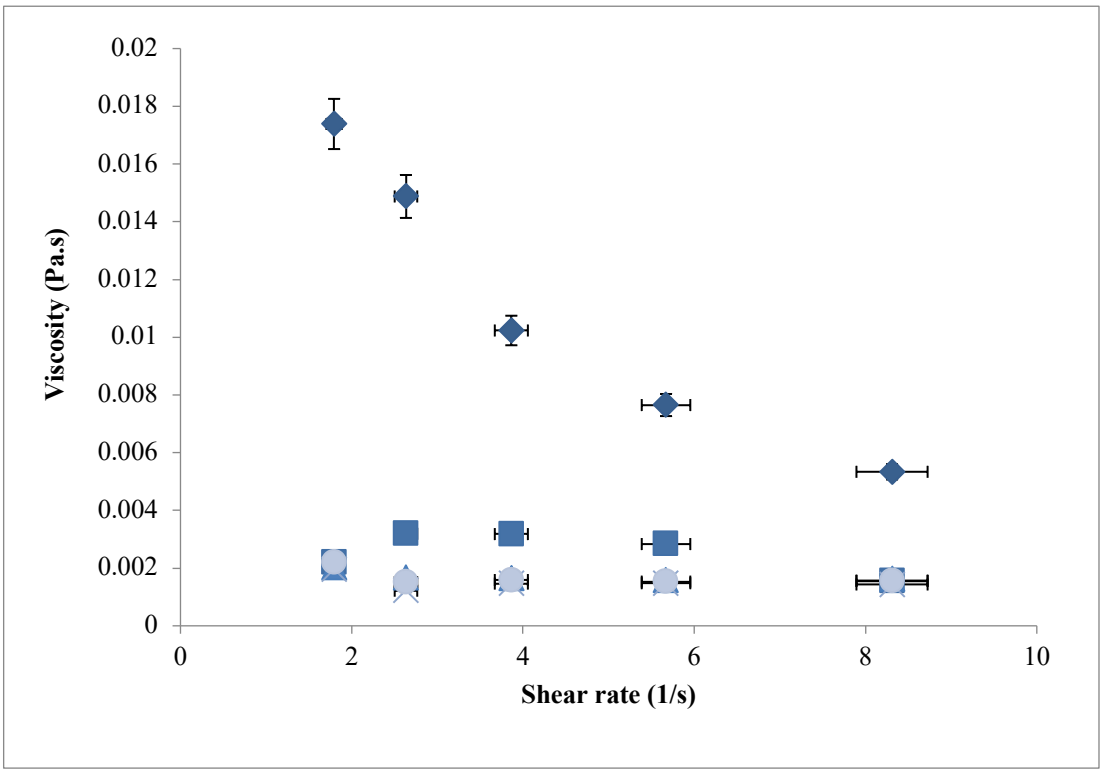

Figure 3.5: Effect of varying shear rate (0-10 /s) on the viscosity of supernatant of micronized and non-micronized sample obtained after centrifugation. (◆Non-micronized, ■: Megatron (cycle 1), Δ: Megatron (cycle 2), ×: Megatron (cycle 3), ●: High pressure throttling system). With the reduction in particle sizes, there was decrease in viscosity of the supernatant obtained after centrifugation, over varying shear rate of range 0-10/s. This was done to verify the trend in the serum as opposed to the purée (containing serum and pulp) to identify whether the serum showed Newtonian behavior, when separated from the pulp. However, it was found that the serum shows same trend as the purée and displays non-Newtonian behavior.

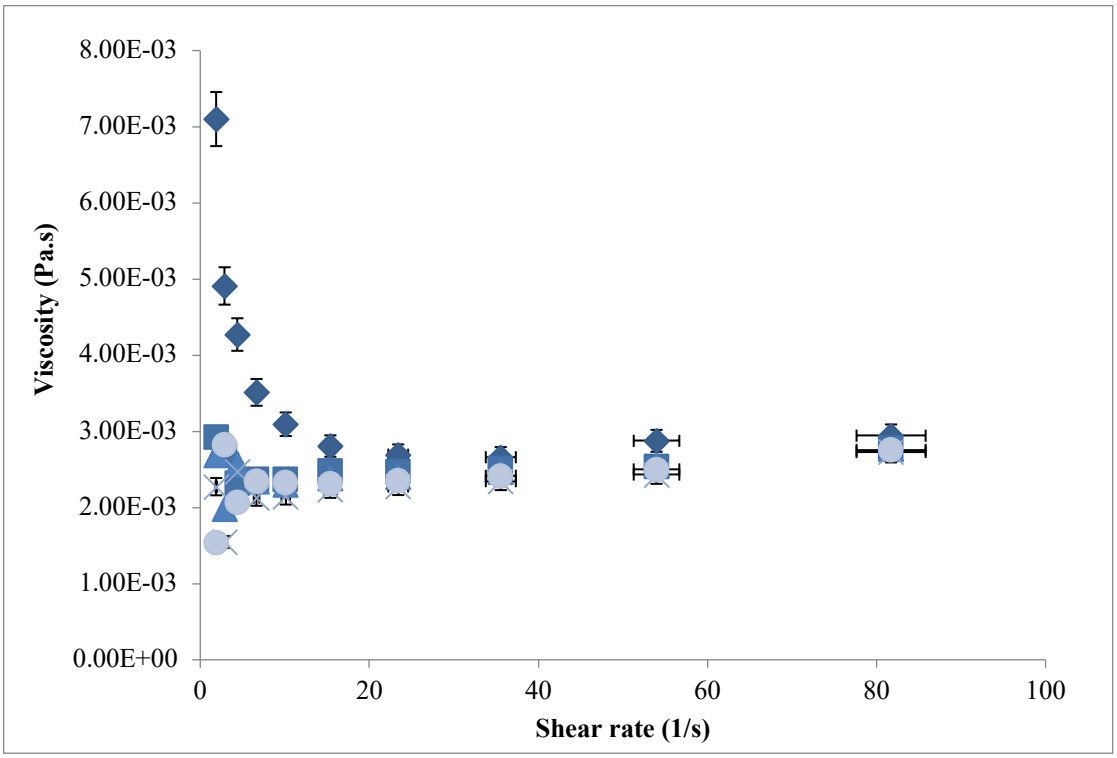

Figure 3.6: Effect of varying shear rate (0-100 /s) on the viscosity of supernatant of micronized and non-micronized sample obtained after centrifugation. (◆Non-micronized, ■: Megatron (cycle 1), Δ: Megatron (cycle 2), ×: Megatron (cycle 3), ●: High pressure throttling system).

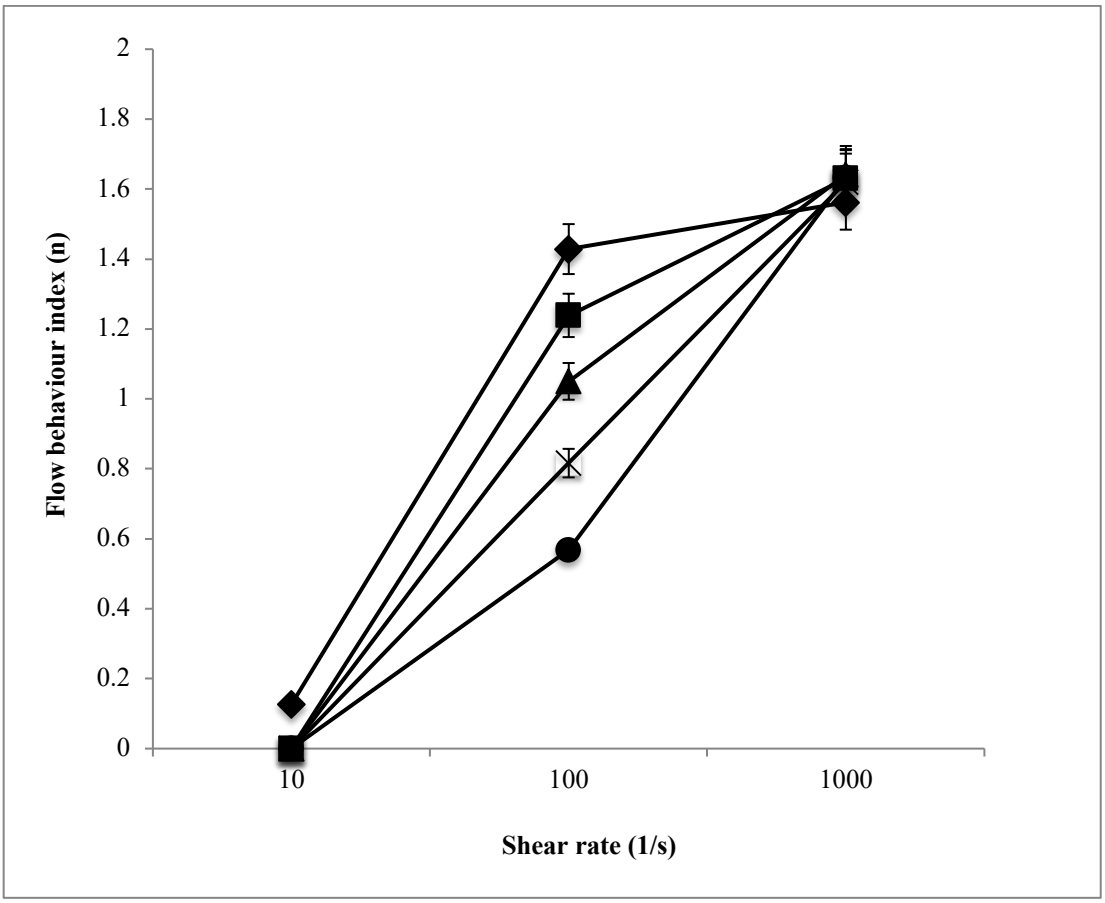

Figure 3.7: Effect of varying shear rates on the flow behavior index of micronized and non-micronized sample obtained after centrifugation. The flow behavior index increases with increasing shear rates showing non-Newtonian behavior. As the fluid becomes more shear thinning, 'n' decreases. For values of n > 1, the apparent viscosity is increasing with increase in shear rates for all methods of micronization and that increase is relatively less for n < 1 (◆Non-micronized, ■: Megatron (cycle 1), Δ: Megatron (cycle 2), ×: Megatron (cycle 3), ●: High pressure throttling system).

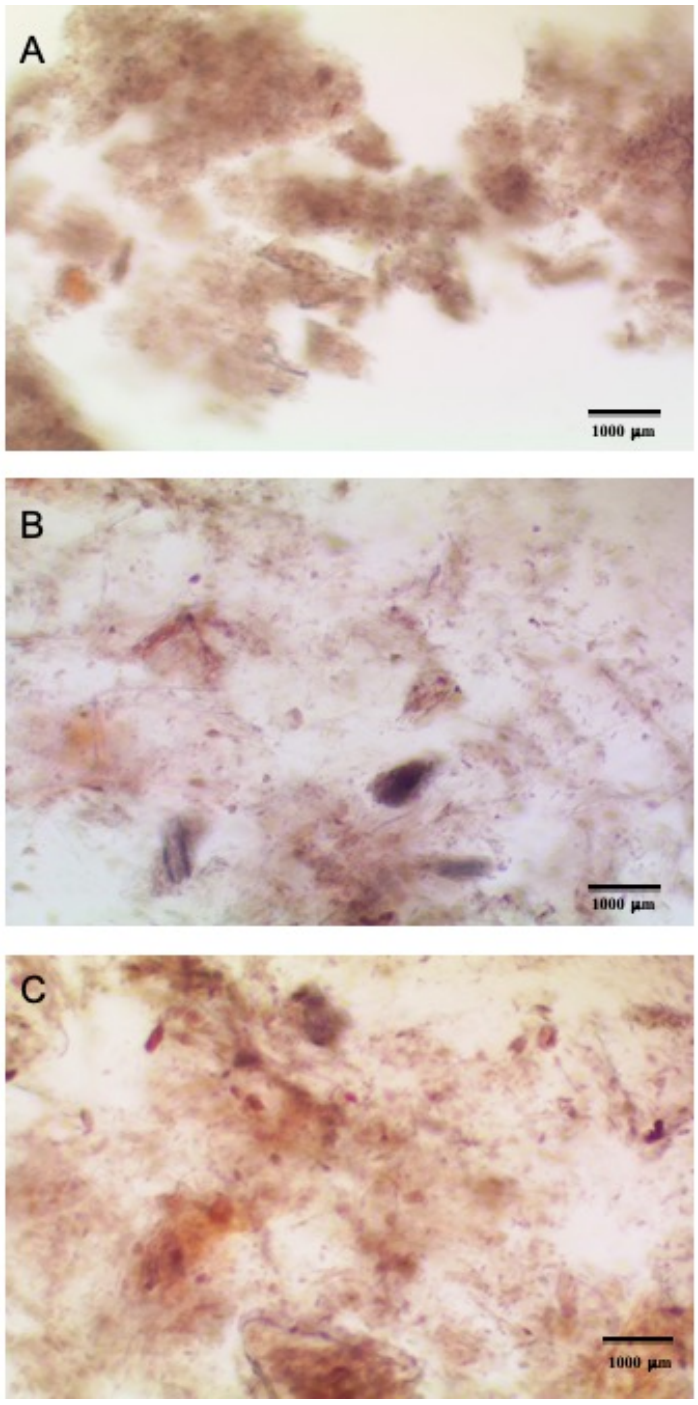

Figure 3.8(a): Photomicrograph of the non-micronized tart cherry purée as observed under light microscope at 10X objective. (b): Photomicrograph of the micronized tart cherry purée obtained from Megatron as observed under light microscope at 10X objective. (c): Photomicrograph of the micronized tart cherry purée obtained from High pressure throttling system as observed under light microscope at 10X objective. Scale bar for all images is 1000 microns.

CHAPTER 4

CHEMICAL PROPERTIES OF TART CHERRY PURÉE AS AFFECTED BY MICRONIZATION

4.1 Introduction

Sour cherry (Prunus cerasus L.) is a ready to eat fruit but is more commonly consumed after processing into various forms. The sour cherry fruit has been known to have significant health benefits, owing to the presence of bioactive phytochemicals, primarily phenolics. Among the polyphenolics, the major portion is due to the presence of anthocyanins which also give the characteristic red color to the cherries (Damar & Ekşi, 2012; Kirakosyan, Seymour, Llanes, Kaufman, & Bolling, 2009). Many researchers have studied the chemical composition of the tart cherry juice to better understand the health benefits (Ekşi, Reicheneder, & Kieninger, 1980; Molnár, Tóth-Markus, & Boross, 2003; Velioğlu & Yıldız, 1996). Sweet and tart cherries have quite a variable range of phenolic content ranging from 28 to 106 mg and from 70 to 241 gallic acid equivalents (GAE)/100 g of edible fresh weight (FW) (Melicháčová, Timoracká, Bystrická, Vollmannová, & Čéry, 2010). Chemical analysis of tart cherries has shown that they can commonly contain 700 mg/100 g of FW, determined as GAE of total phenols (Khoo, Clausen, Pedersen, & Larsen, 2011). Tart cherries contain higher amount of phenolics than the sweet ones. As mentioned earlier, among the phenolics, the most abundant in tart cherries is the anthocyanin, which has anti-neurodegenerative properties (Kim, Ho, Young, Hyun, & Lee, 2005), high anti-oxidative capacity (Šarić et al., 2009), elevated anti-inflammatory and anti-proliferative properties (Kim et al., 2005) improves glucose tolerance (Ataie-Jafari, Hosseini, Karimi, & Pajouhi, 2008), and reduced colon adenoma in an animal model (Kang, Seeram, Nair, & Bourquin, 2003).

Sour cherries are processed into various products, such as jams, frozen fruits and fruit juices for general consumption. However, owing to its characteristic sour taste, the

tart cherry fruit is not 100% processed into juice and therefore requires further processing to make it edible, flavorful and palatable (Akdag, 2011). During these steps of processing, some of which are thermal or involve filtration, there is a potentially significant loss of polyphenolic antioxidants. However, these detrimental effects of processing on the tart cherry antioxidants, with regards to quantity, bio-accessibility and bioavailability, have hitherto not been studied (Kim & Padilla-Zakour, 2010; Kirakosyan et al., 2009).

To address that, our goal of this study was to employ different methods of micronization on tart cherry purée and determine their effect on the chemical properties and total solids content (soluble and insoluble). The secondary goal of our study was to qualitatively confirm the changes in bio-actives with the use of FT-IR and HPLC. The processing techniques targeted to reduce the particle size of the fruit juices/ purées range from thermal to non-thermal methods (Rawson et al., 2011). Previous research on the stability of anthocyanins suggests that thermal methods over 95°C – 105°C can cause the degradation of anthocyanins by the generation of polymeric by-products (Sadilova, Carle, & Stintzing, 2007). Therefore, our homogenization techniques were selected carefully, and continuous thermal profiling was carried out to closely monitor the heating of the sample during the micronization process. One of the techniques of micronization involved the use Megatron® processor, which is a type of rotor/stator homogenizer (Grüneberger, Künniger, Zimmermann, & Arnold, 2014). The Megatron® System MT 5000 pilot plant equipment gives us the major advantage with the product with respect to processing steps. It can be either treated in a single pass or recirculated for secondary processing, thereby simulating a large factory automation setup utilizing low sample and having low energy needs. The Megatron® MT 5000 Rotor/Stator Homogenizer uses a variable speed between

5000 and up to 20000 rpm (depending on the chosen dispersing aggregate and product properties) and has a maximum tip speed of 40 m/s that disperses, emulsifies and reduces the size of solid particles, droplets and gas bubbles to a few microns or less (Goldberg, 2008). Another technique that was used in this study to aid in particle size reduction was the high-pressure homogenization (HPH). A research group reported exceptional results when high-pressure homogenization was employed to reduce the particle size distribution of blueberry beverage (Barba, Esteve, & Frigola, 2013) and it led to aggregation-free sample even after storage stability studies. The results were better than even smaller particles obtained by thermal processing proving that non-thermal processing imparts better properties. Both of the above-mentioned techniques have been found to reduce the particle size in tart cherry purée (Lukhmana, Kong, Kerr, & Singh, 2018). The particle size reduction observed can be attributed to the weakening of membranes in the individual particle due to high pressure. The weakened membrane easily ruptured from shear in the throttling valve, and the particle size distribution was narrowed due to the restricted opening in the micro-metering valve. The cavitation that occurred after depressurization also aided in facilitating reduction of the particle size; and the turbulence at depressurization helped in mixing up the ruptured particles and distributing it evenly (Puppo et al., 2005). Also, since the effect of processing methods on anthocyanin degradation is of significant interest to the authors, it is important to note that since the anthocyanins are located in the vacuoles in plants, HPH is known to affect the membranes in fruit cells, as well as the vacuole membrane, and to enhance cell permeability owing to its aptitude to deprotonate charged groups and disrupt salt bridges and hydrophobic bonds in cell membranes (Nayak & Rastogi, 2010). It has been found in a previous study on

strawberry pulp that HHP made the extraction of anthocyanins from more accessible (Ferrari, Maresca, & Ciccarone, 2012). Recently, an optimization of anthocyanin extraction from red grape skins assisted by HPH was reported (Corrales, García, Butz, & Tauscher, 2009), which demonstrated it to be an effective technology for extraction purposes. The results show that the high-pressure throttling decreased the size of solid particles, homogenized the particles within the suspending fluid and dispersed it evenly.

Therefore, the objective of this study was to determine the effect of micronization on the (1) polyphenol and other essential bio-actives present in the tart cherry purée, and on the (2) solids and secondary plant metabolites that are involved in the antioxidant activity of this fruit.

4.2 Material and methods

4.2.1. Materials

Tart cherry purée was generously provided by Cherry Central Cooperative, Inc. (Traverse City, Michigan), Folin-Ciocalteau (FC) reagent, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, sodium acetate trihydrate, acetic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric (III) chloride hexahydrate, vanillin, sulphuric acid and assay standards, including trolox, gallic acid, ethanol, rutin and aecsin used were all reagent-grade chemicals purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Deionized water (DI) was prepared fresh before use using a Millipore Milli-Q water purification system (Burlington, MA).

4.2.2. Micronization

Samples were prepared using the micronization protocol discussed in Chapter 3 and the micronized samples were then characterized for the particle size reduction (Lukhmana et al., 2018) and stored at -20°C for further analysis. Prior to chemical analysis, the frozen tart cherry samples (micronized and non-micronized) were cold-thawed overnight in the refrigerator (5-10°C) to prevent any thermal degradation of bioactives.

4.2.3. Chemical analyses of tart cherry purée

4.2.3.1. Total Polyphenol content

The TPC of samples was determined using a modified Folin-Ciocalteu method (Singleton & Rossi, 1965). The Folin–Ciocalteu method was employed with modifications (Flores, Singh, & Kong, 2014). A total of 6.5 mL of deionized water, 0.5 mL of Folin-Ciocalteu's phenol reagent, and 1 mL of saturated sodium carbonate solution were added to test tubes containing 2.0 mL of the 10-fold diluted sample. The mixture was vortexed for 15 s using a Digital VWR Mini Vortexer, followed by an incubation period of 40 min at room temperature to allow optimal color development. Absorbance was measured at 760 nm in a 1 cm cuvette using an Evolution 300 UV-Vis Spectrophotometer (Thermo Scientific). The TPC concentration was calculated from a standard curve prepared using gallic acid. Results are presented as μg of gallic acid equivalent (GAE) per mL of sample.

4.2.3.2. Total Flavonoid Content

The flavonoid content was determined using a previously published method (Mihai, Mărghitaş, Bobiş, Dezmirean, & Tămaş, 2010) with some modifications. Sample solutions (0.5 ml) and dilution of standard quercetin were taken separately in test tubes. To each test tube 1.5 ml methanol, 0.1 ml aluminium chloride solution, 0.1 ml potassium acetate solution and 2.8 ml deionized water were added and mixed well. Sample blank for all the dilution of standard quercetin and all the samples were prepared in similar manner. All the prepared solutions were measured for their absorbance, taken at 420 nm against the suitable blank. Results were expressed as μg of quercetin equivalent per mL of sample.

4.2.3.3. Total Monomeric Anthocyanins

Total anthocyanin content was determined by the spectrophotometric method (Esti, Cinquanta, Sinesio, Moneta, & Di Matteo, 2002). The absorbance of the test portion was determined by diluting with pH 1.0 buffer, and pH 4.5 buffer, at both 520 and 700 nm. The diluted test portions are read versus a blank cell filled with distilled water. Anthocyanin pigment concentration was calculated and expressed as cyanidin-3-glucoside equivalents, as follows:

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L)

$$= \frac{\mathrm{A \times MW \times DF \times 10^3}}{\mathrm{e \times L}} \qquad (4.1)$$

where A = (A_{520nm} – A_{700nm}) pH 1.0 – (A_{520nm} – A_{700nm}) pH 4.5;

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; L = pathlength in cm; e = 26,900 molar extinction coefficient, in $L' mol^{-1} cm^{-1}$, for cyd-3-glu; and 10^3 = factor for conversion from g to mg.

4.2.4. Antioxidant activity

4.2.4.1. FRAP Assay

A modified FRAP assay (Flores, Singh, Kerr, Pegg, & Kong, 2014) was conducted. The FRAP reagent consisted of 2.5 ml of 2,4,6-tri(2-pyridyl)-s-triazine solution (10 mM in 40 mM HCl), 2.5 ml of ferric chloride hexahydrate (20 mM) and 25 ml of acetate buffer (300 mM, pH 3.6). The reagent was prepared fresh daily and held in a water bath kept at 37 °C. Each sample (30 µl) was mixed with 1 ml of the reagent in a 1 cm borosilicate cuvette and allowed to react for 4 min before absorbance was measured at λ of 593 nm. FRAP values were calculated from a standard curve generated with 100 µl of various ferrous sulfate heptahydrate solutions (0.1–1mM). Values are presented as nmol of Fe(II) equivalent µg/mL of the sample.

4.2.4.2. DPPH Assay

DPPH method (Hua Wang, Gao, Zhou, Cai, & Yao, 2008) was used with some modifications. A calibration curve was prepared with a standard solution of DPPH at 5, 10, 15, 20 and 25 mg/L. Stock solution was prepared by dissolving 0.0025 g DPPH in 100 mL methanol. 10% w/v of the solutions were made in the distilled water and the diluted samples

were agitated by using a vortex for 1 min. One mL of solution was mixed with 3.9 mL DPPH solution and kept in dark for 1 h. The absorbance was measured at 517 nm against a blank of methanol by using UV-Vis Spectrophotometer. As a positive control, 3.9 mL DPPH solution was added to 100 mL ethanol and the absorbance was measured immediately without leaving it to stand for 60 min. Ascorbic acid was expressed as percent radical scavenging activity/inhibition.

4.2.4.3. Reducing Power Assay

The reducing capability of the sample extracts was measured by the transformation of Fe3+ to Fe2+ in the presence of the extract. Increased absorbance of the reaction mixture indicates increased reducing power (Oyaizu, 2011). Tart cherry solutions (10% w/v) were made in the distilled water and mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min, 2.5 ml of trichloroacetic acid (10%) was then added to the reaction mixture, centrifuged at 3000 rpm for 10 min, 2.5 ml of upper layer of the mixture was mixed with 2.5 ml distilled water and 0.5 ml of $FeCl^3$ solution (0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as positive control.

4.2.5. Total solids content

Total solids of tart cherry purée samples were determined by gravimetric analysis. Prior to drying, 5 mL of the tart cherry purée was added to a small aluminum pan. Samples were placed in the NAPCO Vacuum Oven (Model 5831) at 65°C for 16-18 hours. Dried

samples were removed from the oven and cooled in a desiccator for 30 minutes before weighing. An additional drying process was repeated for 1 hour at the same temperature to ensure complete moisture loss. Total insoluble solids in tart cherry purée were determined using the same method, with addition of a critical step involving vacuum filtration prior to drying and then subtracted from the total solids of tart cherry purée to determine total soluble solids in the samples.

4.2.6 FTIR Spectroscopy

A FTIR spectrometer (A Nicolet 6700 from Thermo Nicolet Corp., Madison, WI) equipped with a deuterated triglycine sulphate (DTGS) as a detector and a KBr/germanium as beam splitter, interfaced to Computer operating under Windows-based, and connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet), was used for FTIR spectra acquisition. A few drops of each sample were positioned in contact with attenuated total reflectance (ATR) on a multi-bounce plate of crystal at controlled ambient temperature (25°C). All FTIR spectra were recorded from 4000 to 650 cm^{-1}, co-adding 32 interferograms at a resolution of 4 cm^{-1} with strong anodization. These spectra were subtracted against background air spectrum. After every scan, a new reference air background spectrum was taken. The ATR plate was carefully cleaned in situ by scrubbing with ethanol twice followed by acetone and dried with soft tissue before filling in with the next sample and made it possible to dry the ATR plate. The plate cleanliness was verified by collecting a background spectrum and compared to the previous one. These spectra were recorded as absorbance values at each data point. The sample measurements were replicated three times.

4.2.7 Identifying the peaks and interpretation of the FT-IR spectral data

FT-IR spectroscopic data were observed for six standards viz. gallic acid, malic acid, citric acid and maltose, fructose and alpha-D-glucose. It was then used to obtain the FT-IR spectra for the control (non-micronized tart cherry purée) and samples (megatron and High-pressure homogenization) obtained by micronization. The data was imported into 'Origin Pro 8' software and the data were used to generate a line plot. The x-axis and y-axis were determined, and the noise cancellation was carried out by smoothening the curve under signal processing. The method chosen for smoothening was 'Savitzky-Golay' method and then the parameters were varied (points of window, polynomial etc.) (Zhao, Tang, Zhang, & Liu, 2014) to find the best fit. Once the best fit was found, the major peaks were identified around the wavenumbers and the literature was reviewed to locate current FTIR databases and more information about the peaks was gained at the molecular level.

4.2.8 RP-HPLC of cherry anthocyanins

Polyphenols were separated using a modular chromatographer HP 1100 (Agilent Technologies, Palo Alto, CA, USA) equipped with a 250 " 2.1 mm Jupiter C18 reversed-phase column, 4 mm particle diameter (Phenomenex, Torrance, CA, USA), maintained at 37°C using a thermostatic oven. A diode array detector (DAD) was used to record UV-Vis spectra every 2 s in the 190–650 nm range. The HPLC separations were monitored by recording the λ = 520nm wavelengths. In order to run the cherry purée samples i.e. non-micronized, megatron-micronized and HPH-micronized samples, we first extracted the cherry samples using 1:1 methanol followed by centrifugation for 5 min at 10,000 g. We then took the supernatant out for analysis and analyzed the samples using HPLC. To run

the HPLC analysis, we used methanol: water in the ratio 88:12 as mobile phase. Next, we arranged the vials with the samples in the sample tray and prepared a wash vial with only methanol. We first purged the columns at 5 mL/min for 5 minutes and then closed the purge valve and ran the instrument for 5 minutes at a flow of 1 mL/minute just to wash the column out. Next, we selected the right method and checked the injection volume to be 50 µL. We then placed the vial set to the same position where it has been positioned in the sample tray and set the wavelength to 520 nm for anthocyanin and the cherry samples. We added at least 2 blanks each at the start and the end of the sequence and between two different samples. Before initiating the sequence, we rechecked the solvent and the sequence as well as all the post run commands. The RP-HPLC was then run for all our tart cherry samples and the data was collected within the Chemstation software.

4.2.9 Statistical Analysis

Micronization was performed in triplicates, and all the chemical assays were conducted in triplicate. The Proc GLM and Proc REG functions of SAS University edition (SAS Inst., Cary, NC) were used to analyze two-way design data and lack-of-fit tests, respectively. Tukey's honestly significant difference was employed as post-hoc test and means were considered significantly different at $p < 0.05$.

4.3 Results and discussion

4.3.1 Micronization

As seen in our previously published study (Lukhmana et al., 2018), we observed that all the micronization methods resulted in smaller particle sizes. Using megatron in

consecutive cycles reduced the particle after every cycle. The smallest particle size was seen when High-pressure homogenization (HPH) was employed on the samples. It has been observed by researchers that particle size reduction leads to higher surface area and a diminution in the mean particle distance, that in turn leads to greater interaction between particles, and better dispersion within the pectin network, therefore developing higher viscosity (Rao, 1992; Tiwari, O'Donnell, & Cullen, 2009). Another study (Sato & Cunha, 2009) analyzed the relation between particle size and rheological properties of jaboticaba pulp. Sieves with different pore sizes were used to obtain variable particle size by passing the sample through them. These various particle sized samples were then added to the serum at the same concentration (21.7% w/ w) and presented as the whole pulp. When the viscosity of these particles was studied, it was found that viscosity increased linearly with particle up to a point but then decreased when particle size became larger. Another group (Ouden & Vliet, 2006) reported similar results in tomato concentrate. Particles size variation also leads to variable compositions in a way that smaller particles may be made of parenchyma tissue whereas the larger particles may contain aggregates of seeds, skin, minor tissues or vascular bundles. The larger particles have a tendency of sedimentation due to shear force arising from internal instability leading to higher viscosity (Sato & Cunha, 2009). The size of the particle and the Inter-particle interaction are two major players that determine the inconsistency of a sample. Usually, higher interaction and smaller particles leads to lower inconsistency. The viscosity follows a trend wherein it increases with increase in particle size up to a point (Beresovsky, Kopelman, & Mizrahi, 1995). Very small particle size also leads to increase in interaction with the sample network, which increases viscosity (Andersen & Jordheim, 2008).

4.3.2 Chemical Analyses of tart cherry purée

The chemical assays assessment for micronized and non-micronized samples is listed in Table 4.2 and discussed individually in the following section.

4.3.2.1 Total Polyphenol content (TPC)

The polyphenol content of micronized and non-micronized samples is shown in Figure 4.1(a). There was an increase in polyphenols as the particle size decreased. Similar results have been reported by Zaiter, Becker, Karam, & Dicko (2016) and other researchers, where particle size reduction gave higher TPC values. Another published research on clear apple juice micronized by ultra-high-pressure technique reported a substantial increase in chlorogenic acid and slight increase in caffeic acid concentrations (Suárez-Jacobo et al., 2011). The high-pressure techniques caused disruption of cell walls and release of bound polyphenols leading to this sudden increase in apple juice (Abid et al., 2013; Kataoka, 2010). The sonochemical reactions also lead to release of hydroxyl radicals that attach themselves to the phenolic group aromatic rings and another study that proved that the addition of second hydroxyl group to the ortho- or para-positions boosts the antioxidant capacity (Kumar et al., 2008). A research group (Aaby, Skrede, & Wrolstad, 2005) reported that the total phenols in strawberry were in the range of 2.30–3.40 g kg−1 gallic acid equivalent (GAE).

As compared with unprocessed pulps, the total phenols POD and PPO are considered to be the main enzymes responsible for phenol decay in processed strawberries and their derived foods. POD and PPO are capable of oxidizing (+)-catechin, inducing (+)-catechin degradation and brown polymer formation (Andersen & Jordheim, 2008).

However, total phenols in strawberry pulps significantly increased after HHP treatment at 500 or 600 MPa for 5 min. Another group reported (Patras, Brunton, Da Pieve, & Butler, 2009) an increase in the total phenols of strawberry purée by 8.3–9.8% and in grape by-products after HPH treatment (Corrales et al., 2009) at 500 or 600 MPa. Molybdenum (Mo6+) and wolfram (W6+) were reduced to Mo2+ or W2+ in the Folin–Ciocalteu method giving higher TPC values due to higher availability of antioxidant anthocynains, amino acids and proteins due to HHP treatment. Another important result that was in-sync with other published research was that TPC was highest in HPH followed by megatron and then non-micronized, following the particle size pattern.

4.3.2.2 Total Flavonoid Content (TFC)

The total flavonoid content for the micronized and non-micronized samples is reported in Figure 4.1(b). The trend for flavonoid content follows the similar pattern as that of polyphenols, i.e. there is increase in TFC values with reduction in the particle size. Hence, high-pressure homogenization had the highest flavonoid content but the difference between values of HPH and megatron is very small. Similar pattern of increased TFC values with decreasing particle size has been observed by other researchers (Gião, Pereira, Fonseca, Pintado, & Malcata, 2009). A study involving orange juice sample micronized with high pressure homogenization also showed a significant improvement in each individual flavanone as compared to non-micronized juice (Plaza et al., 2011). Another study on mandarin leaves extract also found higher values of TFC with decrease in particle size (Ciğeroğlu, Kırbaşlar, Şahin, & Köprücü, 2017). Some studies demonstrated the significant reduction in the flavonol/flavonoid contents of fruits and vegetables during

processing (Hertog, Hollman, Hertog, & Katan, 1992) but that may be attributed to a lot of factors, such as particle size range, temperature, processing and storage conditions. Another study found that particle size reduction by ultra-sonication led to higher values of TFC corroborating our results for tart cherry purée (Das & Eun, 2018)

4.3.2.3. Total Monomeric Anthocyanins

The effect of micronization on total anthocyanins of tart cherry purée are shown in Figure 4.1(c). We observed that there was a significant increase in the release of anthocyanins with particle size reduction. The increase was over 33% in micronized sample, as compared to the control. However, when the two methods of micronization (megatron and HPH) were compared, it was observed that the change in the values was statistically insignificant. Previous literature indicated that the sonication of apple juice did not show any significant ($P > 0.05$) change in the total anthocyanins but they remained stable when compared with non-micronized juice samples (Abid et al., 2013). Previous studies on anthocyanins stability in fruit juice by employing non-thermal treatments has been reported (Tiwari et al., 2009). Another publication on the strawberry juice showed higher retention of anthocyanins when treated with pulse electric field using high frequencies and low pulse widths creating smaller particle sizes (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2009).

The highest stability of Pg-3-glu and Pg-3-rut was found when strawberries were processed at 800 MPa and stored at 4°C. Another group (Kouniaki, Kajda, & Zabetakis, 2004) also reported that cyanidin-3-rutinoside in HHP-treated blackcurrants at 600 MPa was more stable than unprocessed samples, while the lowest loss of delphinidin-3-

rutinoside is at 800 MPa and stored at 5°C. In general, these results showed a high stability and an increase of the monomeric anthocyanins in strawberry subjected to HHP treatments. HPH treatments aided the extraction of monomeric anthocyanins from strawberry pulps. Another study found that extractability of colored pigments in food components was increased at extreme pressures. Anthocyanins are located in the vacuoles in plants, and HPH is known to rupture membranes enhancing cell permeability owing to its aptitude to deprotonate charged groups and disrupt salt bridges and hydrophobic bonds in cell membranes (Nayak & Rastogi, 2010). Thus, HHP assisted the extraction of anthocyanins from strawberry pulps more accessible to solvent. An optimization of anthocyanin extraction from red grape skins assisted by HHP was reported by a research group (Corrales et al., 2009), which demonstrated it to be an effective technology for extraction purposes (Gössinger et al., 2009). Another published study found that there was 40% degradation of Pg-3-glu in strawberry purée pasteurized at 85°C for 10 min (Sadilova et al., 2007). One group showed that Pg-3-glu purified from strawberry decreased after heating at 95 ◦C and suggested that the first step in anthocyanin degradation is the opening of the pyrylium ring; de-glycosylation then occurs. The cleavage products of Pg-3-glu are phloroglucinaldehyde and 4-hydroxybenzoic acid, and the former is the substrate of PPO.

4.3.3 Antioxidant activity

We also studied the antioxidant activity of micronized and non-micronized sample by various assays. Some researchers have applied ultrasound processing to inactivate enzymes responsible for the spoilage of fruit juices such as pectin methylesterase,

peroxidases, and polyphenoloxidases (Kuldiloke, 2002; Lopez, Vercet, Sanchez, & Burgos, 2002; Vercet, Lopez, & Burgos, 1999; Vercet, Sánchez, Burgos, Montañés, & Lopez Buesa, 2002). Another group (Kumar et al., 2008) reported that the antioxidant activity of components such as flavonoids may increase because of the increase in the degree of hydroxylation of molecules due to radicals OH^- formed in ultrasound processing. Another study found that ultrasound processing increased the phenolic content in pineapple juice. Also, a group found that (Lieu & Le, 2010) sonicated samples presented an increase of 114.3% on concentration of total phenolics. The reason for this huge influx in the availability of phenolics was attributed to the random breakdown of cell walls of grape cells that usually contain these phenolics and are set free due to the ultrasonication.

4.3.3.1 FRAP Assay

The FRAP assay, as developed by Benzie and Strain (1996), is based on a different chemical (redox) reaction for measuring antioxidant capacity to that employed by more direct methods, such as the ORAC (oxygen radical absorbance capacity) and TEAC (trolox equivalent antioxidant capacity) assays. The FRAP assay results for micronized and non-micronized tar cherry samples is shown in Figure 4.2(a). As we can see that due to changes in the tissue matrix induced by high hydrostatic pressures, for example disruption of plant cell walls, resulted in the release of metabolites into the extracellular environment thereby increasing FRAP activity for micronized sample as compared to non-micronized. The value was slightly higher for HPH then for megatron micronized samples owing to particle size and effect of high pressure on the sample. Our results were in agreement with another study done on strawberry and blackberry purées wherein HPH gave a boost in the FRAP

values (Patras et al., 2009). The FRAP assay, due to its low cost, speed and technical simplicity, is a useful tool for estimating total reducing/antioxidant power of aqueous vegetable extracts. It has been shown to yield values which correlate closely to those obtained by the TEAC assay for a range of fruits and vegetables (Pellegrini et al., 2018; Proteggente et al., 2002) and the ORAC assay for some vegetables, including carrots and broccoli (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002), and fruits (Aaby et al., 2005). However, the limitations of using a simple in vitro assay system to predict the complex in vivo functionality of antioxidant rich foods must be acknowledged (Collins, 2005).

4.3.3.2 DPPH Assay

The radical scavenging capability of the micronized and non-micronized cherry samples was determined with the DPPH method using a methanol solution of anthocyanins (Brand-Williams, Cuvelier, & Berset, 1995) as the spectrophotometric blank, in order to minimize the interference of anthocyanins at the absorption wavelength $\lambda = 517$ nm (Ge & Ma, 2013). The antiradical activity was expressed as percentage of DPPH· inhibition (% I) exerted by the sample extracts. It has also been seen in studies that antiradical DPPH activity was higher for tart than for sweet cherries (Picariello, Ferranti, De Cunzo, Sacco, & Volpe, 2017). The results for our assay can be seen in Figure 4.2(b). It is clearly seen that the antiradical activity of the sample is higher for micronized sample than non-micronized sample. In fact, we can see that HPH micronized sample has twice the amount of DPPH scavenging activity than the non-micronized sample clearly showing the benefits of micronization. Another study done on tomato purée found a good amount of increase in

%inhibition values for high-pressure processed samples (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2006).

4.3.3.3 Reducing Power Assay

The reducing capability of the micronized and non-micronized tart cherry sample was measured by the transformation of Fe^{3+} to Fe^{2+} in the presence of the extract. Increased absorbance of the reaction mixture indicates increased reducing power (Oyaizu, 2011). In our body, free radicals of different forms are constantly generated for specific metabolic requirement. When the generation of these species exceeds the levels of antioxidant mechanism, they cause extensive damage to the cells leading to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases and extensive lysis (Halliwell & Gutteridge, 1991). The reducing power of tart cherry samples can be seen in Figure 4.2(c), wherein there is definite increase in the values of micronized sample as compared to non-micronized samples. From these results we can clearly conclude that tart cherry and its micronization showed good reducing power activity which can be attributed to tannins and phenolics along with other compounds that may have got released due to cell wall rupture by high pressure homogenization (Rajani, Anandjiwala, Bagul, & Parabia, 2009).

4.3.4. Total solids content

The increase in contents of sugars might be attributed to the breakage of cell wall components due to high-pressure treatment which extracts the sugars from intracellular spaces to the liquid (Hilz, Lille, Poutanen, Schols, & Voragen, 2006). Due to mechanical

effects exerted by shear forces produced by ultrasound, the penetration power of solvent into the sample matrix increases (Aadil et al., 2015). Figure 4.3 shows the amounts of total solids, insoluble and soluble solids in non-micronized and micronized tart cherry sample. As can be seen from the results, the %proportion of soluble solids increases due to processing and reduction in particle size. This is probably due to the high-pressure exerted during HPH converting a fraction of insoluble content into soluble solids. The insoluble content is highest in non-micronized sample of tart cherry for the same reason.

4.3.5 FT-IR spectroscopy

Qualitative analysis of phenolic acids and anthocyanin was done by FT-IR spectroscopy between 400-4500 cm^{-1} wavenumber range on the non-micronized samples of tart cherry purée as obtained from the commercial supplier. This can be seen in the spectrograph in Figure 4.4. The software within the FT-IR instrumentation includes modules for collecting and processing of data, quantitative analysis, generating of own spectra libraries, identification of compounds using own and standard spectra libraries, as well as a bibliography on IR-spectroscopy (Pappas, Takidelli, Tsantili, Tarantilis, & Polissiou, 2011). The bands at 1640–1630, 1604–1585 and 1580– 1562cm^{-1} corresponded to double bond (C5C) stretching. Consequently, the peak at 1640–1630 cm^{-1} can be correlated to the stretching of aromatic C5C in anthocyanins content (Jose, Phadke, & Rao, 1974; Koeppen & Basson, 1966; Merlin et al., 1994). The presence of phenolic compounds gives absorption bands caused by stretching vibrations of the free OH groups (frequency 3670-3580 cm-1), intra- and intermolecular H-bonds in dimers and polymers (frequency 3400-3200 cm-1) (Golubtsova, 2017). The peak around 2900 cm^{-1} is usually associated

with the presence of carbohydrates absorption by valence vibrations of CH_2- groups (Krukowski et al., 2015). The absorption peaks at 1638 and 1444 cm^{-1} corresponded to the aromatic and heterocyclic rings' skeletal vibration in the chromene of cyanidin-3-glucoside (C3G) (Zhao et al., 2016).

Since we were able to identify four major peak regions, we performed FT-IR analysis on non-micronized and micronized samples and the stacked spectra results can be seen in Figure 4.5. All 4 regions, i.e. anthocyanins, carbohydrates and phenolic functional groups were present in all samples. It is clearly seen that the anthocyanins and phenolic group peaks are more prominent in the high-pressure homogenized sample. This is analytical proof of presence of higher amounts of anthocyanins, polyphenols and flavonoids in the micronized samples due to cell wall breakage as we have observed in our previous chemical assays results.

4.3.6 Qualitative analysis of cherry anthocyanins by RP-HPLC

Some researchers have used a DAD detector with simultaneous monitoring and detection of bioactives at multiple wavelengths i.e. λ= 520 nm for anthocyanins, 360 nm for flavonols, 320 nm for hydroxycinnamic acids and 280 nm for general phenolics in various cherry varieties. The comparative analysis of HPLC profiles at multiple wavelengths enables screening of bioactives that belong to the class of colorless polyphenols (favan-3-ols, namely catechin and epicatechin, flavanols and hydroxycinnamic acids**).** Since we were primarily interested in the presence of anthocyanins, we monitored the tart cherry purée at a wavelength of 520 nm. The results indicated appearance of some identifiable peaks with the reduction in particle size.

However, some peaks were non-identifiable and could presumably be the result of the degradation products. The major peaks in the chromatogram were identified based on their retention times (t_R). Some researchers have indicated the presence of hydroxycinnamic acids (such as neochlorogenic, chlorogenic acid and 3-O-pcoumaroylquinic acid) and quercetin derivates at 280 nm (Mozetič & Trebše, 2004; Haibo Wang et al., 1999). Expression of a particular type of caffeoyl derivatives (isomer of chlorogenic acid) have been reported in tart cherries (and not in sweet cherries) (Wojdyło et al., 2014).

However, these are not good candidates to serve as markers, since high fluctuations have been reported in their quantity, based on ripening stage and thermal degradation (Bonerz, Würth, Dietrich, & Will, 2007). Interestingly, kaempferol-3-O-rutinoside (quercetin derivative), has been reported to be exclusively present in tart cherries and is the best candidate qualifying as a marker for cherry flavonoid identification studies. In our study, we observed the appearance of several peaks between t_R of 19 min to 27 min. By comparing these peaks with those reported in the published literature, we identified them as cyanidin derivates (major class of anthocyanins present in tart cherries). Another group, Chaovanalikit & Wrolstad (2004), reported differences in qualitative peculiarities between sweet and tart cherries. Tart cherries exhibit a more complex repertoire as opposed to their sweet counterparts, and specifically have been found to exclusively contain more cyanidin-3-O-rutinoside and cyanidin-3-O-glucoside, whereas sweet cherries only exhibit the peaks corresponding to cyanidin-3-O-glucosylrutinoside. Another peak observed in this study indicated the presence of cyanidin-3-O-xylosylrutinoside at detectable levels with particle size reduction (Table 4.1) and the presence of this branched tri-glycoside in P. cerasus has been previously described (Dekazos, 1970). However, in contrast to some of the previous

studies (Mozetič & Trebše, 2004; Wu & Prior, 2005), we did not find the presence of pelargonidin-derivatives in our results. Also, we did not observe any peak corresponding to degradation compound or pyranoanthocyanins probably due to breakage of hydrogen bonds during elution by HPLC. Another probable reason could be the formation of large molecular weight condensation products of anthocyanins and other phenolics, that got separated in reverse-phase C18 column or during pre-HPLC filtration (Hager, Howard, & Prior, 2008).

Therefore, to summarize, the reduction in particle size led to the appearance of more peaks that were qualitatively identified. Also, there was a small increase in the area under the peaks (Table 4.1), indicating an increase in the amount of the major class of anthocyanins in tart cherries. However, more confirmatory studies need to be done (such as MALDI-TOF MS) to quantitatively determine the differences. Also, the chemo-taxonomical differences between sweet and tart cherries have been linked to higher metabolic biosynthesis of additional anthocyanins and complex genomics in P. cerasus (tetraploid) when compared to P. avium (diploid, 2n = 16) (Dekazos, 1970; Delazar, Khodaie, Afshar, Nahar, & Sarker, 2010; Pantelidis, Vasilakakis, Manganaris, & Diamantidis, 2007).

4.4 Conclusions

The tart cherry purée samples were micronized using various homogenization methods and the two final methods i.e. megatron and high-pressure homogenization were selected based on their particle size distribution. Microparticles generated by both these methods were analyzed for changes in polyphenolic, flavonoid and anthocyanins content.

It was observed that there was a significant increase in the polyphenolic content of micronized samples as compared to the non-micronized sample. The increase was highest in high-pressure homogenized samples, perhaps due to the rupture of cell wall structure and the release of bioactives trapped within the cellular matrix. The flavonoid content followed the same trends of extractability as the polyphenols and was in agreement with the previously published literature. These results indicate a potential increase in the release of polyphenols with particle size reduction. By conducting a comparative study for different micronization methods, we found that the desired particle size of 100 μm was obtained by micronizing the tart cherry purée by high-pressure homogenization (HPH), that resulted in the highest polyphenolic extraction yield of 285.05 GAE μg/ml when compared to the non-micronized sample (166.80 GAE μg/ml), indicating an increase of 70.89%. However, comparative analysis between the two methods indicated that the differences between megatron and high-pressure homogenization (HPH) were not statistically significant, indicating a partial overlap in the particle size distributions obtained by these two methods and therefore, a limited effect of particle size reduction within the range of 100 to 300 μm, on the polyphenol extractability. We also analyzed the micronized samples for antioxidant activity to assess their potency and anti-radical activity that imparts health benefits. The FRAP assay yielded higher activity for the micronized samples and the values for DPPH inhibition for the HPH micronized sample was twice the amount of the non-micronized sample, clearly showing the benefits of micronization. Upon comparison of the total solid (insoluble and soluble) content for the samples, we observed higher soluble solids for megatron treated (7.5%) and high-pressure homogenized samples

(8.1%), as compared to the control (6.25%). The FT-IR spectral analysis and reverse-phase HPLC of the micronized and the non-micronized samples provided information about the presence of functional groups and helped us to qualitatively detect the presence and relative amounts of various polyphenols and anthocyanins in the tart cherry samples. Overall, we were able to corroborate our hypothesis that the particle size reduction leads to a higher extractability of polyphenols and anthocyanins in tart cherry.

4.5 References

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Table 4.1: Retention time and peak area of solvent and anthocyanins released by samples during HPLC analysis.

HPLC peaks	Sample	Retention Time (min)	Solvent/Anthocyanin	Area (muA.s)
1	**Non-Micronized**	1.46	Solvent	52.2
2		13.15	Cyanidin derivatives	1.0
3		20.03	Cyanidin-3-O-Glucoside	1.4
1	**Megatron**	1.48	Solvent	41.1
2		15.38	Cyanidin-3-O-Sophoroside	1.3
3		19.63	Cyanidin-3-O-Xylosylrutinoside	1.6
4		19.94	Cyanidin-3-Rutinoside	1.4
5		20.15	Cyanidin-3-O-Glucoside	0.8
1	**High-Pressure Homogenization**	1.48	Solvent	44.1
2		20.71	Cyanidin-3-O-Glucoside	1.1
3		22.12	Cyanidin-3-Glucosylrutinoside	2.0
4		24.44	Delphinidin-3-O-Rutinoside	1.2
5		25.74	Peonidin-3-O-Rutinoside	2.3

Table 4.2: Chemical assay values for non-micronized and micronized (megatron and high-pressure homogenized) tart cherry sample.

Sample	TPC (Gallic Acid Equivalent in µg/mL)	TFC (Quercitin equivalent in µg/mL)	TMAC (Cyanidin-3-glucoside equivalent in mg/L)	FRAP (Fe^{2+} ion concentration in µg/mL)	DPPH (% inhibition)	RPA (Ascorbic acid equivalent in µg/mL)
Non-Micronized	166.80 ± 12.8[a]	128.66 ± 8.2[a]	59.05 ± 4.9[a]	676.2 ± 65.4[a]	6.09 ± 0.6[a]	8.41 ± 0.8[a]
Megatron	256.52 ± 19.2[b]	186.72 ± 14.2[b]	76.93 ± 6.8[ab]	892.2 ± 74.2[ab]	10.27 ± 1.1[ab]	10.54 ± 0.9[ab]
HPH	285.04 ± 1.4[bc]	194.44 ± 15.4[bc]	101.06 ± 8.2[bc]	916.3 ± 75.1[ab]	12.49 ± 1.5[c]	11.73 ± 1.1[bc]

Columns with same letter are not statistically different at $p < 0.05$.
Abbreviations: TMAC, total monomeric anthocyanin content; FRAP, ferric reducing antioxidant power; TPC, total phenolics content; DPPH, 2, 2-diphenyl-1-picryl-hydrazyl-hydrate; RPA, reducing power assay; TFC, total flavonoid content. (Number of observations = 3)

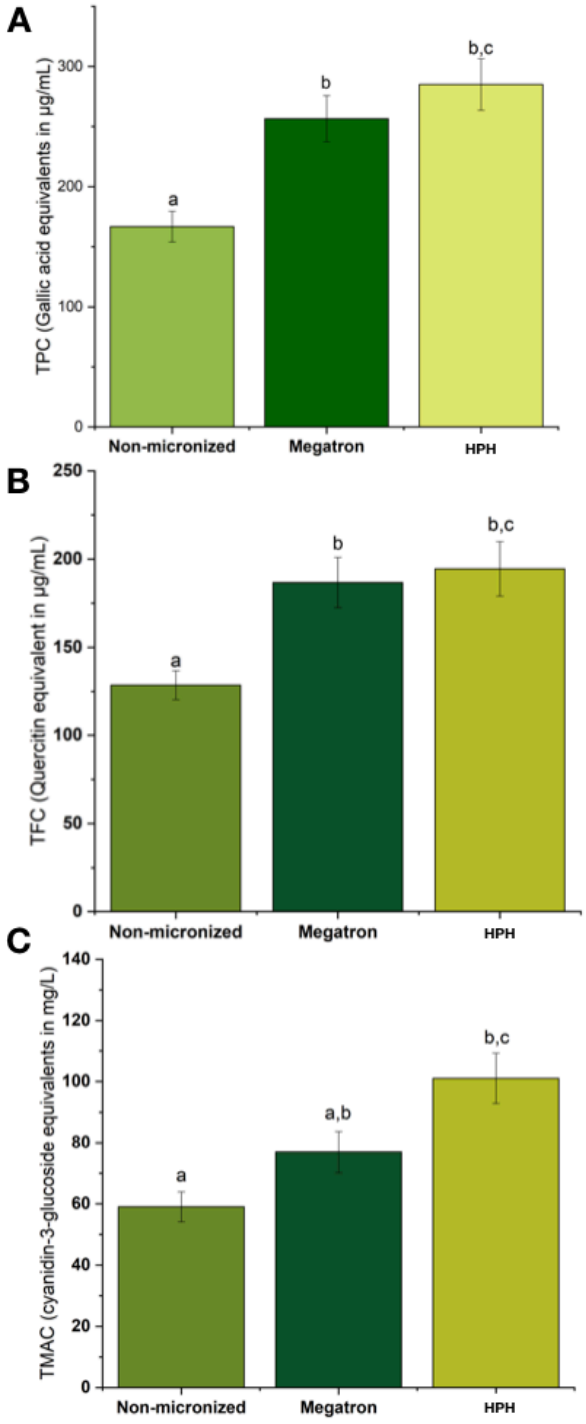

Figure 4.1: Chemical assay values for non-micronized and micronized (megatron and high-pressure homogenized) tart cherry sample viz. a) Total Polyphenolic content, b) Total Flavonoid Content, c) Total Monomeric Anthocyanins

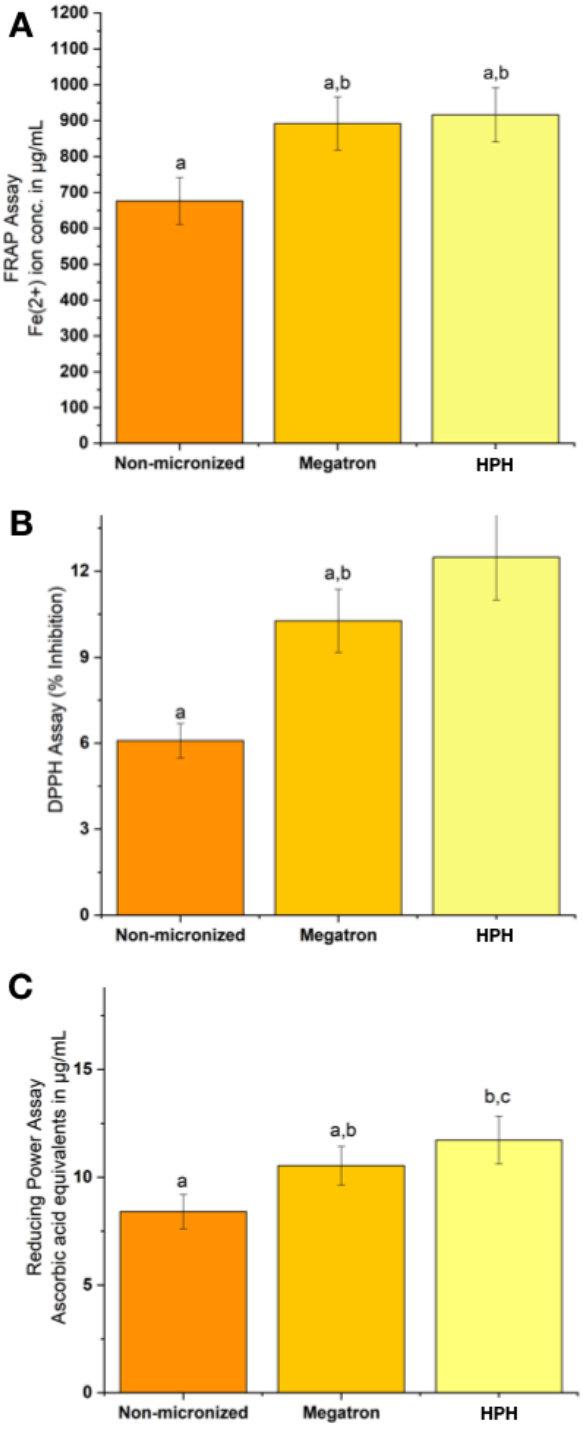

Figure 4.2: Antioxidant assay values for non-micronized and micronized (megatron and high-pressure homogenized) tart cherry sample viz. a) Ferric Reducing Antioxidant Power, b) DPPH scavenging assay, c) Reducing power assay.

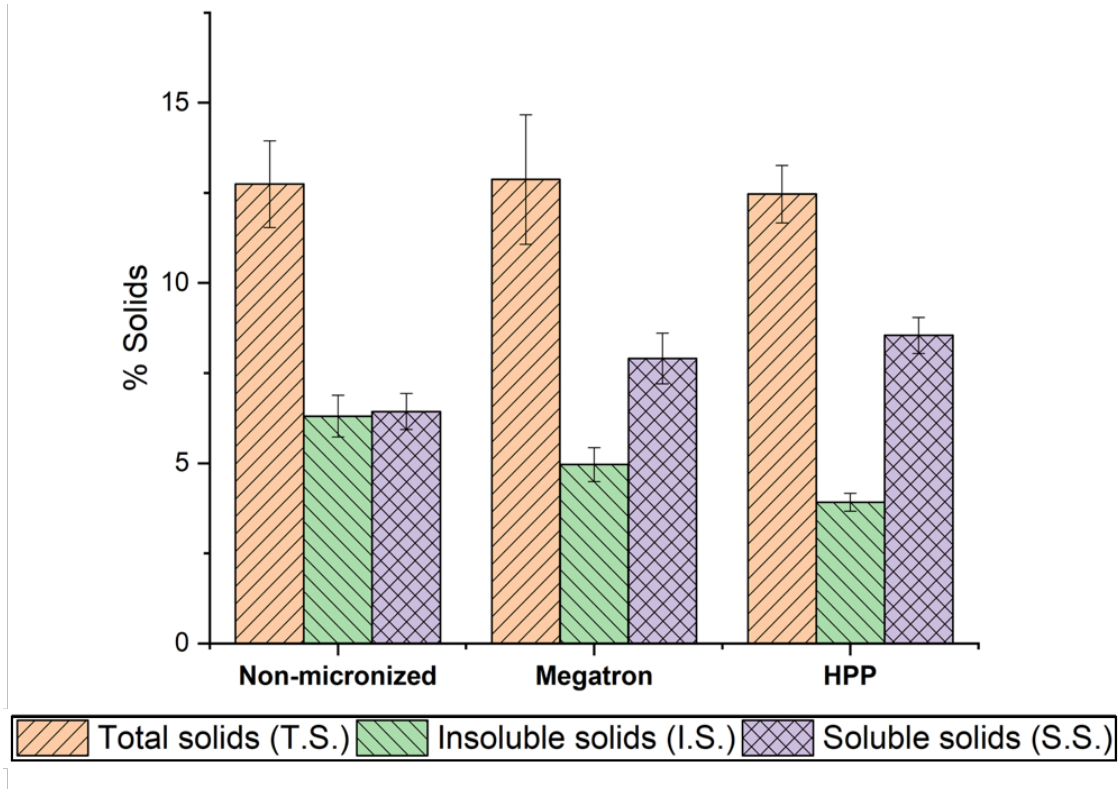

Figure 4.3: Total solids content compared with insoluble and soluble solid values for non-micronized and micronized (megatron and high-pressure homogenized) tart cherry sample.

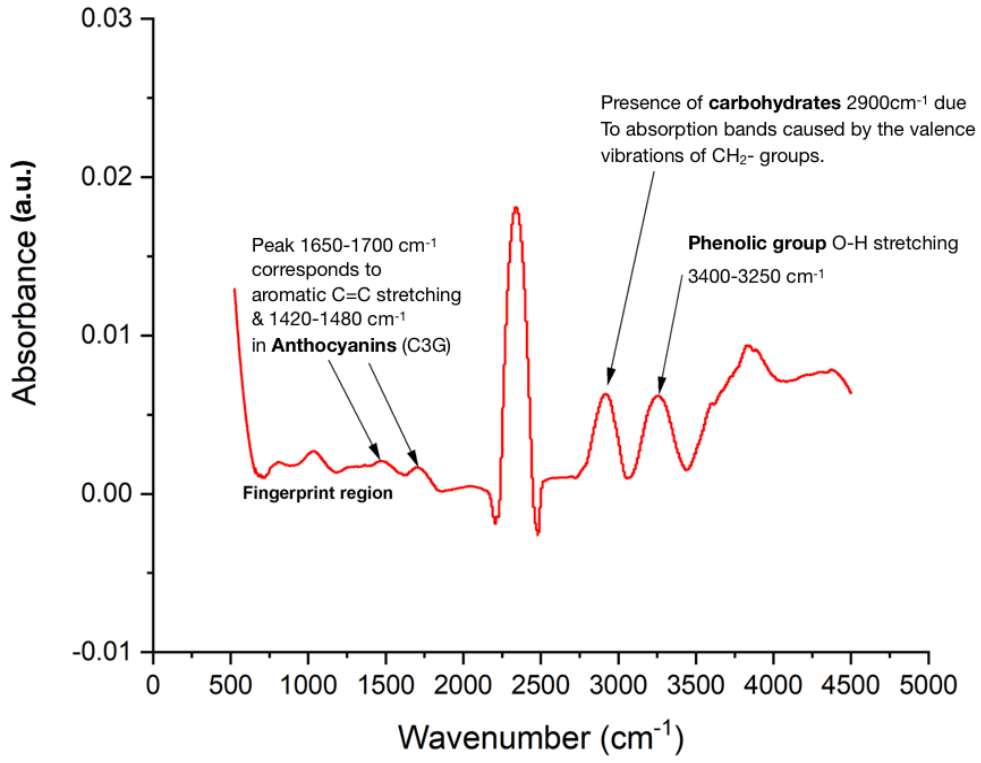

Figure 4.4: FT-IR spectra with peak values for non-micronized tart cherry sample.

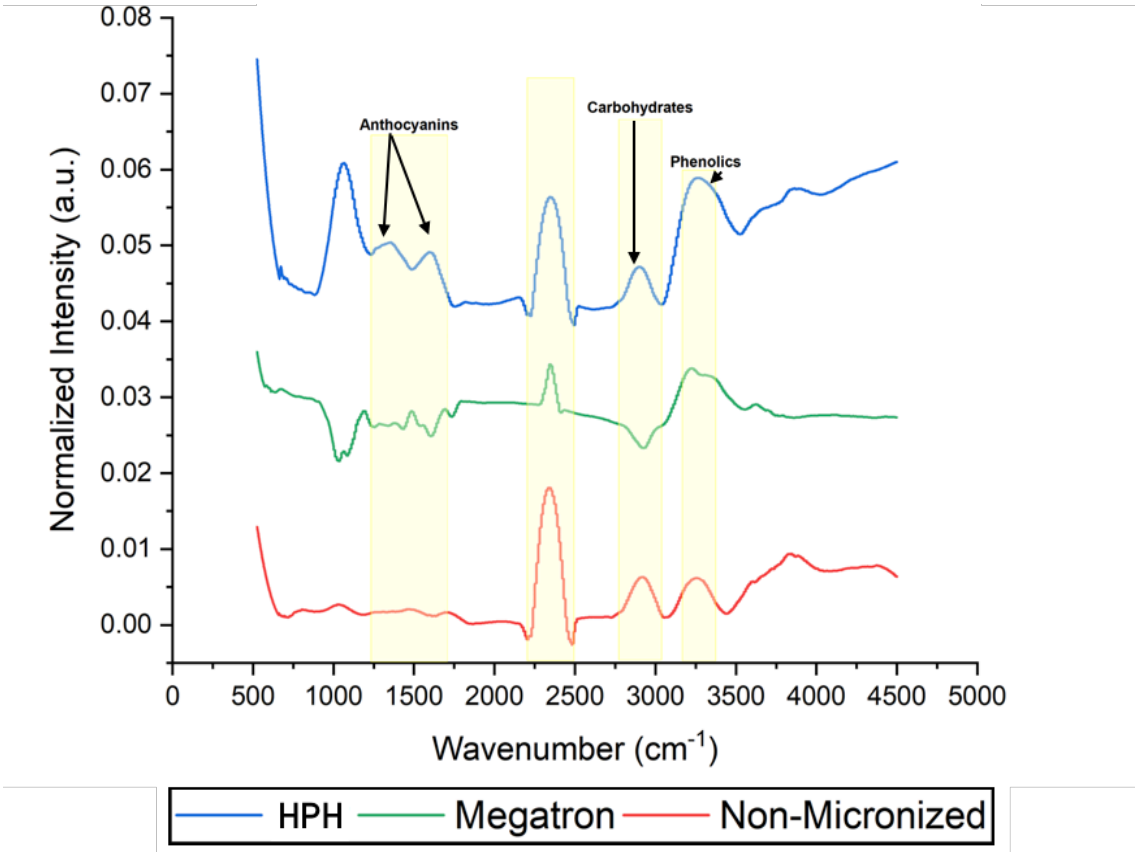

Figure 4.5: FT-IR spectra and peak group values for non-micronized and micronized (megatron and high-pressure homogenized) tart cherry sample.

CHAPTER 5

EFFECT OF MICRONIZATION AND WALL MATERIAL ON THE PROPERTIES OF SPRAY-DRIED TART CHERRY MICROCAPSULES

5.1 Introduction

Tart cherries have low caloric content and provide significant amounts of important nutrients and bioactive food components (BAFC). These include vitamin C, fiber, anthocyanins, quercetin, and carotenoids (Kelley, Adkins, & Laugero, 2018). The anthocyanin content as well as total phenolics is high in tart cherries as compared to other berries and research has indicated that the beneficial health effects may be partially associated with the abundance of anthocyanins, the glycosides of cyanidin (Haibo Wang et al., 1999).

Functional fruit and vegetable juices are often microencapsulated (ME), a technology that entails the packaging of solid, liquid and gaseous materials within small capsules that can be further used to release their contents at controlled rates over prolonged periods of time (Boye & Arcand, 2013). Microencapsulation is of significant interest to the food industry, since it can be used to reduce the cost of production, to increase the stability of compounds, to mask undesirable tastes, and to improve the release properties of compounds in food (Peanparkdee, Iwamoto, & Yamauchi, 2016). Specifically, ME of fruit pomace increases the effectiveness of the natural functional compounds that usually have a lower potency at equivalent levels when compared to synthetic ingredients (Yallapu, Gupta, Jaggi, & Chauhan, 2010). Functional fruits with potential health benefits are being produced by employing microencapsulation techniques like spray drying, spray cooling, extrusion, coacervation, lyophilization and emulsification (Caballero, Trugo, & Finglas, 2003). Since fruit purées have a short shelf life, microencapsulation is used to enhance the storage time, and also to prevent the degradation of polyphenols after the consumption, during different phases of digestion (Phisut, 2012)

Microencapsulation by different drying techniques have been used such as freeze drying, natural convective drying, forced convective drying, microwave drying and spray drying; out of which spray drying is the most popular technique and is widely used for powder-form generation from liquid juices and purées (Phisut, 2012). Spray drying process has been used for decades to encapsulate food ingredients such as anthocyanins, flavonoids and carotenoids (da Costa et al., 2018; Janiszewska-Turak, 2017; Osorio et al., 2010), and it keeps the flavor loss to a minimum. Milk powder, instant coffee, dry creamer, instant soups, baby foods are all examples of spray-dried foods. Spray drying keeps the retail price of such foods low, because the process extends the product's shelf life (Afoakwah, J, Engman, & Hannah, 2012). The major steps in the process of spray drying include the preparation of liquid feed, atomization and droplet contact with the hot air, drying of atomized droplets and the separation of the spray dried powder from the hot air (Verma & Singh, 2015). The liquid feed undergoes atomization in an atomizer at the entrance of drying chamber, when the spray droplets mix with the hot air and the dried particles are generated as a result of the evaporation of the liquid component. Spray dryer performance is recognized by its most important index called the product recovery (Maa, Nguyen, Sit, & Hsu, 1998; Masters, 1991). Spray dried fruit juice powders have several advantages over their liquid counterparts such as reduction in volume and packaging, ease in handling and transportation, and a relatively longer shelf life. Furthermore, they find custom use as flavorings and coloring agents in many food products, due to their stable physical state and a convenient attribute of being a dosable ingredient (Shrestha, Howes, Adhikari, & Bhandari, 2007). However, the spray drying of fruit and vegetable juices that are rich in sugars pose a problem during the process of spray drying, predominantly due to the low

glass transition temperature (Tg) of sugars present in those products (Bhandari, Datta, & Howes, 1997). Besides that, the product may have high stickiness as a result of their high hygroscopicity, low melting point, and high solubility (Goula & Adamopoulos, 2010). The sugars react with the acids to form a sticky substance at the interactive surface between the feed and glass in the drying chamber. This leads to a reduction in product yield and generates a need to optimize the drying conditions. Other factors that affect the product yield and physical properties of the final powder are the choice of wall material and its concentration, drying temperature, atomization speed and pressure and feed flow rate. (Tontul & Topuz, 2017).

The common wall materials used in the encapsulation of fruit purées are maltodextrin, gum Arabic (GA), plant proteins and milk proteins (Tontul & Topuz, 2017). In our study, maltodextrin and gum Arabic (GA) were chosen as wall materials for their encapsulating properties. Maltodextrin is essentially a form of hydrolyzed starch that has a dextrose equivalent (DE) value less than 20 and, is popularly known for its low viscosity at higher concentrations. Hydrolyzed starches of DE values higher than 20 (such as dried glucose syrup) have lower oxygen permeability and pose a problem in terms of being vulnerable to caking during extended storage (Shahidi & Han, 1993). Gum Arabic, produced from the exudates of Acacia trees, is a complex hetero-polysaccharide, known for its high solubility and low viscosity, both of the characteristics that make it a good choice for a carrier material during encapsulation of fruit juices and purées (Turchiuli et al., 2005). Gum Arabic is upto 50% soluble in both cold and hot water and has a ramified and branched structure and protein content (2%), that is responsible for its functional properties. Arabinogalactan fraction present in the gum Arabic (McNamee, O'Riordan, &

O'Sullivan, 1998) contributes towards its film forming properties and thereby makes it a useful carrier material.

The aim of this study was to determine the changes in the physical attributes, morphology and chemical properties of spray-dried tart cherry purée as affected by micronization methods (megatron and high-pressure performance) and wall materials combinations (gum Arabic and maltodextrin).

5.2 Materials and Methods

Folin-Ciocalteau (FC) reagent, sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, sodium acetate trihydrate, acetic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric (III) chloride hexahydrate, vanillin, sulphuric acid and assay standards, including trolox, gallic acid, ethanol, rutin and aecsin used were all reagent-grade chemicals purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). The encapsulating agents, Maltodextrin (STAR-DRI 100) was obtained from Tate & Lyle (Decatur, IL) with dextrose equivalent (DE) value between 9 and 12 and Gum Arabic from acacia tree was obtained from Sigma Life Sciences (St. Louis, MO). Deionized water (DI) was prepared fresh before use using a Millipore Milli-Q water purification system (Burlington, MA).

5.2.1 Sample procurement and preparation of micronized sample

Frozen tart cherry purée was obtained from Cherry Central, Inc, Michigan, USA and was thawed for 12 h at room temperature, followed by 12 h in cold room (4˚C). The sample was divided into small batches in plastic (polyethelene) freezer safe bags and stored

at -20˚C for further studies. Micronization of samples was done in a Megatron MT5000 (Kinematica AG) at 15000 rpm for three cycles (M1, M2, M3; 8-19 min each) followed by high pressure homogenizer (Stansted Fluid Power Ltd.) at 200 MPa. Megatron can generate particle size ~300μm and a high-pressure throttling system can create particle size <100 μm. Temperature of purée during micronization experiments were monitored using a digital thermometer and was kept below 45˚C to prevent any chemical modification of the sample. These micronized cherry samples were compared with the cherry purée obtained by common blending methods, which had particle size 800-1000 μm. The target particle size of the micronized sample was below 300 μm, which was measured using a Malvern Laser Particle Size Analyzer, Mastersizer S with 300 mm lens (Malvern Instruments, Southborough, Mass.). In order to reach the desired particle size distribution, the sample was cooled to 1-2˚C and the micronization process was repeated twice.

5.2.2 Selection of wall material and preparation of emulsions.

Maltodextrin powder (STAR-DRI 100) was obtained from Tate & Lyle (Decatur, IL) with dextrose equivalent (DE) value between 9 and 12, and gum Arabic was obtained from Sigma Life Sciences. Aqueous stock solutions of maltodextrin isolate (MD) and gum Arabic (GA) were each prepared at 30% (w/v) and in varying concentrations maltodextrin and gum Arabic, such as 20% MD: 10% GA (w/v), 15% MD: 15% GA (w/v) and 10% MD: 20% GA (w/v) in deionized water at room temperature (25˚C). The aqueous stock solutions were stirred on a magnetic stirrer (VMS-C7, VWR, Germany) for a duration of 45 min each and then allowed to rehydrate in the refrigerator (4-6˚C) overnight for 15 h. After rehydration of all the five wall material stock solutions, emulsions were prepared by

mixing non-micronized (A), and micronized samples obtained from megatron (B), and high pressure homogenization (C) at a ratio of 1:3 (cherry sample : wall material), resulting in fifteen combinations (1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B, 4C, 5A, 5B, 5C), as shown in Table 5.1.

5.2.3 Rheology of the emulsions

Rheological characterizations of the samples were carried out using a stress-controlled rheometer (TA Instruments, New Castle, DE) controlled with a commercial computer software (Rheology Advantage Data Analysis Software v4.1.2, TA Instruments, New Castle, DE). About 50 mL of sample was loaded into a cup with 28.92 mm diameter. Evaporation was considered negligible due to short durations of measurement (i.e. 20–30 min). The vane geometry fixture was preferred over the cone and plate setup because the pulp in the purée tends to separate easily. Rheology of tart cherry purée microencapsulated using different combinations of wall material was measured at varying shear rates. The instrument was programmed to allow equilibration of the sample to a set temperature before starting the data collection. During pumping, in-pipe flow, mixing and stirring of liquid-like foods, shear rates in the range of 10–1000s^{-1} may be experienced. Therefore, rheological experiments should preferably be done within the shear rate range that encompasses most applications (Steffe, 1996).

Rheology measurements were taken by dividing into two categories; five wall material emulsions with varying concentration of maltodextrin and gum Arabic and fifteen combinations of the mixtures of the tart cherry purée samples with the wall material. The choice of an appropriate model to relate sample viscosity to Brix number and shear rate is

contingent upon the intended application and use of a suitable instrument to determine the model parameters.

For our samples, we employed the Herschel-Bulkley (HB) model, because it is a more accurate model of rheological behavior and is described mathematically as equation 5.1:

$$\tau = \tau_0 + k(\gamma)^n, \quad (5.1)$$

where
τ = shear stress
τ_0 = yield stress
k = consistency factor
γ = shear rate
n = flow index, a power law exponent.

5.2.4 Spray-drying process

Each of the fifteen sample combinations were pumped at 6 ml/min (20% pump speed) via a peristaltic pump to the atomizer of a Model B-290 mini spray-dryer (Büchi Corporation, Flawil, Switzerland) under the following process conditions: 175°C inlet air temperature; 85°C outlet air temperature; 100% aspirator rate (corresponding to a maximum air flow of 35 m^3/h), actual air flow rate of 0.667–1.744 m^3/h, and a nozzle setting of 1 cleaning cycle/min. The resultant powders were stored in polypropylene falcons at -20°C.

The inlet temperature of the dryer was set with a temperature regulator. However, the outlet air temperature depends on the inlet temperature, the pump setting, concentration of the feed and the aspirator setting. Fifteen different experiments (as mentioned in the Table 5.1) were conducted in triplicates.

5.2.5 Physical Characterization of the powder

In order to study the changes in the physical characteristics of the spray dried powder of non-micronized and micronized tart cherry samples, we measure the color, hygroscopicity, encapsulation efficiency, yield, particle size and morphology following protocols and using various instruments.

5.2.5.1 Color measurements

As a rapid estimate of anthocyanin content, the color values (L*, a*, b*) of the spray dried powder of fifteen samples of tart cherry purée were determined using MiniScanEZ 4500L portable spectrophotometer (HunterLab), referring to color space CIE Lab. The colorimeter was calibrated with black and white prior to use, and occasionally between two readings. Samples (2 g) were added to clear plastic dishes (1 cm depth and 3.5 cm diameter). The samples were measured against a white ceramic reference plate (L*= 93.92; a*= 1.03; b* = 0.52). The results were expressed as the mean of triplicates.

5.2.5.2 Hygroscopicity

The hygroscopicity was determined using the gravimetric method. The samples (2–5 g) were placed inside a glass petri dish within a desiccator with a constant relative humidity that was controlled with a supersaturated solution of KI (68.9% at 25°C) (Martínez Navarrete, 1998). To verify the condition for equilibrium between the samples and the environment, the weight of the samples was determined until reaching constant weight (± 0.001 g/g sample). The hygroscopicity was expressed in terms of moisture % (w.b.).

5.2.5.3 Encapsulation efficiency of anthocyanins and product recovery

The method from a previously published study (Idham, Muhamad, & Sarmidi, 2012) was employed with modifications. Fifty milligrams of the spray-dried powder were dissolved in 3 mL of 95% (v/v) ethanol in test tubes, agitated for 1 min with a vortex mixer and centrifuged for 10 min at 3823 x *g*. The supernatant was assayed for surface TMAC as described earlier and reported as mg surface C3G/g powder.

The encapsulation efficiency is defined as follows:

$$\% \text{ Encapsulation efficiency} = \frac{Total\ C3G/g - Surface\ C3G/g}{Total\ C3G/g} \times 100 \qquad (5.2)$$

5.2.5.4 Particle size analysis

The particle size distribution was measured using a Mastersizer® 2,000 laser light diffraction instrument (Malvern Instruments Co.). The particle size was measured based on the protocol mentioned in the section 3.2.2, and expressed as D(V,0.1), D(V,0.5) and D(V,0.9).

5.2.6 Scanning Electron Microscopy

The spray dried powder samples were mounted on SEM stubs with double-sided carbon tape, followed by sputter coating of the samples with a thin layer of Gold-Palladium of about 5 nm. The SEM images were then obtained using a Zeiss AURIGA Crossbeam Scanning Electron Microscope. SEM was operated at an accelerating voltage of 4.0-8.0 kV (Ferrari, Marconi Germer, Alvim, & de Aguirre, 2013).

5.2.7 Chemical Analysis of the spray dried powder

The chemical analysis of the spray dried powder was analyzed and assessed with respect to how the phenols, flavonoids, anthocyanins, reducing power and antioxidant potential fare as affected by the micronization and choice of wall material.

5.2.7.1 Total phenolics content (TPC) assay

The TPC of samples was determined using a modified Folin-Ciocalteu method (Singleton & Rossi, 1965). A total of 6.5 mL of deionized water, 0.5 mL of Folin-Ciocalteu's phenol reagent, and 1 mL of saturated sodium carbonate solution were added to test tubes containing 2.0 mL of the diluted powdered sample. A standard curve was generated with gallic acid and TPC was expressed as g GAE equivalents/mL and used to determine TPC concentration of samples based on the protocol elaborated in the section 4.2.3.1.

5.2.7.2 Ferric reducing antioxidant power (FRAP) assay

A modified FRAP assay was utilized (Benzie & Szeto, 1999). Results were determined using a standard curve prepared using 100 µL of various Trolox solutions (0.1 to 1.0 mM) based on the protocol detailed in section 4.2.4.1

5.2.7.3 DPPH Assay

DPPH method (Hua Wang, Gao, Zhou, Cai, & Yao, 2008) was used with some modifications. A calibration curve was prepared with a standard solution of DPPH at 5, 10,

15, 20 and 25 mg/L. Ascorbic acid was expressed as mg DPPH per 100 g dry matter in triplicate, based on the protocol detailed in the section 4.2.4.2.

5.2.7.4 Reducing Power Assay

The reducing capability of the sample extracts was measured by the transformation of Fe3+ to Fe2+ in the presence of the extract (Benzie & Strain, 1998), based on the protocol detailed in the section 4.2.4.3.

5.2.7.5 Total Monomeric Anthocyanins

Total anthocyanin content was determined by the spectrophotometric method (Esti, Cinquanta, Sinesio, Moneta, & Di Matteo, 2002), based on the protocol detailed in the section 4.2.3.3. Anthocyanin pigment concentration was calculated and expressed as cyanidin-3-glucoside equivalents, as follows:

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L)

$$= \frac{\mathrm{A \times MW \times DF \times 10^3}}{\mathrm{e \times L}} \qquad (5.3)$$

where A = (A_{520nm} – A_{700nm}) pH 1.0 – (A_{520nm} – A_{700nm}) pH 4.5;

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; L = pathlength in cm; e = 26,900 molar extinction coefficient, in $L' mol^{-1} cm^{-1}$, for cyd-3-glu; and 10^3 = factor for conversion from g to mg.

5.2.8 Statistical Analysis

The experimental design was analyzed by an optimization process by testing the variance analysis using two independent factors (micronization method and the choice of wall material) and one dependent factor (for every response variable under study). The statistical design surveyed points located within the levels and also outside them. The Proc GLM and Proc REG functions of SAS 9.3 (SAS Inst., Cary, NC) were used to analyze one-way design data and lack-of-fit tests, respectively. Tukey's honestly significant difference was employed as posthoc test and means were considered significantly different at $p < 0.05$.

5.3 Results and Discussion

5.3.1 Rheology of the core and coating emulsions

The effect of change in the particle size and type of wall material was studied on the tart cherry purée prior to spray drying. Figure 5.1 shows the viscosity vs shear rate (0 – 10 /s) for the rehydrated stock solutions of the wall material maltodextrin (MD) and gum Arabic (GA) at different concentrations and combinations. This study was done prior to the spray drying experiment to analyze the shear behavior of the wall material, and also to predict the rheology of the emulsions that were prepared at a later stage. Also, we were interested in studying the viscosity of the wall material as it is an important parameter (Shahidi and Han, 1993) contributing towards the stability, yield and encapsulation efficiency (Jafari et al., 2008) of the spray drying process. Figure 5.1 shows that there is an overall increase in the viscosity with increasing concentrations of GA. Both MD and GA are common encapsulating agents due to their low viscosity and high solubility (McNamee

et al., 1998). However, when both of them are compared, it was noticed that the MD (30%) solutions (0.0167 Pa.s) yielded a lower viscosity as opposed to the GA (30%) solutions (0.0347 Pa.s). Furthermore, the combination of the two-wall materials at MD:GA (15%: 15%) was relatively less viscous (0.0074 Pa.s) than MD:GA (20%:10% at 0.0131 Pa.s) followed by MD:GA (10%:20% at 0.0204 Pa.s). However, some researchers have reported higher viscosity values for MD as opposed to GA (Jayasundera et al., 2011a; Chong and Wong, 2015; Ho et al., 2015), but it should be noted that such values could be due to the differences in dextrose equivalence (DE) of the MD used. Typically, MD with higher DE exhibits much higher values of viscosity at the same shear rate and in our study, we used MD with DE values between 9 and 12, and that was a contributing factor to the high soluble and less viscous MD stock solutions.

Furthermore, the interactions of the core (non-micronized and micronized tart cherry purée) with the coating material and their effect on the rheology of the emulsions were studied. Figures 5.2 - 5.4 show that the observed behavior was in line with the behavior predicted based on the Figure 5.1. Additionally, changes were observed based on the extent of micronization. Figure 5.2 shows the highest viscosity (0.0574 Pa.s) for the non-micronized emulsion prepared with GA (30%). When compared with the micronized samples, this value was found to be higher than the viscosities of the emulsions prepared with the same wall material with megatron (Figure 5.3) and HPH (Figure 5.4). Similar trends were observed for all the other combinations of wall materials and their treatments with different samples. An exception to this behavior was observed in the Figure 5.4 where the interaction of two wall materials combinations (GA at 30% and MD:GA at 10%:20%) with the core material (HPH) gave higher values than those obtained from megatron

(Figure 5.3). These anomalies from the expected pattern could be explained on the basis of the interaction between the small particle sized HPH treated samples with GA that may have resulted in agglomeration at high GA concentrations.

Previous studies have showed that the lower values of viscosity usually yield in a spray dried product that is more uniform in their particle size distribution, is easier to pass through the nozzle, and results in a higher product yield (Fazaeli et al., 2012). In another study, the authors found a direct correlation between the concentration of maltodextrin in the emulsion and its resultant effect on the product yield (Yousefi et al., 2011). Some researchers also found that concentration of the encapsulating agent can cause a change in the physical characteristics of powder (Caparino et al., 2012). The extent of micronization performed on the starting food material (fruit juices or purées), also has an effect on the viscosity of the resultant emulsion, and that can also be used as a good indicator of the physico-chemical properties of the spray dried powder.

5.3.2 Color Analysis

Color was measured in the micronized and non-micronized samples of tart cherry purée encapsulated with different concentration ratios of maltodextrin (MD) and gum Arabic (GA). The effect of reduction in particle size and choice of wall material on the CIE-L*a*b* parameters, are shown in Table 5.2. There was a significant increase ($P < 0.001$) in the values of L* (lightness) and yellowness (b*) with reduction in particles sizes (non-micronized < megatron < high pressure homogenization) across all wall material concentration ratios. However, when the effect was compared between two wall material treatments, it is important to mention that the L* and b* values increased from MD (30%)

to MD: GA (20%:10%), with the highest values at MD: GA (15%:15%). Since one encapsulating agent may not have all the required properties of a good agent, it is often a good practice to use two compatible wall materials in combination, to achieve the desired physico-chemical results in the end product. Our results were in coherence with the study performed by Nguyen, Mounir, & Allaf (2017) and Ferrari, Marconi Germer, Alvim, & de Aguirre (2013), where they employed two wall materials, MD and GA, and obtained the highest values with least standard deviation when the wall materials were used in an equal ratio. Another research group (Jittanit, Niti-Att, & Techanuntachaikul, 2010) got similar results when they observed a decrease in L* values and an increase in redness (a*) when MD alone was used as wall material for their study on pineapple juice. Also, in our results, we observed the values of L* and b* reduced as we changed the ratio of MD:GA from 1:1 to 1:2, indicating that the interactions that happened when equal parts of wall material are used, yielded in a more homogenized solution, that leads to more surface area and thus better light scattering of the particles. This reason was confirmed by obtaining even lower values for GA (30%). Therefore, owing to the afore-mentioned explanations, the L* and b* values showed a pattern of MD (30%) < MD: GA (20%:10%) < MD: GA (15%:15%) > MD: GA (10%:20%) > GA (30%). However, it is important to notice that the degree of reduction in the values was not equal on either side of MD:GA in the ratio 1:1. It was observed that the higher concentration of MD yielded higher values than the higher concentration of their GA counterparts. A similar trend was observed with chroma (Cab*) values representing a relative saturation and hue (hab*) values representing an angle of the hue in the CIElab color wheel, with the reduction in particle size and the change of wall material concentration ratios. Hutchings (2011) stated that L*, a* and b*, represent food

diffuse reflectance of light, which are dependent on particulate distribution and scattering factors or coefficients. Another study (Saguy & Graf, 1991) showed that in food suspensions, scattering factor is inversely related to particle diameter. Tart cherry purées with varying particle sizes have different structural shapes and arrangements influencing light scattering coefficients and thus appearance.

5.3.3 Physical properties

The physical properties of the spray-dried powder that resulted from non-micronized and micronized tart cherry purée include process yield, encapsulation efficiency, hygroscopicity and particle size distribution, and are discussed in the following sections.

5.3.3.1 Process yield

The selection of spray drying process parameters was carefully done by referring to previous work done on food products with special focus on fruit powders. Some of the key independent factors that have been identified to affect process yield were: inlet air temperature (138–202 °C), feed flow rate (5–25 g/min) and maltodextrin/gum Arabic concentration (0–30%) (Tonon, Brabet, & Hubinger, 2008; Phisut, 2012). The process yield for all 5 variants of wall materials for non-micronized and micronized samples are shown in Table 5.3. From the results, we can clearly see a trend that the maximum yield was obtained when maltodextrin was used alone as a wall material, i.e. MD (30%). Therefore, the process yield values showed a pattern of MD (30%) > MD: GA (20%:10%) > MD: GA (15%:15%) > MD: GA (10%:20%) > GA (30%) for both non-micronized as

well as micronized samples. These results are in accordance with similar studies done by other research groups which also found that increasing of carrier agent concentration in tamarind extract significantly increased the process yield (Cynthia, Don Bosco, & Bhol, 2015; Shrestha et al., 2007; Vissotto, Jorge, Makita, Rodrigues, & Menegalli, 2010). They also concluded that Maltodextrin gave better results than MD-GA than only GA as wall material. The product recovery decreased as the maltodextrin content decreased because of lower total solids in the feed produced lower density which in turn gave a lower viscosity leading to higher values of radial speed, therefore making the droplets to collide with the internal walls of the drying chamber with higher velocity and intensity. This phenomenon creates more deposits on dryer walls and decreases the process yield (Masters, 1991; Tonon et al., 2008). Higher ratios of gum Arabic generally lead to higher viscosity of the feed solution. The higher viscosity values of the solution in turn lower its drying rate (Young, Sarda, & Rosenberg, 2010) therefore the droplets produced do not dry easily. Consequently, the wet powder sticks on chamber walls and subsequently the powder yield is reduced (Quek, Chok, & Swedlund, 2007). Stickiness in the powder was found more in gum Arabic treatment than in maltodextrin treatment.

5.3.3.2 Encapsulation efficiency

The type of coating material has a significant effect on encapsulation efficiency i.e. measure of efficiency of coverage provided by the coating over the core material. The better the coverage, the better the protective effect. This efficiency can vary with the nature of coating material, spray drying conditions, temperature. The encapsulation efficiency of our spray-dried powders with wall materials can be seen in Table 5.4. The encapsulation

efficiency is a very important factor in establishing the stability of the functional ingredient. As can be seen, the efficiency for just maltodextrin as wall material increases drastically from 51% to 75% as the particle size decreases due to micronization. For the non-micronized sample, irrespective of wall material, the encapsulation efficiency is less than 51% i.e. less than half of the sample is properly encapsulated. This clearly shows the importance of micronization and particle size reduction of the tart cherry sample, a process that gives novelty to this project. As observed by another research group, the highest efficiency values were found when only maltodextrin was used as a coating material (Pierucci et al., 2017). It was seen in another study that high encapsulation efficiency was achieved when they used maltodextrin as wall material (Júnior et al., 2017). The use of combination of maltodextrin and gum Arabic with tart cherry samples also gave good results with upto 71% efficiency. The reason behind the higher encapsulation efficiency of MD: GA encapsulated powders is probably due to higher emulsion viscosity of the resultant spray-dried powder which promotes faster drying of resultant powder during exit phase (Premi & Sharma, 2017).

5.3.3.3 Hygroscopicity

Hygroscopicity is used to describe the tendency of a powdered material to readily absorb moisture from the surroundings when exposed to a change in relative humidity. Dry powders can quickly become very problematic if they are highly hygroscopic and pose difficulties in terms of storage and usage. During the process of spray drying, the wall material interacts with the surface of the glass in the drying chamber and depending upon the glass transition temperature converts into coated powder with either low or high

hygroscopicity. Higher values of moisture content were obtained for powders produced with Gum Arabic at a concentration of 30% as seen Table 5.5. Our results were in agreement with those reported by a group (Goula & Adamopoulos, 2005) working on tomato pulp powder, whose hygroscopicity values decreased with an increase in maltodextrin concentration. Another research group (Jaya & Das, 2004) reported that the hygroscopicity of mango powder decreased with increased amount of added maltodextrin. Moreover, maltodextrin with smaller DE values produces powders with less cohesion, higher glass transition temperature, and reduced hygroscopicity, as observed by another research group (W. Wang & Zhou, 2012) for spray-dried soy sauce. Similar results for hygroscopicity were observed by a research group (Kurozawa, Morassi, Vanzo, Park, & Hubinger, 2009) for chicken breast hydrolysate produced with maltodextrin.

5.3.3.4 Particle Size Distribution

An increase in concentration from 15 to 30% of carrier agent resulted in wider curves and an increase in the mean diameter for both GA & MD (data not shown). In a spray-drying system the droplet size usually increases as the feed concentration or viscosity increases (Silva, Kurozawa, Park, & Hubinger, 2012) and the energy available for atomization decreases, resulting in the formation of larger particles (Goula & Adamopoulos, 2005). Same trend was observed by another group working on spray-dried acai with maltodextrin (Tonon et al., 2008).

5.3.4 Chemical Analyses

The spray-dried powder samples with variable wall materials were analyzed for their chemical content by different assays viz. Phenolic content using TPC assay,

Flavonoid content by TFC assay, Monomeric anthocyanins by TMAC assay, Antioxidant potential was computed using DPPH assay and their Redox potential using Reducing power assay.

5.3.4.1. Total Phenolic Content Assay

The total phenolic content of the spray dried powder was assessed for the for the five different combinations of wall material encapsulating micronized and non-micronized samples. As we can see in Figure 5.5 from the histograms that there is definite increase in phenolic content as the particle size decreases. The high-pressure method gives smaller particle size than megatron than the non-micronized sample and that same trend can be seen followed with the phenolic content. Among the wall materials, the 1:1 blend of maltodextrin and gum Arabic brings the high encapsulation efficiency and higher viscosity providing highest retention of phenolic compounds. Use of either maltodextrin separately as carrier material gives lowest results in TPC values. Similar pattern was observed for the sumac powders showing that the increased concentrations of MD decreased TPC of final product. This was attributed to the dilution effect caused by increasing concentration of MD (Caliskan & Nur Dirim, 2013). Another research group observed a significant decrease in TPC content with an increase in MD concentration. They attributed this decrease to the concentration effect of MD (Mishra, Mishra, & Mahanta, 2014).

5.3.4.2 Total Flavonoid Content Assay

To study the flavonoid content of the spray dried samples as affected by wall material and micronization, the TPC assay was performed and the resultant comparative

histogram is shown in Figure 5.6. As we can see from the results, the flavonoids follow the trend of the Phenolics. The best retention of flavonoids was in the 1:1 blend of maltodextrin-gum Arabic and high-pressure micronized samples. Generally, smaller particles result in larger surface area and the blend of MD-GA provides good encapsulation and hence protection of flavonoids against degradation from external factors. Maltodextrin and gum Arabic when used alone as wall materials show a reduction in polyphenols proving that instead of using a single wall material, the use of blend brings the best of properties of both the materials.

5.3.4.3 Total Monomeric Anthocyanin Content Assay

The monomeric anthocyanin content for spray-dried tart cherry powder as affected by micronization and choice of wall material is shown in Figure 5.7. As seen in the results, higher concentration of anthocyanins was found in micronized samples and more so in samples with higher maltodextrin content. Gum Arabic as a wall material shows lower retention of anthocyanins. A research group observed similar results that the powder with GA as wall material had a fine and expanded structure that increased the exposed surface area resulting in lower anthocyanin content. Lowest anthocyanin content was found in waxy starch and highest in MD. Lower anthocyanin content in waxy starch was because the product was collected from the collection tubes due to high stickiness (Papadakis, Gardeli, & Tzia, 2006).

5.3.4.4 DPPH Antioxidant potential assay

The reducing potential as seen in Figure 5.8 was 2-3 times higher for the HPH and megatron micronized sample as compared to the non-micronized sample. Among the carrier agents, other researchers have observed highest antioxidant activity with MD only and the mixture of MD and GA. Increase in GA concentration, however, led to a decreased antioxidant activity (Dag, Kilercioglu, & Oztop, 2017). The blend of equal amounts of maltodextrin-gum Arabic and MD: GA (20%:10%) used as wall material showed good retention of antioxidants proving that spray-drying was a beneficial technique to protect the antioxidants and have better storage stability. Previous researchers have reported results exhibiting higher antioxidant activity in dried powder due to drying and improved accessibility of phenolic compounds (Caliskan & Nur Dirim, 2013).

5.3.4.5 Reducing Power Assay

FRAP is a popular antioxidant assay used for spray dried samples and when ascorbic acid (vitamin C) is employed as a standard in this assay, its known as reducing power assay. The results were reported as μg of ascorbic acid equivalents (AAE) per mL. There was a strong correlation between TPC, TFC and FRAP values as can be seen in Figure 5.9, supporting the fact that phenolics and flavonoids are highly potent antioxidants (Ahmed, Khan, & Saeed, 2015). The reducing power of HPH and Megatron micronized spray-dried sample was higher than non-micronized sample following previous trends. Among the wall material, as seen with Phenolic content and flavonoids, there is maximum reducing power when 1:1 blend of maltodextrin and gum Arabic and MD: GA (20%:10%)

is used. Maltodextrin and gum Arabic when used alone as carrier content seems to lose some reducing potential probably due to their inherent properties.

5.3.5 Microstructure of Spray Dried Particles

The SEM images of various samples can be seen in Figure 5.10. The morphology exhibited by the powders was spherical shape and polydisperse sizes with no observable cracks or fissures. This type of morphology is good since it shows that encapsulated samples have lower permeability thereby shielding and retaining the active ingredient in the core as seen by other research groups as well (Cano-Chauca, Stringheta, Ramos, & Cal-Vidal, 2005; Krishnaiah, Nithyanandam, & Sarbatly, 2014). The variation in size has always been observed in spray dried particles. It corroborates the finding that there was a substantial effect of various wall materials on the morphology of the final product (Cynthia et al., 2015). In the SEM images of samples with MD as wall material, we can see microparticles with smoother surface and fewer teeth or roughness. We can also observe shriveled surface at 30% MD. The differences in size distribution by particle size analyzer and by SEM are due to the particles not being perfectly spherical and or agglomeration and the absence of smaller particles (<1mm) that must have been misplaced during the sample loading on stubs and sputter coating. The particles with GA as wall material are less agglomerated and have larger particle size affected by spray drying temperature as well as sample preparation for SEM as seen by another group studying durian powder (Chin et al., 2010).

5.4 Conclusions

Non-micronized and micronized (megatron and high-pressure homogenization) tart cherry purée samples were subjected to spray drying with five different variations of wall materials combinations of maltodextrin (MD) and gum arabic (GA) viz. MD (30%), MD: GA (20%: 10%), MD: GA (15%: 15%), MD: GA (10%: 20%), and GA (30%). The microcapsules prepared by different wall material treatments were analyzed for differences in the process yield, hygroscopicity, particle size and surface morphology, encapsulation efficiency of anthocyanins and the release properties of bioactives (polyphenols, flavonoids and anthocyanins). Upon studying the changes in the content of bioactives in tart cherry purée as affected by microencapsulation, it was found that the total phenolics and flavonoid content, and the antioxidant activity increased across all wall material conditions with the reduction in particle size. In order to determine the best micronization technique and the wall material combination, the extraction yield of various bioactives in the microcapsules were compared to control and it was found that high-pressure homogenization (HPH) and the blend of maltodextrin and gum arabic in equal ratio (viz. MD: GA (15%: 15%)) resulted in the highest extractability. Upon comparison between the control and HPH samples, we found that the polyphenol extractability increased by 47.35% (from 147.2 to 216.9 GAE μg/ml) and the anthocyanin content increased by 41.52% (from 41.9 to 59.3 cyanidin-3-glucoside μg/ml) in MD : GA (15%: 15%) and MD (30%) formulations respectively, due to the synergistic effect of micronization and wall material combination. The color analysis was inconclusive in terms of the wall material combination, but there was an increase in lightness as affected by the light scattering due

to particle size reduction. SEM images gave us a morphological view of the samples and resulted in microspheres that displayed smoother surface and fewer teeth or roughness with the reduction in particle size. To our knowledge, this is a first report studying the enhanced polyphenol extractability due to spray-drying of tart cherry purée, as affected by the choice of wall material and micronization.

5.5 References

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Table 5.1: Sample preparation and nomenclature based on the method of micronization (A-C) and the choice of wall material (1-5).

Wall Material		Method		
		Non-Micronized (A)	**Megatron (B)**	**High-Pressure Homogenization (C)**
1	30% MD (w/v)	1A	1B	1C
2	20% MD: 10% GA (w/v)	2A	2B	2C
3	15% MD: 15% GA (w/v)	3A	3B	3C
4	10% MD: 20% GA (w/v)	4A	4B	4C
5	30% GA (w/v)	5A	5B	5C

Table 5.2: Effect of tart cherry micronization and the wall material concentration ratio on the composition of color measurements.

Method	Wall	L*	a*	b*	Croma	Hue
Control	MD30	78.33 ± 1.38^{b,c}	8.96 ± 0.56^{a,b}	2.07 ± 0.04^{c}	9.19 ± 0.54^{a,b}	4.25 ± 0.37^{a,c}
	MD20:GA10	80.82 ± 1.96^{a,b,d}	7.80 ± 0.16^{a,c}	2.45 ± 0.03^{b,c}	8.17 ± 0.15^{a,c}	3.08 ± 0.09^{a,d}
	MD15:GA15	80.67 ± 1.06^{b,c}	6.27 ± 0.04^{a,d}	3.17 ± 0.09^{a,c}	7.02 ± 0.02^{a,d}	1.81 ± 0.07^{a,d}
	MD10:GA20	80.55 ± 0.91^{a,b,c}	7.45 ± 0.12^{a,b}	1.71 ± 0.01^{b,d}	7.64 ± 0.11^{a,b}	4.28 ± 0.09^{a,b}
	GA30	77.30 ± 0.30^{b,d}	9.04 ± 0.20^{a}	1.54 ± 0.02^{b,e}	9.17 ± 0.19^{a}	5.83 ± 0.19^{a}
Megatron	MD30	79.12 ± 0.73^{a,b}	8.57 ± 0.13^{a,b}	2.56 ± 0.01^{c}	8.94 ± 0.12^{a}	3.25 ± 0.05^{b}
	MD20:GA10	81.93 ± 1.63^{a,b}	6.42 ± 0.10^{b,c}	2.65 ± 0.04^{a,c}	6.94 ± 0.09^{b}	2.29 ± 0.06^{b,c}
	MD15:GA15	82.84 ± 0.74^{a,b}	4.75 ± 0.07^{b,e}	3.64 ± 0.04^{a,b}	5.98 ± 0.03^{b,d}	1.04 ± 0.04^{b,d}
	MD10:GA20	80.68 ± 0.93^{a,b}	5.51 ± 0.13^{b,d}	2.63 ± 0.19^{a,b}	6.11 ± 0.11^{b,c}	1.94 ± 0.20^{b}
	GA30	78.47 ± 0.31^{a,b,c}	7.78 ± 0.11^{b}	2.36 ± 0.11^{a,d}	8.12 ± 0.70^{b}	3.20 ± 0.21^{a,b}
HPH	MD30	80.73 ± 0.60^{a,c}	7.77 ±. 0.13^{a,c}	3.18 ± 0.03^{b}	8.39 ± 0.12^{a,b}	2.30 ± 0.05^{b,c}
	MD20:GA10	83.77 ± 0.75^{a,b}	5.66 ± 0.05^{c,b}	3.07 ± 0.06^{b}	6.44 ± 0.72^{b,c}	1.66 ± 0.02^{c}
	MD15:GA15	85.05 ± 2.45^{a}	3.19 ± 0.03^{c,d}	4.62 ± 0.03^{a,b}	5.61 ± 0.04^{c}	0.12 ± 0.01^{c,d}
	MD10:GA20	81.68 ± 1.03^{a,b,c,d}	4.75 ± 0.19^{c}	2.71 ± 0.03^{a,c}	5.47 ± 0.15^{c,d}	1.56 ± 0.09^{b,c,d}
	GA30	79.90 ± 0.39^{a,d}	5.82 ± 0.10^{a,c}	2.45 ± 0.03^{a,d}	6.31 ± 0.07^{a,b,c}	2.24 ± 0.07^{a,c}

Values (Means ± standard deviation) in a column not sharing a superscript letter ($^{a, b, c, d}$) are significantly different from each other ($p < 0.05$) (MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)

Table 5.3: Effect of tart cherry micronization and the wall material concentration ratio on the product recovery during the spray drying process.

Product Recovery (g/L)			
Method⇨ / **Wall ⇩**	**Control**	**Megatron**	**HPH**
MD30	$155.346 \pm 0.003^{a,c}$	$169.864 \pm 0.004^{a,b}$	175.155 ± 0.003^{a}
MD20:GA10	$154.574 \pm 0.003^{b,c}$	166.472 ± 0.003^{b}	$167.601 \pm 0.001^{a,b}$
MD15:GA15	148.543 ± 0.002^{c}	$154.176 \pm 0.003^{b,c}$	$163.613 \pm 0.004^{a,c}$
MD10:GA20	$130.728 \pm 0.002^{c,e}$	$152.401 \pm 0.003^{b,d}$	$157.703 \pm 0.002^{a,e}$
GA30	$131.360 \pm 0.001^{c,d}$	$145.566 \pm 0.002^{b,e}$	$149.009 \pm 0.001^{a,d}$

Values (Means ± standard deviation) in a column not sharing a superscript letter ($^{a, b, c, d, e}$) are significantly different from each other ($p < 0.05$) (MD: Maltodextrin, GA: Gum Arabic, number of observations = 3).

Table 5.4: Effect of tart cherry micronization and the wall material concentration ratio on the encapsulation efficiency achieved during the spray drying.

Encapsulation Efficiency (in %)			
Method⇨ / **Wall ⇩**	**Control**	**Megatron**	**HPH**
MD30	$51.438 \pm 0.007^{a,c}$	$64.054 \pm 0.058^{a,b}$	75.557 ± 0.149^{a}
MD20:GA10	$50.119 \pm 0.126^{b,c}$	62.721 ± 0.550^{b}	$72.507 \pm 0.105^{a,b}$
MD15:GA15	$41.612 \pm 0.135^{c,d}$	$53.249 \pm 0.226^{b,d}$	$71.472 \pm 0.213^{a,c}$
MD10:GA20	45.938 ± 0.100^{c}	$54.060 \pm 0.228^{b,c}$	$67.366 \pm 0.122^{a,d}$
GA30	41.699 ± 0.026^{d}	52.622 ± 0.183^{e}	64.977 ± 0.039^{e}

Values (Means ± standard deviation) in a column not sharing a superscript letter ($^{a, b, c, d}$) are significantly different from each other ($p < 0.05$) (MD: Maltodextrin, GA: Gum Arabic, number of observations = 3).

Table 5.5: Effect of tart cherry micronization and the wall material concentration ratio on the hygroscopicity (%) of the spray dried microcapsules.

Hygroscopicity (in %)			
Method⇨ / **Wall ⇩**	**Control**	**Megatron**	**HPH**
MD30	$4.886 \pm 0.068^{a,e}$	$1.927 \pm 0.033^{b,e}$	$1.295 \pm 0.005^{c,e}$
MD20:GA10	$11.079 \pm 0.052^{a,d}$	$8.753 \pm 0.141^{b,d}$	$7.035 \pm 0.047^{c,d}$
MD15:GA15	$12.438 \pm 0.086^{a,c}$	$11.561 \pm 0.153^{b,c}$	7.947 ± 0.045^{c}
MD10:GA20	$16.681 \pm 0.112^{a,b}$	12.401 ± 0.193^{b}	$10.695 \pm 0.191^{b,c}$
GA30	21.689 ± 0.205^{a}	$20.667 \pm 0.116^{a,b}$	$14.500 \pm 0.458^{a,c}$

Values (Means ± standard deviation) in a column not sharing a superscript letter ($^{a, b, c, d, e}$) are significantly different from each other ($p < 0.05$) (MD: Maltodextrin, GA: Gum Arabic, number of observations = 3).

Table 5.6. Chemical assay values of the spray dried tart cherry microcapsules as affected by the changes in the variable concentration ratio of wall material and micronization.

Sample	Wall Material	TPC (Gallic Acid Equivalent in µg/mL)	TFC (Quercitin equivalent in µg/mL)	TMAC (Cyanidin-3-glucoside equivalent in mg/L)
Non-micronized	**MD (30%)**	111.4 ± 4.5	72.2 ± 3.2	41.9 ± 3.6
	MD: GA (20% :10%)	122.3 ± 4.9	75.5 ± 3.4	40.9 ± 4.2
	MD: GA (15% :15%)	147.2 ± 5.9	82.5 ± 3.7	34.9 ± 5.2
	MD: GA (10% :20%)	116.4 ± 4.7	71.4 ± 3.2	37.6 ± 3.6
	GA (30%)	95.7 ± 3.8	68.2 ± 3.1	34.9 ± 4.1
Megatron	**MD (30%)**	128.4 ± 5.1	91.1 ± 4.1	47.8 ± 4.5
	MD: GA (20% :10%)	144.1 ± 5.8	98.5 ± 4.4	46.4 ± 3.1
	MD: GA (15% :15%)	183.4 ± 7.3	109.1 ± 4.9	36.9 ± 3.2
	MD: GA (10% :20%)	141.1 ± 5.6	92.5 ± 4.1	37.6 ± 4.4
	GA (30%)	121.9 ± 4.9	87.0 ± 3.9	36.2 ± 3.3
HPH	**MD (30%)**	154.6 ± 6.2	107.8 ± 4.8	59.3 ± 3.4
	MD: GA (20% :10%)	172.5 ± 6.9	108.8 ± 4.2	52.2 ± 3.8
	MD: GA (15% :15%)	216.9 ± 8.7	139.1 ± 6.2	50.8 ± 3.1
	MD: GA (10% :20%)	168.8 ± 6.8	106.0 ± 4.7	44.1 ± 3.2
	GA (30%)	149.9 ± 6.0	95.3 ± 4.2	41.1 ± 3.6

(MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)

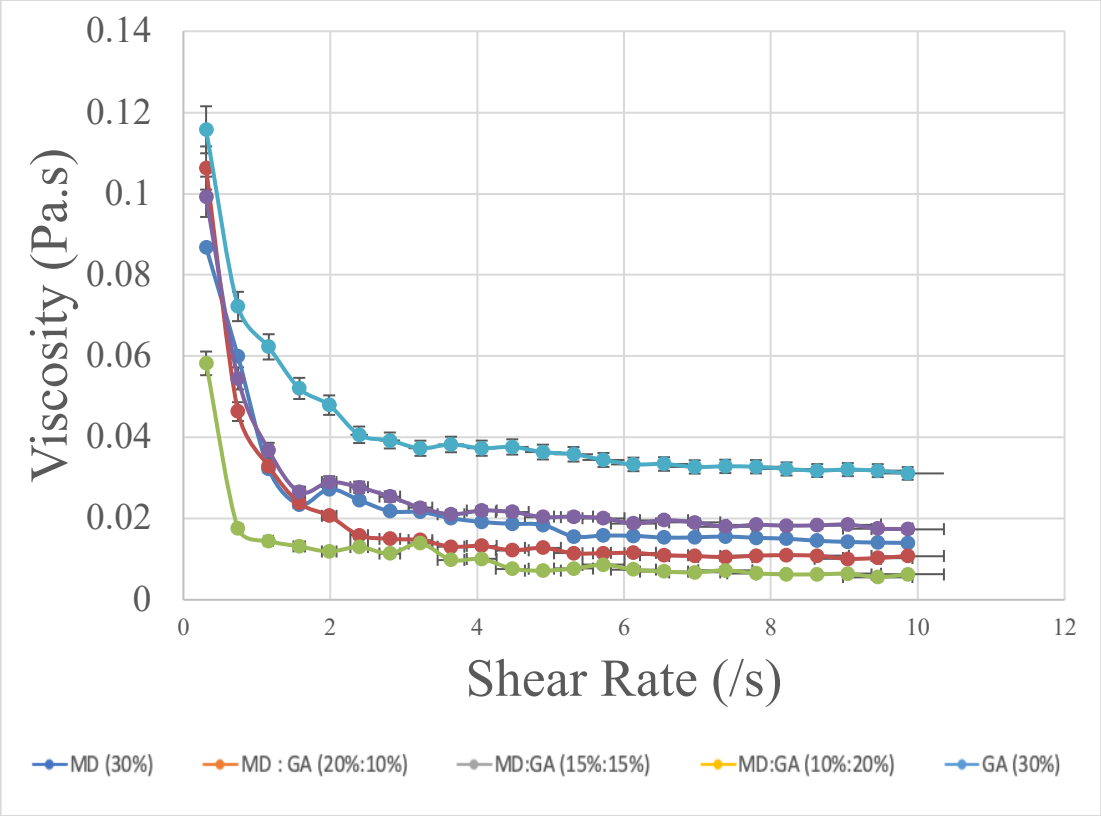

Figure 5.1: Scatter plot illustrating a comparative analysis of the effect of varying shear rate (0-10 /s) on the viscosity of wall material solutions at different concentration ratios.
(MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)

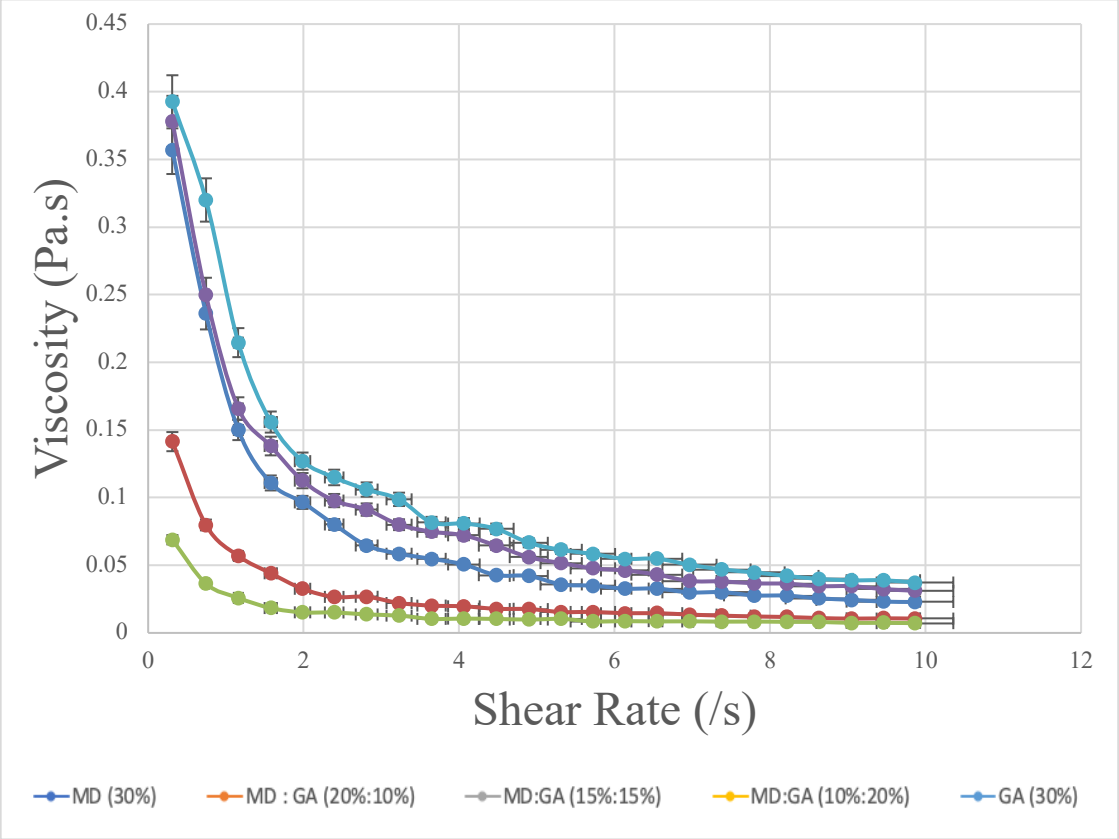

Figure 5.2: Scatter plot illustrating a comparative analysis of the effect of varying shear rate (0-10 /s) on the viscosity of emulsions containing the non-micronized core mixed with different concentration ratios of wall material.
(MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)

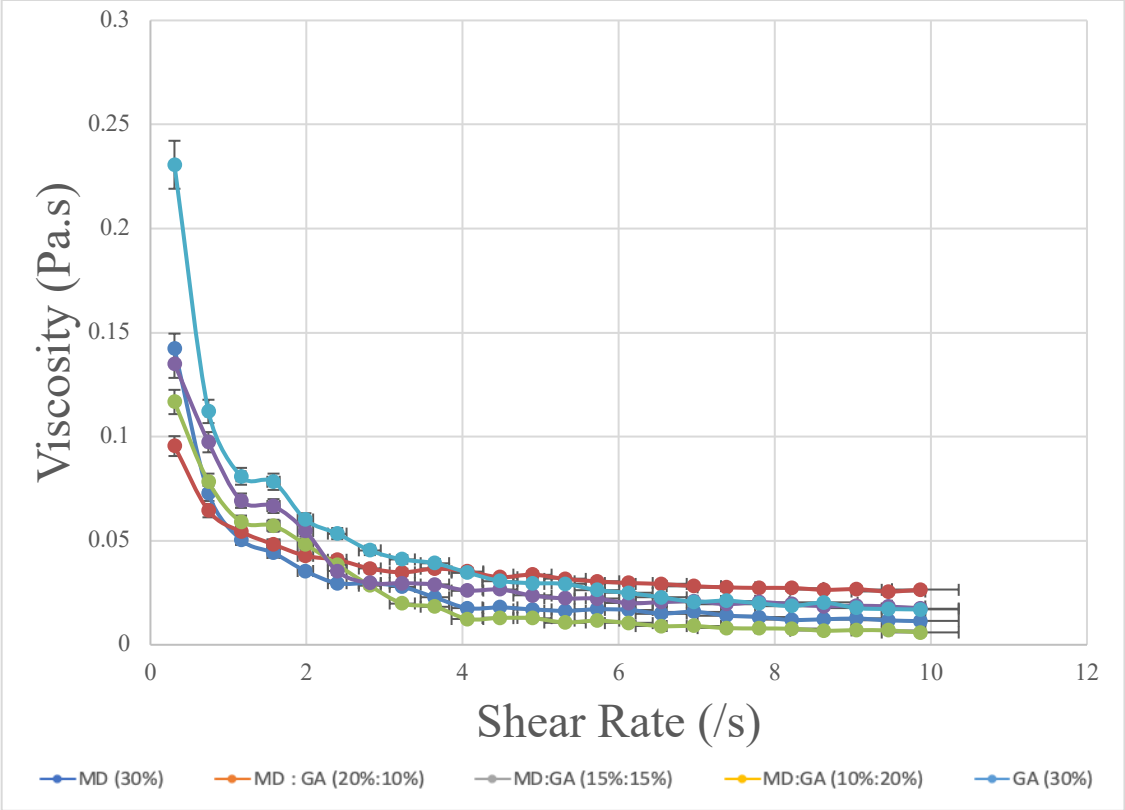

Figure 5.3: Scatter plot illustrating a comparative analysis of the effect of varying shear rate (0-10 /s) on the viscosity of emulsions containing the micronized (megatron) core mixed with different concentration ratios of wall material. (MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)

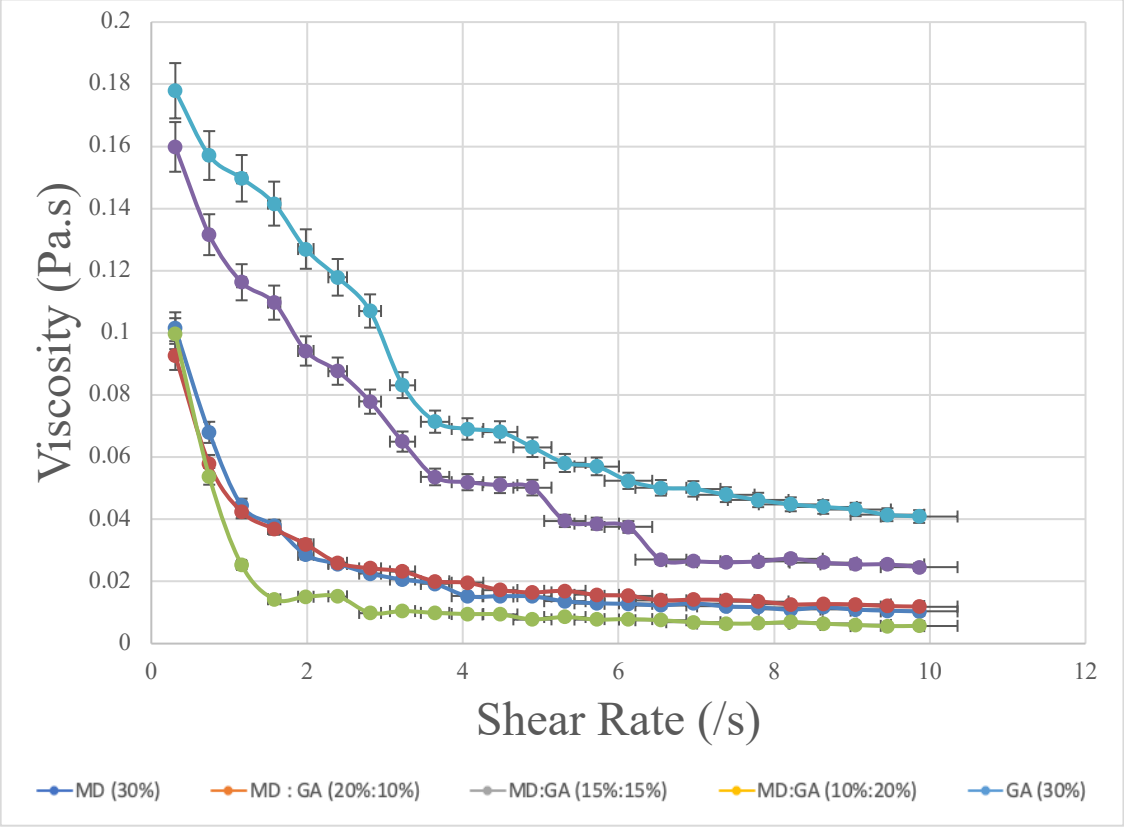

Figure 5.4: Scatter plot illustrating a comparative analysis of the effect of varying shear rate (0-10 /s) on the viscosity of emulsions containing the micronized (HPH) core mixed with different concentration ratios of wall material.
(MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)

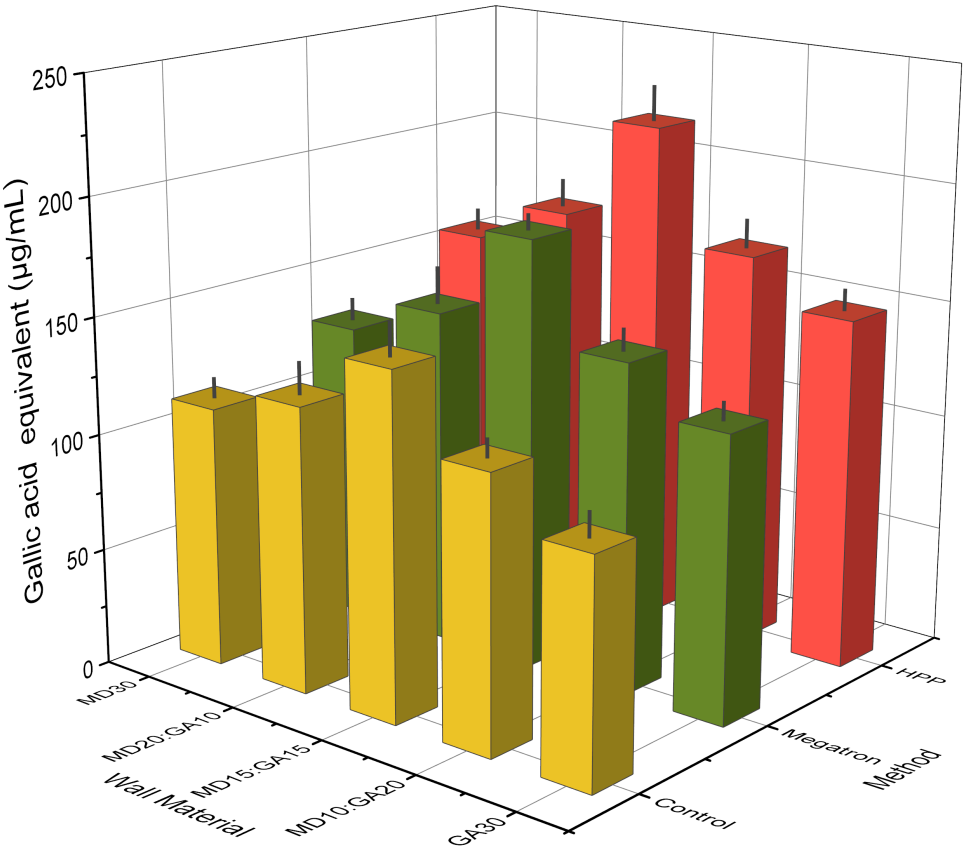

Figure 5.5: 3D Stacked bars plot of total polyphenol content of the spray dried tart cherry microcapsules as affected by the changes in the concentration ratio of wall material and micronization. (Total polyphenol content is expressed as gallic acid equivalents in µg/ml; MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)

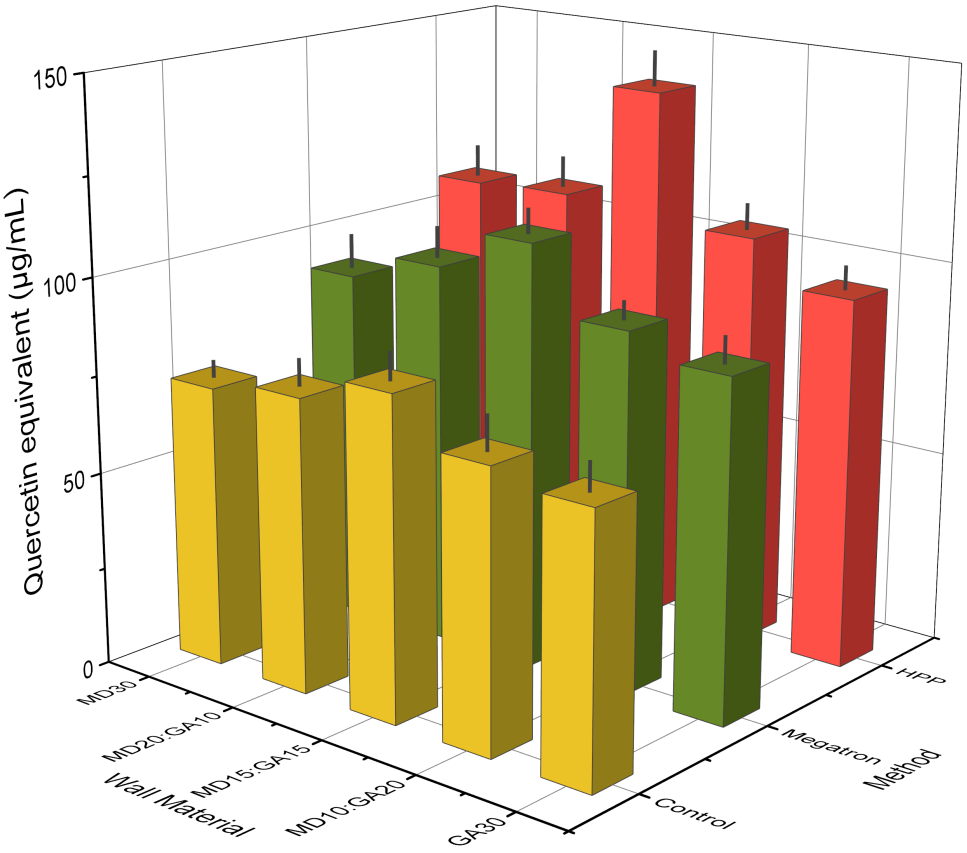

Figure 5.6: 3D Stacked bars plot of total flavonoid content of the spray dried tart cherry microcapsules as affected by the changes in the concentration ratio of wall material and micronization. (Total flavonoid content is expressed as quercetin equivalents in µg/ml; MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)

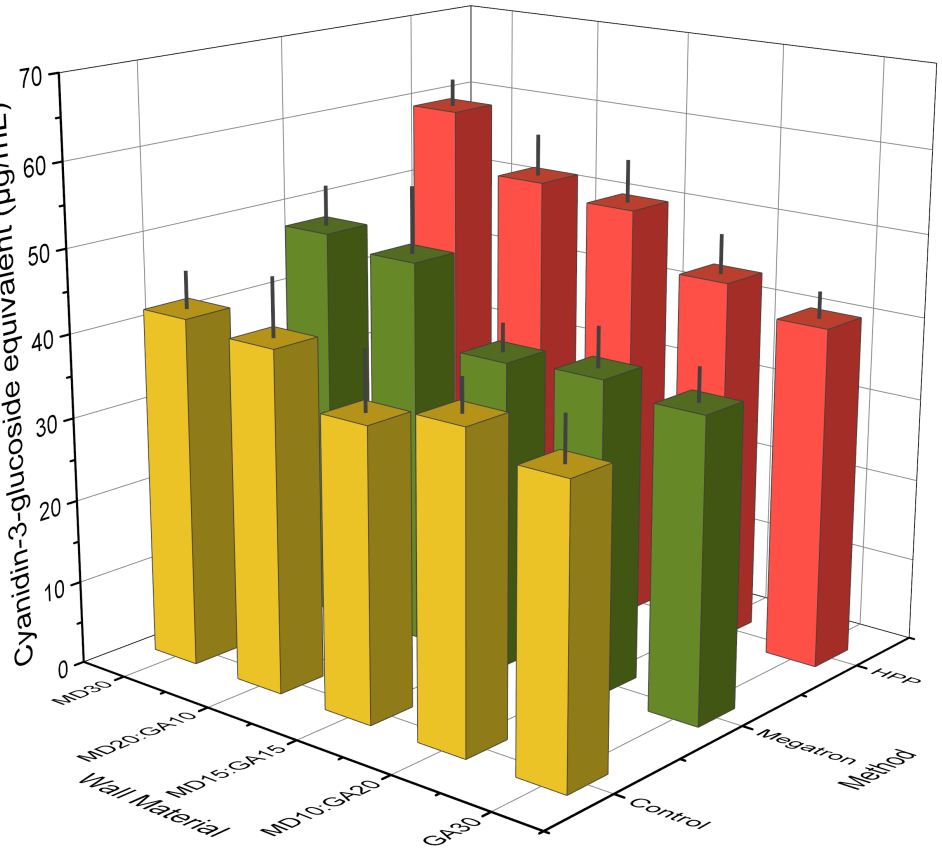

Figure 5.7: 3D Stacked bars plot of total monomeric anthocyanin content of the spray dried tart cherry microcapsules as affected by the changes in the concentration ratio of wall material and micronization. (Total monomeric anthocyanin content is expressed as cyanidin-3-glucoside equivalents in µg/ml; MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)

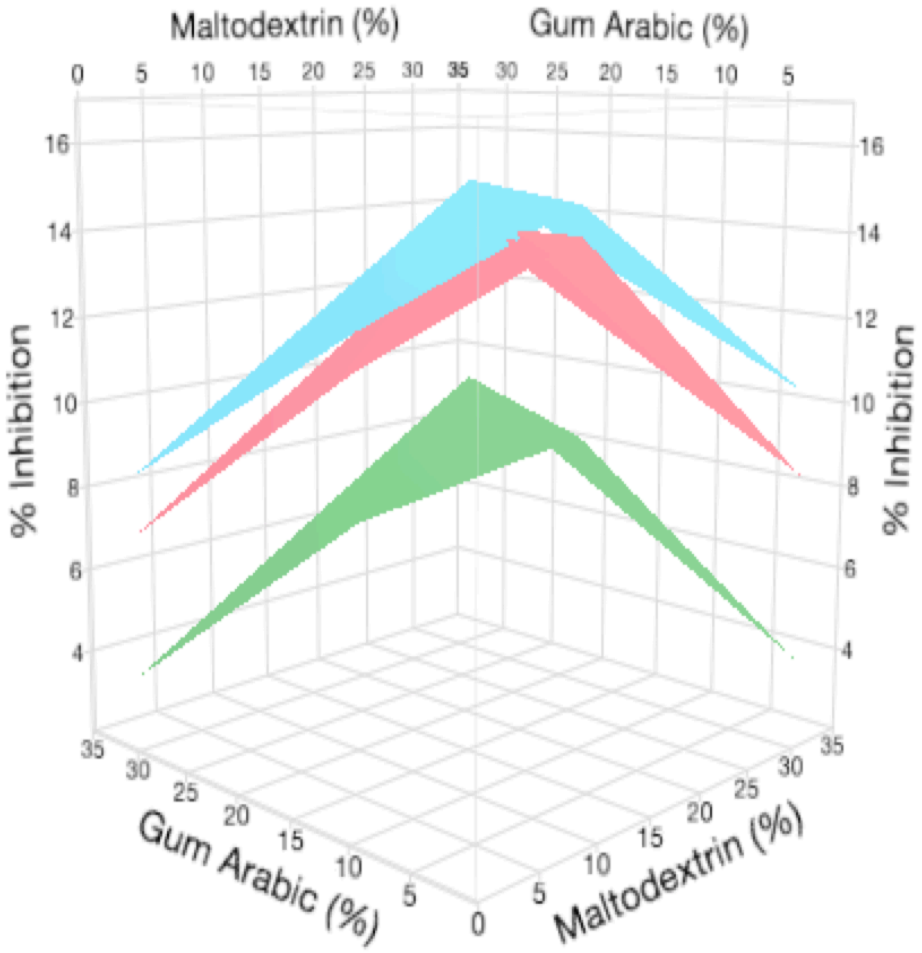

Figure 5.8: Response Surface plot (3D) of DPPH free radical scavenging activity of the spray dried tart cherry microcapsules as affected by the changes in the concentration ratio of wall material and micronization.
(Radical scavenging activity content is expressed as percentage inhibition)
(MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)
: Control : Megatron & : HPH.

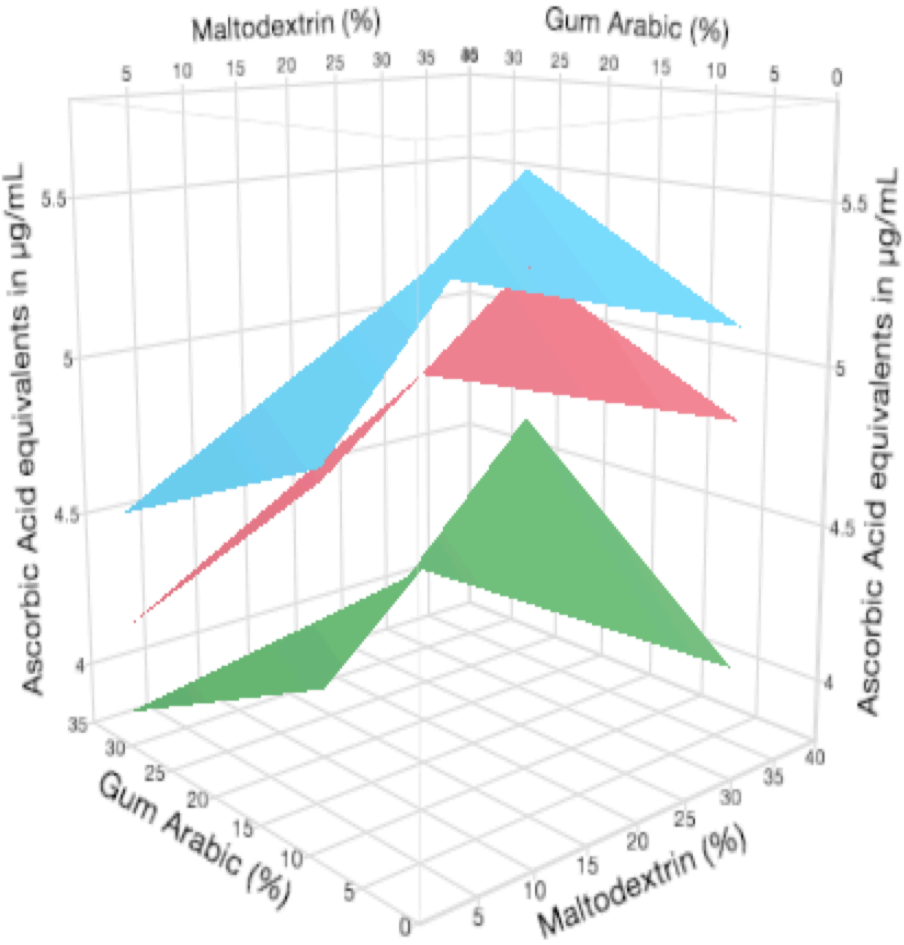

Figure 5.9: Response Surface plot (3D) of antioxidant activity (by reducing power assay) of the spray dried tart cherry microcapsules as affected by the changes in the concentration ratio of wall material and micronization. (Antioxidant activity is expressed as ascorbic acid equivalents in µg/ml)
(MD: Maltodextrin, GA: Gum Arabic, number of observations = 3)
: Control : Megatron & : HPH.

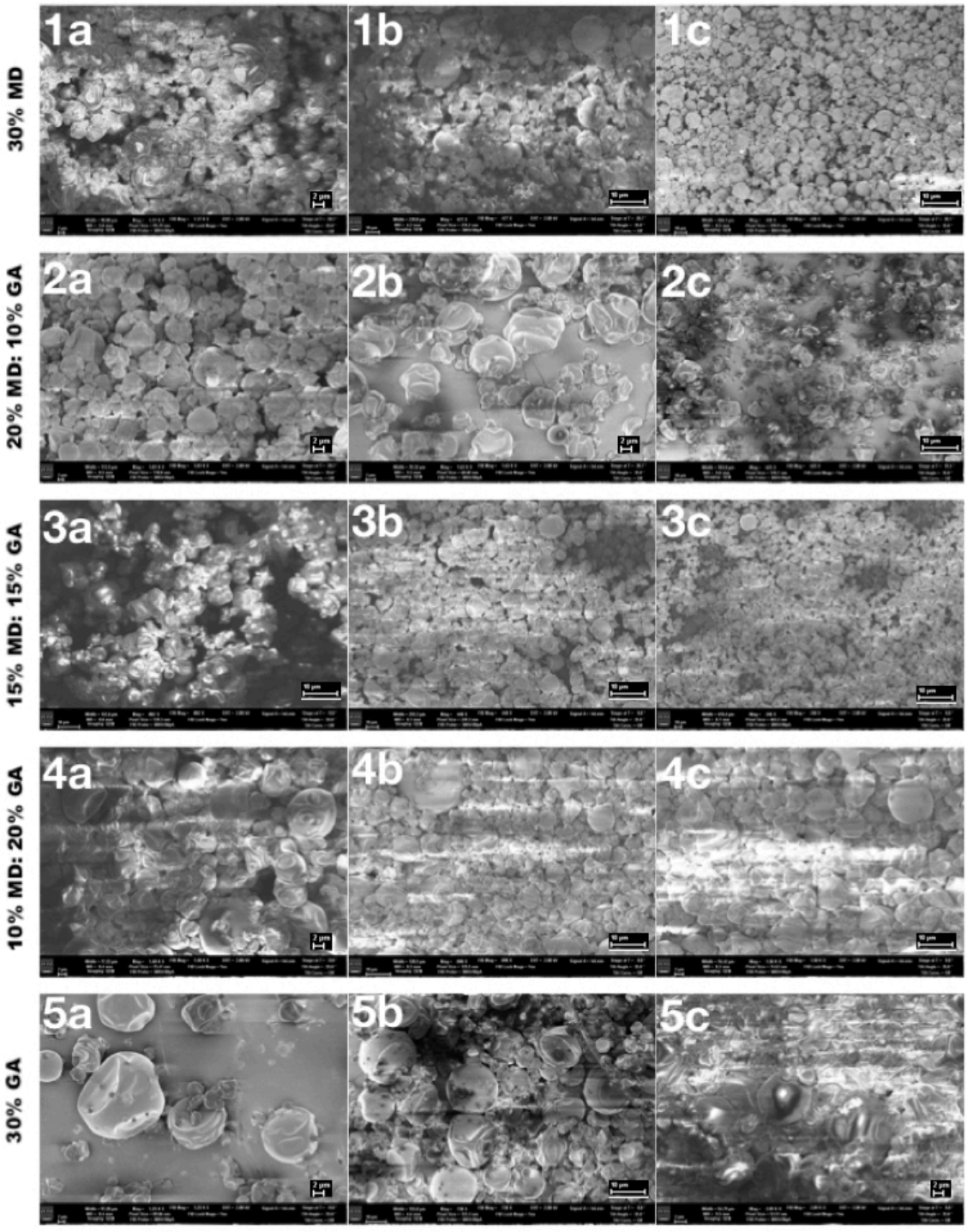

Figure 5.10: SEM micrographs of non-micronized and micronized tart cherry purée, spray dried into a powder form with 5 different combinations of wall material. Scale bar is equal to 2 μm for 1A, 2A, 2B, 4A, 4C, 5A, 5C and is 10 μm for 1B, 1C, 2C, 3A/B/C, 4B, 5B.Non-micronized (A), and Micronized samples obtained from Megatron (B), and High-pressure Homogenization (C) 1: *30% MD (w/v)*, 2: *20% MD: 10% GA (w/v)*, 3: *15% MD: 15% GA (w/v)*, 4: *10% MD: 20% GA (w/v)*, 5: *30% GA (w/v)*.

CHAPTER 6

STORAGE STABILITY AND PHYSICOHEMICAL PROPERTIES OF MICROENCAPSULATED TART CHERRY PURÉE

6.1 Introduction

Microencapsulation is widely used in the food industry to improve product performance, shield the functional food ingredients against degradation (Shahidi & Han, 1993), stabilize the phenolics and for packaging tiny particles or droplets into small capsules, thereby enhancing their storage stability (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Several techniques are available for microencapsulation including spray chilling, spray drying, extrusion, co-crystallization, inclusion complexation, fluidized coating and liposome entrapment (Sliwka, 1975). Owing to its continuous nature and low-cost of processing along with easily manageable equipment, spray-drying is the most commonly used technique producing small and dry powders of high quality (Fang & Bhandari, 2011). Particularly for the heat sensitive functional ingredients in the food, spray drying process proves to be a great option as it is rapid and generally the temperatures in the core is much lower than 100°C (Masters, 1991). In the case of tart cherries, that are rich in polyphenols, which also happen to be heat sensitive phytochemicals; spray drying might be just the right microencapsulation method.

Previous studies have successfully employed this encapsulation technique for numerous polyphenol rich materials viz. black carrot extract (Ersus & Yurdagel, 2007), Hibiscus sabdariffa L. extract (Chiou & Langrish, 2007), soybean extract (Fonseca, Casagrande, Oliveira, Georgetti, & Souza, 2007), grape seed, apple and olive leaf extracts (Kosaraju, Labbett, Emin, Konczak, & Lundin, 2008) and black currant (Bakowska-Barczak & Kolodziejczyk, 2011). It is a procedure to coat materials (like flavor, vitamins etc.) in the form of stabilized micro- and nano-particles in order to protect functional ingredients which are sensitive to oxidation, moisture or light (Jafari, Assadpoor, He, &

Bhandari, 2008). The spray dried powder formulation provides better controlled delivery of ingredients by weight reduction and protects the functional product from bacterial degradation and enhances its storage stability and shelf life by drastically reducing its moisture content (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). The food industry is particularly interested in fruits and vegetables converted to powdered product form, as it provides better nourishment and hydrating properties (García-Gutierrez, González-Maldonado, Ochoa-Martínez, & Medrano-Roldán, 2004). The stability and storage of the powder forms of food is mainly governed by the moisture content (O'hagan, Hasaoidis, Coder, Helsing, & Pokraja, 2005).

Among the wall materials used for encapsulation, gum arabic and maltodextrins are extensively used in the spray drying of fruit juices (Quek, Chok, & Swedlund, 2007) owing to their high solubility and low viscosity. Both these encapsulating agents have high molecular weights that helps to avoid spray drying operational problems in sugar-rich products (fruit juices), such as stickiness on the dryer chamber wall. They also help avoid structural transformations such as collapse and crystallization during the food processing and storage (Carrillo-Navas, González-Rodea, Cruz-Olivares, Barrera-Pichardo, Román-Guerrero, Pérez-Alonso, 2011; Guadarrama-Ledezma et al., 2014). An important thermodynamic tool to determine the interaction between water and food components is water sorption isotherms. When exposed to air at a certain relative humidity, every material can hold a certain amount of water, this can be predicted by a moisture sorption isotherm. This moisture content is subject to external factors like temperature and the environmental relative humidity, as well as internal factors like the composition of the material (García-Pérez, Cárcel, Clemente, & Mulet, 2008).

The inter-relationship between relative humidity at a particular temperature and the equilibrium moisture content of a food product provides vital information used for processing of food in packaging, drying and storage (Kumar & Mishra, 2006; Tonon et al., 2009). To optimize product characteristics, the moisture isotherm data is very useful to select appropriate storage conditions and packaging systems (Labuza, Kaanane & Chen, 1985).

Most interactions of food with water, like the characteristics of water sorption are defined by the composition of the solid part, mainly the carbohydrates and the proteins. Certain time-dependent phenomena like structural transformations and phase transition also affect the sorption properties. Mathematical models based on empirical and/or theoretical criteria are commonly used to present the sorption isotherms and are unique for every material. Recent researchers have cited a large number of isotherm models which have been categorized into various groups; kinetic models based on a multi-layer and condensed film (GAB model), absorbed monolayer of water (BET model), semi-empirical (Halsey model) and purely empirical models (e.g. Oswin and Smith models) (Muzaffar & Kumar, 2016). The temperature at which an amorphous system changes from a glassy to a rubbery state is called the glass transition temperature (Tg) and represents a reference temperature that connects the physical properties of foods with the water content and temperature (J M Aguilera & del Valle, 1995; JoséM M. Aguilera, del Valle, & Karel, 1995; Chuy & Labuza, 1994; Y. H. Roos, 2007; Y. Roos & Karel, 1991). Typical structural occurring in amorphous foods include agglomeration, stickiness, collapse and crystallization alterations all happen when stored at temperatures above Tg (Aguilera et al., 1995; Chuy & Labuza, 1994). It is extremely important to have knowledge of the sorption

properties of food for predicting the physical state of a food because the rate of the deteriorative reactions is affected as the transition occurs (Chuy & Labuza, 1994; Roos, 2007). Thus, the two factors extremely important for predicting the performance of food and its deterioration during processing and storage are water activity and T_g (Roos, 1993).

In Chapter 5, we have used maltodextrin and gum arabic which are widely used as wall material however there is no investigation about the quality changes of tart cherry purée powder during storage. Thus, the objective of the present study was to provide experimental data for sorption characteristics of spray dried tart cherry powder in order to model the sorption isotherms using the selected models and to evaluate the changes in physicochemical properties of spray dried tart cherry powder during storage. It has been reported in the literature that, for some food products, the drying method can affect the sorption isotherms of the final product significantly (Muzaffar & Kumar, 2016). Therefore, there is a knowledge gap there and a clear need to study the moisture sorption isotherms of pure spray-dried tart cherry powders. Permutations of temperature and moisture content in the final powders determine the glass transition temperature of the product and also helps determine the stickiness temperature that needs be avoided during spray drying process in order to reduce the product loss within the glass chamber. Also, although gum arabic is a commonly used encapsulating agent, its contribution to the stability of dehydrated and/or encapsulated juices has not been extensively studied. Therefore, the aim of this work was also to examine the effect of different encapsulating materials on water sorption and chemical characteristics of the tart cherry purée upon storage. Alterations in the color of the encapsulated material caused by moisture sorption and storage were also evaluated.

6.2 Materials and methods

6.2.1 Materials

Folin-Ciocalteau (FC) reagent, sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, sodium acetate trihydrate, acetic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric (III) chloride hexahydrate, vanillin, sulphuric acid and assay standards, including trolox, gallic acid, ethanol, rutin and aecsin used were all reagent-grade chemicals purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). The encapsulating agents, Maltodextrin (STAR-DRI 100) was obtained from Tate & Lyle (Decatur, IL) with dextrose equivalent (DE) value between 9 and 12 and Gum Arabic from acacia tree was obtained from Sigma Life Sciences (St. Louis, MO). Deionized water (DI) was prepared fresh before use using a Millipore Milli-Q water purification system (Burlington, MA).

6.2.2 Microencapsulation by Spray Drying

Microencapsulation by spray drying was done based on the protocol described in section 4.2.2. of Chapter 4. The ratios between MD/GA were selected according to the best ratio of these wall materials presented by previous studies (Ferrari, Marconi Germer, Alvim, & de Aguirre, 2013; Nayak & Rastogi, 2010; Tonon, Brabet, & Hubinger, 2010). All fifteen spray dried powders were packaged to prevent light incidence and stored in polypropylene falcons at -20°C temperature for further experiments.

6.2.3 Storage stability

The samples were kept at three different temperatures of 4°C, 25°C, and 42°C for this study. Control samples were kept at 20°C. A sampling schedule that spanned a total of 4 weeks was made and physicochemical tests (TPC, TFC and FRAP) were conducted at each storage condition. Temperature-dependent properties was investigated by comparing test results at each storage time–temperature combination. Samples (0.5 g) were placed in clear polystyrene covered dishes measuring 35 mm diameter and 10 mm deep and sealed with parafilm.

6.2.4 Chemical analyses

All spray dried powders were dissolved at 0.01 g/mL for about 1 h in deionized water prior to the tests. The non-encapsulated tart cherry purée samples served as control.

6.2.4.1 Total phenolics content (TPC)

A standard curve was generated with gallic acid and TPC was expressed as g GAE equivalents/mL and used to determine TPC concentration of samples based on the protocol elaborated in the section 4.2.3.1.

6.2.4.2 Total flavonoid content (TFC) assay

The flavonoid content was determined using a method previously published (Mihai, Mărghitaş, Bobiş, Dezmirean, & Tămaş, 2010) with some modifications, based on the protocol detailed in the section 4.2.3.2.

6.2.4.3 Ferric reducing antioxidant power (FRAP)

A modified FRAP assay was utilized (Benzie & Szeto, 1999). Results were determined using a standard curve prepared using 100 µL of various Trolox solutions (0.1 to 1.0 mM) based on the protocol detailed in section 4.2.4.1

6.2.4.4 Color measurements

Color values (L*, a*, b*) of all samples of tart cherry purée were determined using MiniScanEZ 4500L portable spectrophotometer (HunterLab), referring to color space CIE Lab. The samples were measured against a white ceramic reference plate (L* = 93.92; a* = 1.03; b* = 0.52). The final data computed were mean of three measurements. Total change in the color, hue and Chroma were calculated according to (Wrolstad, Durst, & Lee, 2005). The color measurements of all samples were taken at three different temperatures of 4°C, 25°C, and 42°C. Measurements were also taken at different % relative humidities by using the saturated solutions of lithium chloride (11%), potassium acetate (22.5%), magnesium chloride (32%), magnesium nitrate (57%) and sodium chloride (75%).

6.2.4.5 Moisture isotherm

The integral method was employed to develop sorption isotherms. Powder samples were placed in a petridish and placed in 5 chambers equilibrated at different % relative humidities (%RH). The chambers were subsequently stored away from light. Saturated salt solutions were used to maintain different %RH: lithium chloride (11%), potassium acetate (22.5%), magnesium chloride (32%), magnesium nitrate (57%) and sodium chloride

(75%). The samples were equilibrated and weighed every week until the weight was optimized. Equilibrium was assumed when the difference between consecutive weights was less than 1 mg. The initial moisture content of the samples was obtained by vacuum drying at 65 °C for 24 h. Moisture content was computed as the ratio between the mass lost during dehydration over the initial mass. Equilibrium moisture content was plotted against equilibrium relative humidity and were analysed using GAB model in the Origin Pro 8.5.1 software (OriginLab Corporation, Northampton, MA).

6.2.4.6 Statistical Analysis

Micronization, spray-drying, and sampling were performed in duplicates, while assays were conducted in triplicate. The Proc GLM and Proc REG functions of SAS University edition (SAS Inst., Cary, NC) were used to analyze two-way design data and lack-of-fit tests, respectively. Tukey's honestly significant difference was employed as post-hoc test and means were considered significantly different at $p < 0.05$.

6.3 Result and Discussion

6.3.1 Color measurements at different temperatures

The values of lightness (L*), redness (a*) and yellowness (b*) color measurement of the spray dried samples taken at three different temperatures of 4°C, 25°C, and 42°C, as seen in Table 6.1, followed a similar trend for the non-micronized sample, in terms of increase in values with increasing temperature for all the wall material variations. The micronization of samples by megatron and by high-pressure methods leads to lower

particle size and an increase in L*, a* and b* values as seen before in our previous study (Lukhmana, Kong, Kerr, & Singh, 2018) mainly due to light scattering. There were some outliers observed and that can be attributed to the wall material. Even with an increase in temperature, the L*, a* and b* values seem to increase, barring a couple of anomalies. The spray drying seem to provide good protection by means of wall material as the values do not increase drastically going from 25°C to 42°C. The increase in values of redness (a*) and yellowness (b*), which is seen as browning, is more enhanced when gum arabic concentration is higher (Manickavasagan et al., 2015). Overall, the wall material does seem to affect the color of the spray dried powders as has been seen in previous studies (Yousefi, Emam-Djomeh, & Mousavi, 2011). The values for L* was higher for GA blended powders and the a* and b* values were higher for MD blended tart cherry powder, which is similar to a study done with spray dried blackberry powder using MD and GA as carrier agents (Ferrari, Germer, Alvim, Vissotto, & de Aguirre, 2012). In another study on spray drying of grapes, similar increase in redness (a*) was observed in the storage temperature studies (Amendola, De Faveri, & Spigno, 2010). The chemical structure of GA that is rich in hydroxyl groups has been known to be susceptible to dehydration and oxidation leading to some degradation of core material (Bertolini, Siani, & Grosso, 2001).

6.3.2 Chemical analysis at varying temperatures.

The spray-dried powder samples with variable wall materials were analyzed for their chemical content by different assays viz. Phenolic content using TPC assay, Flavonoid content by TFC assay and Antioxidant potential was computed using FRAP

assay. All the samples were kept at 3 different temperatures to check their storage stability at those frigid, room and hot temperatures.

6.3.2.1 Total phenolics content (TPC) assay

As can be seen from results in Figure 6.1(a) - 6.1(c), the various tart cherry spray-dried powders have significant differences ($P < 0.05$) in total phenolic content when subject to varying storage temperatures. Among the various samples, it can be clearly seen that the samples spray-dried with an equal blend of MD and GA had the highest phenolic content at all temperatures which was probably due to emulsifying property of GA and density/porosity due to MD as wall materials. Similar results have been obtained by researchers wherein, the use of blend of MD-GA has been found to be best in preservation of vitamin C in acerola powder at temperatures upto 35°C (Carrillo-Navas, H. González-Rodea, D.A. Cruz-Olivares, J., Barrera-Pichardo, J.F.. Román-Guerrero, A. Pérez-Alonso, 2011; Nayak & Rastogi, 2010). Another study found that the combined effect of gum Arabic and maltodextrin as wall material increased the stability of microencapsulated anthocyanins by spray drying (Righetto & Netto, 2006). The samples have highest phenolic content at 4°C, which is frigid refrigerated condition, apt for fruit storage across the board for all samples irrespective of wall material, with a noticeable drop in phenolic content over the 30 days storage period. One of the probable reasons for this reduction even at the optimum temperature maybe due to the precipitation of flavonoids at refrigeration temperature (Gil-Izquierdo, Riquelme, Porras, & Ferreres, 2004; González-Molina, Moreno, & García-Viguera, 2008; Overvad et al., 2012).

Although among themselves, the combination of MD and GA retains highest phenolic activity. Now, at room temperature, 25°C, the phenolic content drops by 25-35% and during storage for 30 days, same as all other samples, its drops further. The rate of oxidation of TPC has been known to be enhanced at room temperature and so reduced the retention of overall phenolic content in the stored samples (Quek et al., 2007).

At higher temperature of 42°C, there is further loss of phenolics, but the wall materials do seem to have a positive effect on retention of phenols. The reduction of phenols at this temperature is higher than at other temperatures but there is another trend that higher level of maltodextrin leads to a reduction in the phenolic content, a trend seen before, primarily due to concentration effect of maltodextrin (Mishra, Mishra, & Mahanta, 2014).

6.3.2.2 Total Flavonoid content (TFC)

The flavonoids have a lot of hydroxyl groups that quickly form hydrogen bonding with the polysaccharide, forming a polysaccharide-flavonoid complex (Bordenave, Hamaker, & Ferruzzi, 2014). Gum Arabic has been used extensively in the wine industry for the same reason (Bordenave et al., 2014). The maltodextrin used in this study had 9–12 dextrose equivalents, which exhibits retentions of flavors and polyphenols and higher yields while having a very low viscosity at high concentration which is opposite of gum Arabic, which has higher viscosity (Bakowska-Barczak & Kolodziejczyk, 2011; Madene, Jacquot, Scher, & Desobry, 2006). Therefore, the highly active interaction between tart cherry samples and wall material solution especially combining the bonding affinity of gum Arabic along with the functional properties of maltodextrin results in better

microencapsulating properties than other wall material combinations used in this study. In the light of our results as seen in Figure 6.2(a) – 6.2(c), gum Arabic could actively link to tart cherry sample in the aqueous solution and was able to retain flavonoids extract throughout the spray drying process.

6.3.2.3 Ferric reducing antioxidant power (FRAP)

The antioxidant capacity is one of major factors we assess and strive to improve, by using various encapsulation methods and materials. To assess the antioxidant potential of the various samples, we perform the FRAP assay. Various studies have indicated that the electron donation capacity of bioactive compounds is associated with the reducing power and therefore the antioxidant activity (Siddhuraju, Mohan, & Becker, 2002; Yen, Duh, & Tsai, 1993). The presence of antioxidants in the sample reduces the ferric ion (Fe^{3+}) to the corresponding ferrous ion (Fe^{2+}) by donating an electron. The reducing power results can be seen in Figure 6.3(a) - 6.3(c), the samples stored at 25°C lost their Ferric reducing activity at faster rate than the sample stored at 4°C, i.e. refrigeration temperature. This observation may be interrelated with the decrease in total phenolic content of the powder observed earlier in the TPC assay. The rate of degradation of ferric ion scavenging activity was relatively higher than to the rate at which there was loss of total phenolic content which supports the findings of previous researcher findings. Researchers have reported in the past that because of them being secondary metabolites, phenolics groups may be broken down into smaller components or utilized into combining to form new constituents that may lead

to lower antioxidant power. Several research groups have reported about this mutual loss of total phenols and FRAP activity (Michalska, Wojdyło, Łysiak, & Figiel, 2017).

6.3.3 Moisture isotherm

Equilibrium moisture content was plotted against equilibrium relative humidity data were fitted by using isotherm models. Data were fitted and modeled using GAB. The three parameters GAB equation derived independently by Guggenheim (Guggenheim, 1966), Anderson (Anderson, 1946), and de Boer (Deboer, 1953) is a multi-molecular, localized, semi-theoretical and homogeneous adsorption model.

$$M = \frac{M_0 C K a_w}{(1-Ka_w)(1-Ka_w+CKa_w)} \quad (6.1)$$

where M is the moisture content (kg/kg dry solid), M_0 is the monolayer moisture content; C and K are constants related to the energies of interaction between the first and further molecules at the individual sorption sites. The data treatment and curve fitting were done using Origin Pro 8.5.1 software (OriginLab Corporation, Northampton, MA).

The GAB model represents a refined extension of the BET theory, postulating that the state of the sorbate molecules in the second and higher layers is equal, but different from that in the liquid-like state. This assumption introduces an additional degree of freedom (an additional constant, K). Lower sorption than that demanded by the BET model is predicted by the GAB isotherm, making it more versatile and successful up to high water activities (i.e. $a_w = 0.9$).

Our spray-dried tart cherry powder had an average of 15% moisture content of dry basis similar to the spray-dried blueberry extract reported in another paper where moisture was 15–20% (Lozada-Ramírez et al., 2011). The final moisture content of the spray-dried product is a major indicator of water diffusion and has been seen in some research results that less than 7% moisture, tend to avoid moisture migration (Pitalua, Jimenez, Vernon-Carter, & Beristain, 2010). The sorption isotherms of spray-dried BBE and corresponding models. Lack-of-fit tests were conducted for the entire water activity range. The Origin pro results showed that GAB isotherms have better values of coefficient of determination, R^2 (0.95-0.99) than other models of moisture isotherms, and therefore is the best fit for our data set (Figure 6.4-6.6).

Furthermore, on the basis of higher R^2 of the linear model and the fitting test, the GAB equation was more suited than the other isotherm equations. The resultant moisture isotherms for the spray dried samples with 5 variations of wall material with MD & GA for the non-micronized, megatron and high-pressure micronized samples can be seen in Figure 6.4, Figure 6.5 and Figure 6.6 respectively. One the trends that was observed in the isotherms was that in both micronized and non-micronized samples, that given some intra-water activity variation, the overall tendency to absorb moisture was highest in GA-only spray dried samples and lowest was in MD-only samples. The water absorption increased as the level of Gum Arabic was increased, or Maltodextrin was decreased. Higher values of moisture content were obtained for powders produced with Gum Arabic at a concentration of 30%. These results were in agreement with those reported (Goula & Adamopoulos, 2008) for tomato pulp powder, whose hygroscopicity values decreased with an increase in maltodextrin concentration. Another research group (Jaya & Das, 2004)

reported that the hygroscopicity of mango powder decreased with increased amount of added maltodextrin. The X_m values for the GA wall material are two to three times that of the MD carrier material, which is attributed to particle size, morphology and presence of hydrophilic groups in the GA molecule, this pattern was also seen in a recently published work (Dev, Annamalai, Orsat, Raghavan, & Ngadi, 2018)

An interesting result from the humidity study was that among all the samples, the non-micronized samples had higher moisture content than the micronized sample as the water activity increased specially at the highest values. The lowest moisture content was seen when maltodextrin was used as the wall material. It has been reported that maltodextrin with similar DE values (9-12) produced powders of spray-dried soy sauce with less cohesion, higher glass transition temperature, and reduced hygroscopicity, as observed by (Wang & Zhou, 2012). Similar results for hygroscopicity for chicken breast encapsulated with maltodextrin were reported by (Kurozawa, Morassi, Vanzo, Park, & Hubinger, 2009). The use of MD & GA as carrier agents improved powder stability, reduced the moisture content, and accordingly reduced the powder's hygroscopicity, improving storage stability as has been seen by another research on mussel protein hydrolysate powder (Silva, Kurozawa, Park, & Hubinger, 2012).

6.3.4 Color measurements at varying relative humidities

The color readings for lightness (L*), redness (a*) and yellowness (b*) values between non-micronized and micronized samples with variation in spray-dried wall material exhibited significant difference ($P \leq 0.05$) during paired t-test at five different relative humidity conditions as seen in Table 6.2, Table 6.3 and Table 6.4. One key result

that could be seen for all samples was that the wall material softened and started to stick to other particles as moved higher up in relative humidity from 11% to 75%. There have been previous studies on the effect of spray dried powder with GA, MD and a blend of both and similar trends were observed in the way that the blend of MD and GA provides the best stability over the range of relative humidity's such as work on blackberry (Delgado-Vargas & Paredes-López, 2010) and on black currant (Bakowska-Barczak & Kolodziejczyk, 2011). Another study on elderberry compared several wall materials and found best results with GA & MD blend (Murugesan & Orsat, 2011). Stability of spray-dried Acai also improved and there was no drastic change in color at various relative humidities (Tonon et al., 2010).

The physical changes in color could be easily seen as the relative humidity increased beyond 32%, as the samples were no more in fine powder form but a rather caking state with darker shade of colors is accompanied by drastic increase in b* values, similar phenomenon was seen and studied by another research group in food powders (Aguilera et al., 1995). In some combinations of wall material like higher MD or higher GA there is certain dip in color values and then a rise again, this can be attributed to similar phenomenon observed in previous studies where the wall material releases water upto a certain water activity and then starts to absorb water again due to crystallization.

6.3.4 Chemical analysis at varying relative humidities

The spray-dried powder samples with variable wall materials were analyzed for their chemical content by different assays, viz. Phenolic content using TPC assay and Antioxidant potential was computed using FRAP assay. Measurements were taken at

different % relative humidities: lithium chloride (11%), potassium acetate (22.5%), magnesium chloride (32%), magnesium nitrate (57%) and sodium chloride (75%).

6.3.4.1 Total phenolics content (TPC)

The spray-dried powder was stored at 5 different relative humidities and the powder was tested for total phenolic content separately. There is rapid loss of phenolic content at higher relative humidity due to higher water activity as seen in Figure 6.7. The stability of the phenolic compounds is affected by a multitude of storage conditions such as light, temperature, oxygen, humidity and pH (Kearsley & Rodriguez, 1981; Singh, Sharma, Bansal, Gupta, & Singh, 2015). Corroborating with present study, similar reactions of oxidation would have taken place at various humidity levels that lead to the consequent reduction in the TPC values (Chua et al., 2018). The combination of MD and GA provides best stability in terms of retention of phenolic compounds at all water activities. Similar to trends seen by other research reports, we also saw that during spray-drying, higher level of maltodextrin effectively encapsulates the phytonutrients of the functional ingredient and guards it against degradation (Mishra et al., 2014; Mishra, Rai, & Mahanta, 2015). The loss of phenolic content was higher at high humidity for all samples.

6.3.4.2 Ferric reducing antioxidant power (FRAP)

FRAP is an extensively used metric for determination of antioxidant potential and has been used for the assessment of the antioxidant and reducing power of tart cherry powders and their stability at 5 different relative humidity conditions. It is known that FRAP assay gives a direct estimation of the antioxidants or reductants existent in the spray-

dried sample based on its ability to reduce the Fe^{3+}/Fe^{2+} couple (Aljadi & Kamaruddin, 2004). As we can see in Figure 6.8 that FRAP values follow a very similar pattern as phenolic contents and reduces as the humidity level increase. There is more loss of antioxidant potential for maltodextrin coated spray powders at higher humidities as compared to gum Arabic. The optimal MD to GA ratio was determined to be 1:1 as can be seen by our results. They also align consistently with process results of the highest values for FRAP as seen by previous research done on aqueous bitter melon extract (Tan, Kha, Parks, Stathopoulos, & Roach, 2015). Because of the encapsulating agent concentration and combination, the result is the formation of a dense and tightly packed continuous phase membrane which gives excellent coverage to solutes, such as the phenolics and flavonoids from the, and thereby prevent the degradation of these bioactive compounds like anthocyanins when exposed to oxidation and humidity (Carneiro, Tonon, Grosso, & Hubinger, 2013; Jafari et al., 2008).

6.4 Conclusions

Knowledge of the sorption properties of foods is critical in determining the storage stability and the shelf life of the dried foods. In our study, non-micronized and micronized (megatron and high-pressure homogenization) tart cherry purée samples were subjected to spray drying with five different variations of wall materials combinations of maltodextrin (MD) and gum arabic (GA) viz. MD (30%), MD: GA (20%: 10%), MD: GA (15%: 15%), MD: GA (10%: 20%), and GA (30%). All the spray dried microcapsules were tested for their storage stability by storing them at three temperatures viz. 4°C, 25°C, and 42°C and five different relative humidity conditions for a period of 30 days. Samples were placed in

five chambers equilibrated at different relative humidities (%RH). Saturated salt solutions were used to maintain different %RH: lithium chloride (11%), potassium acetate (22.5%), magnesium chloride (32%), magnesium nitrate (57%) and sodium chloride (75%). The samples were equilibrated and weighed every week until the weight was stabilized. Upon storage at different temperatures and different relative humidities, it was found that the total phenolics and flavonoid content, and the antioxidant activity decreased slowly across all storage conditions in the order 4°C > 25°C > 42°C, for temperature variations, and 11% > 22.5% > 32% > 57% > 75%, for different relative humidity conditions. Although there was significant polyphenolic degradation over the 30-day storage period, it was lowest in the micronized samples as compared to the control, indicating a positive impact of particle size reduction. Owing to the protective effect of spray drying, the blend of maltodextrin and gum arabic in equal ratio (MD: GA (15%: 15%)) was the best wall material in providing storage stability, as the decline in the bioactives values was the lowest for this combination (92 GAE μg/ml), as opposed to 152 GAE μg/ml and 157 GAE μg/ml for polyphenols in the higher MD (30%) or GA (30%) concentrations respectively. For the storage stability of tart cherry microcapsules, we determined that the most optimum range of temperature was 4°C - 25°C and that of relative humidity was 11% - 22.5% which resulted in the lowest polyphenolic degradation. These results provide a good understanding of the stability of spray dried tart cherry purée at a wide range of storage conditions and the sorption properties could be further studied using the other models, to better predict and optimize the storage stability and in order to develop a food ingredient that promotes health benefits of tart cherry.

6.5 References

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Table 6.1: Color properties of spray-dried tart cherry powder with variation in wall materials measured at three temperature points.[1]

Method	Wall	L*			a*			b*		
	Temperature	4°C	25°C	42°C	4°C	25°C	42°C	4°C	25°C	42°C
Control	MD30	79.21 ± 0.38^{c}	81.23 ± 0.24^{c}	81.98 ± 0.22^{c}	9.87 ± 0.11^{a,e}	10.01 ± 0.81^{a,b}	10.17 ± 0.56^{a,b}	1.98 ± 0.46^{b,c}	2.02 ± 0.12^{b,d}	2.19 ± 0.76^{a,e}
	MD20: GA10	81.12 ± 0.96^{b,d}	84.23 ± 0.28^{a}	83.11 ± 0.79^{b,d}	7.15 ± 0.59^{a,d}	7.71 ± 0.57^{a,c}	7.99 ± 0.16^{a,c}	2.32 ± 0.70^{a,b,d}	2.54 ± 0.72^{a,e}	2.88 ± 0.01^{b,e}
	MD15: GA15	81.28 ± 0.36^{a,c}	83.38 ± 0.24^{b}	84.88 ± 0.46^{a,c}	6.38 ± 0.09^{a,e}	7.24 ± 0.79^{a,d}	7.88 ± 0.04^{a,d}	3.22 ± 0.05^{b,c}	3.45 ± 0.06^{c,e}	3.88 ± 0.66^{c,d}
	MD10: GA20	80.96 ± 0.28^{a,b,c}	84.49 ± 0.26^{b}	82.48 ± 0.20^{a,b,c}	6.72 ± 0.07^{c,e}	7.63 ± 0.35^{a,b}	8.12 ± 0.12^{a,b}	1.77 ± 0.83^{a,b,c}	1.82 ± 0.82^{b,e}	1.93 ± 0.24^{c,e}
	GA30	78.40 ± 0.45^{b,d}	80.53 ± 0.12^{d}	81.23 ± 0.53^{b,d}	8.85 ± 0.51^{c}	7.91 ± 0.66^{a}	9.81 ± 0.20^{a}	1.59 ± 0.26^{b,d}	2.31 ± 0.52^{a,d}	2.83 ± 0.66^{a,d}
Megatron	MD30	82.72 ± 0.73^{a,b}	83.45 ± 0.29^{d}	83.45 ± 0.78^{a,b}	9.54 ± 0.29^{b,d}	9.85 ± 0.32^{a,b}	7.23 ± 0.13^{a,b}	2.45 ± 0.84^{a,b}	2.88 ± 0.45^{c,e}	2.98 ± 0.23^{a,e}
	MD20: GA10	82.13 ± 0.63^{a,b}	85.39 ± 0.45^{b}	86.93 ± 0.29^{a,b}	7.01 ± 0.03^{c,d}	7.04 ± 0.55^{b,c}	5.98 ± 0.10^{b,c}	2.65 ± 0.87^{a,b}	2.95 ± 0.80^{c,d}	3.19 ± 0.39^{b,e}
	MD15: GA15	83.47 ± 0.47^{a,b}	86.61 ± 0.21^{a}	87.52 ± 0.34^{a,b}	5.13 ± 0.66^{c,e}	5.6 ± 0.08^{b,e}	5.78 ± 0.07^{b,e}	3.76 ± 0.21^{a,b}	3.87 ± 0.30^{c,d}	4.01 ± 0.98^{c,e}
	MD10: GA20	81.67 ± 0.38^{b}	84.63 ± 0.27^{c}	86.12 ± 0.38^{b}	5.43 ± 0.39^{b,e}	5.87 ± 0.62^{b,d}	6.55 ± 0.13^{b,d}	2.69 ± 0.34^{a,b}	2.98 ± 0.76^{c,e}	2.99 ± 0.91^{b,e}
	GA30	78.34 ± 0.31^{b,c}	82.76 ± 0.11^{e}	84.76 ± 0.83^{b,c}	7.34 ± 0.32^{a,d}	5.96 ± 0.37^{b}	6.54 ± 0.11^{b}	2.41 ± 0.67^{a,b,c}	2.46 ± 0.26^{b,d}	2.95 ± 0.46^{c,e}
HPH	MD30	84.05 ± 0.60^{a,c}	85.44 ± 0.48^{d}	85.31 ± 0.93^{a,c}	9.32 ±. 0.45^{a,d}	9.54 ±. 0.16^{a,c}	9.54 ±. 0.13^{a,c}	2.98 ± 0.60^{a,c}	3.13 ± 0.38^{c,d}	3.03 ± 0.48^{a,e}
	MD20: GA10	84.10 ± 0.75^{a,b,c}	87.57 ± 0.03^{c}	88.98 ± 0.83^{a,b,c}	5.34 ± 0.10^{c,e}	6.06 ± 0.27^{c,b}	6.87 ± 0.05^{c,b}	3.11 ± 0.28^{a,b}	3.45 ± 0.49^{c,d}	3.66 ± 0.43^{a,d}
	MD15: GA15	85.41 ± 0.68^{a,b}	89.08 ± 0.15^{a}	89.66 ± 0.77^{a,b}	4.27 ± 0.93^{c,d}	5.32 ± 0.86^{c,d}	5.88 ± 0.03^{c,d}	4.71 ± 0.81^{a}	4.82 ± 0.53^{a,e}	4.99 ± 0.91^{b,d}
	MD10: GA20	84.23 ± 0.29^{a}	88.02 ± 0.17^{b}	87.67 ± 0.39^{a}	4.65 ± 0.10^{b}	5.76 ± 0.06^{c}	5.43 ± 0.19^{c}	2.69 ± 0.53^{a,b,c,d}	3.11 ± 0.13^{b,d}	3.23 ± 0.48^{c,d}
	GA30	80.83 ± 0.49^{a,d}	84.73 ± 0.20^{e}	85.87 ± 0.69^{a,d}	5.52 ± 0.82^{a,c,d}	4.83 ± 0.72^{a,c}	5.32 ± 0.10^{a,c}	2.42 ± 0.18^{a,d}	2.78 ± 0.92^{b,d}	3.21 ± 0.20^{b,d}

[1]Columns with same letter are not statistically different at $p < 0.05$. (MD: Maltodextrin, GA: Gum Arabic, number of observations= 3)

Table 6.2: Lightness (L*) measurements at varying relative humidity conditions.[1]

Method	Wall	L*				
	R. Humidity (%)	11	22.5	32	57	75
Control	MD30	74.65 ± 1.16^{a,d}	75.45 ± 0.79^{a,d}	73.28 ± 1.02^{a}	67.75 ± 0.96^{a,c}	43.92 ± 0.29^{a,b}
	MD20: GA10	76.64 ± 1.35^{b,c}	78.6 ± 0.73^{a}	76.42 ± 0.55^{a,c}	69.33 ± 1.27^{a,b}	49.05 ± 1.19^{a,c}
	MD15: GA15	76.79 ± 0.38^{b,d}	79.4 ± 0.04^{b,c}	77.28 ± 0.18^{b,c}	68.71 ± 1.06^{b,c}	44.16 ± 0.05^{a,d}
	MD10: GA20	78.56 ± 1.23^{a,c}	80.15 ± 0.12^{b,d}	79.63 ± 1.22^{b,c}	69.13 ± 0.72^{a,b,c}	39.55 ± 1.45^{a,b}
	GA30	80.4 ± 0.83^{a,b}	78.8 ± 0.47^{b}	78.88 ± 0.37^{d}	73.65 ± 0.18^{b,d}	49.57 ± 0.2^{a}
Megatron	MD30	73.05 ± 1.23^{a,c}	78.21 ± 1.49^{b,c}	77.66 ± 0.3^{b,c,d}	69.38 ± 1.26^{a,b}	47.21 ± 1.5^{a,b}
	MD20: GA10	74.88 ± 0.65^{a,d}	79.5 ± 0.41^{a,d}	80.12 ± 0.15^{b,d}	74.05 ± 1.38^{a,b}	52.62 ± 1.07^{b,c}
	MD15: GA15	76.38 ± 1.2^{a,c}	80.76 ± 1.37^{a,c}	77.82 ± 1.43^{c,d}	72.47 ± 1.1^{a,b}	49.65 ± 1.48^{b,e}
	MD10: GA20	78.18 ± 0.75^{a,c}	81.45 ± 0.41^{c}	79.68 ± 0.15^{b}	70.49 ± 1.46^{a,b}	40.9 ± 0.29^{b,d}
	GA30	80.37 ± 0.85^{b,d}	79.38 ± 0.82^{a,c}	79.81 ± 0.29^{b,c}	76.71 ± 0.79^{a,b,c}	55.9 ± 0.28^{b}
HPH	MD30	71.5 ± 1.44^{c,d}	78.65 ± 1.27^{a,d}	80.27 ± 0.2^{a,d}	69.88 ± 1.18^{a,c}	49.88 ± 0.83^{a,c}
	MD20: GA10	70.33 ± 1.5^{b,d}	81.08 ± 1.06^{a,d}	80.27 ± 0.12^{a,d}	76.81 ± 1.09^{a,b}	55.36 ± 0.11^{c,b}
	MD15: GA15	74.74 ± 0.39^{a,c}	81.53 ± 1.44^{c}	80.39 ± 0.63^{c,d}	74.82 ± 0.51^{a}	52.23 ± 0.42^{c,d}
	MD10: GA20	73.19 ± 0.38^{c,d}	81.68 ± 0.02^{b,d}	80.43 ± 0.96^{c}	72.18 ± 0.79^{a,b,c,d}	49.67 ± 0.82^{c}
	GA30	74.65 ± 1.16^{a,d}	75.45 ± 0.79^{a,d}	73.28 ± 1.02^{a}	78.91 ± 1.33^{b,c}	56.08 ± 0.16^{a,c}

[1]Columns with same letter are not statistically different at $p < 0.05$. (MD: Maltodextrin, GA: Gum Arabic, number of observations= 3)

Table 6.3: Redness (a*) measurements at varying relative humidity conditions.[1]

Method	Wall	a*				
	R. Humidity (%)	11	22.5	32	57	75
Control	MD30	9.52 ± 1.03^{a,c}	12.71 ± 1.12^{a,d}	12.7 ± 0.05^{a,c}	9.88 ± 1.3^{a}	10.42 ± 0.32^{a}
	MD20:GA10	9.32 ± 0.16^{a,c}	9.6 ± 0.1^{b,c}	9.66 ± 0.93^{c}	7.61 ± 0.65^{a,b}	7.14 ± 1.38^{a,b}
	MD15:GA15	9.34 ± 1.31^{c,d}	10.23 ± 0.49^{c,d}	8.42 ± 0.95^{a}	7.15 ± 0.85^{b,c}	6.29 ± 0.7^{b,c}
	MD10:GA20	8.56 ± 1.16^{b,c}	8.4 ± 0.97^{a,c}	7.15 ± 0.07^{b,d}	7.28 ± 0.52^{c}	7.01 ± 0.54^{c}
	GA30	6.91 ± 1.33^{b}	8.36 ± 0.6^{b,d}	6.91 ± 1.19^{c}	7.1 ± 0.16^{a,c}	3.78 ± 1.03^{b}
Megatron	MD30	8.75 ± 1.08^{c}	12.37 ± 0.12^{a,b,d}	11.94 ± 0.59^{a,c}	9.67 ± 0.99^{a,b}	9.41 ± 0.42^{c}
	MD20:GA10	8.14 ± 0.6^{a,c}	9.47 ± 1.07^{a}	8.2 ± 1.32^{c,d}	7.42 ± 0.18^{a}	6.21 ± 0.16^{b,c}
	MD15:GA15	8.87 ± 0.05^{a,d}	9.48 ± 0.59^{a,c}	8.26 ± 0.48^{a,c}	6.9 ± 1.25^{a,c}	6.03 ± 1.13^{a}
	MD10:GA20	7.25 ± 0.08^{b,d}	7.57 ± 1.26^{c}	6.75 ± 1.23^{c,d}	6.9 ± 0.13^{c,d}	4.45 ± 0.65^{a,d}
	GA30	7.45 ± 0.37^{c}	7.94 ± 1.27^{a,d}	6.15 ± 0.05^{a,d}	6.23 ± 1.22^{b}	2.84 ± 1.48^{c,d}
HPH	MD30	11.5 ± 0.28^{a,c}	11.16 ± 1.06^{b,d}	9.76 ± 0.37^{b,c}	8.71 ± 0.43^{d}	7.21 ± 1.47^{a,b}
	MD20:GA10	6.61 ± 1.27^{c}	9.23 ± 1.07^{b,c}	6.9 ± 0.3^{a,c}	7.38 ± 1.34^{a,d}	5.57 ± 0.53^{b,c}
	MD15:GA15	7.9 ± 1.29^{a,d}	8.74 ± 0.81^{a,c}	6.7 ± 0.12^{c,d}	5.85 ± 1.02^{c,d}	2.49 ± 1.09^{b,d}
	MD10:GA20	6.66 ± 1.3^{cd}	7.42 ± 0.93^{c}	6.37 ± 0.35^{d}	6.84 ± 0.83^{b,c}	4.21 ± 1.42^{b}
	GA30	7.99 ± 1.49^{d}	7.13 ± 0.5^{b,c}	5.68 ± 0.95^{b,c}	5.35 ± 1.49^{b}	2.49 ± 0.14^{a,d}

[1]Columns with same letter are not statistically different at $p < 0.05$. (MD: Maltodextrin, GA: Gum Arabic, number of observations= 3)

Table 6.4: Yellowness(b*) measurements at varying relative humidity conditions.[1]

Method	Wall	b*				
	R. Humidity (%)	11	22.5	32	57	75
Control	MD30	-1.79 ± 0.22^{a,d}	-0.38 ± 0.89^{b,c}	0.22 ± 0.03^{a}	4.47 ± 0.69^{c,d}	3.06 ± 0.52^{a}
	MD20:GA10	0.19 ± 0.03^{c}	-0.11 ± 1.31^{a,b,d}	0.18 ± 0.04^{a,c}	4.17 ± 0.51^{b,c,d}	3.44 ± 0.57^{a,b}
	MD15:GA15	0.38 ± 0.05^{b,c}	0.3 ± 0.01^{b,c}	0.77 ± 0.08^{b,c}	3.25 ± 1.02^{b,d}	2.31 ± 0.44^{a,b}
	MD10:GA20	0.99 ± 0.9^{a,c}	0.57 ± 0.08^{a,b,c}	1.2 ± 0.07^{b,c}	4.24 ± 1.14^{c,d}	2.76 ± 0.85^{a,b}
	GA30	2.08 ± 0.09^{c,d}	2.08 ± 0.74^{b,d}	2.66 ± 0.05^{d}	4.8 ± 0.59^{b}	2.99 ± 0.05^{a,b}
Megatron	MD30	-1.18 ± 0.05^{a,b}	-0.33 ± 0.04^{a,b}	0.29 ± 0.02^{b,c,d}	4.54 ± 1.11^{a,d}	3.61 ± 1.03^{a,b}
	MD20:GA10	0.08 ± 0.01^{b,c}	0.35 ± 0.08^{a,b}	0.23 ± 0.17^{b,d}	4.39 ± 0.17^{b,c}	4.31 ± 1.32^{a,b,c}
	MD15:GA15	0.66 ± 0.03^{a,c}	1.04 ± 0.3^{b}	0.94 ± 0.08^{c,d}	3.75 ± 0.52^{b,c}	3.84 ± 1.29^{a,b,c}
	MD10:GA20	1.01 ± 0.06^{c}	1.39 ± 0.08^{a}	1.88 ± 0.07^{b}	4.37 ± 0.97^{c}	2.95 ± 1.02^{a,b,c,d}
	GA30	2.73 ± 0.03^{a,d}	2.84 ± 0.45^{a,b,c}	2.96 ± 0.61^{b,c}	4.84 ± 0.21^{a,c}	3.94 ± 0.72^{a,b,d}
HPH	MD30	-0.88 ± 0.06^{d}	0.13 ± 1.34^{a,c}	0.72 ± 0.04^{a,d}	5.86 ± 0.03^{a,d}	4.21 ± 1.37^{a,c}
	MD20:GA10	-0.17 ± 0.09^{a,c}	0.82 ± 0.08^{a,b}	1.29 ± 0.7^{a,d}	4.48 ± 1.4^{a}	4.64 ± 0.61^{b,c}
	MD15:GA15	1.25 ± 0.08^{c,d}	2.01 ± 0.7^{a}	2.06 ± 0.21^{c,d}	4.69 ± 0.54^{b,c}	4.29 ± 0.51^{b,c}
	MD10:GA20	1.08 ± 0.33^{a,b,c}	2.49 ± 0.57^{a,b,c,d}	2.08 ± 0.05^{c}	5.13 ± 0.73^{d}	5.23 ± 0.05^{b,d}
	GA30	2.85 ± 0.16^{b,d}	2.98 ± 0.07^{b,c,d}	3.31 ± 0.86^{a,c}	6.03 ± 0.52^{a,b,c}	4.8 ± 0.25^{a,c,d}

[1]Columns with same letter are not statistically different at $p < 0.05$. (MD: Maltodextrin, GA: Gum Arabic, number of observations= 3)

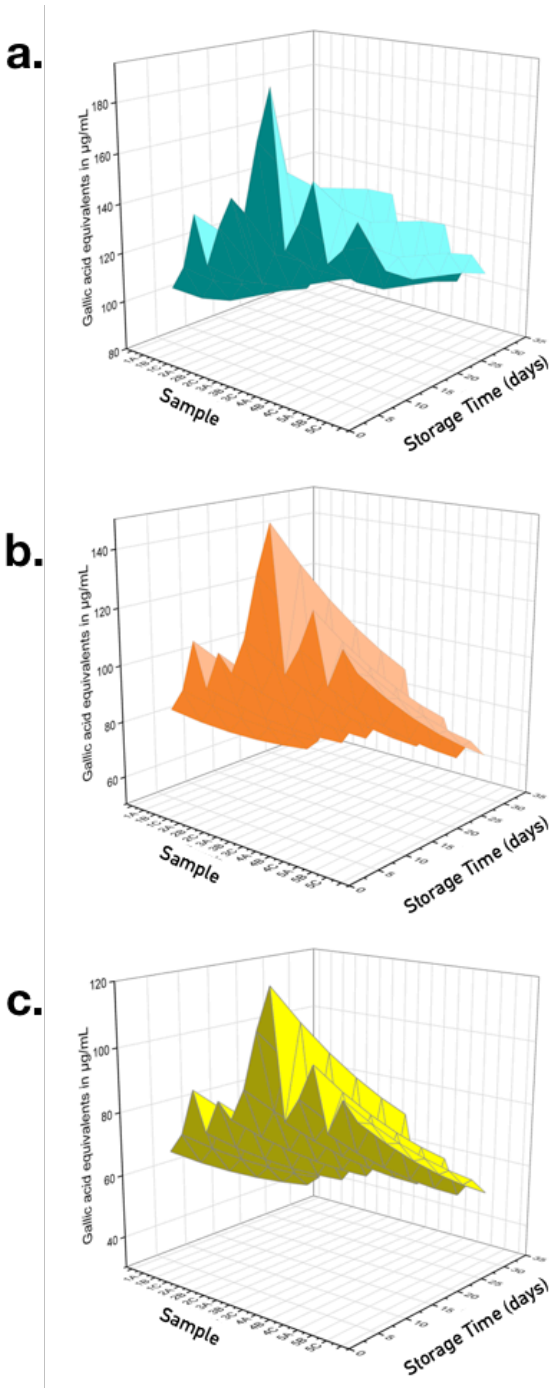

Figure 6.1: 3-D surface plots of total phenolic content (TPC) versus sample-encapsulation ratio of spray dried powder and variable experimental storage temperature at a) 4°C , b) 25°C and c) 42°C.

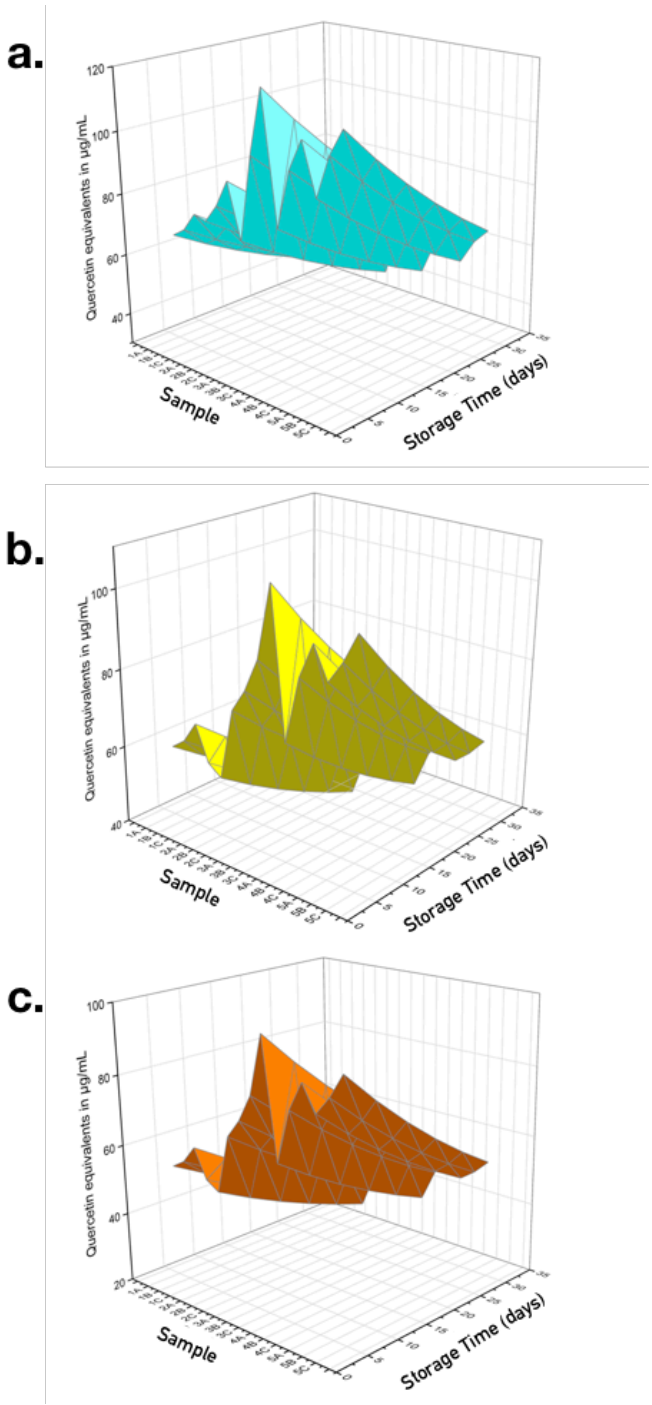

Figure 6.2: 3-D surface plots of total flavonoid content (TFC) versus sample-encapsulation ratio of spray dried powder and variable experimental storage temperature at a) 4°C , b) 25°C and c) 42°C.

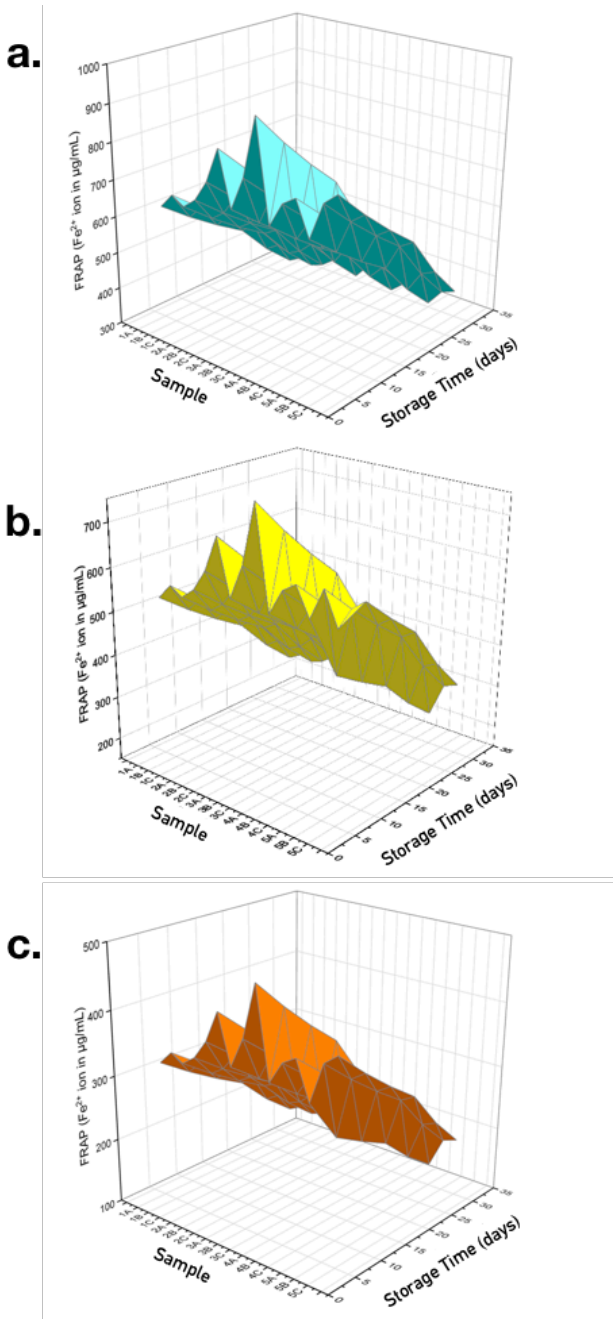

Figure 6.3: 3-D surface plots of FRAP anti-oxidant activity versus sample-encapsulation ratio of spray dried powder and variable experimental storage temperature at a) 4°C , b) 25°C and c) 42°C.

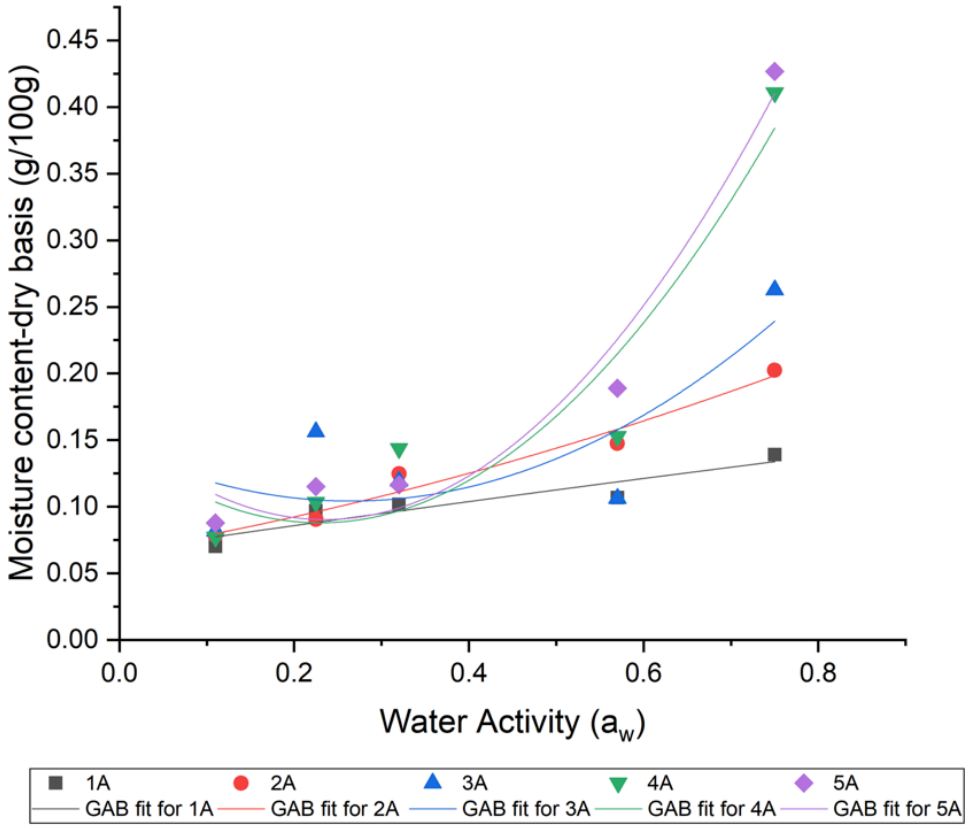

Figure 6.4: Modeling of the experimental moisture sorption isotherm for non-micronized sample based on the GAB equation.

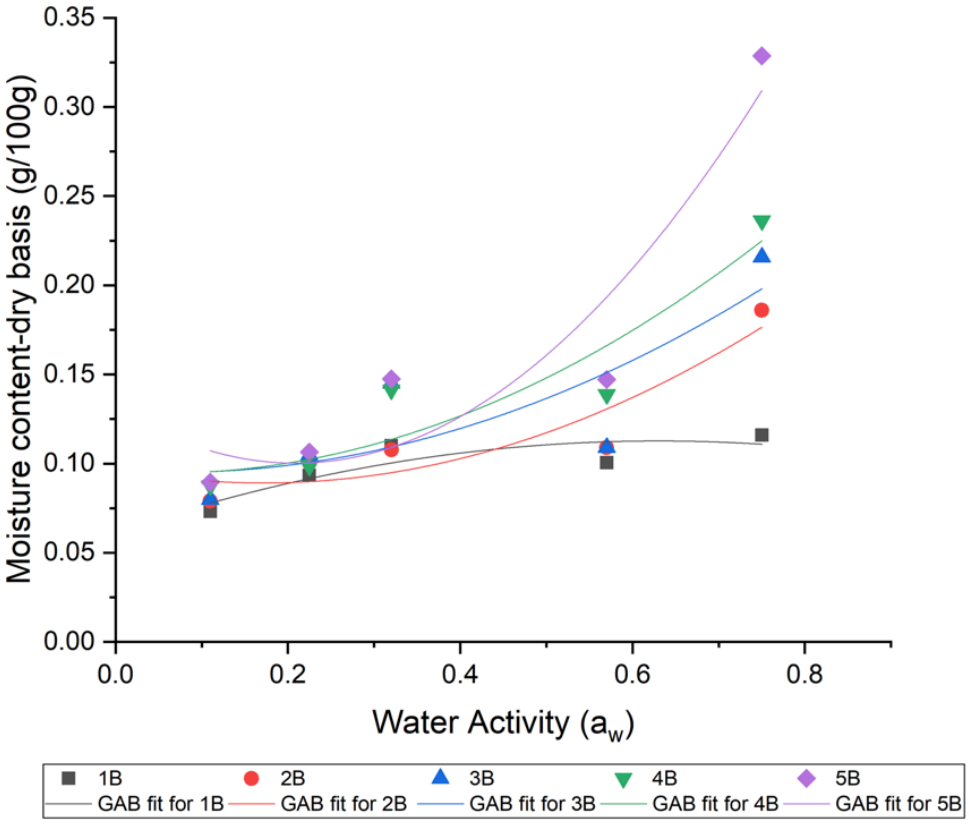

Figure 6.5: Modeling of the experimental moisture sorption isotherm for megatron-micronized sample based on the GAB equation

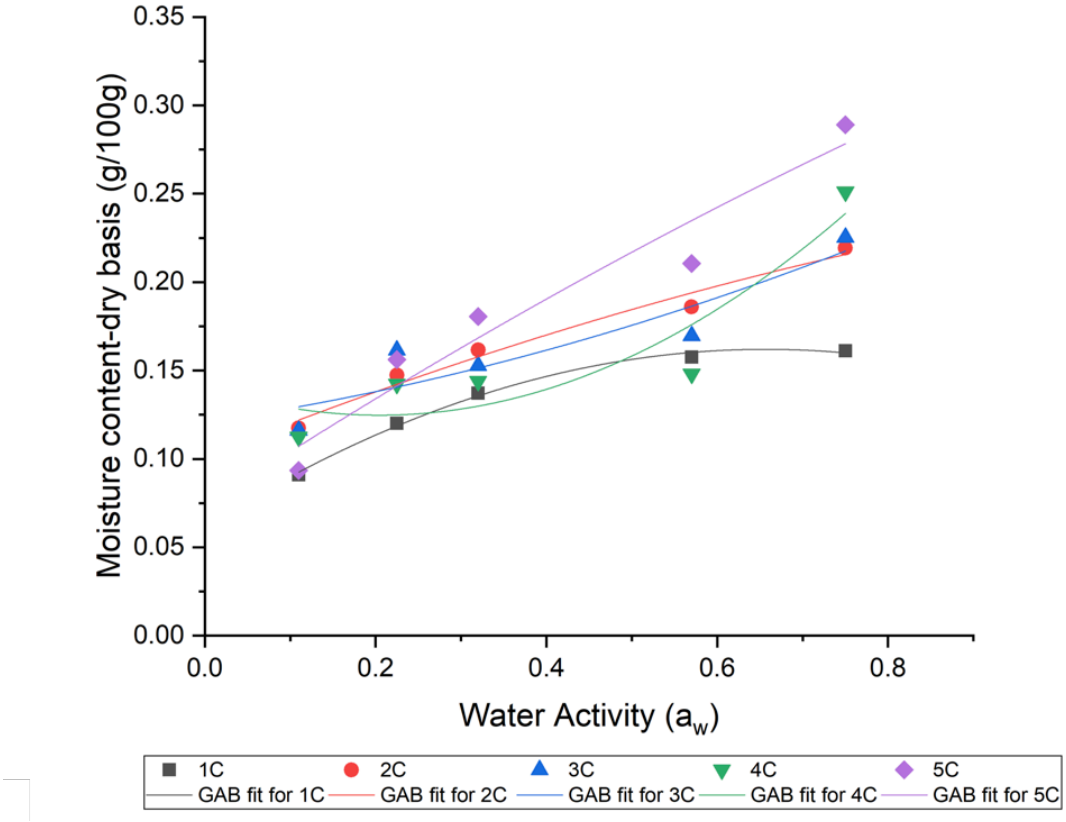

Figure 6.6: Modeling of the experimental moisture sorption isotherm for high-pressure homogenized sample based on the GAB equation

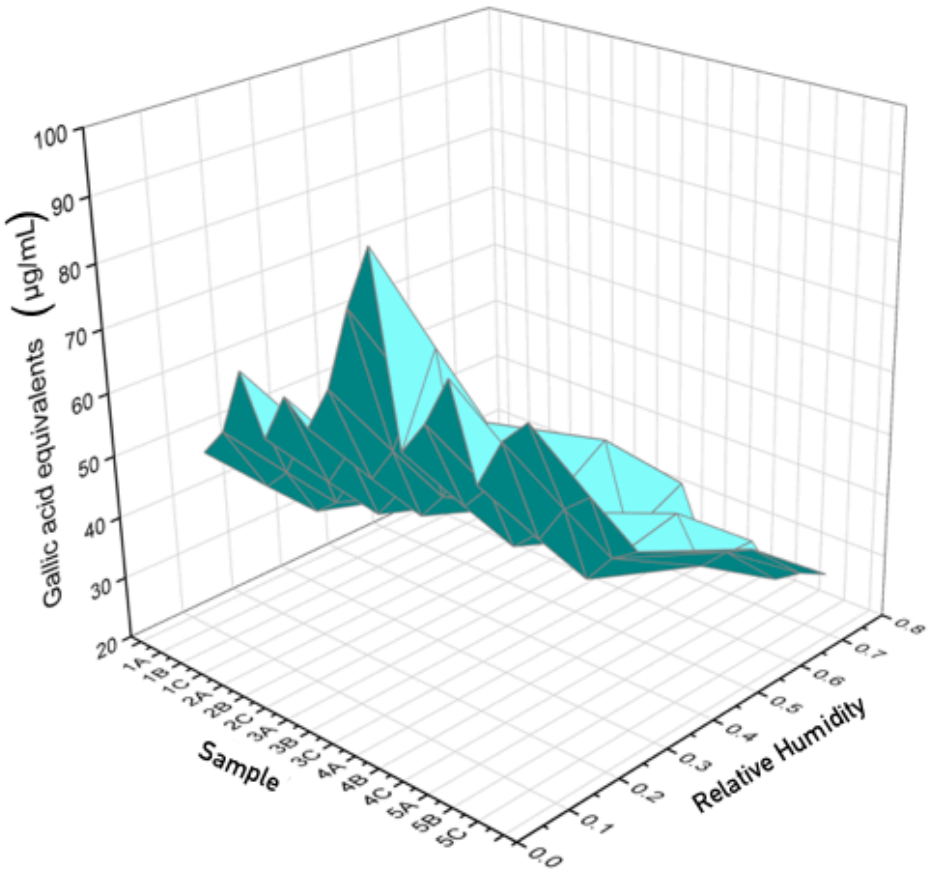

Figure 6.7: Changes in total phenolic content during storage at different relative humidity conditions. (The samples 1A- 5C have been detailed in Table 5.1)

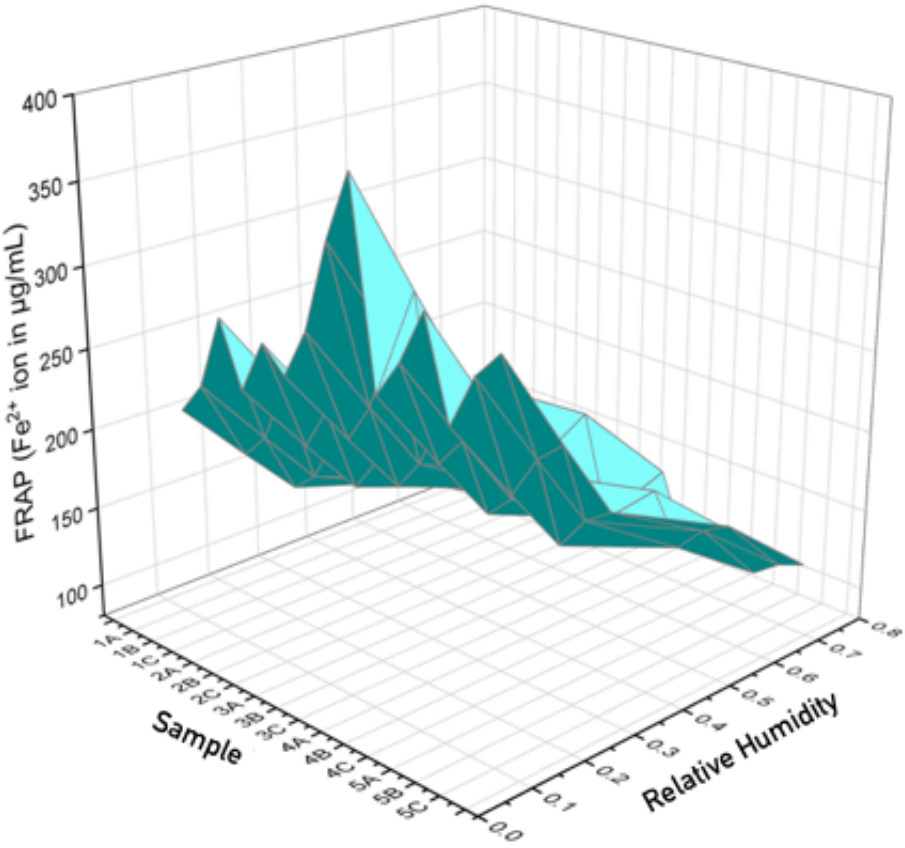

Figure 6.8: Changes in ferric reducing antioxidant power (FRAP) of the spray-dried powder upon storage at different relative humidity conditions. (The samples 1A- 5C have been detailed in Table 5.1)

CHAPTER 7

IN-VITRO DIGESTION OF MICRONIZED TART CHERRY PURÉE AND MICROENCAPSULATED POWDER

7.1 Introduction

Sour cherry (Prunus cerasus L.) fruit is known to possess considerable amounts of phenolic compounds, such as anthocyanins and other antioxidants like hydroxycinnamates, flavonoids, and flavan-3-ols (procyanidins) (Bonerz, Würth, Dietrich, & Will, 2007; Capanoglu, Boyacioglu, De Vos, Hall, & Beekwilder, 2011). Consumption of anthocyanin has been associated to various health-promoting benefits, for example anti-obesity (Kaume, Gilbert, Brownmiller, Howard, & Devareddy, 2012) and cholesterol level and plasma lipid regulation (Zawistowski, Kopec, & Kitts, 2009). Studies of bioactivity, conducted using both in vitro and in vivo techniques, have indicated several health-related impacts of sour cherry, such as tumor growth inhibition (Kang, Seeram, Nair, & Bourquin, 2003) and prevention of certain risk variables for cardiovascular diseases (Ataie-Jafari, Hosseini, Karimi, & Pajouhi, 2008).

Over the last decade, bioavailability trials in humans have shown that the absorption differs extensively based on the type of compound (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005a). Although for some compounds, for example anthocyanins, there may have been an underestimation in the plasma/urine concentration values, owing to instability of the compounds and processing of the sample (Felgines et al., 2018). Absorption of dietary polyphenols is usually very low with total metabolites plasma concentrations usually in the nM range and the time to reach maximum concentration took anywhere from 30 min to several hours depending on the site of absorption (Manach et al., 2005a; Manach, Williamson, Morand, Scalbert, & Rémésy, 2005b). Another factor that determines the bioavailability of polyphenols to peripheral tissues to be high or low, is the intestinal and biliary secretion (Silberberg et al., 2006).

To enhance the understanding of the benefits of fruit polyphenols towards improving human health, more in-depth studies involving analysis of the metabolic fate of the biomolecules are needed. Once the fruits or juices are ingested, these polyphenols undergo a metabolic journey in the gut. We need more studies that are able to analyze their stability within gastric and intestinal passage and gather information about their bioaccessibility and bioavailability. These uptake-based studies will help us understand the dynamics of bioaccessibility better. Currently, there is hardly any literature on in vivo or in vitro digestion studies on tart cherry and its polyphenols.

There have been some studies done on stability and metabolism of some dietary procyanidins in human gastric contents (Bennett et al., 2018), of quercetin in human jejunum (Petri et al., 2003) and of anthocyanins in rat intestine (He, Magnuson & Giusti, 2007) and in pig gastrointestinal tract (Wu, Pittman & Prior, 2004). A commonly used technique to study foods or food components is by employing in vitro methods that simulate the digestion conditions. There have been many reports on the effects of in vitro digestion on dietary polyphenols, e.g. flavonoids and phenolic compounds in orange juice (Gil-Izquierdo, Gil, Ferreres, & Tomás-Barberán, 2001), pomegranate juice (Pérez-Vicente, Gil-Izquierdo, & García-Viguera, 2002) and broccoli (Vallejo, Gil-Izquierdo, Pérez-Vicente, & García-Viguera, 2004) flavan-3-ols in cocoa (Zhu et al., 2002), anthocyanins in raspberry (McDougall, Dobson, Smith, Blake, & Stewart, 2005), quercetin in onions and apples (Boyer, Brown, & Rui, 2004), and isoflavones in soy bread (Walsh, Zhang, Vodovotz, Schwartz, & Failla, 2003).

The results reported by studies have shown that in vitro methods are very useful for evaluating the effects of factors such as food matrix, digestion conditions or interaction

with other compounds, on the stability and properties of polyphenols which may affect their bioaccessibility and final metabolic fate (Aura et al., 2005; Fleschhut, Kratzer, Rechkemmer, & Kulling, 2006). Although these studies are useful but there are many limitations in them like not taking into consideration the effect of light and O_2 during the digestion process, as these are important factors that due to oxidative degradation, could potentially alter the structure and properties of phenolic compounds, (Jorgensen, Marin, & Kennedy, 2004) and polymerization reactions (Talcott & Howard, 1999). It has been reported that in-vivo, the levels of dissolved O_2 usually increases with mastication (Kanner & Lapidot, 2001). Conversely, when it goes into the stomach and then further into the intestine, mucosal membrane takes up the ambient oxygen and is equilibrated across the capillary, reducing the O_2 level even further in the small intestine (He et al., 2002).

Therefore, in vitro systems that emulate low levels of oxygen offer a more realistic scenario of the physiological conditions in the stomach and small intestine (Boyer et al., 2004; Gil-Izquierdo, Riquelme, Porras, & Ferreres, 2004; McDougall et al., 2005; Pérez-Vicente et al., 2002; Vallejo et al., 2004; Walsh et al., 2003; Yokomizo & Moriwaki, 2005; Zhu et al., 2002). Another major challenge of *in vitro* digestion is to be able to simulate and mimic the movement of the sphincter muscle peristalsis and the dynamics of the gastrointestinal compartments (Guerra et al., 2012). Moreover, it is imperative to understand that bioaccessibility and bioavailability, that have a metabolic endpoint cannot be measured totally by any *in vitro* simulated method (Etcheverry, Grusak, & Fleige, 2012). Another disadvantage is the inability of *in vitro* assays to account for host factors that can possibly influence nutrient absorption such as intestinal microorganisms, nutrient status, age, genotype, physiological state (e.g., pregnancy, lactation and obesity), chronic and

acute infectious disease states, secretion of hydrochloric acid, gastric acid, and/or intrinsic factor (Etcheverry et al., 2012; Guerra et al., 2012).

Accordingly, it would seem that the best way forward is to analyze bioaccessibility or bioavailability employing in vivo measurements directly taken from humans or animals (Dyck, Tas, Robberecht, & Deelstra, 1996). However, human trials and animal assays are time consuming, costly, resource intensive, and ethically disputable (Cabañero, Madrid, & Cámara, 2004; Minekus et al., 2014). Therefore, the use of quick and inexpensive *in-vitro* digestion methods simulating gastrointestinal (GI) conditions are being utilized more as they are rapid, safe, and do not have the same ethical restrictions as in vivo methods (Liang et al., 2012). The use of simulated *in-vitro* digestion models allows us to study GI tract digestion in a simplified manner by creating phase-wise conditions such as chemical composition of digestive fluids, pH and residence time periods typical for each compartment (Bornhorst & Singh, 2014). Also, the samples being digested can also be collected at any step and time during the digestion process for analysis at each time point (Etcheverry et al., 2012).

Therefore, in our study, we chose the in-vitro static digestion model to investigate the changes in the content of bioactives of micronized (by megatron and high-pressure homogenization) and non-micronized tart cherry purée. A secondary goal of this study was to see the effect of microencapsulation by employing the method of spray drying and the effect of selection of wall material (gum Arabic and maltodextrin in different combination ratios) on the micronized tart cherry purée during the gastric and intestinal phases of in-vitro digestion. The results of this study should give valuable insight and information about

the polyphenol release properties of tart cherry purée and how its digestion is affected by particle size reduction and spray drying to generate a free-flowing powder.

7.2 Material and methods

7.2.1. Materials

Tart cherry purée was generously provided by Cherry Central Cooperative, Inc. (Traverse City, Michigan), Folin-Ciocalteau (FC) reagent, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, sodium acetate trihydrate, acetic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric (III) chloride hexahydrate, vanillin, sulphuric acid and assay standards, including trolox, gallic acid, ethanol, rutin and aecsin used were all reagent-grade chemicals purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Deionized water (DI) was prepared fresh before use, using a Millipore Milli-Q water purification system (Burlington, MA).

7.2.2 Static in vitro digestion

A static model that uses formulated saliva, gastric, duodenal, and bile juices to simulate digestion in the mouth, stomach and intestines was employed. The pH of the simulated digestive juices was adjusted with 1 M HCl or 1 M NaOH. Tart cherry samples were added to stoppered 300-mL Erlenmeyer flasks and incubated at 37C in a Model 2904-00S water bath (Boekel Scientific, Feasterville, PA) equipped with orbital agitation set at 150 rpm. The samples were digested sequentially as follows: mouth − addition of 30 mL salivary juice and mixing for 5 min; stomach − addition of 60 mL gastric juice and mixing

for 2 h; and intestines − addition of 60 mL duodenal juices and 30 mL bile juices and mixing for 2 h (the composition of juices is described in table 7.1). Deionized water served as control. Duplicate runs were conducted. Aliquots (10 mL) were collected at 1-h intervals for a total of 4 h, centrifuged at 3823 x g for 10 min, filtered at 45 μm and the filtrate stored at −20°C prior to further analyses. Simulated digestion using gastric and intestinal juices was considered complete after 2 h (gastric) and 4 h (gastric and intestinal).

7.2.3 Particle size distribution

The particle-size distribution was measured using Malvern Laser Particle Size Analyzer, Mastersizer S with 300 mm lens (Malvern Instruments, Southborough, Mass.). Tart cherry purée samples were dispersed in deionized water until an obscuration point (10–20%) was obtained in the diffractometer cell at a pump speed of 2020 rpm. An optical model based on the Mie theory of light scattering by spherical particles was applied to calculate the predicted scattering pattern with the refractive indices of the cherry purée and water as follows: real refractive index, 1.47; refractive index of water, 1.33.

The instrument's software calculated the average volume-weighted diameter, D (4,3) = $\Sigma n_i d_i^4 / \Sigma n_i d_i^3$ (where n is the number of particles in a size class of diameter d_i), surface-weighted mean diameter, D(3,2) = $\Sigma n_i d_i^3 / \Sigma n i d i^2$, the diameter below which 90% of the volume of particles are found, D(v,0.9), the diameter below which 50% of the volume of particles are found, D(v,0.5), the diameter below which 10% of the volume of particles are found, D(v,0.1), from the size distribution were calculated by the instrument software.

The measurement of particle size distribution of tart cherry purée after digestion as affected by different methods of particle size reduction was done. Five measurements from three replications were used for the analyses and the analyses were done on the diluted samples in distilled water.

The relative width of the PSD, referred to as the spread, was calculated as:

$$Spread = \frac{\mathrm{D\ (v,0.9) - D\ (v,0.1)}}{\mathrm{D\ (3,2)}} \quad (7.1)$$

7.2.4 Qualitative analysis of cherry anthocyanins by RP-HPLC

To study the anthocyanins, they were separated using a modular chromatographer HP 1100 (Agilent Technologies, Palo Alto, CA, USA) equipped with a 250 " 2.1 mm i.d. Jupiter C18 reversed-phase column, 4 mm particle diameter (Phenomenex, Torrance, CA, USA), maintained at 37C using a thermostatic oven. A diode array detector (DAD) was used to record UV-Vis spectra every 2 s in the 190–650 nm range. The HPLC separations were monitored by recording the l = 520nm wavelengths. In order to run the cherry purée samples i.e. Non-micronized, megatron-micronized and HPH-micronized samples, we first extracted the cherry samples using 1:1 methanol followed by centrifugation for 5 minutes at 10,000 g. We then took the supernatant out for analysis and analyzed the samples using HPLC. To run the HPLC analysis, we used methanol: water in the ratio 88:12 as mobile phase. Next, we arranged the vials with the samples in the sample tray and prepared a wash vial with only methanol. We first purged the columns at 5 mL/min for 5 minutes and then closed the purge valve and ran the instrument for 5 min at a flow of 1 mL/min just to wash the column out. Next, we selected the right method and checked the injection volume to be

50 μL. We then placed the vial set to the same position where it has been positioned in the sample tray and set the wavelength to 520 nm for anthocyanin and the cherry samples. We added at least 2 blanks each at the start and the end of the sequence and between two different samples. Before initiating the sequence, we rechecked the solvent and the sequence as well as all the post run commands. The RP-HPLC was then run for all our tart cherry samples and the data was collected within the Chemstation software.

7.2.5 FT-IR spectroscopy

FTIR spectrometer (A Nicolet 6700 from Thermo Nicolet Corp., Madison, WI) equipped with a deuterated triglycine sulphate (DTGS) as a detector and a KBr/germanium as beam splitter, interfaced to Computer operating under Windows-based, and connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet), was used during FTIR spectra acquisition. A few drops of each sample were positioned in contact with attenuated total reflectance (ATR) on a multi-bounce plate of crystal at controlled ambient temperature (25 °C). All FTIR spectra were recorded from 4000 to 650 cm^{-1}, co-adding 32 interferograms at a resolution of 4 cm^{-1} with strong anodization. These spectra were subtracted against background air spectrum. After every scan, a new reference air background spectrum was taken. The ATR plate was carefully cleaned in situ by scrubbing with ethanol twice followed by acetone and dried with soft tissue before filling in with the next sample and made it possible to dry the ATR plate. The plate cleanliness was verified by collecting a background spectrum and compared to the previous one. These spectra were recorded as absorbance values at each data point. The sample measurements were replicated three times. The software within the FT-IR instrumentation includes modules

for collecting and processing of data, quantitative analysis, generating of own spectra libraries, identification of compounds using own and standard spectra libraries, converting of formats of spectral files, processing of microscopic images, as well as a bibliography on IR-spectroscopy (Pappas, Takidelli, Tsantili, Tarantilis, & Polissiou, 2011). It was used to obtain the FT-IR spectra for the control (non-micronized tart cherry purée), megatron micronized samples and high-pressure throttling system micronized samples. The data was imported into 'Origin Pro 8' software and the data were used to generate a line plot. The x-axis and y-axis were determined, and the noise cancellation was carried out by smoothening the curve under signal processing. The method chosen for smoothening was 'Savitzky-Golay' method and then the parameters were varied (points of window, polynomial etc.) to find the best fit. Once the best fit was found, the major peaks were identified around the wavenumbers and the internet was searched to locate current FTIR databases and more information about the peaks was gained at the molecular level.

7.2.6 Chemical Analyses of tart cherry digesta

7.2.6.1 Total phenolics content (TPC) assay

A standard curve was generated with gallic acid and TPC was expressed as g GAE equivalents/mL and used to determine TPC concentration of samples based on the protocol elaborated in the section 4.2.3.1.

7.2.6.2 Total flavonoid content (TFC) assay

The flavonoid content was determined using a method previously published (Mihai, Mărghitaş, Bobiş, Dezmirean, & Tămaş, 2010) with some modifications, based on the protocol detailed in the section 4.2.3.2.

7.2.6.3 Ferric reducing antioxidant power (FRAP) assay

A modified FRAP assay was utilized (Benzie & Szeto, 1999). Results were determined using a standard curve prepared using 100 µL of various Trolox solutions (0.1 to 1.0 mM) based on the protocol detailed in section 4.2.4.1.

7.2.6.4 Total Monomeric Anthocyanins Content

Total anthocyanin content was determined by the spectrophotometric method (Esti, Cinquanta, Sinesio, Moneta, & Di Matteo, 2002), based on the protocol detailed in the section 4.2.3.3. Anthocyanin pigment concentration was calculated and expressed as cyanidin-3-glucoside equivalents, as follows:

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L)

$$= \frac{\mathrm{A \times MW \times DF \times 10^3}}{\mathrm{e \times L}} \qquad (7.2)$$

where A = (A_{520nm} – A_{700nm}) pH 1.0 – (A_{520nm} – A_{700nm}) pH 4.5;

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; L = pathlength in cm; e = 26,900 molar extinction coefficient, in $L' mol^{-1} cm^{-1}$, for cyd-3-glu; and 10^3 = factor for conversion from g to mg.

7.3 Results and Discussion

7.3.1 Release Properties

In our study, the changes in the release properties of polyphenols in micronized (by megatron and high-pressure homogenization) and non-micronized tart cherry purée were analyzed. A secondary goal of this study was also to see the effect of microencapsulation (by spray drying) and the effect of selection of choice material (gum Arabic and maltodextrin) on the micronized tart cherry purée during in-vitro digestion. The in-vitro digestion was divided into phases: gastric phase for the first two hours (1 h and 2 h), and intestinal phase for the following 2 hours (3 h and 4 h). The amount of total polyphenols, flavonoids, total monomeric anthocyanins and FRAP activity were studied at each hour of digestion and the results were expressed as the gallic acid, quercetin, cyanidin-3-glucoside and ferrous ion equivalents respectively but as fractions of current values as compared to original to study the increase/decrease in values due to various stages of digestion and chemical changes. This will provide us with a better understanding of digestion and metabolism of anti-oxidant activity and how the micronization-microencapsulation affects bioavailability.

7.3.2 Particle Size Distribution

The particle size plays a very important role in the nutrient release kinetics. Therefore, studying the particle size distribution is a quintessential dataset and a performance indicator of sorts for the method employed in vitro. The particle size distribution for both non-micronized sample and micronized sample was taken at four time-stamps during the in vitro simulated digestion i.e. 5 min to assess salivary phase, 1 h and 2

h to assess gastric phase and finally at 3 h and 4 h to assess the intestinal phase as seen in Table 7.2. These results will help study the bioaccessibility of nutrients embedded in a food matrix. They also indicate a good repeatability of the in vitro model.

The non-micronized sample initially had a single peak characterized by a D(v,0.1) value of 52.18 μm, with 90% of the particles smaller than 707.2 μm in the salivary phase. The micronized samples on the other hand retained a single peak in the salivary phase with a D(v,0.1) of 8.86-9.93 μm with 90% of the particles smaller than 193.75 μm. After addition of gastric juice, 90% of the non-micronized samples had size > 700 μm whereas micronized samples reached a size range of ~200 μm. The slight increase in size may be due to depletion flocculation caused by mucin, or the aggregation of lipid droplets due to weakened electrostatic repulsion at low pH and high ionic strength (Espinal-Ruiz, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2016; Zhang, Zhang, Zhang, Decker, & McClements, 2015). Specifically, the concentration of mucin in the gastric phase (1.81 mg/mL) was much higher than that in the salivary phase (0.19 mg/mL). A much greater increase in size was noticed with the addition of intestinal juice, as the D(v,0.9) had increased to 741.46 μm with D(v,0.9) = 270.3 μm for the micronized samples. There was not much difference between the two micronized samples by the end of intestinal phase. This may be due to the addition of bile salts, ion solution and higher pH (pH 7.0) used in this phase, since all these factors were shown to cause an increase in emulsion size (Chang & McClements, 2014). The addition of salt reduces electrostatic repulsion between droplets allowing lipid particles to approach and flocculate. Moreover, replacement of the surfactant from the droplet surface by bile salt can alter the interaction between droplets (Chang & McClements, 2014). An increase in size of micronized samples was similar to increase in

size of Tween 80 stabilized emulsions after intestinal digestion observed by some research group (Espinal-Ruiz et al., 2016).

7.3.3 Analysis of FT-IR spectroscopy

FT-IR spectroscopic data was taken for standards to execute optimization. It was then used to obtain the FT-IR spectra for the control (non-micronized tart cherry purée) and micronized samples by megatron and the high-pressure throttling system. Spectroscopic analysis of polyphenolic and anthocyanins for the non-micronized sample of tart cherry purée was done by FT-IR spectroscopy between 400-4500 cm^{-1} wavenumber range. This can be seen in the spectrograph in Figure 7.1. The bands at 1640–1630, 1604–1585 and 1580– 1562cm^{-1} corresponded to double bond (C=C) stretching. Consequently, the peak at 1640–1630 cm^{-1} can be correlated to the stretching of aromatic C=C in anthocyanins content (Jose, Phadke, & Rao, 1974; Koeppen & Basson, 1966; Merlin et al., 1994). The presence of phenolic compounds gives absorption bands caused by stretching vibrations of the free OH groups (frequency 3670-3580 cm^{-1}), intra- and intermolecular H-bonds in dimers and polymers (frequency 3400-3200 cm^{-1}) (Golubtsova, 2017). The peak around 2900 cm^{-1} is usually associated with the presence of carbohydrates absorption by valence vibrations of CH_2- groups (Krukowski et al., 2015). The absorption peaks at 1638 and 1444 cm^{-1} corresponded to the aromatic and heterocyclic rings' skeletal vibration in the chromene of cyanidin-3-glucoside (C3G) (Zhao et al., 2016).

Now that we were able to identify four major peak regions, we performed FT-IR analysis on non-micronized samples and the stacked spectra results can be seen in Figure 7.2 at 5 min of digestion (salivary phase), 1 h & 2 h mimicking the gastric phase and finally

3 h & 4 h for the intestinal phase. The two major regions, i.e. anthocyanins and flavonoids, and carbohydrates and phenolic functional groups were present in all samples but with varying intensities. In the non-micronized sample, the peak for anthocyanins seems to be lower in intensity as we moved from salivary to gastric to intestinal phase. In Figure 7.3, the FT-IR spectra for megatron micronized sample clearly shows an increase in anthocyanins, flavonoids and polyphenols. The carbohydrate was broken down as the sample moved from salivary to gastric to intestinal phase and the peak sharpened as compared to a broad peak in salivary phase. But it was clearly seen that the anthocyanins and phenolic group peaks were more prominent in the high-pressure homogenized sample as shown in Figure 7.4. This is analytical proof of presence of more anthocyanins, polyphenols and flavonoids within the sample due to cell wall breakage as we have been seeing in our previous chemical assays results.

7.3.4 Analysis of RP-HPLC of tart cherry digesta

The direct identification of anthocyanins in tart cherry was hampered due to intrinsic variability as well as by dearth of literature with related HPLC data. Even when we found a few reports, they reported conflicting data. Some researchers have used a DAD detector with simultaneous monitoring and detection of bioactives at multiple wavelengths i.e. λ= 520 nm for anthocyanins, 360 nm for flavonols, 320 nm for hydroxycinnamic acids and 280 nm for general phenolics in various cherry varieties. The comparative analysis of HPLC profiles at multiple wavelengths enables screening of bioactives that belong to class of colorless polyphenols (favan-3-ols, namely catechin and epicatechin, flavanols and hydroxycinnamic acids**).** In our study, since we were primarily interested in the presence

of anthocyanins, we monitored the tart cherry purée at a wavelength of 520 nm. Our results indicated appearance of some identifiable peaks with the reduction in particle size. However, some peaks were non-identifiable and could presumably be the result of the degradation products. The major peaks in the chromatogram were identified based on their retention times (t_R). Expression of a particular type of caffeoyl derivatives (isomer of chlorogenic acid) have been reported in tart cherries (and not in sweet cherries) (Wojdyło, Nowicka, Laskowski, & Oszmiański, 2014). However, this is not an appropriate candidate to be a marker, since high fluctuations have been reported in their response, based on ripening stage and thermal degradation (Bonerz et al., 2007). Nonetheless, kaempferol-3-O-rutinoside (quercetin derivative), has been reported to be exclusively present in tart cherries and is the best marker for cherry flavonoid identification studies.

In our study, we observed the appearance of several peaks between t_R of 1.9 min to 7 min. By comparing these peaks with those reported in the published literature, we identified them as cyanidin derivates (major class of anthocyanins present in tart cherries). Another group, (Chaovanalikit & Wrolstad, 2004), reported differences in qualitative peculiarities between sweet and tart cherries. Tart cherries exhibit a more complex repertoire as opposed to their sweeter counterparts and have been specifically found to exclusively contain more cyanidin-3-O-rutinoside and cyanidin-3-O-glucoside, whereas sweet cherries only exhibit the peaks corresponding to cyanidin-3-O-glucosylrutinoside. Another peak observed in our study indicated the presence of cyanidin-3-O-xylosylrutinoside at detectable levels with particle size reduction (Table 7.3) and the presence of this branched tri-glycoside in P. cerasus has been previously described (Dekazos, 1970). However, in contrast to some of the previous studies (Mozetič & Trebše,

2004; Wu & Prior, 2005), we did not find the presence of pelargonidin-derivatives in our results. Also, we did not observe any peaks corresponding to degradation compound or pyrano-anthocyanins probably due to breakage of hydrogen bonds during elution when detected by HPLC. Another probable reason could have been the formation of large molecular weight condensation products of anthocyanins and other phenolics, that got separated in reverse-phase C18 column or during pre-HPLC filtration (Hager, Howard, & Prior, 2008). We have listed the presence of solvent or anthocyanin peaks with retention times for 1 h, 2 h for gastric phase digesta and then for 3 h & 4 h for intestinal digesta.

Therefore, to summarize, the reduction in particle size led to the appearance of more peaks that were qualitatively identified. Also, there was a small increase in the area (Table 7.3-7.6) under the peaks, indicating an increase in the amount of the major class of anthocyanins in tart cherries. However, more confirmatory studies need to be done (such as MALDI-TOF MS) to quantitively determine the differences. Also, the chemo-taxonomical differences between sweet and tart cherries have been linked to higher metabolic biosynthesis of additional anthocyanins and complex genomics in P. cerasus (tetraploid) when compared to P. avium (diploid, 2n = 16) (Dekazos, 1970; Delazar, Khodaie, Afshar, Nahar, & Sarker, 2010; Pantelidis, Vasilakakis, Manganaris, & Diamantidis, 2007).

After thorough examination of the HPLC–DAD and HPLC-MS–MS chromatograms from original and digested tart cherry samples, we were not able to detect any new peak(s) that might have suggested the formation of any derivatives after pancreatic digestion although we do see some small blank peaks that might indicate formation of complexes. The number of peaks increased going from 1h to 4 h and from gastric to

intestinal in synchrony with our FRAP results. The availability of anthocyanins is higher in the micronized samples than control sample exhibiting positive effect of micronization. Some of these putative derivatives might not have been detected under our current analytical conditions. Under our analytical conditions, HPLC-MS analysis of a cyanidin-3-glucoside standard solution, both at pH 2.0 and pH 8.0, clearly showed the decrease of the cyanidin-3-glucoside peak under alkaline conditions and the formation of two other peaks with higher retention time indicating formation of cyanidin derivatives. We were not able to clearly identify some of these peaks in the chromatograms from the intestinal digestion of tart cherry. But our HPLC results consistently exhibit anthocyanin derivatives peaks indicating bioavailability in gastric phase and later in intestinal phase in various derivative forms.

7.3.5 Chemical Analyses

7.3.5.1 Total phenolics content (TPC) assay

The phenolic content calculated in previous chapters for the original samples was compared to digested powder samples and liquid micronized samples. The comparison of digested tart cherry samples in terms of fraction of original content indicated that there was a significant increase in the amounts of total polyphenols with particle size reduction, however, the total polyphenols decrease during digestion was significant for non-micronized samples as seen in Figure 7.5. We observed that the fraction of polyphenol obtained in the non-micronized sample increased by a large percentage in the gastric phase at 1 h and 2 h, contrary to a rather significant decline in the intestinal phase at 3 h and 4 h. This is in agreement with the previous study on chokeberry juice by another group (He et

al., 2007). They also found a decrease in total soluble phenolic compounds ranging between 14.1% and 54.2% post digestion of chokeberry juice by HPLC. They attributed this decrease to the decreased phenol stability under mild alkaline conditions. Our results indicate a significant retention of polyphenols during gastric digestion. The results are best for micronized samples and even better for high-pressure micronized samples. Among the powder samples, the best retention of polyphenols is for maltodextrin-gum Arabic blend as wall material. Despite some differences in the incubation conditions, most dietary polyphenols appear to be quite stable during gastric digestion as no significant changes have been reported in the flavonoids and phenolic acid contents of various foods incubated under conditions mimicking those that must occur in the stomach.

Our results, and previous reports, confirm that dietary polyphenols are highly sensitive to the mild alkaline conditions in the small intestine and suggest that, during digestion in the duodenum, a proportion of these compounds may be transformed into different structural forms with different chemical properties. Post-intestinal (after 4 h) vs. post-gastric changes (after 2 h) in the properties of the supernate may be regarded as an estimate of in vitro bioaccessibility of a target bioactive compound (Bouayed, Hoffmann, & Bohn, 2011). As seen in figure 7.9, he concentration ratio of phenolics in micronized samples were higher (>0.80) than in non-micronized (0.59). There was a decrease of the phenolics concentration after intestinal digestion as compared to the concentration after gastric digestion due to pH changes. In the simulated digestion of apples, the majority of the polyphenols was found to be available during the gastric phase of the digestion (Bouayed et al., 2011). Upon treatment with simulated intestinal media, additional phenolics release accounted for less than 10% (Bouayed et al., 2011; Martini, Conte, &

Tagliazucchi, 2017). Similarly, in our study, substantial phenolics release (56-68%) was observed during intestinal digestion of all samples, as also observed by another group (Liang et al., 2012).

7.3.5.2 Total flavonoid content (TFC) assay

We and others (Pérez-Vicente et al., 2002) reported a small increase in anthocyanins after in vitro stomach digestion. This fact finding has been attributed to the lower pH of the sample after the gastric incubation (pH 2.0) than the pH of the fresh initial sample (pH 3.4 for the initial tart cherry purée) which renders an increase of the flavylium cation in the solution (Pérez-Vicente et al., 2002). The high stability under the stomach conditions of flavonoids, as seen in figure 7.6 and figure 7.10, are also comparable to previous reported in vitro (Boyer et al., 2004; Zhu et al., 2002) and in vivo (Bennett et al., 2018). The nature of some chemical derivatives formed from some flavonoids has been known for some time. For example, oxidation of quercetin yields ortho-quinone and transient quinone methide adducts which are likely to play an important role in quercetin bioavailability and bioactivity (Brand et al., 2006). It has also been well described that, at pH > 4.5, the stability of anthocyanins decreases rapidly as the red flavylium cation is transformed into other less stable forms, such as the colourless pseudobase (pH 4–5; M+ = 304), the purple quinoidal base (pH 6–7; M+ = 286) and the yellow chalcone (pH 7–8; M+ = 304), which eventually lead to degradation of the anthocyanins (Staroňová, Nielsen, Roursgaard, & Knudsen, 2012). Some other polyphenol derivatives have been recently described. Dehydrodimers of flavan-3-ols can be formed from the oxidation of (+)-catechin and ()-epicatechin (Sun & Miller, 2003). A dimeric quercetin oxidation product

(Cherviakovsky et al., 2006) anthocyanin-pyruvic acid adducts (Faria et al., 2005) and some putative dimers and phenolic compounds derived from anthocyanidins (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007; Fleschhut et al., 2006) have been reported. Among the phenolic compounds, protocatechuic acid can be formed by spontaneous degradation from cyanidin (Fleschhut et al., 2006). We detected trace quantities of protocatechuic acid (kmax (nm): 259.9/294.2; m/z 153.0) in the original chokeberry juice which increased slightly during the pancreatic digestion (data not shown). Low values of TPC and TFC in the oral phase (after 5 min of digestion) can be explained by the low solubility of these compounds in salivary fluid and to the short period of this reading (Figure 7.6 and figure 7.10).

Similar results were seen by Saikia et al. (2015), in their study on the microencapsulation of phenolic compounds of Averrhoa carambola pomace with maltodextrin by spray and freeze-drying showed in both techniques that the release of phenolic compounds was higher in gastric simulated medium (pH 1.2) than in the simulated intestinal medium (pH 6.8). According to couple of research groups (Saikia, Mahnot, & Mahanta, 2015; Saura-Calixto, Serrano, & Goñi, 2007), the behavior of the microcapsules in a simulated gastrointestinal medium is dependent on the type and property of the coating material used for encapsulation and their resistance or susceptibility to digestive enzymes as well as on the gastrointestinal conditions like pH range which hold true for our results and explains majority of the findings.

7.3.5.3 Ferric reducing antioxidant power (FRAP) assay

To evaluate the influence of micronization and in vitro digestion steps on the antioxidant capacity of tart cherry extracts, we performed the FRAP assay. The antioxidant activity results of micronized and non-micronized tart cherry pomace extracts are shown in Figure 7.8. The antioxidant activity of these extracts is directly related to their phenolic content and types. But it has been known that several factors can affect the antioxidant properties of these compounds due to the chemical transformations that occur from various mechanisms during the gastrointestinal digestion. The antioxidant activity increased strongly for the micronized sample during the digestion process in comparison to the non-micronized material. This increase in activity could be due to the protective effect of the encapsulated micronized sample during the digestive process. When we look at ferric-reducing antioxidant power (FRAP) of digested extracts, both micronized samples during the digestion process exhibited an increase in values. The highest FRAP value was recorded in the gastric and then almost doubled in intestinal phases for both the micronized samples. The activity increased with the digestion steps where the most important FRAP activity was recorded in the intestinal phase for both tart cherry extracts. Our group has published results (Flores, Singh, Kerr, Phillips, & Kong, 2015) that showed that the ferric reducing power increased with digestion steps and was more pronounced in the intestinal phase. Phenolic compounds are a large and diverse group of phytochemicals, which include many different classes of bioactive substances and there are differences in FRAP values, in oral, gastric and intestinal phases. This difference in trends in TPC values and TFC values, when compared to FRAP values is because of this plethoric diversity of the polyphenols present. Again, previous studies have shown that (Manach et al., 2005a) the

effect of the pH is very different for various polyphenolic compounds. Some polyphenols are more anti-oxidant at lower pH whereas some are pro-oxidant, so it is totally dependent on the nature of polyphenols present in the fruit species, in our tart cherry pomace. The highest antioxidant activities were shown by encapsulated extracts, in the intestinal phase, due to the highest phenolics and flavonoids amount released from the microcapsules via the degradation of maltrodextrin-gum arabic coating at neutral pH 7 (data not shown). Another similar study done on pomegranate juice found fall in TPC and TFC values but FRAP values increased significantly in the gastric phase of digestion for all the extracts, perhaps as a result of the observed increase in phenolic compound concentration at this phase(Chen et al., 2016; Correa-Betanzo et al., 2014; Gullon, Pintado, Fernández-López, Pérez-Álvarez, & Viuda-Martos, 2015) . Another study (Wootton-Beard, Moran, & Ryan, 2011) done that analyzed antioxidant activity of 23 commercially available vegetable juice found that FRAP activity either dropped slightly or increased going from gastric to intestinal phase but showed high values during digestion.

7.3.5.4 Total Monomeric Anthocyanins Content

During the in vitro simulation of human digestion and absorption a slight increase in anthocyanin concentration (10%) was observed after stomach digestion (Figures 7.7), due to a general increase in all of the individual anthocyanins, especially cyanidin and delphinidin glycosides. This would be due to the pH (pH 2) resulting after the pepsin digestion, lower than the fresh original juice (pH 3.8), rendering an increase of the flavylium cation in the solution. After the pancreatin bile salt digestion (simulation of small intestine digestion), dialyzed and non-dialyzed fractions were obtained and analyzed. A

significant decrease in anthocyanin concentration was observed, due to a general decrease of all the individual anthocyanins, more marked for delphinidin glycosides (Figure 7.7). Our results on the stability of anthocyanins under acidic conditions agree with those reported for raspberry (McDougall et al., 2005) or pomegranate (Pérez-Vicente et al., 2002) anthocyanins. Thus, the total dialyzed anthocyanin fraction represented only 2.4% (3.38 mg/L of juice), whereas the non-dialyzed fraction was 15.3% (21.60 mg/L of juice). This decrease in the total amount could be partially explained by the transformation of the flavylium cation to the colorless chalcone at the pH of the medium (pH 7.5), as the colorless anthocyanin pseudobases are stable and exist in equilibrium with the colored cationic forms in acidic solutions, but at pH >5 anhydrobases become progressively more stable and increasingly formed until pH 12, when ring fission occurs with formation of ionized chalcones. The initially formed unionized anhydrobases fade rapidly, but those of the 3-glucosides tend to be more persistent than anhydrobases of 3,5-diglucosides.

Nevertheless, it is important to take into consideration that the flavylium form would not be the predominant form in the human body after pancreatin digestion (due to high pH), but the other anthocyanins forms should be considered when other analyses are developed e.g., antioxidant activity tested in nonacidic media. Still, the reason for the high loss of anthocyanins remains unknown, although the possibility was considered that part of the anthocyanins is metabolized to some noncolored forms, oxidized, or degraded into other chemicals, which would escape from the detection under the present conditions. It is also remarkable that no aglycons occurred after intestinal bile digestion, in concordance with previous findings in which anthocyanins are absorbed in glycated forms indicating that no glycoside hydrolysis takes place during digestion. The results here found are also

in concordance with other studies, in human serum, in which a low bioavailability of the ingested anthocyanins, with a quick degradation, oxidation, or excretion of the compounds, is described.

7.4 Conclusions

It has been well documented that tart cherries are a rich source of polyphenols and anthocyanins that have shown bioactive properties. However, little is known of the gastrointestinal fate of tart cherry polyphenols which is imperative to fully understand its bioaccessibility. In our study, the particles micronized by megatron and high-pressure homogenization (HPH) have shown better anti-oxidant capacity, better retention of polyphenols, flavonoids and anthocyanins upto 2 h in the gastric digestion. However, there was a significant loss in the amounts of bioactives thereafter, owing to the instability of flavylium cation at pH >2 and the formation of chalcone base at alkaline pH values. Due to the aforementioned reasons, the changes in the fraction of the original content of polyphenols, flavonoids and anthocyanins during the sequential digestion were in the following order over 4 h of digestion time; 1 h < 2 h > 3 h > 4 h, with the exception of flavonoid bioaccessibility in the non-micronized sample. However, the FRAP assay indicating the fraction of antioxidant activity increased over time; 1 h < 2 h < 3 h < 4 h, for the micronized tart cherry digesta (megatron and HPH). The process of microencapsulation by spray drying them with a coating of maltodextrin-gum arabic has also shown a protective effect in the varying pH environment. The blend of maltodextrin and gum arabic (MD: GA (15%: 15%)) gave the highest values of bioaccessibility in the *in-vitro* digestion studies, perhaps because it combined the best properties of both the emulsions. The fraction

of the total polyphenol and flavonoid content for the digested microcapsules also followed the same sequence as the micronized samples; 1 h < 2 h > 3 h > 4 h. The micronized tart cherry purée microparticles encapsulated with a blend of maltodextrin and gum arabic (MD: GA (15%: 15%)) was the best method that gave highest polyphenol bioaccessibility, retaining 80% of the polyphenols and almost 1.4 times the content of anthocyanins, as compared to the undigested sample, during intestinal and gastric digestion respectively. Thus, our results indicate that employing micronization and microencapsulation is an effective strategy towards improving the polyphenol extractability and bioaccessibility in tart cherry purée.

7.5 References

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Table 7.1: Complete composition of simulated gastrointestinal juices that were used in the in-vitro digestion model.

Enzymes/ Chemicals/ pH	Saliva stock solution	Gastric stock solution	Duodenal stock solution	Bile stock solution
Deionized Water	500 ml	500 ml	500 ml	500 ml
NaCl	58.5 mg	2.752 g	7.012 g	5.259 g
KCl	74.5 mg	0.824 g	0.564 g	0.376 g
$NaHCO_3$/ NaH_2PO_4	1.05 g $NaHCO_3$	0.266 g NaH_2PO_4	3.388 g $NaHCO_3$	5.785 g $NaHCO_3$
Other Salts		0.399 g $CaCl_2.2H_2O$	80 mg KH_2PO_4	
		0.306 g NH_4Cl	50 mg $MgCl_2$	
Urea	0.2 g	0.085 g	0.1 g	0.25 g
HCl conc.	--	6.5 ml	0.180 ml	0.150 ml
Adjuncts				
Enzymes/salts	0.5 g mucin	2.5 g pepsin	9.0 g pancreatin	30 g bile salts
	1.0 g α-amylase	3.0 g mucin	1.5 g lipase	
pH	6.8 ± 0.2	1.3 ± 0.02	8.1 ± 0.2	8.2 ± 0.2

Table 7.2: Particle size distribution (PSD) of non-micronized and micronized tart cherry purée using different methods.

Sample	Size (μm)	Salivary phase	Gastric phase		Intestinal Phase	
		5 mins	1 h	2 h	3 h	4 h
Non-Micronized	D (v, 0.1)	52.18	22.91	31.26	14.8	15.25
	D (v, 0.5)	433.51	508.82	492.71	264.71	285.3
	D (v, 0.9)	707.2	789.24	769.16	741.46	614.96
Megatron	D (v, 0.1)	8.86	33	22.84	11.97	15.34
	D (v, 0.5)	60.7	117.15	88.88	74.18	74.28
	D (v, 0.9)	193.75	263.06	221.51	271.71	238.79
High-Pressure Homogenization	D (v, 0.1)	9.93	28.39	21.42	13.93	12.66
	D (v, 0.5)	49.85	103.75	89.85	69.32	73.92
	D (v, 0.9)	143.79	255.87	208.93	204.67	239.13

Table 7.3: Retention time and area of solvent and anthocyanins released by samples in HPLC during *in-vitro* digestion at 1 hour.

Sample at 1 Hour	Retention Time(min)	Solvent/Anthocyanin	Area
Non-Micronized	1.78	Solvent	14545.2
	4.69	Cyanidin derivative	304.8
	22.06	Cyanidin-3-glucosyl rutinoside	132.9
Megatron	1.85	Solvent	6937.2
	4.71	Cyanidin derivative	39.2
	5.02	Cyanidin-3-glucosyl rutinoside	164.2
	26.71	Cyanidin-3-glucoside	12.2
High-Pressure Homogenization	1.87	Solvent	6241
	4.71	Cyanidin derivative	39
	26.42	Cyanidin-3-glucosyl rutinoside	15.6
	27.35	Cyanidin-3-glucoside	20.3

Table 7.4: Retention time and area of solvent and anthocyanins released by samples in HPLC during *in-vitro* digestion at 2 hours.

Sample at 2 hours	Retention Time(min)	Solvent/Anthocyanin	Area
Non-Micronized	1.84	Solvent	1221.4
	4.71	Cyanidin derivative	204.9
Megatron	1.88	Solvent	6742.3
	4.71	Cyanidin derivative	60.8
High-Pressure Homogenization	1.88	Solvent	6241
	3-4 blank peaks	cyanidin-3-O-xylosylrutinoside & derivatives	--

Table 7.5: Retention time and area of solvent and anthocyanins released by samples in HPLC during *in-vitro* digestion at 3 hours.

Sample at 3 hours	Retention Time(min)	Solvent/Anthocyanin	Area
Non-Micronized	1.89	Solvent	6936.6
	3.08	Cyanidin derivative	500.3
	5.12	Cyanidin-3-glucosyl rutinoside	551.2
Megatron	1.89	Solvent	7246
	3.01	Cyanidin derivative	621.1
	4.64	Cyanidin-3-glucosyl rutinoside	709.2
	6.87	Cyanidin-3-glucoside	235.3
High-Pressure Homogenization	1.88	Solvent	14558.6
	2.68	Cyanidin derivative	715.9

Table 7.6: Retention time and area of solvent and anthocyanins released by samples in HPLC during *in-vitro* digestion at 4 hours.

Sample at 4 hours	Retention Time(min)	Solvent/Anthocyanin	Area
Non-Micronized	1.82	Solvent	3248.5
	4.19	Cyanidin derivative	803.2
	6.86	Cyanidin-3-glucosyl rutinoside	148
Megatron	1.88	Solvent	8395.7
	2.88	Cyanidin derivative	640.7
	3.76	Cyanidin-3-glucosyl rutinoside	180.60
	6.84	Cyanidin-3-glucoside	131.7
High-Pressure Homogenization	1.86	Solvent	7809
	4.71	Cyanidin derivative	811.9

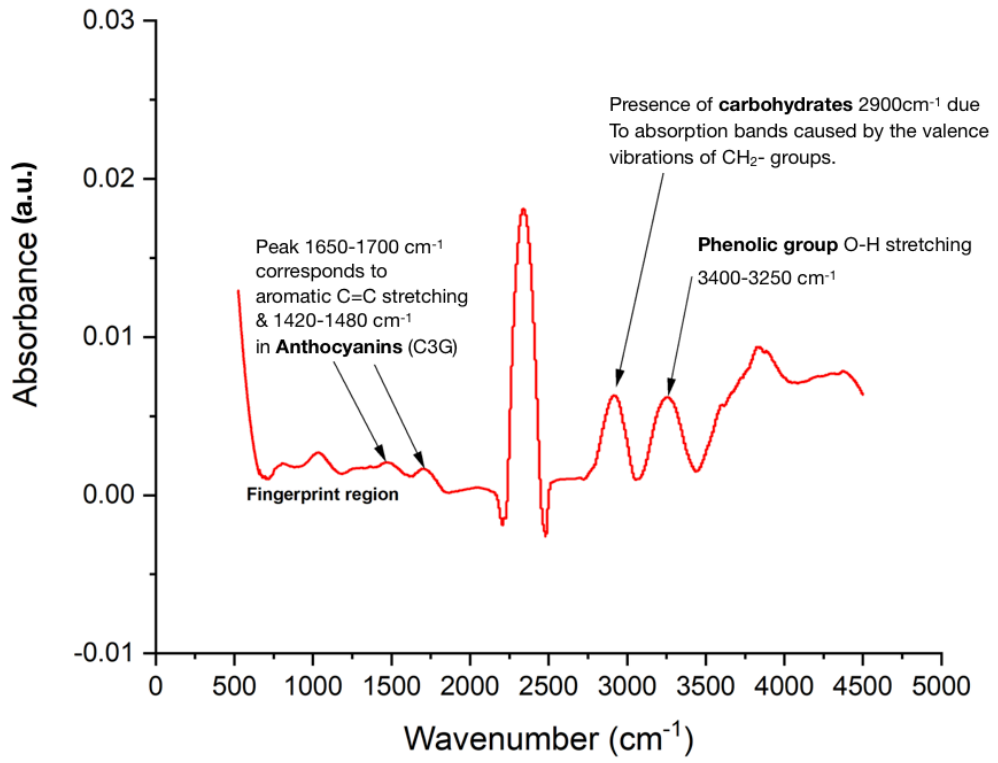

Figure 7.1: FT-IR spectra with peak values for non-micronized tart cherry sample.

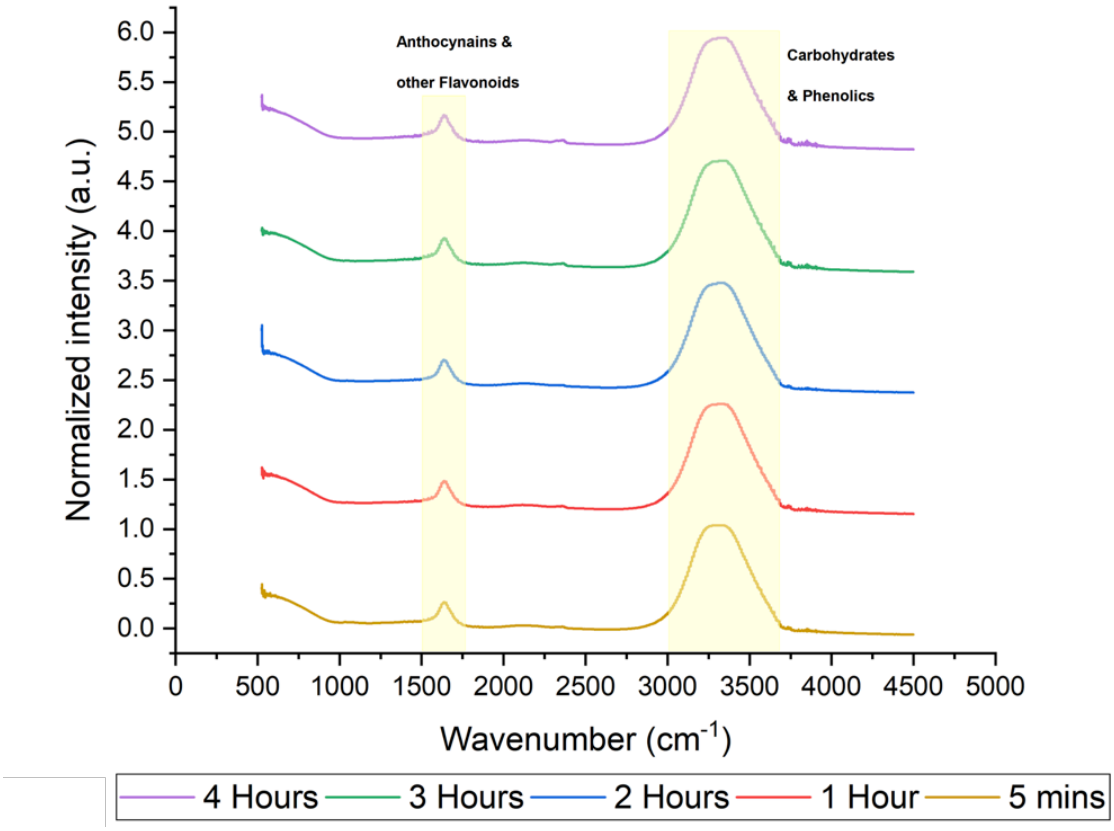

Figure 7.2: FT-IR spectra and peak group values for non-micronized tart cherry digesta at various time intervals.

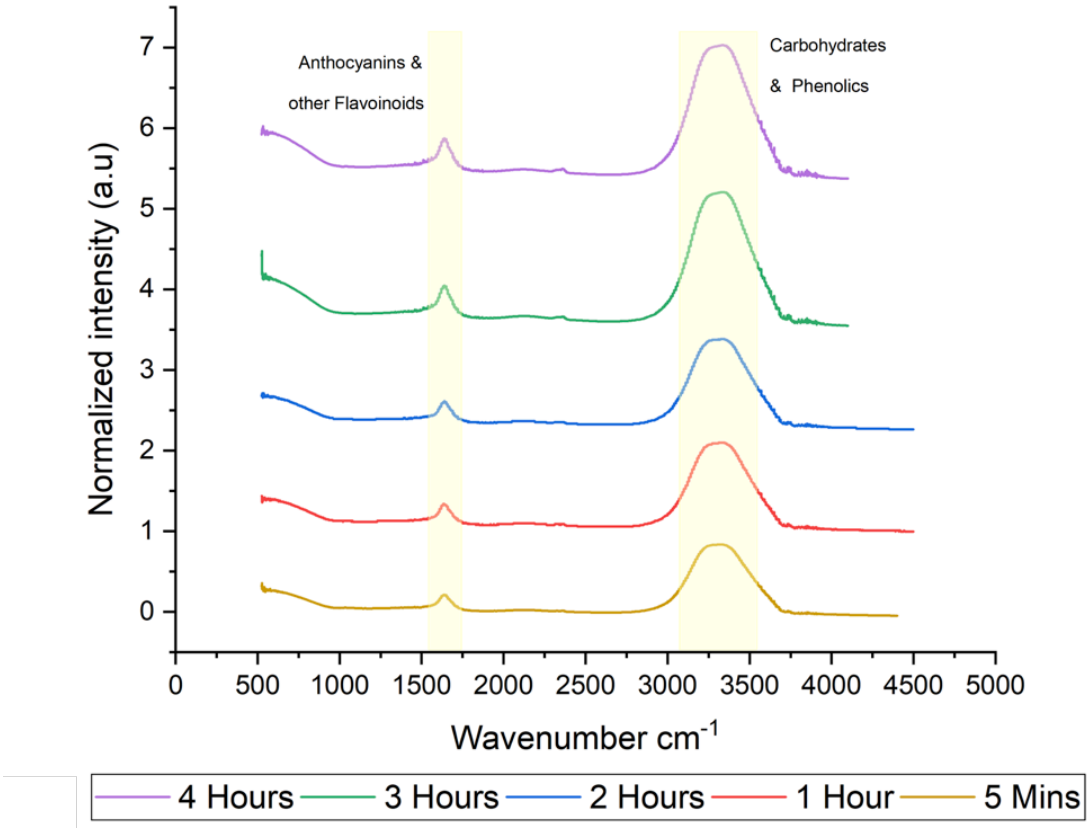

Figure 7.3: FT-IR spectra and peak group values for megatron-micronized tart cherry digesta at various time intervals.

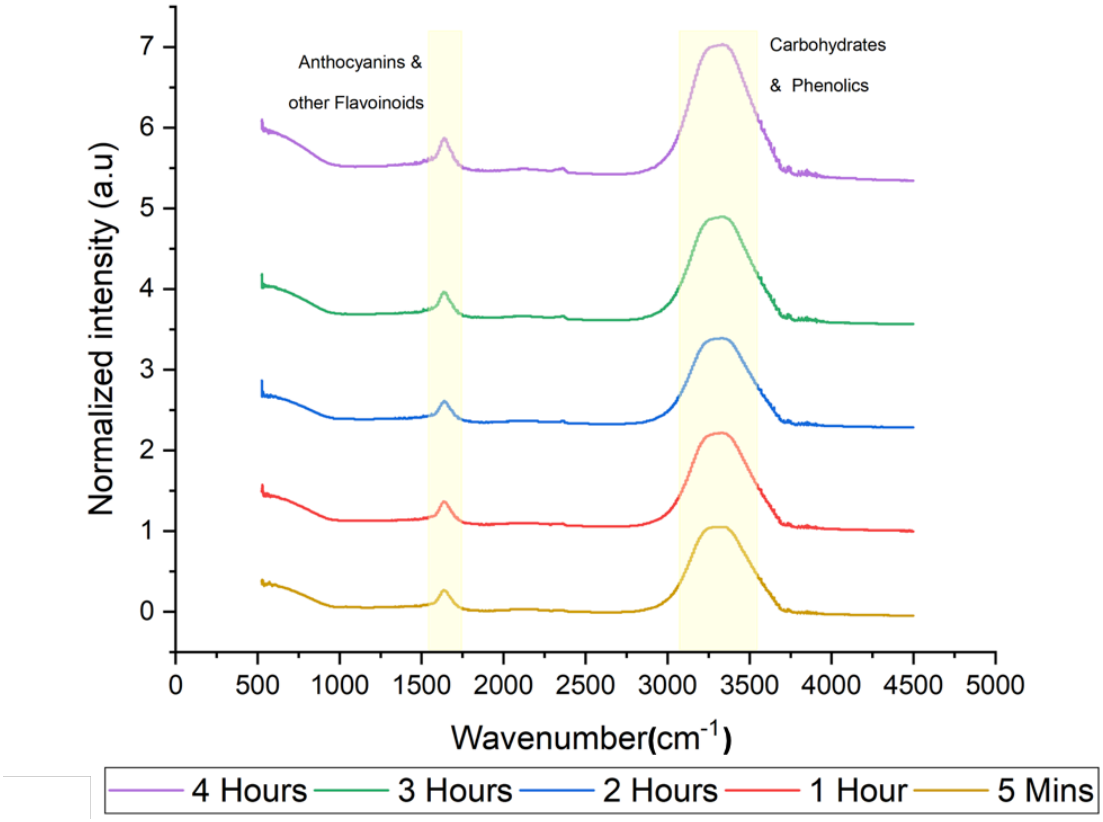

Figure 7.4: FT-IR spectra and peak group values for high pressure-homogenized tart cherry digesta at various time intervals

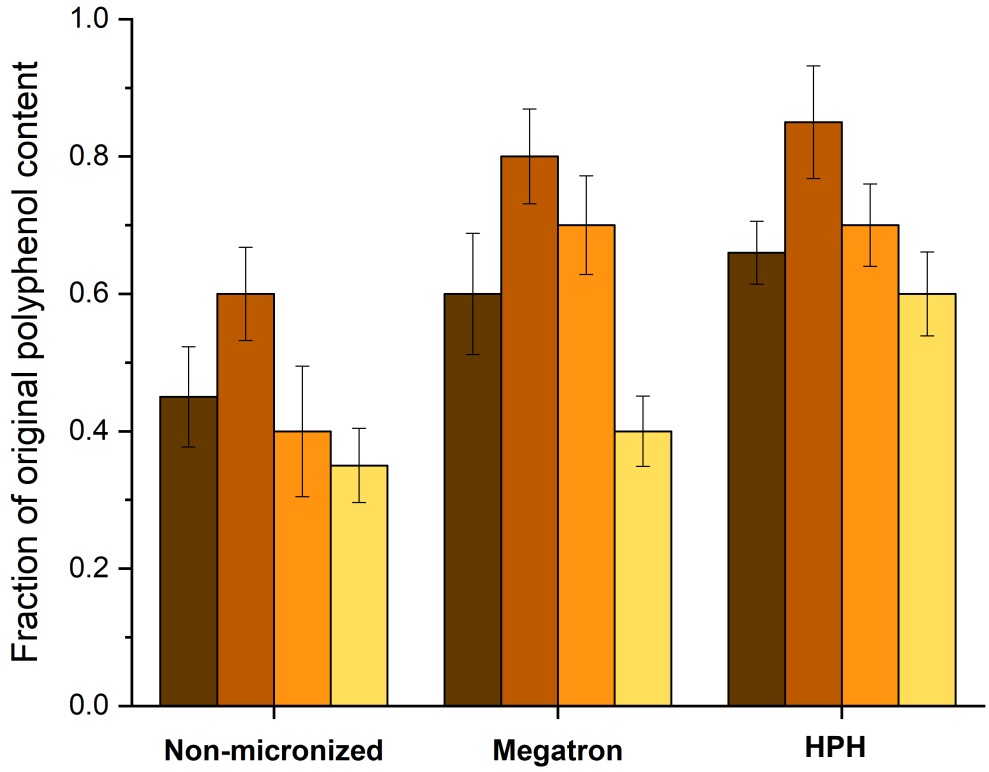

Figure 7.5: Fraction of total polyphenol values at various time intervals for non-micronized and micronized tart cherry digesta samples.

(1 h 2 h 3 h 4 h)

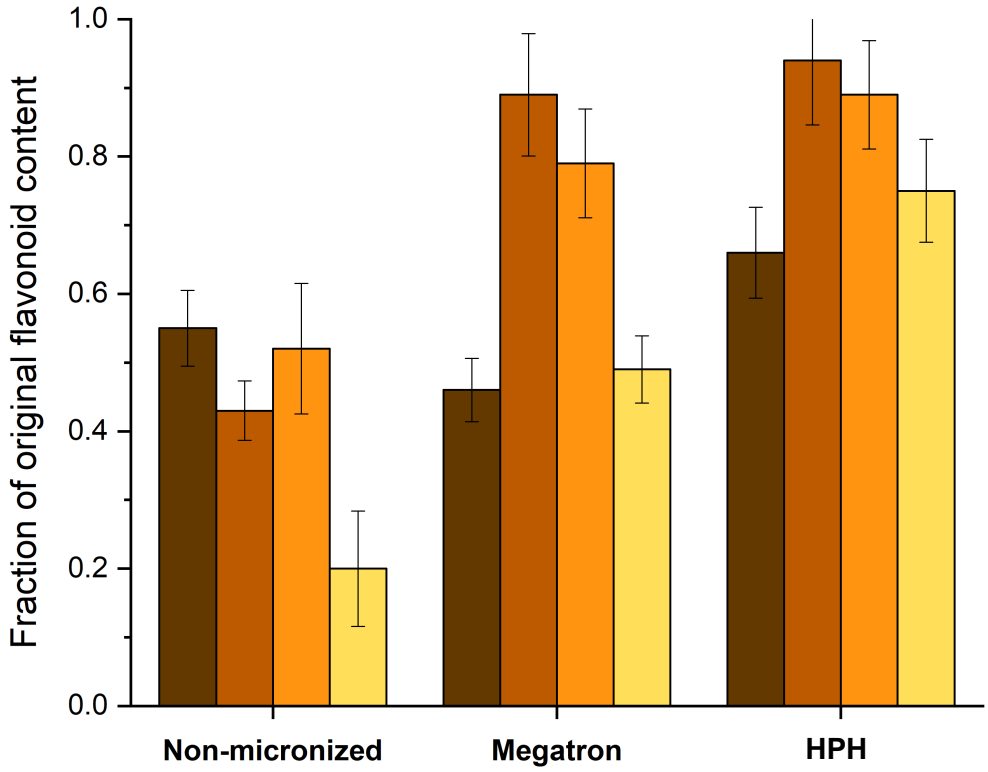

Figure 7.6: Fraction of total flavonoid content at various time intervals for non-micronized and micronized tart cherry digesta samples.

(1 h 2 h 3 h 4 h)

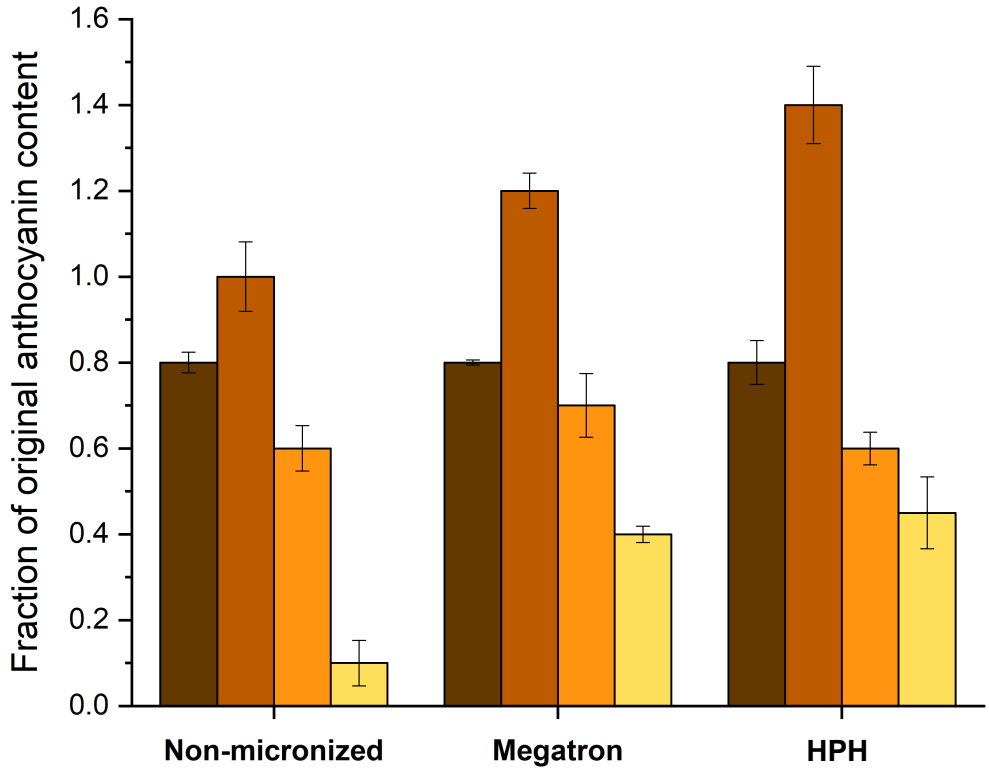

Figure 7.7: Fraction of original total monomeric anthocyanin content at various time intervals for non-micronized and micronized tart cherry digesta samples.

(1 h 2 h 3 h 4 h)

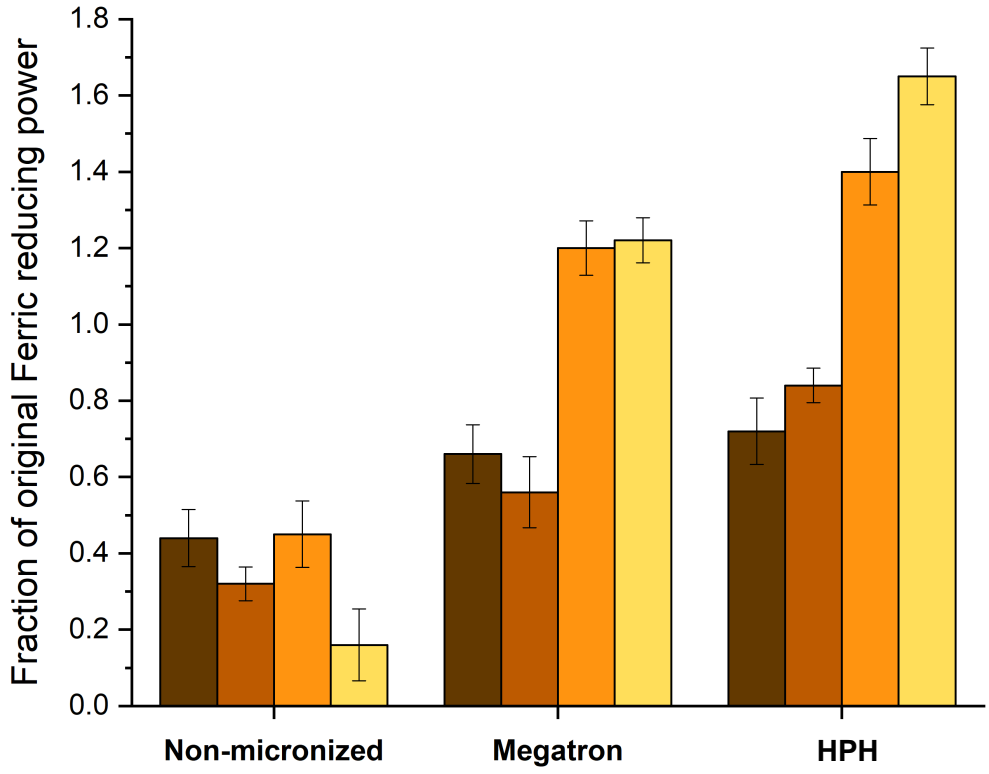

Figure 7.8: Fraction of original ferric reducing power variation at different time intervals for non-micronized and micronized tart cherry digesta samples.

(1 h 2 h 3 h 4 h)

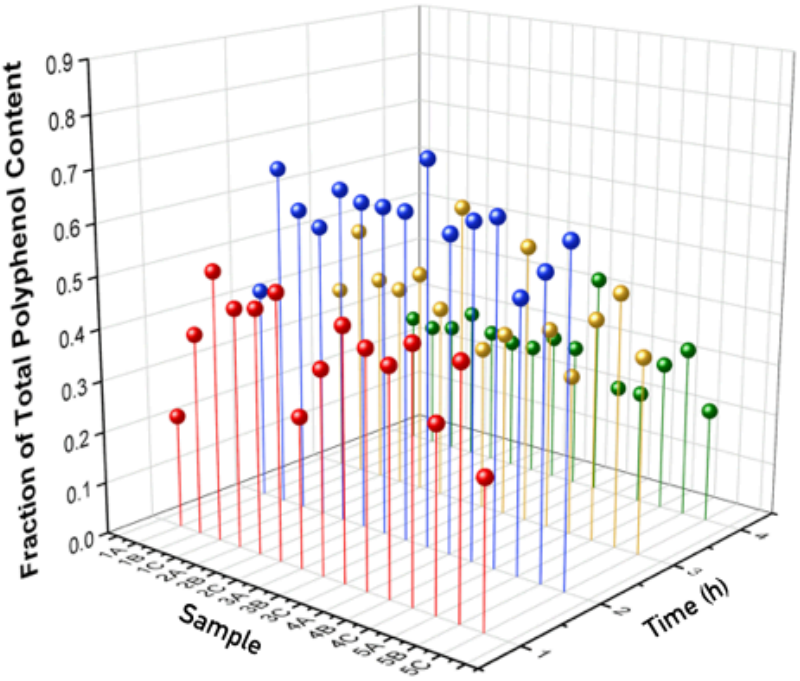

Figure 7.9: Fraction of total polyphenols content at different time intervals for non-micronized and micronized tart cherry powder samples. (The samples 1A- 5C have been detailed in Table 5.1).

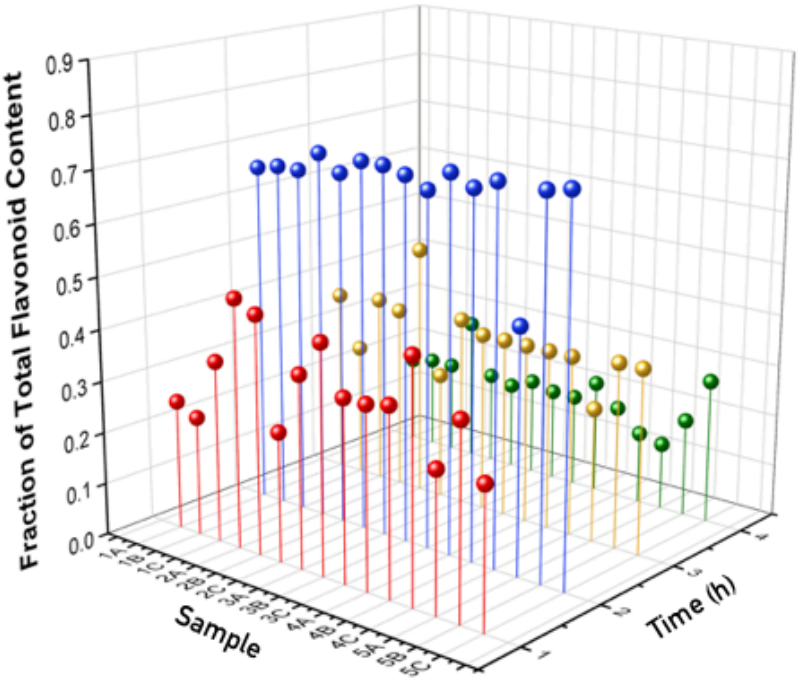

Figure 7.10: Fraction of total flavonoid content at different time intervals for non-micronized and micronized tart cherry powder samples. (The samples 1A- 5C have been detailed in Table 5.1).

CHAPTER 8

SUMMARY AND FUTURE RECOMMENDATIONS

8.1 Summary

Tart cherry (Prunus cerasus) purée, obtained from Cherry Central Cooperative, Inc. (Traverse City, Michigan), was micronized by several particle size reducing methods, and studied for the changes in chemical, rheological and structural properties of the food material. Secondly, these micronized tart cherry purée samples were further microencapsulated by spray drying using two different wall materials (maltodextrin and gum arabic) in varying proportions and the effect of particle size reduction and wall material selection on the physicochemical properties of the microcapsules thus produced, was determined. Thirdly, the stability of these encapsulated tart cherry microparticles upon storage at different temperatures and relative humidity conditions was analyzed using various chemical assays and moisture isotherms. And lastly, both tart cherry purée (homogenized by micronization) and tart cherry spray dried powder (obtained by microencapsulation) were subjected to in-vitro static digestion to simulate the gastric and intestinal phases of digestion in order to study the release properties of polyphenols and anthocyanins.

Different methods of micronization were employed to process the tart cherry purée samples, i.e. hammer mill, super masscolloider, megatron and high-pressure homogenization (HPH) system, as described in Chapter 3. Based on the particle size

distribution (PSD), megatron and HPH were selected as the best methods for further studies. As a result of micronization, the changes in rheology, color and structure indicated a shear thinning behavior in tart cherry purée and a reduction in viscosity with decrease in particle size and reduced agglomeration, as observed with the help of bright field microscope. It was further observed that the tart cherry purée became lighter in color with micronization due to an increase in the surface area of samples that caused more scattering of light. Next, the changes in chemical properties of tart cherry purée upon micronization, as discussed in Chapter 4, were evaluated. A significant increase in the content of polyphenols, flavonoids, and anthocyanins was observed with the particle size reduction due to the breakage of cell wall that causes the polyphenols and the anthocyanins in the vacuoles to release easily.

Furthermore, the tart cherry purée samples (before and after micronization), were encapsulated to produce microcapsules using varying ratios of maltodextrin and gum arabic by the spray drying method (Chapter 5). The phytochemical analysis of the spray dried tart cherry microcapsules indicated a minor loss in the polyphenolic, flavonoid, anthocyanin content, which could be due to the thermal degradation caused by the inlet temperatures during spray drying process. However, the loss was considerably small when compared to the conventional ways of thermal processing. Also, it was observed that the protection yielded to the sample by both the wall materials when present in a 1:1 ratio was the highest as opposed to being used singly or in a 2:1/1:2 ratio. The particle sizes and shapes were further studied by observing the surface morphology of tart cherry powders using scanning electron microscope. Next, the storage stability of the encapsulated tart cherry purée was analyzed (Chapter 6) at different temperatures (4°C, 25°C, 42°C) and different relative

humidity conditions (11%, 22.5%, 32%, 57%, 75%). It was determined that there was a statistically significant loss of polyphenol and anthocyanins with increase in temperature and relative humidity. However, the blend of MD and GA in a 1:1 ratio slowed down the degradation kinetics the best as opposed to the other wall material combinations. Also, it was found that the BET model was the best fit for the moisture isotherm studies. And lastly, with the help of in-vitro static digestion (Chapter 7), the release properties of micronized tart cherry purée (Chapter 3 & 4) and of encapsulated tart cherry powder (Chapter 5) were analyzed. Results showed that microencapsulation may prove to be a viable alternative to the purée, as the fraction of polyphenol release was more for the purée samples in the gastric phase and was much higher for the encapsulated tart cherry powder in the intestine. Therefore, we have demonstrated the importance of tart cherry purée processed with micronization and microencapsulation, to generate microparticles and spray dried microcapsules respectively, in improving the polyphenol extractability and bioaccessibility.

8.2 Future Recommendations

The effect of micronization and microencapsulation showed promising results in enhancing the release of the bioactives present in the tart cherry purée. The innovative and directed use of micronization techniques to reduce the particle size of the sample, appears to be a strategy into the right direction and our results are important to develop new administration strategies which could help protect the antioxidant and anti-inflammatory potential and bioaccessibility of the important bioactives. Since particle size reduction appears to offer favorable outcomes, further studies employing the other non-thermal

processing methods such as ultrasound processing, could be done to compare the efficiency of different methods and their subsequent effect on bioaccessibility. Furthermore, the cost of production is a critical parameter in a food processing facility, and thus, it is important to carefully conduct a cost analysis of the two micronization methods, megatron and high-pressure homogenization (HPH). Based on our study, we can recommend the use of megatron over HPH, despite the further reduction in the particle size, owing to the lower cost of equipment and the process, and also due to the lack of significant differences in the changes of bioactive content. Also, the choice of wall material is a critical factor in spray drying process and more studies involving the use of a larger library of wall materials (such as proteins and nanocellulose) can be pursued in the future. Also, the gastrointestinal dynamic model of digestion could be ensued to simulate the real-time conditions of digestion and to better assess the biomechanism of metabolites present in the tart cherry purée. Furthermore, Caco-2 cell model could be implemented to learn more about the anthocyanin absorption and bioavailability. Since the consumer acceptability is a critical factor in commercialization of a product, sensory and consumer studies are some of the other directions that this study finds future relevance in. And lastly, the effect of micronization on the other nutrients, and the interaction of enhanced tart cherry dietary polyphenols with the gut microbiota could be an interesting area to better understand the digestibility of bioactives. There is a pressing need to focus more on the under-utilized fruits like tart cherry that possess a plethora of health benefits and ensure that technological means are used strategically to maximize their role in disease prevention and the improvement of human health.