

**Encapsulation and real-time release
characteristics of spray dried l-menthol**

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DEDICATION

This dissertation is dedicated to the female persons who have shaped me into who I am:

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ABSTRACT

The objective of this research was to understand the effects of octenyl succinic anhydride (OSAn) substitution level of carbohydrate encapsulation materials on the capsule characteristics and real-time release of l-menthol from spray dried powder. Spray dried l-menthol powder was prepared from modified dextrin and gum acacia of varying levels of OSAn substitution. Physical properties of the microcapsules and encapsulation matrices were determined by: constructing moisture sorption isotherms, particle sizing, density measurement via pycnometry, and by total and surface menthol content of the finished powders. Real-time menthol release and moisture uptake profiles were accomplished by using dynamic vapor sorption (DVS) coupled with either Tenax trapping and gas chromatography (GC) or proton transfer reaction mass spectrometry (PTR-MS).

It was found that as OSAn substitution of carbohydrates increases, total and surface menthol appeared to increase and decrease, respectively. The particle size of the finished spray dried powder was influenced by spray dryer infeed solids concentration with higher solids leading to larger particle size. Microcapsule density did not appear to be affected by OSAn substitution or carrier type.

Menthol release profiles varied greatly with carrier material used. Increased OSAn substitution (up to 3% treatment level) of the carrier material resulted in a significantly greater, but not earlier burst of menthol release. For most samples, the characteristic burst of menthol was followed by a decrease in release which was attributed to an observed matrix collapse. Moisture uptake rates of the powders were related to corresponding menthol release rates. As OSAn substitution increased for gum acacia (*Acacia seyal*), a trend of increased moisture uptake rate with menthol release rate was evident. However, for the dextrans, the moisture uptake rate had no influence on the rate of menthol release.

The degree of OSAn substitution did not affect moisture sorption characteristics for dextrin samples. For gum acacia, increased levels of OSAn substitution resulted in a decreased affinity for moisture in the range of 0.3 to 0.7 a_w .

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Introduction

Encapsulation is: “a process by which one material or mixture of materials is coated or entrapped within another material or system.” [1] The primary reasons flavors are encapsulated are to: convert liquid flavor compounds to free-flowing powders, protect them from chemical reaction (e.g. light induced or oxidation); retain them during storage; and prevent interactions with food components and other flavorings [2-4].

Flavor encapsulation is accomplished using a number of production methodologies. Processes used to encapsulate flavors include but are not limited to: spray drying, extrusion, spray chilling, inclusion complexation, liposome technology, and coacervation; the reader is directed to the referenced comprehensive reviews for more, in-depth information [1, 5-10]. Of the many methods available for flavor encapsulation, spray drying accounts for over 90% of the dried flavors produced [3] likely due to its performance, cost, and availability of equipment [6, 9].

Just as there are numerous processes used to manufacture encapsulated flavors, there are numerous materials used as encapsulating agents. These materials are predominantly carbohydrates (e.g. starches, dextrans, sugars, gums, cyclodextrin, cellulose, and alginates) and proteins (e.g. whey protein and gelatin), but also include other ingredients such as fats and phospholipids [7]. Because the majority of the flavors are produced are by spray-drying, the three most important materials used for encapsulation are gum acacia, maltodextrin, and modified starch. Each of these carrier classes has been studied by numerous authors for strengths and weaknesses as encapsulating matrices. For example: maltodextrins are relatively inexpensive, have good film forming abilities, but have no emulsifying properties; modified starches have

excellent emulsifying properties, but their ability to protect against chemical reactions is disputed; and gum acacias are a good compromise of emulsion stability, flavor retention, and protection [3, 4].

From the above statements, it is clear that scientists and technologists have found ways to adequately encapsulate flavors. However, there is one very significant factor to consider when producing encapsulated flavors – their successful application in a finished food product. Assuming that a perfect flavor encapsulation system has been developed for a particular product based on the aforementioned criteria. All of the benefits and benefits of encapsulation are completely invalidated if the flavor does not impart the desired flavor characteristics in the application for which it is intended. For example, one desired characteristic of chewing gum is flavor longevity; if the encapsulated flavor in the gum is exhausted within 15 seconds of chewing, consumers are probably not going to buy that gum again. The previous example illustrates why recent efforts in flavor research have focused more on encapsulated flavor performance in product instead of flavor protection [8]. This introduces the purpose of the current research – to understand the differences in flavor release from spray dried powders made with different carrier materials.

CHAPTER 1
REVIEW OF LITERATURE

1 Review of Literature

The primary goals of this literature review are to: 1) introduce the encapsulation materials commonly used for spray drying and examine the chemical and physical properties of each; 2) review the mechanisms of volatile release from encapsulated flavor flavorings; 3) introduce the traditional and elaborate upon recent advances of volatile release measurement from encapsulated flavors; and 4) review the literature pertaining to the effects of moisture on the physical characteristics of common encapsulation materials used for spray drying.

1.1 Encapsulation Materials

1.1.1 Gum Acacia

Gum acacia (also known as gum Arabic) is considered the oldest and most recognized natural gum [11]. The gum utilized for flavor encapsulation exudes from the trunk and branches of leguminous plants of *Acacia senegal* and *Acacia seyal* which are indigenous to Sudan, Senegal, Mali, and Nigeria. Gum exudes naturally from *A. seyal* species, whereas gum from *A. senegal* is harvested from ‘wounds’ typically made by superficial incisions into the tree or by stripping the bark. Sun exposure partially dries the exuded gum nodules and the resulting ‘tears’ are harvested by hand [4, 11, 12]. The tears are cleaned, graded, and in many cases further processed by pasteurizing and spray-drying into a very versatile powder [13]. Gum production typically does not exceed 300 grams per tree per year [12].

Table 1.1 illustrates the gross composition of gum *A. senegal*. Gum acacia is comprised of a complex and heterogeneous polysaccharide containing galactose, arabinose, rhamnose, and glucuronic acid and exists as a mixture of calcium, magnesium, and potassium salts [12]. The main carbohydrate fraction is constructed of 1,3-linked β -D-galactopyranosyl units with side chains of two to five 1,3-linked β -D-galactopyranosyl units bonded by 1,6-linkages. Both the main and side chains contain α -L-arabinofuranosyl, α -L-rhamnopyranosyl, β -D-glucuronopyranosyl, and 4-*O*-methyl- β -D-glucuronopyranosyl units [11]. Thevenet [14] published a figure depicting the proposed structure of the arabinogalactan complex.

Table 1.1. Chemical composition of eight *Acacia senegal* samples. Data adapted from Idris, et al., 1998.

| Component | % |
|-----------------|-----------|
| Moisture | 12.5-16 |
| Nitrogen | 0.22-0.39 |
| Protein | 1.5-2.6 |
| Galactose | 39-42 |
| Arabinose | 24-27 |
| Rhamnose | 12-16 |
| Glucuronic acid | 15-16 |

The most widely supported model of the entire structure of gum acacia was proposed by Fincher and Stone [15]. The authors hypothesize that large carbohydrate blocks ($\sim 2.5 \times 10^5$ Da) are individually attached to a polypeptide chain. Interestingly, the polypeptide fraction contains a high content of relatively hydrophobic hydroxyproline and serine amino acid residues [16]. The lesser accepted model of Qi, et al. [17] suggests that the structure of gum acacia is a ‘twisted hairy rope’ composed of a long protein structure (400 amino acid residues) with many smaller carbohydrates ($\sim 6,000$ Da) linked by hydroxyproline. More research studies support the previous model [11]. It is

suspected that heterogeneous structure of gum acacia (hydrophilic polysaccharides coupled with hydrophobic protein) explains the primary reason gum acacia was introduced into the flavor industry; it is an excellent emulsifier and has the ability to form stable flavor emulsions [12]. Nussinovitch [13] provided several studies that attest to the hydrophobic amino acids being either absorbed into or onto the oil phase of emulsions while the arabinogalactan complex has no affinity for the oil.

1.1.2 Dextrin

Dextrin is a term used to classify the group of products produced by acid or enzyme hydrolysis of starch. The most common dextrin products used as flavor encapsulation materials are maltodextrins and corn syrup solids. 21 CFR 184.1444 [18] defines a maltodextrin as a non-sweet nutritive saccharide polymer consisting of D-glucose units linked primarily by α -1-4 bonds with a dextrose equivalent (DE) of less than 20. According to 21 CFR 168.120 and 168.121 [19, 20], corn syrup solids are defined exactly the same way as maltodextrin with the exception that the DE is 20 or higher. Manufacture of these products typically starts with either regular or waxy maize corn starch [21]. They are prepared by partial hydrolysis of starch with suitable acids and/or enzymes, which yields a gamut of hydrolyzed products consisting of D-glucose, maltose, maltose oligosaccharides, maltotriose, linear amylose (α -1-4 bonds), and branched amylopectin (α -1-4 and α -1-6 bonds) degradation products [22]. It is possible to have two products with the same DE, but very different carbohydrate profiles [23].

Typical reaction schemes for acid and enzyme hydrolysis are discussed in detail by Chronakis [22] and generalized below. Acid converted starch is essentially carried out

by treating a suspension of starch with a small amount of strong acid (hydrochloric acid; 0.02 – 0.03 *M*) at fairly high temperatures (135-150°C) for 5 to 8 minutes. Control of DE is typically done by varying reaction temperature at a given time. Shorter reaction times are desired to prevent undesirable reaction products from forming. When the desired DE is reached, the acid is neutralized, the suspension is filtered, decolorized, and concentrated to a desired solids content. Products produced by acid hydrolysis are typically glucose syrups (degree of polymerization (DP) < 5).

Maltodextrin production by enzyme hydrolysis are frequently patented, but generally involve mixing a starch slurry with an enzyme, heating up to and holding the slurry at the starch gelatinization temperature (~75°C), and finally heating to a higher temperature (~105°C) or acidifying (pH~3.5) to deactivate the enzyme. Enzyme activity levels depend on the source (derived from animal, bacteria, fungus, or plant) but are generally highest in acidic conditions. Compared to acid hydrolysis, enzyme hydrolysis can be done at lower temperatures and wider pH ranges. As expected, enzyme or a combination of enzyme and acid hydrolysis is more specific than acid hydrolysis alone.

1.1.3 Modified Starch and Dextrin

Wurzburg [24] gave a generic definition of modified starches as, “any product in which the chemical and/or physical properties of native starch have been altered.” Starch modifications and modification treatments acceptable for food use are outlined in 21 CFR 172.892 [25]. These acceptable modifications include: acid treatment, bleaching, oxidizing, esterifying, etherifying, and enzyme treatment. Fortunately, it is common knowledge in the flavor industry that the most widely used modified starch is one where

1-octenyl succinic anhydride is esterified onto the starch molecule; this modification adds hydrophobic groups to an already hydrophilic polymer. Starches that are modified in this way are known to provide better retention due to their emulsifying capabilities [2, 3].

In a recent review of starch ester preparation, Tessler and Billmers [26] noted that the first paper on starch esterification of starch was published in 1865. Since 1865, hundreds of papers and patents have been published on starch esterification. The authors pointed out that esters can be formed in numerous ways, including but not limited to reactions with: organic anhydrides, vinyl esters, acyl guanidine, N-acylimidazoles, acyl phosphates, and cyclic dibasic acid anhydrides.

Caldwell [27] was the first to react organic anhydrides in water under slightly basic conditions to obtain starch esters. Later, he and Wurzburg [28] filed a groundbreaking patent which marked the beginning of modified starches with emulsion stabilizing properties. Since then, numerous U.S. and European patents featuring emulsifying starches have been granted. When authors need to reference the first works on emulsifying starches, both the Caldwell and Wurzburg patent and a book chapter by Trubiano [29] are often given; Trubiano gives a basic summary of the reaction outlined in the patent. Commercial production is typically a mild base-catalyzed reaction of the reactant and starch in aqueous suspension at room temperature [30].

According to 21 CFR 172.892(d), starch cannot exceed a 1-octenyl succinic anhydride (OSA) treatment level of 3 percent; this quantity corresponds to an approximate degree of substitution (DS) of 0.02 [29]. Shogren et al. [30] believe that the OSA groups are distributed in the interior as well as the exterior of the esterified starch

granules. The authors report that the concentration of OSA groups on the surface of the starch granule is somewhere around 3-4 times that of the bulk.

1.2 Flavor Retention and Release

Some of the practical mechanisms of flavor release in consumer products include: pressure activated, pH sensitive, temperature sensitive, diffusion controlled, and solvent activated (or dissolution) release [31]. However, the methods of release most commonly associated with spray-dried flavors are solvent activated and diffusion controlled release [32]. Solvent activated release depends on the solubilization of the encapsulating wall material to liberate the trapped flavor component. Because most wall materials used for spray drying of flavors are carbohydrates, the solvent of interest is water. A tangible example of this release mechanism in product would be flavor in a cake mix; when water is added to the mix, the wall material dissolves and the flavor is released. The concept of diffusion controlled release, on the other hand, is not quite as simple and will be addressed in the following sections.

1.2.1 Theory of Selective Diffusion

It is commonly accepted that the concept of selective diffusion was introduced by Thijssen in 1965 [33]. He observed that when hot air drying conditions were well chosen, water evaporated quickly from the surface of a fruit juice concentrate, for example, which created a thin film with a very low level of hydration. The film formed during drying demonstrates a selective permeability that allows only water from the interior of the drop to pass through to the outside of the drop, thereby retaining flavor

components within [33]. This film was likened to a “sieve-like layer” that selectively controls the diffusion of moisture and retention of other volatiles [34].

In 1999, Benczédi and Blake [35] composed the following, insightful passage with respect to selective diffusion: “To expect the water to evaporate and not the more volatile flavour components was not logical, but that was what actually happened. It is a tribute to the optimism of those early pioneers that the process has become as well established as it has, even though it was not initially clear why it actually worked and why there was a selective evaporation of the water and not the flavour. Even now, the underlying science of flavour entrapment by spray drying is not fully understood, but it is clear that water behaves in a fundamentally different way from the entrapped flavour molecules with respect to the polymeric support.” This concept has a strong dependence on the water content of the product to be dried. In concentrated solutions, the diffusion coefficients of water and of volatile compounds through the solution behave differently [36]. As water content in a solution decreases, the diffusion coefficients of water and volatile compounds also decrease [37] however, the diffusion coefficients of aroma compounds are typically lower than water [36-38]. The process of spray drying results in very steep concentration gradients at the surface of drying particles (i.e. the film). Once the interfacial concentration of water is low enough such that the diffusion coefficients of the volatile compounds are lower than the water, the film acts as a semi-permeable membrane which allows water to pass while retaining the volatile compounds [37, 38]. Flink and Karel [34] stated that the critical moisture content for a given carbohydrate carrier material to retain volatiles depends on the molecular diameter for each particular volatile compound. Thijssen and Rulkens [38] found that at water concentrations at or

below 10%, carbohydrate and protein systems are permeable to water only. Menting and Hoogstad [39, 40] reported that a critical moisture content of 9% was needed to retain acetone and ethyl acetate in a maltodextrin encapsulation matrix.

1.2.2 The Micro Region Theory

The micro region theory has been said to provide a microscopic view of the macroscopic theory of selective diffusion [34, 37, 41]. It is based on the formation of micro regions containing volatiles within the microstructure of the carbohydrate matrix. These micro regions are supported by carbohydrate-carbohydrate hydrogen bonds. The application of the theory to volatile retention is as follows: as the moisture content of the carbohydrate matrix decreases, the carbohydrate-water hydrogen bond associations decrease until only carbohydrate-carbohydrate associations exist; it is then when the volatiles are sealed into the amorphous structure. The critical amount of moisture content this amorphous carbohydrate structure must contain before structure disruption (and subsequent volatile release) was found to be an amount well in excess of the monomolecular layer value of the matrix as calculated from sorption data by the method of Brunauer, Emmett, and Teller (BET) [42].

1.2.3 Effects of Carrier Phase Transition on Release

Whorton [32, 43] published two reviews on the mechanisms of release of actives from polymeric carbohydrate encapsulation matrices. Phase transitions of carriers play an important role in the formation of a microcapsule and the subsequent release of an active from the microcapsule. The glass transition (T_g) is the temperature at which an

amorphous glass turns rubbery. For proper encapsulation to occur the carrier must form a stable amorphous glass during processing; the glassy carrier entraps flavor compounds and inhibits molecular mobility. Spray-drying and freeze-drying are processes that rapidly dehydrate the matrix and are known to form glassy products [44, 45]. In this glassy state, dissolution and diffusion are responsible for most of the flavor release from microcapsules. It is common to use water soluble wall materials for encapsulation applications in the food industry; dissolution occurs when water solubilizes the encapsulated particle and re-hydrates the wall material which allows for quick release of the active [8]. Diffusional release is dependent on the rate at which an active is able to migrate to the exterior of the encapsulated particle. Diffusion is governed by the physical properties of the wall material (e.g. matrix structure and pore size). King [46] states that volatile mass transfer within dried materials is explained by Fickian diffusion. In short, Fickian diffusion occurs when vacant pores within a glassy matrix (spray-dried or extruded microcapsules) are filled with an active molecule from an adjacent pore. Therefore, active release from glassy matrices is dependant on the diffusion rate through the microcapsule and is typically quite slow. Slow diffusion rates are beneficial for aroma retention in microcapsules; it prevents loss of desirable compounds out of the capsule as well as oxygen intrusion into the capsule.

When plasticizer (water) content increases, glassy microcapsules transition to the rubbery state. Rubbery matrixes have lower viscosity, higher molecular mobility, and higher free volume in the polymer structure than glasses. At this point, there are increases in deteriorative reactions and larger volatile losses as compared to the glassy state [8]. Furthermore, crystallization is believed to start at the transition. Crystallization

occurs when viscosity is sufficiently low for polymers to mobilize into crystalline associations [47]. This leads to a cross-linking effect and reduced areas between polymer chains which forces volatiles from the matrix [8, 32]. Matrix collapse occurs when plasticizer content decreases the viscosity to a point where the polymer matrix cannot withstand the force of gravity [47]. During collapse, the matrix shrinks into a highly viscous, glass-like material. Volatile release increases with increasing plasticizer until matrix collapse which causes a decrease in volatile release [45]. The decrease in volatile release after collapse may be due to a “re-encapsulation” of volatiles and subsequent reduction in diffusion which increases the stability of the matrix [32, 43]. Goubet et al. [47] succinctly summarized the effects of matrix state on aroma release with the following statement: “the amorphous state provides the highest retention, collapse results in losses of aroma, and crystallization leads to the greatest losses of flavors.”

Past flavor research showed that phase change is related to volatile release from glass encapsulated flavors, but the T_g is not the actual point volatile release begins. Levi and Karel [48] postulated that volatile release actually occurs above the T_g . Gunning et al. [49] also noted that aroma release from extruded flavors occurred above the T_g . Blake et al. [50] presented evidence suggesting a poor correlation between T_g and volatile release. They found that some aroma release occurs from an extruded flavor matrix at the T_g , but the bulk of the flavor is released when enough moisture is absorbed to drop the T_g well below ambient temperature. Dronen and Reineccius [51] found a poor relationship between T_g and the onset of volatile release for spray dried model systems. Bohn et al. [52] concluded that benzaldehyde release from extruded flavor matrix increased above $T - T_g$ values of 30°C regardless of %RH exposure.

Bohn et al. [52] elaborated on the complications of linking aroma release to T_g during dynamic humidification of glassy flavors. Consider a particle of an encapsulated flavor in an equilibrium glassy state with water and aroma compounds evenly distributed throughout the particle with a singular, composite T_g . When this particle is exposed to moisture it will hydrate from the outside-in resulting in a moisture gradient. This moisture gradient will result in a gradient of T_g values (T_g decreases as the moisture content increases), and from the discussion above, a gradient of aroma release. From this, flavor release for an entire particle cannot be linked to a single T_g value during a dynamic release process.

1.3 Analysis of Aroma Release from Microcapsules

Measurement of volatile release from dry matrices is possible by a number of techniques. Most commonly, headspace gas chromatography (HS-GC) is used but solvent extraction techniques are also used [53]. Regardless of the measurement technique, these traditional methods for measuring release are extremely time- and labor-intensive [51].

As discussed in the previous section, volatile release from dry matrices is a function of temperature and storage relative humidity (RH). Historically, measurement of aroma release from dry matrices was accomplished under static conditions by storing samples over saturated salts of various RH and measuring the resulting release or retention of aroma compounds [54].

Recently, researchers have taken steps to develop rapid, dynamic methodologies for measurement of volatile release. Blake et al. [50] published a method for rapid

measurement of volatile release. The authors used dynamic vapor sorption atmospheric pressure chemical ionization mass spectrometry (DVS-APCI-MS) to study flavor release. The DVS instrument is normally used for timely creation of moisture sorption isotherms and related phenomena of materials by means of a temperature controlled incubator and controlled humidity gas streams. APCI-MS (a type of atmospheric pressure ionization MS (API-MS)) is a widely used technique to analyze food and flavor volatiles in real-time [55]. Both API-MS and proton transfer reaction MS (PTR-MS) are well suited to analyze gas streams containing moisture (e.g. effluent gas stream from the DVS) [56, 57]. By combining the techniques listed above, Blake et al. [50] monitored the humidity of the sample, change in mass, the state (glassy, rubbery, and liquid collapse) by video camera, and volatile release in real time. Bohn et al. [54] presented a technique that is nearly real-time by using DVS, adsorbent traps, and fast-GC-FID. In this study humidified gas was passed over extruded microcapsules of cherry flavor and the effluent was trapped by Tenax/silica blend and analyzed by fast GC-FID. Although the technique was found to be accurate and precise, it is still not truly a real-time analysis technique. Dronen and Reineccius [51] proposed a methodology using an instrument similar to the DVS called a vapor generator instrument (VGI) coupled with APCI-MS. In this case, the VGI controls temperature and humidity around the encapsulated sample under a stream of nitrogen purge gas. This purge gas exits the VGI and feeds the APCI-MS. Dronen and Reineccius suggested that the method had much potential.

1.4 Effects of moisture on the physical characteristics of encapsulation materials

Moisture sorption isotherm data for carrier materials is useful to understand the physical and chemical changes that may occur during processing, storage, and using flavor materials. When processing spray dried flavors, moisture sorption data can be used to formulate feed emulsions and optimize processing parameters to produce free-flowing powders less prone to stickiness and caking. During storage, moisture sorption data can aid in selecting proper storage conditions to prevent product failure (e.g. caking, chemical reaction, microbial growth, and flavor loss). Finally, moisture sorption data may be useful, in conjunction with glass transition data, when utilizing dried flavors in controlled release applications. The following sections compile moisture sorption and glass transition data of products relevant to the current research; this information has potential to help understand flavor release phenomena.

1.4.1 Gum Acacia

Shotton and Harb [58] determined the equilibrium moisture content of acacia (no specifics on species or form given) stored over a relative humidity (RH) range of 32-100% and temperatures from 25-40°C. These data provided a partial moisture sorption isotherm. The acacia samples were stored in a thermostatically controlled humidity cabinet in which air was circulated over appropriate saturated solutions to obtain specific RH values. Equilibrium was attained when samples showed constant moisture content; moisture content was determined gravimetrically after drying to constant weight in a vacuum oven (10mm Hg and 70°C for approximately 2 hours). Figure 1.1 was adapted from the publication. The authors also noted that at 75% RH the acacia began to cake

and at 80% it swelled. In the high end of the 80% RH range, the acacia began to deliquesce and a sticky paste formed. The authors conclude that temperature had little effect on the equilibrium moisture content. This finding is questionable because the well known Clausius Clayperon equation specifies that water sorption is less favorable at higher temperatures.[59, 60]

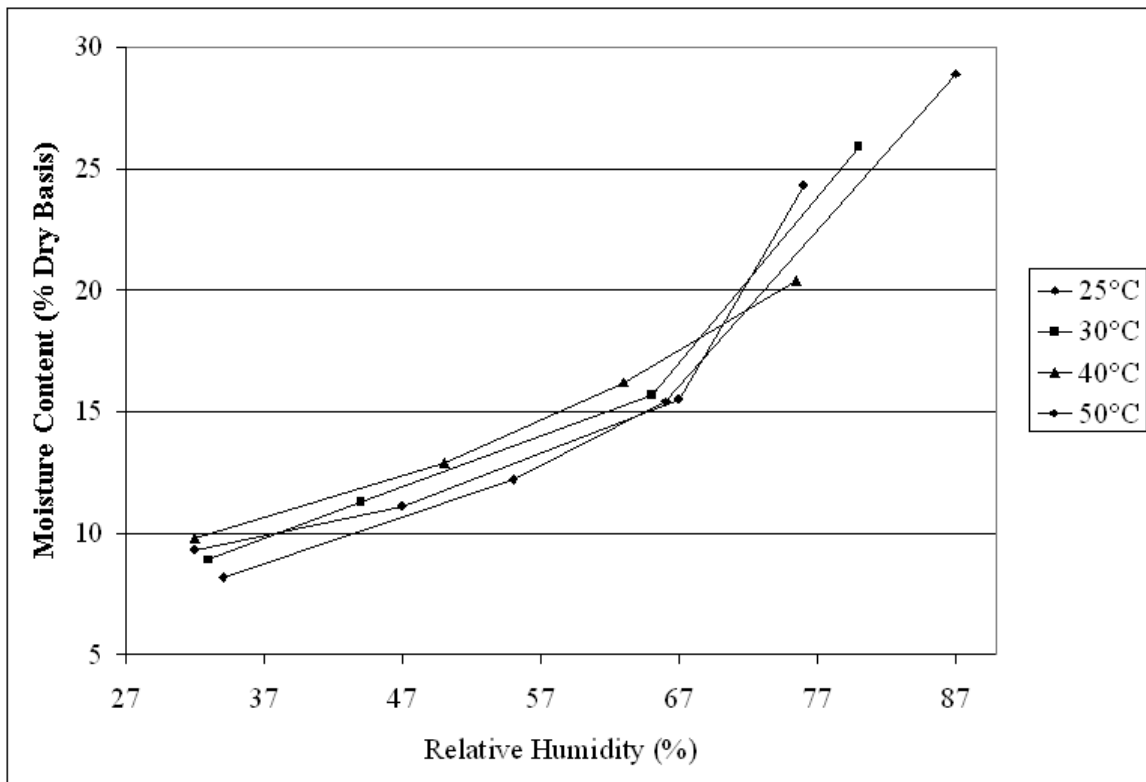


Figure 1.1. Equilibrium moisture content of acacia powder stored between 32-87% RH and 25-50°C. Constructed from the data of Shotton and Harb, 1965.

Phillips et al. [61] used DSC to examine the hydration characteristics of the gum exudate from *Acacia senegal*. The authors mixed samples of the gum with distilled water to obtain final acacia moisture contents between 15 – 489% and analyzed them by DSC. The authors did not observe a thermal transition at the lowest moisture content (15%); the first observable transition the authors noticed was when the product had a moisture content of nearly 50%.

Mothé and Rao [62] studied the thermal properties of gum Arabic containing different gum/water concentrations (from 100/0 to 5/95% w/w) with DSC. The DSC curves indicate an exothermic event, crystalline melting during heating, with an onset temperature around 90°C for the low water content acacia samples (0-40% added water). T_g values from the DSC curves were not given. However, the authors mentioned that “DSC studies are often focused on the first-order transitions,” and recognized that second-order transitions (i.e. T_g) occur just before crystallite melting. Having stated this, the authors presented a figure featuring the DSC thermal curve that shows an arrow depicting where they believe the T_g is located on the curve. The number interpolated from the figure is also 90°C which was their estimated onset for the crystalline melting stage. In a later paper [63], the authors reported the same information – almost verbatim. Unfortunately, there were no additional data on the T_g of gum acacia.

Whorton-Kargel [43] used DSC to examine four different gum acacia products equilibrated at 26% RH. The DSC temperature parameters used for analysis were a bit suspect (20°C/min. scan rate from -50°C to 180°C then rapidly cooled before a second scan of the same parameters). First, the scan rate seems exceedingly fast for adequate determination of samples with barely detectible thermal transitions – the transition may have been lost in the steepness of the scan. Also, the maximum temperature (180°C) of the first scan seemed a bit high; instead of the desired effect of annealing the polymer, this step is more likely to impart thermal damage or scorching which may affect the actual T_g . The author wrote that the, “carriers did not show a distinct glass transition, so the temperature range of T_g was estimated.” Perhaps this is, in part, due to suspect methodology. The reported T_g temperature range for the four products was 70-93°C.

Dronen [64] used DSC to examine gum acacia equilibrated to five different %RH (11, 22, 33, 44, and 54%) at 24°C. Samples were annealed using an appropriate temperature range (dependant on a pre-determined the T_g range) then reheated at 5°C/minute to obtain the T_g . Onset T_g values for the gum (Spraygum IRX 61232) at the aforementioned %RH values were are given in Table 1.2.

Table 1.2 – Onset T_g values for carbohydrate materials equilibrated from 11-54% relative humidity. Data adapted from Dronen, 2004.

| a_w | M250 | M100 | Spraygum IRX 61232 | HiCap |
|-------------|------|------|--------------------|-------|
| 0.11 | 65.7 | 97 | 112.3 | 53.6 |
| 0.22 | 52.7 | 84.6 | 82.1 | 47.4 |
| 0.33 | 47.5 | 76.2 | 71.7 | 33.4 |
| 0.44 | 35.2 | 70.3 | 58.1 | 24.2 |
| 0.54 | 24.8 | 32.1 | 42.2 | 12.3 |

Pérez-Alonso et al. [65] produced moisture sorption isotherms for gum acacia (*Acacia senegal*) stored at 25, 35 and 40°C. Gum samples were dried over P_2O_5 and then re-hydrated over saturated salts ranging in a_w values from 0.11 to 0.85 and the equilibrium moisture content was determined gravimetrically. The authors used the Guggenheim-Anderson-de Boer (GAB) model to describe their data. Calculated monolayer values were 8.11, 9.97, and 11.00 for 25, 35, and 40°C, respectively. Like Shotton and Harb [58], the Clasius Clayperon equation was challenged with the results; Pérez-Alonso et al. saw an increased moisture content with increased temperature. The authors reported that moisture increased with increased temperature below an a_w of 0.75; this result is attributed to the complex nature of moisture adsorption in food polymers. One additional questionable aspect of the research was that the moisture equilibrium time

was not well defined and could be problematic when comparing to other isotherms and modeled data.

1.4.2 Dextrin

Raja et al.[66] evaluated several commercially prepared maltodextrin samples (DEs ranging from 5-25) for a number of physiochemical properties. The authors equilibrated maltodextrin samples over different concentrations of sulfuric acid corresponding to 40, 60, 75, and 90% RH at 28°C; they evaluated the samples for initial moisture content, equilibrium moisture content, and hygroscopicity after a period of storage. Table 1.3 and Figure 1.2 (adapted from the publication) show the initial moisture content and equilibrium moisture contents for the maltodextrin samples. Figure 1.2 represents a partial moisture sorption isotherm constructed from the moisture equilibrium data. Interestingly, their data show the isotherms are basically the same regardless of the DE values, at least for the ranges of RH tested.

Table 1.3 – Initial moisture content and equilibrium moisture content for Maltodextrin samples. Data adapted from Raja et al., 1989.

| Sample | DE | Initial Moisture (%) | RH (%) and Equilibrium Moisture Content (%) | | | | |
|---------------|-----------|-----------------------------|--|-----------|-----------|-----------|----|
| | | | 40 | 60 | 75 | 90 | |
| 1 | 24.9 | 4.10 | 5.87 | 9.57 | 12.77 | 13.33 | * |
| 2 | 25.1 | 6.00 | 5.78 | 9.06 | 12.45 | 12.86 | |
| 3 | 21.4 | 4.18 | 5.96 | 8.99 | 13.01 | 13.19 | ** |
| 4 | 15.5 | 3.57 | 6.2 | 9.66 | 13.11 | 14.24 | |
| 5 | 11.9 | 3.71 | 6.2 | 9.77 | 12.85 | 13.83 | |
| 6 | 5.7 | 3.50 | 6.7 | 9.91 | 13.13 | 13.86 | |
| 7 | 10.3 | 4.10 | 7.5 | 9.71 | 12.47 | 13.37 | |

* Sample became pasty after 6 days of storage at 75% RH

** Sample became pasty after 6 days of storage at 90% RH

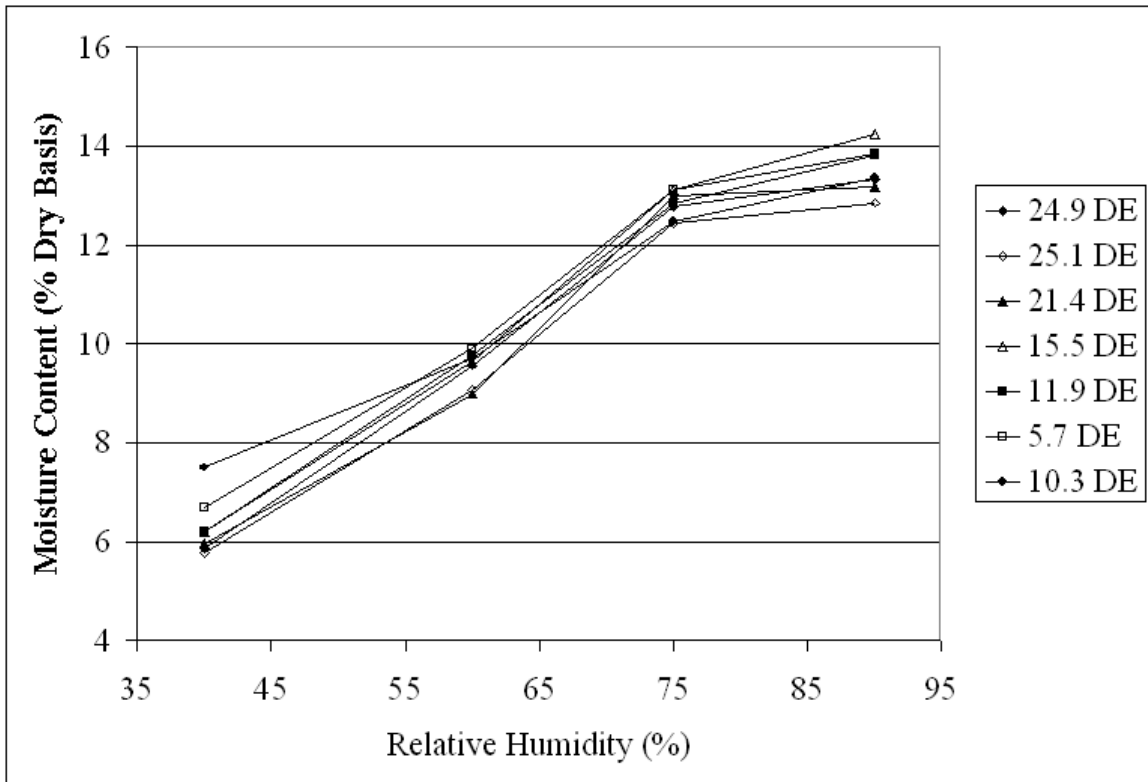


Figure 1.2 – Plot of equilibrium moisture content for maltodextrin samples at various DE values. Constructed from the data of Raja, et al., 1989.

Abramovič and Klofutar[67] presented water adsorption isotherms of six maltodextrin samples ranging from 5 to 25 DE. The authors created the moisture adsorption isotherms across a water activity (a_w) range of 0.1105 to 0.9800. Dry maltodextrin samples (stored over P_2O_5 under vacuum) were equilibrated over saturated salt solutions of known water activity at roughly 25°C for four days and weighed for amount of water adsorbed. The equilibrium time of 4 days is considered very short per standard moisture sorption isotherm methodology [68] and the information gained from this study must be considered carefully. Figure 1.3 depicts data adapted from the publication. The authors were able to model their data with the two most popular moisture sorption isotherm models: the GAB and Brunauer-Emmett-Teller (BET) models, as well as one lesser known model, the Caurie model.

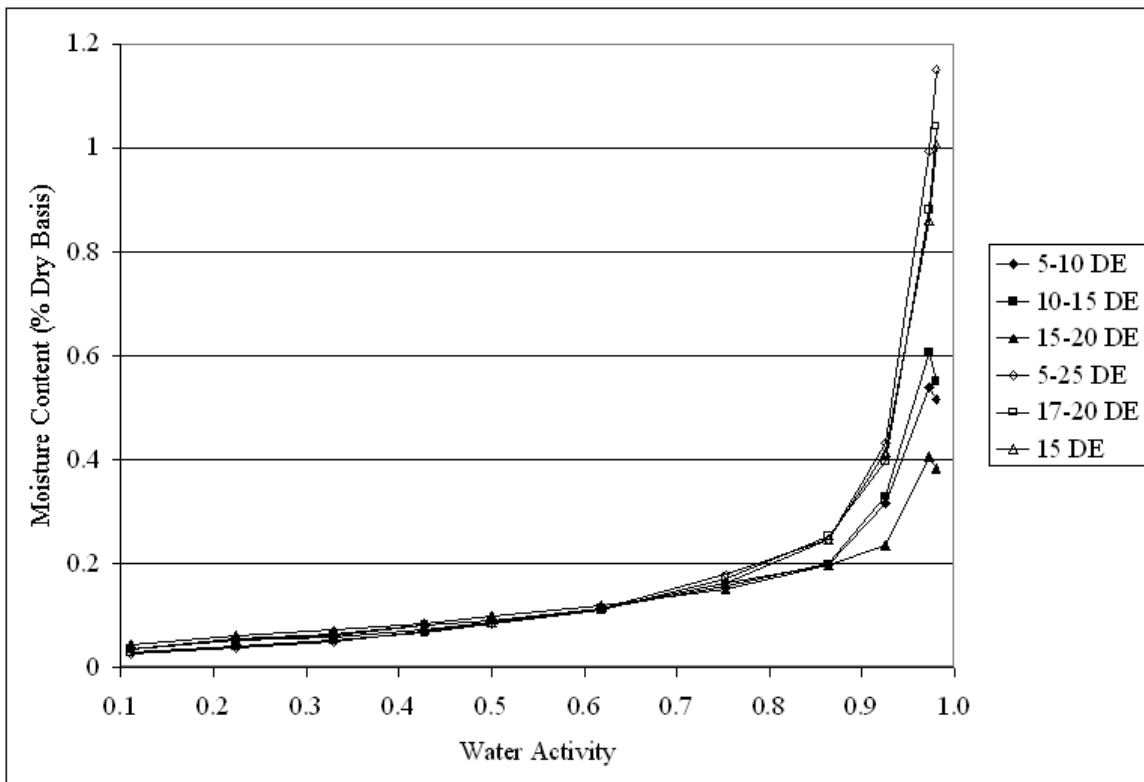


Figure 1.3 – Moisture sorption isotherms for several maltodextrin samples. Constructed from the data of Abramovič and Klofutar, 2002.

Quite possibly the most in-depth publication for this literature review and current research project is one by Roos and Karel[69]. The authors orchestrated a very thorough study on the equilibrium moisture content and T_g (defined at onset of transition curve) of maltodextrins after storage across a range of relative humidity environments. Samples of maltodextrins (5, 10, 15, 20, 25, and 36 DE) and maltose were dissolved in water (20% solutions) and freeze-dried. Then, these samples were dried further over P_2O_5 before controlled re-humidification over saturated salt solutions (11, 23, 33, 43, 52, 75, and 85% RH) and subsequent DSC analysis. Figure 1.4 (adapted from the publication) illustrates the moisture sorption isotherms of the samples; the authors did not model or discuss these data. Figure 1.5 (adapted from the publication) presents the measured T_g s of the samples over the range of %RH. Dry maltodextrins for this study have T_g s between 100-200°C.

The plot clearly shows a plasticizing effect by increasing the moisture content. T_g temperatures appear to drop linearly as the %RH increases from 11-85%, but the overall relationship appears to be sigmoid. Additionally, the T_g s of the maltodextrin samples increase with increasing molecular weight (i.e. T_g increases as DE decreases). These data provide a strong foundation for the discussion on the effects of moisture and phase transition on the release of aroma compounds from glassy matrices which follows.

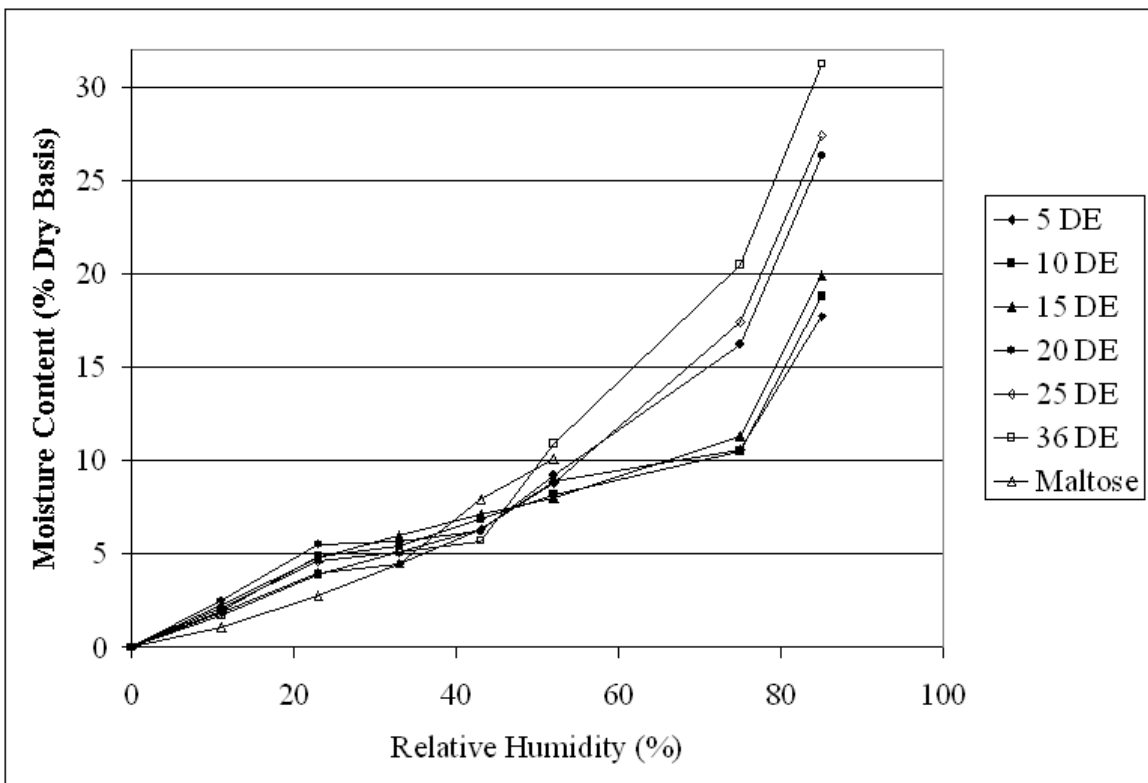


Figure 1.4 – Moisture sorption isotherm for several maltodextrin samples. Constructed from the data of Roos and Karel, 1991.

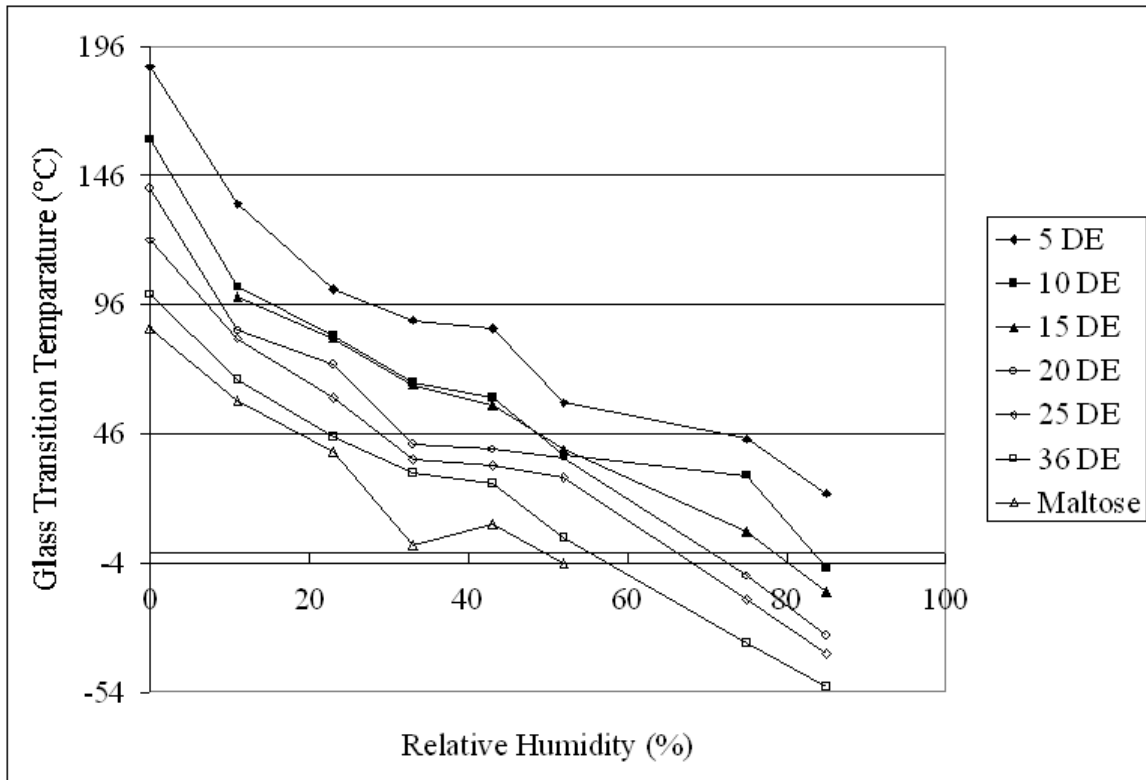


Figure 1.5 – Plot of glass transition temperatures for several maltodextrin samples as affected by moisture equilibrium in a range of %RH values. Constructed from the data of Roos and Karel, 1991.

Using the methodology described in the gum acacia section (section 1.4.1, pages 18-19): Whorton-Kargel [43] reported a T_g range of 62-67°C for a 20 DE dextrin. Despite questionable methodology, this finding was in agreement with Roos and Karel [69]. Dronen [64] reported onset T_g values for 25 and 10 DE maltodextrin products (M250 and M100, respectively) equilibrated to 11, 22, 33, 44, and 54% RH (Table 1.2, page 19). For the 10 DE product, the onset T_g values at 11 and 23% RH are quite similar to the values from Roos and Karel [69] – the similarities end with those two values.

Pérez-Alonso et al. [65] also produced moisture sorption isotherms for maltodextrin (DE 10) stored at 25, 35 and 40°C. Using the same methodology outlined in the gum acacia section, the authors successfully modeled (GAB) the equilibrium moisture content data and reported monolayer values of 12.72, 7.84, and 5.26 for 25, 35,

and 40°C, respectively. Unlike the gum acacia in the same study, the data for the maltodextrin followed the Clarius Clayperon equation below a a_w of 0.75.

1.4.3 Modified Starch and Dextrin

Octenylsuccinated starches are not well characterized by T_g or moisture sorption isotherm data in the literature. Recent searches of the literature revealed that DSC applied to such modified starches is typically for wet systems. [70-72] Only a two sources provided some data on T_g and nothing was found for isotherms. There is a need for further research in this area.

Zasytkin and Porzio [73] characterized the glassy states of spray-dried and extruded flavors encapsulated in octenyl succinic acid anhydride (OSAn)-modified starch blends by the use of modulated DSC (MDSC). Four different carrier blends were composed of three of the following materials in various levels: OSAn-starch, acid-modified OSAn-starch, enzymatically hydrolyzed OSAn-starch, dextrinized OSAn-starch, dextrin, lactose, and dextrose. One of the three components for each formulation was always either lactose or dextrose. Prior to spray-drying, each carrier blend was dispersed in water to a solute level of 40% (w/w) while incorporating either butter flavor or lemonade flavor at two different flavor loads (10 and 20% (w/w)). T_g was characterized by both the onset temperature ($T_{g-onset}$) and a mid-point temperature ($T_{g-midpoint}$) between the onset and end of the transition interval. Because of the complex blends of carriers, the only conclusions that can be drawn are based on the type and concentration of flavor rather than carrier type. The authors speculated that the flavor formulation has a plasticizing effect on the carrier; in particular, they speculated that the

citral in the lemonade flavor has a greater plasticizing effect than the butter flavor. The $T_{g\text{-onset}}$ ranged between 60 to 85°C across the product treatments. Attempts to contact the authors for further information were unsuccessful.

Using the methodology described in the gum acacia section (section 1.4.1, pages 18-19)): Whorton-Kargel [43] reported on two modified starch products. N-lok™ had a T_g range of 48-54°C and Capsul™ had a T_g range of 65-80°C. Dronen [64] reported onset T_g values for HiCap 100™ equilibrated to 11, 22, 33, 44, and 54% RH (Table 1.2, page 19). The modified starches used by Whorton-Kargel and Dronen are commercially prepared by National Starch and Chemical company.

CHAPTER 2
RESEARCH OBJECTIVES

2. Research Objectives

The objectives of this research project are to:

- 1) Produce menthol microcapsules using OSAn-substituted dextrans and gum acacia.
- 2) Determine real-time menthol release and moisture uptake profiles of said microcapsules under humidified nitrogen gas streams.
- 3) Quantify interaction of menthol with aqueous solutions of OSAn-substituted dextrin and gum acacia.
- 4) Determine the physical properties of the encapsulation matrices and microcapsules.
- 5) Assess how changes in menthol release are related to water uptake.
- 6) Assess how changes in menthol emulsion properties are related to OSAn substitution.
- 7) Assess how menthol affinity is related to OSAn-substitution.

Methods used to accomplish objectives:

- 1) To produce the microcapsules, modified dextrin and gum acacia samples of varying levels of OSAn-substitution were emulsified with molten menthol and spray-dried.
- 2) Real-time menthol release and moisture uptake profiles were accomplished using dynamic vapor sorption (DVS) coupled with either Tenax trapping and gas chromatography (GC) or proton transfer reaction mass spectrometry (PTR-MS).
- 3) Interactions between menthol and aqueous solutions of OSAn-substituted dextrin and gum acacia were quantified by static headspace gas chromatography (HS-GC) methodology.

- 4) Physical properties of the microcapsules and encapsulation matrices were done by: constructing moisture sorption isotherms, particle sizing, density measurement via pycnometry, and by determining microcapsule menthol content (surface and total).
- 5) Statistical treatment and individual interpretation of the data obtained with the aforementioned methods were used to address objectives 5 through 7.

CHAPTER 3

Encapsulation and release of menthol. Part 1: The influence of OSAn modification of carriers used to encapsulate l-menthol by spray drying.

This chapter has been accepted for publication in Flavour and Fragrance Journal.

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3.1 Abstract

The objective of this research was to evaluate the effect of octenyl succinic anhydride (OSAn) substitution level of carbohydrate encapsulation matrices on the retention and physical characteristics of spray dried l-menthol microcapsules. It was found that as OSAn substitution of carbohydrates increases, total and surface menthol appeared to increase and decrease, respectively. The particle size of the finished spray dried powder was influenced by spray dryer infeed solids concentration with higher solids leading to larger particle size. Microcapsule density did not appear to be affected by OSAn substitution or carrier type.

Keywords: octenyl succinic anhydride, dextrin, gum acacia, menthol, spray drying, encapsulation

3.2 Introduction

Encapsulation is defined as, “a process by which one material or mixture of materials is coated or entrapped within another material or system”. [1] The primary reasons flavors are encapsulated are to: convert liquid flavor compounds to free-flowing powders, protect them from chemical reaction (e.g. light induced or oxidation); retain them during storage; and prevent interactions with food components and other flavorings. [2-4]

Flavor encapsulation is accomplished using a number of production methodologies. The primary processes used to encapsulate flavors include spray drying, extrusion, inclusion complexation, and coacervation; the reader is directed to the referenced comprehensive reviews of these technologies for more details. [1, 5-10] Of the many methods available for flavor encapsulation, spray drying is the most important method for production of the dried flavors [74] likely due to its simplicity of operation and availability of equipment, manufacturing costs and product performance. [6, 9] Spray drying was chosen as the method of microencapsulation for this research.

Just as there are numerous processes used to manufacture encapsulated flavors, there are numerous materials used as encapsulating agents. These materials include carbohydrates (e.g. starches, dextrans, sugars, gums, cyclodextrins, celluloses, and alginates) and proteins (e.g. whey proteins and gelatin), fats, and phospholipids. [7] Because the majority of the dry flavors produced are by spray-drying or extrusion, the three dominant matrices used for these encapsulation operations are gum acacia, maltodextrins, and modified starch (typically modified with octenyl succinic anhydride

(OSAn)).[3] Strengths and weaknesses of these carrier materials as flavor encapsulating matrices are found in the following references. [3, 4]

For this research, l-menthol was chosen as the active to be encapsulated. l-Menthol is a cyclic terpene alcohol that is responsible for the minty flavor and cooling sensation of food and pharmaceutical products. Of the eight possible menthol isomers (C₁₀H₂₀O, mw = 156.27 g/mol), l-menthol is the most abundant in nature and is assumed when using the term “menthol”. Menthol can be synthesized (usually from other terpenes) or it is found naturally in plants of the *Mentha* species [75]; synthetic menthol was chosen for this research.

Menthol is solid at room temperature (mp = 43°C).[76] Most pure flavoring compounds are liquid and are therefore relatively easy to emulsify into an encapsulation matrix prior to spray drying, this is not the case for menthol. Furthermore, although menthol is a solid at ambient temperatures, it is very volatile. It has an unusual property of forming crystals on the surface of solid pharmaceutical preparations of menthol with various excipients.[77] These surface menthol crystals (coined “whiskers”) are viewed as product defects for functional and aesthetic reasons. These whiskers on the surface of microcapsules imply a lack of encapsulation efficiency and a potential mechanism of menthol loss from product by sublimation. These unique physical properties of menthol make it a challenging model compound for microencapsulation (in particular, spray drying) and published research on menthol microencapsulation is limited.[53, 77]

The present research investigates the effects of various carbohydrate carriers (OSAn-substituted dextrans and gum acacia, un-substituted dextrans and gum acacia) on the characteristics of spray dried menthol microcapsules. It is hypothesized that

increasing OSAn substitution in the matrix will result in microcapsules with increasing total menthol content and decreasing menthol on the surface due to increased emulsifying activity.

3.3 Materials and Methods

3.3.1 Chemicals

Synthetic menthol (l-menthol pellet USP: Takasago International, Rockleigh, NJ, USA) was used as the encapsulation load material. Spectrophotometric grade methyl alcohol (Sigma Aldrich Inc., St. Louis, MO, USA) was used as the dispersant for particle size analysis. Capillary gas chromatography (GC) grade acetone (Sigma Aldrich Inc., St. Louis, MO, USA) was used for total menthol analysis. Capillary GC grade pentane (Sigma Aldrich Inc., St. Louis, MO, USA) was used for surface menthol analysis. Benzyl alcohol (Fisher Scientific, Fair Lawn, NJ, USA) was used as the internal standard for all GC analysis.

3.3.2 Carrier Materials

Ten carrier materials were utilized to produce menthol microcapsules for this research; five starch based and five gum acacia based. Table 3.1 lists the carrier materials and abbreviation codes used to describe them. Further carrier material description follows.

Table 3.1. Carrier materials, identity codes, and formulae used to produce spray dried menthol emulsions.

| Code | Carrier Identity | Carrier Solids Content (%) | Menthol Load (%) | Carrier Mass (kg) | Menthol Mass (kg) | Water Mass (kg) |
|-------------|--|-----------------------------------|-------------------------|--------------------------|--------------------------|------------------------|
| D | Dextrin | 30 | 40 | 2 | 1.33 | 4.67 |
| D1 | 1% OSAn Dextrin | 40 | 40 | 2 | 1.33 | 3.00 |
| D2 | 2% OSAn Dextrin | 40 | 40 | 2 | 1.33 | 3.00 |
| D3 | 3% OSAn Dextrin | 40 | 40 | 2 | 1.33 | 3.00 |
| CAP | Capsul | 40 | 40 | 2 | 1.33 | 3.00 |
| FT | <i>A. seyal</i> (Gum Arabic FT) | 30 | 40 | 2 | 1.33 | 4.67 |
| B2 | 0.67 <i>A. seyal</i> : 0.33 TICAmulsion | 30 | 40 | 2 | 1.33 | 4.67 |
| B1 | 0.33 <i>A. seyal</i> : 0.67 TICAmulsion | 30 | 40 | 2 | 1.33 | 4.67 |
| TA | TICAmulsion | 30 | 40 | 2 | 1.33 | 4.67 |
| GA | <i>A. senegal</i> (Gum Arabic Spray Dry) | 30 | 40 | 2 | 1.33 | 4.67 |

The starch based products are various preparations of dextrinized starch. CAPSUL™ (CAP) (National Starch Inc., Bridgewater, NJ, USA) is a commercially available dextrinized waxy maize starch octenyl succinate. The other four products were: an unmodified dextrin (D) (Amidex 182), and three custom dextrin products that were prepared by treating corn starch with one, two, and three percent OSAn (D1, D2, and D3, respectively). The latter four products were provided by Corn Products Brasil (São Paulo, Brasil).

The gum acacia based products are derived from two species of acacia. Gum Arabic Spray Dry FCC Powder (GA) (TIC Gums, Inc., Belcamp, MD, USA) is a product obtained from the *Acacia senegal* species and is considered an “emulsifying” gum.[78] Gum Arabic FT Powder (FT) (TIC Gums Inc., Belcamp, MD, USA) is obtained from the *Acacia seyal* species and is considered a “non-emulsifying” gum.[78] TICAmulsion A-2010 (TA) (TIC Gums Inc., Belcamp, MD, USA) is a modified gum manufactured by substituting some of the hydroxyl groups on the molecules of *A. seyal* with OSAn.[78] Actual OSAn treatment levels are unknown, but the actual treatment level is suspected to

be no more than 3% as described by the United States of America Code of Federal Regulations for modified starch.[25]

FT and TA were dry blended to create two additional *A. seyal* products with different levels of total octenyl succinic anhydride (OSAn) substitution. FT and TA were blended in ratios (w/w): of 67:33 and 33:67 to create intermediate levels of OSAn substitution between FT and TA. The blends were produced by placing the desired amount of each material in a Hobart mixer (Model #: A-200, Hobart Mfg. Co., Troy, Ohio, USA) and then blending at the lowest speed for 15 minutes with the paddle attachment.

3.3.3 Spray Drying

The selected carrier blends were initially dissolved to solids contents low enough to allow adequate atomization (30% for the gum acacia products and the unmodified dextrin and 40% for all other dextrin products). Each solution was heated to 70°C under constant mixing to insure complete dissolution and then allowed to cool in the refrigerator overnight before use. Immediately before spray drying, each carrier solution was heated to 50°C and menthol was added at 40% of the total solids (i.e. 40:60, menthol mass:carrier mass) as detailed in Table 3.1. Each carrier solution containing molten menthol was homogenized with a bench-top rotor stator high sheer mixer (Model #: 1L.81 Greerco Corp., Hudson, NH, USA) for 5 minutes at 7500 RPM. During spray-drying, the menthol/carrier emulsion was held at 50°C and continuously mixed with the high sheer mixer. Spray drying was done in the pilot plant of the department of Food Science and Nutrition at the University of Minnesota with a Niro Utility dryer (Niro, Inc.,

Columbia, MD, USA). This dryer uses a centrifugal wheel atomizer (24.5 cm diameter rotary vane wheel rotating at 22,400 rpm), is gas heated, has a mass air flow of about 360 kg/h; drying chamber of 1.2 m diameter, 0.75 m cylindrical height, cone angle of 60°; cyclone collector 0.3 m diameter; and powder collection container of 20 l capacity. Operating at an inlet air temperature of 200°C and an exit air temperature of 100°C resulted in a feed rate of ~330 ml/min (varied in order to maintain outlet temperature). After drying, the powders were cooled to ambient temperature and stored in poly bags at room temperature until analysis.

3.3.4 Particle Size of Spray Dried Particles

To obtain the average particle size for the spray dried powders, a Malvern Mastersizer (Malvern Instruments, Ltd., Worcestershire, UK) was utilized. Approximately 0.4g of each menthol powder type was suspended in 10 mL of methyl alcohol. Sample dispersions were shaken immediately before addition into the small volume sample dispersion unit. Particle size (μm) results for each microcapsule are reported as an average of triplicate sampling.

3.3.5 Density of Carrier Materials and Spray Dried Menthol Powder

The densities of the carrier materials and the spray dried menthol powders were calculated as the quotient of mass over absolute volume (as determined by helium pycnometry). Approximately 0.5 to 1 g of carrier material or spray dried menthol powder was measured into the sample cell of a Quantachrome Multipycnometer (Quantachrome Corporation, Boynton Beach, FL, USA) to determine the volume of each powder. The

volume was calculated using the calibrated standard methodology provided with the instrument. Density (g/cm^3) results for each microcapsule are reported as an average of triplicate sampling.

3.3.6 Total Menthol of the Spray Dried Menthol Powder

Total menthol of the spray dried menthol powder was determined using a method similar to Soottitantawat, et al.[53] Briefly, 0.3 g of encapsulated menthol was mixed with 1.7 mL double distilled water in a 20-mL headspace vial, capped, and heated in a water bath at 80°C for 1 minute to dissolve the carbohydrate matrix. Then 8 mL of acetone (with 0.004 g/mL benzyl alcohol as an internal standard) was added to the vial to precipitate the carbohydrate material. The sample vial sat overnight at room temperature to equilibrate and then was centrifuged to separate the carbohydrate material from the extract. A 0.5 μL aliquot of this extract was injected into a Hewlett Packard model 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) with a flame ionization detector (FID) to quantify the menthol extracted from the sample. The GC was equipped with a J&W DB-5 column (30 m, 0.25 mm I.D., 0.5 μm film) operating at a head pressure of 15 psi with a split ratio of 25:1. The GC temperature parameters are as follows: inlet and detector temperature were 250°C and the oven temperature program started at 100°C with no hold time and ramped to 200°C at a rate of $15^\circ\text{C}/\text{minute}$ and held at 200°C for 5 minutes. A standard curve of menthol was used for quantification. Total menthol retention is reported as a percentage of the theoretical load retained after drying. Results for each spray dried menthol powder are reported as an average of triplicate sampling.

3.3.7 Surface Menthol of the Spray Dried Menthol Powder

Surface menthol, i.e. menthol not contained within the spray dried menthol powder, was also determined using a modified version of the method published by Soottitantawat, et al.[53] Briefly, 0.5 g of encapsulated menthol was mixed with 5 mL pentane (with 0.004 g/mL benzyl alcohol as an internal standard) in a 20-mL headspace vial and capped. The sample was put on an orbital shaker (Lab-Line Orbit Shaker #3590, Lab-Line Instruments, Inc., Melrose Park, IL, USA), set at 150 RPM, and allowed to shake for 30 minutes at room temperature. The sample was then allowed to sit to allow the powder to settle. A 0.5 uL aliquot of this extract was injected into the same GC and operating parameters as outlined for the total menthol procedure. A standard curve of menthol was used for quantification. Surface menthol is reported as a percentage of the total menthol measured. Results for each spray dried menthol powder are reported as an average of triplicate sampling.

3.3.8 Statistical Analysis

We used analysis of variance (ANOVA) to determine whether the carrier materials or finished powders differed in any of the aforementioned analyses. Differences among mean values were compared using one-factor ANOVA and the Fisher Least Significant Difference (LSD) method ($\alpha = 0.05$). Design Expert 6.011 (Stat Ease, Inc., Minneapolis, MN, USA) was the software package used for statistical computing.

3.4 Results and Discussion

3.4.1 Influence of Carrier Type on Total and Surface Menthol

The mean total and surface menthol values for all spray dried menthol powders are presented in Figure 3.1. For the custom dextrin products (D1, D2, and D3), average total menthol content was not significantly different regardless of the OSA_n treatment level; approximately 95% of the menthol was retained during drying. These products retained menthol significantly better than the unmodified dextrin (D) which retained about 81% of the menthol. These differences could be explained by the added emulsifying capabilities of the OSA_n-treatment, but also by the formulation (solids content) of the microcapsule emulsion prior to drying. Recall from Table 1 that the carbohydrate content of the infeed emulsion for D was 30% compared to 40% for D1, D2, and D3. Reineccius[79] emphasized the importance of infeed solids for obtaining good volatile retention during spray drying. Soottitawat et al.[53] confirmed this assertion for spray dried menthol reporting increased menthol retention with increased infeed solids concentration. This is primarily explained by the fact that high infeed solids concentrations promotes faster semi-permeable membrane formation at the surface of the emulsion droplet and thus better menthol retention.[38] Therefore, our data do not allow drawing conclusions on the whether OSA_n treatment of carbohydrate carrier affects total menthol content due to confounded variables. However, we can conclude that microcapsules made with 1, 2, and 3% OSA_n treated dextrin do not differ in total menthol content when the solids content of the infeed emulsion is 40%.

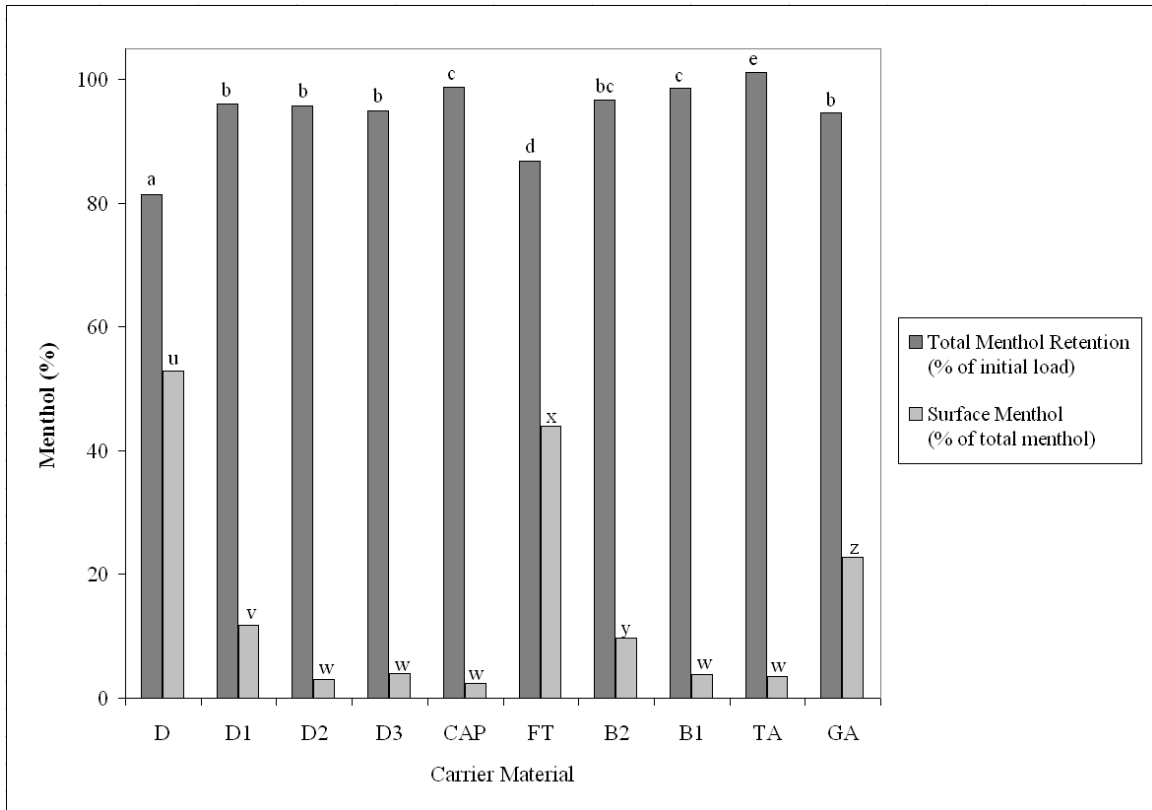


Figure 3.1 – The influence of carrier material on total and surface menthol in finished spray dried menthol powders. Letters a to e indicate Fisher LSD similarities for total menthol and letters u to z are Fisher LSD similarities for surface menthol.

However, the effects of OSA_n treatment of dextrans on surface menthol are more apparent. Figure 3.1 illustrates that increasing emulsifying groups on dextrans decreases the amount of menthol that is extractable from the surface of the spray dried powder. For D, over half of the total menthol was found to be on the surface of the powder. Surface menthol dropped to almost 10% of total menthol when 1% OSA_n treated dextrin was used as a carrier material and to 3% when 2% OSA_n treated dextrin was used. The difference of surface menthol between 2 and 3% OSA_n treatment level was not significant. To conclude, an increase of OSA_n treatment on dextrans increases the amount of menthol actually encapsulated within the microcapsule.

The total menthol load determined for CAP was statistically higher than all the other dextrin products, but was similar to D2 and D3 for surface menthol at about 1% total mass. The chemical composition and particle characteristics of CAP should be quite similar to the OSAn modified dextrans used in this study. Chemical Abstracts Service (CAS) reports the index name for CAPSUL™ (CAS #: 61932-62-5) as hydrogen octenylbutanedioate amyloextrin. The OSAn modified dextrans can also be described by this moniker and therefore, it would seem that reasonable similarities would emerge among CAP and the modified dextrans. Indeed, CAP had similar characteristics to the D2 and D3 products in terms of total menthol retention and surface menthol.

Figure 3.1 also illustrates how OSAn treatment of *A. seyal* (FT) affects menthol retention. Comparisons among the gum acacia products are easier to make because the data are not confounded by infeed solids content as the dextrin samples were. Total menthol retention significantly increases and surface menthol significantly decreases as the proportion of OSAn treated gum acacia increases. Similar to the OSAn modified dextrans, the effects of the two highest substitution levels of OSAn treatment on gum acacia, i.e. medium (B1) and high (TA) are not significantly different for surface menthol.

The emulsifying gum acacia, *A. Senegal* (GA), appears to be an intermediate performer in both total and surface menthol categories between the naturally “non-emulsifying” FT and engineered “emulsifying” TA. It is known that gum acacia from different species exhibits different molecular and structural characteristics and therefore exhibit different functional properties in application.[13, 80, 81] Gum acacia is a hydrocolloid composed of predominantly polysaccharides with a small amount of protein

(comprised mainly of hydrophobic serine and hydroxyproline amino acids).[16] *A. senegal* is known to have a higher protein content than *A. seyal*. [14, 16] Thevenet [14] stated that gum acacia fractions containing protein are responsible for acting as the interface between oil and water phases of emulsions made with gum acacia. From this standpoint, it is rational to accept that GA has significantly higher total menthol and lower surface menthol contents. Also, we suspected that TA performs the best of all because it was designed to do so.

To our knowledge, there is only one journal article that focused on the microencapsulation of menthol by spray drying; the present study will be compared to this publication. Soottitantawat et al.[53] prepared spray dried powders of menthol in CAPSUL and gum acacia (species not reported) at 30% infeed solids and 30% menthol load (recall the present study utilized 40% infeed solids for CAP and D3 and 30% infeed solids for FT and GA with a 40% menthol load for all carriers). The authors reported much lower total menthol retention for their CAPSUL and gum acacia samples as compared to the modified dextrans (CAP and D3) and unmodified gums (FT and GA) in the present study. It is suspected that the primary reason for the differences in their gum acacia samples compared to FT and GA lies in the infeed solids content; they used a lower infeed solids concentration. Another possible reason for the differences among all samples lies in the emulsion homogenization step. Soottitantawat et al.[53] reported a three minute homogenization process at 55°C immediately followed by spray drying at that temperature. The current research utilized a five minute homogenization process at 50°C but also continuous homogenization of the feed emulsion during the drying process. During preliminary trials, in which we did not continuously homogenize during spray

drying, we observed non-emulsified molten menthol floating to the surface. Continuous homogenization ensured that the menthol in the emulsion would stay emulsified and the non-emulsified menthol would at least be suspended in the emulsion. Although total menthol retention in the present study was higher than found by Soottitantawat et al.[53], the surface menthol for their gum acacia sample was much lower than our FT and GA, and much higher for their CAPSUL than our CAP and D3. The differences observed in gum acacia data between these studies may be due to the natural variability of the gum acacias and the fact that we used a 40% menthol load. We cannot offer an explanation for them finding higher surface oil in their modified starch samples unless it again relates to lesser emulsification.

The results of this part of the study show that increasing OSAn substitution (for either carbohydrate type) increases total menthol retention and decreases the surface menthol content of the spray dried powders. We feel that this is most likely due to better emulsification (smaller mean particle size of infeed emulsions) provided by the OSAn modified products. It is known that unmodified dextrans provide little if any emulsification properties and produce coarse and unstable flavor emulsions.[82] Furthermore, emulsions created with OSAn modified dextrans were found to have smaller droplet sizes than unmodified dextrans.[2] Risch and Reineccius[83] found that as emulsion size decreases, total oil increases and surface oil decreases for orange oil spray dried in gum acacia or modified starch matrices. Soottitantawat et al.[84] reported similar findings for a model flavor system in gum acacia, modified starch, and maltodextrin blends. Soottitantawat et al.[53] also observed that modified starch and gum acacia matrices (with intrinsically different levels of emulsifying activity) yielded

different emulsion sizes under the same homogenization conditions and that the emulsion with the smallest droplet size had the highest retention of menthol in the finished powder.

3.4.2 Influence of Carrier Type on Spray Dried Powder Particle Size

Particle size of the resultant powders was influenced by the infeed solids concentration of the feed emulsion into the spray dryer (Figure 3.2). This observation is consistent with previous literature and expectations since higher solids infeeds would shrink less during the drying process. The products formulated at 40% solids (D1, D2, D3, and CAP) had a significantly larger average particle diameters than the products formulated at 30% solids but no differences were found across OSAn treatment levels. OSAn modification might have had a significant effect on powder diameter by altering the viscosity of the infeed material. However, there was little (or an inadequate) difference in viscosity across these samples to show an effect of OSAn treatment levels.

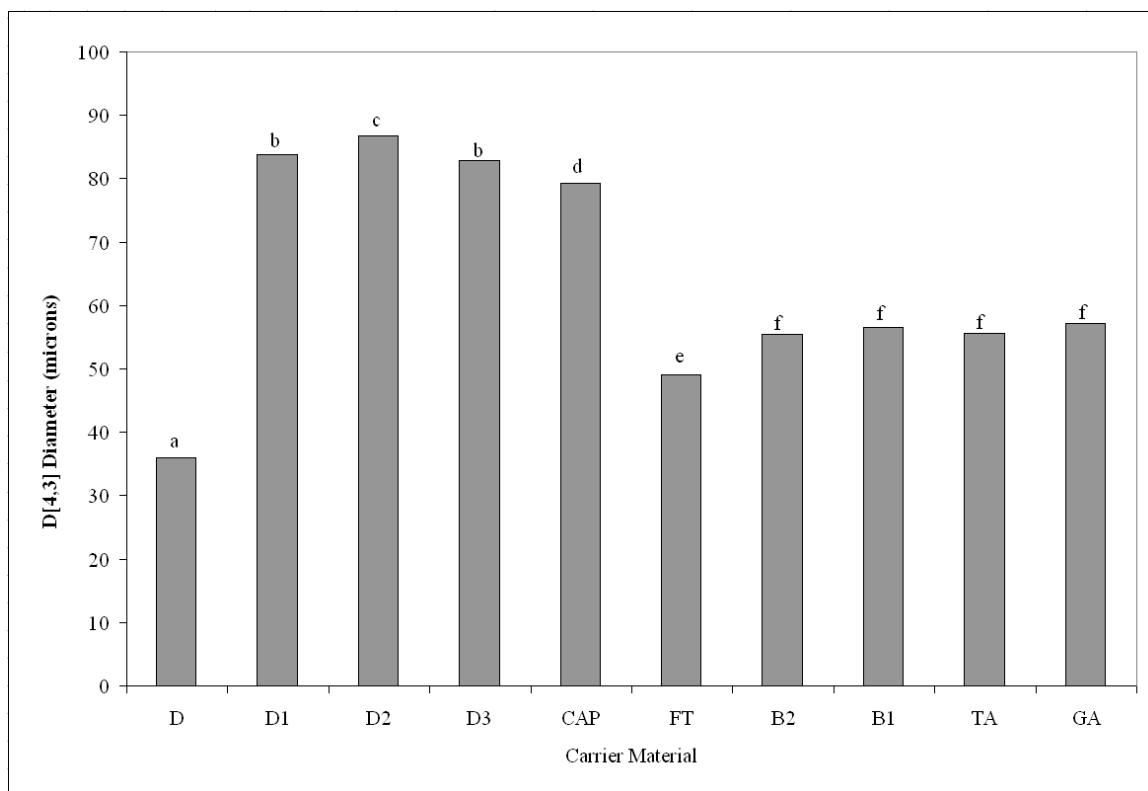


Figure 3.2 – The influence of carrier material on the mean diameter (D[4,3]) of the finished spray dried menthol powders. Letters a to f indicate Fisher LSD similarities.

3.4.3 Spray Dried Powder Density

OSAn treatment did not appear to have an effect on the density of the initial unloaded carrier materials or subsequently produced menthol-loaded, spray dried powders (Figure 3.3). Buffo and Reineccius[85] also observed no noticeable influence of matrix on the density of finished spray dried powders when blends of gum acacia, modified starch, and maltodextrin were varied. The observed statistical differences among the unloaded carrier materials as received in this study are likely due to the different dryer equipment and processing parameters (as the starting material originated from different suppliers).

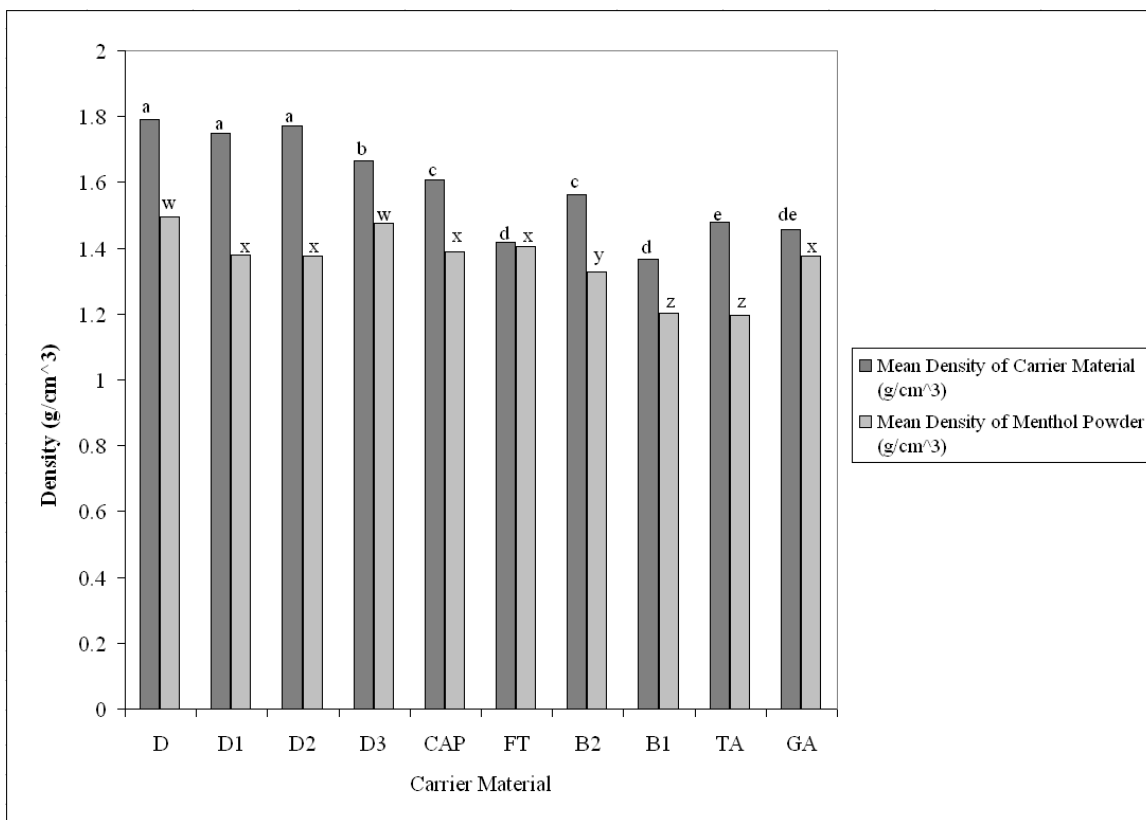


Figure 3.3 – Mean carrier material and finished spray dried menthol powder densities for each carrier type. Letters a to e indicate Fisher LSD similarities for carrier material and letters w to z are Fisher LSD similarities for finished microcapsules.

Upon comparing carrier material density to finished menthol-loaded, spray dried powders; it is clear that the process of our loading and spray drying the carriers affected powder density (Figure 3). In all cases the menthol-loaded, spray dried powders had a lower density than their corresponding unloaded carrier materials. This likely was the result of two factors. First, the addition of menthol (density: 0.90315 g /cm³ [76]) to the carrier materials decreased the overall particle density. For example, a spray dried powder made with CAP (unloaded density: 1.608 g/cm³) at a 40% menthol load has a calculated density of 1.326 g/cm³; the actual measured density of menthol-loaded, spray dried CAP powder is 1.388 g/cm³. Second, spray dried powders will contain some

entrapped air which will also decrease the density of the powder.[86] The amount of air entrapped in the dry powder depends primarily upon the amount of air present in the infeed material and the subsequent atomization process.

3.5 Conclusions

The most significant finding of the current research is that OSAn substitution of hydrocolloids (dextrins and gum acacia) increases the retention of menthol and decrease the surface menthol relative to unmodified products. While commercial products typically have approximately the legal limit of OSAn modification (3% treatment level), it appears that lower levels would suffice in terms of menthol retention during drying (no significant increase in menthol retention between 1, 2 or 3% substitution). However, higher levels of OSAn substitution did reduce the surface menthol: a parameter generally considered important in shelf-life. We assume the improved retention and reduced surface menthol result from enhanced emulsifying capabilities of the OSAn modified carrier materials.

In terms of how OSAn modification affected the physical properties of the resultant spray dried powder, particle size was influenced primarily by infeed solids concentration (higher solids leads to larger particle size) than by OSAn substitution or carbohydrate carrier. The density of the menthol-loaded powders was not affected by OSAn substitution or type of carbohydrate carrier either.

3.6 Acknowledgements

The authors thank Corn Products Inc. and TIC Gums Inc. for their generous product donations. Also, a great deal of gratitude is extended to Joshua Morlock for his time and effort in the laboratory; his hard work made this research a reality.

CHAPTER 4

Encapsulation and release of menthol. Part 2: Direct monitoring of l-menthol release from spray dried powders made with un-modified and OSAn-substituted dextrin and gum acacia carrier materials.

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4.1 Abstract

The objective of this research was to use dynamic “real time” analysis to determine how carrier material affects menthol release characteristics from various spray dried powders. Dynamic menthol release profiles were obtained from spray dried powders as a function of time (0 to 60 minutes) and 90% relative humidity by interfacing a dynamic vapor sorption device to a proton transfer reaction mass spectrometer. The menthol release profile varied greatly with carrier material. Increased octenyl succinic anhydride (OSAn) substitution (up to 3% treatment level) of the carrier material resulted in a significantly greater, but not earlier burst of menthol release. For most samples, the characteristic burst of menthol was followed by a decrease in release which was attributed to an observed matrix collapse. Moisture uptake rates of the powders were related to corresponding menthol release rates. As OSAn substitution increased for gum acacia (*Acacia seyal*), a trend of increased moisture uptake rate with menthol release rate was evident. However, for the dextrans, the moisture uptake rate had no influence on the rate of menthol release. Also, static headspace was used to determine how increased OSAn substitution affects menthol release from aqueous mixtures of dissolved carrier material and menthol. The increased hydrophobicity of the OSAn groups did not affect the volatility of menthol and therefore, interactions between OSAn groups and menthol should not affect dynamic menthol release.

Keywords: octenyl succinic anhydride, dextrin, gum acacia, menthol, spray drying, DVS, purge and trap, PTR-MS

4.2 Introduction

Historically, measurement of aroma release from dry matrices was accomplished by storing samples over saturated salts of various percent relative humidity (%RH) values and measuring the resulting release or retention of aroma compounds.[54] Measurement of volatile release from dry matrices was typically done by a number of analytical techniques. Headspace gas chromatography (HS-GC) is commonly used but solvent extraction techniques are also utilized.[53] These traditional methods for measuring release are extremely time- and labor-intensive.[51] Recently, researchers have been developing rapid, dynamic methodologies for measurement of volatile release. Blake et al.[50] published a method using dynamic vapor sorption atmospheric pressure chemical ionization mass spectrometry (DVS-APCI-MS) to study flavor release. The dynamic vapor sorption (DVS) instrument is normally used as an efficient means to create moisture sorption isotherms by tracking the change in mass of a sample at a given temperature and over a specified range of controlled RH vapor streams. APCI-MS is a widely used technique to analyze food and flavor volatiles in real time.[55] The premise of the DVS-APCI-MS method is to expose encapsulated flavors to humidified gas (via DVS) and analyze the effluent containing released flavor in real time (via APCI-MS). Bohn et al.[54] developed a technique that is nearly real time by using DVS, adsorbent traps, and fast-GC-flame ionization detector (FID). This method also utilizes the DVS for a dynamic sampling process, but continuous, real time monitoring of the sampled effluent was not possible because the adsorbent traps had to be individually analyzed by GC (sampling and analysis time ~ 20 minutes). Dronen and Reineccius[51] proposed a

methodology using an instrument similar to the DVS called a vapor generator instrument (VGI) coupled with APCI-MS.

The current research evaluates the release of menthol from several types of spray dried powders. Two rapid methodologies (DVS-purge and trap (P&T-GC) and DVS-proton transfer reaction mass spectrometry (PTR-MS)) were used to determine menthol release profiles when exposed to air streams at 60% and 90% RH. The method utilizing P&T-GC method was primarily used as a screening tool to determine whether it was worth the time and resources to run the same samples with the DVS-PTR-MS.

It was hypothesized that carrier materials that show an increased moisture uptake rate will show an increased menthol release rate. Variations in moisture uptake were expected to occur by using spray drying carriers containing different levels of octenyl succinic anhydride (OSAn) substitution, and different gum acacias. A second hypothesis was that menthol would interact differently with the selected carrier materials and potentially influence release characteristics. This interaction would have an effect by altering the volatility of the menthol thereby, its release rate. We proposed that as the carrier material increased in OSAn-modification, menthol interaction will also increase and result in lower release rate.

4.3 Materials and Methods

4.3.1 Chemicals

Synthetic menthol (l-menthol pellet USP: Takasago International, Rockleigh, NJ, USA) was used as volatile for study. Capillary GC grade pentane (Cat. #: 4144727-4L, Sigma Aldrich Inc., St. Louis, MO, USA) was used for surface washing of the spray dried powders.

4.3.2 Carrier Materials

Ten carrier materials were utilized to produce spray dried menthol powder for this research; five were starch based and five were gum acacia based (Table 3.1 (page 35) lists the carrier materials and abbreviation codes used to describe them). The starch based products are various preparations of dextrinized starch. CAPSULTM (CAP) (National Starch Inc., Bridgewater, NJ, USA) is a commercially available dextrinized waxy maize starch octenyl succinate. The other four products were: an unmodified dextrin (D) (Amidex 182), and three custom dextrin products that were prepared by treating corn starch with one, two, and three percent OSAn (D1, D2, and D3, respectively). The latter four products (D, D1, D2, and D3) were provided by Corn Products Brasil (São Paulo, Brasil).

The gum acacia based products are derived from two species of acacia. Gum Arabic Spray Dry FCC Powder (GA) (TIC Gums, Inc., Belcamp, MD, USA) is a product obtained from the *Acacia senegal* species and is considered good emulsifier.[78] Gum Arabic FT Powder (FT) (TIC Gums Inc., Belcamp, MD, USA) is obtained from the *Acacia seyal* species and is considered a poor emulsifier.[78] TICAmulsion A-2010 (TA) (TIC Gums Inc., Belcamp, MD, USA) is an OSAn modified gum made from *A. seyal*.[78] Actual OSAn treatment levels are not public information, but the treatment level is suspected to be no more than 3% as described by the United States of America Code of Federal Regulations for modified starch.[25]

FT and TA were dry blended to create two additional *A. seyal* products with different levels of total (OSAn) substitution. FT and TA were blended in ratios (w/w): of 67:33 and 33:67 to create two intermediate levels of OSAn substitution. The blends were

produced by placing the desired amount of each material in a Hobart mixer (Model #: A-200, Hobart Mfg. Co., Troy, Ohio, USA) and then blending at the lowest speed for 15 minutes with the paddle attachment.

4.3.3 Spray Drying

The selected carrier blends were initially dissolved to solids contents low enough to allow adequate atomization (30% for the gum acacia products and the unmodified dextrin and 40% for all other dextrin products). Each solution was heated to 70°C under constant mixing to insure complete dissolution and then allowed to cool in the refrigerator overnight before use. Immediately before spray drying, each carrier solution was heated to 50°C and menthol was added at 40% of the total carrier solids (i.e. 40:60, menthol mass:carrier mass) as detailed in Table 3.1 (page 35). Each carrier solution containing molten menthol was homogenized with a bench-top rotor stator high sheer mixer (Model #: 1L.81 Greerco Corp., Hudson, NH, USA) for 5 minutes at 7500 RPM. During spray-drying, the menthol/carrier emulsion was held at 50°C and continuously mixed with the high sheer mixer. Spray drying was done in the pilot plant of the department of Food Science and Nutrition at the University of Minnesota with a Niro Utility dryer (Niro, Inc., Columbia, MD, USA). This dryer uses a centrifugal wheel atomizer (24.5 cm diameter rotary vane wheel rotating at 22,400 rpm), is gas heated, has a mass air flow of about 360 kg/h; drying chamber of 1.2 m diameter, 0.75 m cylindrical height, cone angle of 60°; cyclone collector 0.3 m diameter; and powder collection container of 20 l capacity. Operating at an inlet air temperature of 200°C and an exit air temperature of 100°C resulted in a feed rate of ~330 ml/min (varied in order to maintain

outlet temperature). After drying, the powders were cooled to ambient temperature and stored in poly bags at room temperature until analysis.

4.3.4 Measurement of Dynamic Menthol Release by DVS-P&T-GC With Tenax Traps

4.3.4.1 Method Overview

This method utilized DVS (Model #: DVS-1, Surface Measurement Systems, Ltd., Alperton, Middlesex, UK) as a vapor generator and mass recording apparatus. Spray dried menthol powder was placed in the DVS and purged with humidified air. The DVS effluent gas was conveyed to a Tenax trap via a copper transfer line (3.175 mm O.D.) maintained at 70°C. The Tenax trap was attached to a pump (SKC Pocket Pump #210, SKC Inc., Eighty Four, PA, USA). The loaded Tenax traps were desorbed in the inlet of a Hewlett Packard model 6890 GC. Figure 4.1 is a simplified depiction of the system. This method was primarily used as a screening tool to determine whether it was desirable to perform further analysis by DVS-PTR-MS. Method detail follows.

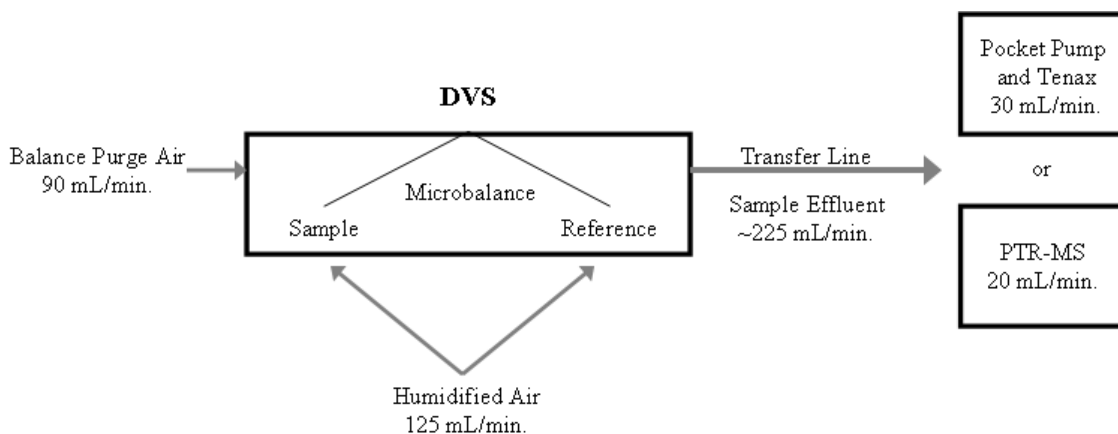


Figure 4.1 – Schematic of DVS coupled to either P&T or PTR-MS. Air inputs into the DVS are indicated as arrows towards the DVS box and output as the transfer line.

4.3.4.2 Adsorbent Traps

Tenax traps were constructed using GC injection port splitless liners (Restek catalog #: 20773, Restek Corporation, Bellefonte, PA, USA) containing 100 mg Tenax TA 60/80 mesh (Restek catalog #: 25550) and stainless steel screens (Supelco catalog #: 28277-U, Supelco, Bellefonte, PA, USA) to hold the Tenax in place; the lower screen was positioned 1 cm from the bottom of the liner. The traps were conditioned at 300°C for 3 hours under a helium flow rate of greater than 60 ml/min prior to use.

4.3.4.3 Sample Pretreatment

Prior to loading the spray dried powder into the DVS, all powders received two pre-treatments: 1) moisture equilibration and 2) a solvent wash to remove surface menthol. For moisture equilibration, each sample was placed in a Pyrex Petri dish and placed in a desiccator containing a saturated solution of lithium chloride at 23°C (approximately 0.11 a_w) for at least 4 weeks prior to analysis. This step was done to ensure that all powders were equilibrated to the same relative humidity prior to analysis.

For surface washing, approximately 0.3-0.7 g of equilibrated powder was weighed into a 20-mL headspace vial; pentane (10 mL) was added to the vial and then capped. The capped vial was placed on the orbital shaker for 15 minutes at 200 rpm. After shaking, the powder was vacuum filtered using a water faucet aspirator and Büchner funnel fitted with filter paper (Catalog #: 1001070, Whatman International Ltd., Maidstone, England). This moisture equilibrated and washed powder was used throughout the menthol release studies.

4.3.4.4 Sampling Parameters

Samples of spray dried powder (~17 mg) were weighed and spread onto a 16mm flat-bottomed quartz pan (Surface Measurement Systems catalog #: QP16VID) and hung onto the balance hook in the sample chamber of the DVS. Extreme care was taken to make sure the sample was evenly distributed over the surface of the pan. The DVS was programmed to purge air over the sample and reference chambers at a combined total flow rate of 125 mL/min. (62.5 mL/min. over the sample and reference pans) at a constant relative humidity (RH) of either 60 or 90%. In addition to the sample flow, a constant flow of air is purged across the DVS balance at a rate of approximately 90 mL/min.; actual measured flow out of the transfer line ranged between 218-223 mL/min. Samples were exposed to this environment for one hour. During purging, the SKC pump drew a portion of the DVS effluent gas through Tenax trap at flow at a rate of 30 mL/min. Traps were changed every 10 minutes for one hour (300 mL of gas sampled).

4.3.4.5 Menthol Release Analysis

To quantify the menthol released from the powders, the Tenax traps were desorbed in the inlet of a Hewlett Packard model 6890 GC-FID equipped with a Flip Top Inlet Sealing System (Agilent part #: 5188-2717). A 0.5 μ L aliquot of pentane (containing 0.006 g/mL benzyl alcohol as a standard) was injected onto the end of the trap (closest to the column) immediately before inserting the trap into the GC inlet. The GC was equipped with a J&W DB-5 column (30 m, 0.25 mm I.D., 0.5 μ m film) (Model #: 122-503E, Agilent Technologies, Inc., Santa Clara, CA, USA) operating at a head

pressure of 15 psi with a split ratio of 25:1. The GC temperature parameters were as follows: inlet and detector temperature were 250°C and the oven temperature program started at 70°C with no hold time and ramped to 200°C at a rate of 25°C/min. with no hold time then the oven ramped to 250°C at a rate of 100°C/min. to 250°C with a hold time of 2 min. A standard curve of menthol was used during quantification. Triplicate samples were run and average menthol content for each powder type and sampling period were calculated. Results were reported as mg of menthol per mg of spray dried powder.

4.3.5 Measurement of Dynamic Menthol Release by DVS-PTR-MS

4.3.5.1 Method Overview

The previously described P&T-GC method is limited to ca. a 10 minute sampling interval (sensitivity) whereas the DVS-PTR-MS methodology records menthol release data in real time. For this method, sample preparation and DVS operation were as described for the P&T-GC method with the following two exceptions; 1) a portion of the DVS effluent was introduced into the PTR-MS instead of a Tenax trap, and 2) the DVS program also included a one hour 11% RH equilibration step prior to the one hour RH treatment step. This additional step established a baseline mass measurement for the powder at 11% RH and insured the removal of any traces of menthol that might remain on the surface of the powder after the solvent wash.

4.3.5.2 PTR-MS Operating Parameters

The PTR-MS sampled the DVS effluent flow at a rate of 20 mL/min. Three ions masses were measured during sampling: 21 (H_3O^{18+}), 37 ($\text{H}_3\text{O}^+\cdot\text{H}_2\text{O}$), and 139 (menthol + H – water: $156 + 1 - 18 = 139$) with a dwell time of 10 sec. on each ion. These dwell

times resulted in a data sampling interval of 30 sec. PTR-MS ion counts were converted into menthol concentrations in parts per billion (ppb)[87] which were in turn converted into mg of menthol released per mg of powder loaded into the DVS.

4.3.6 Menthol/Carrier Interactions by Automated Headspace Sampling

A saturated solution of l-menthol was prepared by placing approximately 10g of menthol in a flask containing 1500g of de-ionized water. This flask was stirred on a magnetic stir plate at 200 rpm for 3 days at room temperature. The saturated menthol solution was compared to a standard curve quantified by GC-FID; the saturated solution contained 435 parts per million (ppm) menthol. Menthol is reported to be soluble in water at 431 ppm [88] and 464 ppm.[89]

Individual solutions of each carrier material (1600 ppm carrier) were also prepared in de-ionized water. The carriers were heated to 70°C and agitated to insure proper hydration. Each carrier solution was mixed with the saturated menthol solution (5 mL of each) in 20-mL headspace vials such that the final concentrations of carrier and menthol in 10 mL water were 800 and approximately 200 ppm, respectively; de-ionized water was used in place of the carrier solution as a reference sample. Triplicate samples of each carrier and eleven reference samples were prepared. The carrier solution vials were randomly loaded into the headspace autosampler tray and the reference vials spaced every four runs to ensure instrument performance and to verify consistent detector response.

An Agilent model 7694 automated headspace sampler coupled to a Hewlett Packard model 5890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA,

USA) (FID detection) was used to monitor menthol in the sample headspace. Operation parameters for the headspace sampler were as follows: pressure: carrier pressure: 1 PSI (~2 mL / min flow through transfer line) and vial pressure: 4 PSI; zone temperatures: oven: 40°C, loop: 65°C, and transfer line: 75°C; event times: GC cycle time: 35 minutes, vial equilibration time: 45 minutes, pressurization time: 0.5 minutes, loop fill time: 0.17 minutes, loop equilibration time: 0.25 minutes, and injection time: 3.5 minutes; and “high” shake intensity. The GC was equipped with a Hewlett Packard HP-5MS column (30 m, 0.25 mm I.D., 0.25 µm film thickness) (Model #: 19091S-433, Agilent Technologies, Inc., Santa Clara, CA, USA) running in splitless mode (purge valve open at 5 minutes) operating at a head pressure of 15 psi with split vent flow of 11 mL/minute. The GC temperature parameters were as follows: inlet and detector temperature were 250°C and the oven temperature program started at 35°C with 4 minute hold time and ramped to 200°C at a rate of 17°C /minute and held at 200°C for 3 minutes.

Headspace menthol peak area is reported as the average of triplicate sampling for each sample type and compared to the average (11 replicates) reference peak area.

4.3.7 Statistical Analysis

We used analysis of variance (ANOVA) to determine whether the finished powders differed for any of the aforementioned analyses. Differences among mean values were compared using one-factor ANOVA and the Fisher Least Significant Difference (LSD) method ($\alpha = 0.05$). Design Expert 6.011 (Stat Ease, Inc., Minneapolis, MN, USA) was the software package used for statistical computing.

4.4 Results and Discussion

4.4.1 Comparison of Menthol Release Profiles Obtained by DVS-P&T-GC and DVS-PTR-MS

DVS-P&T-GC provided total menthol release for each 10 minute period over the 60 minutes of sampling. In order to compare the two methods, data from DVS-PTR-MS were integrated over the corresponding ten minute intervals. For example, Figure 4.2 illustrates the menthol release profiles for sample B1 exposed to an air stream at 90% RH as quantified by both methods. The plots show similar release profiles, but the PTR-MS method indicated more menthol released than the P&T-GC method; this was the case for all samples tested. Menthol breakthrough from the Tenax traps was eliminated as a source of error in experiments conducted during methods development. However, lower quantities of menthol measured from the P&T-GC method may be explained by a number of other reasons. First, menthol loss may have occurred during the dry purge step prior to Tenax desorption. Second, upon introduction of the Tenax liner into the hot inlet, menthol will desorb and may become entrained in the vaporized pentane escaping from the un-sealed Flip Top. Third, there may be an unequal fractionation and loss of menthol during the split due to expansion of the pentane in the liner.

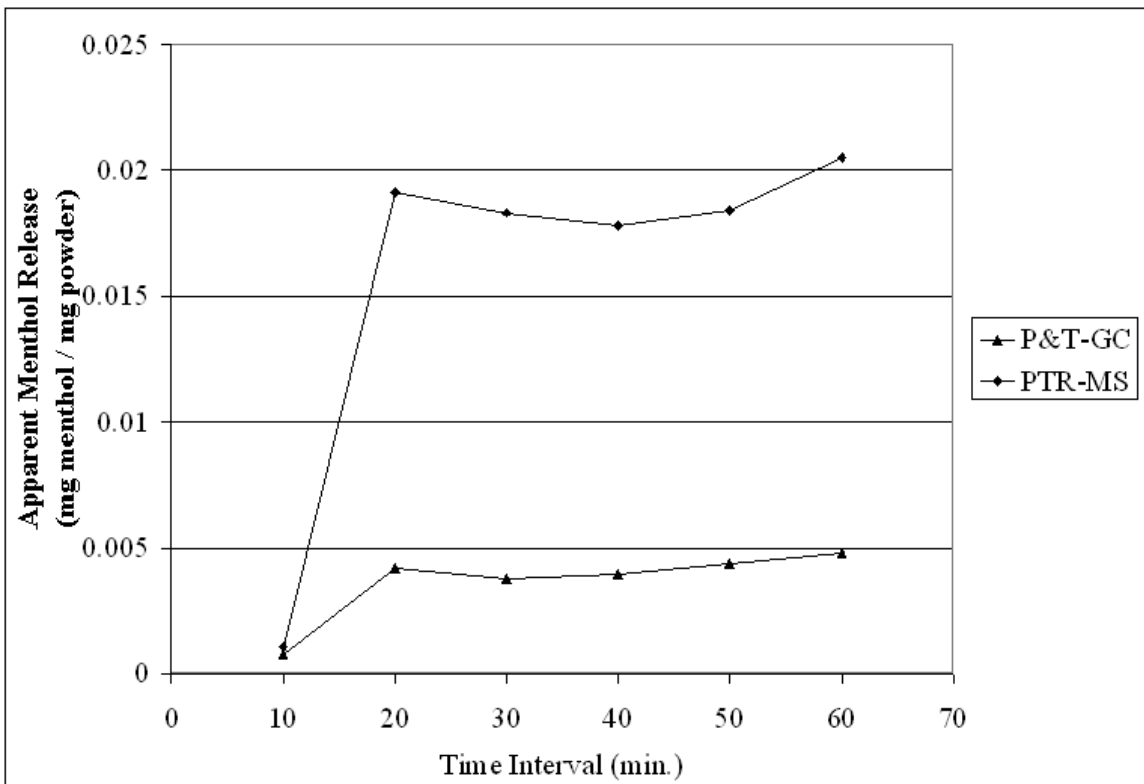


Figure 4.2 – Comparison of mean integrated menthol release from B1 when exposed to a 90% relative humidity air stream quantified DVS-P&T-GC or DVS-PTR-MS.

Regardless of the quantitative differences, the P&T-GC method was a valuable screening tool to decide what samples were to be analyzed using DVS-PTR-MS. All samples were run at 90% RH using DVS-P&T-GC. Considerable menthol release was measured using DVS-P&T-GC method and therefore, we found it desirable to evaluate all samples using the DVS-PTR-MS method. At 60% RH, DVS-P&T-GC data showed no significant menthol release from any of the samples and further experiments on the DVS-PTR-MS were considered a poor use of time and resources.

4.4.2 Effects of Carrier Material on Menthol Release Profile

Menthol release profiles of spray dried powders made using dextrans of differing levels of OSA_n modification (Figure 4.3) or gum acacias (Figure 4.4) are presented. As is evident in these figures, the temporal release profile of menthol varied greatly with flavor carrier. The differences in release profiles among samples are most evident when considering the DVS-PTR-MS data. The DVS-P&T-GC method (Figure 4.2) did not provide as much resolution as the DVS-PTR-MS method (Figures 4.3 and 4.4) thus supporting the use of the real time measurement for such studies.

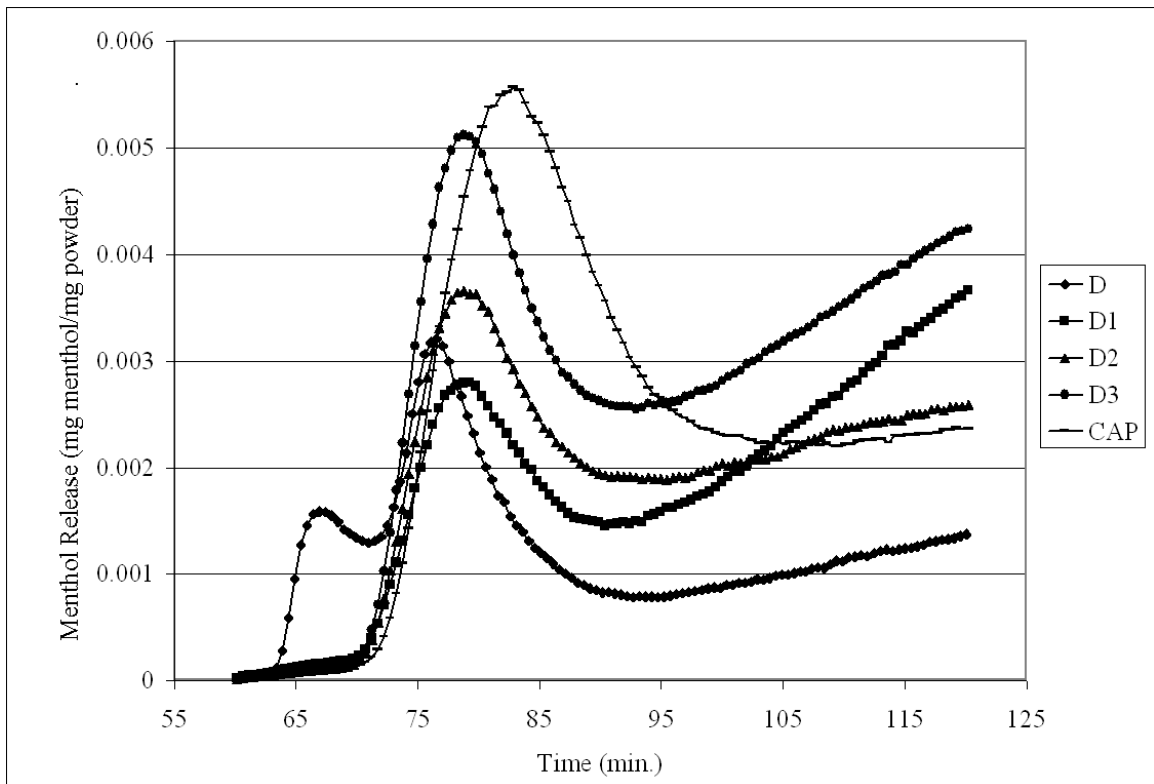


Figure 4.3 – Menthol release profiles obtained by DVS-PTR-MS of dextrin based, spray dried powders exposed to a 90% humidity air stream. D = unmodified dextrin; D1 = 1% OSA_n treatment; D2 = 2% OSA_n; D3 3% OSA_n; and CAP is commercially prepared CAPSULTM.

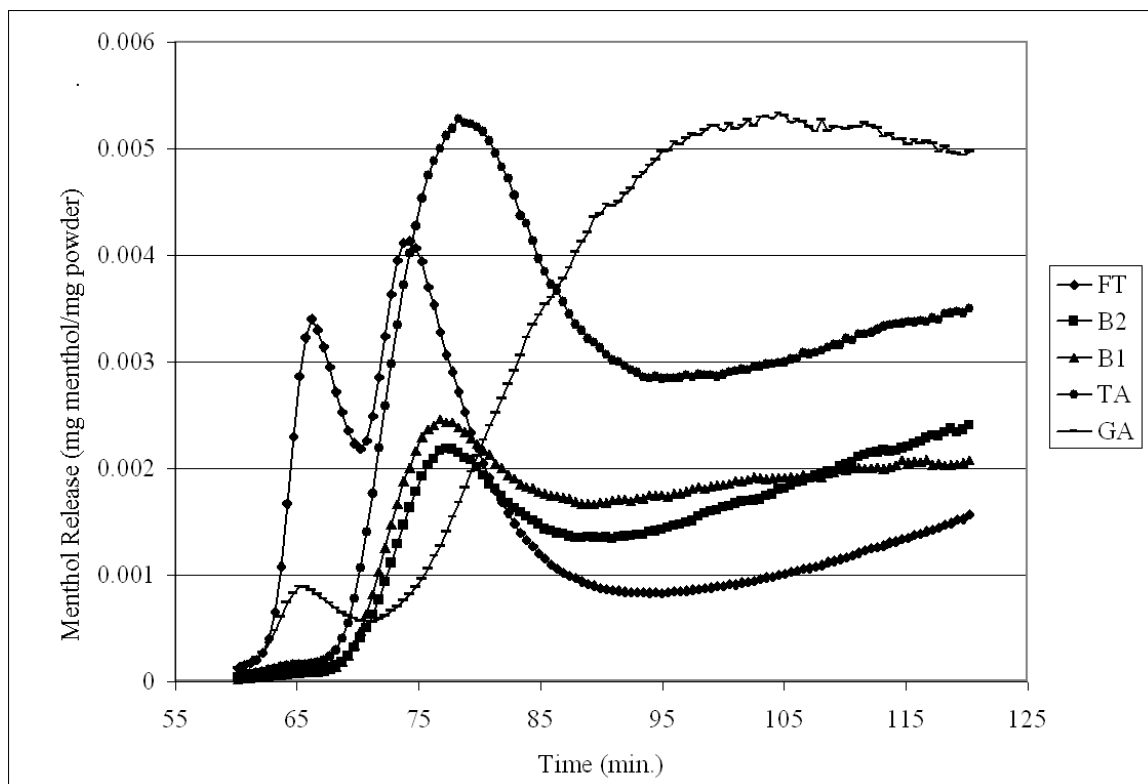


Figure 4.4 – Menthol release profiles obtained by DVS-PTR-MS of gum acacia based, spray dried powders exposed to a 90% humidity air stream. FT = *Acacia seyal*; B2 = 0.67 FT: 0.33 TA; B1 = 0.33 FT: 0.67 TA; TA = TICAmulsion; and GA is *Acacia senegal*.

Effects of OSAn substitution are evident in both series of carrier materials (Table 4.1). As OSAn substitution increased (i.e., $D1 < D2 < D3$ and $B2 < B1 < TA$), the maximum quantity of menthol released at the apex of the release curve also increased. Interestingly, the time taken to reach the apex for D1, D2, and D3 and for B2 and B1 were not significantly different from each other. The higher OSAn substitution resulted in a greater but not earlier burst of menthol release. As expected, CAP had a similar shape to D1, D2, and D3 but with a significantly longer time to the apex and higher maximum menthol released; these differences are likely due to differences in the initial starch, its modification and processing techniques proprietary to each of the starch manufacturing companies. GA exhibited a completely unique release profile compared to the other

samples; the time to reach peak menthol release was much longer than the others but it also had the highest level of total integrated menthol release (Table 4.1).

Table 4.1 – Menthol release events from spray dried powder prepared from various carrier materials.

| | D | D1 | D2 | D3 | CAP | FT | B2 | B1 | TA | GA |
|---|---------------------|----------------------|----------------------|---------------------|---------------------|-----------------------|----------------------|----------------------|----------------------|----------------------|
| Time to Primary Peak (min.) | 16.3 ^a | 19.0 ^b | 18.4 ^b | 19.0 ^b | 22.5 ^c | 14.4 ^d | 17.4 ^e | 17.5 ^e | 18.7 ^b | 43.8 ^f |
| Max Release at Primary Peak (mg / mg) | 0.0034 ^a | 0.0028 ^{ab} | 0.0037 ^{ac} | 0.0047 ^d | 0.0056 ^e | 0.0041 ^{acd} | 0.0022 ^b | 0.0024 ^b | 0.0053 ^{de} | 0.0054 ^{de} |
| Time to Pre-Primary Peak (min.) | 6.7 | - | - | - | - | 6.2 | - | - | - | - |
| Max Release at Pre-Primary Peak (mg / mg) | 0.0017 | - | - | - | - | 0.0034 | - | - | - | - |
| Total Integrated Release (mg / mg) | 0.0818 ^a | 0.1124 ^b | 0.1163 ^b | 0.1657 ^c | 0.1465 ^c | 0.1066 ^{ab} | 0.0912 ^{ab} | 0.0967 ^{ab} | 0.1798 ^c | 0.2139 ^d |

¹ The pre-primary peak is the first menthol release peak in series of two for carrier materials that showed a bi-modal menthol release profile.

² Time to peak quantifies the time taken to reach the apex of the menthol release peak and menthol release is reported as mg of menthol per mg of powder.

³ Letters across each row indicate Fisher LSD similarities among the carrier materials for each event.

Previous research showed that OSA_n modification of the carrier material affects total and surface menthol content of the spray dried products.[90] The differences in menthol concentration due to OSA_n modification may have some effect on, but do not appear to be solely responsible for the differences in menthol release characteristics. For example, D2 and D3 were found to contain statistically similar amounts of total and surface menthol.[90] However, the maximum menthol release peak and total menthol released are statistically different for these two products (Table 4.1).

Also, from Figures 4.3 and 4.4 and Table 4.1 it is clear that the poor emulsifying carriers (D and FT) have very different release profiles than the “emulsifying” carriers. These two products exhibit a bimodal menthol release curve. The bimodal curve was evident when using the DVS-PTR-MS method, it was not apparent with the DVS-P&T-GC method (data not shown). For clarification, if a release curve is bimodal, the first peak is referred to as the “preliminary” peak and the second peak is referred to as the “primary” peak; for a unimodal curve, the release peak is referred to as the “primary” peak. The preliminary release peaks had less menthol release than the primary peaks. Initially, we attributed these peaks to residual surface menthol despite the pentane washing of the powder. However, the 11% RH equilibration step in the DVS program prior to the 90% RH step was included to eliminate any surface menthol. Furthermore, Blake et al.[50] presented a plot with a similar preliminary release peak for a strawberry Durarome[®] (an extruded, amorphous glassy, sucrose-based, strawberry flavor) exposed to a 90% RH gas stream. The preliminary peak observed in Blake et al.[50] is not due to surface flavors because the Durarome[®] manufacturing process eliminates surface volatiles.[54] The reason for a preliminary peak is unknown, but we believe that it is related to the lack of intrinsic emulsifying ability of the encapsulation matrix.

4.4.3 Relationship Between Moisture Uptake and Menthol Release

It is accepted that phase transitions of carriers play an important role in the formation of and the subsequent release of flavor from microcapsules.[32, 43] Recent research attempted to relate real time volatile release from encapsulated flavor powders to the glass transition (T_g) of the powder.[50-52] The researchers found that T_g

(determined by differential scanning calorimetry (DSC)) was not actually the point of flavor release. Instead, the majority of flavor release occurred when enough moisture was absorbed to reduce T_g well below ambient temperatures[50] or at $T-T_g$ values greater than 30°C .[52] Bohn et al.[52] presented a stimulating discussion about linking T_g (determined by DSC) to real time measurement of flavor release. They proposed that when humidifying a powder, water vapor adsorbs to the powder surface and therefore creates a moisture gradient to the particle center which results in a corresponding T_g gradient. As T_g decreases, the powder becomes increasingly rubbery. It is understood that rubbery matrixes have lower viscosity, higher molecular mobility, and higher free volume than the corresponding glasses and therefore, result in higher aroma release rates.[45] Consequently, at a given point in time, the T_g gradient will result in flavor release at different rates throughout the powder.

For the aforementioned studies that addressed real time volatile release, T_g was determined by DSC. The DSC provides a mean sample T_g at an equilibrium a_w , not just the T_g of the portion of the powder associated with flavor release. Therefore, relationships between dynamic flavor release phenomena and static T_g measurements are not necessarily observed.

Instead of pursuing the relationship between static T_g and dynamic menthol release, a different approach was taken to link the effects of moisture on real time menthol release from spray dried powders. Moisture uptake rates of the spray dried powders during hydration were obtained from the DVS outputs and related to the rates of menthol release. The theory of selective diffusion implies that diffusion coefficients of volatile compounds increase with increased water concentration;[36-38] from this, it is

logical that moisture uptake rate will correlate well with real time release. Figure 4.5 depicts menthol release from and moisture uptake of spray dried TA. The linear portions of the menthol release curve immediately before the release apex were defined by linear models. These models were optimized by maximizing the R^2 value by adjusting the number of data points and position of the model on the curve ($R^2 > 0.98$). Linear models were also fit to the moisture uptake curve over the same time period ($R^2 > 0.98$).

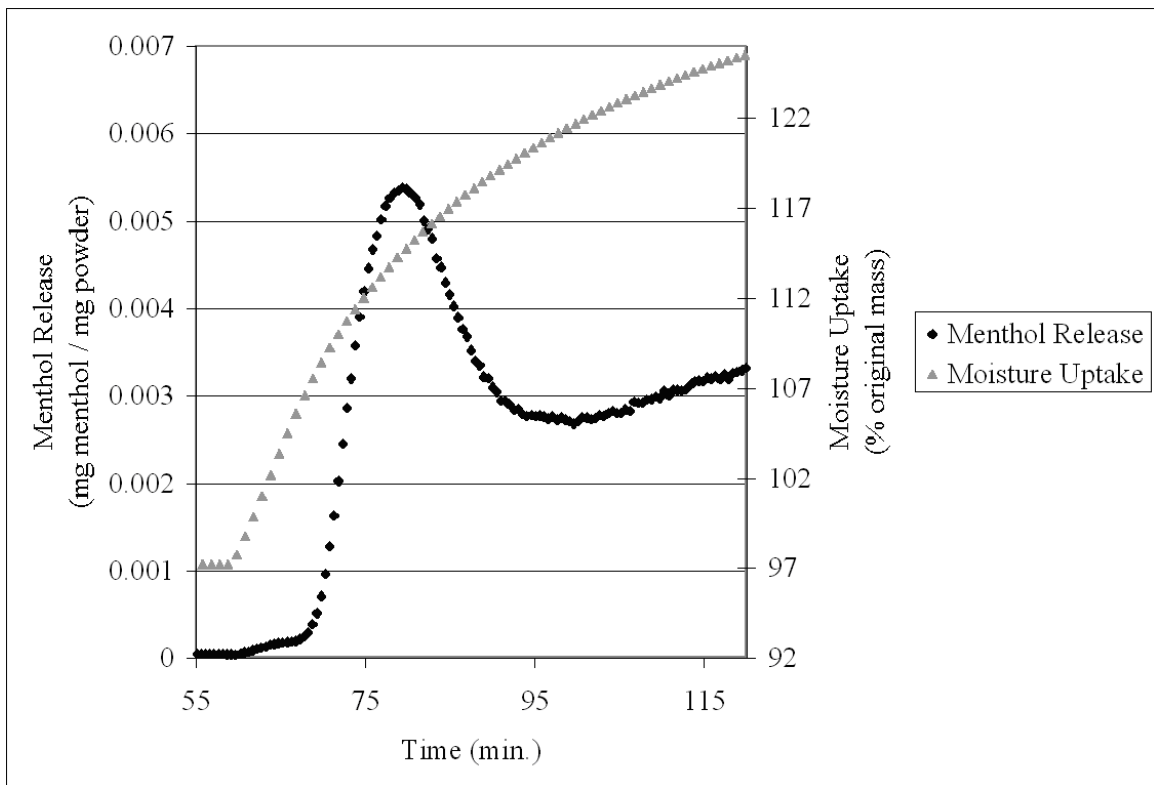


Figure 4.5 – Relationship between menthol release and moisture uptake profiles for spray dried TA powder.

Menthol release rate was plotted against water uptake rate for corresponding carrier materials; interesting trends emerged (Figure 4.6). While there appears to be a slight relationship between moisture uptake and rate of release irrespective of the carrier type, the carrier type clearly had an influence on menthol release rate. In Figure 4.6,

attention to the OSAn treated carriers is drawn by lines connecting B2, B1, and TA and D1, D2, and D3. As OSAn substitution increased in the acacia series, a trend of increased moisture uptake rate with menthol release rate was evident. However, for the dextrans, the moisture uptake rate had no influence on the rate of menthol release. Instead, a greater menthol release rate was observed for the highest OSAn substitution. This result is not surprising as OSAn-substitution may affect T_g and possibly the release of flavor from powders. Sagar et al.²³ and Aburto et al.[91, 92] observed that fatty-acid esters acted as an internal plasticizer (and lower T_g) when substituted onto starch molecules. Therefore, an OSAn substituted starch or gum acacia product is expected to have a greater release of menthol when compared to a product with no substitution. Another possible reason for greater menthol release with increased OSAn substitution is related to increased steric effects of the OSAn. Bulky OSAn groups may decrease intracapsule carbohydrate interactions (i.e. hydrogen bonding[34, 41]) which would increase porosity and ultimately result in greater menthol release rates.[93]

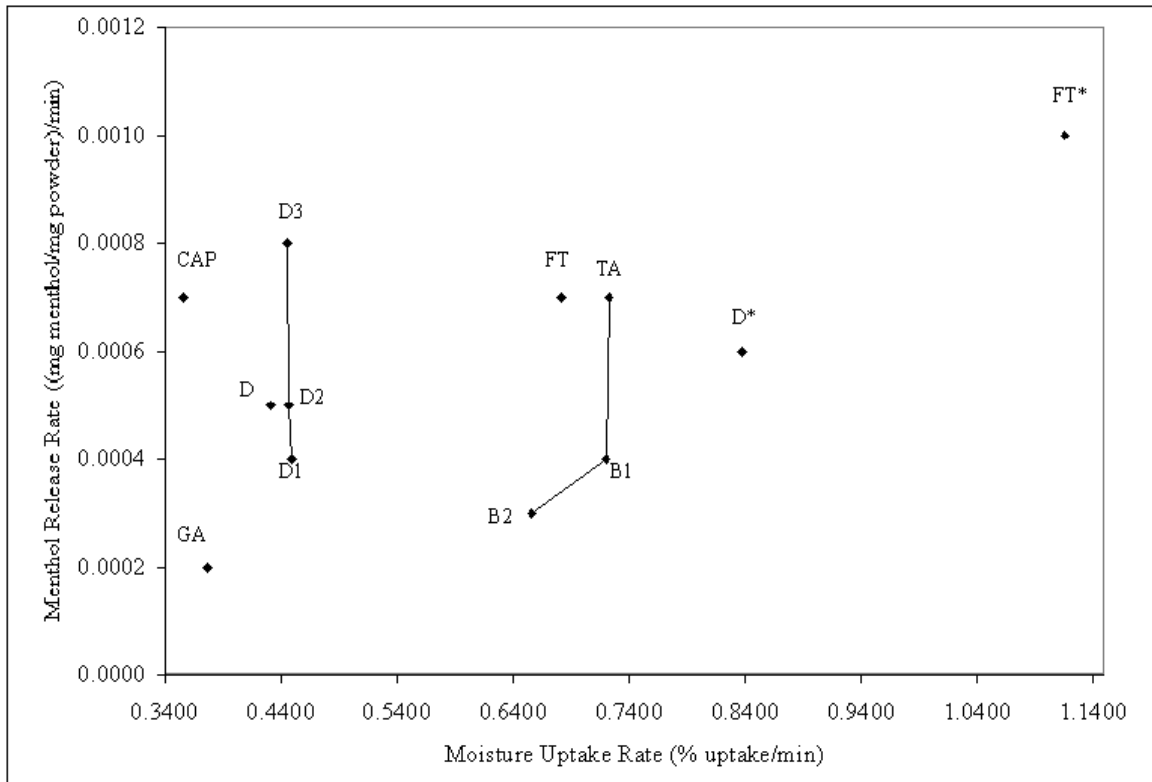


Figure 4.6 - Relationship between the rate of menthol release prior to release maxima, and the rate of moisture uptake in spray dried menthol for different carrier materials. Note: carrier materials labeled with an asterisk (*) correspond to the rates of menthol release and moisture uptake immediately preceding the “preliminary” release peak for those carrier materials exhibiting a bi-modal menthol release profile.

The appearance of each powder was noted every minute of the 60 minute 90% RH humidification process. All samples initially had a rough and dry appearance. During humidification, the appearance changed at nearly the same time (+/- 1 minute) of the menthol release apex for all carrier materials (except for GA which did not change). The rough appearance of the powders started to smooth and the surface appeared shiny or wet. Upon completion of the 60 minute humidification period, all powders (except GA) had a shiny, smooth, plastic appearance and the entire powder mass deliquesced. This change in appearance suggested that the matrix collapsed and explains the shape of the menthol release curve. In general, collapse occurs when plasticizer content decreases the matrix viscosity to a point where the polymer matrix cannot withstand the force of

gravity and shrinks into a glass-like material.[47] During humidification, volatile release increases until matrix collapse which then causes a decrease in volatile release.[45] This decrease in volatile release may be due to a “re-encapsulation” of volatiles and subsequent reduction of diffusion.[32, 43]

4.4.4 Menthol/Carrier Interactions

The purpose of this experiment was to investigate if the carrier materials (specifically the OSA_n substituted products) interacted with menthol in a manner that would reduce its volatility. It is well documented that aroma release may be decreased by interactions with carbohydrates.[94-103] This may be due to either changing the polarity of the aqueous continuous phase making it a better solvent for menthol or through chemical binding to the menthol. It is intuitive to expect a lower real time release of menthol from a spray dried powder if menthol is better solubilized or chemically bound to the carrier material. In other words, we could expect that adding hydrophobic OSA_n groups (LogP = ~4.45) [104] to dextrin or gum acacia will reduce the vapor pressure of the hydrophobic menthol (LogP = ~3.38)[104] and result in lower detectable menthol in the headspace. However, our data do not support this hypothesis. Figure 4.7 illustrates that headspace menthol peak area for the reference sample (i.e. in water, without carrier) is not significantly different from the samples containing carrier materials. Comparable results for a similar experiment were reported by Secouard et al.[100] These results implied that the considerably different real time menthol release profiles were not affected by association interactions between the carrier materials and menthol.

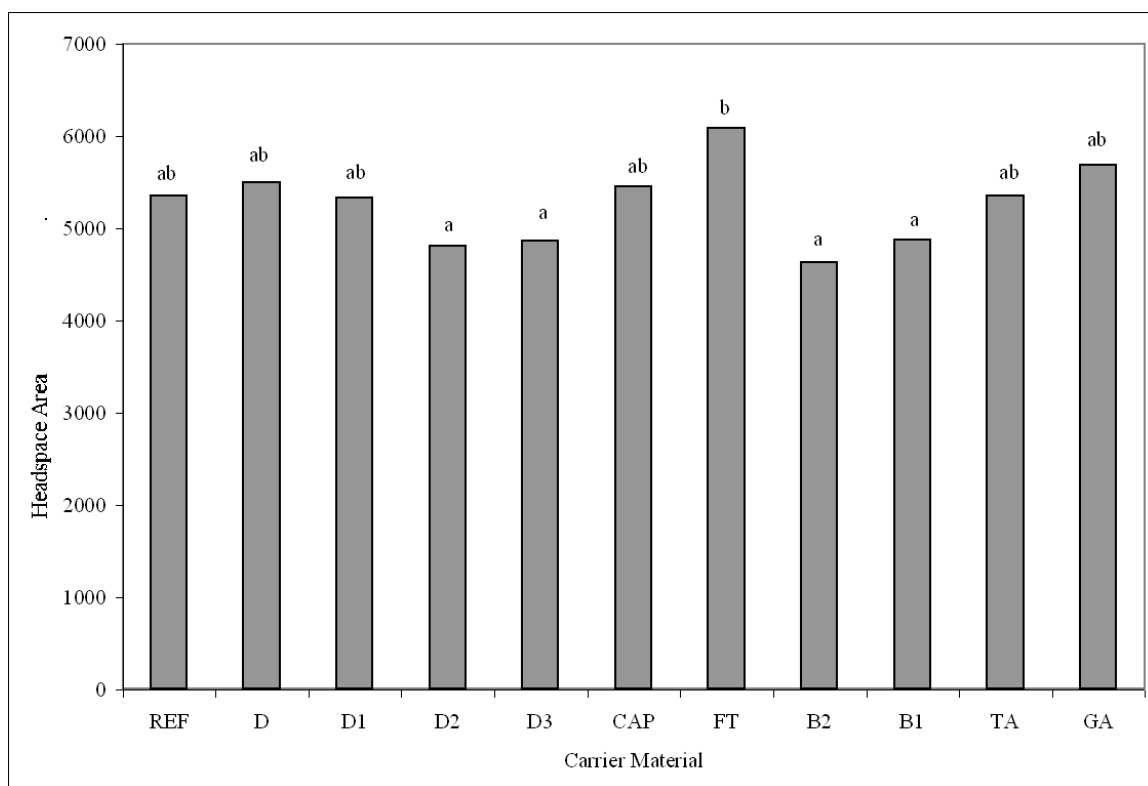


Figure 4.7 – Menthol content in headspace above aqueous solutions of carrier materials (800 ppm carrier material and 200 ppm menthol). REF contains no carrier material. Letters a to b indicate Fisher LSD similarities.

4.5 Conclusions

The current research utilized DVS- P&T-GC and DVS-PTR-MS to acquire information on the real time menthol release characteristics from spray-dried, unmodified or OSAn-substituted dextrin and gum acacia products. DVS- P&T-GC methodology proved to be a useful screening tool to select samples for the more precise DVS-PTR-MS analysis. DVS-PTR-MS was a very effective instrument to obtain menthol release profiles from encapsulated products. The resolution provided by the DVS-PTR-MS allowed for visualizing small differences among the menthol release curves due to carrier material. It was apparent that increased OSAn treatment increased the total menthol release with little or no change in primary release time. The results of

this research have merit in flavor application and could lead to producing custom blends of spray dried flavors that provide product specific temporal release profiles.

4.6 Acknowledgements

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CHAPTER 5

Moisture sorption isotherms for un-modified and OSAn-substituted dextrin and gum acacia used as carrier materials for spray dried encapsulation of flavoring materials.

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5.1 Abstract

Moisture sorption isotherms of ten un-modified and octenyl succinic anhydride substituted dextrin and gum acacia samples were determined gravimetrically via equilibration over saturated salt solutions (a_w ranged from 0.11 to 0.85) at 23 and 35°C. The degree of octenyl succinic anhydride substitution did not affect moisture sorption characteristics for dextrin samples. For gum acacia, increased levels of octenyl succinic anhydride substitution resulted in a decreased affinity for moisture in the range of 0.3 to 0.7 a_w . The sorption data were well described by the BET and GAB models. Monolayer values for all ten products at 23°C ranged from 0.0470 to 0.0759 and 0.0454 to 0.0859 g water/g solids for BET and GAB models, respectively; at 35°C, these ranges were 0.0407 to 0.0700 and 0.0400 to 0.0797 g water/g solids for BET and GAB models, respectively.

Keywords: octenyl succinic anhydride, dextrin, gum acacia, moisture sorption isotherms

5.2 Introduction

Moisture affects the properties of spray dried flavoring materials in a number of ways. Moisture affects the quality (i.e. caking, flowability, etc.) of finished flavor powders as well as the retention and release of flavor volatiles from these powders. For example, the theory of selective diffusion depends on decreasing moisture content to ensure the retention of flavors in hot air dried matrices.[33, 37, 38] Also, the micro region theory is used to explain effects of moisture on volatile retention and release at a microscopic level. This theory is based on volatiles being entrapped in micro regions of an amorphous structure comprised of carbohydrate-carbohydrate hydrogen-bonds that can be disrupted by moisture contents above the monomolecular layer value of the matrix.[34, 41] Moreover, water acts as a plasticizer to carbohydrates and can initiate phase transitions from a glassy to rubbery state, which can induce crystallization, and possibly collapse thereby causing various release phenomena of encapsulated flavors.[32]

It is therefore reasonable to assume that an understanding of moisture sorption properties of common carrier materials could potentially benefit the flavor manufacturer and end user alike. The carbohydrates primarily used as carrier materials for spray drying of flavoring materials are gum acacia, maltodextrin, and modified starch (typically modified with octenyl succinic anhydride (OSAn)).[3] The choice of which carrier material to use for flavor encapsulation is based on a number of functional strengths and weaknesses (e.g. protection of flavor during storage, encapsulation efficiency, etc.); a summary of these characteristics can be found in the noted references.[3, 4] A few researchers have published moisture sorption isotherms or equilibrium moisture content data to be interpreted as such for gum acacia [58, 105], maltodextrin [66, 67, 69, 106],

and OSA_n-modified starch [107]. However, to our knowledge, there are no published moisture sorption isotherm data for carrier materials of controlled OSA_n-modification.

The objective of this study was to generate moisture sorption isotherms for unmodified and OSA_n-substituted starch and gum acacia based carrier materials. This was accomplished by determining the static equilibrium moisture contents of each carrier material over a range of relative humidity (%RH) values (a_w ranged from 0.11 to 0.85). Additionally, the data was modeled with the Brunauer-Emmett-Teller (BET) and Gugenheim-Anderson-de Boer (GAB) equations.

5.3 Materials and Methods

5.3.1 Chemicals

Methyl alcohol (Optima grade, Fisher Scientific, Fair Lawn, NJ, USA), anhydrous sodium sulfate (Fisher Scientific, Fair Lawn, NJ, USA), and the chemicals comprising the pyridine free reagents kit (Photovolt Instruments Inc., Minneapolis, MN, USA) for the Karl Fischer titration system were used for Karl Fischer moisture analysis. Salts for the saturated salt solutions were also obtained from Fisher Scientific.

5.3.2 Carrier Materials

Ten carrier materials were utilized for this research; five were starch based and five were gum acacia based. Table 5.1 lists the carrier materials and abbreviation codes used to describe them. The starch based products are variations of dextrinized starch. CAPSULTM (CAP) (National Starch Inc., Bridgewater, NJ, USA) is a commercially available dextrinized waxy maize starch octenyl succinate. The other four products were:

an unmodified dextrin (D) (Amidex 182), and three custom dextrin products prepared by treating corn starch with one, two, and three percent OSA_n (D1, D2, and D3, respectively).

Table 5.1 – Carrier material abbreviation codes and identities.

| Code | Carrier Identity |
|-------------|--|
| D | Dextrin |
| D1 | 1% OSA Dextrin |
| D2 | 2% OSA Dextrin |
| D3 | 3% OSA Dextrin |
| CAP | Capsul |
| FT | <i>A. seyal</i> (Gum Arabic FT) |
| B2 | 0.67 <i>A. seyal</i> : 0.33 TICAmulsion |
| B1 | 0.33 <i>A. seyal</i> : 0.67 TICAmulsion |
| TA | TICAmulsion |
| GA | <i>A. senegal</i> (Gum Arabic Spray Dry) |

The gum acacia based products are derived from two species of acacia. Gum Arabic Spray Dry FCC Powder (GA) (TIC Gums, Inc., Belcamp, MD, USA) is a product harvested from the *Acacia senegal* species. Gum Arabic FT Powder (FT) (TIC Gums Inc., Belcamp, MD, USA) is harvested from the *Acacia seyal* species. TICAmulsion A-2010 (TA) (TIC Gums Inc., Belcamp, MD, USA) is a modified gum made by treating *A. seyal* with OSA_n.^[78] Actual OSA_n treatment levels are not published, but the treatment level is suspected to be no more than 3% as given by the United States of America Code of Federal Regulations for modified starch.^[25]

FT and TA were dry blended to create two additional *A. seyal* products with different levels of total (OSA_n) substitution. Ratios (w/w) of 67:33 and 33:67 of FT and TA were blended to create intermediate levels of OSA_n substitution. These blends were produced by placing the proper amount of each gum acacia product in a Hobart mixer

(Model #: A-200, Hobart Mfg. Co., Troy, Ohio, USA) and then blending at the lowest speed with the paddle attachment for 15 minutes.

5.3.3 Initial Moisture Content of the Carrier Materials

Initial moisture contents of each carrier material were determined with Karl Fischer methodology using the Aquatest CMA instrument (Photovolt Instruments Inc., Minneapolis, MN, USA). Between 0.2 and 0.9 g of each carrier material were weighed into 20-mL headspace vials. To insure an instrument response that was in operational range, greater masses were used for powders that had lower moisture contents (as determined by previous trials). Approximately 10 g of methyl alcohol dried over anhydrous sodium sulfate was carefully weighed into the vial that contained the carrier material and tightly capped. The capped vials were placed on an orbital shaker (Lab-Line Orbit Shaker #3590, Lab-Line Instruments, Inc., Melrose Park, IL, USA) set for 200 rpm and left to shake overnight at room temperature. Aliquots of these extracts were weighed and injected into the Aquatest CMA instrument. Moisture content of each carrier material were calculated using the output from the Aquatest CMA and the data recorded during extract preparation. Methyl alcohol blanks were also run to correct for residual moisture in the solvent and for moisture absorbed during normal extraction procedures. Initial moisture content (g water/g solids) results for each carrier are calculated as an average of triplicate samples.

5.3.4 Moisture Sorption Isotherms of Carrier Materials

Moisture sorption isotherms were prepared for all carrier materials according to Bell and Labuza.[60] Triplicate samples of approximately 0.3 g were weighed into 20 mL scintillation vials (Research Products International Corp., Mount Prospect, IL, USA) and placed in desiccators containing the following saturated salt solutions at 23°C and 35°C (a_w at 23°C, a_w at 35°C):[108] lithium chloride (0.113, 0.113); potassium acetate (0.225, 0.216); magnesium chloride (0.327, 0.321); potassium carbonate (0.432, 0.432); magnesium nitrate (0.533, 0.500); cobalt chloride (0.669, 0.586); sodium chloride (0.754, 0.752); potassium chloride (0.847, 0.830). After 21 days equilibrium time, samples were re-weighed. Data from the static desiccators and initial moisture content data were used to construct “working” isotherms.[60] Isotherms were plotted using the equilibrium moisture contents of the products. BET and GAB models were fit using a Microsoft Excel spreadsheet based on the Water Analyzer Series – Isotherm/BET/GAB Program.[109]

5.4. Results and Discussion

5.4.1 Moisture Sorption Isotherms

The equilibrium moisture content data for all carrier materials stored over the saturated salt solutions at 23°C and 35°C are shown in Tables 5.2 and 5.3, respectively. These data were plotted in Figures 5.1-5.4 to identify trends in the raw data; all four plots have the same scales to aid in visualizing differences among the results. In general, the gum acacia samples appeared to be more hygroscopic than the dextrin samples, particularly at water activity values greater than 0.7. The moisture sorption isotherms for

all of the dextrin-based carrier materials are similar in shape to each other; the same is true for the gum acacia-based carrier materials. For the dextrin samples, the variations in water adsorption affinity do not appear to be strongly aligned with OSA_n-treatment. Furthermore, there are several points where the isotherms from different products cross each other. These differences are suspected to be due to differences in the dextrinizing process which was a batch treatment for each product. On the other hand, differences in the gum acacia carrier materials were very clear and trends were obvious. Between the water activity range of 0.3 to 0.7, acacia blends with increased OSA_n result in a decreased affinity for moisture. This result is intuitive due to the hydrophobic nature of OSA_n. Because variation of OSA_n level for the acacia products was accomplished by blending two FT and TA, these incredibly clear trends are likely explained by forming two weight-averaged (mass balance) isotherms for B2 and B1 in between FT and TA. Nonetheless, it still appears that a decrease in OSA_n content will result in powder that is more hygroscopic.

Table 5.2 – Equilibrium moisture content for the dextrin and gum acacia carrier materials equilibrated at 23°C. Note: a_w values of saturated salt solutions are adjusted for temperature.

| Moisture Content (g water / g solids) | | | | | | | | | | |
|---------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| a_w | D | D1 | D2 | D3 | CAP | FT | B2 | B1 | TA | GA |
| 0.113 | 0.0364 | 0.0427 | 0.0374 | 0.0438 | 0.0564 | 0.0591 | 0.0542 | 0.0429 | 0.0444 | 0.0638 |
| 0.225 | 0.0570 | 0.0685 | 0.0574 | 0.0599 | 0.0753 | 0.0744 | 0.0721 | 0.0673 | 0.0641 | 0.0930 |
| 0.327 | 0.0743 | 0.0857 | 0.0764 | 0.0823 | 0.0833 | 0.0850 | 0.0852 | 0.0730 | 0.0645 | 0.1103 |
| 0.432 | 0.0862 | 0.1039 | 0.0925 | 0.0943 | 0.0921 | 0.1037 | 0.0961 | 0.0874 | 0.0819 | 0.1248 |
| 0.533 | 0.1033 | 0.1188 | 0.1106 | 0.1060 | 0.1100 | 0.1282 | 0.1167 | 0.1065 | 0.0988 | 0.1472 |
| 0.669 | 0.1262 | 0.1351 | 0.1284 | 0.1230 | 0.1286 | 0.1637 | 0.1538 | 0.1405 | 0.1339 | 0.1812 |
| 0.754 | 0.1456 | 0.1590 | 0.1530 | 0.1523 | 0.1543 | 0.2195 | 0.2145 | 0.2117 | 0.2085 | 0.2350 |
| 0.847 | 0.1913 | 0.2038 | 0.2012 | 0.1948 | 0.1901 | 0.3372 | 0.3249 | 0.3326 | 0.3370 | 0.3385 |

Table 5.3 – Equilibrium moisture content for the dextrin and gum acacia carrier materials equilibrated at 35°C.
 Note: a_w values of saturated salt solutions are adjusted for temperature.

| Moisture Content (g water / g solids) | | | | | | | | | | |
|---------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| a_w | D | D1 | D2 | D3 | CAP | FT | B2 | B1 | TA | GA |
| 0.113 | 0.0343 | 0.0438 | 0.0351 | 0.0432 | 0.0506 | 0.0513 | 0.0430 | 0.0403 | 0.0322 | 0.0505 |
| 0.216 | 0.0466 | 0.0550 | 0.0479 | 0.0494 | 0.0645 | 0.0626 | 0.0591 | 0.0518 | 0.0495 | 0.0792 |
| 0.321 | 0.0627 | 0.0737 | 0.0587 | 0.0634 | 0.0738 | 0.0724 | 0.0695 | 0.0604 | 0.0524 | 0.0903 |
| 0.432 | 0.0802 | 0.0938 | 0.0874 | 0.0813 | 0.0853 | 0.0954 | 0.0856 | 0.0723 | 0.0683 | 0.1136 |
| 0.500 | 0.0943 | 0.1000 | 0.0945 | 0.0902 | 0.0955 | 0.1075 | 0.0992 | 0.0857 | 0.0787 | 0.1299 |
| 0.586 | 0.1094 | 0.1199 | 0.1097 | 0.1111 | 0.1176 | 0.1344 | 0.1241 | 0.1138 | 0.1046 | 0.1553 |
| 0.752 | 0.1402 | 0.1509 | 0.1402 | 0.1424 | 0.1499 | 0.2041 | 0.1978 | 0.1928 | 0.1967 | 0.2191 |
| 0.830 | 0.1810 | 0.1860 | 0.1833 | 0.1752 | 0.1687 | 0.2899 | 0.2763 | 0.2765 | 0.2885 | 0.2918 |

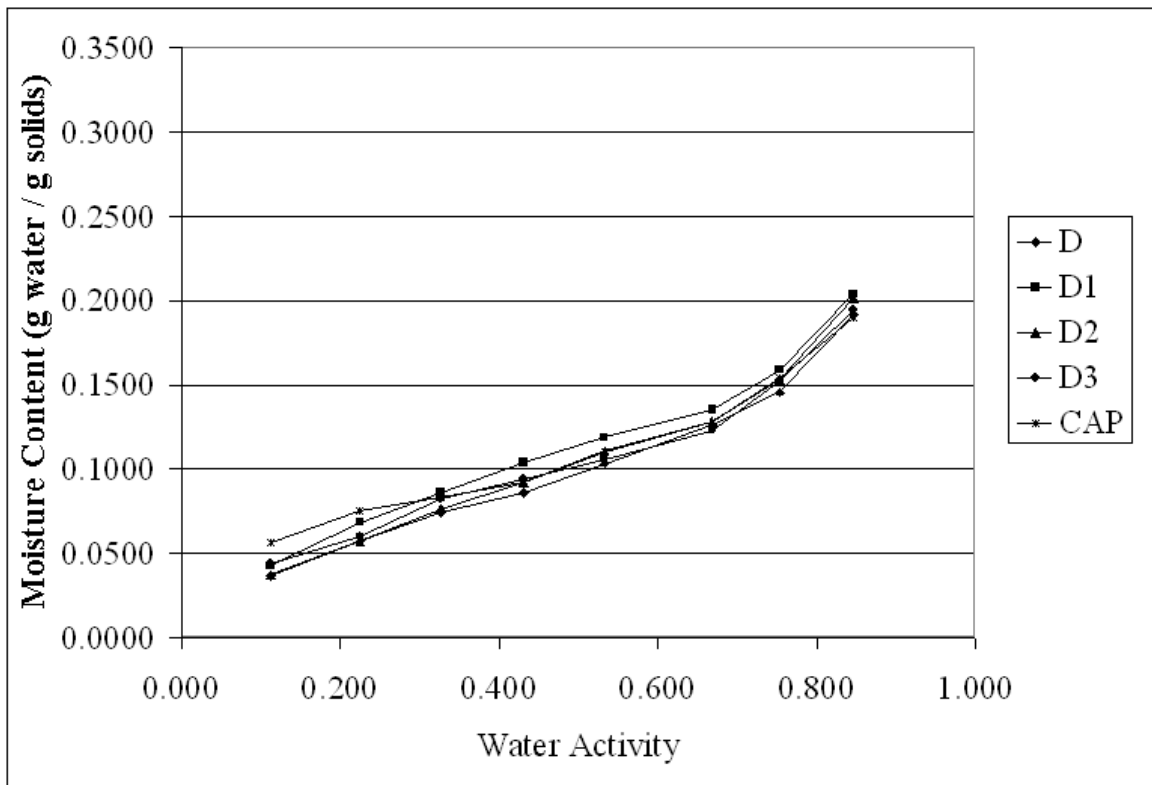


Figure 5.1 – Comparison of the moisture adsorption isotherms for starch based products at 23°C.

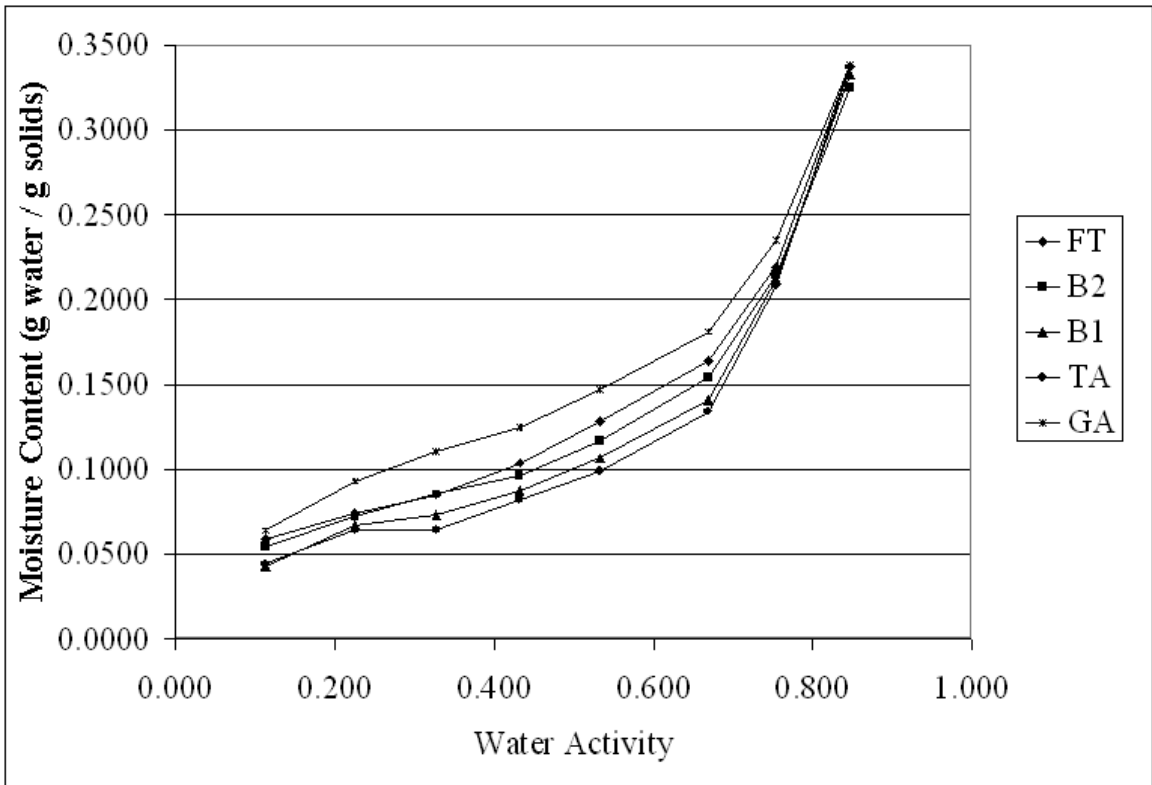


Figure 5.2 – Comparison of the moisture adsorption isotherms for gum acacia based products at 23°C.

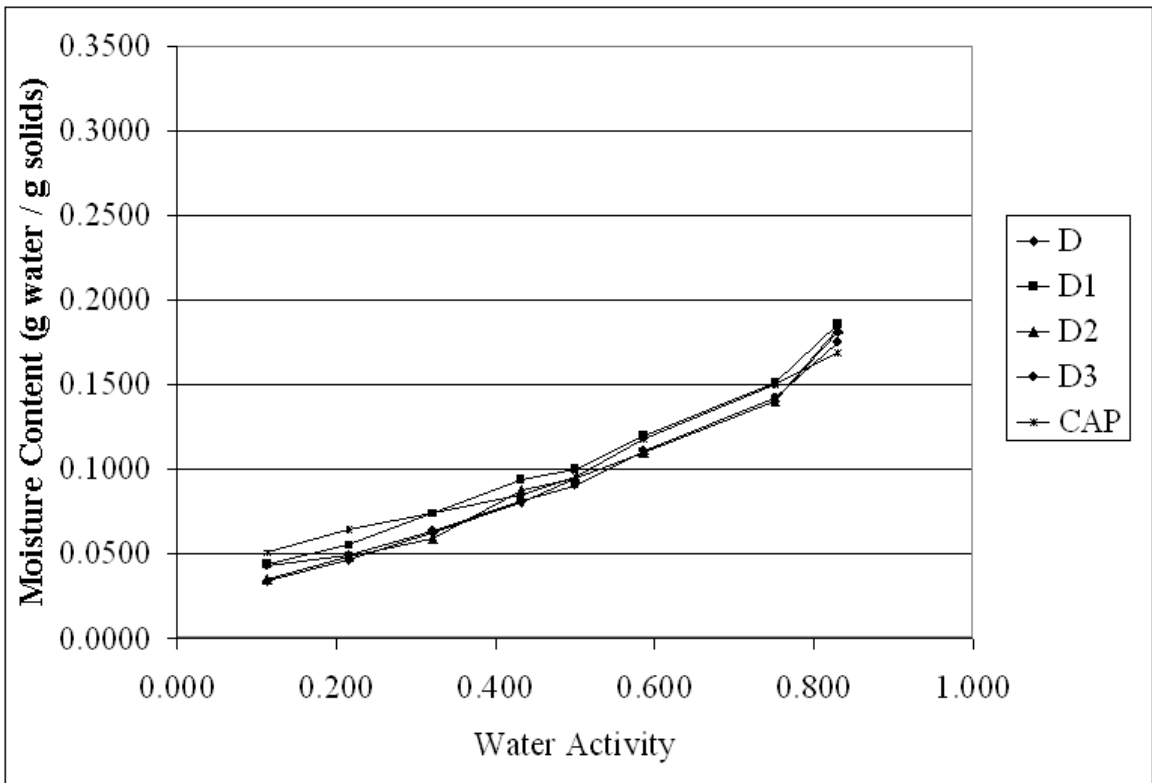


Figure 5.3 – Comparison of the moisture adsorption isotherms for starch based products at 35°C.

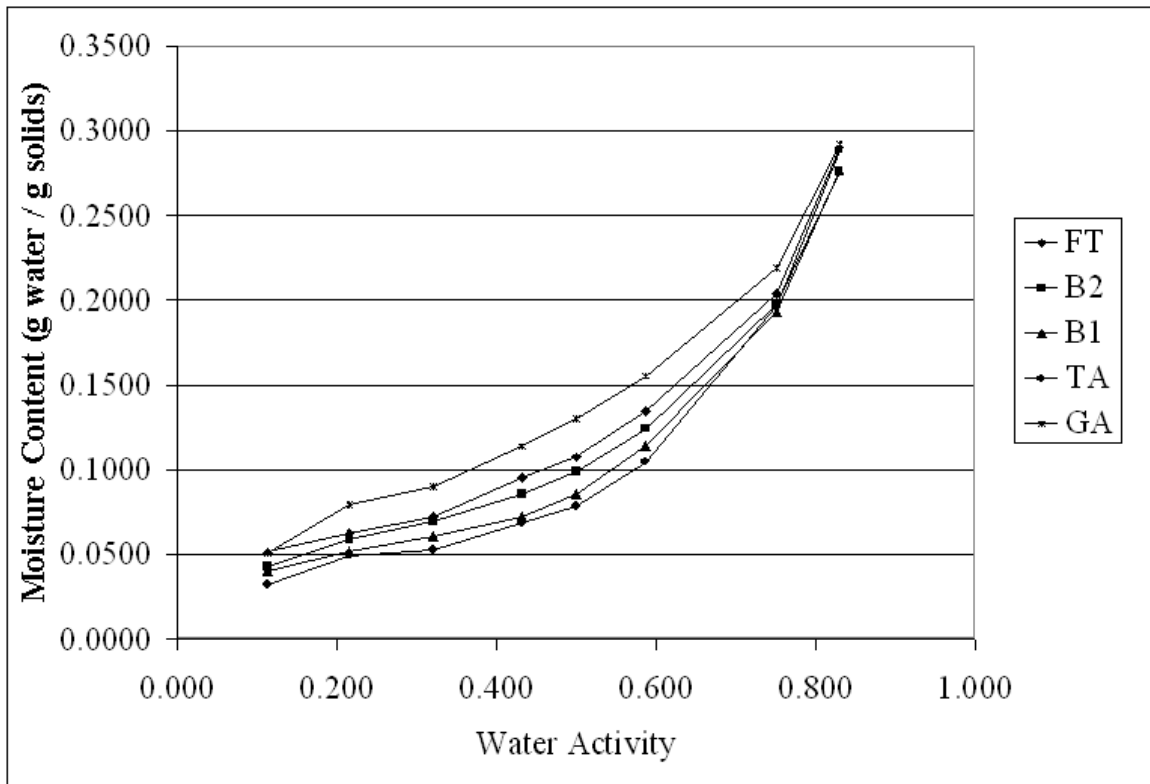


Figure 5.4 – Comparison of the moisture adsorption isotherms for gum acacia based products at 35°C.

The effect of temperature on sorption isotherms was quite evident. In comparing Figures 5.1 and 5.3 and Figures 5.2 and 5.4, all products adsorbed less water at 35°C than at 23°C. This finding was expected as it is commonly accepted that as temperature increases at a given water activity (a_w) the amount of sorbed water decreases based on the Clausius Clayperon equation.[59, 60] Iglesias and Chirife [110] commented that at higher temperatures, water sorption is less favorable and foods become less hygroscopic. Also, as temperature varies at a given a_w , so does molecular excitation and attraction which leads to a variation in amount of water sorbed.[111, 112] Other researchers have reported similar findings in other comparable food products [113, 114] with one exception; Perez-Alonso [65] et al. reported that moisture decreased with increased temperature for dextrin (dextrose equivalence of 10) below an a_w of 0.75 but the opposite

for *A. senegal*. The authors attribute this result to the complexity of moisture adsorption in food polymers.

5.4.2 BET and GAB Modeling of Sorption Data

The BET and GAB equations are commonly used for description of moisture sorption isotherms. The data from the current experiment were inputted into an Excel spreadsheet based on the Water Analyzer Series program provided by Dr. Ted Labuza to determine the BET and GAB models. Tables 5.4 and 5.5 summarize the output of the spreadsheet; the output includes the calculated monolayer value (m_0) and sorption constants C and K for the GAB model as well as m_0 and constant C for the BET model for all products at 23°C and 35°C, respectively. According to the mean relative deviation modulus (%P-value)[115, 116], the majority of the carrier material sorption data were explained by the models relatively well. The %P-value was less than 5 (a value considered to be a good fit) for all GAB models except D2 at 35°C with an average value of 3.41 and a range of 1.28 to 5.54. However, the BET models had more products with a %P-value greater than 5 (CAP, B1, and TA at 23°C and D2 and TA at 35°C) with an average %P-value of 3.63, and a range of 1.07 to 6.24.

Table 5.4– Estimated GAB and BET model parameters for all carrier materials at 23°C.

| 23° C | GAB | | | | BET | | |
|-------|---------|---------|-------|----------|---------------------|-------|----------|
| | m_0^a | C | K | %P-value | m_0^a | C | %P-value |
| D | 0.0702 | 10.115 | 0.753 | 2.12 | 0.0559 | 11.87 | 3.42 |
| D1 | 0.0859 | 10.787 | 0.688 | 3.37 | 0.0669 | 11.28 | 2.57 |
| D2 | 0.0758 | 9.124 | 0.742 | 3.06 | 0.0605 | 9.79 | 1.47 |
| D3 | 0.0696 | 14.925 | 0.755 | 3.74 | 0.0596 | 14.56 | 2.96 |
| CAP | 0.0649 | 52.257 | 0.776 | 2.42 | 0.0589 ^b | 58.93 | 3.41 |
| FT | 0.0619 | 48.275 | 0.959 | 1.92 | 0.0601 | 54.58 | 1.07 |
| B2 | 0.0596 | 95.327 | 0.961 | 4.18 | 0.0569 | 69.13 | 3.60 |
| B1 | 0.0518 | 34.169 | 0.992 | 4.64 | 0.0526 | 30.37 | 5.41 |
| TA | 0.0454 | 121.245 | 1.022 | 4.70 | 0.0470 | 68.19 | 5.85 |
| GA | 0.0807 | 30.236 | 0.885 | 4.01 | 0.0759 | 32.52 | 4.84 |

Notes:

^a Monolayer values represented as g water / g solids.

^b In order to obtain a positive C value for CAP using BET modeling, the water activity range used was from 0.11 to 0.32 (three points); all other data in the table used four points.

Table 5.5 – Estimated GAB and BET model parameters for all carrier materials at 35°C.

| 35° C | GAB | | | | BET | | |
|-------|---------|--------|-------|----------|---------|--------|----------|
| | m_0^a | C | K | %P-value | m_0^a | C | %P-value |
| D | 0.0648 | 8.395 | 0.790 | 3.74 | 0.0510 | 10.52 | 2.02 |
| D1 | 0.0714 | 11.654 | 0.759 | 3.56 | 0.0578 | 13.43 | 3.32 |
| D2 | 0.0654 | 8.523 | 0.790 | 5.54 | 0.0538 | 8.99 | 6.24 |
| D3 | 0.0577 | 15.113 | 0.824 | 4.46 | 0.0480 | 21.70 | 4.70 |
| CAP | 0.0631 | 26.434 | 0.774 | 2.62 | 0.0495 | 228.81 | 3.40 |
| FT | 0.0574 | 25.649 | 0.970 | 2.55 | 0.0552 | 29.30 | 2.92 |
| B2 | 0.0536 | 20.749 | 0.976 | 1.28 | 0.0513 | 25.76 | 1.71 |
| B1 | 0.0435 | 33.910 | 1.026 | 2.86 | 0.0423 | 55.31 | 1.74 |
| TA | 0.0400 | 21.493 | 1.050 | 4.82 | 0.0407 | 23.01 | 5.27 |
| GA | 0.0797 | 13.163 | 0.878 | 2.52 | 0.0700 | 16.84 | 4.08 |

Notes:

^a Monolayer values represented as g water / g solids.

The calculated GAB m_0 values for the dextrin based carrier materials ranged from 0.0649 to 0.0859 g water/g solids at 23°C and 0.0577 to 0.0714 g water/g solids at 35°C. The BET m_0 was less than the GAB m_0 for all products except for B1 and TA at 23°C and TA at 35°C. Collectively, the m_0 values for dextrin samples are comparable to the dextrin of Pérez-Alonso et al. [65] who reported 0.0735 g water/g solids at 25°C, but higher than Abramovič and Klofutar [67] who reported a range of 0.0463 to 0.0522 g

water/g solids across a range of dextrans (dextrose equivalence (DE) range from 5 to 25). The GAB m_0 values calculated for the gum acacia samples in the current research were from 0.0454 to 0.0807 g water/g solids at 23°C and 0.0400 to 0.0797 g water/g solids at 35°C. Pérez-Alonso et al. [65] reported a m_0 value of 0.0811 g water/g solids for *A. Senegal*; this value was nearly identical to *A. Senegal* (GA) in the current research. However, as a group, the m_0 values for gum acacia samples in the current research were lower than Pérez-Alonso et al. [65]

The aforementioned GAB m_0 comparisons must be considered with caution. Both Pérez-Alonso et al.[65] and Abramovič and Klofutar[67] determined true adsorption isotherms of the carbohydrate materials by gravimetric methods with samples stored over saturated salt solutions; however, the time taken for moisture equilibration was either not well defined [65] or quite short [67]. Wolf et al. [68] commented that an equilibration period of at least 14 days is recommended to achieve equilibrium moisture content; this time is longer if the a_w of the salt solution is greater than 0.65. Furthermore, Bell and Labuza recommended an equilibration time of between 10 to 21 days or a sample mass change of no more than 2 mg/g of dry sample when sampled once every 7 days. Because these criteria were not met or defined in the previous studies [65, 67], the resultant calculated GAB m_0 values may not be indicative of the true sorption phenomena of the carbohydrate materials and therefore cannot be compared to the current study with complete confidence.

Other than the custom dextrin products (D, D1, D2, and D3) the calculated values for the C constant ranged considerably at 23°C. Conversely, at 35°C, the C constant range was far tighter than at 23°C. All C constants were within the normal range of

$1 < C < 200$. [117] However, it should be noted that for the GAB model, when K constant values equal 1, the GAB equation reduces to the BET equation and at values above 1, the isotherm is physically unsound.[118] Three of the products have K values that are just slightly (negligibly) greater than 1.

Linearity of the BET model depends on the maximum a_w value used to model the data. In the literature, the maximum reported a_w value declared acceptable for BET modeling ranges from 0.35 to 0.75.[118, 119] However, the BET modeling criteria used for the data in this paper was recommended by Bell and Labuza[60]; the authors recommend that if available, four a_w values less than 0.45 should be used to calculate m_o . The total range of a_w values used for BET modeling for this research was from 0.11 to 0.43. Using this a_w range, the calculated BET m_o values for the dextrin based carrier materials ranged from 0.0559 to 0.0669 g water/g solids at 23°C and 0.0480 to 0.0578 g water/g solids at 35°C. The calculated m_o values for dextrin samples were in consensus with other researchers who used the BET equation for dextrin products.[67, 110] It should be noted that the aforementioned criteria used to generate BET models was not successful for CAP at 23°C; the C value was negative. In order to obtain a positive C value for CAP at 23°C, the BET modeling utilized a_w range of 0.11 to 0.32. Using the un-modified criteria, the BET model parameters were 0.0534, -167.04, and 6.06 for m_o , C, and %P-value, respectively. The BET m_o values calculated for the gum acacia samples in the current research were from 0.0470 to 0.0759 g water/g solids at 23°C and 0.0407 to 0.0700 g water/g solids at 35°C. All of the BET m_o values were less at 35°C than at 23°C, as expected since the excess enthalpy decreases with increasing moisture content. A decrease in m_o may be due to temperature induced physical or chemical

changes that lead to a reduction of active adsorption sites. [112, 120, 121] Alternatively, this may be due to water molecules being in a higher energy state which would allow a detachment from adsorption sites. [112]

5.5 Conclusions

The objective of this study was to generate moisture sorption isotherms for unmodified and OSAn-substituted starch and gum acacia used as carrier materials for spray dried flavoring materials. In general, gum acacia appeared to be more hygroscopic than the dextrin based samples. Furthermore, it appeared that varying the degree of OSAn-substitution for dextrin samples did not affect moisture sorption characteristics. However, for gum acacia samples, increased OSAn-substitution resulted in a decreased affinity for moisture in the range of 0.3 to 0.7 a_w . GAB modeling indicated that m_o values were typically higher for dextrin than for gum acacia samples; BET m_o values could not be generalized as easily.

Moisture sorption isotherm data for carrier materials is useful for processing, storing, and using flavor materials. When processing spray dried flavors, moisture sorption data can be used to formulate feed emulsions and optimize processing parameters to produce free-flowing powders less prone to stickiness and caking. During storage, moisture sorption data can aid in selecting proper storage conditions to prevent product failure (e.g. caking, chemical reaction, microbial growth, and flavor loss). Finally, moisture sorption data may be useful, in conjunction with glass transition data, when using dried flavors in controlled release applications.

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CHAPTER 6
FINAL DISCUSSION

6. Final Discussion

Flavor scientists and technologists are well aware of the benefits of using modified carbohydrates for encapsulating flavoring materials; numerous scientific publications and continued new product launches from starch and gum suppliers testify to this fact. However, to our knowledge, there is little or no (public) information that relates the degree of OSAn substitution of starches or gums to encapsulation performance. Furthermore, there are no publications that address flavor/aroma release phenomena from such products. In addition, only one study by Sootitawat et al. [53] utilized l-menthol as model for spray dried encapsulation studies. The results contained in this dissertation expanded the base of knowledge for the aforementioned research areas. The most significant findings of this research follow.

The current research considered the effects of OSAn substitution level on the retention and physical characteristics of spray dried l-menthol. The results confirmed that emulsifying ability of carrier materials improved encapsulation effectiveness. It was found that as OSAn substitution of carbohydrates increases, total and surface menthol appeared to increase and decrease, respectively. However, increased OSAn substitution may have a greater effect on decreasing surface menthol content than increasing total menthol content; this would indicate OSAn substitution may be directly related to improvements in shelf-life stability.

This research also capitalized on the creativity of researchers who first explored the use of real-time flavor release analysis via hyphenated instrument couplings (e.g. VGI-APCI-MS and DVS-APCI-MS). Two methods (DVS-P&T-GC and DVS-PTR-MS) were developed to explore the menthol release characteristics from various starch and

gum acacia based carrier materials. It was found that increased OSAn substitution of the carrier material increased the total menthol release with little or no change in the primary release time. Furthermore, there appeared to be a slight relationship between moisture uptake rate and the menthol release rate. However, for dextrans, increased menthol release rate appeared to be strongly related to increased OSAn substitution levels rather than moisture uptake rate.

As with most research projects, this one created results which led to more unanswered questions and thus, future research opportunities. Two possibilities follow:

1) It was discovered that different levels of OSAn substitution resulted in quite distinct differences in menthol release profiles. Speculations of why these differences existed were discussed, but the mechanisms were not experimentally confirmed.

2) It was found that moisture uptake rate may be used as a tool to understand menthol release rate. In the process of determining this it was also discovered that increased OSAn substitution was correlated to increased menthol release rate for the dextrin products and not necessarily so for the gum acacia products. Again, potential reasons why this occurred were presented but not confirmed.

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