CHARACTERIZATION OF VOLATILES IN COMMERCIAL AND SELF-PREPARED RUM ETHERS AND COMPARISON WITH KEY AROMA COMPOUNDS OF RUM

BY

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THESIS

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Abstract

Rum ether is a distillate of wood extractives, so named as a result of its purported similarity in flavor to rum; however, despite it being used widely throughout the flavor industry, no work is publicly available that delves into the aroma characteristics of rum ether or explores how they compare to those of rum. With these goals in mind, two popular rums were subjected to aroma extract dilution analysis (AEDA) in order to establish the aroma profile for typical gold or white rum. Both commercial and self-prepared samples of rum ether were then subjected to aroma analysis for comparison with these results. Ten commercial samples obtained from a number of flavor companies were analyzed by direct injection gas chromatography-olfactometry (GCO) in order to establish an understanding of traits common to most or all commercial rum ethers. These served as a guide when using scaled-down industrial methods for distillation of two rum ethers. In both, the feasibility was assessed of replacing pyroligneous acid, a traditional rum ether ingredient, with liquid smoke, one that is more widely available and safer for use in food. Self-prepared ethers were found to be comparable to commercial samples despite this ingredient substitution and underwent AEDA for more direct comparison with rum. Ultimately, a number of compounds, including ethyl isobutyrate, ethyl butyrate, and guaiacol, were found to be essential to both rum and rum ether. Not all compounds were aligned: isoamyl alcohol and β-damascenone were found to be extremely important in rum but not present in rum ether, while ethyl acrylate and ethyl 3-butenoate were important rum ether aroma contributors that are nowhere to be found in rum. Rum ether therefore currently possesses a solid foundation of rum-like odor notes, especially wood extractives and short-chain esters, but could benefit from the addition of certain fermentation-derived compounds and the removal of several unpleasant off-notes. Methods for eliminating these discrepancies could be the basis for much future work.

Table of Contents

Chapter One:

Introduction

Fenaroli's Handbook of Flavor Ingredients defines rum ether as "the mixture resulting from the oxidation and the hydration of ethyl alcohol." In practice, it is produced by the distillation of pyroligneous acid, more commonly known as wood alcohol, and ethanol (Burdock 2009). The production of rum ether is vastly different from that of rum in both materials and process; its name was derived from its purported similarity in flavor to rum. Rum ether is first mentioned in old perfumery handbooks (Piesse 1857), and as early as the late 19th century it was also cited as a means of supplementing rum flavor in liquors (*Western Druggist* 1885). In the years since, it has moved beyond the world of alcoholic beverages into baked goods and confectionary products (Burdock 2009). It has been given Generally Recognized as Safe (GRAS) status by the Flavor and Extract Manufacturers Association (FEMA) – in fact, it was among the original set of substances given this designation in 1965 (Hall and Oser 1965). Additionally, it is considered "all natural," an especially important distinction in today's food climate, where natural flavors have a marked commercial advantage. Its long history of use and status as a natural flavor should make rum ether an ideal means of creating a rummy note in a variety of food products; assuming, of course, that it truly does mimic the flavor of rum. However, despite its long-running and widespread use, no research currently exists in the public domain to support this assumption.

Only limited research is available that even establishes the exact composition of rum flavor. This is a surprising gap in the literature considering that rum was the most popular

beverage in the American colonies (Barr 1999) and that, after seeing a dip in demand, it now finds itself in the liquor category (molasses-based spirits) with the greatest worldwide growth (Delevante 2004). Although studies can be found in the published literature that examined rum flavor, these typically did so in a peripheral way, such as in comparison to a primary liquor of interest (Ng 1999; de Souza et al. 2006) or as a medium for the analysis of a specific and narrow class of volatile compounds (Timmer et al. 1971, Pino et al. 2002). One of the most thorough analyses of rum volatiles was conducted several decades ago (Maarse and ten Noever de Brauw 1966), using technology that was appropriate at the time, but that has since been replaced by more sensitive instrumentation with the ability to obtain much more detailed information. The other most rum-focused work contains a thorough listing of rum volatiles, but includes all volatiles – not just those with an aroma – leaving the discussion of aroma to only the few most potent odorants (Pino 2012). In establishing the similarity of rum ether to rum, it would be helpful to first have a more complete understanding of the key aroma compounds in rum. This information would additionally be useful in its own right as a way to better understand a beverage that has been both popular and historically important for centuries.

This research aimed to first determine the aroma profile and key aroma compounds of both white and gold rum through the use of aroma extract dilution analysis (AEDA). In AEDA, serially diluted aroma extracts are subjected to analysis by gas chromatography-olfactometry (GCO); all aroma-active compounds – those that can be smelled, even faintly – are part of the aroma profile, while those compounds that continue to be sensed in even the most dilute extracts are considered potent or key odorants. Once a characteristic aroma profile was established for rum, a wide range of commercially available rum ether samples was assessed in order to determine which compounds were most common among all samples. Additionally, rum ethers

were distilled in the laboratory using traditional methods but with one ingredient substitution, replacing historically used pyroligneous acid with liquid smoke, a more widely available and less toxic product that is also derived from wood. These rum ethers were analyzed in the same manner as the commercial products and compared to both rum and other rum ethers to determine the feasibility of using liquid smoke in rum ether production. Self-made rum ethers also provided the opportunity to determine points in the rum ether production process that could be altered to create a product more reminiscent of rum. With this information, it was possible to determine whether rum ethers are, or could be made, similar to rum in volatile composition, or whether the resemblance is purely qualitative.

Objectives

- Determine what aroma compounds are most important in giving rum its distinctive flavor using a combination of headspace, direct injection, and AEDA GCO as well as GC-MS confirmation.
- Distill rum ethers on a bench scale in a consistent way using liquid smoke as a replacement for pyroligneous acid.
- Characterize the aroma profile of rum ethers using a combination of direct injection and aroma extract dilution analysis GCO with GC-MS confirmation and compare with the key aroma compounds found in rum.
- Identify potential changes that could be made to rum ether distillation in order to produce a rum ether that more closely mirrors true rum flavor.

1.1 References

Barr, A. *Drink: A Social History of America*. Carroll & Graf Publishers, Inc.: New York, 1999.

Burdock, G.A. *Fenaroli's Handbook of Flavor Ingredients*. 6th ed. CRC Press: Boca Raton, FL, 2009.

Delevante, M.P. "Rum." *Distilled Spirits: Tradition & Innovation*. Bryce, J.H.; Stewart, G.G., Eds. Nottingham University Press: Nottingham, UK, 2004. 209-14.

Hall, R.L., and B.L. Oser. "Recent progress in the consideration of flavor ingredients under the Food Additives Amendment. 3. GRAS Substances." *Food Technology.* 1965, *19*, 151-97.

Maarse, H.; ten Noever de Brauw, M.C. "The Analysis of Volatile Components of Jamaica Rum." *Journal of Food Science.* 1966, *31*, 951-5.

Ng, L-K. "Analysis of Vodkas and White Rums by SPME-GC-MS." *Applications of Solid Phase Microextraction*. Pawliszyn, J.; Smith, R.M. Eds. Royal Society of Chemistry: Cambridge, UK, 1999. 393-406.

Piesse, G.W.S. *The Art of Perfumery*. Lindsay and Blakiston: Philadelphia, 1857.

Pino, J.A.; Tolle, S.; Gök, R.; Winterhalter, P. "Characterisation of odor-active compounds in aged rum." *Food Chemistry.* 2012, *132*, 1436-1441.

Pino, J., et al. "Headspace solid-phase microextraction of higher fatty acid ethyl esters in white rum aroma." *Journal of Chromatography A.* 2002, *954*, 51-7.

de Souza, M.D.C.A.; Vásquez, P.; del Mastro, N.L.; Acree, T.E.; Lavin, E.H. "Characterization of Cachaca and Rum Aroma." *Journal of Agricultural and Food Chemistry.* 2006, *54*, 485-8.

Timmer, R.; ter Heide, R.; Wodden, H.J.; de Valois, P.J. "Phenolic Compounds in Rum." *Journal of Food Science.* 1971, *36*, 462-3.

Western Druggist. Vol. 7. G.P. Engelhard & Co.: Chicago, 1885.

Chapter Two:

Literature Review

2.1. Rum

2.1.1 History

The English first happened upon the Caribbean island of Barbados in 1607 (Gately 2008), and by 1625 the first British settlements had been built there. The land was initially used for attempts at growing tobacco, followed by cotton and indigo, but no crop seemed to take to the land. It was not until the 1640s, when sugarcane was introduced, that the plantations really began to thrive. Soon sugar was being refined on the island and exported for use throughout Europe (Barr 1999).

In addition to sugar, the refining process created a sticky byproduct known as molasses. At first glance, the settlers deemed it worthless and used it as animal slop or trash. Before long, however, it was discovered that addition of water created an easily fermentable material; furthermore, the resulting alcohol was found to be very well-suited to distillation. With that, rum was born (Gately 2008). Although distillation of sugarcane-based beverages was not new – the Portuguese had for years been making alcohol from sugarcane juice – the ability to do the same with an otherwise unmarketable byproduct held great novelty. This meant that the British could use a single crop of sugarcane for production of both sugar and rum, and within a decade of the discovery they were the richest people in the New World (Barr 1999).

Which is not to say that rum was an instant success. The first recorded mention of rum is found in a description of Barbados from 1651, where it is described as "hot, hellish, and terrible" (Gately 2008). It just so happened, however, that the nearby continent was quickly filling with colonists who were desperate enough for a replacement libation that they would try anything, no matter how hellish. For while beer was the drink of choice in Britain, American soil proved to be unwelcoming to wheat and barley, the raw ingredients necessary to prepare it; importing these from overseas was prohibitively expensive (Park 1985). Additionally, while the population density was high enough in Britain to consume large quantities of beer before it could spoil, the colonies were so spread out that most taverns could not reasonably expect to finish a keg before it went bad. With water considered unsafe to drink, rum seemed the best solution (Barr 1999).

Before long, rum was the most consumed beverage in the colonies. By the height of its popularity in the late eighteenth century, the two million colonists were drinking eight million gallons of the liquor each year, or four gallons per capita. Approximately half of this was imported from the West Indies, while the other half was distilled stateside using imported molasses. As a result, the two most imported commodities from the Caribbean were by far rum and molasses. A common myth exists that this was one leg of a construct known as the triangle trade, where sugar and molasses were sent from the islands to the colonies, from whence rum would be sent to Africa. Finally, Africa would send slaves back to the Indies, completing the "triangle." But although African slaves were certainly brought to the Americas, only a small amount of rum was actually involved in this trade; the bulk of demand for rum originated in the colonies themselves (Park 1985).

Some other rum-related lore, specifically that related to pirates, contains a bit more truth. American colonists were not the only ones who had grown to like the beverage; its use had also

spread back to Europe and even onto the high seas. Sir Henry Morgan, a famous English privateer, made rum a part of his brand to the point that he died not from cannon fire or walking the plank but from overconsumption of alcohol. Today, one of the most popular brands of rum appropriately – or perhaps inappropriately – bears his name. Rum was so deeply engrained in pirate culture that it was even mentioned in their governing articles, which recognized the right of every man to "strong liquors" (Gately 2008).

Rum's importance in colonial times truly cannot be overstated. By many accounts, it was one of the first sparks of contention between the British and their colonies that led eventually to the Revolutionary War. The Molasses Act of 1733 placed a tax on molasses imported from non-British colonies (Barr 1999). This dealt a huge blow to domestic distilleries, which relied on cheap molasses from French islands in the Caribbean to remain profitable. Rum production was at the time the most lucrative colonial business in New England, with at least one distillery in every major city, and the Molasses Act threatened the very foundation on which that industry was built (Park 1985). Although many more grievances would accumulate by the start of the war, this first insult was not easily forgotten. Upon conclusion of the Revolutionary War, John Adams is noted as having said "I know not why we should blush to confess that molasses was an essential ingredient in American independence. Many great events have proceeded from much smaller causes" (Gately2008).

By this time, colonists had forgotten much of their anger over molasses import taxes, as their sights had been set on a newly popular drink. During the course of the war, imports had been impeded significantly by the British Navy, and many distilleries had switched over to whiskey. Although the end of the war brought the opportunity to pick back up on rum, whiskey was cheaper and could be made from domestic raw materials, something the newly independent

country took a lot of pride in (Barr 1999). By 1790, per capita rum consumption was down from its four gallon peak to only one gallon, and by the mid-1850s, rum comprised only five percent of all American spirit consumption (Park 1985). It maintained some popularity in Europe throughout the nineteenth century, but it still seemed that perhaps rum's best days were behind it (Wilson 2006).

Recently, however, rum has experienced a renaissance. With the introduction of Bacardi white rum in the 1970s, many who had always thought of rum as a dark beverage began to view it as an alternative to vodka in mixed drinks (Park 1985). In the years since, many brands of rum, like Bacardi and Captain Morgan, have become household names, and new premium rums are now widely being seen as competitive with premium spirits in other categories (Delevante 2004). In 2001, rum was able to capture 11.9% of the U.S. distilled spirit market (Corrigan 2004), and this figure seems to be growing: sugarcane-based spirits experienced the largest growth of any spirit category in 2004 (Delevante 2004). So although it would be decidedly impossible for rum to recapture its colonial monopoly in today's liquor market, it is reemerging as a beverage worth watching and, perhaps, studying.

2.1.2. Production

What constitutes rum varies significantly across the globe; with more than twenty standards of identity, it is more ambiguously defined than any other spirit (Delevante 2004). In the United States, a beverage must meet three criteria in order to be classified as rum: it must be made solely of distillates of sugar cane or its byproducts, it must be distilled at less than 190° proof and bottled at greater than 80° proof, and it must have the taste and aroma characteristic of rum (United States). Other countries may contain different requirements regarding ethanol

concentration or include additional criteria, most commonly a minimum length of aging (Delevante 2004).

Despite the diversity of standards, it is generally agreed upon that the raw material for rum should be sugar cane derivatives, including molasses, cane juice, and cane syrup. Molasses is the most popular starting material, both for its ability to create a flavorful spirit and for the economic benefits of using an otherwise unpopular sugar byproduct (Lehtonen and Suomalainen 1977). Typical molasses composition is as follows:

Table 2.1. Composition of molasses (adapted from Persad-Doodnath 2008)

Water	**15-25%**
Organic Solids	**65-70%**
Fermentable sugars	35-55%
Unfermentable sugars	2-9%
Other organics (protein, starch, acid)	1-33%
Inorganic Solids	**10-15%**

One of the most important factors here is the ratio of fermentable matter – the fermentable sugars sucrose, fructose, and glucose – to the remaining solids, which for an ideal fermentation should be always greater than 1 and preferably greater than 1.2 (Persad-Doodnath 2008).

In preparation for fermentation, the molasses is diluted with pure water to a sugar concentration (w/v) of 10-12% (Lehtonen and Suomalainen 1977). Insufficient dilution will lead to a fatal concentration of ethanol for the yeast, which will die before all sugars have been converted. In addition to being diluted, the molasses may also be heated to remove microorganisms that might compete with the chosen yeast culture or centrifuged to separate out

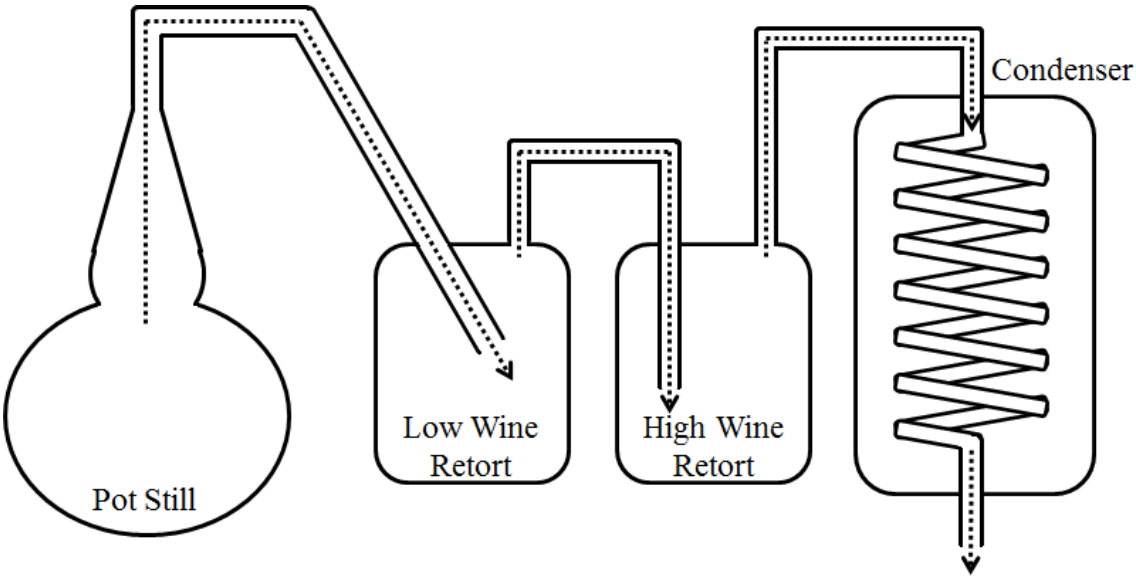
colloidal matter (Persad-Doodnath 2008). Finally, yeast is added to the dilute molasses substrate to begin fermentation. In the case of large-scale producers, this is usually a single, carefully selected culture from the *Saccharomyces* genus for light rums or the *Schizosaccharomyces* genus for heavier ones (Lehtonen and Suomalainen 1977). Smaller, artisan producers are more likely to still use a mixture of wild yeast strains or the yeast residue remaining from an earlier batch (Buglass 2011).

Fermentation now begins. Although more extreme conditions are occasionally used, most fermentation is conducted at a temperature of 30-33°C and a pH between 5.5 and 5.8. This process is usually allowed to continue for one to three days (Lehtonen and Suomalainen 1977), at which point all fermentable sugars will have been converted. Most sugars – about 90% – are converted into ethanol, while the rest are utilized for cell growth and production of glycerol. The finished fermented molasses has an ethanol concentration of 6-9%. In addition to ethanol, many of rum's characteristic aroma compounds are formed during the fermentation process: alcohol oxidizes to form aldehydes, which oxidize to form acids. Meanwhile, alcohols and acids undergo esterification, and amino acids react to form fusel oils or sulfur compounds (Persad-Doodnath 2008).

Fermentation is followed by distillation, which serves to concentrate both the alcohol and desirable aroma compounds while removing any unpleasant congeners. Although this step does not contribute as heavily as fermentation to the formation of aroma compounds, it is essential in determining their concentrations in the finished rum (Lehtonen 1977). Two types of distillation are still commonly used: continuous distillation and pot-still distillation.

Pot-still distillation (Figure 2.1) is a more traditional, batch method of distillation and is still used by many smaller distilleries, especially those making heavier, darker rums. The fermented mixture, or beer, is placed in the pot still. Both the low and high wine retorts are filled with the tail end cuts from previous distillations. These are more concentrated in alcohol than the beer – the beer is only 6-9% alcohol, while the low wines are approximately 30% and the high approximately 75% - but are still less concentrated than the desired finished product. Heat is applied to the pot still, causing evaporation of the beer. Upon hitting the retort filled with cooler low wines, the water in the beer condenses; the heat from this condensation is enough to evaporate much of the ethanol in the mixture. This process of condensation and evaporation is repeated in the high wine retort. Finally, this vapor, now highly concentrated in ethanol, moves to the condenser, is cooled to the point of condensation, and is collected. The initial condensate is collected as headings and typically discarded. The first fraction, at about 90% ethanol, is collected to be aged into rum. As the ethanol begins to be exhausted, second and third fractions are collected to replenish the high and low wine retorts, respectively (Buglass 2011).

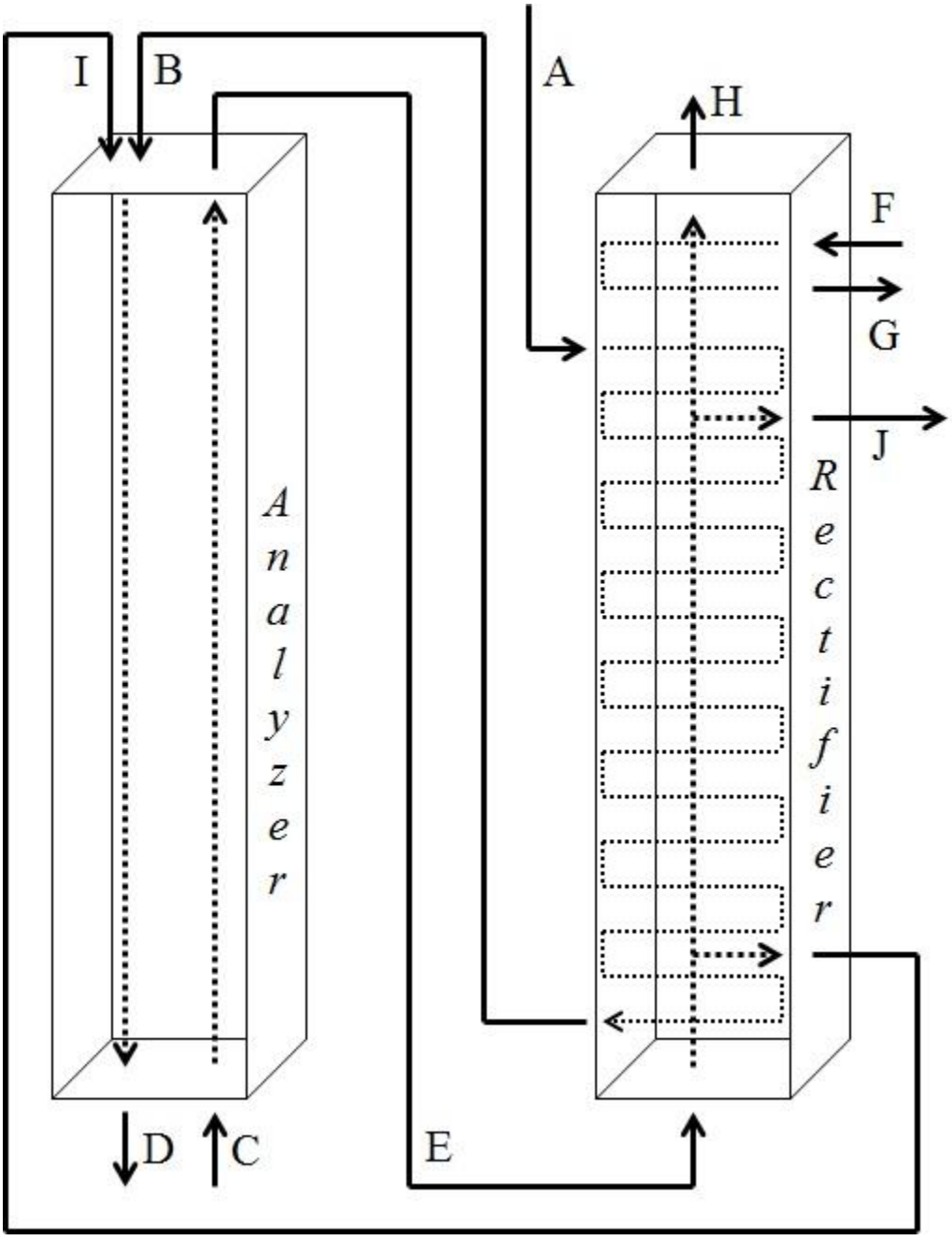
Figure 2.1. Pot-still distillation of rum



Continuous distillation (Figure 2.2) is a more modern alternative to the pot-still method and is common in large-scale operations. A typical continuous distillation set-up contains two columns: the analyzer, where initial concentration of ethanol occurs, and the rectifier, where fractionation takes place. The process begins when the beer (A) is fed through a series of coils running through the rectifier, allowing the hot vapors formed later in the distillation to warm the beer almost to boiling. This improves efficiency – rather than heating the beer exclusively with fresh steam, the heat energy already being released by another part of the distillation is captured and utilized. This hot beer (B) is then fed into the first column, the analyzer. Steam (C) is fed from the bottom of the column. As the steam flows upward, there is an exchange of heat between the steam and the hot beer. The steam heats the more volatile components of the beer, particularly ethanol, to the point of evaporation, while the beer cools much of the steam to the point of condensation. The spent beer (D), now consisting primarily of water, exits the bottom of the column, while the more volatile components (E), now highly concentrated in ethanol, are collected and fed into the second column, or rectifier. The rectifier has a heat gradient – cool at the top and warm at the bottom – created by a flow of cool water (F) through a coil at the top of the column; this serves to absorb much of the heat of the vapors without actually contacting and diluting them, and exits the column significantly warmer (G). As the concentrated beer vapor flows towards the top of the rectifier, the gradually decreasing temperature creates condensation of increasingly volatile components. The most volatile components (H) remain vapor and are collected at the top and discarded, as they typically contain undesirable compounds such as methanol; the least volatile components (I) condense almost immediately upon entering the rectifier and are recycled back into the analyzer column. Only the heart (J), a cut out of the middle of the rectifier column taken slightly below the top, is collected to be aged and sold as

rum. Despite only requiring two columns, this rum fraction can be very concentrated in ethanol, often reaching all the way to the azeotrope at around 95% ethanol (Buglass 2011).

Figure 2.2. Continuous (Coffey still) distillation of rum



The primary differences between pot-still and continuous distillation come down to a trade-off: volume of flavor vs. efficiency. A pot-still distillation consumes significantly more energy than the alternative, but it captures more of the volatiles and can therefore result in stronger, more complexly flavored rum. The continuous still can create a larger volume more quickly and in a less energy-intensive way, but often excludes many aroma compounds in the process. This explains why a pot-still is favored for dark, heavy rums designed to be drunk neat, where depth of flavor is key, and continuous distillation is preferred for light rums, which benefit from a cleaner flavor.

The collected distillate from either method then undergoes an aging process, as it is harsh and lacking in complexity of flavor when fresh (Persad-Doodnath 2008). The mixture is put into charred, oak barrels for typically at least one year; barrels purchased from Bourbon producers are popular, as rum has no rules about reuse of barrels, while Bourbon requires that they be used only once (Buglass 2011). During aging, the aroma profile of the rum is altered in three major ways: constituents of the wood are extracted into the rum, oxygen seeping in through the pores of the barrel participates in oxidation reactions, and unstable components of the rum react with one another to produce more stable molecules (Persad-Doodnath 2008). Although more complexity is developed, it is at the expense of product, as about 10% of the volume is lost with every year of aging, so a balance must be struck between quality and quantity of rum produced (Buglass 2011).

Once rum has been aged for the desired length of time, only two steps remain before it is ready to be bottled and sold. First, an employee of the distillery trained in creating well-rounded rum flavors mixes together several different distillates – perhaps some of different age, ethanol content, or method of fermentation or distillation (Buglass 2011). The purpose is to create not

only balanced flavor, but also a consistent product from year to year. Although blenders now have the aid of chemical analysis to confirm consistency, in traditional rum-making, it was essential that they be well-trained to maintain quality in every batch. Often blenders have at their disposal small amounts of rum aged for a very long time – twenty years or more – to lend complexity to much younger spirits (Blue 2004). Finally, the color of the blend may be altered to influence perception – rums advertised as dark but not aged sufficiently to take on a naturally golden color can be tinted with caramel, while those aged in wood but sold as white rums can undergo charcoal filtration to remove coloration (Buglass 2011). The rum is now finally ready to be bottled, purchased, and, most importantly, consumed.

2.1.3. Aroma Research

A fairly significant body of work is in existence on the volatile compounds in rum. Studies of rum flavor first appeared in the literature during the 1960s, and additional work has steadily been published in the literature until as recently as a few weeks ago. In fact, rum research provides a fairly complete view of the evolution of flavor analysis techniques, with the first thorough analysis of rum volatiles utilizing solvent extraction and capillary-column gas-liquid chromatography (Maarse and ten Noever de Brauw 1966), and the most recent research harnessing the power of solid phase microextraction (Pino 2007) and aroma extract dilution analysis (Pino et al. 2012), two of the more modern developments in flavor research.

With such an abundance of work available, one might presume that a full characterization of rum volatiles would at this point be redundant, but in fact none of the currently published work provides a thorough profile of rum aroma by current standards. Much of what is available

does not make complete characterization its aim, choosing to focus instead on a single group of compounds such as fatty acids (Nykänen et al. 1968), phenols (Timmer et al. 1971), nitrogen-containing compounds (Wobben et al. 1971), or fatty acid ethyl esters (Ng 1999; Pino et al. 2002). Each of these fills in some holes in the knowledge, but even in combination these studies do not provide a complete picture of the complex chemistry responsible for rum aroma.

Four articles exist that come closest to providing this picture. A synthesis of those compounds identified within at least two of the four, ranked by retention index on a DB5 column, can be found in Table 2.2. The 1966 work of Maarse and ten Noever de Brauw is one of the first places in the literature where rum aroma is mentioned at all. For this analysis, the aroma compounds of dark Jamaican rum were extracted using a mix of pentane and ether, and the extracts were analyzed using a combination of gas chromatography with either infrared or mass spectrometry. A total of sixty-five compounds were identified. The primary setback to using this work today is that the technology has evolved significantly over the past five decades, and many of the techniques used are now seen as somewhat dated. The same can be said of the rum research published in 1970 by Liebich et al., which identified more than three times as many compounds; although it used the best technology available at the time, we now know that many of those analysis methods can create artifacts not present in the original product. Additionally, without the aid of more recently developed olfactometry techniques, both of these researchers are unable to provide more than a simple, unranked list of all compounds that are thought to have an impact on the overall aroma (Maarse and ten Noever de Brauw 1966, Liebich et al. 1970). This leaves open the possibility that many of the compounds listed are volatile but odorless, contributing nothing to the overall rum flavor. Two more recent contributions are available as well. In the work of de Souza et al., published in 2006, more modern extraction techniques are

paired with olfactometric methods to obtain a list of rum aroma compounds ordered by importance of their contribution to the aroma. Unfortunately, the goal of the work is to compare the most important compounds in rum with those in cachaça, so only the few largest aroma contributors are discussed, passing over the more minor components (de Souza et al. 2006). Finally, in some of the most recent rum research, published in 2012, Pino et al. use a combination of the olfactometric method of aroma extract dilution analysis, or AEDA, with GC-MS to create both an exhaustive list of volatile components in rum and a short exploration of the most potent odorants. However, the longer list includes many odorless compounds, while the shorter one focuses on only a small number of very potent odorants, making it hard to distinguish those compounds that might be weaker contributors to the aroma profile from those that contribute nothing at all.

Table 2.2. Commonly identified rum volatiles

Compound	**RI (DB5)***	**Type**	**A**	**B**	**C**	**D**
ethanol	435[D]	aldehyde	X	X		
propanal	[A]	aldehyde	X	X		
ethyl formate	504[D]	ester	X	X		X
1-propanol	568[D]	alcohol	X	X		X
ethyl acetate	606[D]	ester	X	X		X
isobutanol	624[D]	alcohol	X	X		X
3-methylbutanal	630[D]	aldehyde		X		X
diacetyl	644[C]	ketone		X	X	X
acetic acid	645[D]	acid	X			X
propanoic acid	668[D]	acid	X	X		X
2-butanol	676[D]	alcohol	X	X		X
2-pentanone	687[D]	ketone		X		X
ethyl propionate	715[D]	ester	X	X		X
propyl acetate	719[D]	ester	X	X		
diethyl acetal	730[C]	acetal	X	X	X	X
2-methyl-1-butanol	736[D]	alcohol	X	X		
3-methyl-1-butanol	740[D]	alcohol	X	X		X
ethyl isobutyrate	758[C]	ester	X	X	X	X

Table 2.2 (cont.)

Compound	RI (DB5)*	Type	A	B	C	D
1-pentanol	[A]	alcohol	X	X		
isobutyl acetate	769[D]	ester		X		X
ethyl butyrate	803[C]	ester	X	X	X	X
butyl acetate	[A]	ester	X	X		
coffee furanone	814[D]	lactone	X	X		
butyric acid	820[D]	acid	X	X		X
furfural	836[D]	aldehyde	X	X		
ethyl 2-methylbutanoate	850[C]	ester	X	X	X	X
ethyl 3-methylbutanoate	856[D]	ester	X	X		
butanol	[A]	alcohol	X	X		
isoamyl acetate	880[D]	ester	X	X		X
propyl butyrate	[A]	ester	X	X		
ethyl valerate	900[D]	ester	X	X		X
pentanoic acid	910[D]	acid	X	X		X
2-acetylfuran	912[D]	ketone	X	X		X
benzaldehyde	962[D]	aldehyde	X	X		X
ethyl hexanoate	1000[D]	ester	X	X		X
hexyl acetate	1008[D]	ester	X	X		
ethyl lactate	[A]	ester	X	X		
caproic acid	[A]	acid	X	X		
isoamyl butyrate	[A]	ester	X	X		
guaiacol	1088[C]	phenol		X	X	X
ethyl heptylate	1097[D]	ester	X	X		
2-phenylethyl alcohol	1111[C]	alcohol			X	X
ethyl benzoate	1173[D]	ester	X	X		
diethyl succinate	1179[D]	ester	X	X		X
4-methyl guaiacol	1191[D]	phenol	X	X		X
ethyl octanoate	1195[D]	ester	X	X		
β-phenethyl acetate	1255[C]	ester		X	X	X
octanoic acid	1279[D]	acid	X	X		X
4-ethylguaiacol	1279[C]	phenol		X	X	X
ethyl nonanoate	1296[D]	ester	X	X		
eugenol	1368[C]	phenol	X	X	X	X
β-damascenone	1383[C]	ketone			X	X
ethyl decanoate	1396[D]	ester	X	X		X
vanillin	1407[C]	phenol		X	X	X
ethyl laurate	1594[D]	ester		X		X

A: Maarse et al. 1966 ; B: Liebich et al. 1970 ; C: de Souza et al. 2004 ; D: Pino et al. 2012

Esters account for about half of the commonly identified compounds in rum (Table 2.2); alcohols come in a far second, and the remainder of compounds are approximately evenly divided between acids, aldehydes, ketones, and phenols. This does not necessarily indicate that esters are the most important or even the most abundant aroma compounds, only that they are the most diverse group. It would seem, however, that esters are considered to be some of the most important distinguishing compounds in rum, with the existence of ester number, or mg ester per 100 mL ethanol, as an established classification method for different types of rum (Lehtonen and Suomalainen 1977).

2.2. Rum Ether

2.2.1. History

There is a notable lack of information available in the case of rum ether, in particular where its history is concerned. Mentions of the substance can be found in books dating back to at least 1857, at which time a short formula was published in George William Septimus Piesse's exhaustively titled work *The Art of Perfumery and Method of Obtaining the Odors of Plants: With Instructions for the Manufacture of Perfumes for the Handkerchief, Scented Powders, Odorous Vinegars, Dentifrices, Pomatums, Cosmetiques, Perfumed Soap, Etc.: with an Appendix on the Colors of Flowers, Artificial Fruit Essences, Etc., Etc.* Here it is described as a reaction product of black oxide of manganese, sulfuric acid, alcohol, and strong acetic acid that was responsible for the "peculiar" flavor of rum (Piesse 1857). Similar descriptions can be found in other manuals from around the same time, but it is difficult to locate anywhere an account of how or when rum ether was first discovered.

2.2.2. Production

A modern formula for rum ether is given in Fenaroli's *Handbook of Flavor Ingredients*:

Ethanol (95%)	12-25 kg
Manganese Dioxide	2-5 kg
Pyroligneous Acid (12% Acetic Acid)	10-16 kg
Sulfuric Acid (66 °Be)	3-8 kg

One at a time and in order, each ingredient is stirred into a flask attached to a distillation apparatus. Once everything has been added, the mixture is slowly heated; everything distilling between 60°C and 100°C is collected. This mixture is then further rectified, with only the portion boiling between 65°C and 87°C being used for the finished rum ether. The finished product has been noted for its qualitative similarity to rum, and has thus been incorporated into a number of sweets and beverages to lend rummy notes. The concentration of use varies greatly depending on the product. In ice cream, for example, its use generally remains close to 100 ppm, while in alcoholic beverages it may reach two or three percent of the finished product (Burdock 2010).

2.2.3. Aroma Research

A search of the literature reveals no relevant work in regards to rum ether. The limited information available again comes from handbooks, which list the bulk constituents of a typical rum ether: water, ethyl alcohol, ethyl acetate, methanol, ethyl formate, acetone, acetaldehyde, and formaldehyde. No source is provided for this information, and nothing is included about the more minor constituents that are responsible for the flavor of rum ether, making it a compelling subject (Burdock 2010).

2.3. Aroma Analysis Techniques

2.3.1. Liquid-Liquid Continuous Extraction

In flavor analysis of food and beverage volatiles, it is often necessary to remove only the portion responsible for the aroma – the volatile compounds – from the sample matrix and concentrate these to make them detectable by laboratory instruments (Plutowska and Wardencki 2007). One way of doing this is liquid extraction. In liquid-liquid continuous extraction (LLCE), an immiscible solvent with a low boiling point is continuously refluxed through the sample of interest, slowly transferring the volatiles in the sample matrix into the solvent. Only a tiny fraction of the volatile components will be transferred with each drop of solvent falling through the sample, but with repetition of this process for up to a day, the extraction of volatiles is significant (MacNamara and Hoffman 1998). The cycle of continuous evaporation and condensation allows for a more complete transfer of volatiles than traditional liquid extraction methods. This addresses one of the biggest problems with solvent extraction – extraction bias. In traditional extraction, those compounds that have high affinity for the solvent matrix will be disproportionately represented in the finished extract. The prolonged contact in LLCE allows for those compounds with a lower affinity for the solvent to be more fully extracted. Although extraction bias cannot be eliminated entirely – some compounds will still be over- or underrepresented – LLCE takes a step in the right direction (Buglass 2011).

2.3.2. Aroma Extract Dilution Analysis

It is often necessary in flavor analysis to not merely identify volatile compounds but to offer some suggestions about which ones might be the biggest contributors to the flavor. One

way to do this is to determine both odor detection thresholds and concentrations of each compound in the sample, but this can be extremely time-consuming and may not be necessary in all cases. For some samples, it is sufficient to use a dilution technique such as aroma extraction dilution analysis (AEDA) or combined hedonic aroma response measurement (CHARM). Of these, AEDA is the more commonly used method (Plutowska and Wardencki 2007). AEDA was first detailed by Dr. Werner Grosch in 1993. In his method, he describes how a flavor extract is serially diluted and each dilution analyzed by gas chromatography-olfactometry. Each compound is then assigned a flavor dilution (FD) factor that represents the most dilute sample in which it was detected, e.g., a compound sensed only until a 1:32 dilution would have an FD factor of 32. Dr. Grosch describes the drawbacks of AEDA as well. Because it depends on detection in an extract, extraction bias can skew the FD factors; additionally, many people may differ in their sensitivity to certain compounds and therefore not all assign the same FD factor to the same compound. Despite these setbacks, AEDA remains a very popular method for analysis of relative importance of volatiles in a given sample (Plutowska and Wardencki 2007).

2.4. References

Barr, A. *Drink: A Social History of America*. Carroll & Graf Publishers, Inc.: New York, 1999.

Blue, A.D. "Rum." *The Complete Book of Spirits: A Guide to Their History, Production, and Enjoyment*. HarperCollins: New York, 2004. 69-103.

Buglass, A.J. *Handbook of Alcoholic Beverages: Technical, Analytical and Nutritional Aspects.* John Wiley and Sons: Sussex, UK, 2011.

Burdock, G.A. *Fenaroli's Handbook of Flavor Ingredients*. 6th ed. CRC Press: Boca Raton, FL, 2009.

Corrigan, J. "The distilled spirits industry: where to now?" *Distilled Spirits: Tradition & Innovation*. Bryce, J.H.; Stewart, G.G. Eds. Nottingham UP: Nottingham, UK, 2004. 245-56.

Delevante, M.P. "Rum." *Distilled Spirits: Tradition & Innovation*. Bryce, J.H.; Stewart, G.G., Eds. Nottingham University Press: Nottingham, UK, 2004. 209-14.

Gately, I. *Drink: A Cultural History of Alcohol*. Gotham Books: New York, 2008.

Grosch, W. *"*Detection of potent odorants in foods by aroma extract dilution analysis.*" Trends in Food Science and Technology.* 1993, *4.3*, 68-73.

Lehtonen, M.; Suomalainen, H. "Rum." *Alcoholic Beverages.* Rose, A.H., Ed. Academic: New York, 1977. 595-633.

Liebich, H.M.; Koenig, W.A.; Bayer, E. "Analysis of the flavor of rum by gas-liquid chromatography and mass spectrometry." *Journal of Chromatographic Science.* 1970, *8*, 527-33.

Maarse, H.; ten Noever de Brauw, M.C. "The Analysis of Volatile Components of Jamaica Rum." *Journal of Food Science.* 1966, *31*, 951-5.

MacNamara, K.; Hoffmann, A. "Gas Chromatographic Technology in Analysis of Distilled Spirits." *Instrumental Methods in Food and Beverage Analysis*. Wetzel, D.; Charalambous, G., Eds. Elsevier Science: Amsterdam, 1998. 303-346.

Ng, L-K. "Analysis of Vodkas and White Rums by SPME-GC-MS." *Applications of Solid Phase Microextraction*. Pawliszyn, J.; Smith, R.M. Eds. Royal Society of Chemistry: Cambridge, UK, 1999. 393-406.

Nykänen, L.; Puputti, E.; Suomalainen, H. "Volatile Fatty Acids in Some Brands of Whisky, Cognac, and Rum." *Journal of Food Science.* 1968, *33*, 88-92.

Park, P. "The Supply Side of Drinking: Alcohol Production and Consumption in the United States Before Prohibition." *Contemporary Drug Problems.* 1985, *12*, 473-509.

Persad-Doodnath, V. "From sugar to rum – the technology of rum making." *Distilled Spirits: Production, Technology, & Innovation*. Bryce, J.H.; Piggott, J.R.; Stewart, G.G. Eds. Nottingham University Press: Nottingham, UK, 2008. 159-67.

Piesse, G.W.S. *The Art of Perfumery*. Lindsay and Blakiston: Philadelphia, 1857.

Pino, J.A.; Tolle, S.; Gök, R.; Winterhalter, P. "Characterisation of odor-active compounds in aged rum." *Food Chemistry.* 2012, *132*, 1436-1441.

Pino, J.A. "Characterization of rum using solid-phase microextraction with gas chromatography–mass spectrometry." *Food Chemistry*. 2007, *104*, 421-8.

Pino, J., et al. "Headspace solid-phase microextraction of higher fatty acid ethyl esters in white rum aroma." *Journal of Chromatography A.* 2002, *954*, 51-7.

Plutowska, B.; Wardencki, W. “Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – A review.” *Food Chemistry.* 2008, *107*, 449-63.

de Souza, M.D.C.A.; Vásquez, P.; del Mastro, N.L.; Acree, T.E.; Lavin, E.H. "Characterization of Cachaca and Rum Aroma." *Journal of Agricultural and Food Chemistry*. 2006, *54*, 485-8.

Timmer, R.; ter Heide, R.; Wodden, H.J.; de Valois, P.J. "Phenolic Compounds in Rum." *Journal of Food Science.* 1971, *36*, 462-3.

United States. Office of the Federal Register. "Title 27, Part 5.22." *Electronic Code of Federal Regulations*. National Archives and Records Administration. <http://ecfr.gpoaccess.gov/>.

Wilson, C. A. *Water of Life*. Prospect Books: London, 2006.

Wobben, H.J.; Timmer, R.; ter Heide, R.; de Valois, P.J. “Nitrogen Compounds in Rum and Whiskey.” *Journal of Food Science.* 1971, *36*, 464-5.

Chapter Three:

Characterization of Rum Aroma

3.1. Introduction

Although research into the flavor profile of rum can be found in the literature, none of what is currently available provides a complete picture of rum aroma. Much of the work focused only on specific classes of aroma compounds, and only two researchers have attempted to study not just the presence or absence of compounds but their relative importance. Even these two studies looked only at the twenty most potent odorants in their respective samples, which captures the essential notes but still leaves out more than half of detectable rum volatiles (de Souza et al. 2006, Pino et al. 2012).

In order to determine how rum ether is both similar to and different from rum, it was important to have a characterization of rum aroma that was complete, one that included not just the primary rum volatiles, but the minor ones as well. For direct comparison of rum and rum ether data, it was also necessary to have collected information on both in a consistent fashion. With these goals in mind, it was deemed necessary to determine the odorants in both white and gold variants of the most commonly found rum brand. While this decision was made primarily for its necessity in rum ether analysis, the research was worthwhile in its own right as a contribution to the current knowledge of rum, one that both confirms what is already available and adds to the discussion.

A combination of gas chromatography-olfactometry and gas chromatography-mass spectrometry were used to identify the odor-active compounds in rum extracts. Dilution analysis was employed for determining which of these were most and least important in the overall aroma. These techniques produced a clearer profile of rum volatiles than was otherwise available; this profile was essential in evaluating rum ether.

3.2. Materials and Methods

3.2.1. Materials

Two commercial rums, Bacardi Gold and Bacardi Superior, were chosen as representative samples based on their widespread popularity in the U.S. (Longfield 2011). Both products were reported to contain 40% alcohol by volume and fit the U.S. standard of identity for rum ("Liquors" 2011). Bacardi is mentioned for the purpose of being thorough but was in no way affiliated with this research.

Dichloromethane and anhydrous sodium sulfate were both purchased from Fisher Scientific (Fair Lawn, NJ).

Aroma standards were obtained from the following sources: Baker (Phillipsburg, NJ): 9, 14; Bedoukian (Danbury, CT): 44; Fisher Scientific: 23; Fluka (Switzerland): 31, 45; Sigma Aldrich Co. (St Louis, MO): 1,2, 4-6, 10, 11, 13, 17-20, 22, 25, 26, 28-30, 33-36, 38, 39, 41, 43, 46.

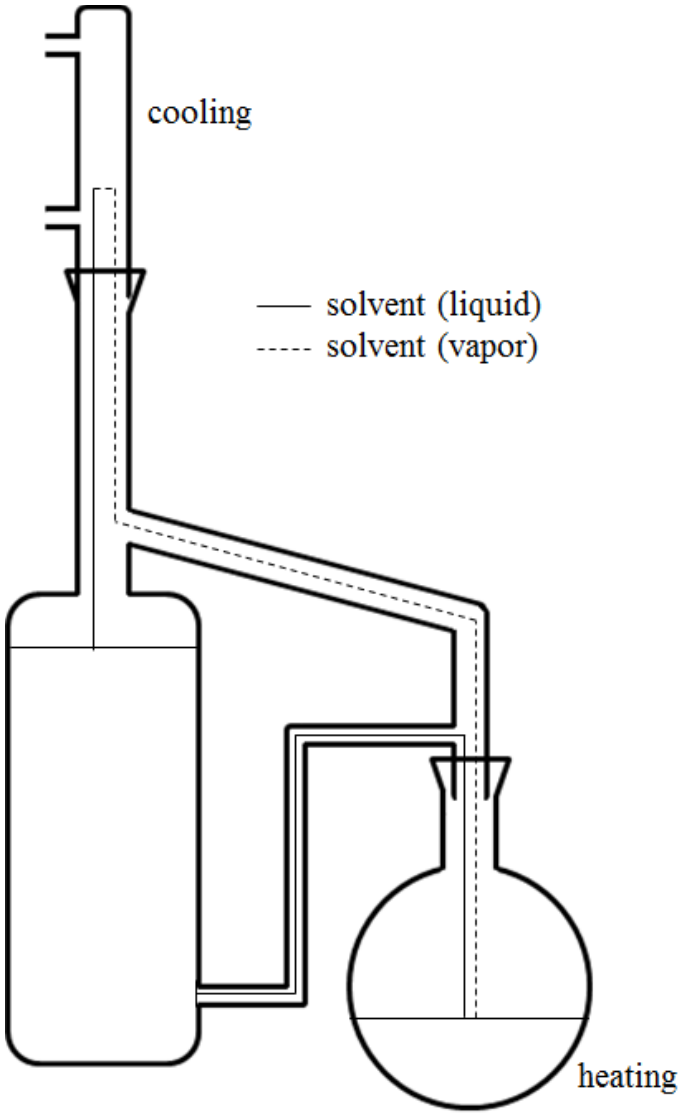
3.2.2. Static Headspace Analysis

Gas chromatography-olfactometry analysis of decreasing static headspace volumes (GCO-H) was modeled after the method described by Zhou et al. for buckwheat honey (2002). A

sample of 10.0 mL of rum was diluted with 10.0 mL of odor-free water to obtain an ethanol concentration of 20% (v/v). The diluted sample was placed in a 250 mL flask fitted with a septum and the headspace allowed to equilibrate with stirring in a warm water bath at 40°C for 30 minutes. After equilibration, a sample of the headspace of 25 mL, 5 mL, 1 mL, or 0.2 mL was withdrawn with a gastight syringe for analysis by GCO.

3.2.3. Liquid-Liquid Continuous Extraction

Figure 3.1. Liquid-liquid continuous extraction (LLCE) set-up



Aroma extracts were obtained by continuous liquid-liquid extraction (LLCE, Figure 3.1). A continuous extraction apparatus (#Z562440; Sigma Aldrich, St Louis, MO) was attached to both a 7-inch-long condenser and a 300 mL receiving flask. The condenser was cooled to a temperature of 4° C, while the flask was heated to a temperature appropriate for steady evaporation of solvent. To the system were added 150 mL of dichloromethane as the solvent, 100 mL of rum, and 425 mL of deodorized water.

Dichloromethane was refluxed through the diluted rum for 18 hours. The dichloromethane fraction was collected and dried over anhydrous sodium sulfate. This extract was then purified using solvent-assisted flavor evaporation (SAFE).

3.2.4. Solvent-Assisted Flavor Evaporation

Solvent-assisted flavor evaporation (SAFE), a method for high vacuum distillation, was used on the dichloromethane extracts as a means of separating volatile compounds from non-volatiles present. SAFE was applied using the technique described by Song et al. (2008), which is based on the method given by Engel et al. (1999). The SAFE set-up was similar to that used by Engel; it comprised a high-vacuum pump, a turbo-pump, a receiving trap, and a waste trap. The distillation lasted two hours and was maintained at a low pressure of roughly 10^{-5} torr throughout this time.

3.2.5. Gas Chromatography-Olfactometry

Gas chromatography-olfactometry (GCO) analysis was completed using an Agilent 6890 Gas Chromatograph (Agilent, Santa Clara, CA) outfitted with a Gerstel Olfactometry Detection Port and CIS-4 Programmable Temperature Vaporizer (PTV) inlet (Mulheim an der Ruhr, Germany), as well as a Flame Ionization Detector. Two columns were used for all samples: a

polar RTX-Wax and non-polar RTX-5, both from Restek (Bellefonte, PA). Both columns were 15 m in length, with an inner diameter of 0.53 mm and a film thickness of 1 μm.

Total GCO runtime was 38.5 min. Helium, with a flow rate of 21.8 mL/min, was used as the carrier gas. Cold splitless injection was used. For vapor (headspace) samples, the CIS-4 inlet was programmed to -120°C for injection and held there for 0.10 min, at which point it was increased to a final temperature of 250°C at a rate of 12°C/s. For liquid (extract) samples, the inlet was cooled to -50°C prior to injection and held at this temperature for 0.10 min, at which point it was increased to a final temperature of 260°C at a rate of 10°C/s. For both vapor and liquid samples, the GC oven was initially brought to 40°C, held for 5.00 min, increased at 10.0°C/min until reaching 225°C, and finally held at this temperature for 15.00 min.

The exit flow from the column was split between the Flame Ionization Detector and the Olfactometry Detection Port. Each sample was tested by two individuals, who recorded for each aroma an elution time, one or more descriptors, and a perceived odor strength. In addition to the samples, a series of standard alkanes ranging from 6 to 28 carbons was injected in order to calculate retention indices.

3.2.6. Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted using an Agilent 6890 GC with Gerstel CIS-4 PTV inlet paired with a Hewlett-Packard 5973 mass spectrometer. Samples were analyzed using both a polar RTX-Wax and a non-polar RTX-5 column (Restek), both with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 μm. The National Institute of Science and Technology (NIST) database was used for comparison against experimental mass spectra.

GC-MS analysis lasted 71.25 minutes. Helium, with a flow rate of 1.0 mL/min, was used as the carrier gas. Samples were injected in cold splitless mode at an initial inlet temperature of -50°C. This was held for 0.10 min, at which point it was increased to a final temperature of 260°C at a rate of 12°C/s. The GC oven was programmed to hold at 40°C for 5 min, increase by 4°C/min to 225°C, and then hold this temperature for an additional 20 min. The entirety of the gas chromatograph's outlet flow was directed to the mass spectrometer for analysis in scan mode.

3.2.7. Identification of Volatiles

Volatile compounds were identified using a combination of GCO and GC-MS data and confirmed using authentic standards. For each volatile detected using GCO, a retention index was calculated using the elution time of both the volatile and the surrounding standard alkanes. The formula was as follows:

$$RI = RI_n + (RI_N - RI_n)\frac{RT - RT_n}{RT_N - RT_n}$$

where RI indicates retention index and RT retention time, and the subscripts indicate the target compound (no subscript), the alkane directly preceding this compound (n), and the alkane directly following this compound (N). Each alkane is assigned a retention index equal to its carbon chain length multiplied by 100, e.g. hexane has a retention index of 600.

Each compound was tentatively identified using its retention indices on both a polar and non-polar phase, as well as the odor impressions recorded. These identifications were considered positive if they could be confirmed using at least one mass spectra database match on the GC-

MS (either polar or non-polar phase) as well as a match in both retention index and odor impression to an authentic standard on the GCO.

3.2.8. Aroma Extract Dilution Analysis

Relative importance of volatiles was determined using Aroma Extract Dilution Analysis (AEDA, Grosch 1993). A series was prepared of increasing 1:3 extract dilutions in dichloromethane, ranging from a concentrated extract to a dilution of 1:2187, at which point no additional volatiles were detected. Each dilution was analyzed using GCO. Each of the compounds identified in the extracts was assigned a flavor dilution (FD) factor that corresponded to the last dilution at which it was detected. Those compounds with the highest FD factor were considered most crucial in creating characteristic rum aroma.

3.3. Results

3.3.1. Static Headspace Analysis

Nine compounds were identified using static headspace analysis of gold rum; six of these were also detected in white rum (Table 3.1). The compound with the highest FD factor in both rum samples was acetaldehyde. More than half of the compounds identified were esters.

Table 3.1. Compounds identified by GCO-H and their FD factors for gold and white rum

Compound	RI (Wax)	RI (DB5)	Aroma	FD[a] (Gold)	FD[a] (White)
1. acetaldehyde	619	<500	sweet, pungent	4	4
2. 2-methyl propanal	779	545	dark chocolate	3	3
3. ethyl propanoate	884	733	fruity	2	2
4. 3-methyl butanal	904	656	dark chocolate	3	2
5. ethyl isobutyrate	945	761	fruity	3	2
7. ethyl butyrate	1028	813	fruity	1	--
8. ethyl 2-methylbutyrate	1046	861	blueberry	1	--
9. ethyl 3-methylbutyrate	1065	861	blueberry	1	1
23. β-damascenone	1910	1396	applesauce	1	--

a: FD factors are $\log_5$ and were found on a wax column.

3.3.2. Aroma Extract Dilution Analysis

In total, forty-four compounds were detected in the undiluted gold rum extract by AEDA; of these, thirty-six were smelled in the white rum extract (Table 3.2). No additional aroma compounds were found in the white rum. Thirty-one of the forty-four volatiles were able to be positively identified. Of the remaining thirteen, three were tentatively identified, and ten remain unidentified. More than a third of the odorants were esters, with the remainder comprising acids, alcohols, and various carbonyls. Both gold and white rum had detectable odorants through a dilution of 1:729, which corresponded to a $\log_3$ FD factor of 7. In gold rum, both ethyl propanoate and phenethyl alcohol could be found at this dilution, while in white rum only ethyl propanoate was sensed.

Table 3.2. Compounds identified by AEDA and their FD factors for gold and white rum

Compounds	RI (Wax)	RI (DB5)	Aroma	FD[a] (Gold)	FD[a] (White)
2. 2-methyl propanal	<900	<700	malty, dark chocolate	3	3
4. ethyl propanoate	926	715	caramel, fruity	7	7
5. 3-methyl butanal	933	<700	malty	4	4
6. ethyl isobutyrate[b]	974	758	butterscotch	5	1
9. 1-propanol	1003	705	dirty	2	1
10. ethyl butyrate	1036	806	sweet, fruity	4	1
11. ethyl 2-methylbutyrate	1054	844	berry	2	2
13. ethyl 3-methylbutyrate	1068	856	fruity, berry	4	4
14. isobutanol	1100	<700	malty	3	1
U1. *unknown*	1127	747	fruity	4	2
17. isoamyl acetate[c]	1135	--	banana	1	2
U3. *unknown*	1188	925	berry	2	4
18. isoamyl alcohol	1215	732	malty	6	6
U4. *unknown*	1224	943	pool water	1	1
19. ethyl hexanoate	1232	995	fruity	1	--
20. 2,5-dimethyl pyrazine	1327	906	woody	3	2
21. 2-acetyl-1-pyrroline[d]	1339	928	popcorn	3	3
U6. *unknown*	1417	1102	fruit	1	1
22. ethyl octanoate	1424	1190	solvent, fresh	2	--
23. acetic acid	1437	<700	vinegar	5	4
U7. *unknown*	1551	1070	berry	1	1
25. propanoic acid	1564	--	fruity, sweaty	1	--
26. butyric acid	1621	801	cheesy	1	1
28. 3-methylbutyric acid	1665	873	sweaty	1	1
29. 2-methylbutyric acid[d]	1673	873	cheesy	1	--
30. 2-methylpentanoic acid	1745	979	sweaty	1	1
U11. *unknown*	1804	--	minty	1	--
31. β-damascenone	1816	1390	apple juice	6	2
33. guaiacol	1843	1093	spearmint	5	3
34. (E)-oak lactone	1881	1339	honey	3	2
35. phenethyl alcohol	1899	1115	floral	7	6
36. (Z)-oak lactone	1947	1330	woody!	6	6
38. 4-ethylguaiacol	2014	1284	brown spice	3	2
39. p-cresol[d]	2070	1088	overrun motor	3	--
U15. *unknown*	2096	1224	dirty	5	2
U16. *unknown*	2102	1354	floral	1	--
41. 4-ethylphenol	2168	1175	burnt motor	3	3
43. eugenol	2176	1365	sweet brown spice	1	2
44. decanoic acid[c]	2257	--	bandage	1	2
U19. *unknown*	2333	1460	floral	3	1
U20. *unknown*	2437	1473	fruity, juicy	1	--
45. vanillin	2539	1407	marshmallow	5	4
46. ethyl vanillate	2613	1574	floral	3	2

a: FD factors are $\log_3$ and were found on a wax column. b: The butterscotch aroma was prolonged and potentially was the result of multiple odorants, including 2,3-butanedione, which was also detected by mass spectrometry. c: Compound only tentatively identified (mass spectral data unable to be matched).

3.4. Discussion

Static headspace analysis identified only one compound – acetaldehyde – that was not also detected by AEDA. All other compounds found by GCO-H had extract FD factors of at least 2. Although the identification of acetaldehyde provides one additional piece of information with regards to rum flavor, this was deemed not significant enough to justify subjecting all remaining samples – the commercial and self-prepared rum ethers – to GCO-H.

The aroma profile determined by AEDA shows a great deal of agreement with the literature available on rum aroma. Of the compounds identified, all but four had been previously written about as rum odorants; the most significant odorants – all those in gold rum with a flavor dilution (FD) factor of at least 4 – had all been featured in at least two significant rum papers (those appearing in Table 2.2).

In addition to agreement on identity of compounds, the data agreed with the information available on their importance. In de Souza et al. (2006), β-damascenone was found to be the most important odorant. Although it was not the most important in our samples, it was an important odorant, at least for gold rum. Many of the others documented in this work were found to be major contributors in these samples as well, including ethyl 2-methylbutyrate, ethyl isobutyrate, vanillin, ethyl butyrate, and phenethyl alcohol. In the more recently published Pino et al. (2012), AEDA was used to identify the most important odorants (those detected in at least a 1:32 dilution) in rum aged fifteen years. Although this data would not be expected to have perfect agreement with AEDA data for gold and white rums, which are aged for a much shorter length of time, one would expect some overlap to be present, and it is. All five of the volatiles

found by Pino et al. to be most important in aged rum – ethyl butyrate, ethyl hexanoate, β-damascenone, (Z)-oak lactone, and vanillin – were found in both gold and white rum extracts, and in all cases but ethyl hexanoate were also seen as important in these.

It is also worth taking the time to compare rum to literature data for other alcoholic beverages. There is bound to be some overlap between rum volatiles and those of other fermented, distilled beverages; those compounds found in rum but not in other beverages could be seen as especially important for setting rum apart from other similar products. Although the literature contains limited AEDA data for other liquors, there is some information available. As might be expected by the common production step of barrel aging, both whiskey and rum share many wood extractives. In 2008, Poisson and Schieberle reported many compounds in bourbon whiskey that were also found in rum, including guaiacol, vanillin, and both oak lactones. However, whiskey appears to contain additional wood-derived lactones not found in rum, likely as a result of the lengthier aging time demanded of most whiskeys. Where whiskey fell short in comparison to rum was in ester content, especially branched esters. Tequila analysis by AEDA also reported far fewer detectable esters than rum (Benn and Peppard 1996). This would indicate that esters are an important group of compounds to look at when determining whether or not a model rum system is indeed rum-like, since they appear to set rum apart from other spirits.

This is a logical distinction between rum and other liquors. The step that most differentiates rum from other beverages is fermentation, as both the raw materials – molasses or sugar cane juice – and yeast used in the fermentation step differ from those used for whiskey or tequila. This is where one would expect the biggest distinctions between the various distilled beverages to emerge; it would make sense then that the compounds responsible for setting rum apart would be formed in this step. Research would indicate that, as expected, formation of esters

is influenced most heavily by the fermentation stage; in this step, yeast facilitates the formation of fatty acids, which then undergo esterification. One study additionally found that rum had a higher concentration of many of the short chain fatty acids than whiskey or cognac (Lehtonen and Suomalainen 1977). This would explain the high dilution factors for their derivative esters, odorants like ethyl propanoate, ethyl isobutyrate, and ethyl butyrate, as well as the higher ester content in general with respect to other alcohols.

It is important as well to compare the rums not only to the literature but to one another in order to understand the variation that exists among this particular product. In comparing the two rums, it is clear that white was very similar to gold in overall profile, but simply less strong in many compounds, which is in line with what is known about the rum production process. Since both rums are from the same brand and one that produces rum in mass quantities, it is likely that neither were aged for more than a couple of years. The gold rum, however, was likely aged a short while longer, leading to more overall complexity. The white rum has also almost certainly undergone charcoal filtration to remove any coloration, which can remove some aroma compounds in addition to pigmentation. The biggest difference between the two samples is found with β-damascenone, which has an FD factor of 6 in the gold rum extract, making it one of the five most potent odorants, but only an FD factor of 2 in the white rum extract. Because β-damascenone reportedly has a very low odor threshold (Pino et al. 2012), even a small difference in concentration could dramatically impact detection of this compound. It is also the product of degradation of plant extractives, so a shorter aging time could be responsible for such a difference. The next chapter will examine how these two rums compare to rum ether samples, both commercial and self-prepared.

3.5. References

Benn, S.M.; Peppard, T.L. “Characterization of Tequila Flavor by Instrumental and Sensory Analysis.” *Journal of Agricultural and Food Chemistry*. 1996, *44*, 557-66.

Engel, W.; Bahr, W.; Schieberle, P. “Solvent assisted flavour evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices.” *European Food Research and Technology*. 1999, *209*, 237-41.

Grosch, W. *“*Detection of potent odorants in foods by aroma extract dilution analysis.*” Trends in Food Science and Technology.* 1993, *4.3*, 68-73.

Lehtonen, M.; Suomalainen, H. “Rum.” *Alcoholic Beverages.* Rose, A.H., Ed. Academic: New York, 1977. 595-633.

"Liquors." *Code of Federal Regulations: Title 27 - Alcohol, Tobacco Products and Firearms*. U.S. Government Printing Office. http://www.gpo.gov/fdsys/pkg/CFR-2011-title27-vol1/xml/CFR-2011-title27-vol1-sec5-22.xml.

Longfield, David. “Millionaires 2011: Rum and Cachaça.” *Drinks International.* 2011, *7*, 33.

Pino, J.A.; Tolle, S.; Gök, R.; Winterhalter, P. “Characterisation of odor-active compounds in aged rum.” *Food Chemistry.* 2012, *132*, 1436-1441.

Poisson, L.; Schieberle, P. “Characterization of the key aroma compounds in an American Bourbon whisky by quantitative measurements, aroma recombination, and omission studies.” Journal of Agricultural and Food Chemistry. 2008, *56*, 5820-6.

Song, H.; Cadwallader, K.R.; Singh, T.K. “Odour-active compounds of Jinhua ham.” *Flavour and Fragrance Journal*. 2008, *23*, 1-6.

de Souza, M.D.C.A.; Vásquez, P.; del Mastro, N.L.; Acree, T.E.; Lavin, E.H. "Characterization of Cachaca and Rum Aroma." *Journal of Agricultural and Food Chemistry*. 2006, *54*, 485-8.

Zhou, Q.; Wintersteen, C.L.; Cadwallader, K.R. “Identification and quantification of aroma-active components that contribute to the distinct malty flavor of buckwheat honey.” *Journal of Agricultural and Food Chemistry* 2002, *50*, 2016-21.

Chapter Four:

Characterization of Rum Ether Aroma

4.1. Introduction

Rum ether is an interesting and enigmatic substance. It has been used for nearly two centuries, but its origins remain a mystery. It is produced from an unusual combination of ingredients that would seem unfit for human consumption, yet someone saw it a suitable addition to foods long before chemical analysis confirmed this was true, and it has since been given the Flavor and Extract Manufacturers Association's (FEMA) Generally Recognized as Safe (GRAS) status and an "all natural" designation (Hall and Oser 1965). Its ingredients furthermore are completely unrelated to the raw materials used for rum, and still it has been universally recognized as mimicking rum. And although it is widely used by flavor chemists for creating rum-like notes, there is no information available in the literature that confirms its rummy character.

The current body of knowledge contains only mentions of the components of rum ether that make up its bulk phase; its odor-active compounds go completely undocumented. It would be valuable for the flavor industry to have a thorough characterization of rum ether. Not only could this confirm the underlying assumption that has driven the use of rum ether – that it is similar to rum – but it could also help with understanding what the similarities actually are and how to compensate for the differences.

To this end, ten commercial rum ethers were analyzed by direct injection gas chromatography-olfactometry (GCO) and GC-MS to determine those aroma compounds that were common among commercial products. Two rum ethers were also prepared in the lab with a widely available wood extractive – liquid smoke – replacing the traditionally used pyroligneous acid; these were compared with commercial products to determine the fitness of liquid smoke as a substitute ingredient. Self-prepared rum ether volatiles were also analyzed using extractive methods, in particular aroma extract dilution analysis (AEDA). The results of AEDA were compared with rum results obtained in the same manner in order to ultimately determine how rum ether aroma components compare to those of rum.

4.2. Materials and Methods

4.2.1. Materials

Ten commercially available rum ethers were obtained from a number of flavor houses and ingredient supply companies. Of these, seven rum ethers – Advanced Biotech 1206 (Paterson, NJ), Bell 1075ATF (Northbrook, IL), Berje 71169 (Bloomfield, NJ), Fleurchem (Middletown, NY), Mission RU-107 (Foothill Ranch, CA), Ungerer 200048 (Lincoln Park, NJ), Wild FALJ509 (Erlanger, KY) – were labeled as natural and the remaining three - Advanced Biotech 1440, Bell 129.11840, Virginia Dare 23635 (Brooklyn, NY) – as artificial or imitation rum ethers.

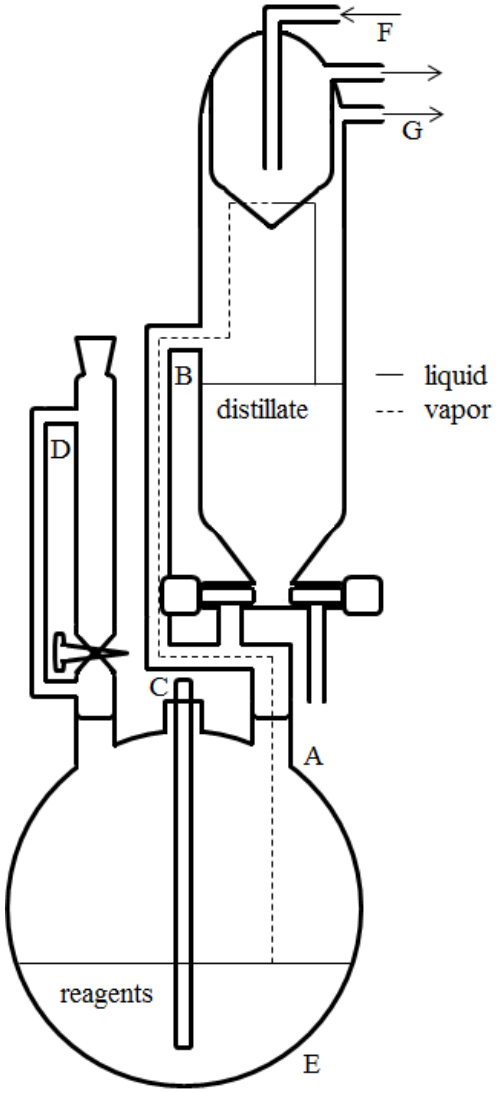
For production of self-prepared rum ethers, liquid smoke was obtained from Red Arrow Products (Manitowoc, WI). Manganese (IV) oxide came from Sigma Aldrich (St. Louis, MO). The remaining components – absolute ethanol, glacial acetic acid, sulfuric acid, and activated carbon – were acquired from Fisher Scientific (Fair Lawn, NJ).

Dichloromethane and anhydrous sodium sulfate were both purchased from Fisher Scientific.

Aroma standards were obtained from the following sources: Baker (Phillipsburg, NJ): 3; Fisher Scientific: 23; Fluka (Switzerland): 46; Mallinckrodt (St. Louis, MO): 37; Sigma Aldrich Co.: 1, 2, 4-7, 10-13, 16, 22, 34, 36, 38, 32-36, 40-42; TCI (Portland, OR): 27.

4.2.2. Rum Ether Distillation

Figure 4.1. Apparatus for bench scale distillation of rum ether



The distillation system for rum ether (Figure 4.1) consisted of a three-necked 3000 mL flask (A) attached to a solvent repurification distillation apparatus (B, Kimble-Chase, Vineland, NJ), a thermometer for monitoring distillation temperature (C), and a drop funnel for addition of chemicals (D). The flask was held in a mantle that provided both heat and magnetic stirring (E). The solvent repurification distillation apparatus was maintained at 4.0°C throughout the process by pumping coolant through the top (F). Ventilation of extremely volatile compounds – those that would not condense at 4°C – was also provided at the top (G).

Chemicals were added to the flask with stirring in the following order and amounts: 375 mL liquid smoke, 5.25 g activated carbon, 0.65 g manganese dioxide, 333.75 mL absolute ethanol diluted with 41.25 mL deodorized water, 72 mL glacial acetic acid diluted with 50 mL deodorized water, 17 mL sulfuric acid. Once all components had been added and temperature equilibrated, the mantle was turned on and the heat was slowly increased until the thermometer registered 75°C.

At this point, one of two paths was taken. For original method rum ether, distillate began to be collected as soon as the target temperature of 75°C was reached. For reflux rum ether, the reaction mixture was allowed to reflux through the system for 24 hrs once the target temperature was reached; after 24 hrs, distillate was collected. In both cases, the target distillate volume was 375 mL.

4.2.3. Liquid-Liquid Continuous Extraction (LLCE)

Aroma extracts of self-prepared rum ethers were obtained by continuous liquid-liquid extraction (LLCE, Figure 3.1). A continuous extraction apparatus (#Z562440; Sigma Aldrich, St Louis, MO) was attached to both a 7-inch-long condenser and a 300 mL receiving flask. The

condenser was cooled to a temperature of 4° C, while the flask was heated to a temperature appropriate for steady evaporation of solvent. To the system were added 150 mL of dichloromethane as the solvent, 2 mL of rum ether, and 523 mL of deodorized water.

Dichloromethane was refluxed through the diluted rum ethers for 18 hours. The dichloromethane fraction was collected and dried over anhydrous sodium sulfate. This extract was then purified using solvent-assisted flavor evaporation (SAFE).

4.2.4. Solvent-Assisted Flavor Evaporation

Solvent-assisted flavor evaporation (SAFE), a method for high vacuum distillation, was used on the dichloromethane extracts as a means of separating volatile compounds from non-volatiles present. SAFE was applied using the technique described by Song et al. (2008), which is based on the method given by Engel et al. (1999). The SAFE set-up was similar to that used by Engel; it comprised a high-vacuum pump, a turbo-pump, a receiving trap, and a waste trap. The distillation lasted two hours and was maintained at a low pressure of roughly 10^{-5} torr throughout this time.

4.2.5. Gas Chromatography-Olfactometry

Gas chromatography-olfactometry (GCO) analysis was completed using an Agilent 6890 Gas Chromatograph (Agilent, Santa Clara, CA) outfitted with a Gerstel Olfactometry Detection Port and CIS-4 Programmable Temperature Vaporizer (PTV) inlet (Mulheim an der Ruhr, Germany), as well as a Flame Ionization Detector. Two columns were used for all samples: a polar RTX-Wax and non-polar RTX-5, both from Restek (Bellefonte, PA). Both columns were 15 m in length, with an inner diameter of 0.53 mm and a film thickness of 1 μm.

Total GCO runtime was 38.5 min. Helium, with a flow rate of 21.8 mL/min, was used as the carrier gas. Cold splitless injection was used for both extract analysis of self-prepared rum ethers and direct injection of all samples, including commercial rum ethers. The inlet was cooled to -50°C prior to injection and held at this temperature for 0.10 min, at which point it was increased to a final temperature of 260°C at a rate of 10°C/s. For both vapor and liquid samples, the GC oven was initially brought to 40°C, held for 5.00 min, increased at 10.0°C/min until reaching 225°C, and finally held at this temperature for 15.00 min.

The exit flow from the column was split between the Flame Ionization Detector and the Olfactometry Detection Port. Each sample was tested by two individuals, who recorded for each aroma an elution time, one or more descriptors, and perceived odor strength. In addition to the samples, a series of standard alkanes ranging from 6 to 28 carbons was injected in order to calculate retention indices.

4.2.6. Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted using an Agilent 6890 GC with Gerstel CIS-4 PTV inlet paired with a Hewlett-Packard 5973N mass spectrometer. Samples were analyzed using both a polar RTX-Wax and a non-polar RTX-5 column (Restek; Bellefonte, PA), both with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 μm.

GC-MS analysis lasted 71.25 minutes. Helium, with a flow rate of 1.0 mL/min, was used as the carrier gas. Samples were injected in cold splitless mode at an initial inlet temperature of -50°C. This was held for 0.10 min, at which point it was increased to a final temperature of 260°C at a rate of 12°C/s. The GC oven was programmed to hold at 40°C for 5 minutes, increase by

4°C/min to 225°C, and then hold this temperature for an additional 20 min. The entirety of the gas chromatograph's outlet flow was directed to the mass spectrometer for analysis in scan mode.

4.2.7. Identification of Volatiles

Volatile compounds were identified using a combination of GCO and GC-MS data and confirmed using authentic standards. Tentative identification of compounds was done by comparison of mass spectra of unknown against those in the National Institute of Science and Technology (NIST) database. For each volatile detected using GCO, a retention index was calculated using the elution time of both the volatile and the surrounding standard alkanes. The formula was as follows:

$$RI = RI_n + (RI_N - RI_n)\frac{RT - RT_n}{RT_N - RT_n}$$

where RI indicates retention index and RT retention time, and the subscripts indicate the target compound (no subscript), the alkane directly preceding this compound (n), and the alkane directly following this compound (N). Each alkane is assigned a retention index equal to its carbon chain length multiplied by 100, e.g. hexane has a retention index of 600.

Each compound was tentatively identified using its retention indices on both a polar and non-polar phase, as well as the odor impressions recorded. These identifications were considered positive if they could be confirmed using at least one mass spectra database match on the GC-MS (either polar or non-polar phase) as well as a match in both retention index and odor impression to an authentic standard on the GCO.

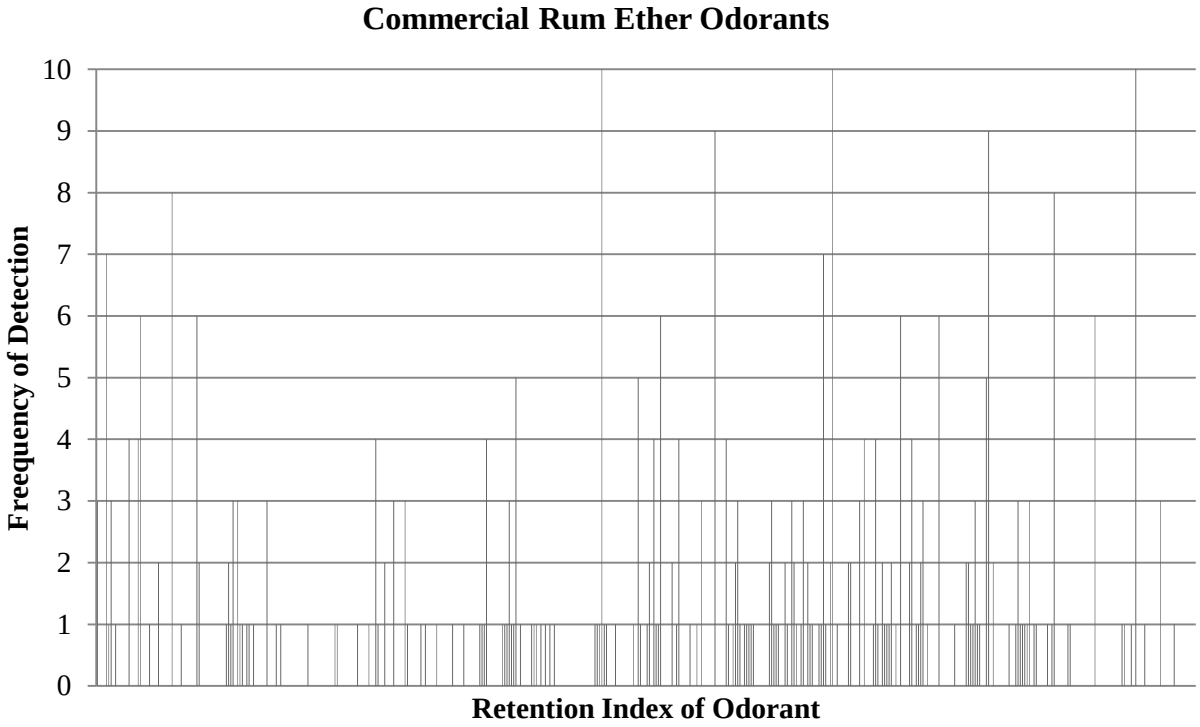
4.2.8. Aroma Extract Dilution Analysis

Relative importance of volatiles in self-prepared samples was determined using Aroma Extract Dilution Analysis (AEDA, Grosch 1993). A series was prepared of increasing 1:3 extract dilutions in dichloromethane, ranging from a concentrated extract to a dilution of 1:2187, at which point no additional volatiles were detected. Each dilution was analyzed using GCO. Each of the compounds identified in the extracts was assigned a flavor dilution (FD) factor that corresponded to the last dilution at which it was detected. Those compounds with the highest FD factor were considered most crucial in creating characteristic rum ether aroma.

4.3. Results

4.3.1. Analysis of Commercial Rum Ethers

Figure 4.2. Frequency of detection of all odorants sensed in commercial rum ether samples



More than two hundred distinct aroma compounds were detected through direct injection GCO analysis of the ten commercial samples (Figure 4.2). However, more than one hundred of these were unique to a single rum ether sample, and many others were found in only a small fraction of samples. In order to determine what could be considered typical of commercial rum ethers, the focus was narrowed to those compounds found in at least half of all commercial samples (Table 4.1).

Table 4.1. Most commonly detected odorants in commercial rum ether samples

Compound	RI (Wax)	RI (DB5)	Aroma	Detection Frequency
1. acetaldehyde	<900	<700	yogurt	7
3. ethyl acetate	908	<700	pungent	6
4. ethyl propanoate	965	709	butterscotch	8
8. ethyl acrylate	1006	<700	plasticky	6
23. acetic acid	1438	<700	vinegar	10
24. 2-acetylfuran	1495	--	cooling	5
25.propanoic acid	1532	<700	cheesy	6
26. butyric acid	1623	827	cheesy	9
32. 3-methyl-1,2-cyclopentanedione	1846	1047	maple	7
33. guaiacol	1846	1099	smoky	10
37. phenol	1983	980	phenolic	6
39. p-cresol	2069	1089	mechanical	6
41. 4-ethylphenol	2168	1175	bandage	5
42. sotolon[a]	2175	1114	maple	9
U19. *unknown*	2332	--	floral	8
U20. *unknown*	2431	1458	sweet	6
45. vanillin	2537	1409	vanilla	10

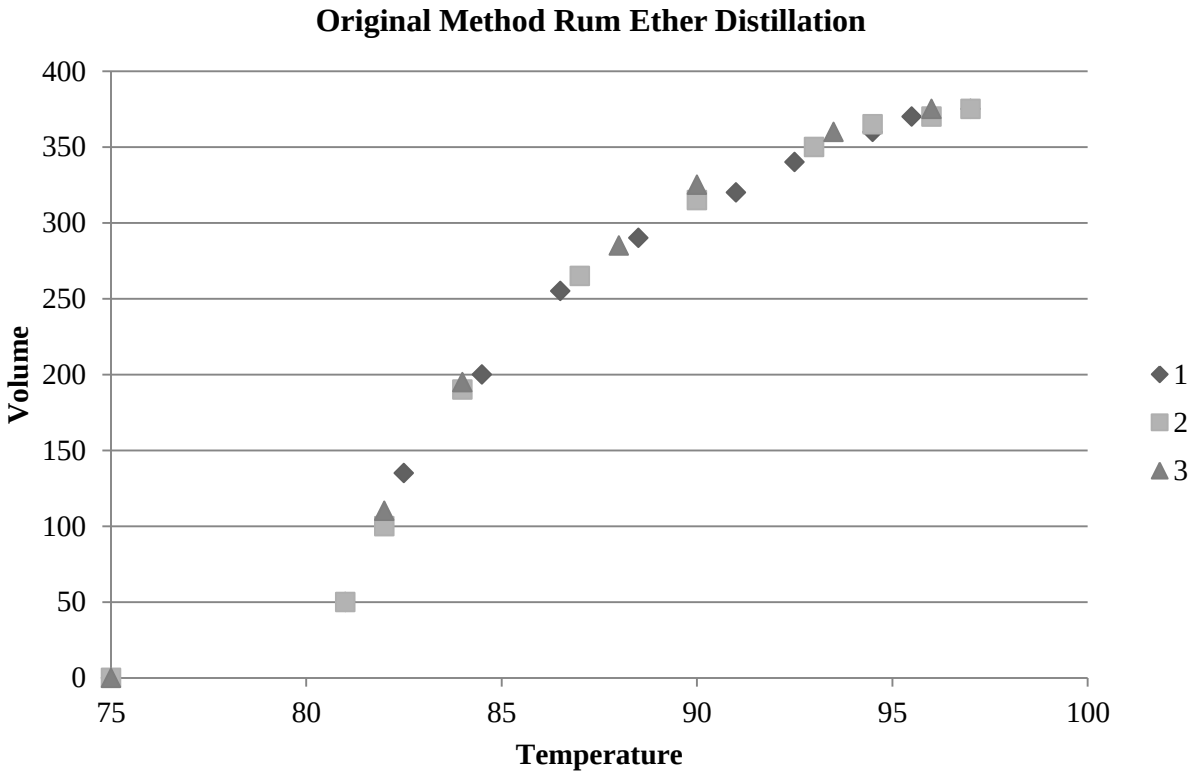
a: Sotolon was not detectable by GC-MS, likely due to its low odor threshold (Grosch 2007).

A total of seventeen compounds were found in five or more commercial rum ether samples, with three being found in all ten. Some combination of most or all of these compounds that included the ubiquitous rum ether trio of acetic acid, guaiacol, and vanillin could therefore be considered to be one indicator of a rum ether.

4.3.2. Consistency of Self-Prepared Rum Ethers

Three rum ethers were distilled using the original (non-refluxed) method to test for consistency. During distillation, information was collected periodically about the temperature of the reaction and the volume of distillate (Figure 4.3). Although not all three rum ethers distilled in an identical length of time, all appeared to follow a nearly identical curve with regards to total volume collected for a given distilling temperature.

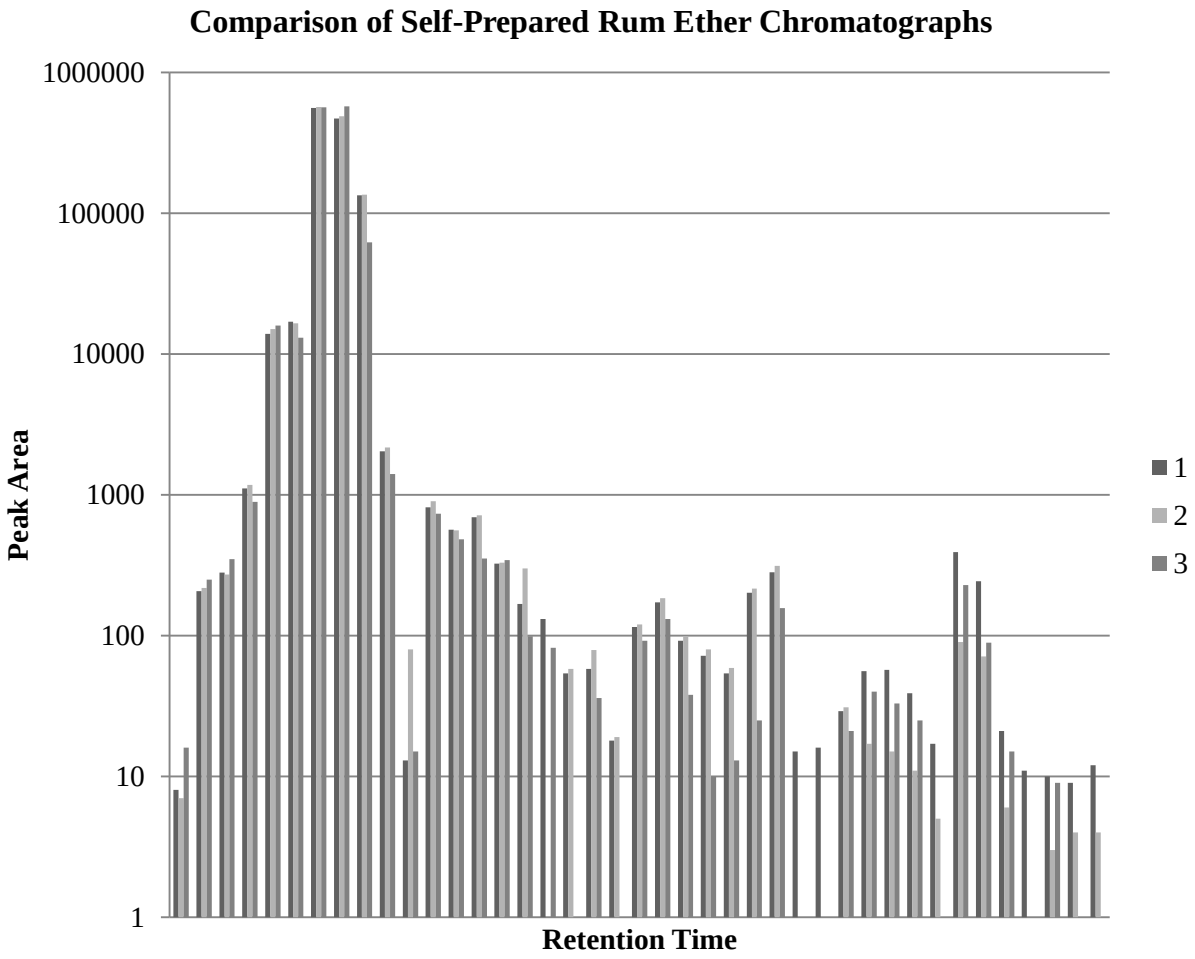
Figure 4.3. Total collected volume of distillate vs. distillation temperature for three preparations of original method rum ether



In addition to collecting distillation parameters, all three ethers were analyzed by direct injection GC in order to compare the finished composition. The chromatographs for all three appeared nearly identical by comparison of peak times and areas (Figure 4.4); any peaks that were found in one or two ethers but not all three had an area less than 100, at which point the

instrument could have been dismissing some peaks as noise. The method was deemed to produce consistent results, and one of the three rum ethers was selected for the remainder of analysis.

Figure 4.4. Comparison of chromatographic data for three self-prepared ethers made by original method



4.3.3. Comparison of Self-Prepared Rum Ethers to Commercial Samples

Self-prepared rum ethers were analyzed by direct injection GCO using a method identical to that developed for commercial samples. This information was compared to the list of seventeen common odorants in commercial rum ethers (Table 4.3) for fitness of self-prepared samples as rum ethers.

Table 4.2. Common rum ether odorants in self-prepared samples.

Compound	RI (Wax)	RI (DB5)	Aroma	Original Method	24 Hr Reflux
1. acetaldehyde	<900	<700	yogurt	--	X
3. ethyl acetate	908	<700	pungent	X	X
4. ethyl propanoate	965	709	butterscotch	X	X
8. ethyl acrylate	1006	<700	plasticky	X	X
23. acetic acid	1438	<700	vinegar	X	X
24. 2-acetylfuran	1495		cooling	X	X
25.propanoic acid	1532	<700	cheesy	--	--
26. butyric acid	1623	827	cheesy	--	X
32. 3-methyl-1,2-cyclopentanedione	1846	1047	maple	--	X
33. guaiacol	1846	1099	smoky	X	X
37. phenol	1983	980	phenolic	--	X
39. p-cresol	2069	1089	mechanical	X	X
41. 4-ethylphenol	2168	1175	bandage	X	X
42. sotolon[a]	2175	1114	maple	X	--
U19. *unknown*	2332		floral	X	--
U20. *unknown*	2431	1458	sweet	X	--
45. vanillin	2537	1409	vanilla	X	X

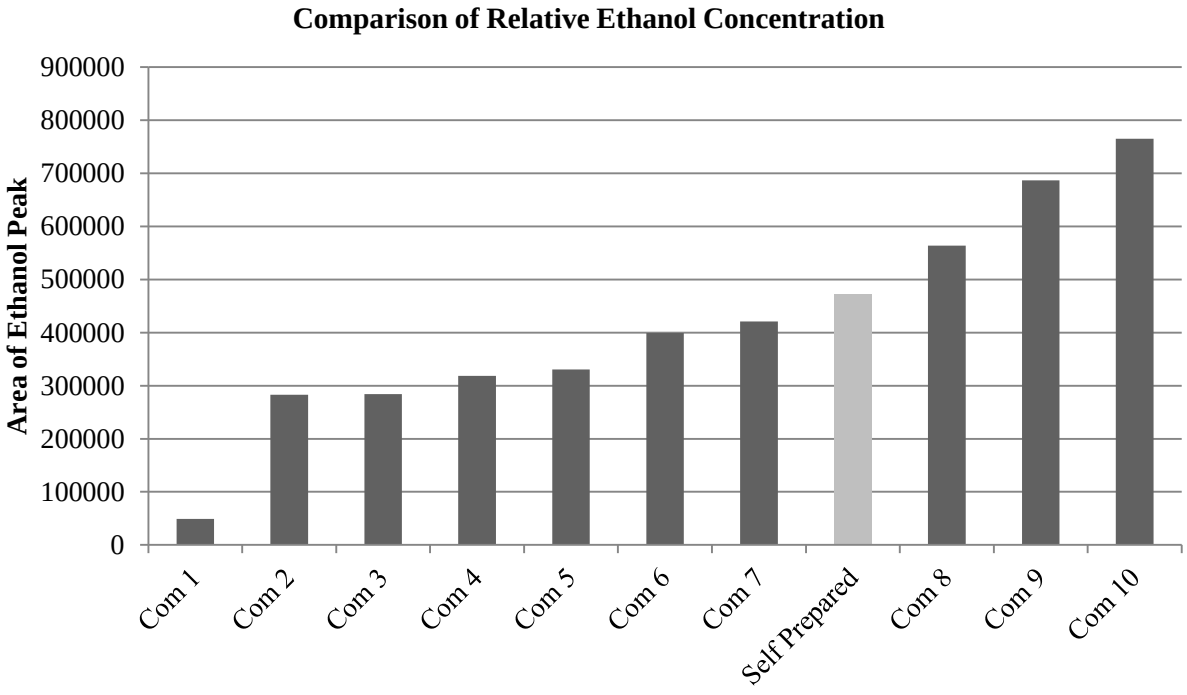
a: Sotolon was not detectable by GC-MS, likely due to its low odor threshold (Grosch 2007). b: By virtue of being unknown, these compounds were matched only by retention indices and odor impression.

Both samples contained thirteen out of seventeen compounds, including the three essential rum ether compounds: acetic acid, guaiacol, and vanillin. The thirteen odorants were not an identical group, however; propanoic acid could not be detected in either, but the identity of the other three missing compounds differed between the two samples. For this reason, both samples were considered important for further analysis.

In addition to presence of odorants, bulk composition was considered. The most abundant compound asides from water was ethanol, which was present in high concentrations in all commercial products. Ethanol concentration was not quantified, but by comparison of GC peak area (Figure 4.5), it is clear that the original method self-prepared ether falls within the range of concentrations found in commercial samples. It is therefore comparable to commercial

ethers in both aroma profile and bulk composition and was selected for further analysis by aroma extract dilution analysis (AEDA).

Figure 4.5. Comparison of relative ethanol concentration in commercial and self-prepared rum ethers



4.3.4. Aroma Extract Dilution Analysis

A total of 31 odorants were detected in the rum ether obtained by the original method; nearly a third more, or 40, were found in the sample that had been refluxed for 24 hours (Table 4.3). Most of the 31 original formula rum ether compounds were also found in the reflux rum ether, and in most cases where the dilution factors were not the same, the refluxed rum ether had the higher FD factor. The most important odorant in both samples was ethyl acrylate.

Table 4.3. AEDA analysis of both self-prepared rum ether extracts

Compound	RI (Wax)	RI (DB5)	Aroma	FD[a] (Original)	FD[a] (24 Hr)
1. acetaldehyde	<900	<700	yogurt	1	2
2. 2-methyl propanal	<900	<700	dark chocolate	2	2
3. ethyl acetate	919	<700	nail polish	4	3
4. ethyl propanoate	922	715	butterscotch	1	1
5. 3-methyl butanal	937	<700	dark chocolate	3	1
6. ethyl isobutyrate	967	751	butterscotch	6	7
7. 2,3-butanedione	975	<700	butter	1	1
8. ethyl acrylate[b]	1000	703	plastic	7	8
10. ethyl butyrate	1033	806	fruity	6	7
11. ethyl 2-methyl butyrate	1052	847	berry	1	3
12. 2,3-pentanedione	1058	<700	butter	2	2
13. ethyl 3-methylbutyrate	1068	859	blueberry	5	6
15. ethyl 3-butenoate[b]	1101	776	plastic	4	4
16. ethyl pentanoate	1127	906	berry	--	3
U2. *unknown*	1166	847	dark chocolate	2	2
U3. *unknown*[c]	1183	928	blueberry	3	3
U5. *unknown*	1315	1135	blueberry	6	5
22. ethyl octanoate	1424	1194	berry	3	5
23. acetic acid	1439	<700	vinegar	3	2
24. 2-acetylfuran[d]	1489	--	minty	4	4
26. butyric acid	1620	839	cheesy	--	1
27. ethyl benzoate	1631	1149	honey	--	1
28. 3-methylbutyric acid	1655	855	sweaty	1	1
U8. *unknown*	1661	1260	woody	1	4
U9. *unknown*	1715	--	hay	--	1
U10. *unknown*	1782	--	mechanical failure	1	2
32. 3-methyl-1,2-cyclopentanedione	1823	1061	maple	--	4
33. guaiacol	1839	1091	smoky	3	6
34. (E)-oak lactone	1881	1287	spicy	4	1
35. phenethyl alcohol[d]	1900	--	floral	--	3
36. (Z)-oak lactone	1937	1331	maple	1	1
U12. *unknown*	1949	--	overrun motor	--	2
37. phenol	1988	981	earwax	--	1
U13. *unknown*	2019	1487	minty	--	3
40. p-cresol	2061	1083	overrun motor	1	4
U14. *unknown*	2117	1463	grape cough syrup	--	3
41. 4-ethylphenol	2164	1174	bandage	2	5
42. sotolon[e]	2176	1115	maple	2	--
U17. *unknown*	2196	--	woody	--	1
U18. *unknown*	2300	--	grape juice	--	1
U19. *unknown*[c]	2331	--	floral	1	--
U20. *unknown*[c]	2430	--	sweet	1	--
46. vanillin	2528	1412	marshmallow	2	3

a: FD factors are $\log_3$ and were found on a wax column. b: Both "plastic" notes are tentative identifications; aroma standards were not available to be matched. c: As a result of being unidentified, those unknowns numbered in parallel with rum unknowns are only tentatively matched as the same compound. d: Compound only able to be tentatively identified due to not being detected on DB5 column. e: Sotolon was not detectable by GC-MS, likely due to its low odor threshold (Grosch 2007).

4.4. Discussion

The seventeen odorants detected in at least half of commercial rum ethers represent only a small subset – less than ten percent – of all detected odorants in these products. This would seem to indicate that rum ether should be treated not as a uniform product with a singular identity, but as a class of products. This is a positive sign for anyone with the end goal of creating a product more similar to rum, since the rum ethers currently available represent a wide range of compounds which could, in theory, be selected for by small modifications to the current process without falling outside the range of what is considered normal for rum ether. Only three odorants – acetic acid, guaiacol, and vanillin – appear in all commercial products and could therefore be treated as essential to rum ether, but since these are all also found in rum, they are desirable in a product that aims to mimic rum.

If acetic acid, guaiacol, and vanillin are a positive trio to have in any rum ether, ethyl acetate, ethyl acrylate, and phenol could be considered their rum ether foils – compounds that are not only not found in rum, but that have odors that are both strong and unpleasant. However, unlike the other three, these are not universally found in rum ethers. Each is only found in six of ten commercial samples, or slightly more than half. This could therefore be a good focus in initial development of rum ethers, since it would seem that not all methods currently in use create these potential off-odors.

For in-lab preparation of rum ethers, liquid smoke was substituted for a more traditional ingredient, pyroligneous acid. Both are made by collecting volatiles during the burning of wood, but while liquid smoke is produced with the end goal of being used in food, pyroligneous acid is a byproduct of charcoal production that happened to find a home in the making of rum ether. It

used to be widely and cheaply available for use as an energy source, but as it has gradually been replaced by petrochemicals, it has become a less economically viable product to the point that it is no longer easy to come by. Additionally, it was not designed for use in food, so it contains a number of components that make it a "highly polluting noxious corrosive liquid," including a very high percentage of methanol (FAO 1987). Although one would hope this would be removed before the finished product made it into food, one handbook claims that rum ether must only have less than five percent combined methanol and formaldehyde content (Burdock 2010). Both producers and consumers could benefit from the replacement of pyroligneous acid with something easier to find and safer; liquid smoke seems a suitable alternative.

When compared with commercial rum ethers, those made with liquid smoke fit in quite well. Both contain thirteen out of the seventeen commonly detected odorants, but only one – propanoic acid – is not found in either, so all but one of the common odorants could still be produced by the liquid smoke substitution. Acetic acid, guaiacol, and vanillin are found in both. Although each variation is missing four common odorants, this is not necessarily a red flag; in fact, none of the commercial products actually contains all seventeen. This places the self-prepared ethers squarely in the realm of the commercial products.

AEDA of these self-prepared ethers using a method identical to that used for rum allowed for a direct comparison of the two substances. One would expect there to be some overlap, both because of the nearly universal recognition of qualitative similarities and because both rum and rum ether seem to obtain a significant part of their aroma from wood; this expectation is met. Many of the compounds found to be important in the AEDA of rum ether were similarly important in rum, including ethyl isobutyrate, ethyl butyrate, and ethyl 3-methylbutyrate. It is also worth noting that all three of these are esters, compounds that, as discussed in Chapter 3, are

widely considered some of the most important and characteristic in rum. Even many of the less potent esters, for instance ethyl 2-methylbutyrate and ethyl octanoate, are found in both. Less important to the overall aroma but still worth noting are the wood extractives shared by rum and rum ether, compounds like (E)- and (Z)-oak lactones, guaiacol, and vanillin. Acetic acid, one of the most important rum odorants, is also found in rum ether, a finding that is not surprising when one considers that it is one of the raw ingredients.

Perhaps a more interesting focus is where rum and rum ether are not similar. This could be potentially useful information for anyone aiming to minimize the differences between the two products. The first set of differences is made up of compounds unique to rum. This includes three of the most key odorants in rum: isoamyl alcohol, which has a malty aroma, β-damascenone, which gives the impression of applesauce, and phenethyl alcohol, which contributes a floral, rosy smell. These make up three of the four most important odorants. Phenethyl alcohol was tentatively identified in one of the self-prepared samples but not the other, while the other two compounds were not found at all. This information alone could be enough to make a marked difference in the similarity of rum ether to rum. Two of these – isoamyl alcohol and phenethyl alcohol - were found in a small number of the commercial samples and could possibly be incorporated into rum ether through development of the proper methodology. However, for the well-informed flavor chemist, another, easier option exists. In cases where rum ether is being used as one of many flavors, a simple supplementation with one or more of these three compounds, all of which are available individually as natural flavors, could enhance the finished product in a way that makes it more true-to-rum. The product could still be labeled as all natural if desired, and would be set apart from products using less well-rounded flavors.

A second set of differences between rum and rum ether is less easily solved. This set comprises the compounds found in rum ether but not rum, and it is arguably the biggest challenge facing rum ether. Although all three of the biggest contributors to this category are esters, they are not the desirable fruity esters for which rum is famed. These esters – ethyl acetate, ethyl acrylate, and ethyl 3-butenoate – contribute pungent chemical and plastic notes in the AEDA conducted on self-prepared ethers. Ethyl acetate and ethyl acrylate especially are important because they are found within the ranks of the seventeen common rum ether odorants. Luckily, the variation among commercial samples indicates that composition of a rum ether can be altered without it losing its identity entirely; this is reinforced by the samples produced with liquid smoke, which show some variation even with only a minor change in methodology.

4.5. References

Burdock, G.A. *Fenaroli's Handbook of Flavor Ingredients*. 6th ed. CRC Press: Boca Raton, FL, 2009.

Engel, W.; Bahr, W.; Schieberle, P. "Solvent assisted flavour evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices." *European Food Research and Technology*. 1999, *209*, 237-41.

FAO Forestry Department. "Recovery of by-products from hardwood carbonization." *Simple technologies for charcoal making.* Food and Agriculture Organization of the United Nations: Rome, 1987.

Grosch, W. *"*Detection of potent odorants in foods by aroma extract dilution analysis.*" Trends in Food Science and Technology.* 1993, *4.3*, 68-73.

Grosch, W. "Gas Chromatography-Olfactometry of Aroma Compounds." *Flavours and Fragrances: Chemistry, Bioprocessing, and Stability.* Berger, R.G., Ed. Springer: Berlin, 2007. 363-78.

Song, H.; Cadwallader, K.R.; Singh, T.K. "Odour-active compounds of Jinhua ham." *Flavour and Fragrance Journal*. 2008, *23*, 1-6.

Chapter Five:

Conclusions

The objectives of this research were to characterize both rum and rum ether for the purpose of better understanding how rum ether, an important but poorly understood flavoring agent, relates to its namesake. The end goal was to be able to create a useful understanding of rum ether's strengths and weaknesses as a substitute for more expensive rum extracts; this will allow for anyone using it as a flavor to highlight the strengths while compensating for the weaknesses.

A number of compounds were able to be identified in both rum and rum ether, including quite a few that were common to both. A more thorough definition of what makes a rum ether was established, and two rum ethers were selected for thorough characterization and comparison with rum. Additionally, rum ethers were prepared on a bench scale in the lab to better understand the process and determine if liquid smoke was a suitable alternative to pyroligneous acid as a raw material.

However, this research merely scratched the surface of what is unknown about rum ether as a flavor. Although odorants were ranked by apparent potency according to AEDA, a more rigorous analysis could be applied that employed quantification of odorant concentrations and odor thresholds. Comparing these with the same information about rum could provide new parallels or discrepancies that AEDA missed.

Additionally, throughout the last chapter, the possibility of altering rum ether composition by changes in process conditions is mentioned. A cursory attempt at this was made, but a huge amount of time could be dedicated exclusively to documenting how small changes in production method are reflected in the finished product. What was written about here certainly proves that these small changes are important to the final composition, but not how these changes manifest themselves.

Finally, sensory analysis is an important final step in much flavor research. In order to do a meaningful sensory study, it would be necessary to create a rum ether that much more closely follows rum, as could be done with quantification of odorants, or a series of rum ethers that varied significantly less than products currently available, as could be done by studying rum ether process conditions. As it stands now, the rum and rum ether are far from confusable – a sensory study is not needed to confirm this – and the products available vary so greatly that it would be impossible to pinpoint which specific compounds are responsible for making one rum ether more or less rummy than another.

The current research hopes to be able to provide a starting point for additional explorations of rum ether, an initial dive into the depths of its mysteries. However, truly understanding this product will require further study.