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Stability Testing of Mint Oils through Gas Chromatography Analysis

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CERTIFICATE OF ORAL DEFENSE OF HONORS THESIS

Sarah Y. Wolf, having been admitted to the Carl and Winifred Lee Honors College in Fall 2003, successfully presented the Lee Honors College Thesis on May 8, 2007.

The title of the paper is:

"Stability Testing of Mint Oils"

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Stability Testing of Mint Oils Through Gas Chromatography Analysis

Lee Honors College Thesis by Sarah Wolf

Western Michigan University

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Presented on 5/08/07

Abstract

In this experiment, the stability of mint oil was measured through gas chromatography analysis. Eight different oils were tested. Levels of key components of mint oil, including menthol and menthone for peppermint oil, and carvone and limonene for spearmint oil, was compared over time. Several packaging materials were immersed in oils in order to determine their effects, if any, on the composition of the oils. Additionally, oil was subjected to either 25 degree or 40 degree Celsius temperatures to determine if temperature affected the oil composition.

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I. Introduction

Background information:

Peppermint and spearmint oil has been used throughout history as a flavor for food as well as for medicinal purposes. In ancient times, mint plants were spread on the floor of homes to release their fragrance. In the 10^{th} century A.D. mint oil was used as a Japanese eye wash (Landing, 1969). Commercial cultivation of mint oil began in Europe after the plants became popular in gardens. Before the 1800's, mint plants arrived in the United States, brought by colonists (Landing, 1969).

Both peppermint and spearmint are part of the family Lamiaceae, formally called Labiatae. This name refers to the two-lipped flower petals. This family contains a wide variety of genera and species, including other common herbs such as basil and oregano. The genus *Mentha* includes mint plants such as spearmint (*Mentha spicata*) and peppermint (*Mentha piperita* and *arvensis*) (Spencer, Dowd, Faas, n.d.). Mint oil, and other essential oils, are created and stored in the plant's trichomes, or specialized cells. Mint oil is grown in only specific areas of the United States including Washington, Oregon, Idaho, Wisconsin, Indiana, Michigan, Montana, and South Dakota. These separate states are grouped together to form growing regions based on the environmental conditions.

Mint Agriculture and growing regions:

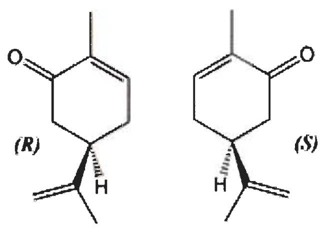
Midwest peppermint and spearmint is grown in Wisconsin, Indiana, and Michigan. The soil in this area is exposed to 33 inches per year with moderate light intensity and high humidity with a growing season of 120-150 days. Oregon mint in the Willamette region is grown in sandier soil with 39 inches of rain per year. Average temperature and light intensity is lower than in Michigan. Because most of natural rainfall occurs in the winter, fields are irrigated by sprinkler system. Madras, Oregon and Northern California grown mint oil is another popular growing region. The soil in this area is very sandy and rainfall is slight. The light intensity varies with a photoperiod of 14-16 hours. Idaho mint plants are grown in sandy soil with similar conditions to Madras/N. California mint plants. The final region of mint growing occurs in Washington in Kennewick and Yakima. The sandy volcanic soil receives little rainfall and the growing season is very long, up to 180 days. Often these regions are grouped into two general segments called Midwestern and Far Western.

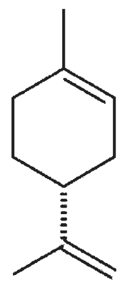
Growing conditions and the ancestry of the species of plant heavily affect the odor and taste characteristics of mint oil and flavor. Photoperiod, precipitation, soil type, and winter conditions all have an influential effect. In a study by Burbott, Loomis (1967), photoperiod increased the levels of menthols and menthones in peppermint. Short photoperiods produced high levels of menthofuran.

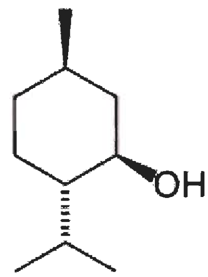
The oils from mint are obtained from steam distillation. Mature plants are swathed, or cut, into piles called windrows (A.M. Todd, Overview of mint agriculture). After curing for up to three days, windrows are chopped into mint hay and transported to a distillery. Steam is filtered through the hay and oil is released from the mint glands. Once vaporized, mint oil is carried to a still and cooled. Once in liquid form, the oil is separated from the more dense water.

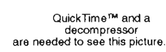
Distillation of mint oil yields several groups of compounds. Low boilers and heads consist mostly of monoterpene hydrocarbons. The body of the oil consists of oxygenated compounds. Tails include higher molecular weight compounds. Tails and heads are usually removed to increase the solubility of the oil, as well as improve the taste and smell of the product.

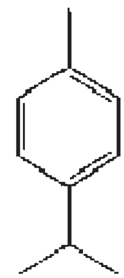
Basic structures of Mint Oil Components:











Carvone **Limonene** **Menthol** **Menthofuran** **P-cymene**

Overview of Experimental Purpose

At A.M. Todd, all mint oil is arbitrarily given an expiration date of 12 months. However, no documentation of why this assigned time period exists since there is no analytical data that supports this assumption. The purpose of this study is to provide necessary data to support the 12-month expiration date claim. Analysis of oil was done by measurement of various components of mint oil through gas chromatography. A change in the composition of mint oil over time would indicate that the mint oil was not chemically stable. Additionally this study will investigate the possible effects of several packaging material on the stability of the mint oil. Various packaging materials used at A.M. Todd to store mint oil has not been directly studied to determine their effects, if any, on the composition of peppermint and spearmint oils. This experiment attempted to explore this question by immersing various materials in mint oils and analyzing the results. Additionally, the effect of high temperature versus room temperature on mint oil components was investigated. Previously, it was unclear if higher temperature testing would affect mint oil in an accelerated fashion, or if room temperature tested mint oil would perform similar to high temperature oils at a slower rate. By comparing results for high temperature and room temperature, an attempt was made to correlate the changing levels of key components with exposure temperature. By comparing the data from accelerated and regular testing, future testing of mint oils in regular conditions can be eliminated.

Peppermint oil composition varied less drastically than spearmint oil composition in accelerated testing. Regular tested mint oil, both peppermint and spearmint, exhibited less fluctuation in levels of components than in accelerated testing (40 degree oven testing). A loss of heads in all mint oils resulted in erratic color changes and composition changes in some samples. Fluctuations in trends of collected data were attributed to the loss of heads in mint oils. Oils did not show any dramatic changes in composition over time, indicating that the shelf life of mint oil is stable. Since peppermint oil was more stable than spearmint oil, indicated by less fluctuation in composition over time, this oil's shelf life could potentially be extended beyond the arbitrary shelf life date of 12 months. To increase the shelf life, additional testing would be required to evaluate changes in the oil's organoleptic qualities over time.

II. Materials and Methods

Characteristics of Mint oils used:

Eight different oils were tested in this experiment: four spearmint and four peppermint. Spearmint oils included type numbers: 200100, 200717, 206470, and 200420. Each type number refers to a particular mix or blend of oil. 200100 is 100% natural oil of spearmint. 200717 contains a 14.5 percent head cut and a 2 percent tail cut removed. 206470 contains a 20.5 percent head cut, and a 2.5 percent tail cut. Finally, 200420 contains a 3.5 percent head cut and a 0.5 percent tail cut. Peppermint oils tested include 100100 which is 100% natural oil of peppermint, 100133 with a 3 percent head cut, and 100186 which has a 12.5 percent head cut and 2 percent tail cut. Finally, 1102003 is an engineered mint oil containing 48.73 percent *Mentha arvensis* and 33.5 percent *Mentha piperita* with 17.7 percent other mint components. Different types of mint oil were used to determine if the composition affected the stability of the oils.

All eight mint oil samples were obtained from A.M. Todd inventory. A.M. Todd keeps mint oil stored in drums with temperatures fluctuating in summer and winter months. However, drums are tightly sealed, stored indoors, and are protected from sunlight. Prior to being subjected to the stability testing conditions, oil samples were held in aluminum containers with a tightly sealing lid. Around 5 pounds of each oil were used.

Instruments used to obtain data:

Gas chromatography was used to analyze the components of mint oil. Each oil was run on one of two Varian 8100 Autosampler using a pre-calibrated 15 minute run.

Each machine contains a DB Wax column 15 meter with .25 micron film. All spearmint and peppermint oil is run at A.M. Todd using this particular method of analysis. Pre-set methods for processing the chromatograms were used for spearmint and peppermint oil. A Perkin Elmer 888-PE Autosystem XL Gas Chromatograph-Mass Spectrometer with a SPB5 column was also used for the analysis of oil. Only particular samples were run on the GC-MS because no further results were obtained from this instrument. Furthermore, it was ascertained that no further information could be gained from running samples on both the GC and GC-MS. The two Varian GC instruments showed some variability when running identical mint oils. To show the extent of variability, 15 vials of the same sample of mint (200717) were run consecutively on each machine. These GC results were then compared. At A.M. Todd, both instruments are used interchangeably when examining the contents of mint oils or flavors.

Procedure:

During testing, amber vials were used to store all mint oils in either 40 or 25 degree Celsius temperature. These vials prevent UV rays from reaching the oil. Vials were manually filled and labeled. Four different materials were added to vials: galvanized metal, blue plastic, white plastic, and epoxy liner. These materials were added to test their effects on the mint oil composition through time. All four materials are used by A.M. Todd to store mint oils and flavors. Materials were obtained from A.M. Todd storage containers. Galvanized metal was cut from previously used drums, plastics were cut using shears, and epoxy liner was obtained by peeling the lining out of containers. Small pieces (around ½ inch X ½ inch) of each material was cut and placed in amber vials. A control was used which contained no added material. 32 vials containing each material were used for each oil, with a total of 160 total vials used for each oil.

Accelerated testing refers to samples placed in the 40 degree Celsius oven. Regular testing refers to samples placed in room temperature, or 25 degrees Celsius. A total of 16 vials per oil were exposed to either environment. Tops to the amber vials were made of plastic, and are designed to prevent reaction of the mint oil with the material on the cap. The tops for the accelerated testing were screwed on lightly, due to the experimenter's uncertainty of reaction in high temperatures. The tops for regular testing were screwed on tightly. Head space in each vial of oil was not measured, but each vial was filled almost to capacity.

Time span for this experiment ran from June, 2006 to April, 2007. Each oil was obtained and started at different times in an attempt to stagger the workload. For the accelerated testing, a small sample of oil was taken from a random vial containing each material (galvanized metal, blue plastic, white plastic, epoxy, and control) for a total of five vials. These were run on a gas chromatograph and then put into a refrigerator. Vials were sampled every 15 days. Physical constants were measured every 30 days for accelerated testing. For the regular testing, samples of oil were collected every 3 months, or 90 days. Physical constants were measured every time samples of oil were collected for regular testing. Color of the oil was noted during collection.

Physical constants included measurement of specific gravity, optical rotation, and refractive index. Specific gravity measures the relative density of the sample to water at 25 degrees Celsius. Optical rotation of peppermint oil is generally -30 to -40 degrees, while for spearmint it is -50 to -60 degrees. The amount of rotation depends on (l)-

menthol and (l)-carvone levels present in peppermint and spearmint oil respectively. Refractive index measures the speed of light through a sample and is taken at 20 degrees Celsius.

The computer program Adage was used to organize the chromatograph data. Each of the eight mint oils were given a particular lot name using this format: ST *(type of material) (REG/ACC) (Type number).* Type of materials were abbreviated: GM for galvanized metal, BP for blue plastic, WP for white plastic, EPOXY for epoxy, and CTRL for control. REG referred to 25 degree Celsius testing, while ACC referred to 40 degree Celsius testing. ST was used to indicate that the sample was being used for stability. Type numbers were assigned using the seven-digit system utilized by A.M. Todd to name its mint oil inventory. Within Adage, stability families were created to indicate the type of material and its regular or accelerated status. Then, each lot number was assigned to its respective stability family.

Initially, samples were going to be tested for a total of 9 trials, or 120 days including an initial run. Some mint oils were run for longer than this, however, and natural oil of peppermint and spearmint ran for 15 trials, or 210 days total. The regular testing of mint oils were run for as long as possible, however time limitations only allowed between one and four trials total.

III. Results

Major peaks of interest in Peppermint and Spearmint:

Major peaks of interest in GC chromatograms include menthol and menthone in peppermint. P-cymene is also of notable interest in peppermint because it is a measure of oxidation. When present in quantities higher than 0.2%, it potentially has a negative impact upon the odor and taste quality of the oil. Menthofuran is another peak of interest because its presence in mint oil decreases the value of the oil. Total heads is a summed group of peaks that is of interest for both oils. These peaks consist of a combination of peaks that show up early on the chromatogram. They have low boiling points and evaporate easily, especially in high temperature conditions.

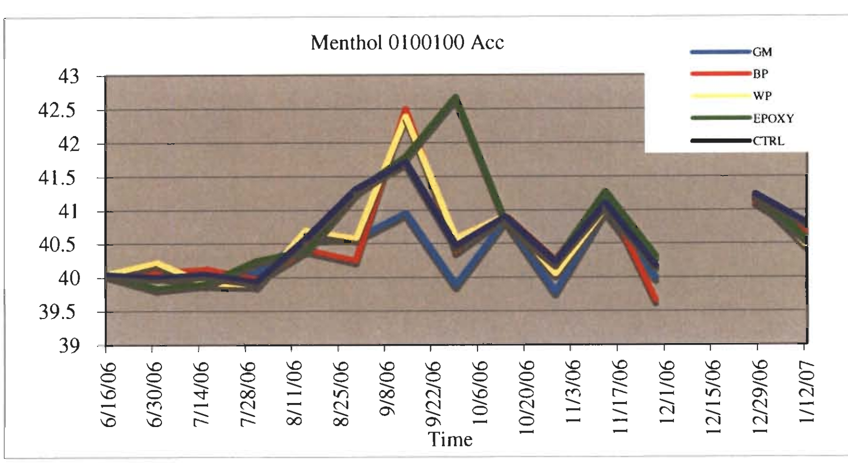
Major peaks of interest in spearmint oil include the terpenes, Carvone and limonene. Pure *Mentha spicata* contains at least 51% R-(-)-Carvone. Limonene has a characteristic odor of oranges and is a primary odor constituent in citrus fruits. Terpenes refer to a class of hydrocarbons and are derived from subunits called isoprenes with the molecular formula C_5H_8. Terpenes are the primary constituents of essential oils.

The addition of galvanized metal, blue plastic, white plastic, and epoxy lining did not cause any detected changes in the mint composition in either accelerated or regular conditions. Therefore, the data presented is based on a generalized overview of all 5 mint oils exposed to each material because all oils with various materials behaved very similarly. Diethyl phthalate, a substance found in plastic products, can be detected using a GC-MS and has a retention time of 39.67 minutes. No additional peaks were detected in 200100 or 100100 indicating that diethyl phthalate did not leach out of the packaging materials used in this experiment.

For accelerated testing of all types of oil, samples were collected every 15 days, and physical constants were measured every 30 days. For accelerated testing, T0 refers to the initial testing time at time zero, and T1-T14 refers to each subsequent test every 15 days. For regular testing, samples were collected every 90 days, with physical constants being measured every testing period. For regular testing, T0 refers to the initial testing time at time zero, and T1-T3 refers to each test every 90 days. Some data is missing from each oil type because of time limitations in the experiments.

Natural Oil of Peppermint, 0100100, Accelerated and Regular test results:

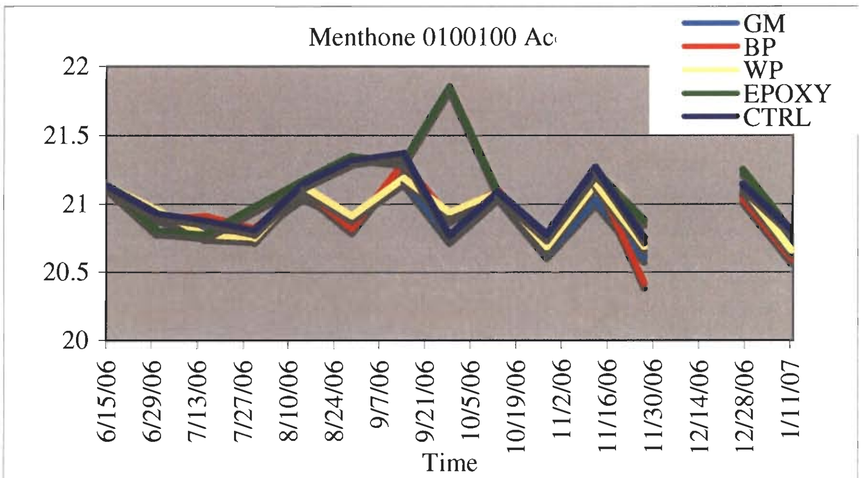
Both natural oil of peppermint and natural oil of spearmint were tested from June 2006 to January 2007. In natural oil of peppermint, 100100, the levels of components did not vary significantly. Menthol levels in accelerated testing started at 40.05% and varied less than 1.2% for all materials. Some single variations occurred, including the increase of menthol to 42.67% in oil with epoxy liner at T7 (see Figure 1).



Menthol Acc T0=40.05%	**7/1/06**	**1/12/07**
GM	40.04	40.71
BP	40.08	40.66
WP	40.22	40.48
EPOXY	39.84	40.57
CTRL	40.01	40.81

Figure 1. Menthol levels for Natural Oil of Peppermint, Accelerated testing

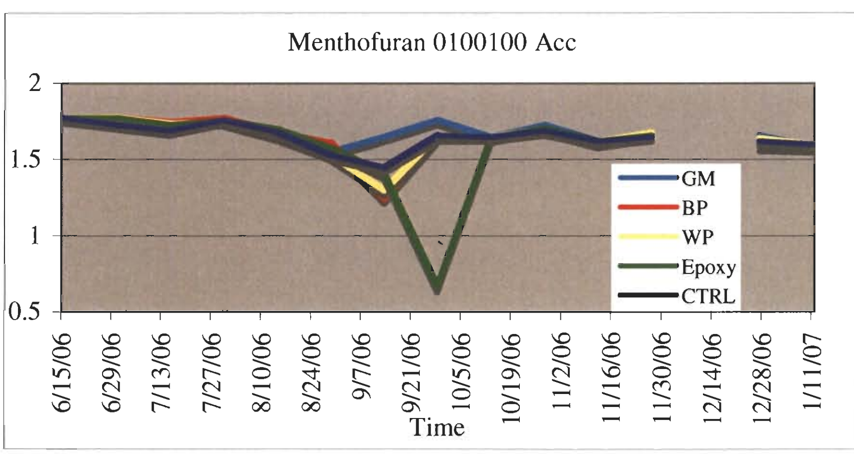
Menthone levels for accelerated conditions decreased slightly from 21.13% to 20.6% (see Figure 2).



Menthone Acc T0=21.13%	6/30/06	1/11/07
GM	20.91	20.72
BP	20.90	20.60
WP	20.96	20.66
EPOXY	20.81	20.80
CTRL	20.93	20.81

Figure 2. Menthone levels for Natural Oil of Peppermint, Accelerated testing

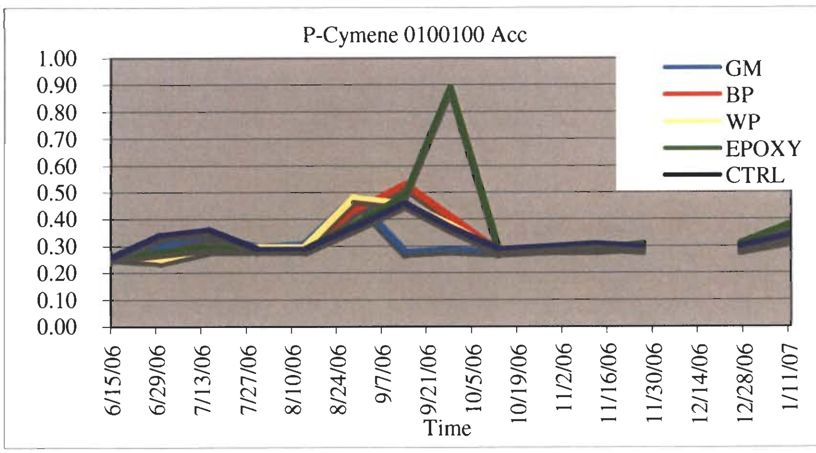
The trend for menthofuran decreased slightly from 1.77% at T0 to between 1.58% and 1.60% in T14. However, some oils showed a dramatic decrease to 0.67% in T7 epoxy oil, and 1.30% in T6 white plastic oil (see Figure 3).



Menthofuran Acc T0=1.77%	6/30/06	1/11/07
GM	1.75	1.59
BP	1.78	1.59
WP	1.78	1.60
EPOXY	1.77	1.58
CTRL	1.73	1.60

Figure 3. Menthofuran levels for Natural Oil of Peppermint, Accelerated testing

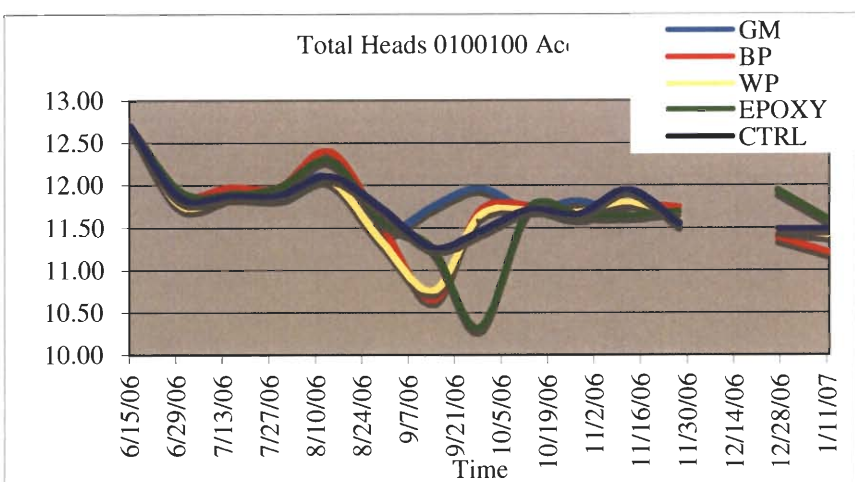
Levels of p-cymene increased slightly in accelerated testing. Initial levels began at 0.26% and varied from 0.25%-0.47% depending on the date of testing. In T7 of epoxy, p-cymene levels increased to 0.89% (see figure 4).



P-cymene T0=0.26%	6/30/06	1/11/07
GM	0.30	0.33
BP	0.27	0.32
WP	0.25	0.32
EPOXY	0.28	0.38
CTRL	0.34	0.34

Figure 4. P-cymene levels for Natural Oil of Peppermint, Accelerated testing

Total heads levels decreased slightly from 12.69% to 11.4%-11.6% (see figure 5).

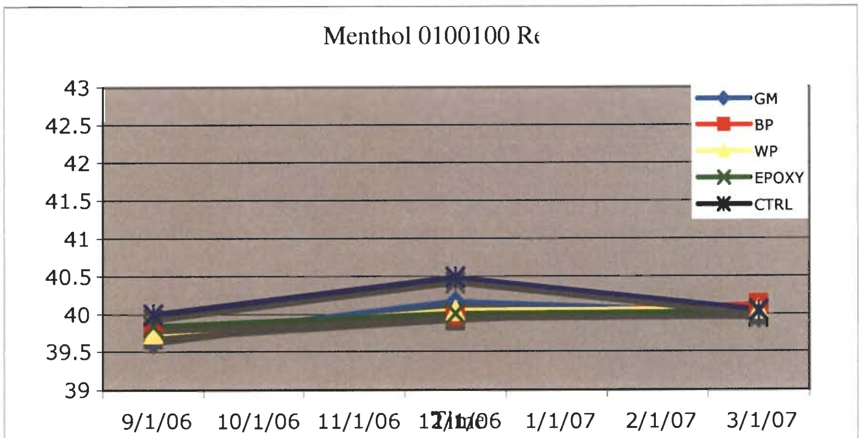


Total Heads T0=12.69%	6/30/06	1/11/07
GM	11.83	11.43
BP	11.91	11.21
WP	11.77	11.41
EPOXY	11.93	11.60
CTRL	11.86	11.49

Figure 5. Total heads levels for Natural Oil of Peppermint, Accelerated testing

Physical constants, including optical rotation, specific gravity, and refractive index, did not change significantly for accelerated testing in natural oil of peppermint. The optical rotation for accelerated testing at T0 was -25 degrees and varied between -25 and -24.6 degrees throughout testing. Refractive index varied from 1.4611 to 1.4617. Specific gravity varied between 0.8998 and 0.9007 throughout testing. Epoxy testing at T6 had a specific gravity of 0.9072 and a refractive index of 1.4631. No other sample at any time showed such fluctuation.

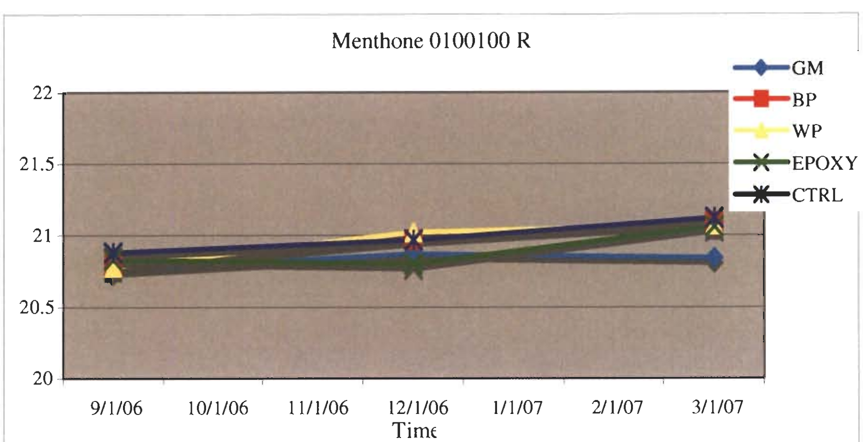
Components of natural oil of peppermint in regular testing varied less than in accelerated testing. Menthol levels ranged from 39.69% to 40.49% (see figure 6).



Menthol Reg T0=40.05%	9/13/06	3/12/07
GM	39.69	40.02
BP	39.75	40.13
WP	39.75	40.07
EPOXY	39.85	40.03
CTRL	40.00	40.05

Figure 6. Menthol levels for Natural Oil of Peppermint, Regular testing

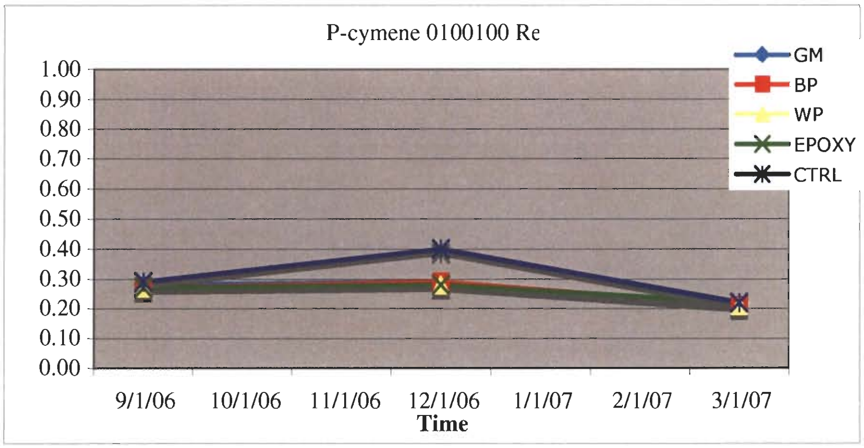
Menthone levels ranged from 20.76% to 21.13% (see figure 7).



Menthone Reg T0=21.13%	9/13/06	3/12/07
GM	20.76	20.84
BP	20.79	21.09
WP	20.78	21.07
EPOXY	20.83	21.06
CTRL	20.88	21.12

Figure 7. Menthone levels for Natural Oil of Peppermint, Regular testing

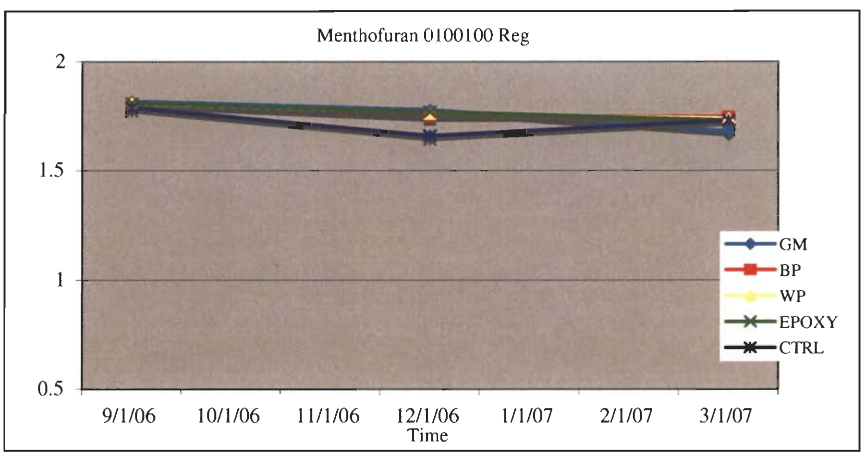
P-cymene levels started off at 0.26% and increased slightly in T1 and T2 to 0.28% but then decreased in T4 to 0.22% (see figure 8).



P-Cymene T0=.26%	9/13/06	3/12/07
GM	0.28	0.21
BP	0.27	0.21
WP	0.27	0.21
EPOXY	0.27	0.22
CTRL	0.29	0.22

Figure 8. P-cymene levels for Natural Oil of Peppermint, Regular testing

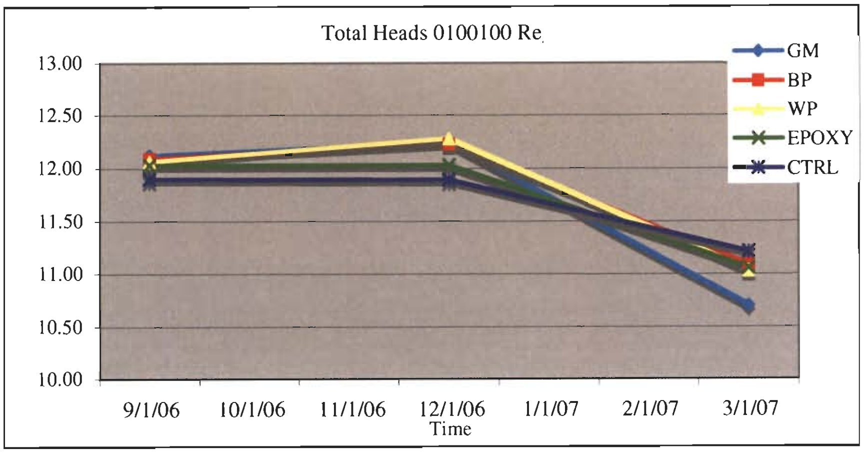
Menthofuran varied from 1.59% to 1.81% (see figure 9).



Menthofuran T0=1.77%	9/13/06	3/12/07
GM	1.82	1.68
BP	1.81	1.75
WP	1.81	1.74
EPOXY	1.81	1.73
CTRL	1.79	1.73

Figure 9. Menthofuran levels for Natural Oil of Peppermint, Regular testing

Total heads for regular testing showed a downward trend from 12.69% at T0 to 11.21% in the control and 10.70% in oil exposed to galvanized metal (see figure 10).



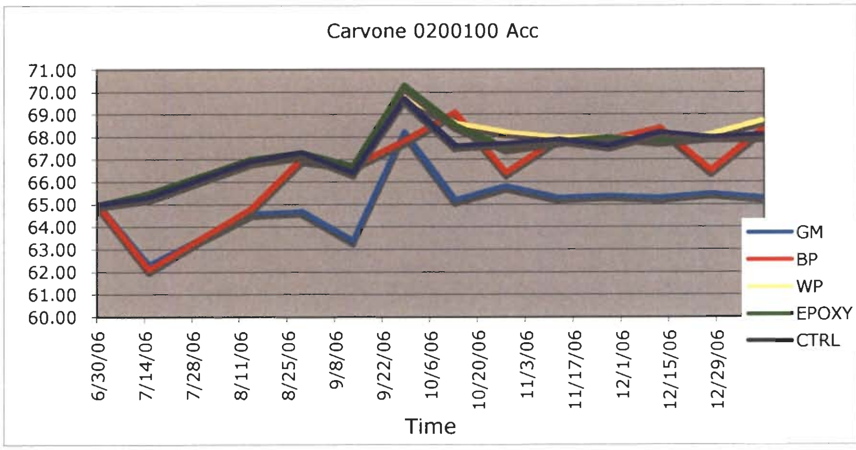
Tot Heads T0=12.69%	**9/13/06**	**3/12/07**
GM	12.12	10.70
BP	12.09	11.09
WP	12.06	11.03
EPOXY	12.03	11.06
CTRL	11.90	11.21

Figure 10. Total Heads for Natural Oil of Peppermint, Regular testing

Physical constants for regular testing were very similar to accelerated testing results. Optical rotation varied from -25.4 to -24.7 degrees. Refractive index varied from 1.4609 to 1.4614. Specific gravity varied from 0.8991 to 0.8999 throughout testing. None of the physical constants showed a clear upward or downward trend.

Natural oil of Spearmint, 0200100, Accelerated and Regular test results:

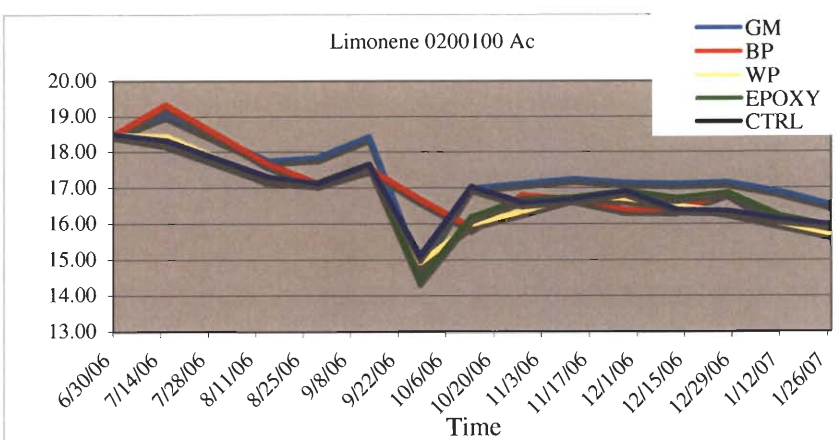
Total carvone levels in accelerated testing of natural oil of spearmint increased slightly with all four materials and in the control. The initial level of carvone at T0 was 65% and increased to 68.1% in the control. In the other materials, carvone levels at T14 varied from 65.5% in galvanized metal to 68.1% in the plastics (see figure 11).



Carvone Acc T0=65%	7/15/06	1/26/07
GM	62.30	65.50
BP	62.10	68.10
WP	65.40	67.90
EPOXY	65.50	68.10
CTRL	65.30	67.60

Figure 11. Carvone levels Natural Oil of Spearmint, Accelerated testing

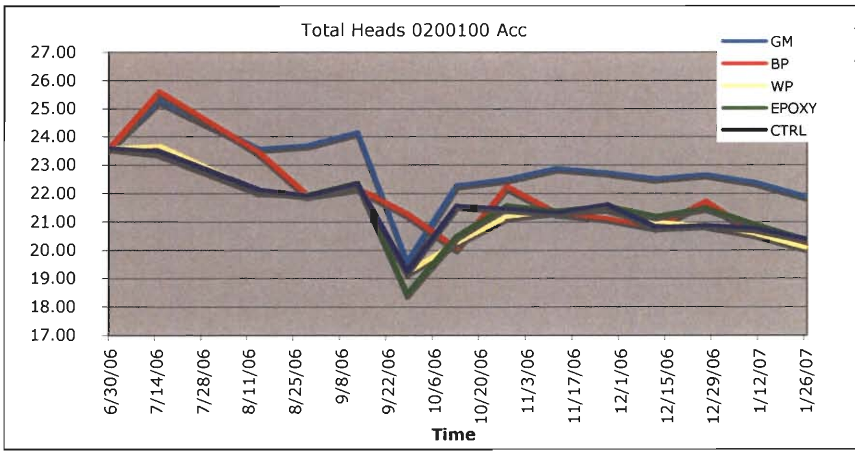
Limonene levels decreased consistently throughout all materials used. Initial levels were 18.5% and decreased to levels between 16.56% and 15.71% (see figure 12).



Limonene Acc T0=18.5%	7/15/06	1/26/07
GM	19.07	16.54
BP	19.34	15.80
WP	18.45	15.71
EPOXY	18.26	15.95
CTRL	18.33	15.98

Figure 12. Limonene Natural Oil of Spearmint, Accelerated testing

Total heads also decreased from 23.61% to 20.41% in the control (see figure 13).

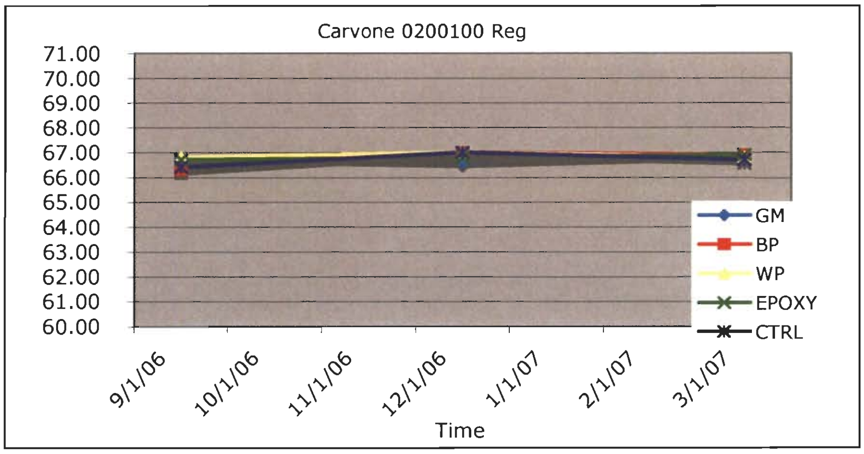


Total heads		
T0=23.61%	7/15/06	1/26/07
GM	25.29	21.90
BP	25.61	20.16
WP	23.68	20.09
EPOXY	23.41	20.38
CTRL	23.48	20.41

Figure 13. Total Heads Natural Oil of Spearmint, Accelerated testing

Physical constants for accelerated testing did not vary significantly. Optical rotation at T0 was -56.7 degrees and decreased slightly in each subsequent testing, between -54.2 and -55 degrees. Refractive index ranged from 1.4868 to 1.4898 but mostly stayed within the range of 1.4884-1.4891. Specific gravity increased slightly in the control from 0.9270 to 0.9306.

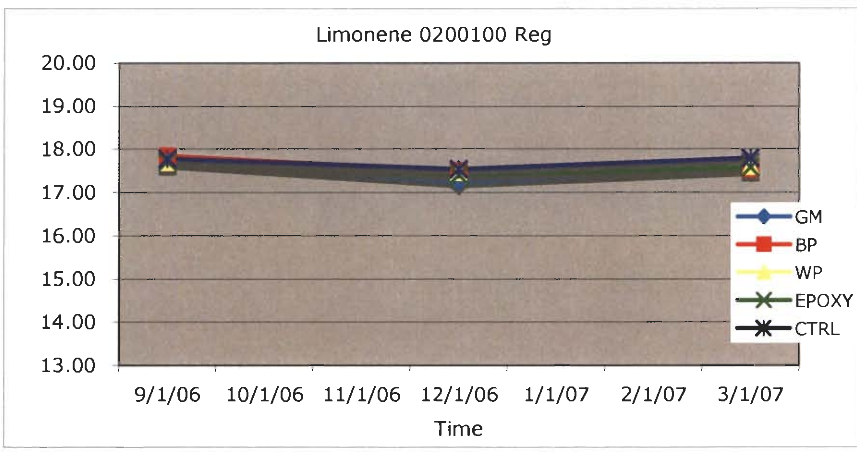
In regular tested oils, carvone, limonene, and total head levels changed to a lesser degree than in accelerated testing. Carvone levels at T0 were 65% and increased at most to 67% in the plastics at T2 (see figure 14).



Carvone		
T0=65%	9/28/06	3/27/07
GM	66.80	66.90
BP	66.30	66.90
WP	66.80	66.80
EPOXY	66.70	66.90
CTRL	66.40	66.70

Figure 14. Carvone Natural Oil of Spearmint, Regular testing

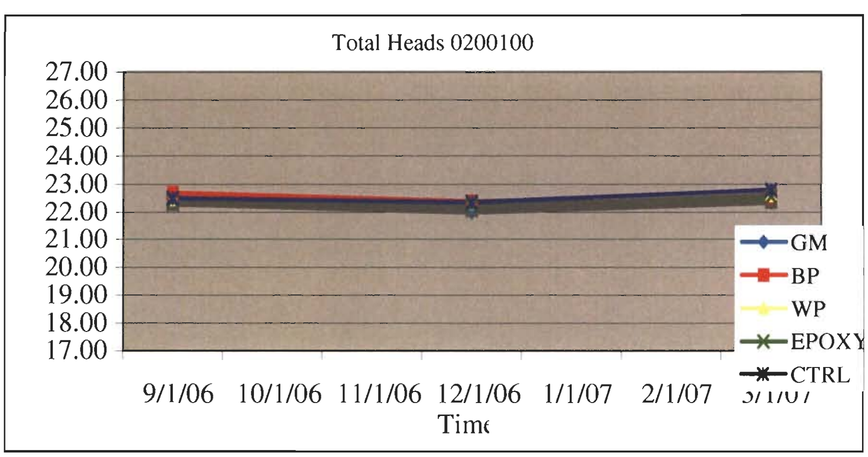
Limonene decreased from 18.5% to 17.79% in the control (see figure 15).



Limonene		
T0=18.5%	9/28/06	3/27/07
GM	17.69	17.52
BP	17.84	17.54
WP	17.71	17.61
EPOXY	17.72	17.57
CTRL	17.78	17.79

Figure 15. Limonene levels Natural Oil of Spearmint, Regular testing

Total heads decreased from 23.61% to 22.78% in the control (see figure 16).



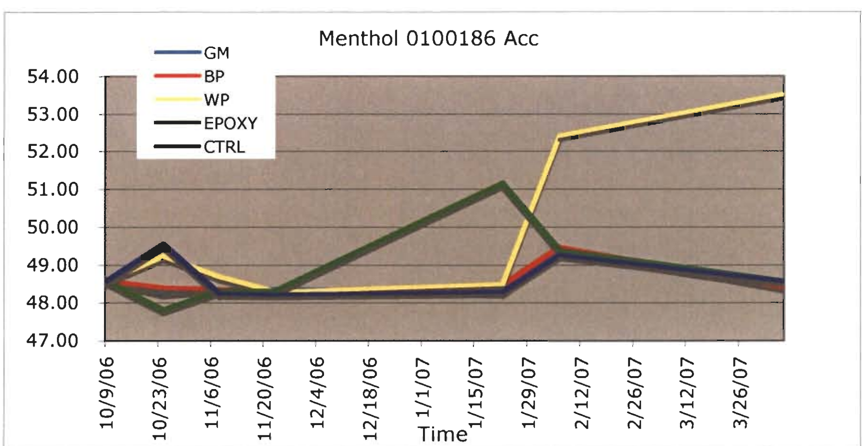
Total Heads		
T0=23.61%	9/28/06	3/27/07
GM	22.45	22.49
BP	22.68	22.52
WP	22.46	22.61
EPOXY	22.42	22.55
CTRL	22.50	22.78

Figure 16. Total Heads levels Natural Oil of Spearmint, Regular testing

Refractive index varied from 1.4884 to 1.4891. Specific gravity varied from 0.9263 to 0.9281. Optical rotation remained between -56 to -57 degrees.

Redistilled oil of Peppermint, Farwest Terpeneless, 0100186 Accelerated and Regular test results:

100186 testing began on September 9, 2006 and continued through April 2007. 100186 showed almost no variability in menthol levels. At T0, menthol was 48.59%, and this level varied less than 1% throughout testing (see figure 17). Menthone levels showed little change as well, and varied from 21.67% to 21.19% for all materials. White plastic oil at T8 showed an increase in menthol to 53.51% and menthone to 22.81%. Epoxy oil at T7 increased similarly. Both of these oils did not stay at these high levels, and at the next testing decreased back down to levels similar to the other oils (see figure 18).



Menthol		
T0=48.59%	10/24/06	4/6/07
GM	48.26	48.46
BP	48.40	48.41
WP	49.23	53.51
EPOXY	47.82	48.59
CTRL	49.55	48.58

Figure 17. Menthol levels 0100186, Accelerated testing

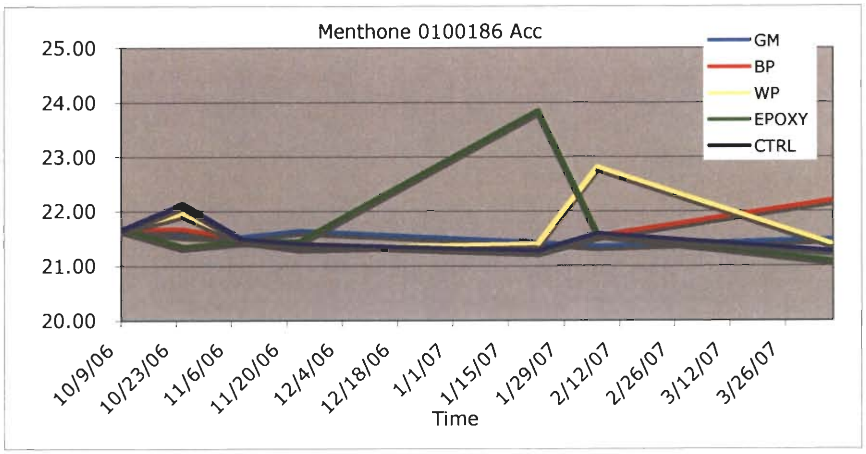


Figure 18. Menthone levels 0100186, Accelerated testing

Menthone		
T0=21.67%	10/24/06	4/6/07
GM	21.57	21.49
BP	21.68	22.19
WP	21.96	21.40
EPOXY	21.36	21.10
CTRL	22.13	21.27

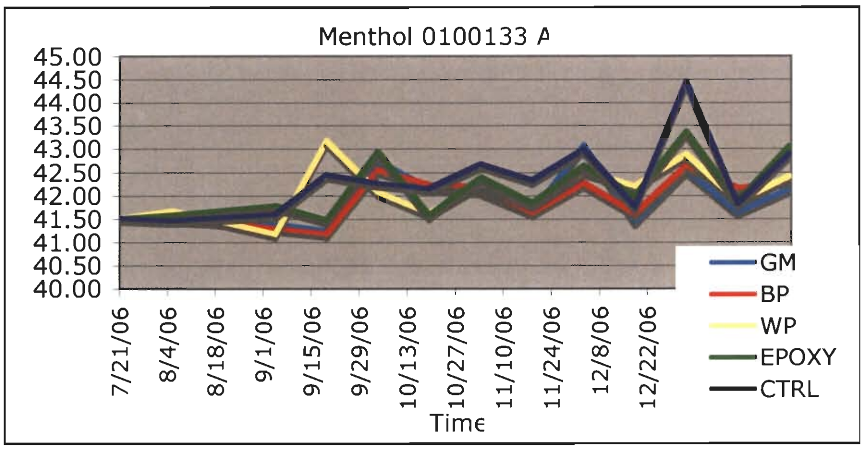
Menthofuran levels decreased very slightly from 1.68% to around 1.58% in all oils. P-cymene levels stayed constant throughout testing at 0.02-0.04% for all oils, however in white plastic oil p-cymene levels increased to 0.09% and 0.10% in T8 and T12.

Optical rotation for accelerated testing of 100186 stayed within the range of -26.8 to -27.8 degrees. Refractive index fluctuated from 1.4601 to 1.4631 in all oils. In the control and blue plastic oil, refractive index remained between 1.4608 and 1.4612. Specific gravity fluctuated from .9015 to .9070 in the control oil. In epoxy oil, specific gravity increased to 0.9275 at T6 but then dropped down to initial levels during subsequent testing.

100186 regular testing only occurred twice in September 2006 and April 2007. Levels between the two dates changed very slightly. Menthol levels remained almost constant at 48.59%, menthone levels remained close to 21.67%, menthofuran levels decreased slightly from 1.68% to 1.58%, p-cymene levels remained constant between 0.03% to 0.02%. Optical rotation fluctuated between -27.8 to -27.5 degrees, refractive index stayed within the range of 1.4610 and 1.4607. Specific gravity for all oils changed from .9015 to .9017.

Special Crystal White, 0100133, Accelerated and Regular test results:

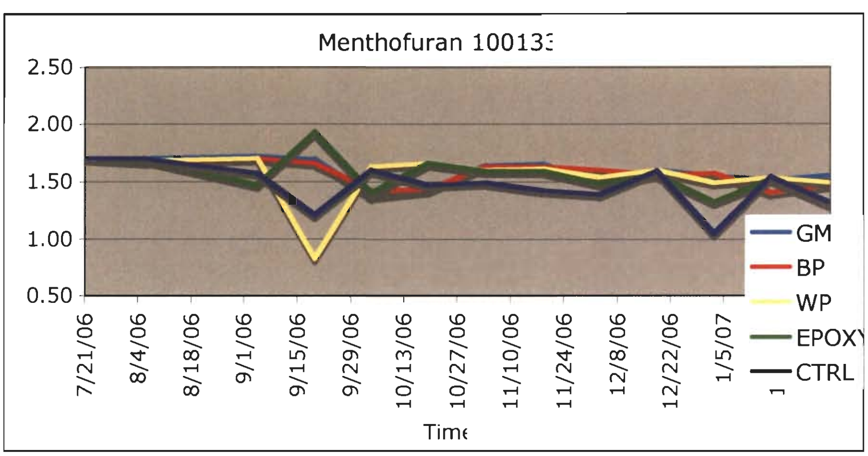
100133 testing began in July 2006 and continued through February 2007. Menthol levels increased slightly from 41.5% to around 42.5% with some variances from this range seen in each oil. Control oil reached 44.45% in T11 but decreased again in T12 (see figure 19).



Menthol T0=41.5%	8/5/06	2/1/07
GM	41.49	42.13
BP	41.54	42.30
WP	41.67	42.41
EPOXY	41.59	43.05
CTRL	41.47	42.89

Figure 19. Menthol levels 0100133, Accelerated testing

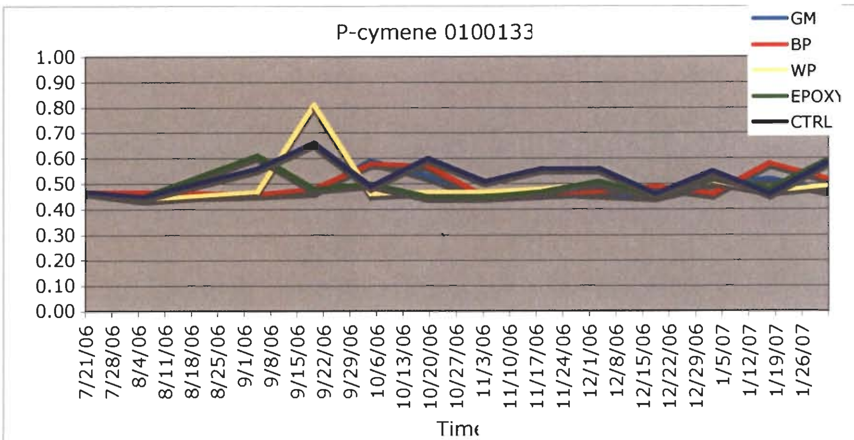
Menthone levels slightly decreased from 23.45% to 23.25%. Slight differences in menthone occurred in white plastic and epoxy oil with levels decreasing below 23.0% and increasing to almost 24%. Menthofuran levels decreased from 1.70% to a range between 1.55% and 1.3% (see figure 20).



Menthofuran T0=1.70%	8/5/06	2/1/07
GM	1.70	1.55
BP	1.67	1.46
WP	1.68	1.49
EPOXY	1.69	1.30
CTRL	1.70	1.32

Figure 20. Menthofuran levels, 100133 Accelerated testing

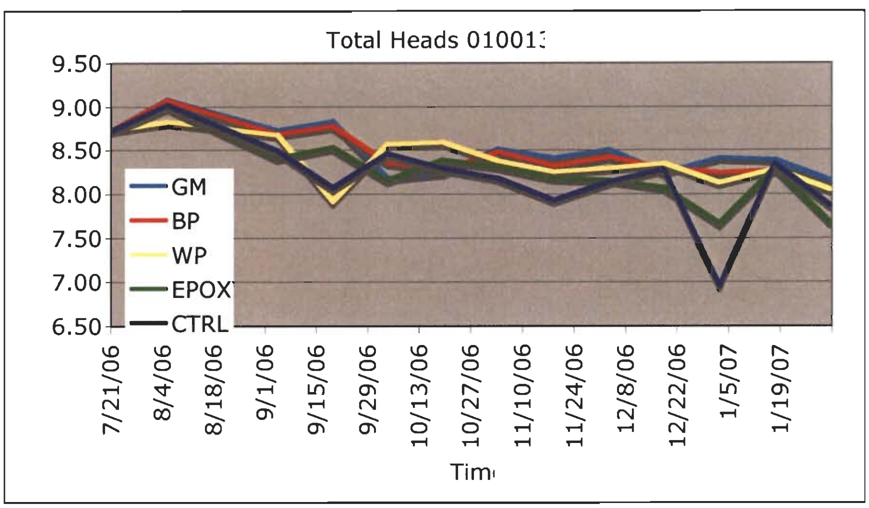
P-cymene increased slightly from 0.47% at T0 to a maximum of 0.59% in all oils except white plastic. White plastic oil showed an increase of p-cymene to 0.81% at T4 but decreased in the following tests (see figure 21).



P-cymene T0=0.47%	8/5/06	2/1/07
GM	0.45	0.47
BP	0.47	0.52
WP	0.44	0.49
EPOXY	0.45	0.59
CTRL	0.45	0.58

Figure 21. P-cymene level 0100133, Accelerated testing

Total heads decreased less than 1% for all materials (see figure 22).

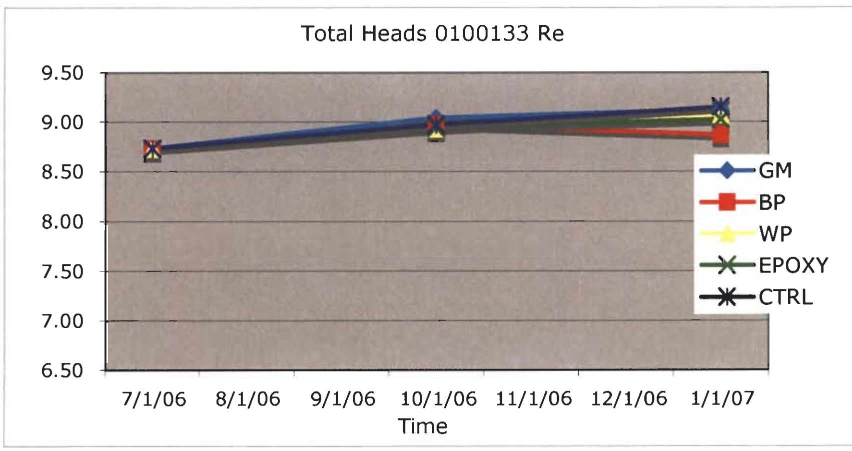


Total Heads	8/5/06	2/1/07
GM	9.08	8.16
BP	9.07	8.05
WP	8.83	8.05
EPOXY	9.00	7.68
CTRL	9.02	7.87

Figure 22. Total Heads 0100133, Accelerated testing

Physical constants for accelerated 100133 testing did not show any trends. Refractive index varied from 1.4595 to 1.4628 with most oils staying above a refractive index of 1.4600. Optical rotation for all oils stayed within a range of -23.8 to -24.5 degrees. Specific gravity for all oils began at 0.9021 and increased slightly throughout testing with the highest value being 0.9057 in white plastic oil at T4.

100133 regular testing began in July 2006 and continued to January 2007 with a total of 3 trials tested. Menthol and menthone levels remained constant throughout testing with both components staying within a 1% range of the initial values. P-cymene decreased around .03% in all oils from .47% initially. Total heads increased slightly in all oils from 8.73% with a rise of up to .3% at T2 (see figure 23).



Total Heads	10/18/06	1/16/07
GM	9.04	9.13
BP	8.97	8.87
WP	8.93	9.07
EPOXY	8.94	9.02
CTRL	8.97	9.15

Figure 23. Total Heads levels, 0100133 Regular testing

All physical constants remained constant throughout testing. Optical rotation stayed within a narrow range of -24.3 to -24.7 degrees, refractive index remained within 1.4603 and 1.4607, and specific gravity remained within the range of .9021-.9017.

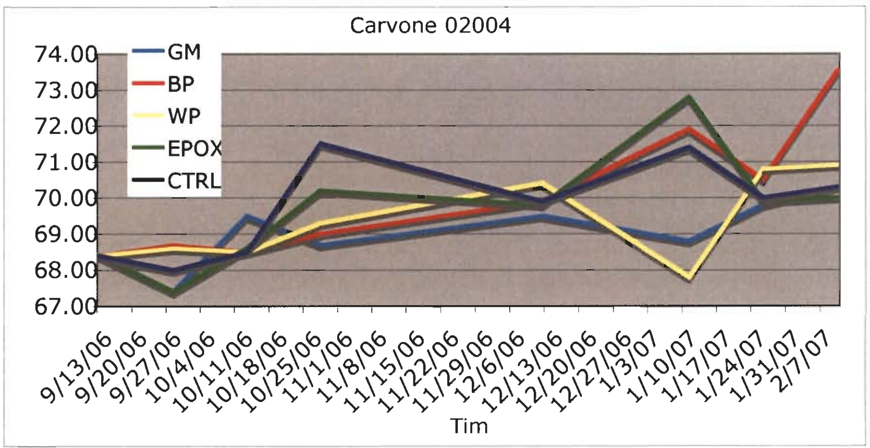
Pushing Envelope Willamette, 1102003, Accelerated and Regular test results:

1102003 accelerated testing began in December 2006 and ended in March 2007. 1102003 accelerated testing did not show any significant changes in the levels of menthol, menthone, and menthofuran. Menthol levels for all materials increased less than 2% during T3-T6 and then decreased again to almost initial levels for T7 testing. Menthone levels did not fluctuate more than 1% from the initial level for all materials. Menthofuran levels remained very similar and did not vary more than .1% from 0.51%. P-cymene levels did not change significantly. At T0, p-cymene was 0.14% and fluctuated slightly in the subsequent trials. Total heads decreased slightly between T2-T6 and then increased for T7 testing back near the initial levels. Physical constants were measured twice for this particular peppermint oil. Specific gravity remained in a narrow range of 0.8971-0.8978, refractive index from 1.4594 to 1.4600, and optical rotation from -23.5 to -23.8 degrees.

1102003 regular testing was measured a total of two times, with the initial sample taken in December 2006 and the next sample in March 2007. Menthol levels did not change significantly, with the most change in galvanized metal oil from 41.59% to 40.9%. Menthofuran did not change more than 0.01% from initial levels, p-cymene decreased by 0.03% in all five oils, menthone levels did not change more than 0.2% from initial levels, and total heads decreased in all oils by less than 1%. Physical constants for this oil were not taken initially and therefore no comparison can be made from T0 to T1. However, T1 physical constants between the oils immersed with five different materials did not vary amongst themselves.

Rectified oil of Spearmint, 0200420, Accelerated and Regular test results:

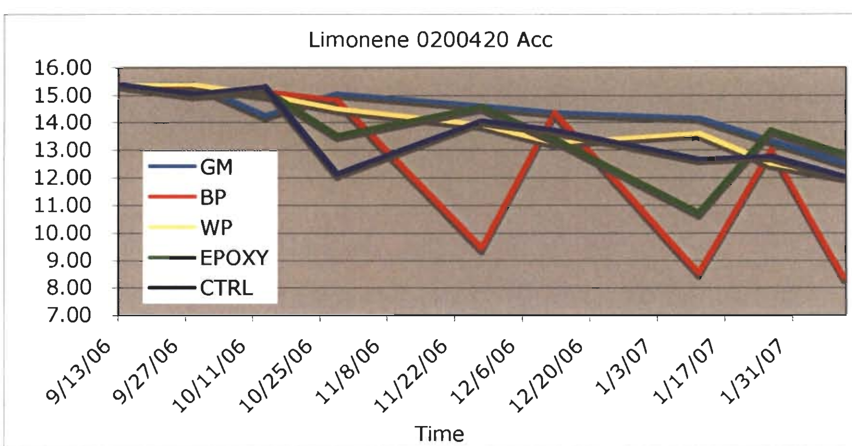
200420 accelerated testing began in September 2006 and ended February 2007. Carvone levels for all materials increased from 68.4% to a minimum of 70%, although blue plastic oil increased to 73.5% during the last trial T10 (see figure 24).



Carvone	9/28/06	2/10/07
GM	67.40	70.30
BP	68.70	73.50
WP	68.60	70.90
EPOXY	67.40	70.00
CTRL	68.00	70.30

Figure 24. Carvone levels 0200420, Accelerated testing

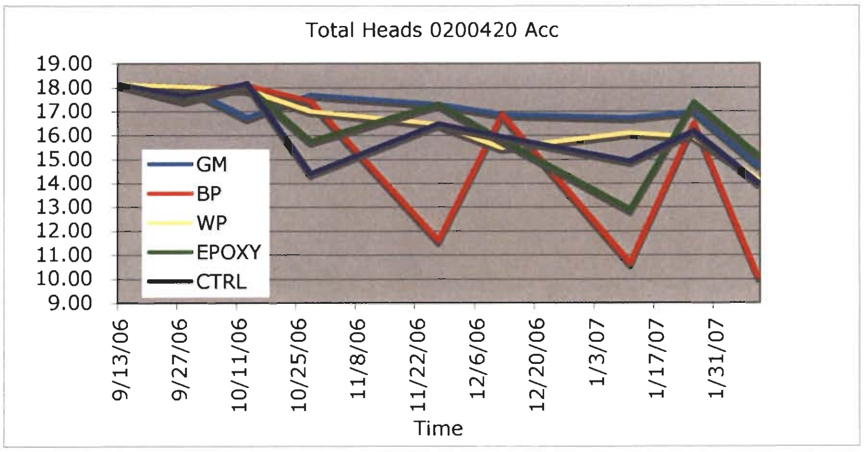
Limonene levels decreased throughout testing for all materials from 15.38% initially to a maximum of 12.86% at T10 for all oils, however some oils in trials before T10 decreased to as low as 8.54% in blue plastic at T8 and 8.37% at T10 (see figure 25)



Limonene	9/28/06	2/10/07
GM	15.41	12.52
BP	15.28	8.37
WP	15.38	12.02
EPOXY	15.01	12.86
CTRL	15.05	12.04

Figure 25. Limonene levels 0200420, Accelerated testing

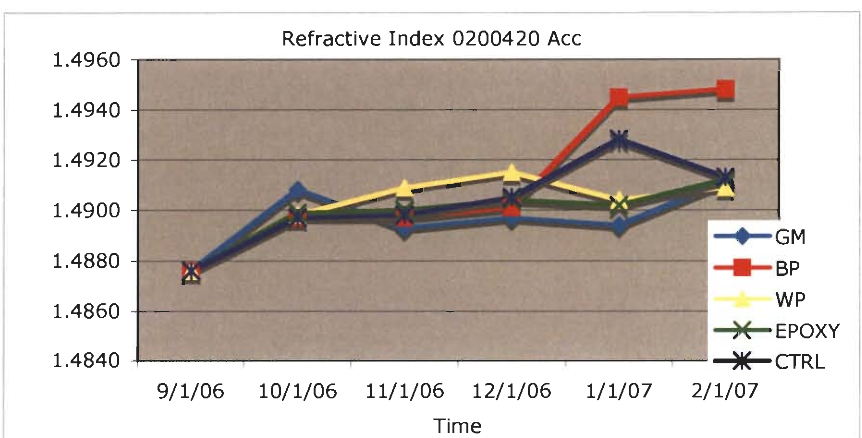
Total heads for 200420 decreased from 18.14% initially to a maximum of 15.16% at T10. Again, there were fluctuations depending on the time and the material added to the oil. In blue plastic oil at T10, total heads were 10.10%. However, at T9 with blue plastic oil, the total heads were 16.5% (see figure 26).



Total Heads	9/28/06	2/10/07
GM	18.07	14.76
BP	17.94	10.10
WP	18.05	14.16
EPOXY	17.46	15.16
CTRL	17.67	14.02

Figure 26. Total Heads levels 0200420, Accelerated testing

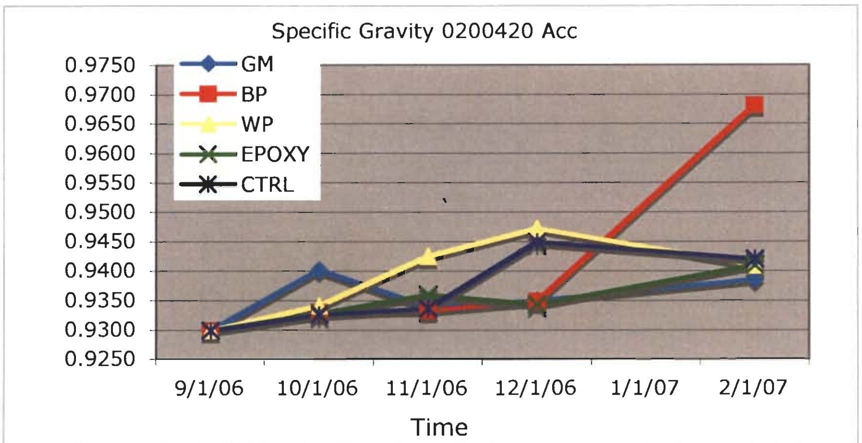
Refractive index for accelerated testing of 200420 increased from T0 at 1.4876 to above 1.4890 for all oils containing each material. The most change in refractive index was seen in blue plastic oil, which increased to 1.4948 in the last trial T10 (see figure 27).



Refr. Indx	10/13/06	2/10/07
GM	1.4908	1.4911
BP	1.4897	1.4948
WP	1.4898	1.4909
EPOXY	1.4899	1.4912
CTRL	1.4897	1.4913

Figure 27. Refractive Index levels 0200420, Accelerated testing

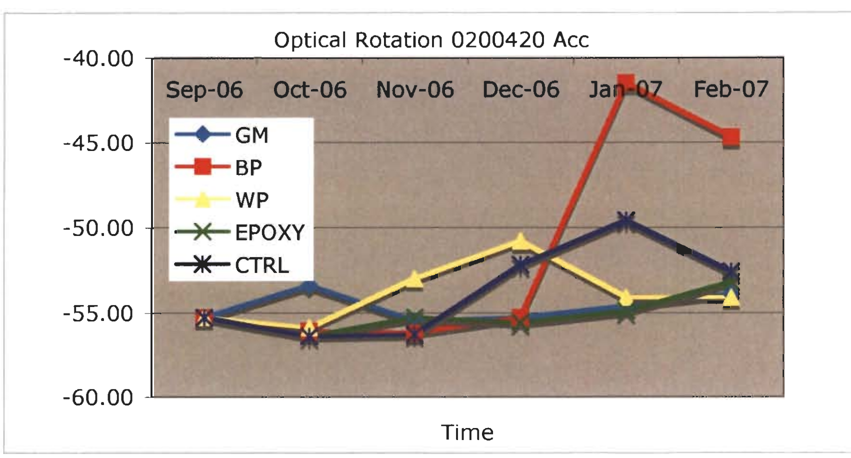
Specific gravity also showed an upward trend over time for all oils from 0.9299 at T0 to above .9400. The specific gravity of some oils increased dramatically for particular trials including an increase to 0.9744 in blue plastic at T8 (see figure 28).



Specific G.	10/13/06	2/10/07
GM	0.9402	0.9385
BP	0.9330	0.9681
WP	0.9341	0.9409
EPOXY	0.9328	0.9410
CTRL	0.9326	0.9420

Figure 28. Specific Gravity levels 0200420, Accelerated testing

Optical rotation showed a decreasing trend, which fluctuated more than previous oil types. Blue plastic showed the most instability over time, with the initial reading at -55.3 degrees and the reading at T8 at -41.5 degrees. Other oils had an ending optical rotation at T10 in the -53 degree range (see figure 29).



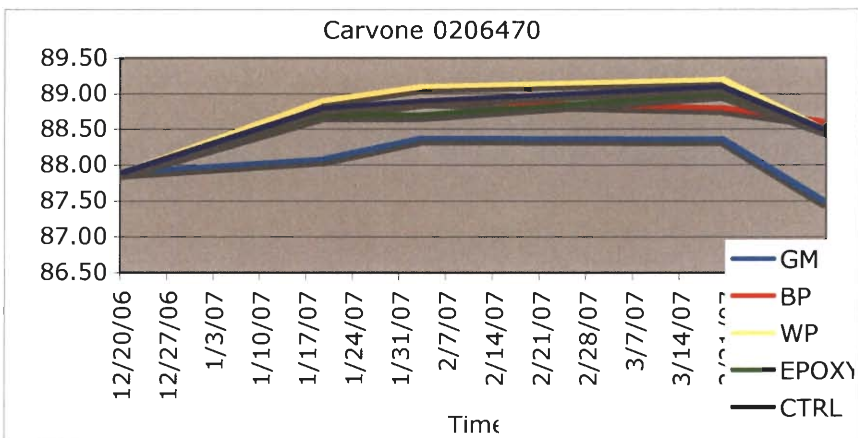
Optical R.	10/13/06	2/10/07
GM	-53.40	-53.90
BP	-56.10	-44.70
WP	-55.90	-54.10
EPOXY	-56.40	-53.20
CTRL	-56.40	-52.60

Figure 29. Optical Rotation levels 0200420, Accelerated testing

Regular testing of 200420 started in September 2006 and ended in March 2007, with two total trials taking place. The carvone, limonene, and total heads at T0 and T2 were compared and showed no fluctuation. Similarly, optical rotation did not change significantly between T0 and T2 testing. Specific gravity increased from 0.9299 initially to a minimum of 0.9317 in galvanized metal at T2, and a maximum of 0.9323 in blue plastic at T2. Refractive index also increased from 1.4876 initially to a minimum of 1.4896 at T2 in all oils.

Rectified oil of Spearmint, Farwest Scotch Terpeneless, 0206470, Accelerated and Regular test results:

Accelerated testing of 206470 started in December 2006 and ended in April 2007 and showed almost no changes from T0 to T8. Initially, carvone levels were 87.9% and did not fluctuate over 89.1% throughout testing (see figure 30).



Carvone	1/19/07	4/4/07
GM	88.08	87.51
BP	88.70	88.60
WP	88.90	88.50
EPOXY	88.70	88.50
CTRL	88.80	88.50

Figure 30. Carvone levels 0206470 Accelerated testing

Limonene levels did not fluctuate more than 0.2% from the initial level at 0.51%. Total heads at T0 was 0.60% and did not fluctuate more than 0.14%. Physical constants were tested twice for 206470. Optical rotation decreased from -53.2 degrees to -47.8 degrees in galvanized metal oil, and from -53 degrees to -49.3 degrees in the control oil. Optical rotation in epoxy, blue plastic, and white plastic oils did not fluctuate by more than 1.1 degrees (see figure 31).

Opt Rot	1/19/07	3/20/07
GM	-53.20	-47.80
BP	-52.80	-53.30
WP	-52.30	-53.40
EPOXY	-53.00	-49.30
CTRL	-53.00	-53.20

Figure 31. Optical Rotation 0206470 Accelerated testing

Refractive index increased by less than 0.02 from the first value at T2 to the final value at T6. Specific gravity increased very slightly as well from the initial value at T2 to the final value at T6.

Regular testing of 206470 began in December 2006 and ended in March 2007. Two trials of 206470 were taken total. Initial and final levels for oils containing each material remained constant. Physical constants could not be compared because values were only recorded for one trial. However, the physical constants taken at T1 compare with constants from earlier trials of the accelerated testing.

Redistilled oil of Spearmint, 0200717, Accelerated and Regular test results:

Accelerated testing of 200717 began in December 2006 and ended in April 2007. Carvone levels did not change more than 1.4% from the initial level of 85.5%. Limonene levels did not change more than 0.7% from the initial level of 8.10%. Total heads decreased less than 1% throughout testing. Physical constants were tested twice at T2 and T8. Optical rotation did not fluctuate more than 0.4 degrees from -56.70 degrees initially. Refractive index also remained steady and remained in the range of 1.4947 to 1.4954. Specific gravity remained within the range of 0.9456 and 0.9468 for all materials.

Regular testing of 200717 began in December 2006 and ended in March 2007 with two total trials recorded. The initial carvone level was 85.4% and decreased less than 0.5% for all materials. Limonene levels decreased by less than 0.16% from 8.1%

initially. Total heads decreased by less than 0.14% from an initial level of 9.71%. Physical constants were tested twice and did not show any significant fluctuation. Optical rotation stayed within a range of -56.7 to -57.1 degrees. Refractive index remained within a range of 1.4942 to 1.4947. Specific gravity remained in a range between 0.9450 and 0.9456.

Overall Trends:

The peppermint mint oils tested, 100100, 100186, 1102003, and 100133, showed less variation in composition through analysis with gas chromatography than did spearmint oils. Spearmint oils tested, 200100, 200717, 206470, and 200420, showed more variation in their composition when analyzed with gas chromatography. Overall trends in peppermint oils included a relatively stable level of menthol without general increasing or decreasing trends. 100186 proved to vary the most in menthol levels with a maximum increase of menthol from 48.59% to 53.51%. However, this change in menthol was not indicative of a trend and most of 100186 oils remained at fairly constant levels.

P-cymene levels showed a slight upward trend in all peppermint oils, except for 100186, at accelerated testing levels. P-cymene levels for regular testing did not indicate any trends. Trends for menthone levels decreased very slightly in 100100 and 100133 only. 100186 and 1102003 levels of menthone did not show any trends. Accelerated testing of menthofuran indicated slight downward trends in 100133, 100186 and 100100 oils, but did not show any trend for 1102003. Total heads decreased slightly for all oils in accelerated testing. Total heads did not show any variance in regular testing.

The dates of 6/15/06, 9/13/06, and 12/12 or 12/27/06 were compared for 100100 regular and accelerated peppermint oil (see figures 32-35). The levels of menthofuran changed slightly more in the accelerated than in the regular testing. The menthol levels in the regular testing oil did not change, while the menthol levels in accelerated testing oil increased slightly. P-cymene levels fluctuated for both accelerated and regular tested oil, but accelerated oil exhibited the overall highest level of p-cymene. Total heads decreased more significantly in the accelerated tested oil than in the regular tested oil.

Trends for key peaks in Natural oil of Peppermint, Accelerated and Regular tests:

100100				
Menthol	6/15/06	9/13/06	12/12/06	3/12/07
Regular	40.05	40.00	40.49	40.05
Accelerated	40.05	41.71	41.23	

Figure 32. Menthol levels of accelerated and regular testing, Natural Oil of Peppermint 0100100

100100				
Menthofuran	6/15/06	9/13/06	12/12/06	3/12/07
Regular	1.77	1.79	1.66	1.73
Accelerated	1.77	1.45	1.62	

Figure 33. Menthofuran levels of accelerated and regular testing, Natural Oil of Peppermint

100100				
P-cymene	6/15/06	9/13/06	12/12/06	3/12/07
Regular	0.26	0.29	0.40	0.22
Accelerated	0.26	0.46	0.30	

Figure 34. P-Cymene levels of accelerated and regular testing, Natural Oil of Peppermint

100100				
Total Heads	6/15/06	9/13/06	12/12/06	3/12/07
Regular	12.69	11.90	11.89	11.21
Accelerated	12.69	11.27	11.49	

Figure 35. Total Heads levels of accelerated and regular testing, Natural Oil of Peppermint

The spearmint oils tested displayed more variations in the composition over accelerated testing. Trends in carvone levels increased for all four spearmint oils, with the greatest increasing trend in 200100. Limonene levels in all four spearmint oils decreased over time in accelerated testing. Total head trends for accelerated testing decreased in 200420 and 200100, but did not seem vary significantly in 200717 and 206470. The total heads for 200420 in accelerated conditions varied the most. The initial value for total heads at T0 was 18.14%. Final values fell to between 10.1% to 15.15%.

The dates of 6/30/06, 9/28/06, and 12/27/06 were compared for 0200100 regular and accelerated spearmint oil (see figures 36-38). The levels of carvone, limonene, and total heads changed more significantly in accelerated oil as compared to regular oil. Both oils exhibited the same trends, but accelerated oil trends showed a greater degree of change.

Trends for key peaks in Natural oil of Spearmint, Accelerated and Regular tests:

200100 **Carvone (%)**	6/30/06	9/28/06	12/27/06	3/27/07
Regular	65.00	66.40	67.00	66.70
Accelerated	65.00	67.60	68.10	

Figure 36. Carvone levels of accelerated and regular testing, Natural Oil of Spearmint

200100 **Limonene (%)**	6/30/06	9/28/06	12/27/06	3/27/07
Regular	18.50	17.78	17.53	17.79
Accelerated	18.50	17.03	16.14	

Figure 37. Limonene levels of accelerated and regular testing, Natural Oil of Spearmint

200100 **Total Heads (%)**	6/30/06	9/28/06	12/27/06	3/27/07
Regular	23.61	22.50	22.35	22.78
Accelerated	23.61	21.58	20.77	

Figure 38. Total Heads levels of accelerated and regular testing, Natural Oil of Spearmint

IV. Discussion

All peppermint and spearmint oils tested showed more erratic levels of components in the accelerated testing. Therefore, the stability of peppermint and spearmint oils decreased in the 40 degree Celsius oven compared to the 25 degree Celsius temperature. Galvanized metal, blue plastic, white plastic, and epoxy materials immersed in peppermint and spearmint oil over both accelerated and regular testing conditions failed to influence the composition of the mint oils. Additionally, mint oil color did not change significantly over time.

Trends were hard to compare between accelerated and regular tested oils because of a discrepancy in the sampling of the data. Oils in 25 degree Celsius conditions were only tested every three months, while oils in 40 degree Celsius conditions were tested every 15 days. However, data was compared using total fluctuations from T0 for both regular and accelerated oils.

Peppermint oil tested under regular conditions did not show any major variances, which suggests that these oils are stable in room temperature. Peppermint oil in accelerated testing was thought to exhibit variable behavior compared with regular testing because the oil was subject to increased evaporation. This hypothesis is suggested because the tops of vials for accelerated testing were left unscrewed for safety reasons. Oils contain low boiling components of oils which are presumed to evaporate easily. With increased temperature, evaporation occurred more readily. This is shown from the data by the decrease in total heads for only accelerated tested oils. Regular testing data supports the hypothesis of evaporation of heads. Tops of vials for regular testing were screwed down tightly, which prevented low boilers from evaporating. Overall, no significant trends were found in any peppermint oils. Changes occurred more readily in peppermint oils tested in accelerated conditions than in regular conditions. Because of a lack of trends in key components of peppermint oil using gas chromatography analysis, peppermint oil was found to be stable. Stability was not affected by the addition of galvanized metal, blue plastic, white plastic, and epoxy materials to the oils. Stability was not affected greatly by accelerated conditions.

Among the peppermint oil, no single oil proved to be least or most stable. All oils showed some small fluctuations in components in both accelerated and regular testing. Some oils in accelerated testing showed dramatic increases or decreases in components during a particular testing period. These sudden and temporary changes in composition were not indicative of any trends and were not exclusive among any particular oils or oils immersed in particular materials.

Spearmint oil tested under accelerated conditions showed consistent trends among the four spearmint oils tested. The spearmint oils were less stable than the peppermint oils shown by the significant trends in spearmint components, including: carvone, limonene, and total heads. Spearmint oil under regular condition was measured few times, but starting and ending values for key peaks did not vary significantly. Only 200100 regular testing showed trends because testing was performed from T0 to T3. Other oils were not tested for such a prolonged period because of time restraints. In natural oil of spearmint oil, carvone showed an increasing trend. Limonene and total heads showed a decreasing trend. Although the trends for accelerated and regular testing of 200100 were similar, regular tested oil varied to a smaller degree than accelerated tested oil.

The hypothesis for the discrepancy between regular and accelerated tested oil is the same as for peppermint oil. Because tops to the vials were not screwed down tightly, evaporation of the low boiling heads occurred. In spearmint oil, however, this caused a greater increase in the amount of variability shown in the data as compared with peppermint oil. This suggests that spearmint oil is less stable than peppermint oil when the oil exposed to the air because the vial top is not sealed tightly.

Comparison of accelerated and regular tested oils at the same time intervals (figures 32-38) indicated that accelerated tested oil components vary more rapidly than the regular oil component counterparts. One of the goals of this experiment was to correlate the accelerated time with equivalent regular time in order to expedite stability analysis of oils in the future. However, the trends for accelerated tested oils changed too rapidly, and the regular tested oils were not measured often enough to elucidate an equivalent ratio of component changes between accelerated versus regular time in mint oil.

Suggestions for future experiments

Many improvements could be made to enhance results in a future experiment. The amount of oil added to each vial was not a standardized amount, which could have affected the head space (amount of air) above the oil. The level of tightness of the tops to the vials was also not measured or standardized, so some tops could have been tighter or looser than others. This could have affected the ability of air to pass between the oil and the surrounding environment. Therefore, the amount of evaporation of the oils could not be controlled. Tops to the vials were not screwed on tightly in the 40 degree Celsius oven, which caused evaporation of some components of oil, particularly low boilers.

Tops to the vials in 25 degree Celsius conditions were screwed on tightly, but again, the level of tightness was not measured or standardized.

Variation in the data from gas chromatography could have occurred based on the instrument used to run a particular sample. Two gas chromatography machines were used to run vials. Mint oil 0200717 was run fifteen consecutive times on both machines in order to test the machine variance between runs. As shown in figure 39, the machines do not run samples identically. Thus, the components measured and compared in the stability testing experiment have variability due to the specific machine used to run the samples.

Variation between chromatograms used:

GC-1	Limonene (%)	Carvone (%)	GC-2	Limonene (%)	Carvone (%)
1	8.22	84.19	1	8.10	84.76
2	8.22	84.16	2	8.12	84.85
3	8.28	84.37	3	8.10	84.85
4	8.17	84.44	4	8.10	84.89
5	8.16	84.01	5	8.11	84.85
6	8.24	83.92	6	8.11	84.85
7	8.25	83.91	7	8.06	84.66
8	8.28	84.42	8	8.12	84.95
9	8.26	84.04	9	8.12	84.93
10	8.19	84.61	10	8.08	84.85
11	8.19	84.49	11	8.07	84.75
12	8.18	83.94	12	8.08	84.88
13	8.19	84.50	13	8.08	84.88
14	8.24	84.00	14	8.12	84.85
15	8.19	84.53	15	8.10	84.83
AVG	**8.21**	**84.23**		**8.098**	**84.84**

Figure 39. Results from GC-1 and GC-2, comparison of two key peaks from 200717

Comparing accelerated and regular tested oils proved to be difficult due to the different methods used to collect these oils. Regular tested oils were only collected every three months, while accelerated oil was tested every 15 days. The time period for this experiment lasted less than one year, and therefore only few samples from regular testing were collected. Without time constraints, the longer periods of testing for regular conditions would not be as unfavorable. Because of time constraints in this experiment, only 100100 and 200100 regular and accelerated oils were compared for longer than two trials.

Only the composition of the mint oils were tested and compared. Odor and taste characteristics, which are used to measure the organoleptic qualities of mint oils, were not tested. Gas chromatography has limitations and cannot measure the leaching of chemicals from materials into the oil.

V. Conclusion

This experiment tested the stability of mint oils over time with four various packaging materials immersed in the oil. The oils were exposed to 25 degree and 40 degree Celsius temperatures. Composition of the mint oils was tested through analysis by gas chromatography. Levels of key peaks were compared in each mint oil over time. Regular tested and accelerated tested oils behaved differently, with regular oil composition varying less over time. One of the reasons for the heightened variance in accelerated tested oils was because the tops to vials containing the oils were left partially unscrewed. This caused an evaporation of the lower boiling components, or the heads. Future experiments could involve the use of instruments capable of detecting components that leach out of packaging materials immersed in the oils. Also, more time is needed to evaluate the regular tested oil over a longer period of time. However, the composition of mint oils was found to be stable based on their composition levels in regular testing. This finding suggests that mint oil, especially the more stable peppermint oils, is stable over time and indicates that the 12-month expiration date of mint oils could be extended.

References:

Burbott, A.J., Loomis, W.D. (1966). Effects of Light and Temperature on the Monoterpenes of Peppermint. *Plant Physiology, 42,* 20-28.

Landing, J.E. (1969). American Essence: A History of the Peppermint and Spearmint Industry in the United States. Kalamazoo, MI: A.M. Todd Foundation.

Soheil, S. M., Croteau, R.B. (2003). Menthofuran regulates essential oil biosynthesis in peppermint by controlling a downstream monoterpene reductase. *Plant Biology, 100,* 14481-14486.

Spencer, J.S., Dowd, E., Faas, W. (n.d). The Genuineness of Mint Essential Oils: A GC, GC-MS Study on Differentiating Essential Oils from *Mentha Piperita* vs. *Mentha Arvensis.* A.M. Todd Company, Kalamazoo MI.