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Conditions Associated with Clostridium sporogenes Growth as a Surrogate for Clostridium botulinum in Non-thermally Processed Canned Butter

Reed H. Taylor
Brigham Young University - Provo

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Reed Hoggan Taylor

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Master of Science

Dr. Frost M. Steele
Dr. Lynn V. Ogden
Dr. Michael L. Dunn
Dr. Michelle A. Lloyd

Department of Nutrition, Dietetics, and Food Science

Brigham Young University

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ABSTRACT

Conditions Associated with *Clostridium sporogenes* Growth as a Surrogate for

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Shelf-stable canned butter is currently available in retail stores, and many home-preservationists promote home-canning of butter. Non-cultured butter is a low-acid canned food, which would presumably require thermal processing. The lack of a thermal process step in canned butter products raises questions of potential safety, because they are hermetically sealed and generally exhibit anaerobic growth conditions, which are optimal for *Clostridium botulinum* growth. Without thermal processing, low-acid canned foods (LACF) must have inhibitory factors present to prevent *C. botulinum* growth. Some potential intrinsic inhibitory factors, or "hurdles", within butter include: reduced water activity (a_w), acidity (pH) in cultured products, elevated salt content, and the micro-droplet nature of the aqueous phase in the butter emulsion. It was hypothesized that a normal intact butter emulsion would have sufficient "hurdles" to prevent *C. botulinum* growth, while a broken butter emulsion would result in a larger aqueous phase that would allow for growth.

Butter was prepared using a batch churn method with either inoculated or uninoculated cream. Butter samples with four different salt amounts (0, 0.8, 1.6, & 2.4% added NaCl) were prepared and placed in coated aluminum cans for storage. Samples were stored for 1 or 2 week periods at either 22°C or 41°C and then plated for *C. sporogenes* growth. Samples stored at 41°C showed a significant increase over those stored at 22°C. This growth increase occurred due to incubation near the optimal growth temperature for *C. sporogenes* and damage to emulsion structure. Furthermore, sodium chloride (NaCl) addition was found to have a significant effect on *C. sporogenes* growth, with 0.8 % NaCl promoting more growth than 0%, but with decreases in growth beyond 0.8%. Uninoculated control plates were also found to have bacterial growth. This growth was attributed to other anaerobic bacteria present within the cream.

It was concluded that removal of the butter structure "hurdle" could result in *C. botulinum* growth even at elevated salt levels and therefore home preparation of canned butter is not advisable. It is also possible that commercially canned butter, if heat abused, could potentially allow for *C. botulinum* growth and therefore consumption is not recommended.

Keywords: [Canned Butter, *Clostridium botulinum, Clostridium sporogenes*]

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INTRODUCTION

Justification for the study

Many organizations have emphasized the need for emergency food storage, including the American Red Cross (American National Red Cross 2009) and the U.S. Dept. of Homeland Security (U.S. Department of Homeland Security 2010). While this has been an emphasis for years, recent natural disasters throughout the world demonstrate once again the importance of having an emergency food supply.

In emergency situations it remains important to maintain a diet with a balance of nutrients, including all types of macromolecules. The Dietary Guidelines for Americans states that the recommended daily intake of fat is 20 – 35% of calories consumed (Dietary Guidelines for Americans 2005). Fats, in emergency situations, can provide needed energy and variety to the diet.

The primary lipid sources currently used in food storage include oils and shortenings. Liquid oils and shortenings have moderate shelf stability; however both have limitations in versatility of application. Shortenings and oils typically are used in baking or frying applications or need some preparation prior to consumption. However, butter can be consumed directly (on crackers or bread) thereby increasing the palatability of other products with minimal preparation. Therefore, canned butter provides a lipid source with increased versatility of application over other products.

Currently a canned butter product is imported and sold in the United States as a shelf-stable lipid product for food storage. There has also been interest in certain circles of home preservationists to prepare and can butter for storage in the home, as demonstrated by articles and instructions available on various internet sites. For example, the National Center for Home Food Preservation (NCHFP) has addressed the issue of canning butter in the Frequently Asked Questions section of their website (Andress and Nummer 2006). One particular process for canning butter described online involves heating and melting the butter prior to canning.

The potential benefits and applications of a canned butter product have been discussed above; however, there are also safety concerns associated with canned butter. The NCHFP has addressed some of these concerns on their website, indicating that most proposed processes for storing canned butter would not prevent growth of *Clostridium botulinum* and other pathogens in butter stored at room temperature (Andress and Nummer 2006).

The primary concern with "canning" butter and other similar products is the potential for outgrowth of *C. botulinum* and subsequent toxin production. Processing requirements for canned goods differ based upon the nature of the food product. Canned butter is considered a "Low Acid Canned Food". The U.S. Food and Drug Administration (FDA) defines low-acid canned foods as any food, other than alcoholic beverages, with a pH > 4.6 and a water activity > 0.85, excluding tomatoes and tomato products having a finished equilibrium pH < 4.7 (FDA 2009b, Cole and Oh 2003). Canned butter exhibits the above characteristics and therefore it is currently not possible to ensure safe production without thermally processing the canned butter to ensure destruction of *C. botulinum* spores, according to FDA process requirements. In the absence of thermal

processing, canned butter must have other inhibitory parameters in place, to ensure a safe finished product (Cole and Oh 2003).

Hurdle technology

In many instances, it is possible to use a combination of non-thermal environmental and compositional parameters to ensure safe production of food products, where thermal processing will damage the desired finished product. Optimization of factors such as sodium chloride concentration (% NaCl), acidity (pH), redox potential, and preservatives can help minimize bacterial growth in foods (Chung and Murdock 1991). This concept was first described by Leistner in 1978 and is known as "hurdle technology" (Leistner 2000). Hurdle technology is the principle of utilizing various conditions within a food product that can act in combination to prevent microbial growth and preserve product quality (Leistner 2000, Leistner and Gorris 1995).

Bacterial growth is affected by a variety of intrinsic factors associated with butter, including acidity (pH), water activity (a_w), NaCl concentration, and butter structure. Butter is an emulsion that is composed of a continuous lipid phase with water droplets suspended throughout. The structure of these aqueous droplets affects the microbial stability of the butter product.

Potentially the combination and interaction of these factors could inhibit bacterial growth in canned butter. Each of these intrinsic factors has cut-off points at which *C. botulinum* can no longer grow and where spore outgrowth is inhibited. Values for these cut-off points are group specific and Group I *C. botulinum* is the group of primary concern in canned butter. Group I *C. botulinum* is strongly inhibited below pH 4.6, below a_w of 0.94, and at sodium chloride

concentrations above 10% (Simjee 2007). With respect to emulsion droplet size, it has been shown that bacterial growth is limited when aqueous droplet diameters are less than 20 µm (Wehr and Frank 2004).

Previously published typical values for butter (Voysey and others 2009), corroborated by our own preliminary results, indicate that levels for each of these intrinsic factors was near the limit for *C. botulinum* growth, but all were still within ranges that would allow for *C. botulinum* growth (Table 1). It is possible that, even though these intrinsic factors are individually not at levels that would inhibit *C. botulinum* growth, these sub-optimal intrinsic factors could produce a combined inhibitory effect against *C. botulinum.*

If in fact these four intrinsic factors do combine to inhibit growth of *C. botulinum,* what would happen if one of these hurdles was removed? Butter emulsion structure is a factor known to be significantly affected by storage conditions. For example, canned butter stored in a cool basement, maintains intact emulsion structure; however, the same butter stored in a hot warehouse or other location with fluctuating temperatures, breaks down and loses its structure resulting in separation of aqueous and lipid phases. Also, current methods described online for canning butter involve heating the butter which results in a separation of phases and damage of butter structure. The inhibitory effect of butter structure due to small aqueous droplet size would thus be eliminated. This possibility raises the question of whether the other three "hurdles" alone could prevent outgrowth and toxin production by *C. botulinum*.

Emulsion structure of butter

Butter is produced through a process of churning cream, an oil-in-water emulsion, until phase reversion occurs, producing a finished butter product that is a dispersion of water/solutes, air, fat crystals, and fat globules in oil (Frede and Buchheim 1994). This dispersed system is considered to be a water-in-oil emulsion. Therefore, structurally it is composed of a continuous lipid phase with aqueous droplets, containing water-soluble solutes, suspended throughout (Charley and Weaver 1998, Fennema 1996). The structure of butter contributes to the microbial stability due to the fact that microbial growth within water-in-oil emulsions is confined within the small aqueous droplets, which provide little room for growth (Wilson and others 2002). It has been demonstrated that aqueous droplet sizes < 20 μm are inhibitory to bacterial growth (Wehr and Frank 2004). Also, properly prepared butter will have less than 5% of water droplets with diameters larger than 10um (Lund and others 2000).

If butter undergoes heat-abuse, the water droplets can coalesce to form a much larger aqueous phase. This larger aqueous phase provides a more suitable environment for microbial survival and proliferation. Verrips and Zaalburg (1980) and Verrips and others (1980) demonstrated that control of microbial growth in butter is related to prevention of coalescence of aqueous droplets. This is due to the fact that there are limited growth compounds necessary for microbial survival available in these small droplets and the water-soluble growth compounds are not able to migrate through the lipid phase from one aqueous droplet to another. If emulsion stability is compromised and coalescence of aqueous droplets occurs, microbial growth will increase. However, if the emulsion structure remains intact microbial growth is limited.

Therefore, breaking of the emulsion structure can remove one of the hurdles and provide an environment more suitable for microbial growth.

Clostridium sporogenes: surrogate for *Clostridium botulinum*

To determine the ability of a food to support growth of spoilage organisms or pathogens, microbiological challenge testing is often used (FDA 2009a). Studies that involve highly regulated, dangerous organisms, like *Clostridium botulinum*, often use a surrogate organism to prevent exposure to dangerous pathogens. *Clostridium sporogenes* is traditionally used as a surrogate organism for *Clostridium botulinum* (Mah and others 2008). *C. sporogenes* is a useful surrogate for *C. botulinum* due to the fact that it is essentially a non-toxic variant of proteolytic (Group I) *C. botulinum* (Zhu and others 2008). *C. sporogenes* is often used as a surrogate based on its successful use in thermal processing and high pressure inactivation studies (Mah and others 2008, Bull and others 2009).

Botulinum toxin is one of the most potent neurotoxins produced in nature (Arnon and others 2001). There are seven different serotypes of botulinum neurotoxin designated A-G (Turton and others 2002, Roxas-Duncan and others 2009). All serotypes of botulinum toxin are zinc metaloprotease enzymes that function by binding a nerve synapse, translocating into the neuronal vesicle, and cleaving proteins necessary for acetylcholine release. Inhibition of acetylcholine release at the neuromuscular junction results in flaccid paralysis of vital organs (Chen and others 1997).

As a result of the extreme toxicity of Botulinum toxin, *C. sporogenes* is used for challenge studies. *C. sporogenes* has been shown to exhibit increased resistance to heat and

pressure as compared to *C. botulinum* (Zhu and others 2008); therefore, determining proper conditions for limiting growth and survival of *C. sporogenes* would also likely ensure *C. botulinum* growth would be limited.

Aqueous droplet size and its effects on bacterial growth

The majority of commercial butter production occurs by a continuous process; however, for small scale production of butter, batch processes are typically used. There is evidence that water droplet size and distribution will vary based on processing method (Voysey and others 2009); therefore, batch processed butter and continuous processed butter will likely have differing aqueous droplet sizes on average. As previously stated, aqueous droplet sizes < 20 µm in diameter do not harbor bacteria; however, above 20 µm, there is increasing potential for microbial growth as droplet size increases (Wehr and Frank 2004). It is therefore important to monitor droplet size of batch produced butter to determine how bacterial growth may be affected.

Study Objective

The objective of this study was to better understand the parameters affecting *C. botulinum* growth and to specifically determine the effect of removing/altering hurdles on *C. botulinum* survival and growth. The study focused on two "hurdles" in particular: % NaCl and butter structure. Experimental parameters were designed to target the effects of altering butter structure as well as varying salt content on overall survival and growth of *C. sporogenes.* This design allows for determination of the microbial safety of canned butter and an increased understanding of parameters affecting bacterial growth.

MATERIALS AND METHODS

Growth and purification of *C. sporogenes* spores

Spore growth

Clostridium sporogenes, strain NCA 3679, ATCC #7955 was purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). Lyophilized *C. sporogenes* was reanimated as described on the Product Information Sheet ATCC 7955. The vial of purified *C. sporogenes* was rehydrated using 500 µl reinforced clostridial medium (RCM) and transferred to a test tube containing 6 ml of RCM which was then incubated anaerobically at 37°C for 24 hours (hrs). Production and purification of the *C. sporogenes* spores was performed as described by Yang and others (2009). For sporulation, 550 µl of the *C. sporogenes* growth culture was added to eight flasks containing sporulation media [3% trypticase peptone, 1% peptone, and 1% ammonium sulfate]. The flasks were heat shocked in a water bath at 80°C for 15 minutes (min) and then incubated anaerobically at 30°C for 7 days. The flasks were gently swirled after 2 days and 4 days. Completion of sporulation was determined by phase contrast microscopy and classified as the point at which sporulation had reached 90% or greater (Yang and others 2009).

Spore purification

Upon completion of sporulation, purification of the spores was performed as described by Yang and others (2009). Contents of the flasks were poured into centrifuge bottles and the spore suspensions were then centrifuged at 12,850 *g* for 15 min at 4°C. The supernatant was discarded and the spores were washed once by resuspending the pellet in 40 ml sterile deionized water. The samples were then centrifuged again under the conditions described above and the supernatant

was discarded. The pellet was resuspended in 40 ml of 1X Phosphate Buffered Saline (PBS) containing 500 µg/ml lysozyme (Sigma, St. Louis, MO, USA), sonicated for 5 min to release the spores from the mother cells, and incubated for 2 hrs in a 37°C water bath to enable lysozyme to digest any vegetative cells present. To remove vegetative cell debris, the spore suspension was centrifuged and the pellet was washed 8 times with 40 ml sterile ddH_2O, followed by centrifugation at 2000 *g*. After the supernatant was removed following the final wash step, 20 ml of sterile ddH_2O was added to the pellet in each conical tube and the spore pellet was mixed to ensure a homogeneous spore mixture. All conical tube spore contents were then combined to attain a consistent spore count. The purified spores were then aliquoted into 2 ml cryovials and stored at 4°C (Yang and others 2009).

Determination of spore counts and spore culturability

A hemocytometer was used to determine the count of the purified *C. sporogenes* spores. Dilutions (10^{-2}, 10^{-3}, & 10^{-4}) of purified spore samples were made and analyzed on a phase-contrast microscope as described by Yang and others (2009).

To determine spore culturability, duplicate spore samples were diluted and plated on reinforced clostridial agar (RCA) (38 g Reinforced Clostridial Media/L; 15 g Bacto™ agar/L; Becton Dickinson and Company, Sparks, MD, USA). The plates were incubated anaerobically at 37°C for 72 hrs (Nygaard and Hostmark 2008). Plate counts were determined following incubation.

Anaerobic incubation

Anaerobic incubation conditions were created using a sealed incubator with a vacuum pump attached. This allowed removal of ambient air from the chamber and subsequent flushing

of the chamber with an anaerobic gas mixture (90% N_2, 5% CO_2, 5% H_2). Palladium catalyst (Sigma-Aldrich, St. Louis, MO, USA) was placed in a culture dish in the incubator as an oxygen scavenger. Each time the incubator was opened, fresh palladium catalyst was used. Also Drierite (W.A. Hammond Drierite Company, Xenia, OH, USA) was placed in the incubator to control excess moisture. The plates were placed in the incubator and the chamber ambient air was removed by vacuum pump. The chamber was then flushed with the anaerobic gas mixture three times. Anaerobic indicator strips (BR0055B Oxoid Ltd, UK) were used to confirm anaerobic conditions were achieved.

Experimental Design

The experimental design targets two of the key hurdles involved in bacterial inhibition, specifically butter structure and aqueous % NaCl. As discussed above, intact butter structure is inhibitory to bacterial growth (Verrips and Zaalberg 1980, 1980). However, if a shelf stable butter product was stored at an elevated temperature (e.g. warehouse during summer), the butter emulsion would likely be broken resulting in an environment that is more supportive of bacterial growth.

The butter structure hurdle was addressed by including two different storage temperatures. Room temperature (22°C) samples would retain the emulsion structure over the storage period. However, 41°C was sufficient heat treatment to break the emulsion and result in complete separation of the lipid phase and the aqueous phase. Sample cans were stored for one or two week periods and the bacterial counts were determined in duplicate for each can. The entire experimental design shown in Figure 1 was performed in duplicate.

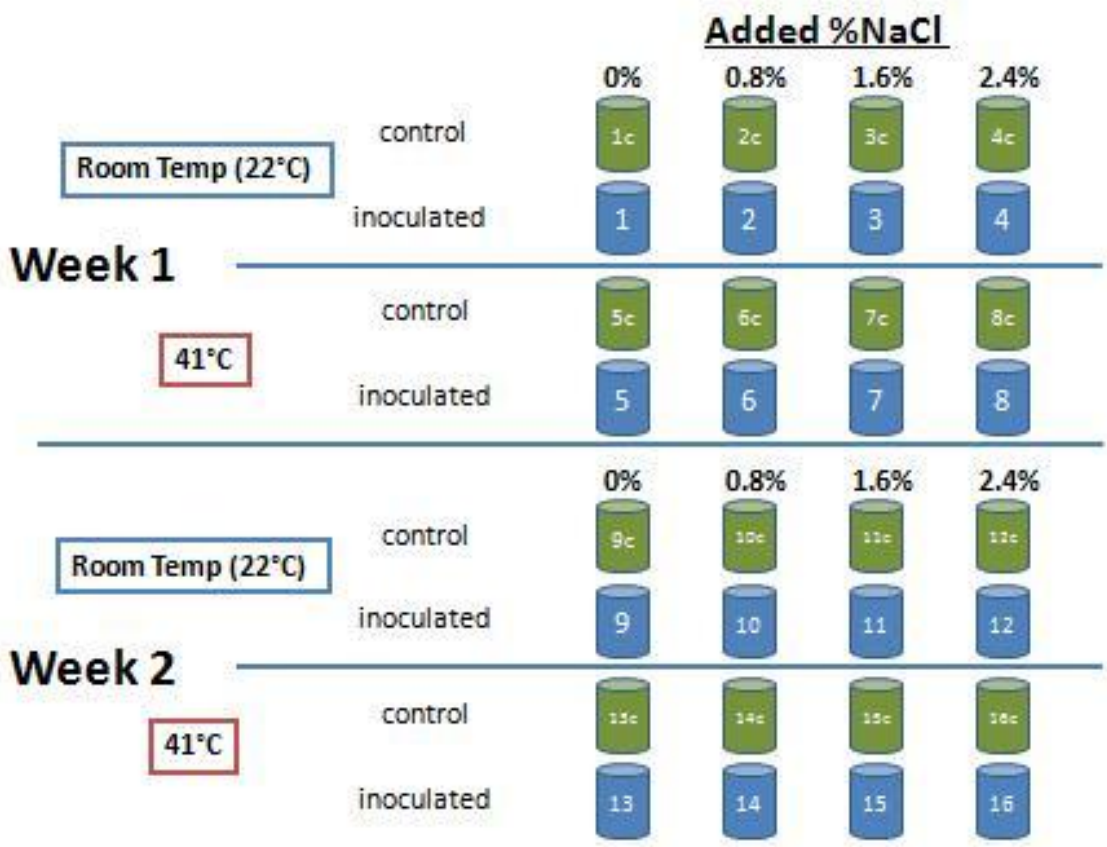

Figure 1: Experimental Design of canned butter analysis. The top can of each section designated with a number and the letter "c" following are control cans that contained butter prepared with uninoculated cream. Cans on the second row of each section, with only a number designation, are cans that had butter prepared with *C. sporogenes* inoculated cream. Temperature storage conditions for each can (22°C or 41°C) are listed to the left of each set of cans. Storage time at each temperature designation (1 week or 2 weeks) is shown to the left side, with all 4 upper rows being stored for 1 week and the bottom 4 rows being stored for 2 weeks. Added % NaCl (0, 0.8, 1.6, and 2.4%) is listed at the top of each column of cans. This entire experimental design was replicated.

Data was collected for each sample to ensure potential confounding variables were accounted for. Each canned butter sample was analyzed for: $\%O_2$ headspace and *C. sporogenes* count. The canned butter samples were then taken and the aqueous fraction was isolated as described above. The aqueous fraction was then analyzed for: pH, a_w, and % NaCl.

Batch churn butter production

Butter was produced by a batch churn process using pasteurized cream provided by Deseret Dairy (Salt Lake City, UT, USA). The butter was produced using a method adapted from Wood and others (1975) and Britten and others (2008). The cream was transported from Deseret Dairy and stored at 4°C. Prior to churning, the cream was allowed to warm slightly until it reached a temperature of 6 to 9°C. The cream was churned to butter using a Hobart A120T mixer (Hobart Corporation, Troy, OH, USA). All bowls, attachments, and instruments used during the butter making process were autoclaved prior to use to minimize potential contamination. The cream was mixed with the whisk attachment for 1 min on setting 2 and then 5-7 min on setting 3 (until the emulsion broke). Once the emulsion was broken and the cream separated into butter and buttermilk fractions, the buttermilk fraction was discarded. The remaining butter fraction was then mixed using the beater attachment for 30 seconds on setting 1 to remove any remaining buttermilk from the solid butter fraction. The butter was then washed 3-4 times by adding 250 ml of chilled autoclaved distilled water and mixing with the beater attachment until the wash water was clear. The wash residue was discarded each time. After the final wash step the butter was pressed together using cheese cloth to extract excess moisture present in the butter.

To simulate cream contaminated with *C. botulinum*, cream was inoculated with purified *C. sporogenes* spores at a concentration of 3.0×10^4 cfu/ml. It has been demonstrated that in inoculation studies the butter production process can result in lower than estimated counts of microorganisms based on the initial inoculum, as a result of lethality caused by mechanical shear as well as procedures for diluting and plating samples that can result in lethality for previously sub-lethally injured bacteria (Verrips and others 1980). These studies were not performed on *C. sporogenes* and therefore the direct applicability is unknown, however typically spores are more

resilient to a variety of challenges and therefore reduction may not be as prevalent as demonstrated with other bacterial forms.

In previous inoculation studies with water-in-oil emulsions, high levels of bacterial inoculations (> 10^{10} cfu/ml) have been used. However, such high inoculations often affected the emulsion stability (Verrips and others 1980). As a result of potential effects on emulsion stability, as well as the desire to mimic a typical contamination scenario, 3.0 x 10^4 cfu/g was determined as the optimal inoculums.

After inoculation, the cream was churned to butter following the protocol described above. The only process change for inoculated samples involved added precautions taken to ensure proper collection and disposal of by-products produced during the production of inoculated samples.

To determine the actual amount of the initial inoculum that ended up in the butter as opposed to the buttermilk, samples of each batch of butter were diluted and plated on reinforced clostridial agar. After incubation was complete the counts were determined and recorded.

Salt incorporation

Following butter formation, salt was incorporated into the butter product. Morton iodized table salt (Morton, Chicago, IL, USA) was finely ground using a coffee grinder (Hamilton Beach/Proctor-Silex, Inc., Washington, NC, USA) and dried at 100°C overnight. Dried salt was kept in sealed bottles in a desiccator prior to use.

After batch formation, the butter was separated into four 375 g fractions and salt amounts were added based on the sample cans corresponding to that particular batch of butter. The four

salt variables were 0 g (0%), 3.0 g (0.8%), 6.0 g (1.6%), and 9.0 g (2.4%); which were measured and incorporated into the 375 g butter fraction by mixing thoroughly with a sanitized rubber spatula.

Flushing, Canning, and Storage of Butter

Following salt addition, butter samples were placed into a sanitized No. 300 can coated with universal enamel. Care was taken to ensure butter samples were pressed down into the can to eliminate any air voids present. The can was flushed with N_2 gas and sealed with a ZPT-30J Ageless oxygen absorber (Mitsubishi Gas Chemical America, New York, NY) taped on the inside to help create an anaerobic environment. Based on the experimental design, the cans were stored at either 22°C +/- 2°C or 41°C +/- 2°C.

Sampling, dilutions and plating of *C. sporogenes*

Collecting a sample

Sampling of the butter product was performed as described in section 3.073 of Standard Methods for the Examination of Dairy Products (SMEDP) (Wehr and Frank 2004). The described method was adapted for sampling product from a no. 300 can. For 22 °C samples, a sterile stainless steel trier (lab scoop) was used to remove a plug of butter the length of the can, the butter sample was placed in a 50-ml conical tube; this was continued at approximately three locations within the can until a 20 g sample was obtained. The samples stored at 41°C had separate aqueous and lipid fractions; therefore the samples were mixed with a sterile stainless steel spatula to collect a representative sample. After mixing the two phases, a 20 g sample was poured into a 50-ml conical tube.

Diluting the sample

Dilutions were performed according to protocol 9.060 in the SMEDP (Wehr and Frank 2004). In the standard method, dilution blanks of 99 ml are used, however 9 ml dilution tubes with autoclaved 0.1% peptone were used for this study. Dilutions for 22°C stored samples were 10^{-1} and 10^{-2} while dilutions for 41°C stored samples were 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. The specified dilutions for each temperature variable were based on expected growth counts as determined by preliminary experiments.

Plating and Incubation

Plates were prepared by pouring RCA into disposable Petri dishes (92 x 16 mm) and allowing them to setup. Samples were plated using the spread plate technique, in which a sample pipetted onto the prepared plate is spread over the surface of the plate using a sterile bent glass rod. To plate a 10^{-1} final dilution, 1 ml of a 10^{-1} sample dilution tube was distributed across 3 separate plates. Following anaerobic incubation as described previously, the colonies on the 3 plates were counted and the sum of the 3 plates was calculated. One hundred µl aliquots from the 10^{-1} to 10^{-5} dilutions were plated on RCA to achieve final plate dilutions from 10^{-2} to 10^{-6} respectively. All dilutions were plated in duplicate and plates were incubated under anaerobic conditions.

Confirmation of *C. sporogenes* colony growth using MIDI analysis

RCA plates containing purified *C. sporogenes* spores and RCA plates with presumed *C. sporogenes* colonies from inoculated butter samples were analyzed by MIDI (Microbial IDentification Inc.) analysis. The identification of isolated colonies was conducted by the Microbial Identification System (MIS), which evaluates the whole cell fatty acid profile using

gas chromatography (MIDI Inc, Newark, USA). Extractions of fatty acid methyl esters (FAME) from colony samples were performed according to MIDI instructions and were analyzed on a 6890 Series Gas Chromatograph (Agilent Technologies) using the MIDI MIS software (MIDI Inc) (Slabbinck and others 2008)(Yilmaz 2009).

Emulsion stability determination: Confocal Microscopy

Confocal microscopy analysis was used to obtain both qualitative and quantitative data for comparison of droplet size between batch-churned butter produced in the research lab and butter produced by a continuous process in a commercial facility. Six butter samples produced by a continuous process at a local dairy were compared with six butter samples produced in the BYU lab; both continuous and batch processed butters were produced using cream obtained from Deseret Dairy.

Slides were prepared using a method adapted from Van Dalen (2002); an updated method was provided by Han Blonk through personal communication. Nile Red dye crystals (N3010, Sigma-Aldrich, St. Louis, MO) were placed on a clean microscope slide. A sample of the butter product was taken using a sterile stainless steel spatula and the butter sample was placed on top of the Nile Red crystals positioned on the microscope slide. A cover slip was placed on the butter and the butter was pressed flat with the coverslip, ensuring the thickness of the butter was maintained at a consistent thickness of 1mm. The samples were then placed in a foil covered container to exclude light and stored at 4°C for 48 hrs to allow dye penetration throughout the lipid phase of the sample. The samples were analyzed with the confocal microscope to determine overall droplet size.

The Olympus FluoView FV 300 confocal laser scanning microscope (Olympus America Inc., PA, USA) with FluoView software was used for image generation with the following settings: Laser – He-ne Green 543, Dye - Trit C, Kalman setting – 3. The 40 x objective was used for all sample images and Z-stack images were taken to obtain images at varying depths throughout the butter.

Quantitative analysis of slide data was performed using ImageJ software (Abramoff and others 2004). Aqueous phase droplets appeared as dark circles, while the lipid phase appeared as a continuous mass of red. ImageJ software was used to count the number of aqueous droplets and determine the average area of the aqueous droplets. Image scale was set based on the 50 μm measure bar for each image. Images were converted to binary 8-bit images and adjustments were made to brightness/contrast and image threshold values to ensure optimal image resolution. Droplets with an area $< 2\mu m^2$ were not counted to ensure black pixels that may or may not be actual droplets were rejected; acceptable droplets had an area $\geq 2\ \mu m^2$. Total droplet number as well as average droplet area data was collected for each image.

Confocal microscopy was used for its ability to take layered images of the butter sample. This enabled selection of images that were in the center of the butter sample as opposed to images of the topmost layer pressed against the glass coverslip. The ability to select inner layers ensured the droplets observed were actual aqueous droplets within the butter structure as opposed to distorted droplets pressed against the glass coverslip.

Incubation temp vs. emulsion structure effects

There are two temperature-related factors that could potentially affect *C. sporogenes* counts; the first being the effect of temperature on the butter emulsion structure, and the second being the proximity of the storage temperature (41°C) to the optimal growth temperature for *C. sporogenes* (35-40°C) (Simjee 2007). The experimental design mentioned above does not address the question of specifically which of these two factors could be causing the change in *C. sporogenes* counts. To determine this, a small secondary experiment was performed. In this experiment, cream was inoculated and churned to butter as described above; however the storage conditions were altered. One batch of *C. sporogenes* inoculated butter was produced and the batch was separated into five 300 g fractions. Salt was then incorporated into the separate fractions with two cans containing 0% NaCl, and three cans containing 0.8%, 1.6%, and 2.4% respectively, as shown in Figure 2. The samples were then canned according to the methods mentioned in this document. All five cans were then heated at 41°C for 18 hrs to break the emulsion. Four cans, one from each salt percentage, were removed from the 41°C storage and stored at 22°C, while the final can (0% NaCl) was kept at 41°C for the entire 1 week storage period. Cans were then checked for $\%O_2$ headspace, opened, and plated for *C. sporogenes* growth.

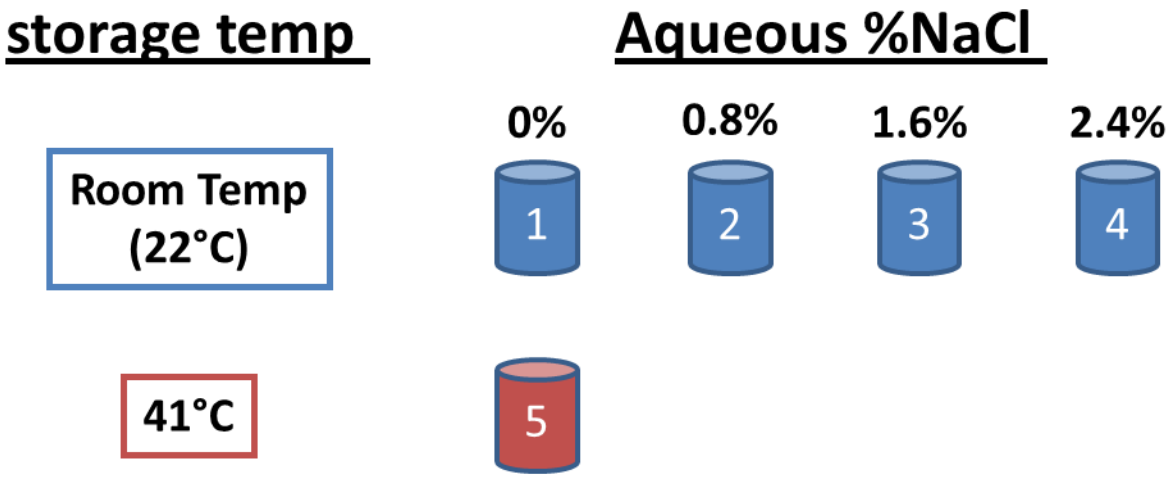

Figure 2: Experimental design of secondary experiment to determine whether incubation temperature or emulsion structure had a larger effect on C. sporogenes growth. Samples were all incubated initially at 41°F for 18 hrs to break the emulsion. Following initial incubation sample cans were stored according to the conditions shown above.

Separation of aqueous fraction of butter

Separation of the aqueous fraction was adapted from a protocol used by Britten and others (2008). The butter was placed in a beaker and placed on a hotplate to melt the butter sample. The melted butter sample was transferred to a clean separatory funnel surrounded by hotplates to ensure the sample remained warm enough to prevent solidification of the melted butter. The butter sample was then left in the separatory funnel to allow the aqueous phase to settle to the bottom of the funnel. After the majority of the aqueous phase had settled to the base of the separatory funnel (~ 15 min), the aqueous phase was emptied into a sterile 50-ml conical tube. The 50-ml conical tube was then centrifuged at 3000 rpm for 10 min to separate any residual lipid phase from the aqueous fraction. The lipid layer formed on the top was removed and the aqueous layer was transferred to a sterile 50-ml conical tube using a disposable transfer pipette. The separated aqueous fraction was then stored at 4°C until analysis.

Measurement of potential hurdles

$\%O_2$ headspace measurement

Illinois Instruments 6500 headspace Oxygen analyzer (Illinois Instruments, Johnsburg, IL, USA) was used to determine $\%O_2$ in the headspace of the canned butter samples. Adhesive septa were placed on the can lid and a can puncture attachment was used to puncture the can for sample removal. A sample was removed using a 50 ml syringe which was then injected into the sample port of the headspace analyzer. Duplicate samples were taken from each sample can and the headspace value was recorded.

Water activity measurement

Water activity (a_w) of each sample was determined using the Aqua Lab Water Activity Meter Series 3 (Decagon, Pullman, WA, USA). The a_w was measured following the protocol described in the AquaLab Water Activity Meter Operator's Manual (Decagon 2009) using sample cups, lids, and verification standards as specified by Decagon.

pH measurement

Measurement of pH was performed as described in SMEDP 15.022.9.4.4 (Wehr and Frank 2004). To determine the pH value of butter, it is necessary to measure the pH of the isolated aqueous phase. Therefore, separation and isolation of the aqueous phase (as described above) was performed prior to pH measurement. Duplicate butter samples were analyzed for each treatment and average pH values were determined.

%NaCl determination

The %NaCl was determined using the AOAC Official Method 960.29: Salt in Butter – Titrimetric method (Horwitz 2003). Samples were analyzed for salt content for the entire butter product as well as for the aqueous fraction of the butter. 5 g samples were weighed into an Erlenmeyer flask and the weight was recorded (±10 mg). 100 ml of boiling water was added to each flask to melt the butter sample and the samples were allowed to cool to 50-55°C. 2 g of K2CrO4 was added to each sample and the samples were titrated with 0.1M $AgNO_3$ following the AOAC official method. Duplicate samples for every can sample were taken and averaged to obtain the final %NaCl of each sample.

Sensory panel

Consumer acceptance of butter containing varying salt amounts was evaluated in the Brigham Young University Sensory Laboratory to determine the threshold of salt levels that consumers found acceptable. A 53-member consumer panel was recruited from a database of university employees and students with approximately equal representation among age categories from age 20-60 years. Approval for use of human subjects was granted by the Institutional Review Board and panelists provided informed consent prior to participation.

Butter fractions with 5 different added salt percentages (0.8, 1.6, 2.4, 3.2, & 4.0%) were made in the BYU lab. Butter production and salt incorporation was performed following the methods described above in the methods section; butter samples for the consumer panel were uninoculated and prepared using Good Manufacturing Practices. Samples were prepared for the consumer panel by placing 2 g (± 0.2 g) of butter into ½ oz sample cups labeled with three-digit sample blinding codes (0.8%-**275**, 1.6%-**841**, 2.4%-**439**, 3.2%-**128**, & 4.0%-**506**) and lids were

placed on the sample cups. Samples were prepared the day prior to the sensory panel and stored at 4°C overnight. The samples were removed from the refrigerator and allowed to equilibrate at room temperature (22°C) 2 hrs prior to the consumer panel. The 5 samples were presented side-by-side with squares (4 cm x 4 cm) of white bread. An unsalted cracker and water were provided to enable panelists to refresh their sense of taste between each sample. Panelists were provided 5 taster spoons to spread each entire 2 g sample over the squares of bread.

Questions were presented one-at-a-time on a computer screen and data were collected using Compusense®5 (version 5.2) software (Compusense Inc., Guelph, Ontario, Canada). Panelists evaluated appearance, overall flavor, salt flavor, and overall acceptability using a discrete 9-point hedonic scale where 9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely. Determination of appropriate salt level was done using a Just-about-right ideality question in which 5 = Definitely too high, 1 = Definitely too low, and 3 = Just-about-right. Panelists were also asked to rank the 5 samples in order of preference with 1 being the most liked and 5 being the least liked. Panelists were instructed to use a bite of unsalted cracker and sip of bottled water to refresh their sense of taste between samples. Panelists were compensated monetarily for their time.

Statistical Analysis

SAS 2007 (version 9.2) software (SAS Inc., Cary, NC) was used to perform an analysis of covariance (ANCOVA) using initial bacterial counts and headspace as covariates. The ANCOVA was performed on the experimental data to determine what variables had a significant effect on the *C. sporogenes* counts. The reduced model was used for the analysis. Analysis of Variance (ANOVA) was also performed using SAS with Tukey adjusted post-hoc pairwise

comparisons to determine the effects of storage temperature and salt content on pH. Bar charts for bacterial counts were generated with XL STAT (version 2008.7.03) (Addinsoft, Paris, France). Basic statistical data including means, standard deviations, and t-tests were obtained using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA).

RESULTS AND DISCUSSION

Initial Hurdle measurements

Commercial canned butter samples were tested to determine the initial values of the four "hurdles" (pH, aw, %NaCl, aqueous droplet diameter). It was observed that three of the" hurdle" values (pH, a_w, and % NaCl).were within ranges that individually would allow for *C. botulinum* growth (Table 1). While three of the hurdle values are within ranges that would allow for *C. botulinum* growth, they are near the threshold of inhibition and it is likely that the combination of all four "hurdle" values provide inhibition.

Hurdles in Canned butter	Canned butter initial values	*C. botulinum* growth inhibited
pH	6.06 ± 0.14	< 4.6*
a_w	0.94 ± 0.007	< 0.94*
Aq. %NaCl (%)	9.26 ± 0.55	> 10*
droplet diam. (µm)	5.46 ± 0.30	< 20**

Table 1: Initial canned butter hurdle measurements and *C. botulinum* inhibition thresholds. Values measured for pH, a_w, and Aq. % NaCl are all at levels that individually would not completely inhibit *C. botulinum*. The observed average droplet diameter would be inhibitory to *C. botulinum*. The < 20 µm inhibition level reported for droplet diameter applies to all bacteria and not just *C. botulinum*. *(Simjee 2007); **(Wehr and Frank 2004)

Canned butter sample results

Anaerobic plate count data were collected for each batch of butter produced. Control batches with no added *C. sporogenes* inoculum resulted in < 10 cfu/g, based on no colonies

observed at a 10^{-1} dilution. The initial average count, for batches of butter produced with *C. sporogenes* inoculated cream, was $1.2 \times 10^2 \pm 2.8 \times 10^1$ cfu/g.

The headspace $\%O_2$ was measured for each can sample and results were generally less than 2% O_2 (Table 5 of appendix). The 22°C stored samples typically had higher $\%O_2$ levels, compared to the 41°C stored samples and the levels of oxygen present seemed to show a correlation with the level of microbial growth within the sample. Therefore, it is likely that the increased temperature conditions allowed for an increase in bacterial growth which resulted in decreased oxygen levels due to bacterial consumption.

Bacterial counts in canned butter after storage under the particular treatment condition were recorded and results are shown graphically in **Error! Not a valid bookmark self-reference.** (raw data shown in Table 5 of the appendix).

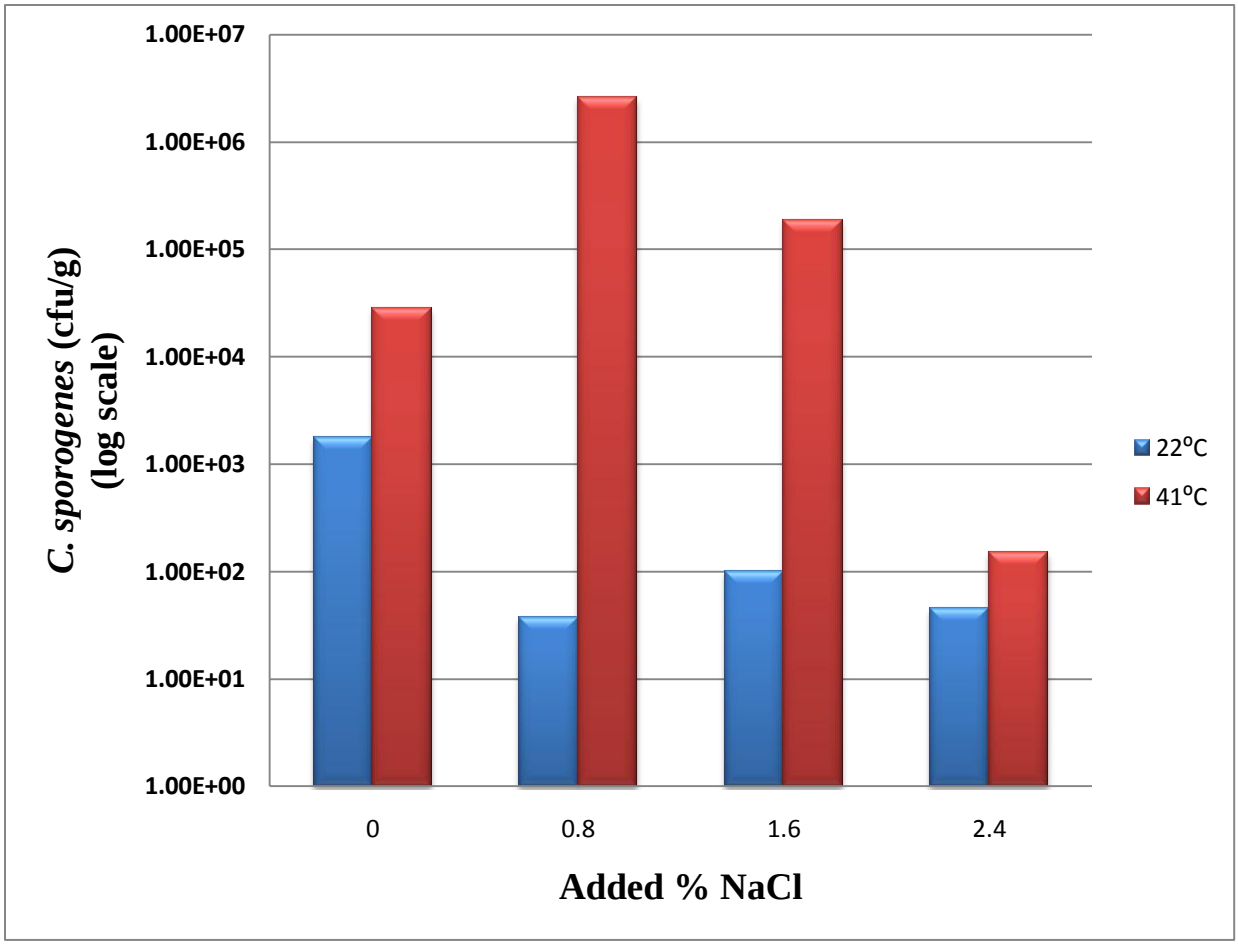

Figure 3: *C. sporogenes* growth at varying added salt percentages (0, 0.8, 1.6, & 2.4%). Different storage temperatures are distinguished by different colored columns; the blue column (left side of each pair) is 22°C storage, while the red column (right side of each pair) is 41°C storage. Samples stored at 41°C demonstrate an increase in *C. sporogenes* counts with the largest increase observed at 0.8% added NaCl and a subsequent decrease at 1.6% and 2.4%. 22°C samples showed little change with regards to initial batch inoculum values of 1.2 x 10^2 cfu/g, with the no salt sample showing a slight increase.

Sodium chloride (NaCl) addition was found to have a significant effect on *C. sporogenes* growth, with 0.8% NaCl promoting more growth than 0% but with decreases in growth beyond 0.8%. It was expected that the highest *C. sporogenes* counts would be observed in samples with 0% NaCl, with the bacterial counts decreasing as % NaCl increased. One possible explanation for this unexpected increase could be competitive bacteria present within the sample. MIDI analysis revealed the presence of other possible bacteria within the canned butter samples including, *Escherichia coli*, *Clostridium oceanicum*, *Coprococcus eutactus*, *Fusobacterium rusii*,

Capnocytophaga sputigena, *Prevotella intermedia*, *Bifidobacterium breve*, and *Clostridium cadaveris*. It is possible that in the 0% NaCl samples, competitive bacteria present within the sample outcompeted *C. sporogenes*; however as the salt content increased to 0.8% NaCl, the competitive organisms were inhibited and *C. sporogenes* counts increased. *C. sporogenes* counts decreased slightly at 1.6% and more significantly at 2.4%. *C. sporogenes* counts at 2.4% NaCl were very close to the original inoculation level.

One indication of the growth of other bacteria in the 41°C, 0% NaCl samples is pH which was significantly lower in the 41°C, 0% NaCl samples compared to all other temperature and salt combinations ($p < 0.0001$). Table 2 indicates the pH value obtained with different percentages of % NaCl added. The mean pH value for the 0% NaCl sample incubated at 41°C was 4.55, whereas the mean pH values for the other salt percentages were closer to original pH of the butter (6.06 ± 0.14). Therefore, it appears as though competitive bacteria present within the butter samples are outcompeting *C. sporogenes* at 0% added NaCl and producing acid as a bi-product, which lowers the pH of the butter and results in decreased *C. sporogenes* counts. However, as the salt levels increase *C. sporogenes* appears to be more salt tolerant and is able to outcompete the competitive bacteria at higher salt levels as it is able to survive up to aqueous salt percentages of 10% which would correspond to around 1.6% added NaCl (Voysey and others 2009).

% Added NaCl	22°C pH	41°C pH
0	6.49 ± 0.69	4.55 ± 0.20
0.8	6.80 ± 0.13	5.90 ± 0.33
1.6	6.87 ± 0.09	5.90 ± 0.46
2.4	6.74 ± 0.12	6.13 ± 0.37

Table 2: pH values of butter samples with varying salt percentages stored at 22°C and 41°C. It was observed that 41°C, 0% NaCl samples had a lower pH compared to other samples. This was attributed to competitive organisms present that acidified the butter.

Therefore, at 0.8% added NaCl, *C. sporogenes* could survive while the competitive bacteria are inhibited, resulting in increased *C. sporogenes* levels due to increased salt tolerance.

It was observed that there was still some *C. sporogenes* growth at the 2.4% added NaCl levels. In the majority of samples, high salt concentrations proved inhibitory; however, there were some samples where *C. sporogenes* survived. Therefore, it is possible that cream contaminated with *C. sporogenes* and subsequently churned to butter could allow for survival and growth even at high added salt percentages.

It is noteworthy that titration of the aqueous fraction of the butter showed that actual levels of aqueous % NaCl were often lower than target salt addition levels (Table 3).The lower values observed in amount of salt incorporation is probably related to weighing, mixing, and analytical variability; and the significantly lower aqueous levels as compared to target levels, are possibly due to retention of salt in the fat phase. It was also observed that levels of salt varied within the same canned butter sample, indicating that salt incorporation was not entirely homogenous. Therefore, the *C. sporogenes* growth occurring at high salt percentages is probably due to the fact that the overall level of salt incorporated was variable and sometimes not sufficient to be completely inhibitory. In a commercial process these inconsistencies could be corrected through standardized processing and incorporation of salt in a slurry.

Added % NaCl	Target Aq% NaCl	Actual Aq % NaCl
0	0	0.16 ± 0.03
0.8	4	3.38 ± 0.28
1.6	8	6.11 ± 0.77
2.4	12	8.98 ± 0.79

Table 3: Comparison of added % NaCl, the target final aqueous % NaCl, and the actual measured aqueous % NaCl. The added % NaCl values represent salt percentages in reference to the total butter weight. Whereas the aqueous % NaCl for both target and actual represent salt percentages in reference to just the aqueous fraction of the butter.

Statistical analysis

Analysis of variance (ANOVA) using SAS (version 9.2) (SAS Inc., Cary, NC, 2007) was performed on the experimental results. An initial analysis was performed to identify confounding variables; potential confounding variables of consideration included % O_2 headspace, a_w, and pH. Of these potential confounding variables, % O_2 headspace was the only variable that exhibited a significant effect on the plate count results ($p = 0.034$). Therefore, a_w and pH were left out of further analysis. The specific factors that were analyzed in the second step of the ANCOVA were the bacterial plate count, headspace, % NaCl, treatment (inoculated or not), storage temperature, storage time (1 week or 2 weeks) and interactions among any of the factors. It was observed that % NaCl, storage temperature, and the combination of the two had a significant effect on *C. sporogenes* growth with p values of 0.0004, 0.0002, and 0.003 respectively. It was determined that the length of storage, whether 1 week or 2 weeks, did not have a significant effect ($p = 0.605$) on *C. sporogenes* growth.

It is of note that, whether the samples were inoculated or not did not have a significant effect on the bacterial count ($p = 0.749$). The reason for this is the fact that some of the control samples exhibited growth on the RCA plates. The results of the control sample plating data are shown in Figure 4. Initially it was thought that contamination may have occurred during dilutions and plating; however, colony growth on different dilutions was indicative of actual bacterial presence in the sample, as opposed to contamination during plating. It was therefore, concluded that there may have been some other anaerobic bacteria present.

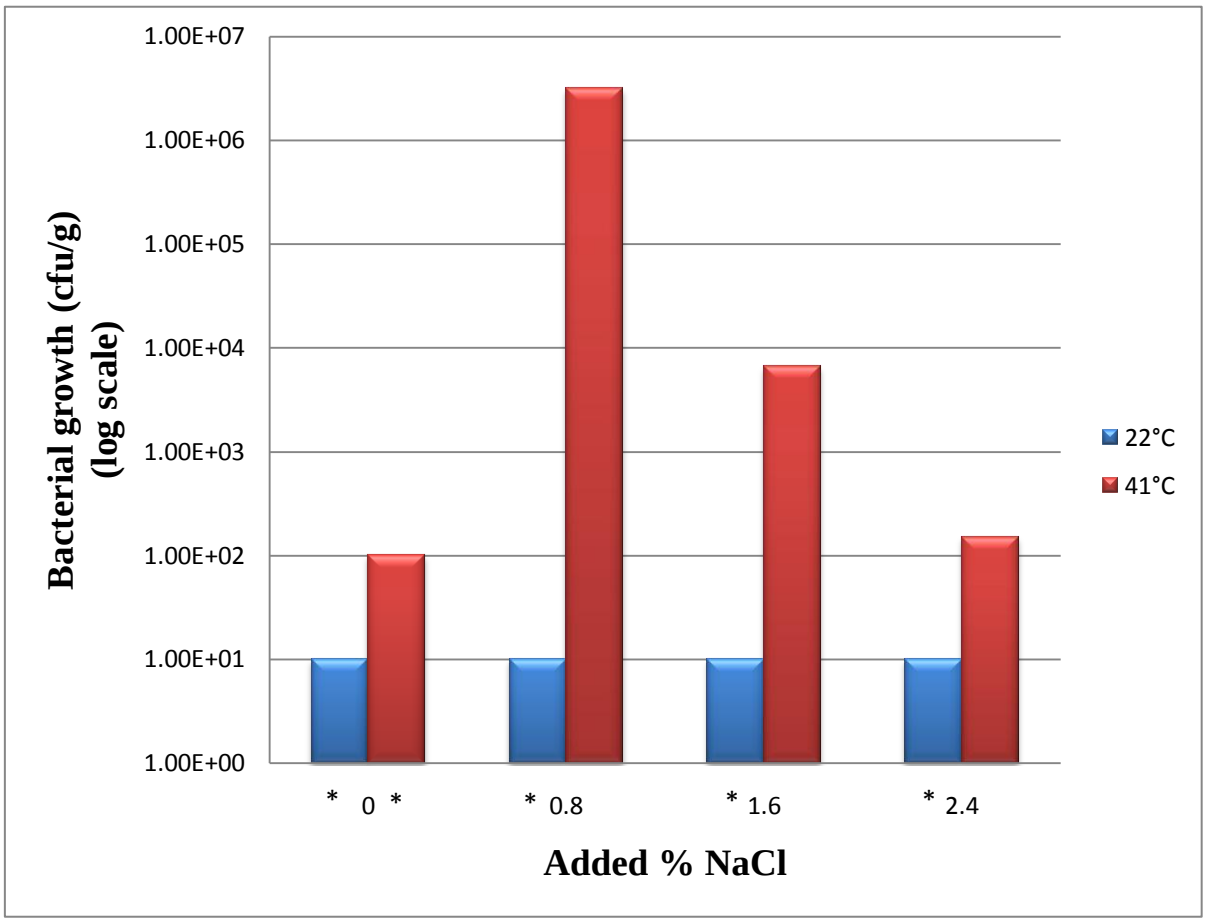

Figure 4: Bacterial growth with colony formation similar to that of *C. sporogenes* from control (uninoculated) butter samples. Columns marked with an asterisk indicate values at the limit of differentiation based on the dilution plated. Reported values for these columns are <10 cfu/g for the 22°C samples and <100 cfu/g for the 41°C sample.

One potential contaminating organism in the butter is the bacteria *Clostridium tyrobutyricum*. This particular bacteria is an anaerobic, lactate-fermenting, sporeformer, which is found in milk products (Le Bourhis and others 2007, Klijn and others 1995, Dasgupta and Hull 1989). Spores of *C. tyrobutyricum* survive pasteurization and cause a defect in cheese known as "late blowing" (Dasgupta and Hull 1989). Optimal pH for growth of *C. tyrobutyricum* is 5.8 (Le Bourhis and others 2007), which is close to the average pH values measured for the butter produced in this study. This is one potential bacterial species that may be present within the pasteurized cream; however further identification procedures, such as 16S rRNA analysis, would

need to be performed to definitively confirm the presence of *C. tyrobutyricum*, or to identify other possible anaerobic bacteria present in butter.

Storage Temperature effects

Samples stored at room temperature (22°C) did not show a large increase in overall *C. sporogenes* growth at any of the particular salt percentages. The general levels of *C. sporogenes* remained somewhat constant, relative to the initial *C. sporogenes* counts of the butter. It appears that the maintenance of butter structure (small aqueous droplet size) helped limit *C. sporogenes* growth due to limited space and/or limited nutrient availability. Another possible factor is that the incubation temperature was below the optimal range (35-40°C) for *C. sporogenes* growth (Simjee 2007).

Butter samples stored at 41°C for the specified storage period resulted in broken emulsions; and increased growth was observed, specifically at the lower salt percentages. There was a significant increase in the number of *C. sporogenes* colonies at 0.8% added NaCl, with decreasing counts as % added NaCl increased, as stated previously. It appears that the resulting increase in combined aqueous phase volume in the broken emulsions at 41°C provided a suitable environment for *C. sporogenes* growth. Incubation of the samples at 41°C also provided an incubation temperature closer to the optimal growth temperature for *C. sporogenes*. Based on the experimental design it is not possible to differentiate which of the factors (breaking the emulsion or optimal growth temperature) was responsible for the increase in *C. sporogenes* growth. It is likely that both factors play a role and have an impact on overall growth; however to further identify which factor may have played a larger role, a small secondary experiment was performed

The secondary experiment involved incubating the canned butter samples at 41°C for 18 hrs to break the emulsion structure. Cans were stored at either 22°C or 41°C for 1 week and samples were taken and plated. It was observed that the sample that was stored at 41°C for 1 week had much higher *C. sporogenes* counts (1.1 x 10^6 cfu/ml) compared to the equivalent sample stored at 22°C, which had little to no growth (< 10 cfu/ml) (Figure 5). The high counts observed in the 41°C samples are likely due to a combination of both the broken emulsion and the fact that the incubation temperature is nearer to the optimum for *C. sporogenes* growth. For the 22°C samples, it appears that breaking the emulsion alone did not have a large effect on *C. sporogenes* growth. It is likely that both factors play a role in bacterial growth and based on the scale of the experiment it is not possible to definitively state that breaking of the emulsion did not have an effect on *C. sporogenes* growth. This experiment was a small scale secondary experiment and therefore to definitively determine the interaction of these

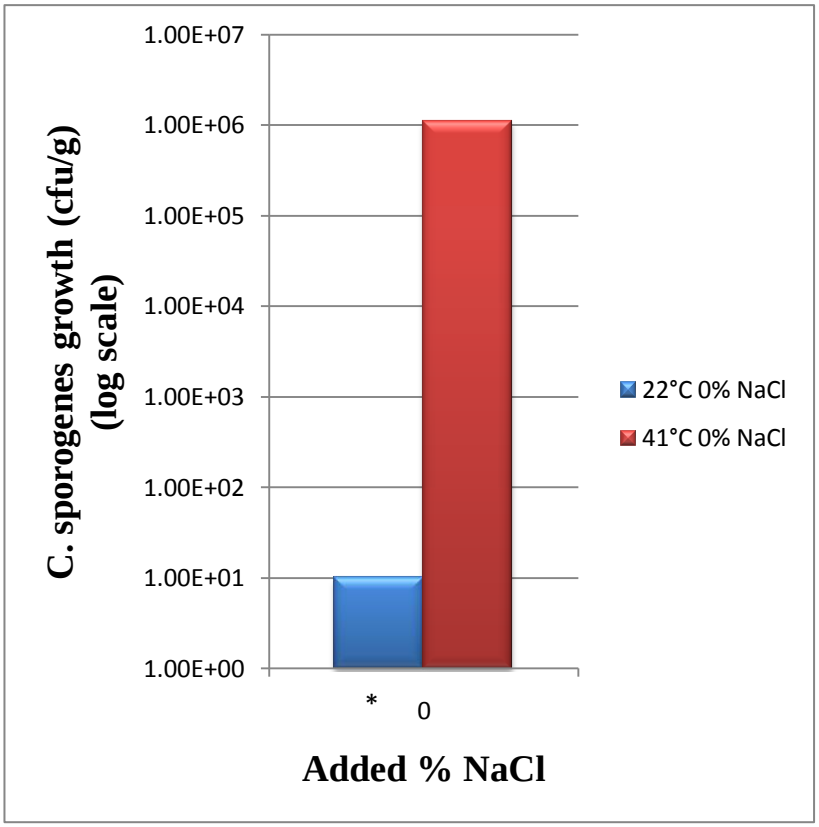

Figure 5: Effects of breaking the butter emulsion vs. optimal incubation temperature. Both samples were heated at 41°C for 18 hrs to break the emulsion, then stored at the temperatures listed. *C. sporogenes* growth is much larger in the sample stored for the entire time at 41°C. Columns marked with an asterisk indicate values at the limit of differentiation based on the dilution plated; the reported value for the column is <10 cfu/g.

factors it is suggested that further analysis be performed incorporating these factors into a large scale experimental design.

MIDI confirmation of *C. sporogenes*

C. sporogenes colonies were confirmed through MIDI identification and associated analysis of Fatty Acid Methyl Esters (FAME) produced by each bacterial species. Purified *C. sporogenes* colonies, were correctly identified as *C. sporogenes* by the MOORE6 anaerobic library.

Plates contained spherical colonies with a cottonlike appearance which are typical of *C. sporogenes* (Nygaard and Hostmark 2008). However, the plates also exhibited additional colony growth beyond the typical *C. sporogenes* colonies, including small round colonies as well as colonies with a distinct peak rising out of the center. Definitive identification of these colonies was not achieved; however, some colonies were analyzed with MIDI analysis and potential bacterial species were determined. Some potential species identified include *Escherichia coli*, *Clostridium oceanicum, Coprococcus eutactus, Fusobacterium rusii, Capnocytophaga sputigena, Prevotella intermedia, Bifidobacterium breve,* and *Clostridium cadaveris*. Sim Index values produced give an indication of how well the gas chromatography peaks correspond to those found in the particular database being used for comparison. Some of the Sim Index values were lower than would be considered ideal. Therefore, confirmation of these colonies through 16S rRNA analysis could provide added evidence of specific bacterial species present within canned butter.

Butter aqueous droplet size

Qualitative analysis of confocal images was performed on batch and continuous processed samples (n=6). Observational analysis lead to the conclusion that aqueous droplet sizes appeared to be slightly larger in the batch churned samples from the BYU lab; however there did not appear to be a large discrepancy between the batch and continuous processed samples. It appears that batch processed butter samples tend to have a larger ratio of large to small droplets; whereas continuous processed butter samples have fewer large droplets.

Quantitative analysis of droplet size was performed by determining the diameter of the aqueous droplets as well as the average diameter and number of droplets over 20 μm using ImageJ analysis. Average adjusted droplet diameters were calculated from values of a measured slice of the droplet based on personal communication with Malan (Malan 2010). This was performed to obtain an accurate estimate of the true diameter of the droplets as opposed to the diameter of one slice of the droplet alone. Statistically, mean droplet diameter for batch and continuous processed samples was compared using a T-test (one sided; unpaired-equal variance). Average droplet diameter for batch churned butter produced in the BYU lab was 5.46 μm; while average droplet diameter for butter produced through continuous processing by a local dairy was 5.44 μm (Figure 6). The one-sided T-test p-value for comparison of mean area was 0.459; therefore there is no significant difference between average adjusted droplet diameters of batch churned butter produced by the BYU research team and butter produced by a commercial facility through continuous processing.

The droplet sizes of the batch churned butter were generally observed to be small enough to inhibit microbial growth. As was mentioned previously, the threshold for support of bacterial

growth is a diameter > 20 µm (Wehr and Frank 2004). Therefore, by counting the number of droplets larger than 20 µm and calculating the average diameter of those droplets, it is possible to obtain another indicator of bacterial growth potential. Image analysis of batch churned butter resulted in an average of 36.5 ± 9.3 droplets per microscopic field with a diameter > 20 µm, with an average diameter of those droplets at 57.4 µm. Continuous processed butter had an average of 38 ± 10.9 droplets per field with a diameter > 20 µm, with an average diameter of those droplets at 52.1 µm. T-test comparisons of both droplet number and average large droplet size resulted in one-tailed p-values of 0.40 and 0.180 respectively. Therefore, there was also no significant difference between batch and continuous processed butter with regards to the number of droplets per field > 20 µm in diameter and the average diameter of those droplets. This provides increased evidence of the similarity in structure of butter samples produced by these two processing methods, and confirms the ability to make comparisons between samples produced by these two methods.

One consideration that should be taken into account is the number of sample images that were analyzed to generate the data mentioned above. Six sample images for each process type (batch or continuous) were analyzed using ImageJ software. To obtain a representative sample size for complete analysis and comparison of butter structure, an increased number of samples and replicate images from each sample should be taken. The goal of confocal image analysis in this particular study was to provide general evidence to compare samples of the two processing methods; therefore the smaller sample size was deemed sufficient for the aims of this study.

A. BYU Lab – Batch Process

B. Deseret Dairy – Continuous Process

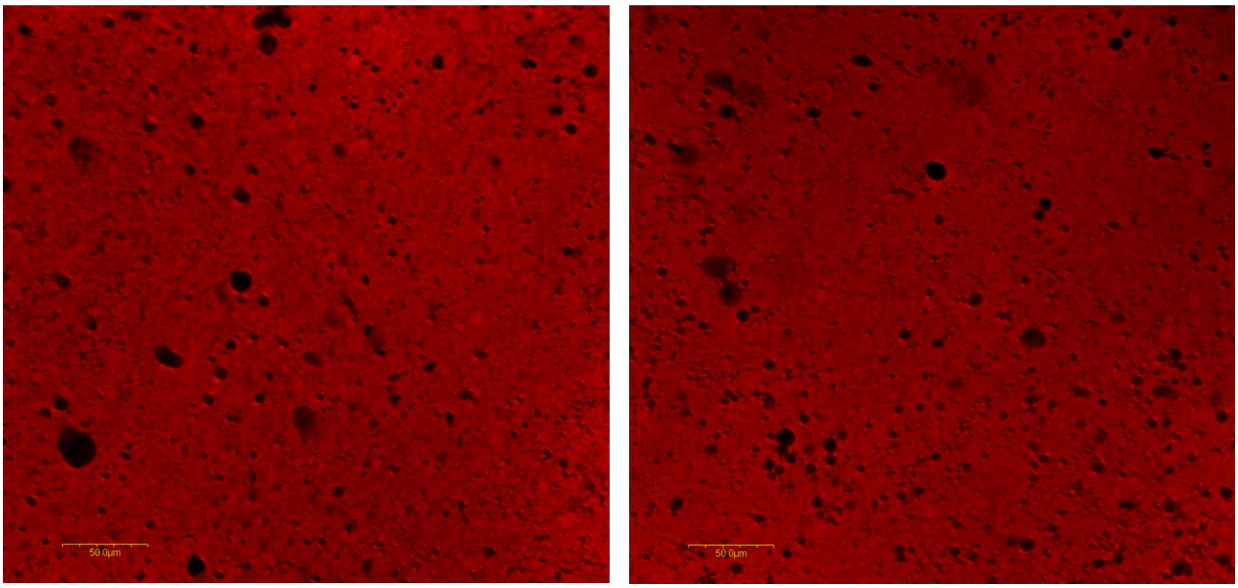

Figure 6: Confocal microscopy images of butter stained with Nile red dye; lipid fraction is seen as red continuous phase and aqueous droplets are seen as dark circles. (A) Butter produced in BYU lab by batch process, average corrected droplet diameter = 5.46 µm. (B) Butter produced by Deseret Dairy utilizing a continuous process; Average corrected droplet diameter = 5.44 µm. T-test analysis demonstrates no significant difference between mean average relative droplet area for batch process and continuous processed butter.

It was observed during the butter making and salt incorporation process that, after storage times, butter with higher salt levels resulted in a more coarse emulsion. Confocal microscopy images were also taken of butter with different salt levels incorporated (Figure 7). It is apparent that as a general trend increased salt results in increased droplet size and coarseness of the emulsion structure. However in this case, 1.6% added NaCl had the largest droplet diameter as opposed to the 2.4% added NaCl image. It is of note that only one image was taken at each salt percentage and therefore representative sample images were not obtained. Therefore, with only one image taken it is difficult to make any significant conclusions other than a qualitative observation of butter structure at each salt percentage.

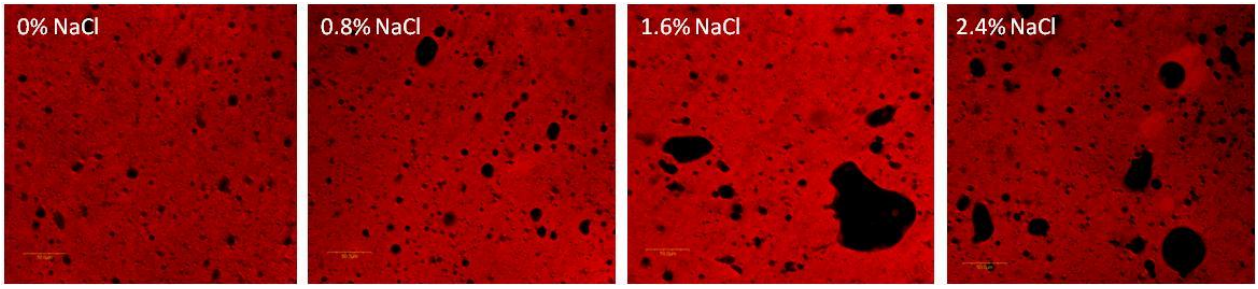

Figure 7: Confocal microscopy images of butter produced in the BYU research lab with varying salt amounts. It was observed in the small sample size presented that average droplet areas increased at 1.6% and 2.4% compared to the 0.8% NaCl sample.

Sensory Evaluation

The primary objective of the sensory evaluation was to determine what salt level was considered optimal, however more specifically, to determine if salt levels that appeared to be inhibitory to *C. sporogenes* were acceptable to consumers. Higher, more inhibitory salt levels were also evaluated. Discrete 9-pt Hedonic scale questions included overall acceptability, appearance, overall flavor, and salt flavor. The mean hedonic score ranges were 5.68 to 7.40 for overall acceptability, 6.96 to 7.53 for appearance, 5.21 to 7.38 for overall flavor, and 3.87 to 7.06 for salt flavor. Consistently, 1.6% salt and 0.8% salt had the highest hedonic score values and neither was significantly different from the other (Table 4). An ideality question for "Level of Salt" was also included to determine what salt contents were considered optimal to consumers. 1.6% salt was determined to be ideal, whereas 0.8% salt was considered not salty enough and 2.4, 3.2, and 4.0% salt were all considered too salty. Ideality statistics were performed based on a method described by Stone and Sidel (1985). Panelists ranked samples in order of preference by assigning a score of 1 to the most liked sample and continuing to rank samples based on preference until all five samples had been ranked. Rank sum values were determined by calculating the total of all rank values and it was observed that 1.6%, 0.8%, and 2.4% had the lowest rank sum values respectively; however, none of the three were significantly different from

another. Therefore 1.6%, 0.8%, and 2.4% were the most preferred samples with no significant difference among them.

	% NaCl Added					
	0.8%	**1.6%**	**2.4%**	**3.2%**	**4.0%**	Critical value
Overall acceptance	7.32[a]	7.40[a]	6.77[ab]	6.57[b]	5.68[c]	cr = 0.695
Appearance acceptance	7.53[a]	7.47[a]	7.45[a]	7.13[ab]	6.96[b]	cr = 0.40
Overall Flavor acceptance	7.38[a]	7.36[a]	6.43[b]	6.40[b]	5.21[c]	cr = 0.79
Salt flavor acceptance	7.06[a]	6.89[a]	5.66[b]	5.04[b]	3.87[c]	cr = 1.049
Level of Salt Ideality (ideal = 3.00)	2.74 Not enough salt	2.96 Just about right	3.77 Too salty	4.00 Too salty	4.49 Too salty	N.A.
Ranking (rank sum)	119[a]	116[a]	147[ab]	185[bc]	228[c]	cr = 44.4

Table 4: Mean acceptance, ideality, and rank sum scores of salted butter. Common superscripts in the same column indicate no significant difference (p > 0.05). Salt ideality values listed are mean ideality scores for each sample. Conclusions for ideality were determined based on a method described by Stone and Sidel and are listed below the mean ideality scores. Rank sum values are a total sum of the rank value (1-5) that panelists assigned to each butter, with 1 being most liked and 5 being least liked. Therefore the butter with the lowest rank sum value was most preferred by panelists and the butter sample with the highest rank sum value was least preferred.

Typical salted butter contains between 1.5% and 2% NaCl which would be around 9.4 – 12.5% NaCl of the aqueous phase (Voysey and others 2009). It is not surprising that consumers found 0.8, 1.6, and 2.4% NaCl samples as the most preferred samples due to the fact that typical butter consumed would likely be in those ranges. It was the goal of the sensory study to determine the maximum % NaCl added that consumers would consider acceptable and make suggestions for proper processing based on consumer acceptance and *C. sporogenes* growth potential. The maximum % NaCl that was considered acceptable for ranking and overall acceptance was 2.4%. 2.4% NaCl was considered inhibitory to *C. sporogenes* however survival of *C. sporogenes* still occurred at 2.4% NaCl in some samples. Therefore, to ensure production

of an organoleptically acceptable product, it is not advisable to go beyond 2.4% added NaCl; however, salt levels should be maintained close to that level to ensure sufficient salt is present to provide inhibition against *C. botulinum*.

CONCLUSIONS

Intrinsic "hurdles" associated with butter such as acidity (pH), water activity (a_w), NaCl concentration, and butter structure help provide inhibitory barriers for bacteria. While three of the measured "hurdle" values (pH, aw, & %NaCl) in butter are within ranges that individually could allow for *C. botulinum* growth, a combination of the three "hurdles" near inhibition thresholds and the fourth "hurdle" (butter structure) potentially could provide sufficient inhibition against *C. botulinum*. However, heating the butter until the emulsion broke and removal of the butter emulsion structure "hurdle" resulted in increased growth of *C. sporogenes*. Therefore, if the butter was heat abused and the emulsion was broken, *C. botulinum* growth could occur.

The confocal microscopy results suggest that there was no statistical difference between batch and continuous processed samples as far as aqueous droplet size is concerned. Therefore, data obtained for batch produced samples used for this study can be applied to continuous processed samples as well.

Through ANCOVA statistical analysis, it was determined that storage temperature, % NaCl, and a combination of the two, were the factors that had a significant effect on the overall bacterial counts. It was noteworthy that whether the samples were inoculated with *C. sporogenes* or not did not have a significant effect on bacterial growth on the RCA plates. It was determined that this was likely due to the presence of other anaerobic bacteria within the cream prior to churning. *Clostridium tyrobutyricum* was suggested as a possible candidate due to its presence in milk, ability to survive pasteurization, and growth parameters similar to those observed in this

study. However, further identification would need to be performed to confirm presence of *C. tyrobutyricum* or other possible anaerobic sporeformers within butter samples.

It was observed that canned butter produced with 0.8% added NaCl had the highest levels of *C. sporogenes* growth, with a subsequent decrease in *C. sporogenes* counts observed at 1.6 and 2.4%. The samples with 0% added NaCl had lower *C. sporogenes* counts than anticipated. This was attributed to acidification of the butter by competitive bacteria present based on decreased pH values observed in the 41°C, 0% NaCl samples. A potential future direction for this project could include the possibility of canning cultured butter. Cultured butter would result in acidification of the butter and could produce a pH below the 4.6 limit for *C. botulinum* growth, potentially resulting in a safe canned butter product.

It was also noted that *C. sporogenes* survival was observed in the 2.4% added NaCl samples which when considered as percentage of the aqueous phase would be above 12% aqueous NaCl. This is higher than the 10% aqueous NaCl level deemed inhibitory in the literature (Simjee 2007). Titration of the aqueous phase of these samples demonstrated that canned butter samples designated in this study as 2.4% added NaCl typically had aqueous salt levels lower than the 12% target. Therefore, it is possible that if appropriate target salt levels were achieved complete inhibition of *C. sporogenes* could occur.

One important question addressed in this study was the effect of butter structure, and the breaking of butter structure, on *C. botulinum* growth. Canned butter samples stored at 41°C (broken emulsion) had a significant increase in *C. sporogenes* counts compared to canned butter stored at 22°C (intact emulsion). The increase in *C. sporogenes* was associated with the optimal

incubation temperature as well as a broken emulsion structure facilitating growth. The specific balance and interaction of these two factors should be investigated further.

Sensory analysis demonstrated that preferred salt percentages by consumers were 0.8, 1.6, and 2.4%. Therefore, a canned butter product should not have more than 2.4% added NaCl for consumer preference; however, salt content should be maintained high enough to ensure inhibition of *C. botulinum*.

Current online methods for canning butter involve a step in which the butter is heated until separation of the aqueous and lipid phases occurs. This heat step is performed to ensure a proper vacuum seal is formed in the butter jars. However, this step can actually promote growth of *C. botulinum* due to butter emulsion structure damage by the heat, resulting in an increase in aqueous phase volume. This increased aqueous phase can then allow for *C. botulinum* growth. Therefore, based on results of this study the preparation and canning of butter in a home process setting is strongly discouraged due to the potential for *C. botulinum* growth and toxin production.

Commercially canned butter samples may also potentially undergo heat abuse through transport in a hot truck, storage in a hot warehouse, or abusive consumer storage practices. Food products must be able to remain safe even if exposed to conditions resulting in abuse and product damage. Due to the possibility that heat abuse could occur and potentially result in growth of *C. botulinum* and toxin production it is also not recommended to consume commercially canned butter. With further investigation it is possible that regulatory agencies could provide additional direction in the future for possible safe production of a canned butter product.

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APPENDIX

Combined Hurdle Data

Target Aq % NaCl	Actual Aq % NaCl	storage (weeks)	headspace (%O2)	Aq pH	Aq. aw	Storage temp (°C)	initial batch ct. (cfu/g)	final count (cfu/g)
0	0.14	1	4.98	5.47	1.00	22	9.00E+01	7.25E+01
4	3.02	1	4.14	6.75	0.98	22	9.25E+01	6.50E+01
8	5.74	1	1.74	6.78	0.97	22	1.53E+02	3.00E+01
12	10.68	1	1.27	6.70	0.93	22	1.24E+02	6.75E+01
0	0.12	1	1.32	4.51	1.00	41	1.53E+02	2.63E+02
4	3.64	1	0.00	6.10	0.98	41	1.28E+02	2.69E+06
8	6.98	1	0.30	5.95	0.96	41	1.08E+02	2.50E+02
12	9.16	1	1.07	6.29	0.94	41	9.40E+01	2.50E+02
0	0.15	2	0.81	6.70	1.00	22	1.20E+02	3.51E+03
4	3.59	2	1.70	6.64	0.98	22	1.28E+02	1.00E+01
8	4.70	2	1.55	6.95	0.97	22	1.33E+02	1.70E+02
12	9.29	2	1.05	6.60	0.94	22	1.23E+02	2.25E+01
0	0.21	2	0.00	4.29	1.00	41	1.08E+02	5.60E+04
4	3.18	2	0.79	5.41	0.98	41	9.25E+01	2.50E+06
8	6.60	2	0.15	6.47	0.96	41	1.23E+02	3.77E+05
12	8.71	2	1.22	5.61	0.94	41	1.33E+02	5.00E+01
0	0.13	1	1.37	6.79	1.00	22	< 10	< 10
4	3.42	1	2.37	6.90	0.98	22	< 10	< 10
8	6.12	1	2.87	6.79	0.96	22	< 10	< 10
12	8.11	1	2.39	6.88	0.95	22	< 10	< 10
0	0.18	1	1.02	4.74	1.00	41	< 10	< 100
4	3.21	1	0.00	5.99	0.98	41	< 10	5.90E+05
8	6.42	1	1.61	5.82	0.96	41	< 10	1.33E+04
12	8.69	1	0.55	6.48	0.94	41	< 10	< 100
0	0.18	2	1.30	7.01	1.00	22	< 10	< 10
4	3.82	2	1.09	6.91	0.98	22	< 10	< 10
8	6.83	2	1.70	6.94	0.96	22	< 10	5.00E+00
12	8.35	2	0.77	6.78	0.94	22	< 10	< 10
0	0.17	2	0.24	4.67	1.00	41	< 10	< 100
4	3.14	2	0.00	6.11	0.98	41	< 10	5.66E+06
8	5.50	2	1.39	5.35	0.97	41	< 10	< 100
12	8.84	2	2.11	6.12	0.94	41	< 10	3.00E+02

Table 5: Combined data for replicate canned butter samples. Data for each row is an average value determined by combining duplicate samples from the 2 experimental designs. Values shown as < 10 or < 100 exhibited no growth on the highest dilution and therefore are reported as < (dilution factor).

Sample #	Batch	% NaCl added	initial ct. (cfu/g)	Storage (weeks)	Storage temp (°C)	Inoculate (y/n)	Headspace (%O2)	C. sporogenes (cfu/g)	aw	pH	Titration (% NaCl)
1	2	0	6.00E+01	1	22°C	y	9.00	< 10	0.996	6.57	0.12
2	2	4	6.00E+01	1	22°C	y	1.42	9.50E+01	0.978	6.83	3.42
3	8	8	1.45E+02	1	22°C	y	1.01	5.00E+00	0.96	6.85	6.32
4	7	12	1.20E+02	1	22°C	y	1.33	3.50E+01	0.92	6.64	11.20
5	8	0	1.45E+02	1	41°C	y	0.00	< 100	0.998	4.42	0.12
6	1	4	9.50E+01	1	41°C	y	------	2.30E+06	0.971	5.68	4.33
7	1	8	9.50E+01	1	41°C	y	------	< 100	0.949	5.93	7.39
8	2	12	6.00E+01	1	41°C	y	1.61	< 100	0.938	6.66	9.08
9	7	0	1.20E+02	2	22°C	y	0.64	6.30E+03	0.997	6.53	0.16
10	1	4	9.50E+01	2	22°C	y	1.36	1.00E+01	0.972	6.5	4.35
11	8	8	1.45E+02	2	22°C	y	0.84	1.75E+02	0.964	6.75	5.53
12	7	12	1.20E+02	2	22°C	y	0.56	3.50E+01	0.926	6.58	10.45
13	1	0	9.50E+01	2	41°C	y	0.00	2.50E+04	0.999	4.18	0.30
14	2	4	6.00E+01	2	41°C	y	0.00	5.00E+06	0.976	5.97	3.55
15	7	8	1.20E+02	2	41°C	y	0.30	3.50E+03	0.951	6.43	7.59
16	8	12	1.45E+02	2	41°C	y	0.40	< 100	0.946	5.65	8.22
1c	3	0	< 10	1	22°C	n	1.01	< 10	0.996	6.76	0.15
2c	5	4	< 10	1	22°C	n	0.56	< 10	0.975	6.9	4.09
3c	5	8	< 10	1	22°C	n	2.11	< 10	0.953	6.83	7.25
4c	6	12	< 10	1	22°C	n	3.36	< 10	0.942	6.85	9.23
5c	4	0	< 10	1	41°C	n	2.03	< 100	0.999	5.14	0.23
6c	6	4	< 10	1	41°C	n	0.00	1.45E+05	0.982	6.38	3.31
7c	4	8	< 10	1	41°C	n	1.34	< 100	0.956	6.1	6.96
8c	6	12	< 10	1	41°C	n	0.49	< 100	0.946	6.61	8.54
9c	4	0	< 10	2	22°C	n	0.06	< 10	0.999	7.08	0.22
10c	5	4	< 10	2	22°C	n	0.64	< 10	0.982	6.9	3.25
11c	3	8	< 10	2	22°C	n	0.48	< 10	0.965	6.95	5.78
12c	5	12	< 10	2	22°C	n	0.58	< 10	0.938	6.77	9.16
13c	6	0	< 10	2	41°C	n	0.00	< 100	1	4.37	0.15
14c	3	4	< 10	2	41°C	n	0.00	1.20E+05	0.981	5.5	2.92
15c	3	8	< 10	2	41°C	n	0.13	< 100	0.962	5.38	5.92
16c	4	12	< 10	2	41°C	n	1.98	6.00E+02	0.922	6.71	10.59

Table 6: Data collected for each canned butter sample (samples 1-16c). Values shown as < 10 or < 100 exhibited no growth on the highest dilution and therefore are reported as < (dilution factor).

Sample #	Batch	% NaCl added	initial ct. (cfu/g)	Storage (weeks)	Storage temp (°C)	Inoculate (y/n)	Headspace (%O2)	C. sporogenes (cfu/g)	aw	pH	Titration (% NaCl)
17	10	0	1.20E+02	1	22°C	y	0.95	1.45E+02	1.002	4.37	0.16
18	16	4	1.25E+02	1	22°C	y	6.85	3.50E+01	0.984	6.66	2.62
19	15	8	1.60E+02	1	22°C	y	2.47	5.50E+01	0.971	6.71	5.16
20	9	12	1.28E+02	1	22°C	y	1.20	1.00E+02	0.935	6.76	10.15
21	15	0	1.60E+02	1	41°C	y	2.63	5.25E+02	1.001	4.6	0.11
22	15	4	1.60E+02	1	41°C	y	0.00	3.07E+06	0.99	6.51	2.95
23	10	8	1.20E+02	1	41°C	y	0.30	5.00E+02	0.962	5.96	6.57
24	9	12	1.28E+02	1	41°C	y	0.53	5.00E+02	0.94	5.91	9.24
25	18	0	1.20E+02	2	22°C	y	0.98	7.10E+02	0.999	6.87	0.13
26	15	4	1.60E+02	2	22°C	y	2.03	1.00E+01	0.981	6.78	2.83
27	18	8	1.20E+02	2	22°C	y	2.27	1.65E+02	0.977	7.14	3.86
28	16	12	1.25E+02	2	22°C	y	1.53	1.00E+01	0.949	6.62	8.14
29	18	0	1.20E+02	2	41°C	y	0.00	8.70E+04	0.996	4.39	0.12
30	16	4	1.25E+02	2	41°C	y	1.57	1.00E+03	0.981	4.85	2.80
31	16	8	1.25E+02	2	41°C	y	0.00	7.50E+05	0.969	6.51	5.62
32	18	12	1.20E+02	2	41°C	y	2.05	1.00E+02	0.941	5.57	9.20
17c	13	0	< 10	1	22°C	n	1.73	< 10	0.999	6.81	0.11
18c	11	4	< 10	1	22°C	n	4.18	< 10	0.983	6.89	2.75
19c	14	8	< 10	1	22°C	n	3.63	< 10	0.969	6.75	5.00
20c	11	12	< 10	1	22°C	n	1.41	< 10	0.956	6.9	6.98
21c	12	0	< 10	1	41°C	n	0.00	< 100	1.002	4.34	0.13
22c	13	4	< 10	1	41°C	n	0.00	1.04E+06	0.985	5.59	3.11
23c	13	8	< 10	1	41°C	n	1.87	2.65E+04	0.963	5.53	5.88
24c	11	12	< 10	1	41°C	n	0.61	< 100	0.942	6.35	8.84
25c	17	0	< 10	2	22°C	n	2.54	< 10	0.998	6.93	0.15
26c	17	4	< 10	2	22°C	n	1.53	< 10	0.971	6.91	4.38
27c	17	8	< 10	2	22°C	n	2.91	1.00E+01	0.947	6.92	7.89
28c	14	12	< 10	2	22°C	n	0.95	< 10	0.951	6.78	7.54
29c	17	0	< 10	2	41°C	n	0.49	< 100	0.998	4.97	0.19
30c	13	4	< 10	2	41°C	n	0.00	1.12E+07	0.983	6.71	3.36
31c	14	8	< 10	2	41°C	n	2.64	< 100	0.973	5.31	5.08
32c	14	12	< 10	2	41°C	n	2.24	< 100	0.956	5.52	7.10

Table 7: Data collected for each canned butter sample (samples 17-32c). Values shown as < 10 or < 100 exhibited no growth on the highest dilution and therefore are reported as < (dilution factor).

Research Design

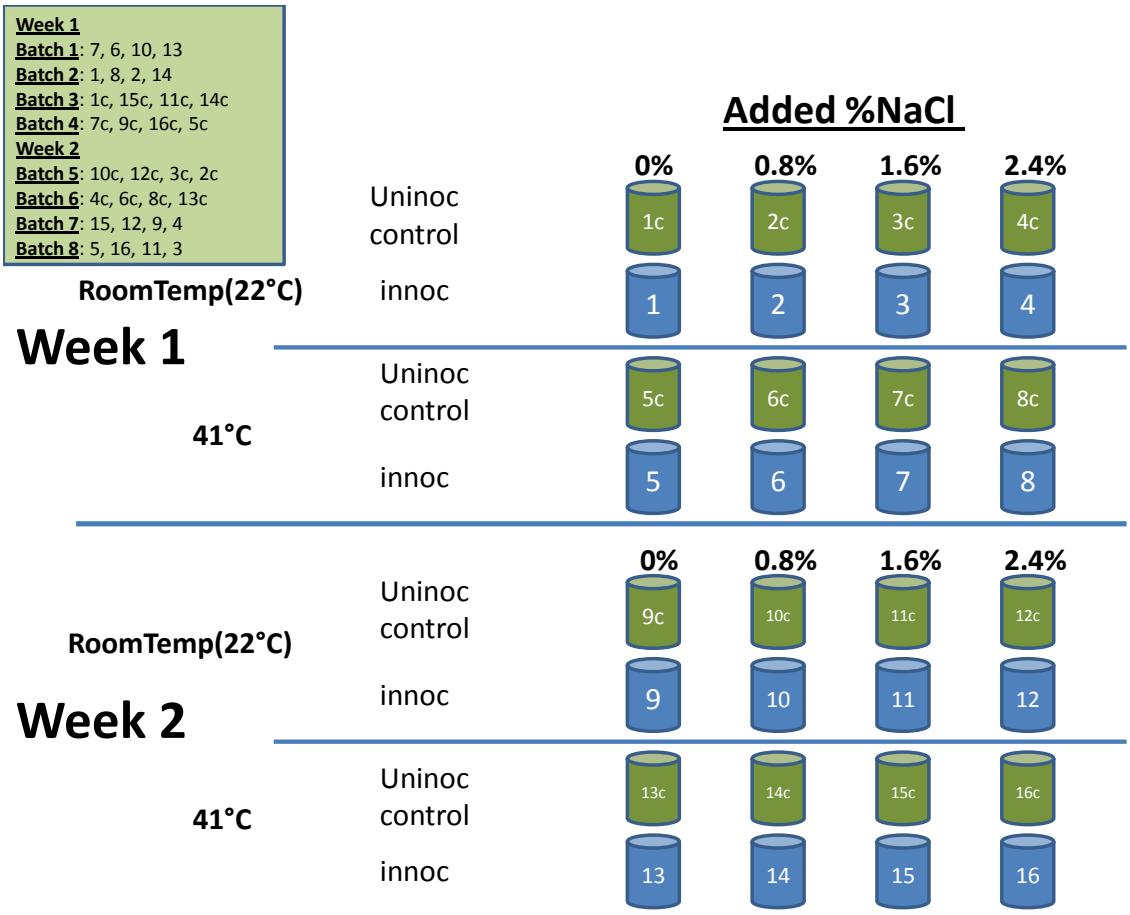


Figure 8: Experimental Design of canned butter analysis. The top can of each section designated with a number and the letter "c" are control cans that contained butter prepared with uninoculated cream. Cans on the second row of each section, with only a number designation, are cans that had butter prepared with *C. sporogenes* inoculated cream. Temperature storage conditions for each can (22°C or 41°C) are listed to the left of each set of cans. Storage time at each temperature designation (1 week or 2 weeks) is shown to the left, with all 4 upper rows being stored for 1 week and the bottom 4 rows being stored for 2 weeks. Aqueous % NaCl (None, 0.8, 1.6, and 2.4%) is listed at the top of each column of cans. Sample cans chosen for each batch was performed by an online random sequence generator, to ensure randomization of the samples. This entire experimental design was performed in duplicate; duplicate experimental design follows in Figure 9.

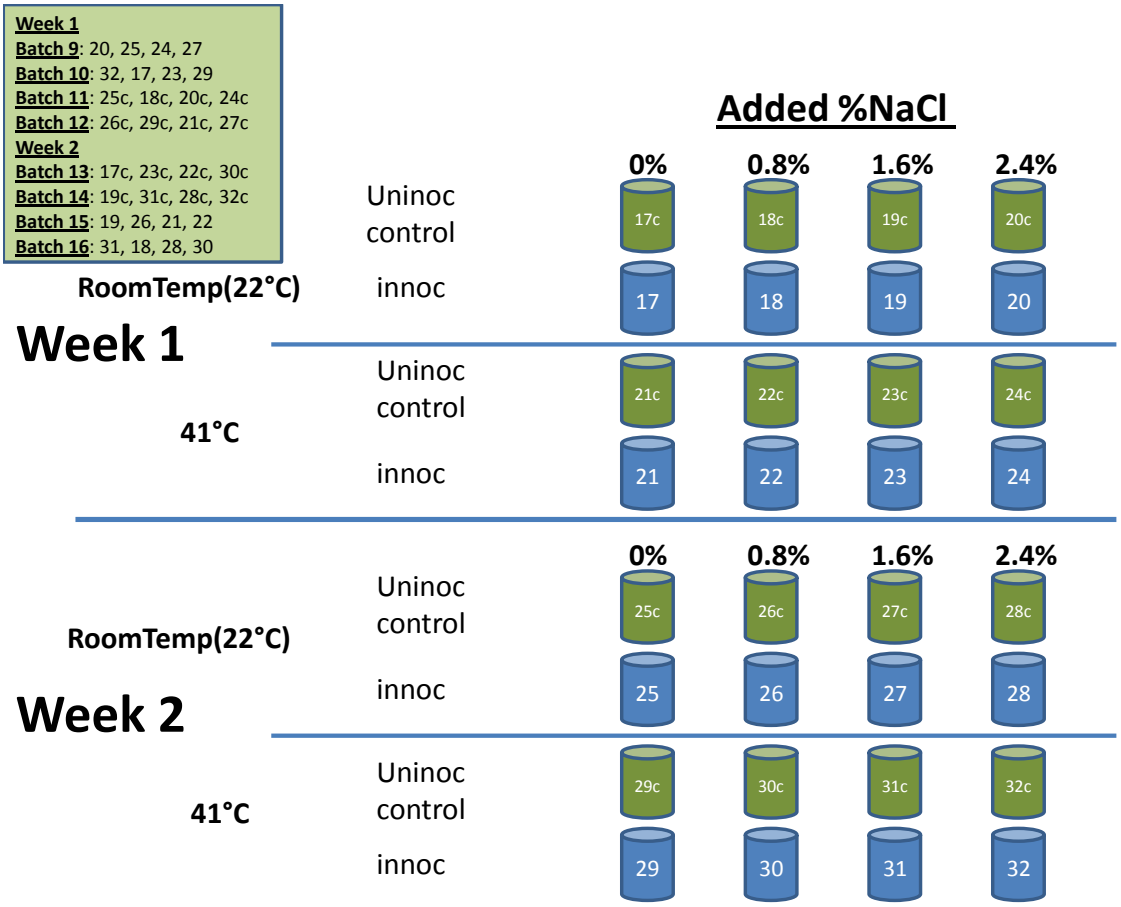


Figure 9: Replicate experimental design of canned butter analysis. The top can of each section designated with a number and the letter "c" are control cans that contained butter prepared with uninoculated cream. Cans on the second row of each section, with only a number designation, are cans that had butter prepared with *C. sporogenes* inoculated cream. Temperature storage conditions for each can (22°C or 41°C) are listed to the left of each set of cans. Storage time at each temperature designation (1 week or 2 weeks) is shown to the left, with all 4 upper rows being stored for 1 week and the bottom 4 rows being stored for 2 weeks. Added % NaCl (0, 0.8, 1.6, and 2.4%) is listed at the top of each column of cans. Sample cans selected for each batch were determined by an online random sequence generator, to ensure randomization of the samples.

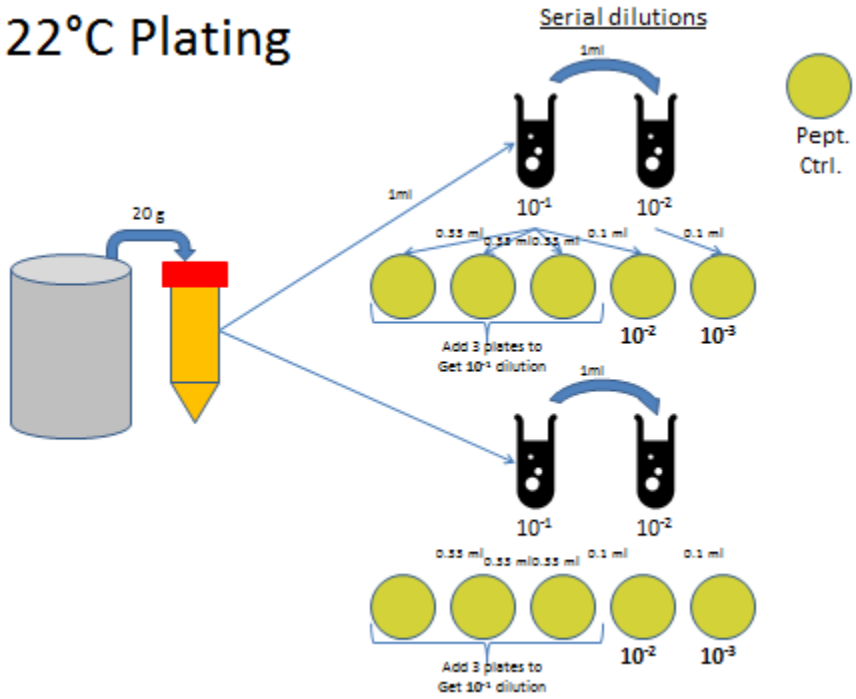


Figure 10: Dilutions and plating of 22°C samples.

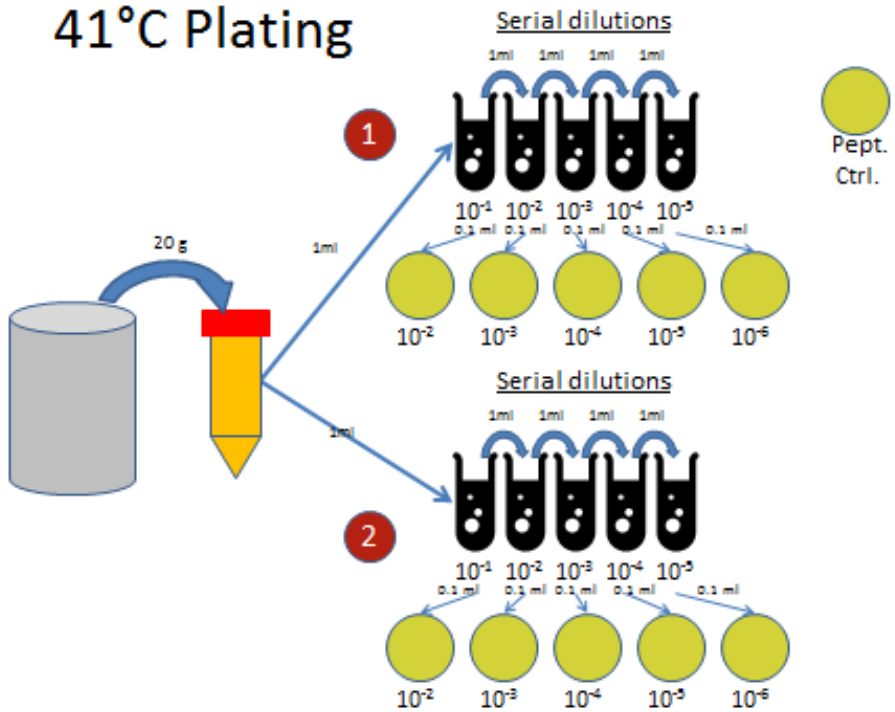


Figure 11: Dilutions and plating of 41°C samples

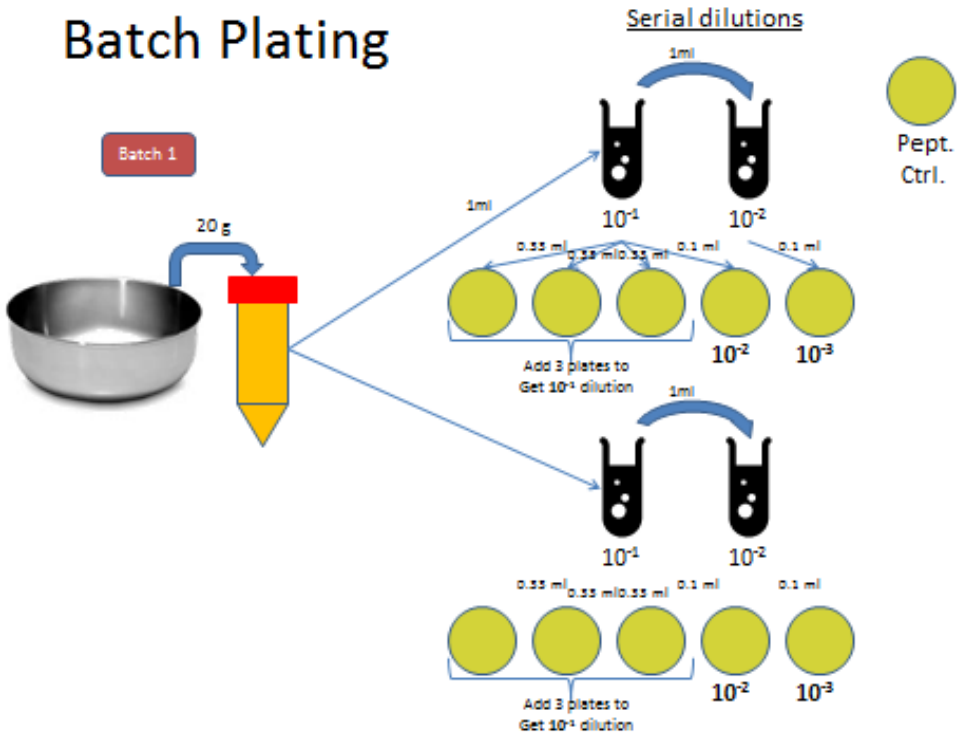


Figure 12: Dilutions and plating of butter batches

Ballot for Butter Sensory Analysis

Name_____ Signature_____
(Sign after reading consent form)

Welcome to the Food Science Sensory Laboratory. A copy of the form titled "Consent to Be a Research Subject" is posted in each booth. Please read it carefully before continuing. By signing your name above, you acknowledge that you have read and understand the consent form, and desire of your own free will and volition to participate in this study. You may withdraw at any time without penalty. Please inform the receptionist if you wish to withdraw.

In this session, you will evaluate **FIVE** samples of **BUTTER** (served with a piece of bread) side by side. **BUTTER** used in this panel refers to real dairy butter**, NOT** margarine or a vegetable oil spread.

Please read all instructions and questions carefully. Before you receive the samples, please answer the following five questions by checking the appropriate circles. Keep in mind that you are evaluating the **BUTTER**, NOT THE BREAD.

- What is your age category?
  - O Under 20
  - O 20 - 29 years
  - O 30 - 39 years
  - O 40 - 49 years
  - O 50 - 60 years
  - O Over 60

- What is your gender?
  - O Female
  - O Male

- What is your attitude about **BUTTER**?
  - O I like it
  - O I neither like nor dislike it
  - O I dislike it

- **HOW OFTEN** do you consume BUTTER?

  - O More than once a week
  - O Once a week to every two weeks
  - O Once every two weeks to once a month
  - O Once a month to once every three months
  - O Less than once every three months

- For butter consumed as a spread (not used in baking applications), do you generally prefer unsalted or salted butter?

  O unsalted
  O salted

Locate the set of lights to the right of the computer screen and press the button next to the green **"READY"** light to indicate that you are ready to receive your samples. Please be patient; they will arrive shortly.

Please fill in the code numbers on the top of the columns in the same order left to right as they are arranged in front of you. Please evaluate the samples as they are placed in front of you, from left to right.

Please take the samples and spread the **ENTIRE CONTENTS OF EACH CUP** evenly over the small squares of bread provided, being sure to keep each coded sample separate and clearly identified. **PLEASE USE A DIFFERENT TASTER SPOON TO SPREAD EACH SAMPLE.**

If at any time during the test you need more sample or any other help **press the button next to the "HELP" LIGHT** to the right of the screen

You may now **TASTE THE SAMPLES**. Use a bite of cracker and a sip of water between samples to refresh your sense of taste.

Please remember that you are evaluating the **BUTTER**, not the bread.

- **EVERYTHING CONSIDERED**, how do you feel about the **OVERALL ACCEPTABILITY** of each butter?

	Sample #'s (please write in the numbers)				
	____	____	____	____	____
Like extremely	O	O	O	O	O
Like very much	O	O	O	O	O
Like moderately	O	O	O	O	O
Like slightly	O	O	O	O	O
Neither like nor dislike	O	O	O	O	O
Dislike slightly	O	O	O	O	O
Dislike moderately	O	O	O	O	O
Dislike very much	O	O	O	O	O
Dislike extremely	O	O	O	O	O

- How much do you like or dislike the **APPEARANCE** of each butter?

	Sample #'s (please write in the numbers)				
	____	____	____	____	____
Like extremely	O	O	O	O	O
Like very much	O	O	O	O	O
Like moderately	O	O	O	O	O
Like slightly	O	O	O	O	O
Neither like nor dislike	O	O	O	O	O
Dislike slightly	O	O	O	O	O
Dislike moderately	O	O	O	O	O
Dislike very much	O	O	O	O	O
Dislike extremely	O	O	O	O	O

- How much do you like or dislike the **FLAVOR** of each butter?

Like extremely	O	O	O	O	O
Like very much	O	O	O	O	O
Like moderately	O	O	O	O	O
Like slightly	O	O	O	O	O
Neither like nor dislike	O	O	O	O	O
Dislike slightly	O	O	O	O	O
Dislike moderately	O	O	O	O	O
Dislike very much	O	O	O	O	O
Dislike extremely	O	O	O	O	O

- How much do you like or dislike the amount of **SALT FLAVOR** in each butter?

Like extremely	O	O	O	O	O
Like very much	O	O	O	O	O
Like moderately	O	O	O	O	O
Like slightly	O	O	O	O	O
Neither like nor dislike	O	O	O	O	O
Dislike slightly	O	O	O	O	O
Dislike moderately	O	O	O	O	O
Dislike very much	O	O	O	O	O
Dislike extremely	O	O	O	O	O

- How do you feel about the **LEVEL OF SALT** in each butter?

	Sample #'s (please write in the numbers)				
	____	____	____	____	____
Definitely too high	O	O	O	O	O
Slightly too high	O	O	O	O	O
Just about right	O	O	O	O	O
Slightly too low	O	O	O	O	O
Definitely too low	O	O	O	O	O

- Please **RANK** the samples in order of preference by writing the sample code in the appropriate space.

_____	_____	_____	_____	_____
Liked best	Liked 2nd Best	Liked 3rd Best	Liked 4th Best	Liked Least

If you have any additional brief comments, please write them here including the individual sample code of the sample you are commenting on.

_____ _____
_____

_____ _____
_____

_____ _____
_____

_____ _____
_____

_____ _____
_____

You are finished.
Please place the samples and tray in the pass-through compartment and **PRESS THE BUTTON BY THE "FINISHED" LIGHT**.
Please give this questionnaire to the receptionist.
THANK YOU!

Sensory Analysis: Compusense output

Project: BUTTER REVISED

Question Number: 2
Question Type: Multiple Choice (Demographic)
Question Title: Age

Choices

Number	Value	Choices
1	[6]	Under 20
2	[5]	20 - 29 years
3	[4]	30 - 39 years
4	[3]	40 - 49 years
5	[2]	50 - 60 years
6	[1]	Over 60

Crosstabulation

Sample	1 [6]	2 [5]	3 [4]	4 [3]	5 [2]	6 [1]	Total
n/a		10	12	11	14	6	53
TOTALS		10	12	11	14	6	53

Percentage Crosstabulation

Sample	1 [6]	2 [5]	3 [4]	4 [3]	5 [2]	6 [1]	Total
n/a		18.9	22.6	20.8	26.4	11.3	100

Project: BUTTER REVISED

Question Number: 3
Question Type: Multiple Choice (Demographic)
Question Title: Gender

Choices

Number	Value	Choices
1	[2]	Female
2	[1]	Male

Crosstabulation

Sample	1 [2]	2 [1]	Total
n/a	29	24	53
TOTALS	29	24	53

Percentage Crosstabulation

Sample	1 [2]	2 [1]	Total
n/a	54.7	45.3	100

Project: BUTTER REVISED

Question Number: 4
Question Type: Multiple Choice (Demographic)
Question Title: Attitude

Choices

Number	Value	Choices
1	[3]	I like it
2	[2]	I neither like nor dislike it
3	[1]	I dislike it

Crosstabulation

Sample	1 [3]	2 [2]	3 [1]	Total
n/a	53			53
TOTALS	53			53

Percentage Crosstabulation

Sample	1 [3]	2 [2]	3 [1]	Total
n/a	100			100

Project: BUTTER REVISED

Question Number: 5
Question Type: Multiple Choice (Demographic)
Question Title: How often

Choices

Number	Value	Choices
1	[5]	More than once a week
2	[4]	Once a week to every two weeks
3	[3]	Once every two weeks to once a month
4	[2]	Once a month to once every three months
5	[1]	Less than every three months

Crosstabulation

Sample	1 [5]	2 [4]	3 [3]	4 [2]	5 [1]	Total
n/a	40	8	2	3		53
TOTALS	40	8	2	3		53

Percentage Crosstabulation

Sample	1 [5]	2 [4]	3 [3]	4 [2]	5 [1]	Total
n/a	75.5	15.1	3.8	5.7		100

Project: BUTTER REVISED

Question Number: 6
Question Type: Multiple Choice (Demographic)
Question Title: Salt preference

Choices

Number	Value	Choices
1	[2]	unsalted
2	[1]	salted

Crosstabulation

Sample	1 [2]	2 [1]	Total
n/a	13	40	53
TOTALS	13	40	53

Percentage Crosstabulation

Sample	1 [2]	2 [1]	Total
n/a	24.5	75.5	100

Project: BUTTER REVISED

Question Number: 7
Question Type: Category / Hedonics
Question Title: Overall acceptability
Attribute Number: 1
Attribute Title: Q#7.1
Design: T=5, K=5, B=60

Products

Products	Code	Name
1 - 275	275	0.8% NaCl
2 - 841	841	1.6% NaCl
3 - 439	439	2.4% NaCl
4 - 128	128	3.2% NaCl
5 - 506	506	4.0% NaCl

Scale Parameters

Value	Descriptor
9	Like Extremely
8	Like Very Much
7	Like Moderately
6	Like Slightly
5	Neither Like or Dislike
4	Dislike Slightly
3	Dislike Moderately
2	Dislike Very Much
1	Dislike Extremely

Note: Numbers shown in brackets are the 'values' associated with the category selected.

Crosstabulation

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 275	7	21	13	8	3		1			53
2 - 841	8	22	11	9	1	2				53
3 - 439	9	14	7	13	3	5	1		1	53
4 - 128	5	17	10	9	1	7	2	2		53
5 - 506	4	8	12	8	1	10	5	4	1	53
TOTALS	33	82	53	47	9	24	9	6	2	265

Percentage Crosstabulation

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 275	13.2	39.6	24.5	15.1	5.7		1.9			100
2 - 841	15.1	41.5	20.8	17.0	1.9	3.8				100
3 - 439	17.0	26.4	13.2	24.5	5.7	9.4	1.9		1.9	100
4 - 128	9.4	32.1	18.9	17.0	1.9	13.2	3.8	3.8		100
5 - 506	7.6	15.1	22.6	15.1	1.9	18.9	9.4	7.6	1.9	100

Counts, Medians, Means and SD's

Sample Number	Count	Total	Median	Mean	Standard Deviation
1 - 275	53	388.00	8.00	7.32	1.237
2 - 841	53	392.00	8.00	7.40	1.214
3 - 439	53	359.00	7.00	6.77	1.804
4 - 128	53	348.00	7.00	6.57	1.886
5 - 506	53	301.00	6.00	5.68	2.191

This is a Complete Block Design.

Analysis of Variance

This analysis does not compensate for missing data or lack of balance.

	D.F.	Sum of Squares	Mean of Squares	F Value	p-value
Samples	4	101.985	25.496	15.29	0.0000
Judges	52	413.260	7.947	4.77	0.0000
Error	208	346.815	1.667		
Total	264	862.060	3.265		
Standard Error (SEM) =	0.177				

Multiple comparison tests may appear below. Tukey's HSD controls for maximum experimentwise error rate and can be used without F protection. Standard practice recommends that LSD and Duncan's be considered only if the ANOVA p-value is deemed acceptable to control for experimentwise error rates (under the complete null hypothesis). If automatic significance is selected, an available significance level is chosen for the multiple comparison test based on the observed p-value.

Tukey's HSD = 0.695 (5% Significance Level)

Sample	Mean		Significantly Different Than Sample
2 - 841	7.40	a	4 5
1 - 275	7.32	a	4 5
3 - 439	6.77	ab	5
4 - 128	6.57	b	5
5 - 506	5.68	c	

Project: BUTTER REVISED

Question Number: 8
Question Type: Category / Hedonics
Question Title: appearance
Attribute Number: 1
Attribute Title: Q#8.1
Design: T=5, K=5, B=60

Products

Products	Code	Name
1 - 275	275	0.8% NaCl
2 - 841	841	1.6% NaCl
3 - 439	439	2.4% NaCl
4 - 128	128	3.2% NaCl
5 - 506	506	4.0% NaCl

Scale Parameters

Value	Descriptor
9	Like Extremely
8	Like Very Much
7	Like Moderately
6	Like Slightly
5	Neither Like or Dislike
4	Dislike Slightly
3	Dislike Moderately
2	Dislike Very Much
1	Dislike Extremely

Note: Numbers shown in brackets are the 'values' associated with the category selected.

Crosstabulation

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 275	11	20	12	6	4					53
2 - 841	9	22	11	8	2	1				53
3 - 439	8	22	15	3	4	1				53
4 - 128	9	17	14	3	5	5				53
5 - 506	9	16	11	6	4	6	1			53
TOTALS	46	97	63	26	19	13	1			265

Percentage Crosstabulation

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 275	20.8	37.7	22.6	11.3	7.6					100
2 - 841	17.0	41.5	20.8	15.1	3.8	1.9				100
3 - 439	15.1	41.5	28.3	5.7	7.6	1.9				100
4 - 128	17.0	32.1	26.4	5.7	9.4	9.4				100
5 - 506	17.0	30.2	20.8	11.3	7.6	11.3	1.9			100

Counts, Medians, Means and SD's

Sample Number	Count	Total	Median	Mean	Standard Deviation
1 - 275	53	399.00	8.00	7.53	1.170
2 - 841	53	396.00	8.00	7.47	1.170
3 - 439	53	395.00	8.00	7.45	1.170
4 - 128	53	378.00	7.00	7.13	1.520
5 - 506	53	369.00	7.00	6.96	1.664

This is a Complete Block Design.

Analysis of Variance

This analysis does not compensate for missing data or lack of balance.

	D.F.	Sum of Squares	Mean of Squares	F Value	p-value
Samples	4	13.079	3.270	5.92	0.0002
Judges	52	362.626	6.974	12.62	0.0000
Error	208	114.921	0.553		
Total	264	490.626	1.858		
Standard Error (SEM) =	0.102				

Multiple comparison tests may appear below. Tukey's HSD controls for maximum experimentwise error rate and can be used without F protection. Standard practice recommends that LSD and Duncan's be considered only if the ANOVA p-value is deemed acceptable to control for experimentwise error rates (under the complete null hypothesis). If automatic significance is selected, an available significance level is chosen for the multiple comparison test based on the observed p-value.

Tukey's HSD = 0.40 (5% Significance Level)

Sample	Mean		Significantly Different Than Sample
1 - 275	7.53	a	5
2 - 841	7.47	a	5
3 - 439	7.45	a	5
4 - 128	7.13	ab	
5 - 506	6.96	b	

Project: BUTTER REVISED

Question Number: 9
Question Type: Category / Hedonics
Question Title: Overall flavor
Attribute Number: 1
Attribute Title: Q#9.1
Design: T=5, K=5, B=60

Products

Products	Code	Name
1 - 275	275	0.8% NaCl
2 - 841	841	1.6% NaCl
3 - 439	439	2.4% NaCl
4 - 128	128	3.2% NaCl
5 - 506	506	4.0% NaCl

Scale Parameters

Value	Descriptor
9	Like Extremely
8	Like Very Much
7	Like Moderately
6	Like Slightly
5	Neither Like or Dislike
4	Dislike Slightly
3	Dislike Moderately
2	Dislike Very Much
1	Dislike Extremely

Note: Numbers shown in brackets are the 'values' associated with the category selected.

Crosstabulation

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 275	8	18	17	8	1		1			53
2 - 841	8	22	12	8		1	2			53
3 - 439	7	11	14	8		7	5		1	53
4 - 128	9	11	10	7	2	8	4	2		53
5 - 506	4	8	8	7	1	10	6	6	3	53
TOTALS	36	70	61	38	4	26	18	8	4	265

Percentage Crosstabulation

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 275	15.1	34.0	32.1	15.1	1.9		1.9			100
2 - 841	15.1	41.5	22.6	15.1		1.9	3.8			100
3 - 439	13.2	20.8	26.4	15.1		13.2	9.4		1.9	100
4 - 128	17.0	20.8	18.9	13.2	3.8	15.1	7.6	3.8		100
5 - 506	7.6	15.1	15.1	13.2	1.9	18.9	11.3	11.3	5.7	100

Counts, Medians, Means and SD's

Sample Number	Count	Total	Median	Mean	Standard Deviation
1 - 275	53	391.00	7.00	7.38	1.164
2 - 841	53	390.00	8.00	7.36	1.360
3 - 439	53	341.00	7.00	6.43	2.005
4 - 128	53	339.00	7.00	6.40	2.097
5 - 506	53	276.00	6.00	5.21	2.437

This is a Complete Block Design.

Analysis of Variance

This analysis does not compensate for missing data or lack of balance.

	D.F.	Sum of Squares	Mean of Squares	F Value	p-value
Samples	4	168.400	42.100	19.56	0.0000
Judges	52	465.457	8.951	4.16	0.0000
Error	208	447.600	2.152		
Total	264	1081.457	4.096		
Standard Error (SEM) =	0.201				

Multiple comparison tests may appear below. Tukey's HSD controls for maximum experimentwise error rate and can be used without F protection. Standard practice recommends that LSD and Duncan's be considered only if the ANOVA p-value is deemed acceptable to control for experimentwise error rates (under the complete null hypothesis). If automatic significance is selected, an available significance level is chosen for the multiple comparison test based on the observed p-value.

Tukey's HSD = 0.79 (5% Significance Level)

Sample	Mean		Significantly Different Than Sample
1 - 275	7.38	a	3 4 5
2 - 841	7.36	a	3 4 5
3 - 439	6.43	b	5
4 - 128	6.40	b	5
5 - 506	5.21	c	

Project: BUTTER REVISED

Question Number: 10
Question Type: Category / Hedonics
Question Title: Salt Flavor
Attribute Number: 1
Attribute Title: Q#10.1
Design: T=5, K=5, B=60

Products

Products	Code	Name
1 - 275	275	0.8% NaCl
2 - 841	841	1.6% NaCl
3 - 439	439	2.4% NaCl
4 - 128	128	3.2% NaCl
5 - 506	506	4.0% NaCl

Scale Parameters

Value	Descriptor
9	Like Extremely
8	Like Very Much
7	Like Moderately
6	Like Slightly
5	Neither Like Nor Dislike
4	Dislike Slightly
3	Dislike Moderately
2	Dislike Very Much
1	Dislike Extremely

Note: Numbers shown in brackets are the 'values' associated with the category selected.

Crosstabulation

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 275	10	14	16	3	6	2	1		1	53
2 - 841	8	17	13	5	4	1	2	3		53
3 - 439	10	8	7	4	2	8	5	7	2	53
4 - 128	7	6	6	3	4	8	10	5	4	53
5 - 506	3	6	4	1	4	7	6	8	14	53
TOTALS	38	51	46	16	20	26	24	23	21	265

Percentage Crosstabulation

Sample	1 [9]	2 [8]	3 [7]	4 [6]	5 [5]	6 [4]	7 [3]	8 [2]	9 [1]	Total
1 - 275	18.9	26.4	30.2	5.7	11.3	3.8	1.9		1.9	100
2 - 841	15.1	32.1	24.5	9.4	7.6	1.9	3.8	5.7		100
3 - 439	18.9	15.1	13.2	7.6	3.8	15.1	9.4	13.2	3.8	100
4 - 128	13.2	11.3	11.3	5.7	7.6	15.1	18.9	9.4	7.6	100
5 - 506	5.7	11.3	7.6	1.9	7.6	13.2	11.3	15.1	26.4	100

Counts, Medians, Means and SD's

Sample Number	Count	Total	Median	Mean	Standard Deviation
1 - 275	53	374.00	7.00	7.06	1.703
2 - 841	53	365.00	7.00	6.89	1.888
3 - 439	53	300.00	6.00	5.66	2.645
4 - 128	53	267.00	4.00	5.04	2.594
5 - 506	53	205.00	3.00	3.87	2.696

This is a Complete Block Design.

Analysis of Variance

This analysis does not compensate for missing data or lack of balance.

	D.F.	Sum of Squares	Mean of Squares	F Value	p-value
Samples	4	373.411	93.353	24.60	0.0000
Judges	52	638.649	12.282	3.24	0.0000
Error	208	789.389	3.795		
Total	264	1801.449	6.824		
Standard Error (SEM) =	0.267				

Multiple comparison tests may appear below. Tukey's HSD controls for maximum experimentwise error rate and can be used without F protection. Standard practice recommends that LSD and Duncan's be considered only if the ANOVA p-value is deemed acceptable to control for experimentwise error rates (under the complete null hypothesis). If automatic significance is selected, an available significance level is chosen for the multiple comparison test based on the observed p-value.

Tukey's HSD = 1.049 (5% Significance Level)

Sample	Mean		Significantly Different Than Sample
1 - 275	7.06	a	3 4 5
2 - 841	6.89	a	3 4 5
3 - 439	5.66	b	5
4 - 128	5.04	b	5
5 - 506	3.87	c	

Project: BUTTER REVISED

Question Number: 11
Question Type: Category / Hedonics
Question Title: Level of Salt
Attribute Number: 1
Attribute Title: Q#11.1
Design: T=5, K=5, B=60

Products

Products	Code	Name
1 - 275	275	0.8% NaCl
2 - 841	841	1.6% NaCl
3 - 439	439	2.4% NaCl
4 - 128	128	3.2% NaCl
5 - 506	506	4.0% NaCl

Scale Parameters

Value	Descriptor
5	Definitely too high
4	Slightly too high
3	Just about right
2	Slightly too low
1	Definitely too low

Note: Numbers shown in brackets are the 'values' associated with the category selected.

Crosstabulation

Sample	1 [5]	2 [4]	3 [3]	4 [2]	5 [1]	Total
1 - 275		6	29	16	2	53
2 - 841		9	35	7	2	53
3 - 439	11	21	19	2		53
4 - 128	18	24	10		1	53
5 - 506	36	10	5	1	1	53
TOTALS	65	70	98	26	6	265

Percentage Crosstabulation

Sample	1 [5]	2 [4]	3 [3]	4 [2]	5 [1]	Total
1 - 275		11.3	54.7	30.2	3.8	100
2 - 841		17.0	66.0	13.2	3.8	100
3 - 439	20.8	39.6	35.9	3.8		100
4 - 128	34.0	45.3	18.9		1.9	100
5 - 506	67.9	18.9	9.4	1.9	1.9	100

Counts, Medians, Means and SD's

Sample Number	Count	Total	Median	Mean	Standard Deviation
1 - 275	53	145.00	3.00	2.74	0.711
2 - 841	53	157.00	3.00	2.96	0.678
3 - 439	53	200.00	4.00	3.77	0.824
4 - 128	53	217.00	4.00	4.09	0.838
5 - 506	53	238.00	5.00	4.49	0.891

This is a Complete Block Design.

Analysis of Variance

This analysis does not compensate for missing data or lack of balance.

	D.F.	Sum of Squares	Mean of Squares	F Value	p-value
Samples	4	117.683	29.421	63.01	0.0000
Judges	52	66.166	1.272	2.73	0.0000
Error	208	97.117	0.467		
Total	264	280.966	1.064		
Standard Error (SEM) =	0.093				

Multiple comparison tests may appear below. Tukey's HSD controls for maximum experimentwise error rate and can be used without F protection. Standard practice recommends that LSD and Duncan's be considered only if the ANOVA p-value is deemed acceptable to control for experimentwise error rates (under the complete null hypothesis). If automatic significance is selected, an available significance level is chosen for the multiple comparison test based on the observed p-value.

Tukey's HSD = 0.368 (5% Significance Level)

Sample	Mean		Significantly Different Than Sample
5 - 506	4.49	a	4 3 2 1
4 - 128	4.09	b	2 1
3 - 439	3.77	b	2 1
2 - 841	2.96	c	
1 - 275	2.74	c	

Project: BUTTER REVISED

Question Number: 12
Question Type: Ranking
Question Title: Ranking the samples
Design: T=5, K=5, B=60

Products

Products	Code	Name
1 - 275	275	0.8% NaCl
2 - 841	841	1.6% NaCl
3 - 439	439	2.4% NaCl
4 - 128	128	3.2% NaCl
5 - 506	506	4.0% NaCl

Crosstabulation

Sample	1	2	3	4	5	Total
1 - 275	18	17	8	7	3	53
2 - 841	17	20	9	3	4	53
3 - 439	9	10	22	8	4	53
4 - 128	6	4	10	24	9	53
5 - 506	3	2	4	11	33	53
TOTALS	53	53	53	53	53	265

Percentage Crosstabulation

Sample	1	2	3	4	5	Total
1 - 275	34.0	32.1	15.1	13.2	5.7	100
2 - 841	32.1	37.7	17.0	5.7	7.6	100
3 - 439	17.0	18.9	41.5	15.1	7.6	100
4 - 128	11.3	7.6	18.9	45.3	17.0	100
5 - 506	5.7	3.8	7.6	20.8	62.3	100

Friedman Analysis of Rank

This procedure is valid for Complete Block Experimental Designs with no missing data only.
This is a Complete Block Design.

Calculated Friedman Statistic	Degrees of Freedom	p-value
68.15	4	0.000

Critical values corresponding to specific levels of significance:
10%=7.78 5%=9.49 1%=13.28

The samples differ at the 10% level. (68.15 >= 7.78)

The samples differ at the 5% level. (68.15 >= 9.49)

The samples differ at the 1% level. (68.15 >= 13.28)

Tukey's HSD = 44.432 (5% Significance Level)

Sample	Rank Total		Significantly Different Than Sample
5 - 506	228.00	a	3 1 2
4 - 128	185.00	ab	1 2
3 - 439	147.00	bc	
1 - 275	119.00	c	
2 - 841	116.00	c	

Combined results

	Acceptance				Ideality	Ranking
Aq. %NaCl	**Overall Acceptance**	**Appearance**	**Overall Flavor**	**Salt Flavor**	**Level of Salt**	**Rank sum**
4	7.32^{a}	7.53^{a}	7.38^{a}	7.06^{a}	2.74	119^{a}
8	7.40^{a}	7.47^{a}	7.36^{a}	6.89^{a}	2.96	116^{a}
12	6.77^{ab}	7.45^{a}	6.43^{b}	5.66^{b}	3.77	147^{ab}
16	6.57^{b}	7.13^{ab}	6.40^{b}	5.04^{b}	4.00	185^{bc}
20	5.68^{c}	6.96^{b}	5.21^{c}	3.87^{c}	4.49	228^{c}

ANCOVA Statistical Output

The SAS System

1 11:22 Friday, September 17, 2010

The Mixed Procedure

Model Information

Data Set	WORK.GOOD
Dependent Variable	lcount
Covariance Structure	Variance Components
Subject Effect	Batch
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Batch	18	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Dimensions

Covariance Parameters	2
Columns in X	5
Columns in Z Per Subject	18
Subjects	18
Max Obs Per Subject	4

Number of Observations

Number of Observations Read	64
Number of Observations Used	62
Number of Observations Not Used	2

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	342.51137275	
1	3	340.63296786	0.00001266
2	1	340.63144778	0.00000001
3	1	340.63144659	0.00000000

Convergence criteria met.

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
Batch	Batch	2.9978
Residual		15.4251

Fit Statistics

-2 Res Log Likelihood	340.6
AIC (smaller is better)	344.6
AICC (smaller is better)	344.9
BIC (smaller is better)	346.4

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
licount	1	41	7.14	0.0107
headspace	1	41	9.65	0.0034
WA	1	41	1.24	0.2726
ph	1	41	0.01	0.9152

Upon initial analysis the headspace is the only variable of the possible confounding variables that has a significant effect on the count. Therefore the water activity and pH variables were eliminated from the analysis.

The Mixed Procedure

Model Information

Data Set	WORK.GOOD
Dependent Variable	lcount
Covariance Structure	Variance Components
Subject Effect	Batch
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Batch	18	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Dimensions

Covariance Parameters	2
Columns in X	3
Columns in Z Per Subject	18
Subjects	18
Max Obs Per Subject	4

Number of Observations

Number of Observations Read	64
Number of Observations Used	62
Number of Observations Not Used	2

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	353.88133865	
1	3	351.13047901	0.00004711
2	1	351.12456533	0.00000014
3	1	351.12454871	0.00000000

Convergence criteria met.

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
Batch	Batch	3.5437
Residual		14.9081

Fit Statistics

-2 Res Log Likelihood	351.1
AIC (smaller is better)	355.1
AICC (smaller is better)	355.3
BIC (smaller is better)	356.9

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
licount	1	43	6.67	0.0133
headspace	1	43	10.50	0.0023

The Mixed Procedure

Model Information

Data Set	WORK.GOOD
Dependent Variable	lcount
Covariance Structure	Variance Components
Subject Effect	Batch
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Batch	18	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
treatment	2	control treat
temp	2	22 41
time	2	1 2

Dimensions

Covariance Parameters	2
Columns in X	22
Columns in Z Per Subject	46
Subjects	18
Max Obs Per Subject	4

Number of Observations

Number of Observations Read	64
Number of Observations Used	62
Number of Observations Not Used	2

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	323.74666666	
1	2	322.64361407	0.00000000

Convergence criteria met.

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
Batc(trea*temp*time)	Batch	5.0381
Residual		12.4381

Fit Statistics

-2 Res Log Likelihood	322.6
AIC (smaller is better)	326.6
AICC (smaller is better)	326.9
BIC (smaller is better)	328.4

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
licount	1	13	0.18	0.6805
headspace	1	13	4.06	0.0652
nacl	1	13	1.55	0.2348
treatment	1	39	0.44	0.5095
temp	1	39	5.08	0.0299
time	1	39	0.27	0.6045
treatment*temp	1	39	0.49	0.4863
treatment*time	1	39	0.44	0.5112
temp*time	1	39	0.86	0.3601

When the data is analyzed with the exact measured %NaCl, the %NaCl does not appear to exhibit a significant effect on the overall count. Dr. Eggett thought that using the exact values was forcing it to fit to a specific value and that would be affecting significance. His suggestion was to reanalyze the data using the estimated amount of %NaCl estimated (0,0.8,1.6,2.4) because this is how someone would make the butter.

The Mixed Procedure

Model Information

Data Set	WORK.GOOD
Dependent Variable	lcount
Covariance Structure	Variance Components
Subject Effect	Batch
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Batch	18	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
treatment	2	control treat
temp	2	22 41

Dimensions

Covariance Parameters	2
Columns in X	9
Columns in Z Per Subject	34
Subjects	18
Max Obs Per Subject	4

Number of Observations

Number of Observations Read	64
Number of Observations Used	62
Number of Observations Not Used	2

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	337.68555169	
1	3	334.84655294	0.00020683
2	2	334.82402327	0.00000184
3	1	334.82380680	0.00000000

The Mixed Procedure

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
Batch(treatmen*temp)	Batch	3.8427
Residual		11.6785

Fit Statistics

-2 Res Log Likelihood	334.8
AIC (smaller is better)	338.8
AICC (smaller is better)	339.1
BIC (smaller is better)	340.6

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
licount	1	24	0.14	0.7144
headspace	1	24	4.34	0.0481
nacl	1	24	2.43	0.1319
nacl*nacl	1	24	4.04	0.0558
treatment	1	31	0.43	0.5173
temp	1	31	5.54	0.0251

The Mixed Procedure

Model Information

Data Set	WORK.GOOD
Dependent Variable	lcount
Covariance Structure	Variance Components
Subject Effect	Batch
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Batch	18	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
treatment	2	control treat
temp	2	22 41
time	2	1 2
NaCl_added	4	0 4 8 12

Dimensions

Covariance Parameters	2
Columns in X	49
Columns in Z Per Subject	46
Subjects	18
Max Obs Per Subject	4

Number of Observations

Number of Observations Read	64
Number of Observations Used	62
Number of Observations Not Used	2

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	239.31468595	
1	1	239.31468595	0.00000000

The Mixed Procedure

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
Batc(trea*temp*time)	Batch	0
Residual		8.2619

Fit Statistics

-2 Res Log Likelihood	239.3
AIC (smaller is better)	241.3
AICC (smaller is better)	241.4
BIC (smaller is better)	242.2

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
licount	1	2	0.00	0.9527
headspace	1	2	0.80	0.4649
NaCl_added	3	2	9.83	0.0938
treatment	1	39	0.19	0.6629
temp	1	39	20.55	<.0001
time	1	39	0.63	0.4338
treatment*temp	1	39	0.16	0.6934
treatment*time	1	39	0.84	0.3653
temp*time	1	39	0.11	0.7466
treatment*NaCl_added	3	2	2.18	0.3298
temp*NaCl_added	3	2	10.73	0.0865
time*NaCl_added	3	2	1.20	0.4841

The Mixed Procedure

Model Information

Data Set	WORK.GOOD
Dependent Variable	lcount
Covariance Structure	Variance Components
Subject Effect	Batch
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Batch	18	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
treatment	2	control treat
temp	2	22 41
NaCl_added	4	0 4 8 12

Dimensions

Covariance Parameters	2
Columns in X	19
Columns in Z Per Subject	34
Subjects	18
Max Obs Per Subject	4

Number of Observations

Number of Observations Read	64
Number of Observations Used	62
Number of Observations Not Used	2

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	279.16360841	
1	3	278.92624516	0.00000577
2	1	278.92570558	0.00000000

The Mixed Procedure

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
Batch(treatmen*temp)	Batch	0.7283
Residual		7.9045

Fit Statistics

-2 Res Log Likelihood	278.9
AIC (smaller is better)	282.9
AICC (smaller is better)	283.2
BIC (smaller is better)	284.7

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
licount	1	20	0.00	0.9573
headspace	1	20	2.26	0.1480
NaCl_added	3	20	9.71	0.0004
treatment	1	31	0.10	0.7485
temp	1	31	17.89	0.0002
temp*NaCl_added	3	20	10.03	0.0003

Based on analysis, the interaction between temperature and %NaCl together appear to have a significant effect on the bacterial count observed. The treatment (inoculated or not) did not demonstrate a significant effect on the overall count, however the storage temperature and %NaCl added do have a significant effect.

Least Squares Means

Effect	temp	NaCl added	Estimate	Standard Error	DF	t Value	Pr > |t|
NaCl_added		0	2.9922	0.7462	20	4.01	0.0007
NaCl_added		4	7.3102	0.7693	20	9.50	<.0001
NaCl_added		8	4.0234	0.8116	20	4.96	<.0001
NaCl_added		12	1.9223	0.7518	20	2.56	0.0188
temp	22		2.2699	0.5986	31	3.79	0.0006
temp	41		5.8542	0.5968	31	9.81	<.0001
temp*NaCl_added	22	0	2.8131	1.0591	20	2.66	0.0152
temp*NaCl_added	22	4	2.0244	1.0763	20	1.88	0.0746
temp*NaCl_added	22	8	2.5787	1.1299	20	2.28	0.0336
temp*NaCl_added	22	12	1.6634	1.0678	20	1.56	0.1350
temp*NaCl_added	41	0	3.1713	1.0733	20	2.95	0.0078
temp*NaCl_added	41	4	12.5961	1.1610	20	10.85	<.0001
temp*NaCl_added	41	8	5.4680	1.1286	20	4.85	<.0001
temp*NaCl_added	41	12	2.1811	1.0409	20	2.10	0.0491

Tukey Adjusted Post-hoc Pairwise Comparison

The GLM Procedure

Class Level Information

Class	Levels	Values
Target	4	0 4 8 12
temperature	2	22 41

Number of Observations Read	32
Number of Observations Used	32

The GLM Procedure

Dependent Variable: Aq_pH Aq pH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	16.27477188	2.32496741	18.24	<.0001
Error	24	3.05875000	0.12744792		
Corrected Total	31	19.33352188			

R-Square	Coeff Var	Root MSE	Aq_pH Mean
0.841790	5.786916	0.356998	6.169063

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Target	3	4.51285312	1.50428437	11.80	<.0001
temperature	1	9.76820000	9.76820000	76.64	<.0001
Target*temperature	3	1.99371875	0.66457292	5.21	0.0065

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Target	3	4.51285313	1.50428438	11.80	<.0001
temperature	1	9.76820000	9.76820000	76.64	<.0001
Target*temperature	3	1.99371875	0.66457292	5.21	0.0065

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Target	Aq_pH LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
0	5.52062500	0.12621802	<.0001	1
4	6.34750000	0.12621802	<.0001	2
8	6.37812500	0.12621802	<.0001	3
12	6.43000000	0.12621802	<.0001	4

Least Squares Means for effect Target
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Aq_pH

i/j	1	2	3	4
1		0.0006	0.0004	0.0002
2	0.0006		0.9982	0.9666
3	0.0004	0.9982		0.9912
4	0.0002	0.9666	0.9912	

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

temperature	Aq_pH LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1= LSMean2 Pr > |t|
22	6.72156250	0.08924962	<.0001	<.0001
41	5.61656250	0.08924962	<.0001	

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Target	temperature	Aq_pH LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
0	22	6.49000000	0.17849924	<.0001	1
0	41	4.55125000	0.17849924	<.0001	2
4	22	6.79625000	0.17849924	<.0001	3
4	41	5.89875000	0.17849924	<.0001	4
8	22	6.86250000	0.17849924	<.0001	5
8	41	5.89375000	0.17849924	<.0001	6
12	22	6.73750000	0.17849924	<.0001	7
12	41	6.12250000	0.17849924	<.0001	8

Least Squares Means for effect Target*temperature
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Aq_pH

i/j	1	2	3	4	5	6	7	8
1		<.0001	0.9201	0.3127	0.8124	0.3032	0.9730	0.8222
2	<.0001		<.0001	0.0004	<.0001	0.0004	<.0001	<.0001
3	0.9201	<.0001		0.0292	1.0000	0.0280	1.0000	0.1806
4	0.3127	0.0004	0.0292		0.0161	1.0000	0.0489	0.9846
5	0.8124	<.0001	1.0000	0.0161		0.0153	0.9996	0.1099
6	0.3032	0.0004	0.0280	1.0000	0.0153		0.0468	0.9825
7	0.9730	<.0001	1.0000	0.0489	0.9996	0.0468		0.2694
8	0.8222	<.0001	0.1806	0.9846	0.1099	0.9825		

Plate

A1 – Reed's C. sporogenes purified spore plate

- Pick 1 colony — sporogens

A2 – Savalis C. sporogenes purified spore plate

- Pick 1 colony — sporogens

B – Room temp / N_2 + O_2 Abs / No Salt

- Pick 1 smooth colony
- Pick 1 rough colony — sporogens

C – Room temp / N_2 + O_2 Abs / 12% NaCl

- Pick 1 large colony — ? ecoli
- Pick 1 small colony — Clost.

D – 37°C / N_2 + O_2 Abs / No Salt

- Pick 1 rough colony — ecoli 301
- Pick 1 smooth colony — ecoli 292

E – 37°C / N_2 + O_2 Abs / 12% NaCl

- Pick 1 smooth colony
- Pick 1 rough colony — ? 109

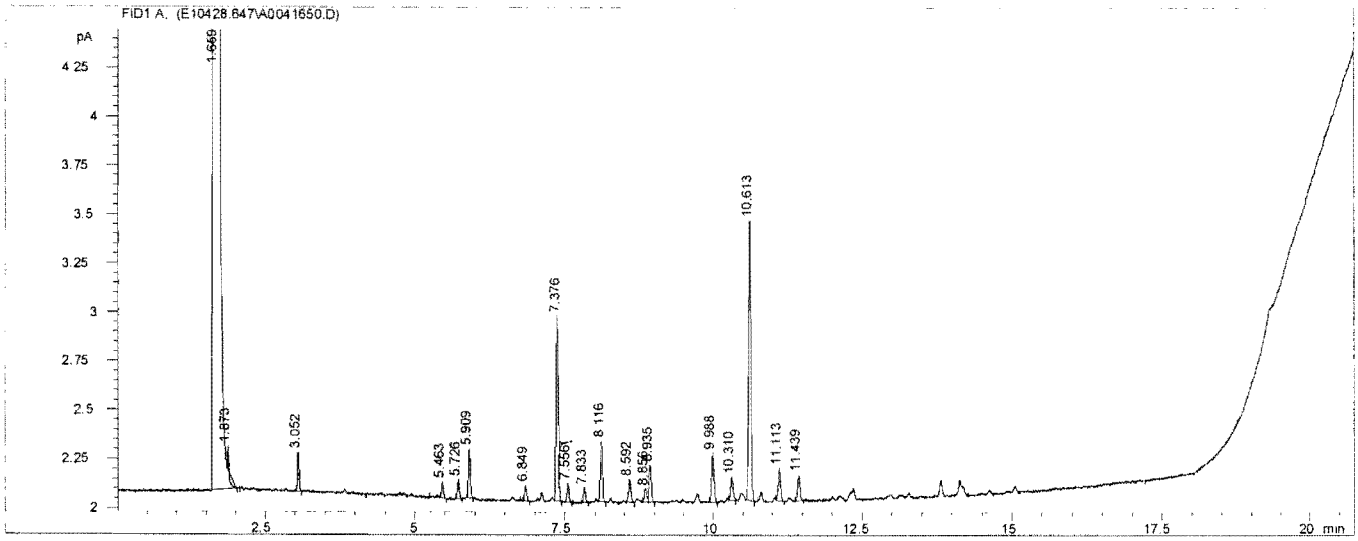
Volume: DATA File: E104286.47A Samp Ctr: 4 ID Number: 1650
Type: Samp Bottle: 3 Method: MOORE6
Created: 4/28/2010 4:44:00 PM
Sample ID: A1 04.28.10 EM

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.623E+8	0.029	----	7.020	SOLVENT PEAK	----	< min rt	
1.873	38	0.008	----	7.437		----	< min rt	
3.052	672	0.027	1.190	9.737	unk 9.740	3.63	ECL deviates -0.003	
5.463	356	0.034	----	12.546		----		
5.726	412	0.034	----	12.771		----		
5.909	1119	0.035	1.015	12.927	Sum In Feature 1	5.16	ECL deviates -0.003	13:1 at 12-13
6.849	354	0.035	0.993	13.618	14:0 iso	1.60	ECL deviates 0.000	Reference 0.000
7.376	4595	0.038	0.983	13.999	14:0	20.53	ECL deviates -0.001	Reference -0.002
7.556	482	0.037	0.980	14.115	13:0 iso 3OH	2.15	ECL deviates 0.001	
7.833	368	0.037	0.976	14.293	14:1 w7c DMA	1.63	ECL deviates 0.002	
8.116	1525	0.040	0.972	14.474	14:0 DMA	6.74	ECL deviates 0.002	Reference 0.001
8.592	692	0.045	0.966	14.780	Sum In Feature 4	3.04	ECL deviates 0.000	15:2
8.856	349	0.040	0.962	14.950	16:0 aldehyde	1.53	ECL deviates -0.001	Reference -0.003
8.935	999	0.041	0.962	15.000	15:0	4.36	ECL deviates 0.000	Reference -0.001
9.988	1393	0.044	0.951	15.627	16:0 iso	6.02	ECL deviates 0.000	Reference -0.001
10.310	670	0.041	0.949	15.819	16:1 w7c	2.89	ECL deviates 0.001	
10.613	7845	0.043	0.946	15.999	16:0	33.74	ECL deviates -0.001	Reference -0.003
11.113	911	0.044	0.943	16.285	16:1 w7c DMA	3.90	ECL deviates 0.000	
11.439	718	0.038	0.941	16.472	16:0 DMA	3.07	ECL deviates 0.001	Reference 0.000
----	1119	---	----	----	Summed Feature 1	5.16	13:1 at 12-13	14:0 aldehyde
----	-----	---	----	----		----	11:1 2OH	
----	692	---	----	----	Summed Feature 4	3.04	UN 14.762 15:2 ? FA	15:2
----	-----	---	----	----		----	15:1 w8c	

ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 8
Total Response: 23459 Total Named: 22691
Percent Named: 96.73% Total Amount: 22003
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.241	Clostridium-sporogenes
	0.200	Treponema-denticola


FID1 A, (E104286.47\A041650.D)
pA
4.25
4
3.75
3.5
3.25
3
2.75
2.5
2.25
2
1.669
1.879
3.052
5.463
5.726
5.909
6.849
7.376
7.556
7.833
8.116
8.592
8.856
8.935
9.988
10.310
10.613
11.113
11.439
2.5
5
7.5
10
12.5
15
17.5
20 min

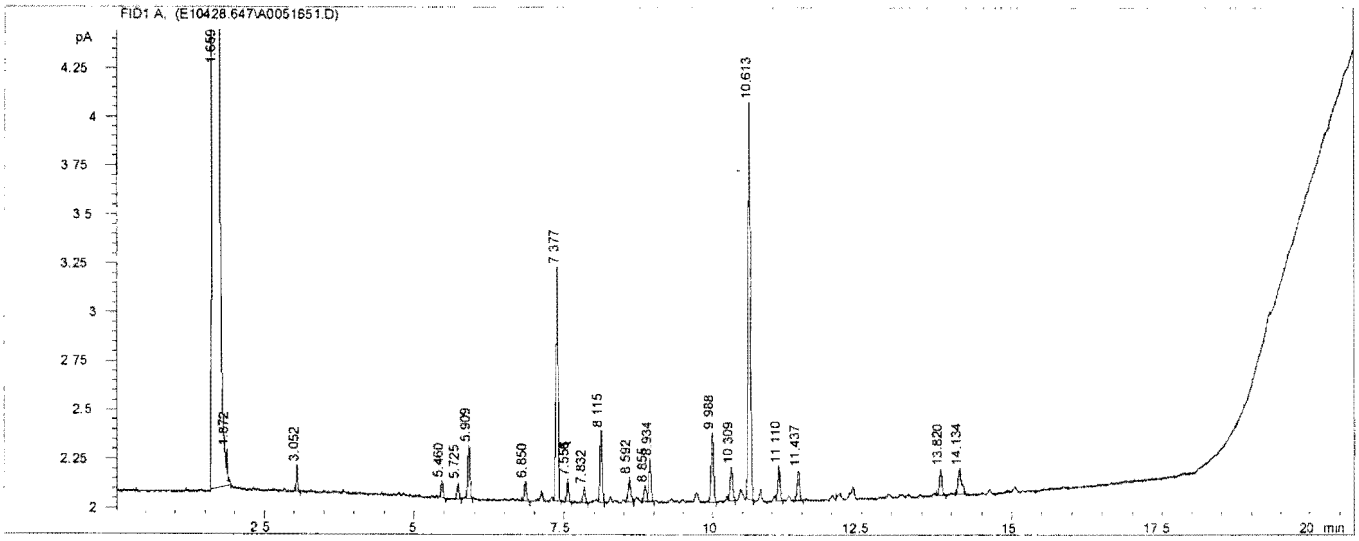
Volume: DATA File: E104286.47A Samp Ctr: 5 ID Number: 1651
Type: Samp Bottle: 4 Method: MOORE6
Created: 4/28/2010 5:08:52 PM
Sample ID: A2 04.28.10 EM

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.625E+8	0.029	----	7.019	SOLVENT PEAK	----	< min rt	
1.872	289	0.022	----	7.435		----	< min rt	
3.052	474	0.027	1.190	9.738	unk 9.740	1.90	ECL deviates -0.002	
5.460	372	0.035	----	12.542		----		
5.725	337	0.032	----	12.769		----		
5.909	1171	0.034	1.015	12.928	Sum In Feature 1	4.00	ECL deviates -0.002	13:1 at 12-13
6.850	485	0.039	0.993	13.619	14:0 iso	1.62	ECL deviates 0.001	Reference 0.000
7.377	5751	0.038	0.983	13.999	14:0	19.03	ECL deviates -0.001	Reference -0.001
7.556	536	0.036	0.980	14.115	13:0 iso-3OH	1.77	ECL deviates 0.001	
7.832	394	0.035	0.976	14.292	14:1 w7c DMA	1.29	ECL deviates 0.001	
8.115	1827	0.039	0.972	14.473	14:0 DMA	5.98	ECL deviates 0.001	Reference 0.000
8.592	743	0.044	0.966	14.780	Sum In Feature 4	2.42	ECL deviates 0.000	15:2
8.855	421	0.040	0.962	14.949	16:0 aldehyde	1.36	ECL deviates -0.002	Reference -0.003
8.934	1129	0.040	0.962	15.000	15:0	3.65	ECL deviates 0.000	Reference -0.002
9.988	1958	0.043	0.951	15.628	16:0 iso	6.27	ECL deviates 0.001	Reference -0.001
10.309	969	0.044	0.949	15.818	16:1 w7c	3.09	ECL deviates 0.000	
10.613	11397	0.043	0.946	16.000	16:0	36.31	ECL deviates 0.000	Reference -0.002
11.110	983	0.043	0.943	16.285	16:1 w7c DMA	3.12	ECL deviates 0.000	
11.437	835	0.043	0.941	16.472	16:0 DMA	2.65	ECL deviates 0.001	Reference -0.001
13.820	769	0.046	0.929	17.823	Sum In Feature 10	2.41	ECL deviates -0.001	18:1 w7c
14.134	999	0.053	0.927	18.000	18:0	3.12	ECL deviates 0.000	Reference -0.002
----	1171	---	----	----	Summed Feature 1	4.00	13:1 at 12-13	14:0 aldehyde
----	-----	---	----	----		----	11:1 2OH	
----	743	---	----	----	Summed Feature 4	2.42	UN 14.762 15:2 ? FA	15:2
----	-----	---	----	----		----	15:1 w8c	
----	769	---	----	----	Summed Feature 10	2.41	18:1 w7c	unknown 17.834

ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 9
Total Response: 31550 Total Named: 30841
Percent Named: 97.75% Total Amount: 29704
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.265	Treponema-denticola
	0.230	Clostridium-botulinum-type B, proteolytic 2


FID1 A, (E10428.647\A0051651.D)
pA
4.25
4
3.75
3.5
3.25
3
2.75
2.5
2.25
2
1.669
1.872
3.052
5.460
5.725
5.909
6.850
7.377
7.558
7.832
8.115
8.592
8.858
8.934
9.988
10.309
10.613
11.110
11.437
13.820
14.134
2.5
5
7.5
10
12.5
15
17.5
20 min

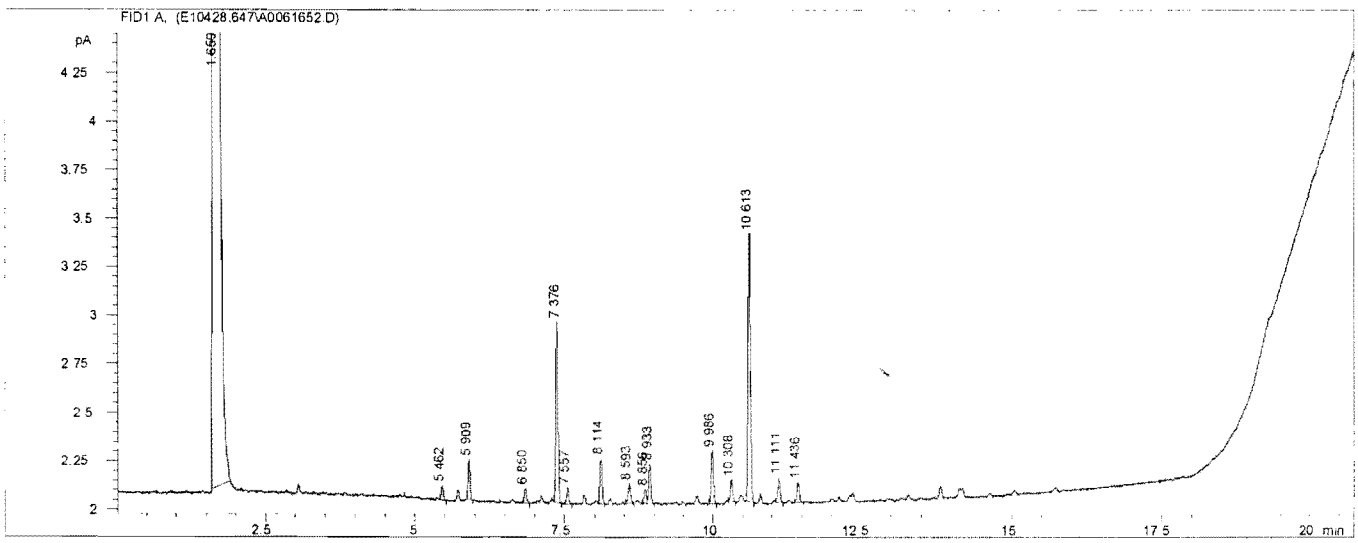
Volume: DATA File: E104286.47A Samp Ctr: 6 ID Number: 1652
Type: Samp Bottle: 5 Method: MOORE6
Created: 4/28/2010 5:33:24 PM
Sample ID: B rough 04.28.10 EM

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.559E+8	0.029	----	7.016	SOLVENT PEAK	----	< min rt	
5.462	342	0.034	----	12.545		----		
5.909	854	0.032	1.015	12.925	Sum In Feature 1	4.38	ECL deviates -0.005	13:1 at 12-13
6.850	399	0.039	0.993	13.618	14:0 iso	2.00	ECL deviates 0.000	Reference 0.001
7.376	4513	0.038	0.983	13.999	14:0	22.41	ECL deviates -0.001	Reference -0.002
7.557	397	0.038	0.980	14.115	13:0 iso 3OH	1.96	ECL deviates 0.001	
8.114	1157	0.038	0.972	14.474	14:0 DMA	5.68	ECL deviates 0.002	Reference 0.000
8.593	599	0.041	0.966	14.781	Sum In Feature 4	2.92	ECL deviates 0.001	15:2
8.856	367	0.038	0.962	14.950	16:0 aldehyde	1.78	ECL deviates -0.001	Reference -0.003
8.933	1062	0.040	0.962	15.000	15:0	5.16	ECL deviates 0.000	Reference -0.002
9.986	1494	0.042	0.951	15.627	16:0 iso	7.18	ECL deviates 0.000	Reference -0.003
10.308	668	0.041	0.949	15.818	16:1 w7c	3.20	ECL deviates 0.000	
10.613	7760	0.044	0.946	16.000	16:0	37.09	ECL deviates 0.000	Reference -0.003
11.111	726	0.044	0.943	16.285	16:1 w7c DMA	3.46	ECL deviates 0.000	
11.436	581	0.041	0.941	16.471	16:0 DMA	2.76	ECL deviates 0.000	Reference -0.002
----	854	---	----	----	Summed Feature 1	4.38	13:1 at 12-13	14:0 aldehyde
----	-----	---	----	----		----	11:1 2OH	
----	599	---	----	----	Summed Feature 4	2.92	UN 14.762 15:2 ? FA	15:2
----	-----	---	----	----		----	15:1 w8c	

ECL Deviation: 0.002 Reference ECL Shift: 0.002 Number Reference Peaks: 8
Total Response: 20920 Total Named: 20578
Percent Named: 98.37% Total Amount: 19798
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.220	Treponema-denticola



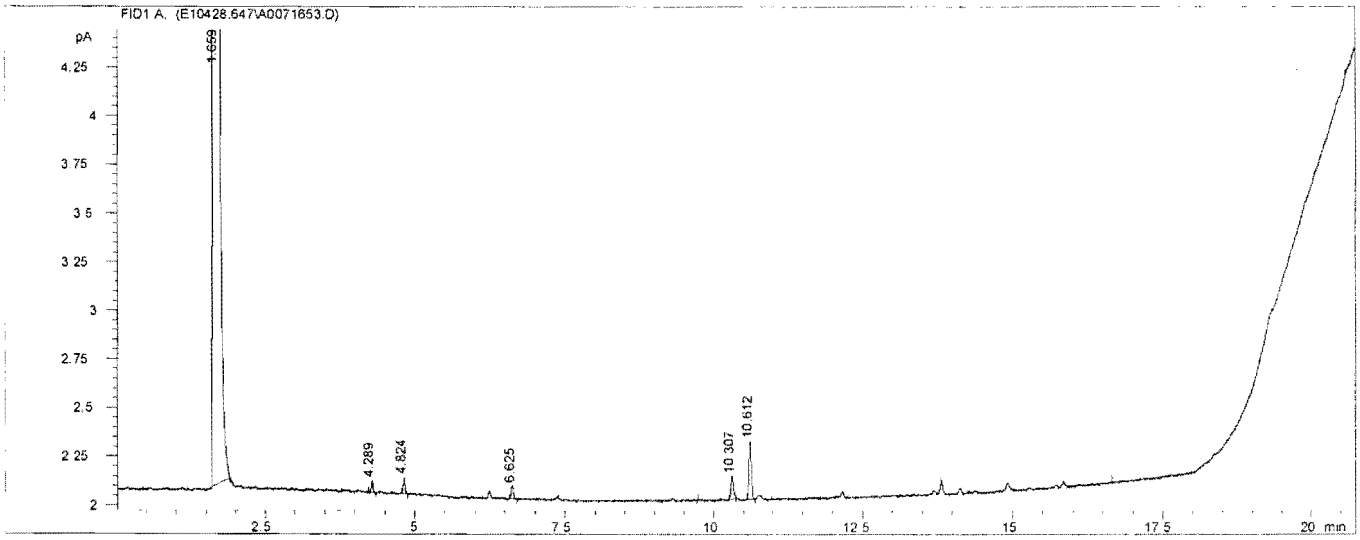
Volume: DATA File: E104286.47A Samp Ctr: 7 ID Number: 1653
Type: Samp Bottle: 6 Method: MOORE6
Created: 4/28/2010 5:58:02 PM
Sample ID: B smooth 04.28.10 EM

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.615E+8	0.029	----	7.024	SOLVENT PEAK	----	< min rt	
4.289	222	0.032	1.081	11.426	10:0 3OH	7.65	ECL deviates 0.004	
4.824	352	0.033	1.053	12.000	12:0	11.83	ECL deviates 0.000	Reference -0.001
6.625	353	0.039	0.998	13.458	Sum In Feature 2	11.25	ECL deviates 0.002	12:0 3OH
10.307	712	0.047	0.949	15.818	16:1 w7c	21.58	ECL deviates 0.000	
10.612	1577	0.043	0.946	16.000	16:0	47.68	ECL deviates 0.000	Reference -0.003
----	353	---	----	----	Summed Feature 2	11.25	12:0 3OH	13:0 DMA

ECL Deviation: 0.002 Reference ECL Shift: 0.002 Number Reference Peaks: 2
Total Response: 3215 Total Named: 3215
Percent Named: 100.00% Total Amount: 3130
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.144	Neisseria-mucosa



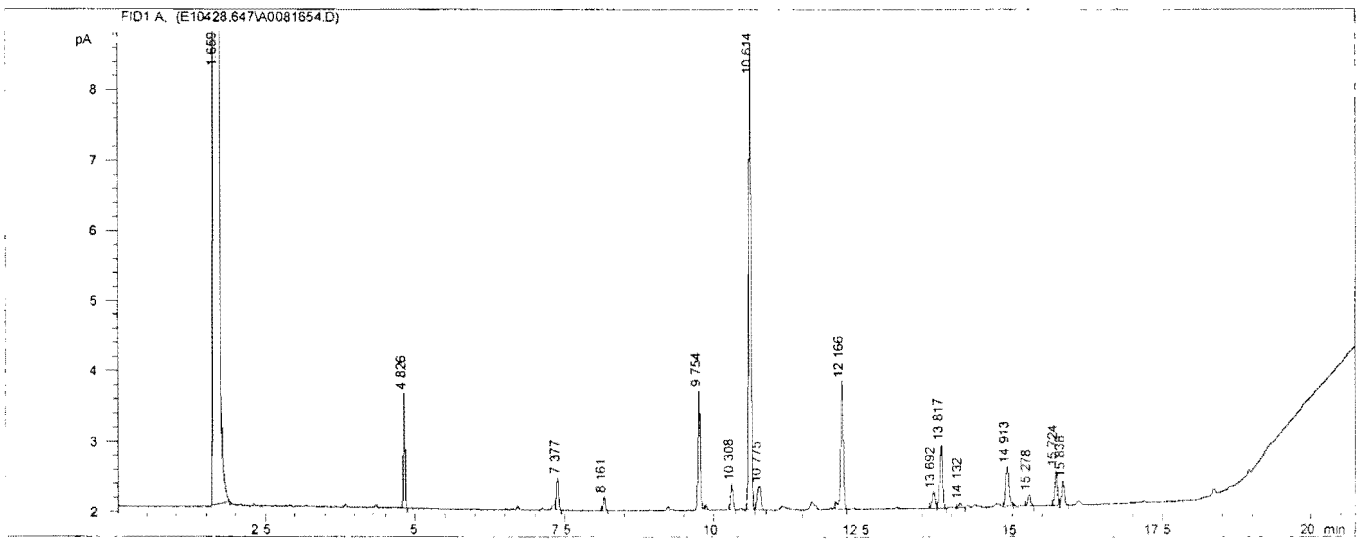
Volume: DATA File: E104286.47A Samp Ctr: 8 ID Number: 1654
Type: Samp Bottle: 7 Method: MOORE6
Created: 4/28/2010 6:22:47 PM
Sample ID: C large 04.28.10 EM

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.625E+8	0.029	----	7.025	SOLVENT PEAK	----	< min rt	
4.826	6397	0.031	1.053	12.000	12:0	8.90	ECL deviates 0.000	Reference 0.000
7.377	2244	0.037	0.983	14.000	14:0	2.92	ECL deviates 0.000	Reference -0.002
8.161	1014	0.039	----	14.504		----		
9.754	9504	0.045	0.953	15.489	Sum In Feature 5	11.98	ECL deviates 0.001	14:0 3OH
10.308	2066	0.043	0.949	15.818	16:1 w7c	2.59	ECL deviates 0.000	
10.614	37130	0.045	0.946	16.000	16:0	46.44	ECL deviates 0.000	Reference -0.002
10.775	2977	0.062	----	16.092		----		
12.166	11710	0.050	0.937	16.888	17:0 cyclopropane	14.49	ECL deviates -0.001	
13.692	1529	0.049	----	17.749		----		
13.817	5850	0.050	0.929	17.819	Sum In Feature 10	7.18	ECL deviates -0.005	18:1 w7c
14.132	381	0.040	0.927	17.996	18:0	0.47	ECL deviates -0.004	Reference -0.003
14.913	4284	0.058	----	18.438		----		
15.278	1065	0.046	0.922	18.645	Sum In Feature 12	1.30	ECL deviates 0.011	19:0 iso
15.724	3075	0.047	0.920	18.897	19 cycloprop. 11,12	3.74	ECL deviates -0.007	Reference -0.004
15.838	2259	0.049	----	18.961		----		
----	9504	---	----	----	Summed Feature 5	11.98	15:0 DMA	14:0 3OH
----	5850	---	----	----	Summed Feature 10	7.18	18:1 w7c	unknown 17.834
----	1065	---	----	----	Summed Feature 12	1.30	unknown 18.622	19:0 iso

ECL Deviation: 0.004 Reference ECL Shift: 0.006 Number Reference Peaks: 6
Total Response: 91483 Total Named: 79421
Percent Named: 86.82% Total Amount: 75664

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.277	Escherichia-coli



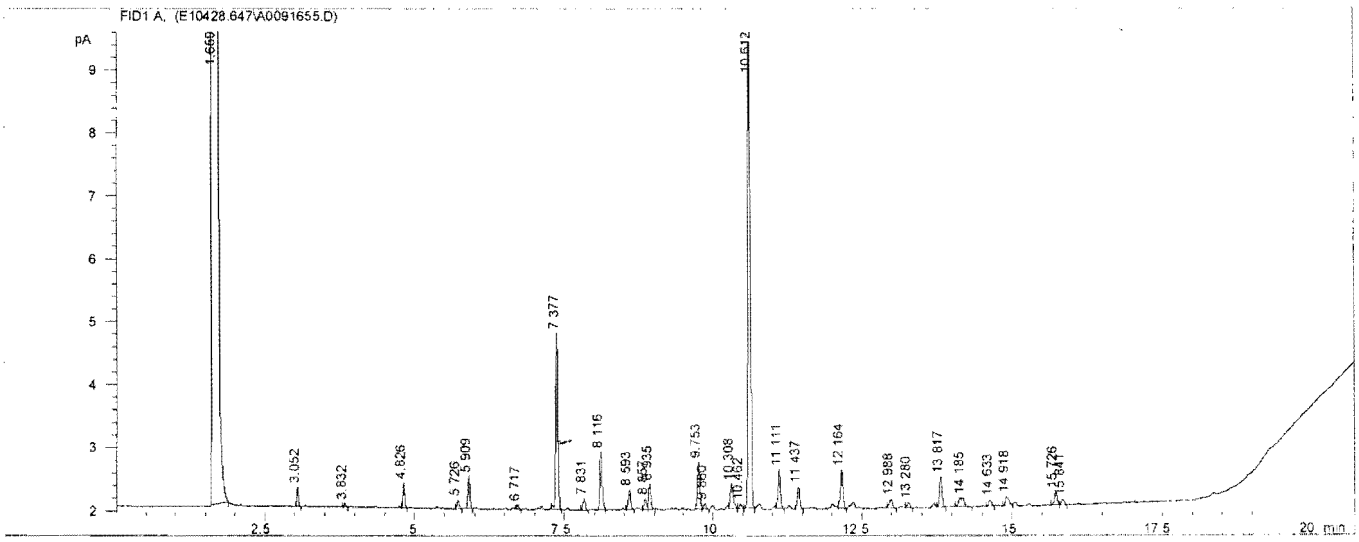
Volume: DATA File: E104286.47A Samp Ctr: 9 ID Number: 1655
Type: Samp Bottle: 8 Method: MOORE6
Created: 4/28/2010 6:47:27 PM
Sample ID: C small 04.28.10 EM

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.626E+8	0.029	----	7.017	SOLVENT PEAK	----	< min rt	
3.052	1058	0.027	1.190	9.737	unk 9.740	1.37	ECL deviates -0.003	
3.832	215	0.030	----	10.913		----		
4.826	1587	0.032	1.053	12.000	12:0	1.82	ECL deviates 0.000	Reference 0.000
5.726	598	0.034	----	12.772		----		
5.909	2254	0.035	1.015	12.929	Sum In Feature 1	2.50	ECL deviates -0.001	13:1 at 12-13
6.717	341	0.034	----	13.523		----		
7.377	13739	0.039	0.983	13.999	14:0	14.75	ECL deviates -0.001	Reference -0.002
7.831	959	0.040	0.976	14.291	14:1 w7c DMA	1.02	ECL deviates 0.000	
8.116	4763	0.041	0.972	14.474	14:0 DMA	5.06	ECL deviates 0.002	Reference 0.001
8.593	1778	0.044	0.966	14.780	Sum In Feature 4	1.87	ECL deviates 0.000	15:2
8.857	894	0.039	0.962	14.950	16:0 aldehyde	0.94	ECL deviates -0.001	Reference -0.002
8.935	2107	0.042	0.962	15.000	15:0	2.21	ECL deviates 0.000	Reference -0.001
9.753	4454	0.047	0.953	15.487	Sum In Feature 5	4.64	ECL deviates -0.001	14:0 3OH
9.860	505	0.042	----	15.551		----		
10.308	2581	0.051	0.949	15.818	16:1 w7c	2.67	ECL deviates 0.000	
10.462	383	0.037	0.948	15.910	16:1 w5c	0.40	ECL deviates 0.001	
10.612	43262	0.047	0.946	15.999	16:0	44.71	ECL deviates -0.001	Reference -0.003
11.111	3469	0.045	0.943	16.285	16:1 w7c DMA	3.57	ECL deviates 0.000	
11.437	1970	0.043	0.941	16.472	16:0 DMA	2.02	ECL deviates 0.001	Reference -0.001
12.164	3931	0.048	0.937	16.888	17:0 cyclopropane	4.02	ECL deviates -0.001	
12.988	977	0.053	0.932	17.355	17:0 cyclo DMA	0.99	ECL deviates 0.001	
13.280	487	0.042	0.931	17.520	16:0 3OH	0.50	ECL deviates -0.002	
13.817	2871	0.049	0.929	17.822	Sum In Feature 10	2.91	ECL deviates -0.002	18:1 w7c
14.185	1747	0.081	----	18.030		----		
14.633	618	0.043	0.925	18.284	18:1 w7c DMA	0.62	ECL deviates -0.001	
14.918	975	0.048	----	18.445		----		
15.726	1369	0.050	0.920	18.904	19 cycloprop. 11,12	1.38	ECL deviates 0.000	Reference -0.003
15.841	557	0.048	----	18.969		----		
----	2254	---	----	----	Summed Feature 1	2.50	13:1 at 12-13	14:0 aldehyde
----	-----	---	----	----		----	11:1 2OH	
----	1778	---	----	----	Summed Feature 4	1.87	UN 14.762 15.2 ? FA	15:2
----	-----	---	----	----		----	15:1 w8c	
----	4454	---	----	----	Summed Feature 5	4.64	15:0 DMA	14:0 3OH
----	2871	---	----	----	Summed Feature 10	2.91	18:1 w7c	unknown 17.834

ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 8
Total Response: 100447 Total Named: 95510
Percent Named: 95.09% Total Amount: 91561

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.347	Clostridium-oceanicum


FID1 A, (E10428.647\A0091655.D)
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1.669
3.052
3.832
4.826
5.726
5.909
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7.377
7.831
8.116
8.593
8.935
9.753
10.308
10.632
11.111
11.437
12.164
12.988
13.280
13.617
14.185
14.633
14.918
15.726
15.941
2.5
5
7.5
10
12.5
15
17.5
20 min

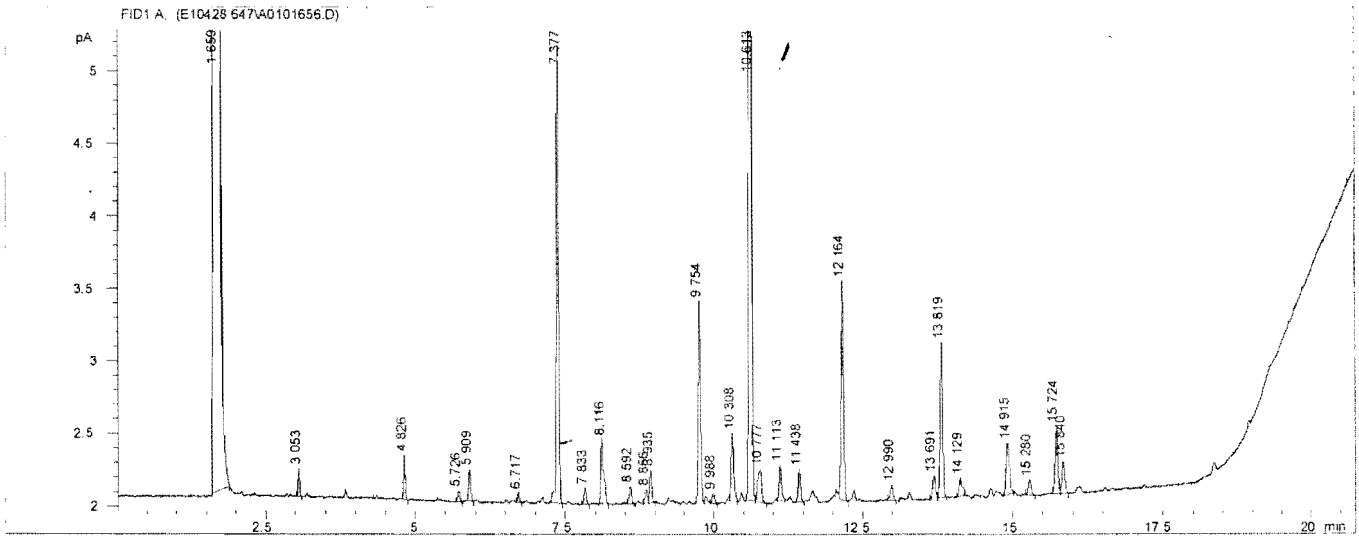
Volume: DATA File: E104286.47A Samp Ctr: 10 ID Number: 1656
Type: Samp Bottle: 9 Method: MOORE6
Created: 4/28/2010 7:12:06 PM
Sample ID: D rough 04.28.10 EM

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.621E+8	0.029	----	7.024	SOLVENT PEAK	----	< min rt	
3.053	673	0.027	1.190	9.742	unk 9.740	0.78	ECL deviates 0.002	
4.826	1222	0.032	1.053	11.999	12:0	1.25	ECL deviates -0.001	Reference 0.000
5.726	334	0.033	----	12.772		----		
5.909	995	0.036	1.015	12.929	Sum In Feature 1	0.98	ECL deviates -0.001	13:1 at 12-13
6.717	315	0.034	----	13.523		----		
7.377	15916	0.039	0.983	14.000	14:0	15.17	ECL deviates 0.000	Reference -0.001
7.833	578	0.040	0.976	14.293	14:1 w7c DMA	0.55	ECL deviates 0.002	
8.116	3012	0.049	0.972	14.475	14:0 DMA	2.84	ECL deviates 0.003	Reference 0.001
8.592	686	0.044	0.966	14.780	Sum In Feature 4	0.64	ECL deviates 0.000	15:2
8.856	515	0.037	0.962	14.950	16:0 aldehyde	0.48	ECL deviates -0.001	Reference -0.003
8.935	1131	0.040	0.962	15.000	15:0	1.05	ECL deviates 0.000	Reference -0.001
9.754	7707	0.044	0.953	15.488	Sum In Feature 5	7.13	ECL deviates 0.000	14:0 3OH
9.988	400	0.044	0.951	15.627	16:0 iso	0.37	ECL deviates 0.000	Reference -0.002
10.308	2895	0.048	0.949	15.817	16:1 w7c	2.66	ECL deviates -0.001	
10.613	49082	0.045	0.946	15.999	16:0	45.04	ECL deviates -0.001	Reference -0.002
10.777	1976	0.061	----	16.093		----		
11.113	1476	0.047	0.943	16.286	16:1 w7c DMA	1.35	ECL deviates 0.001	
11.438	1219	0.044	0.941	16.471	16:0 DMA	1.11	ECL deviates 0.000	Reference -0.001
12.164	9041	0.047	0.937	16.887	17:0 cyclopropane	8.21	ECL deviates -0.002	
12.990	668	0.044	0.932	17.353	17:0 cyclo DMA	0.60	ECL deviates -0.001	
13.691	1001	0.048	----	17.748		----		
13.819	6317	0.047	0.929	17.820	Sum In Feature 10	5.69	ECL deviates -0.004	18:1 w7c
14.129	934	0.053	0.927	17.994	18:0	0.84	ECL deviates -0.006	Reference -0.005
14.915	2743	0.057	----	18.439		----		
15.280	701	0.050	0.922	18.646	Sum In Feature 12	0.63	ECL deviates 0.012	19:0 iso
15.724	2940	0.049	0.920	18.897	19 cycloprop. 11,12	2.62	ECL deviates -0.007	Reference -0.004
15.840	1466	0.052	----	18.963		----		
----	995	---	----	----	Summed Feature 1	0.98	13:1 at 12-13	14:0 aldehyde
----	-----	---	----	----		----	11:1 2OH	
----	686	---	----	----	Summed Feature 4	0.64	UN 14.762 15:2 ? FA	15:2
----	-----	---	----	----		----	15:1 w8c	
----	7707	---	----	----	Summed Feature 5	7.13	15:0 DMA	14:0 3OH
----	6317	---	----	----	Summed Feature 10	5.69	18:1 w7c	unknown 17.834
----	701	---	----	----	Summed Feature 12	0.63	unknown 18.622	19:0 iso

ECL Deviation: 0.004 Reference ECL Shift: 0.005 Number Reference Peaks: 11
Total Response: 115944 Total Named: 108109
Percent Named: 93.24% Total Amount: 103121

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.364	Escherichia-coli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4.5
4
3.5
3
2.5
2
1 659
3 053
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5 726
5 909
6 717
7 377
7 833
8 116
8 592
8 856
8 935
9 754
9 988
10 308
10 777
10 813
11 113
11 438
12 164
12 990
13 691
13 819
14 129
14 915
15 280
15 724
16 340
2 5
5
7 5
10
12 5
15
17 5
20 min

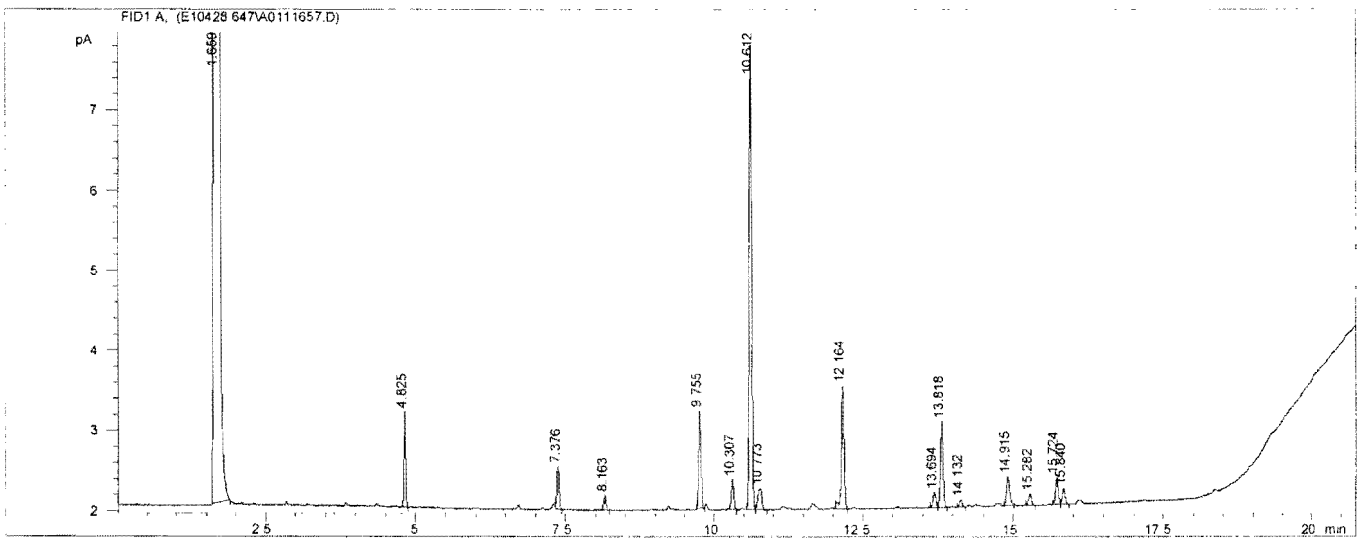
Volume: DATA File: E104286.47A Samp Ctr: 11 ID Number: 1657
Type: Samp Bottle: 10 Method: MOORE6
Created: 4/28/2010 7:36:56 PM
Sample ID: D smooth 04.28.10 EM

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.622E+8	0.029	----	7.019	SOLVENT PEAK	----	< min rt	
4.825	4777	0.031	1.053	12.000	12:0	7.72	ECL deviates 0.000	Reference -0.001
7.376	2621	0.038	0.983	14.000	14:0	3.95	ECL deviates 0.000	Reference -0.002
8.163	920	0.040	----	14.505		----		
9.755	6652	0.043	0.953	15.489	Sum In Feature 5	9.73	ECL deviates 0.001	14:0 3OH
10.307	2124	0.044	0.949	15.818	16:1 w7c	3.09	ECL deviates 0.000	
10.612	32925	0.045	0.946	16.000	16:0	47.81	ECL deviates 0.000	Reference -0.003
10.773	2376	0.066	----	16.092		----		
12.164	9949	0.050	0.937	16.889	17:0 cyclopropane	14.30	ECL deviates 0.000	
13.694	1280	0.050	----	17.754		----		
13.818	6699	0.049	0.929	17.824	Sum In Feature 10	9.55	ECL deviates 0.000	18:1 w7c
14.132	538	0.044	0.927	18.000	18:0	0.77	ECL deviates 0.000	Reference -0.003
14.915	2718	0.059	----	18.445		----		
15.282	926	0.049	----	18.653		----		
15.724	2181	0.049	0.920	18.904	19 cycloprop. 11,12	3.08	ECL deviates 0.000	Reference -0.004
15.840	1329	0.050	----	18.969		----		
----	6652	---	----	----	Summed Feature 5	9.73	15:0 DMA	14:0 3OH
----	6699	---	----	----	Summed Feature 10	9.55	18:1 w7c	unknown 17.834

ECL Deviation: 0.000 Reference ECL Shift: 0.003 Number Reference Peaks: 5
Total Response: 78014 Total Named: 68466
Percent Named: 87.76% Total Amount: 65166

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.292	Escherichia-coli



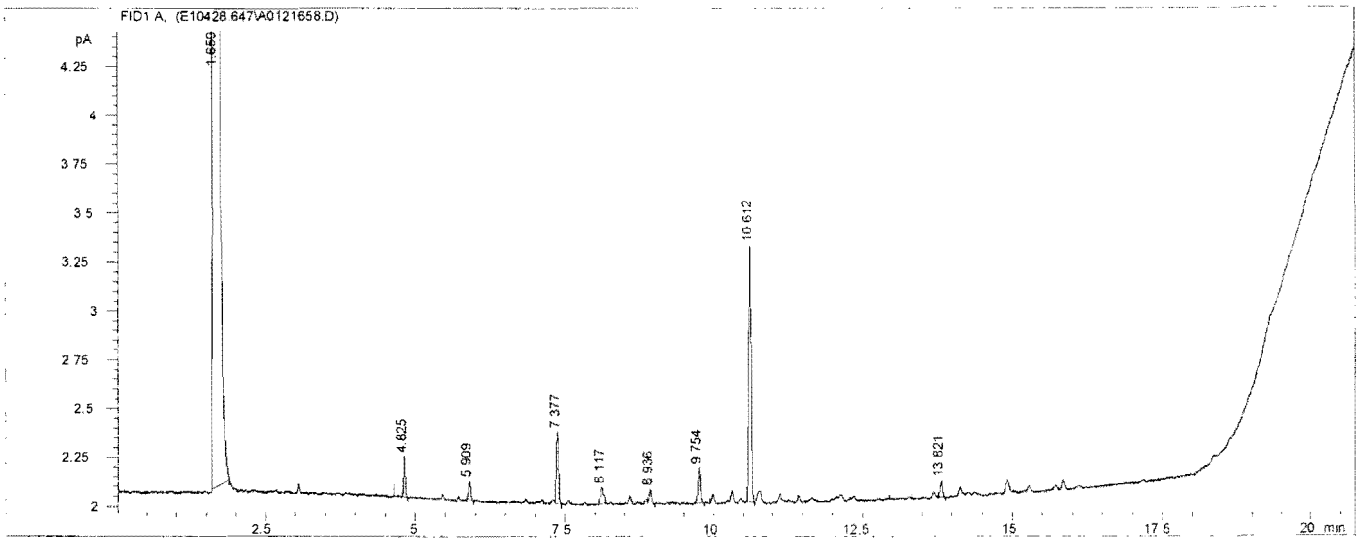
Volume: DATA File: E104286.47A Samp Ctr: 12 ID Number: 1658
Type: Samp Bottle: 11 Method: MOORE6
Created: 4/28/2010 8:01:37 PM
Sample ID: E rough 04.28.10 EM

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.645E+8	0.029	----	7.019	SOLVENT PEAK	----	< min rt	
4.825	829	0.030	1.053	12.000	12:0	7.22	ECL deviates 0.000	Reference -0.001
5.909	413	0.033	1.015	12.927	Sum In Feature 1	3.47	ECL deviates -0.003	13:1 at 12-13
7.377	1790	0.038	0.983	13.998	14:0	14.55	ECL deviates -0.002	Reference -0.001
8.117	535	0.048	0.972	14.474	14:0 DMA	4.30	ECL deviates 0.002	Reference 0.002
8.936	301	0.037	0.962	15.000	15:0	2.39	ECL deviates 0.000	Reference 0.000
9.754	1034	0.045	0.953	15.488	Sum In Feature 5	8.15	ECL deviates 0.000	14:0 3OH
10.612	7195	0.043	0.946	16.000	16:0	56.29	ECL deviates 0.000	Reference -0.003
13.821	474	0.042	0.929	17.823	Sum In Feature 10	3.64	ECL deviates -0.001	18:1 w7c
----	413	---	----	----	Summed Feature 1	3.47	13:1 at 12-13	14:0 aldehyde
----	-----	---	----	----		----	11:1 2OH	
----	1034	---	----	----	Summed Feature 5	8.15	15:0 DMA	14:0 3OH
----	474	---	----	----	Summed Feature 10	3.64	18:1 w7c	unknown 17.834

ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 5
Total Response: 12572 Total Named: 12572
Percent Named: 100.00% Total Amount: 12097
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.109	Coprococcus-eutactus

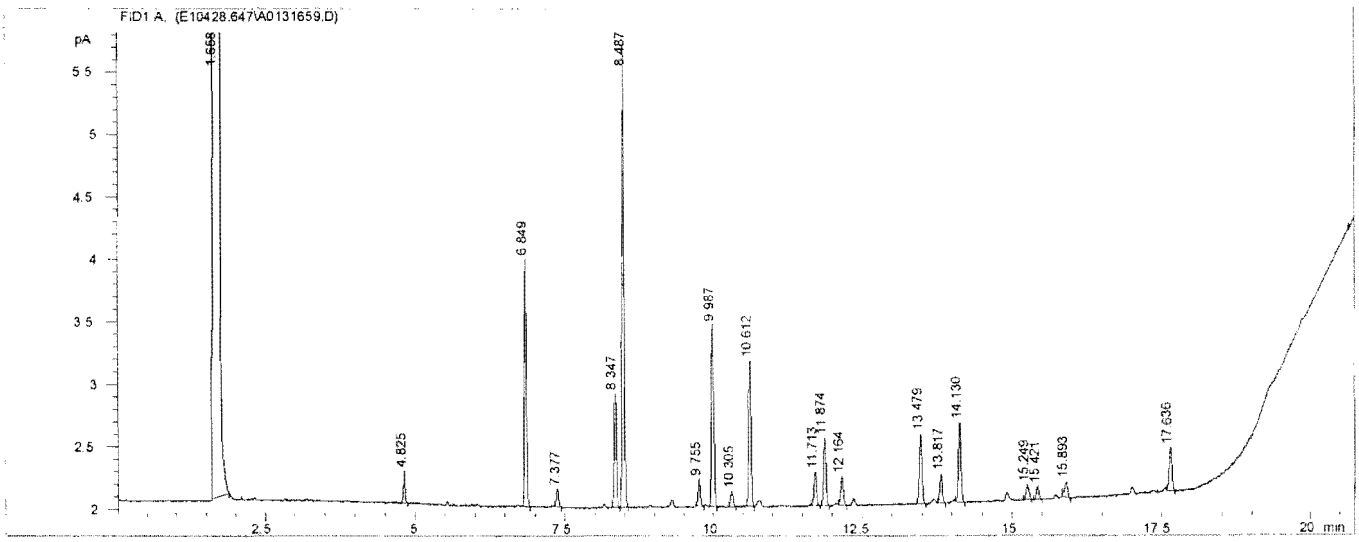


Volume: DATA File: E104286.47A Samp Ctr: 13 ID Number: 1659
Type: Samp Bottle: 12 Method: MOORE6
Created: 4/28/2010 8:26:09 PM
Sample ID: E smooth 04.28.10 EM

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.658	3.62E+8	0.029	----	7.017	SOLVENT PEAK	----	< min rt	
4.825	1034	0.030	1.053	12.000	12:0	1.61	ECL deviates 0.000	Reference 0.000
6.849	9339	0.036	0.993	13.619	14:0 iso	13.73	ECL deviates 0.001	Reference 0.000
7.377	687	0.037	0.983	14.000	14:0	1.00	ECL deviates 0.000	Reference -0.001
8.347	4648	0.040	0.969	14.623	15:0 iso	6.66	ECL deviates 0.000	Reference -0.002
8.487	19141	0.040	0.967	14.713	15:0 anteiso	27.38	ECL deviates -0.001	Reference -0.003
9.755	1118	0.041	0.953	15.489	Sum In Feature 5	1.58	ECL deviates 0.001	14:0 3OH
9.987	7942	0.043	0.951	15.627	16:0 iso	11.18	ECL deviates 0.000	Reference -0.002
10.305	717	0.044	0.949	15.817	16:1 w7c	1.01	ECL deviates -0.001	
10.612	6524	0.043	0.946	16.000	16:0	9.13	ECL deviates 0.000	Reference -0.003
11.713	1638	0.047	0.939	16.631	17:0 iso	2.28	ECL deviates 0.001	Reference -0.002
11.874	3131	0.045	0.938	16.723	17:0 anteiso	4.35	ECL deviates 0.000	
12.164	1420	0.045	0.937	16.889	17:0 cyclopropane	1.97	ECL deviates 0.000	
13.479	3395	0.047	0.930	17.632	18:0 iso	4.67	ECL deviates 0.000	Reference -0.003
13.817	1355	0.045	0.929	17.823	Sum In Feature 10	1.86	ECL deviates -0.001	18:1 w7c
14.130	3975	0.048	0.927	18.000	18:0	5.45	ECL deviates 0.000	Reference -0.004
15.249	810	0.048	0.922	18.634	Sum In Feature 12	1.11	ECL deviates 0.000	19:0 iso
15.421	616	0.043	0.922	18.732	19:0 anteiso	0.84	ECL deviates 0.002	
15.893	913	0.053	0.920	19.000	19:0	1.24	ECL deviates 0.000	Reference -0.004
17.636	2195	0.048	0.911	20.000	20:0	2.96	ECL deviates 0.000	Reference -0.005
----	1118	---	----	----	Summed Feature 5	1.58	15:0 DMA	14:0 3OH
----	1355	---	----	----	Summed Feature 10	1.86	18:1 w7c	unknown 17.834
----	810	---	----	----	Summed Feature 12	1.11	unknown 18.622	19:0 iso

ECL Deviation: 0.001 Reference ECL Shift: 0.003 Number Reference Peaks: 13
Total Response: 70598 Total Named: 70598
Percent Named: 100.00% Total Amount: 67592

*** No Matches found in MOORE6



Volume: DATA File: E104286.47A Samp Ctr: 1 ID Number: 1
Type: Calib Bottle: 1 Method: MOORE6
Created: 4/28/2010 3:31:47 PM
Sample ID: Lot 40350 04.28.10 EM

Profile Comment: Good peak matching. Peak position matching error (RMS) is 0.0019.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.998	MIDI Calibration Mix 1

Volume: DATA File: E104286.47A Samp Ctr: 2 ID Number: 1
Type: Calib Bottle: 1 Method: MOORE6
Created: 4/28/2010 3:54:43 PM
Sample ID: Lot 40350 04.28.10 EM

Profile Comment: Good peak matching. Peak position matching error (RMS) is 0.0012.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.998	MIDI Calibration Mix 1

Volume: DATA File: E104286.47A Samp Ctr: 3 ID Number: 1649
Type: Blank Bottle: 2 Method: MOORE6
Created: 4/28/2010 4:19:29 PM
Sample ID: Blank 04.28.10 EM

Profile Comment:
*** Library match not attempted

Volume: DATA File: E104286.47A Samp Ctr: 4 ID Number: 1650
Type: Samp Bottle: 3 Method: MOORE6
Created: 4/28/2010 4:44:00 PM
Sample ID: A1 04.28.10 EM

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.241	Clostridium-sporogenes
	0.200	Treponema-denticola

Volume: DATA File: E104286.47A Samp Ctr: 5 ID Number: 1651
Type: Samp Bottle: 4 Method: MOORE6
Created: 4/28/2010 5:08:52 PM
Sample ID: A2 04.28.10 EM

Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.265	Treponema-denticola
	0.230	Clostridium-botulinum-type B, proteolytic 2

Volume: DATA File: E104286.47A Samp Ctr: 6 ID Number: 1652
Type: Samp Bottle: 5 Method: MOORE6
Created: 4/28/2010 5:33:24 PM
Sample ID: B rough 04.28.10 EM

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.220	Treponema-denticola

Volume: DATA File: E104286.47A Samp Ctr: 7 ID Number: 1653
Type: Samp Bottle: 6 Method: MOORE6
Created: 4/28/2010 5:58:02 PM
Sample ID: B smooth 04.28.10 EM

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.144	Neisseria-mucosa

Volume: DATA File: E104286.47A Samp Ctr: 8 ID Number: 1654
Type: Samp Bottle: 7 Method: MOORE6
Created: 4/28/2010 6:22:47 PM
Sample ID: C large 04.28.10 EM

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.277	Escherichia-coli

Volume: DATA File: E104286.47A Samp Ctr: 9 ID Number: 1655
Type: Samp Bottle: 8 Method: MOORE6
Created: 4/28/2010 6:47:27 PM
Sample ID: C small 04.28.10 EM

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.347	Clostridium-oceanicum

Volume: DATA File: E104286.47A Samp Ctr: 10 ID Number: 1656
Type: Samp Bottle: 9 Method: MOORE6
Created: 4/28/2010 7:12:06 PM
Sample ID: D rough 04.28.10 EM

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.364	Escherichia-coli

Volume: DATA File: E104286.47A Samp Ctr: 11 ID Number: 1657
Type: Samp Bottle: 10 Method: MOORE6
Created: 4/28/2010 7:36:56 PM
Sample ID: D smooth 04.28.10 EM

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.292	Escherichia-coli

Volume: DATA File: E104286.47A Samp Ctr: 12 ID Number: 1658
Type: Samp Bottle: 11 Method: MOORE6
Created: 4/28/2010 8:01:37 PM
Sample ID: E rough 04.28.10 EM

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.109	Coprococcus-eutactus

Volume: DATA File: E104286.47A Samp Ctr: 13 ID Number: 1659
Type: Samp Bottle: 12 Method: MOORE6
Created: 4/28/2010 8:26:09 PM
Sample ID: E smooth 04.28.10 EM

*** No Matches found in MOORE6

MIDI Data

E103184.82A [1607] 1R 03.18.10 AB

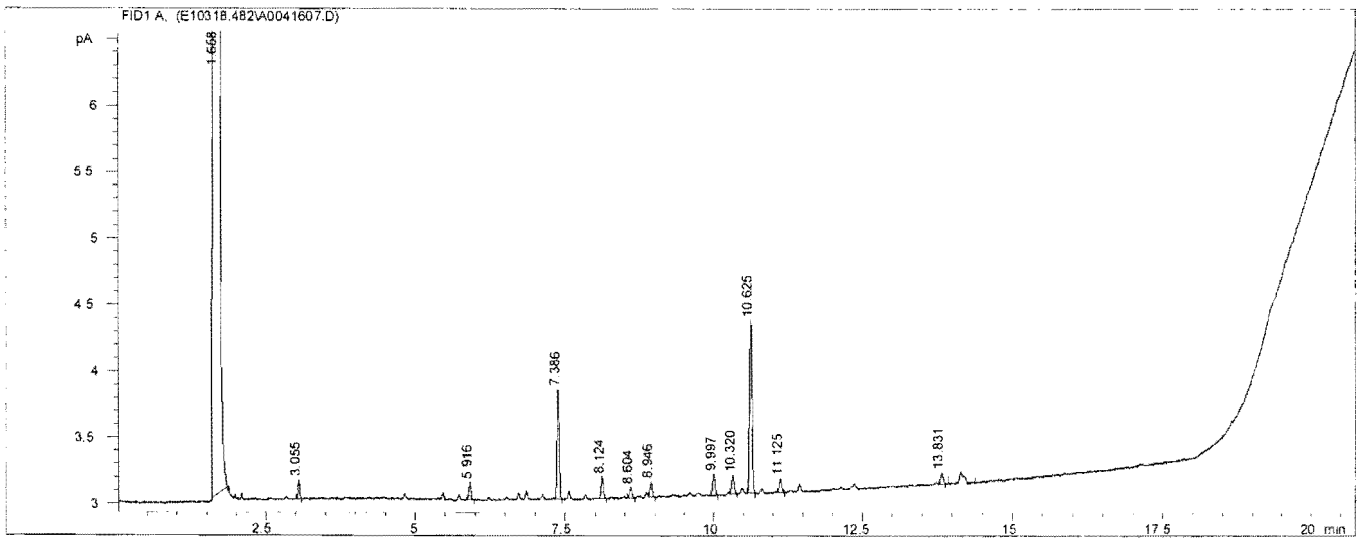
Volume: DATA File: E103184.82A Samp Ctr: 4 ID Number: 1607
Type: Samp Bottle: 3 Method: MOORE6
Created: 3/18/2010 12:46:30 PM
Sample ID: 1R 03.18.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.658	3.646E+8	0.029	----	7.021	SOLVENT PEAK	----	< min rt	
3.055	524	0.028	1.202	9.738	unk 9.740	3.86	ECL deviates -0.002	
5.916	571	0.036	1.012	12.928	Sum In Feature 1	3.54	ECL deviates -0.002	13:1 at 12-13
7.386	4051	0.038	0.979	14.000	14:0	24.27	ECL deviates 0.000	Reference -0.003
8.124	817	0.040	0.968	14.473	14:0 DMA	4.84	ECL deviates 0.001	Reference 0.000
8.604	474	0.046	0.961	14.781	Sum In Feature 4	2.79	ECL deviates 0.001	15:2
8.946	542	0.034	0.957	15.000	15:0	3.18	ECL deviates 0.000	Reference -0.001
9.997	885	0.044	0.947	15.626	16:0 iso	5.13	ECL deviates -0.001	Reference -0.002
10.320	756	0.041	0.945	15.819	16:1 w7c	4.37	ECL deviates 0.001	
10.625	7223	0.044	0.942	16.001	16:0	41.66	ECL deviates 0.001	Reference -0.002
11.125	618	0.046	0.939	16.288	16:1 w7c DMA	3.55	ECL deviates 0.003	
13.831	499	0.043	0.926	17.824	Sum In Feature 10	2.83	ECL deviates 0.000	18:1 w7c
----	571	---	----	----	Summed Feature 1	3.54	13:1 at 12-13	14:0 aldehyde
----	-----	---	----	----		----	11:1 2OH	
----	474	---	----	----	Summed Feature 4	2.79	UN 14.762 15:2 ? FA	15:2
----	-----	---	----	----		----	15:1 w8c	
----	499	---	----	----	Summed Feature 10	2.83	18:1 w7c	unknown 17.834

ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 5
Total Response: 16960 Total Named: 16960
Percent Named: 100.00% Total Amount: 16340
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.243	Clostridium-oceanicum
	0.203	Clostridium-botulinum-type B, proteolytic 2

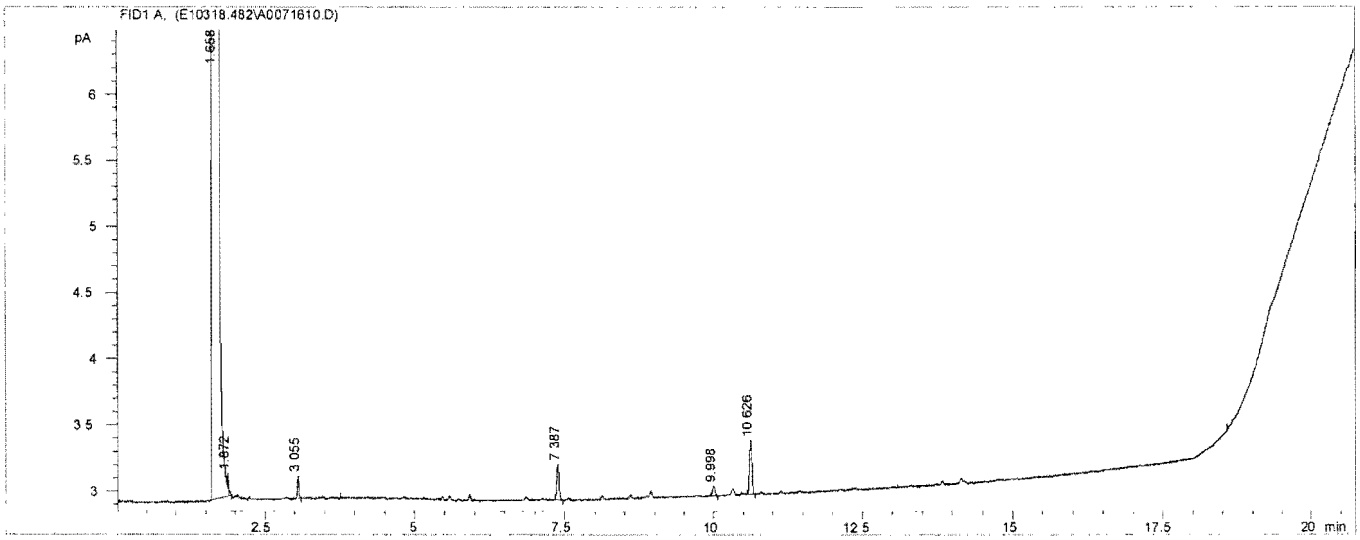


Volume: DATA File: E103184.82A Samp Ctr: 7 ID Number: 1610
Type: Samp Bottle: 6 Method: MOORE6
Created: 3/18/2010 2:00:34 PM
Sample ID: 3R 03.18.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.658	3.587E+8	0.029	----	7.021	SOLVENT PEAK	----	< min rt	
1.872	272	0.021	----	7.438		----	< min rt	
3.055	570	0.026	1.202	9.738	unk 9.740	14.98	ECL deviates -0.002	
7.387	1365	0.040	0.979	14.000	14:0	29.21	ECL deviates 0.000	Reference -0.002
9.998	431	0.045	0.947	15.627	16:0 iso	8.93	ECL deviates 0.000	Reference -0.002
10.626	2275	0.044	0.942	16.000	16:0	46.87	ECL deviates 0.000	Reference -0.001

ECL Deviation: 0.001 Reference ECL Shift: 0.001 Number Reference Peaks: 3
Total Response: 4641 Total Named: 4641
Percent Named: 100.00% Total Amount: 4574
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

*** No Matches found in MOORE6



looks similar to
1R 03.18.10 AB just lemke are too low

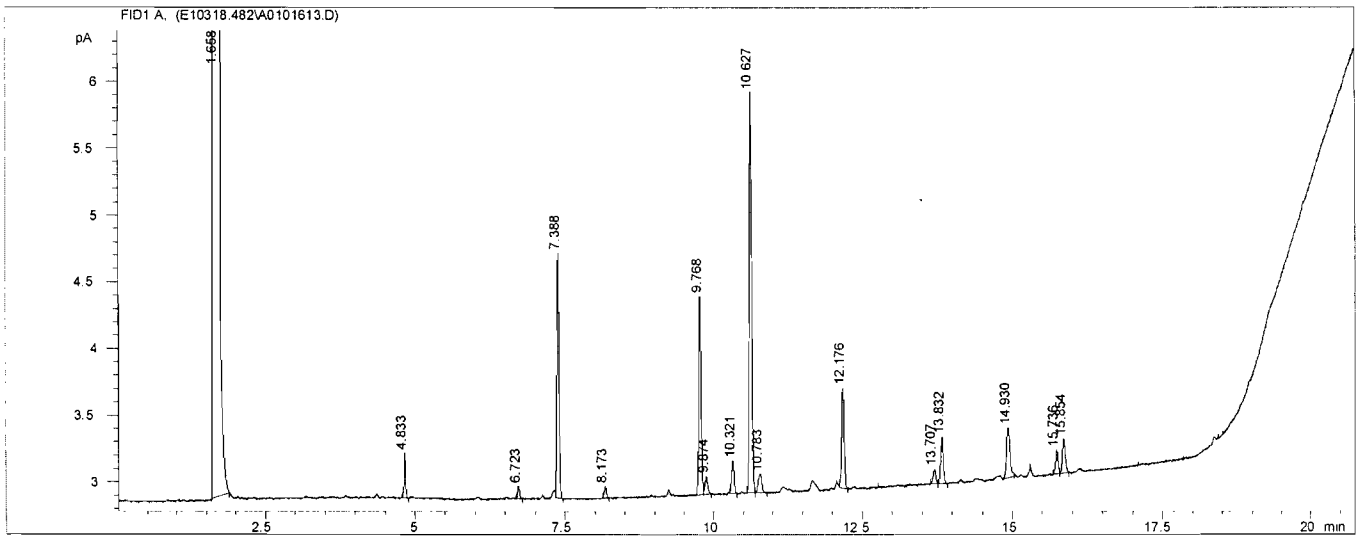
Volume: DATA File: E103184.82A Samp Ctr: 10 ID Number: 1613
Type: Samp Bottle: 9 Method: MOORE6
Created: 3/18/2010 3:14:32 PM
Sample ID: 4S 03.18.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.658	3.601E+8	0.029	----	7.020	SOLVENT PEAK	----	< min rt	
4.833	1360	0.032	1.052	12.000	12:0	3.36	ECL deviates 0.000	Reference 0.000
6.723	423	0.034	----	13.520		----		
7.388	8896	0.038	0.979	14.000	14:0	20.47	ECL deviates 0.000	Reference -0.001
8.173	437	0.033	----	14.504		----		
9.768	8363	0.043	0.949	15.490	Sum In Feature 5	18.66	ECL deviates 0.002	14:0 3OH
9.874	804	0.044	----	15.553		----		
10.321	1394	0.044	0.945	15.819	16:1 w7c	3.09	ECL deviates 0.001	
10.627	16946	0.044	0.942	16.001	16:0	37.53	ECL deviates 0.001	Reference 0.000
10.783	1158	0.062	----	16.091		----		
12.176	4448	0.046	0.933	16.889	17:0 cyclopropane	9.76	ECL deviates 0.000	
13.707	677	0.046	----	17.755		----		
13.832	2181	0.046	0.926	17.825	Sum In Feature 10	4.75	ECL deviates 0.001	18:1 w7c
14.930	2994	0.058	----	18.447		----		
15.736	1105	0.049	0.918	18.903	19 cycloprop. 11,12	2.39	ECL deviates -0.001	Reference -0.004
15.854	1693	0.049	----	18.970		----		
----	8363	---	----	----	Summed Feature 5	18.66	15:0 DMA	14:0 3OH
----	2181	---	----	----	Summed Feature 10	4.75	18:1 w7c	unknown 17.834

ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 4
Total Response: 52877 Total Named: 44692
Percent Named: 84.52% Total Amount: 42549
Profile Comment: Percent named is less than 85.00.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.282	Escherichia-coli



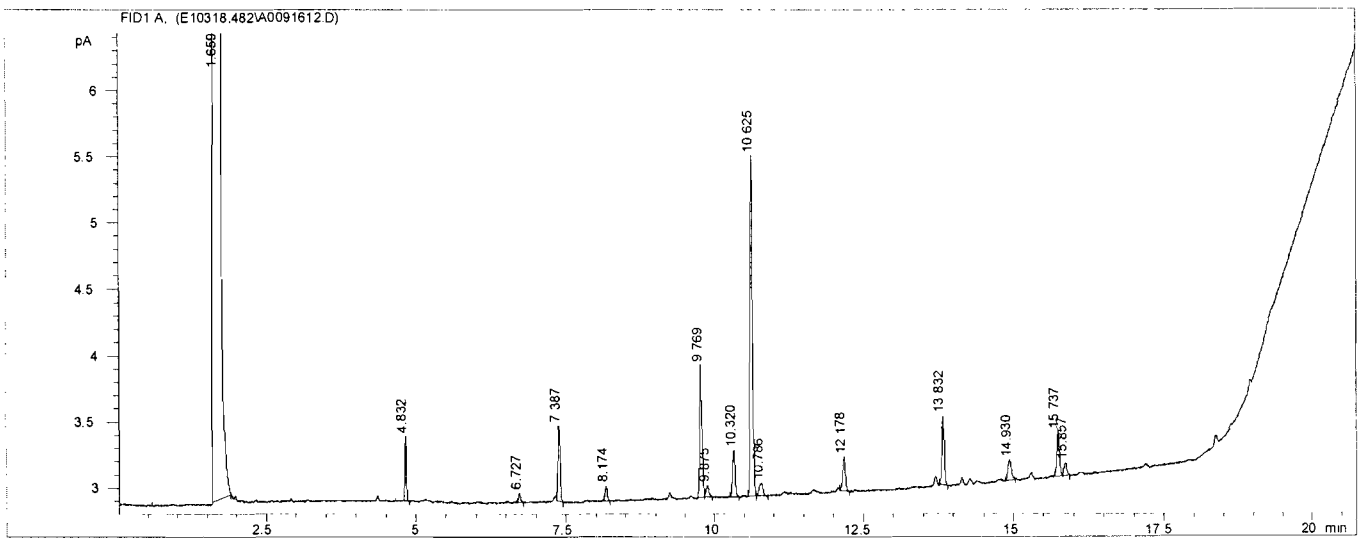
Volume: DATA File: E103184.82A Samp Ctr: 9 ID Number: 1612
Type: Samp Bottle: 8 Method: MOORE6
Created: 3/18/2010 2:49:58 PM
Sample ID: 4R 03.18.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.598E+8	0.029	----	7.022	SOLVENT PEAK	----	< min rt	
4.832	1932	0.031	1.052	12.000	12:0	6.28	ECL deviates 0.000	Reference -0.001
6.727	336	0.035	----	13.524		----		
7.387	2869	0.039	0.979	14.000	14:0	8.68	ECL deviates 0.000	Reference -0.002
8.174	602	0.040	----	14.505		----		
9.769	5704	0.045	0.949	15.491	Sum In Feature 5	16.73	ECL deviates 0.003	14:0 3OH
9.875	561	0.043	----	15.554		----		
10.320	2093	0.047	0.945	15.819	16:1 w7c	6.11	ECL deviates 0.001	
10.625	14583	0.043	0.942	16.001	16:0	42.46	ECL deviates 0.001	Reference -0.002
10.786	829	0.056	----	16.093		----		
12.178	1613	0.051	0.933	16.890	17:0 cyclopropane	4.65	ECL deviates 0.001	
13.832	3092	0.046	0.926	17.825	Sum In Feature 10	8.85	ECL deviates 0.001	18:1 w7c
14.930	1228	0.054	----	18.446		----		
15.737	2197	0.049	0.918	18.904	19 cycloprop. 11,12	6.24	ECL deviates 0.000	Reference -0.003
15.857	604	0.046	----	18.971		----		
----	5704	---	----	----	Summed Feature 5	16.73	15:0 DMA	14:0 3OH
----	3092	---	----	----	Summed Feature 10	8.85	18:1 w7c	unknown 17.834

ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 4
Total Response: 38242 Total Named: 34084
Percent Named: 89.13% Total Amount: 32364
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.482	Escherichia-coli



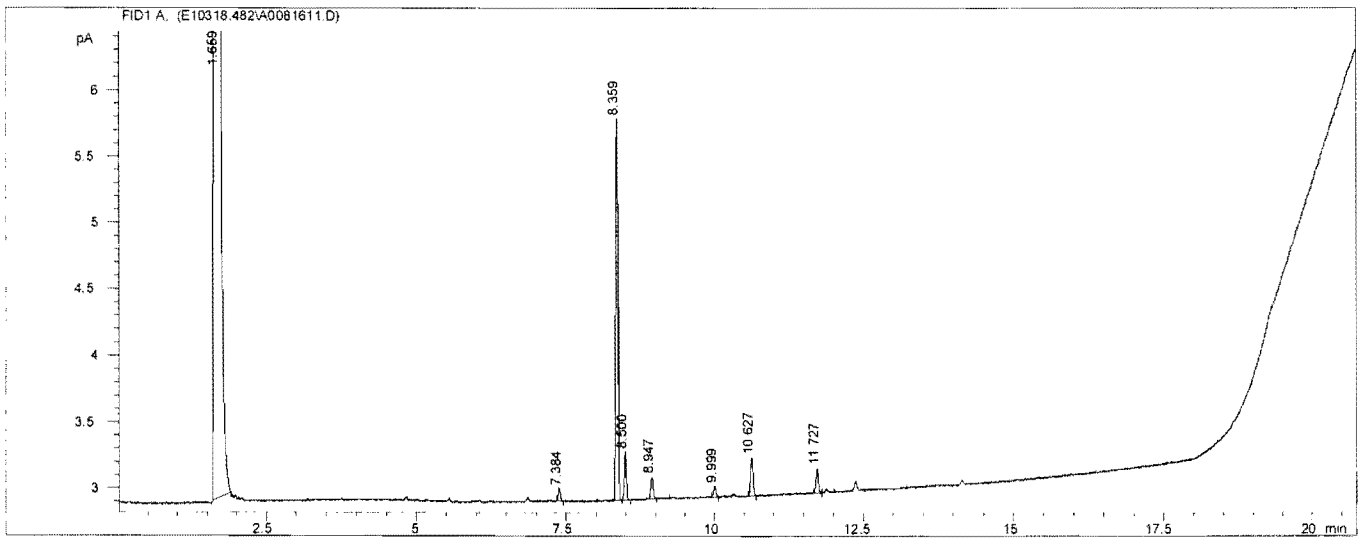
Volume: DATA File: E103184.82A Samp Ctr: 8 ID Number: 1611
Type: Samp Bottle: 7 Method: MOORE6
Created: 3/18/2010 2:25:12 PM
Sample ID: 3S 03.18.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.659	3.579E+8	0.029	----	7.030	SOLVENT PEAK	----	< min rt	
7.384	440	0.034	0.979	13.999	14:0	2.15	ECL deviates -0.001	Reference -0.004
8.359	14460	0.039	0.964	14.624	15:0 iso	69.59	ECL deviates 0.001	Reference -0.001
8.500	1867	0.038	0.963	14.714	15:0 anteiso	8.97	ECL deviates 0.000	Reference -0.001
8.947	907	0.042	0.957	15.000	15:0	4.33	ECL deviates 0.000	Reference -0.001
9.999	451	0.042	0.947	15.626	16:0 iso	2.13	ECL deviates -0.001	Reference -0.001
10.627	1666	0.046	0.942	16.000	16:0	7.83	ECL deviates 0.000	Reference 0.000
11.727	1070	0.042	0.936	16.630	17:0 iso	5.00	ECL deviates 0.000	Reference 0.000

ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 7
Total Response: 20861 Total Named: 20861
Percent Named: 100.00% Total Amount: 20039
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.520	Fusobacterium-rusii-GC subgroup C (4)
	0.422	Capnocytophaga-sputigena



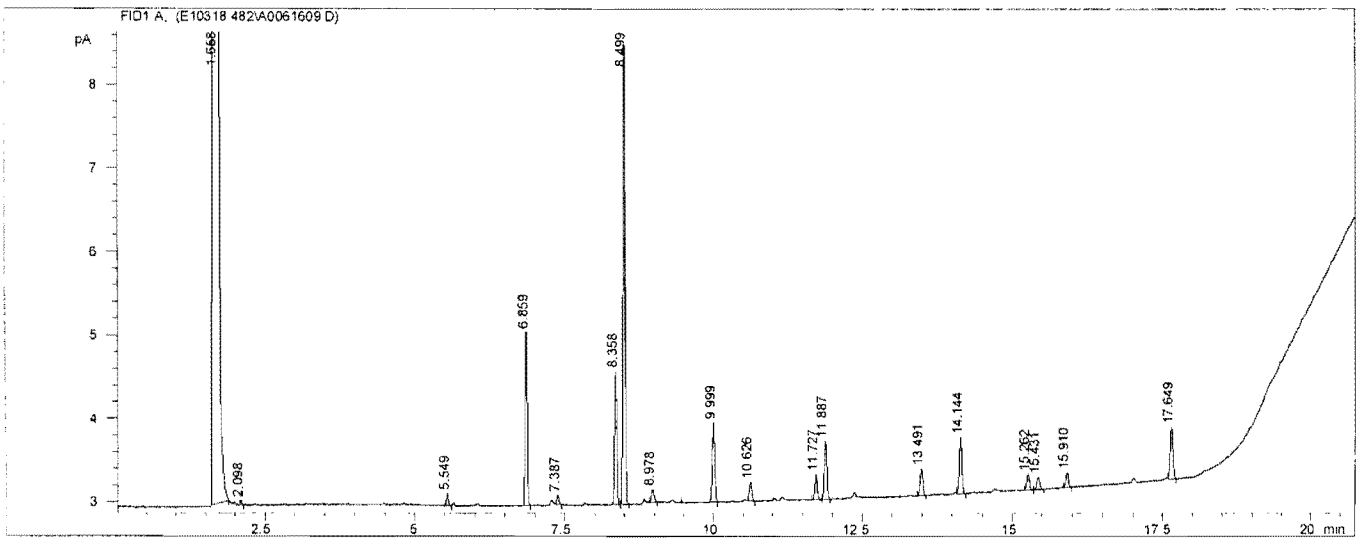
Volume: DATA File: E103184.82A Samp Ctr: 6 ID Number: 1609
Type: Samp Bottle: 5 Method: MOORE6
Created: 3/18/2010 1:35:57 PM
Sample ID: 2S 03.18.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.658	3.636E+8	0.030	----	7.018	SOLVENT PEAK	----	< min rt	
2.098	142	0.020	----	7.874		----	< min rt	
5.549	602	0.034	1.025	12.614	13:0 iso	0.88	ECL deviates 0.000	Reference -0.001
6.859	9742	0.037	0.990	13.619	14:0 iso	13.75	ECL deviates 0.001	Reference 0.000
7.387	513	0.035	0.979	14.000	14:0	0.72	ECL deviates 0.000	Reference -0.001
8.358	8201	0.041	0.964	14.623	15:0 iso	11.28	ECL deviates 0.000	Reference -0.001
8.499	28539	0.041	0.963	14.713	15:0 anteiso	39.18	ECL deviates -0.001	Reference -0.002
8.978	1021	0.050	----	15.019		----		
9.999	5281	0.043	0.947	15.627	16:0 iso	7.14	ECL deviates 0.000	Reference -0.001
10.626	1294	0.045	0.942	16.000	16:0	1.74	ECL deviates 0.000	Reference -0.001
11.727	1759	0.049	0.936	16.631	17:0 iso	2.35	ECL deviates 0.001	Reference -0.001
11.887	4038	0.045	0.935	16.723	17:0 anteiso	5.38	ECL deviates 0.000	
13.491	1876	0.045	0.928	17.632	18:0 iso	2.48	ECL deviates 0.000	Reference -0.002
14.144	4293	0.048	0.925	18.000	18:0	5.66	ECL deviates 0.000	Reference -0.002
15.262	1188	0.050	0.920	18.634	Sum In Feature 12	1.56	ECL deviates 0.000	19:0 iso
15.431	983	0.051	0.920	18.730	19:0 anteiso	1.29	ECL deviates 0.000	
15.910	1090	0.047	0.918	19.001	19:0	1.43	ECL deviates 0.001	Reference -0.002
17.649	3989	0.049	0.909	20.000	20:0	5.17	ECL deviates 0.000	Reference -0.003
----	1188	---	----	----	Summed Feature 12	1.56	unknown 18.622	19:0 iso

ECL Deviation: 0.000 Reference ECL Shift: 0.002 Number Reference Peaks: 13
Total Response: 74408 Total Named: 73387
Percent Named: 98.63% Total Amount: 70114

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.245	Prevotella-intermedia
	0.236	Staphylococcus-epidermidis
	0.217	Staphylococcus-warneri



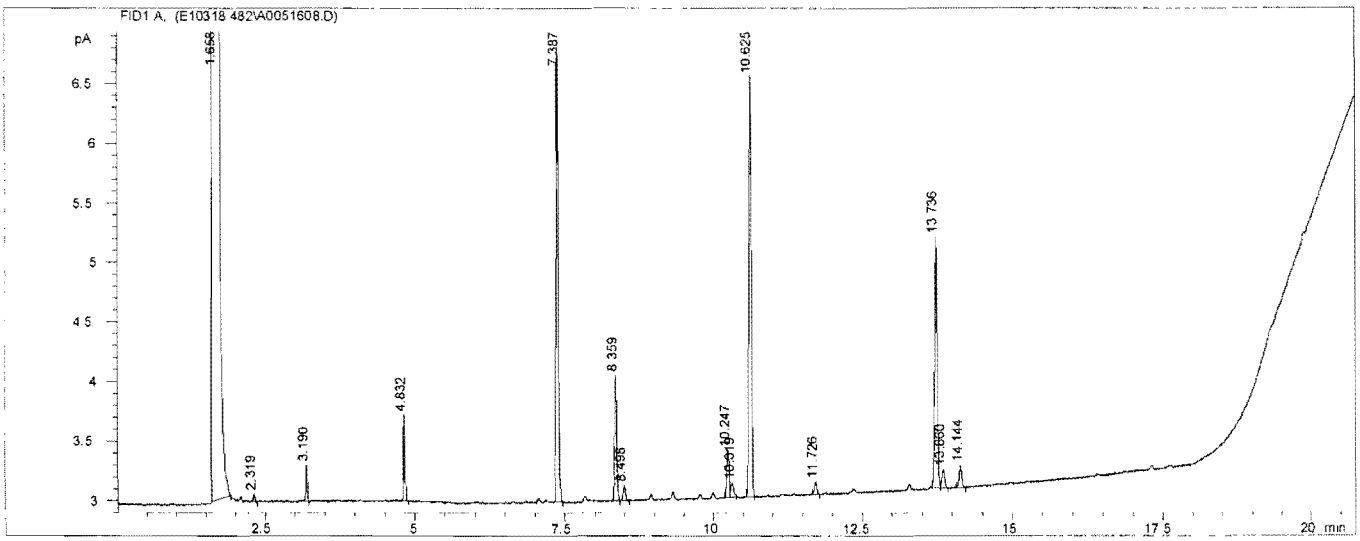
Volume: DATA File: E103184.82A Samp Ctr: 5 ID Number: 1608
Type: Samp Bottle: 4 Method: MOORE6
Created: 3/18/2010 1:11:13 PM
Sample ID: 1S 03.18.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.658	3.636E+8	0.030	----	7.019	SOLVENT PEAK	----	< min rt	
2.319	236	0.024	----	8.304		----	< min rt	
3.190	969	0.025	1.180	10.000	10:0	1.78	ECL deviates 0.000	Reference 0.000
4.832	2827	0.031	1.052	12.000	12:0	4.62	ECL deviates 0.000	Reference -0.001
7.387	18711	0.039	0.979	14.000	14:0	28.44	ECL deviates 0.000	Reference -0.002
8.359	5390	0.040	0.964	14.624	15:0 iso	8.07	ECL deviates 0.001	Reference -0.001
8.498	720	0.040	0.963	14.713	15:0 anteiso	1.08	ECL deviates -0.001	Reference -0.002
10.247	2243	0.044	0.945	15.775	16:1 w9c	3.29	ECL deviates 0.001	
10.319	735	0.044	0.945	15.818	16:1 w7c	1.08	ECL deviates 0.000	
10.625	19784	0.045	0.942	16.000	16:0	28.95	ECL deviates 0.000	Reference -0.001
11.726	627	0.046	0.936	16.631	17:0 iso	0.91	ECL deviates 0.001	Reference -0.001
13.736	12972	0.049	0.927	17.770	18:1 w9c	18.66	ECL deviates -0.001	
13.860	1002	0.047	0.926	17.840	Sum In Feature 10	1.44	ECL deviates 0.006	unknown 17.834
14.144	1165	0.047	0.925	18.000	18:0	1.67	ECL deviates 0.000	Reference -0.002
----	1002	---	----	----	Summed Feature 10	1.44	18:1 w7c	unknown 17.834

ECL Deviation: 0.002 Reference ECL Shift: 0.001 Number Reference Peaks: 8
Total Response: 67147 Total Named: 67147
Percent Named: 100.00% Total Amount: 64397

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.297	Bifidobacterium-breve-GC subgroup B
	0.256	Clostridium-cadaveris



Volume: DATA File: E103184.82A Samp Ctr: 1 ID Number: 1
Type: Calib Bottle: 1 Method: MOORE6
Created: 3/18/2010 11:34:43 AM
Sample ID: Lot R-506201 03.18.10 AB

Profile Comment: Good peak matching. Peak position matching error (RMS) is 0.0019.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.998	MIDI Calibration Mix 1

Volume: DATA File: E103184.82A Samp Ctr: 2 ID Number: 1
Type: Calib Bottle: 1 Method: MOORE6
Created: 3/18/2010 11:57:09 AM
Sample ID: Lot R-506201 03.18.10 AB

Profile Comment: Good peak matching. Peak position matching error (RMS) is 0.0016.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.998	MIDI Calibration Mix 1

Volume: DATA File: E103184.82A Samp Ctr: 3 ID Number: 1606
Type: Blank Bottle: 2 Method: MOORE6
Created: 3/18/2010 12:21:51 PM
Sample ID: BLANK 03.18.10 AB

Profile Comment:
*** Library match not attempted

Volume: DATA File: E103184.82A Samp Ctr: 4 ID Number: 1607
Type: Samp Bottle: 3 Method: MOORE6
Created: 3/18/2010 12:46:30 PM
Sample ID: 1R 03.18.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.243	Clostridium-oceanicum
	0.203	Clostridium-botulinum-type B, proteolytic 2

Volume: DATA File: E103184.82A Samp Ctr: 5 ID Number: 1608
Type: Samp Bottle: 4 Method: MOORE6
Created: 3/18/2010 1:11:13 PM
Sample ID: 1S 03.18.10 AB

Matches:

see if she can match back to previous runs

Library	Sim Index	Entry Name
MOORE6 6.00	0.297	Bifidobacterium-breve-GC subgroup B
	0.256	Clostridium-cadaveris

Volume: DATA File: E103184.82A Samp Ctr: 6 ID Number: 1609
Type: Samp Bottle: 5 Method: MOORE6
Created: 3/18/2010 1:35:57 PM
Sample ID: 2S 03.18.10 AB

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.245	Prevotella-intermedia
	0.236	Staphylococcus-epidermidis
	0.217	Staphylococcus-warneri

Volume: DATA File: E103184.82A Samp Ctr: 7 ID Number: 1610
Type: Samp Bottle: 6 Method: MOORE6
Created: 3/18/2010 2:00:34 PM
Sample ID: 3R 03.18.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
*** No Matches found in MOORE6

Volume: DATA File: E103184.82A Samp Ctr: 8 ID Number: 1611
Type: Samp Bottle: 7 Method: MOORE6
Created: 3/18/2010 2:25:12 PM
Sample ID: 3S 03.18.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.520	Fusobacterium-rusii-GC subgroup C (4)
	0.422	Capnocytophaga-sputigena

Volume: DATA File: E103184.82A Samp Ctr: 9 ID Number: 1612
Type: Samp Bottle: 8 Method: MOORE6
Created: 3/18/2010 2:49:58 PM
Sample ID: 4R 03.18.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.482	Escherichia-coli

Volume: DATA File: E103184.82A Samp Ctr: 10 ID Number: 1613
Type: Samp Bottle: 9 Method: MOORE6
Created: 3/18/2010 3:14:32 PM
Sample ID: 4S 03.18.10 AB

Profile Comment: Percent named is less than 85.00.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.282	Escherichia-coli

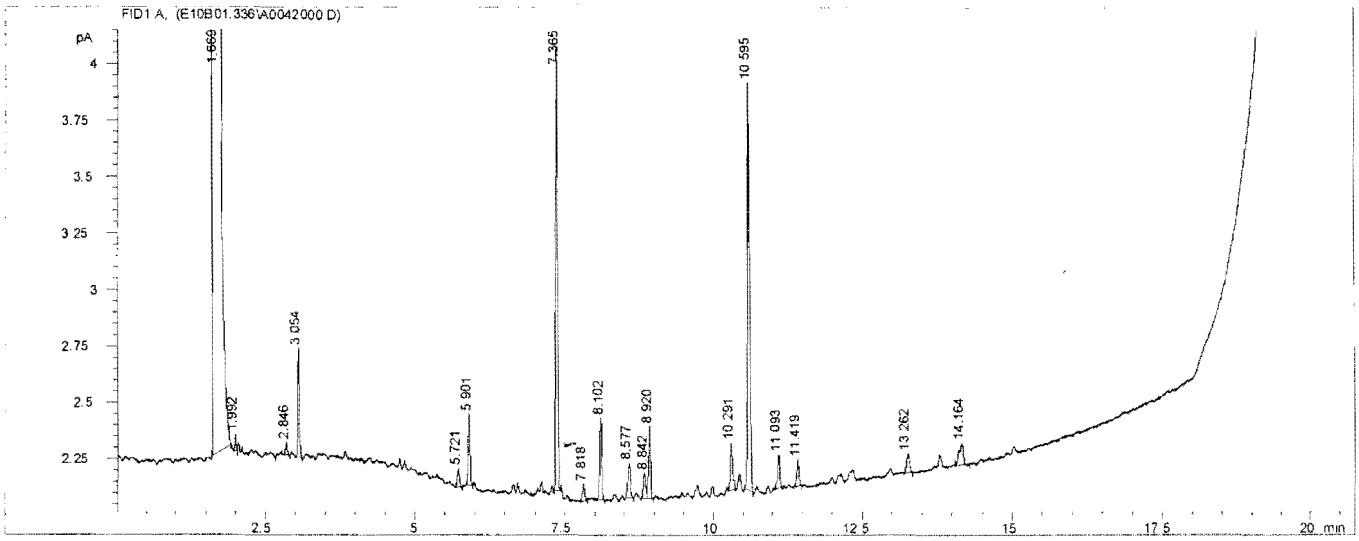
Volume: DATA File: E10B013.36A Samp Ctr: 4 ID Number: 2000
Type: Samp Bottle: 3 Method: MOORE6
Created: 11/1/2010 9:15:58 AM
Sample ID: 1R 11.01.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.669	3.401E+8	0.028	----	7.023	SOLVENT PEAK	----	< min rt	
1.992	212	0.022	----	7.657		----	< min rt	
2.846	178	0.026	----	9.330		----		
3.054	1663	0.027	1.260	9.738	unk 9.740	7.08	ECL deviates -0.002	
5.721	367	0.035	----	12.774		----		
5.901	1332	0.034	1.012	12.929	Sum In Feature 1	4.55	ECL deviates -0.001	13:1 at 12-13
7.365	9221	0.037	0.973	13.999	14:0	30.29	ECL deviates -0.001	Reference -0.003
7.818	367	0.040	0.965	14.291	14:1 w7c DMA	1.19	ECL deviates 0.000	
8.102	1714	0.037	0.960	14.474	14:0 DMA	5.56	ECL deviates 0.002	Reference -0.001
8.577	885	0.042	0.954	14.780	Sum In Feature 4	2.85	ECL deviates 0.000	15:2
8.842	555	0.037	0.950	14.950	16:0 aldehyde	1.78	ECL deviates -0 001	Reference -0.003
8.920	1596	0.040	0.949	15.001	15:0	5.11	ECL deviates 0.001	Reference -0.001
10.291	1271	0.047	0.937	15.817	16:1 w7c	4.02	ECL deviates -0.001	
10.595	10010	0.043	0.934	15.999	16:0	31.59	ECL deviates -0.001	Reference -0.004
11.093	748	0.040	0.931	16.285	16:1 w7c DMA	2.35	ECL deviates 0.000	
11.419	651	0.044	0.929	16.472	16:0 DMA	2.04	ECL deviates 0.001	Reference -0.002
13.262	507	0.046	0.920	17.520	16:0 3OH	1.57	ECL deviates -0.002	
14.164	901	0.066	----	18.029		----		
----	1332	---	----	----	Summed Feature 1	4.55	13:1 at 12-13	14:0 aldehyde
----	-----	---	----	----		----	11:1 2OH	
----	885	---	----	----	Summed Feature 4	2.85	UN 14.762 15:2 ? FA	15:2
----	-----	---	----	----		----	15:1 w8c	

ECL Deviation: 0.001 Reference ECL Shift: 0.002 Number Reference Peaks: 6
Total Response: 31964 Total Named: 30519
Percent Named: 95.48% Total Amount: 29610
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.158	Clostridium-botulinum-type B, proteolytic 2
	0.147	Leptotrichia-D37
	0.135	Clostridium-sporogenes


FID1 A, (E10B01.336\A0042000.D)
pA
4
3.75
3.5
3.25
3
2.75
2.5
2.25
1.669
1.992
2.846
3.054
5.721
5.901
7.365
7.818
8.102
8.577
8.842
8.920
10.291
10.595
11.093
11.419
13.262
14.164
2.5
5
7.5
10
12.5
15
17.5
20 min

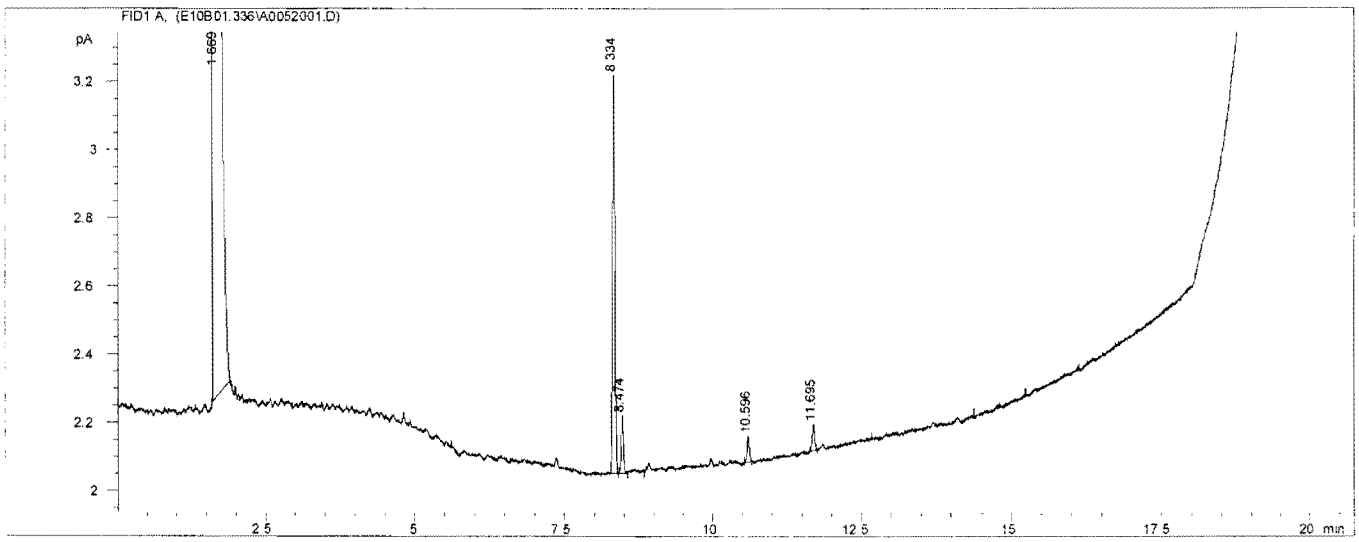
Volume: DATA File: E10B013.36A Samp Ctr: 5 ID Number: 2001
Type: Samp Bottle: 4 Method: MOORE6
Created: 11/1/2010 9:40:45 AM
Sample ID: 1S 11.01.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.669	3.348E+8	0.028	----	7.025	SOLVENT PEAK	----	< min rt	
8.334	6031	0.040	0.957	14.623	15:0 iso	77.77	ECL deviates 0.000	Reference -0.002
8.474	919	0.044	0.955	14.714	15:0 anteiso	11.83	ECL deviates 0.000	Reference -0.003
10.596	427	0.041	0.934	15.999	16:0	5.37	ECL deviates -0.001	Reference -0.003
11.695	402	0.042	0.928	16.630	17:0 iso	5.03	ECL deviates 0.000	Reference -0.002

ECL Deviation: 0.000 Reference ECL Shift: 0.002 Number Reference Peaks: 4
Total Response: 7779 Total Named: 7779
Percent Named: 100.00% Total Amount: 7420
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.440	Fusobacterium-rusii-GC subgroup C (4)
	0.373	Capnocytophaga-sputigena

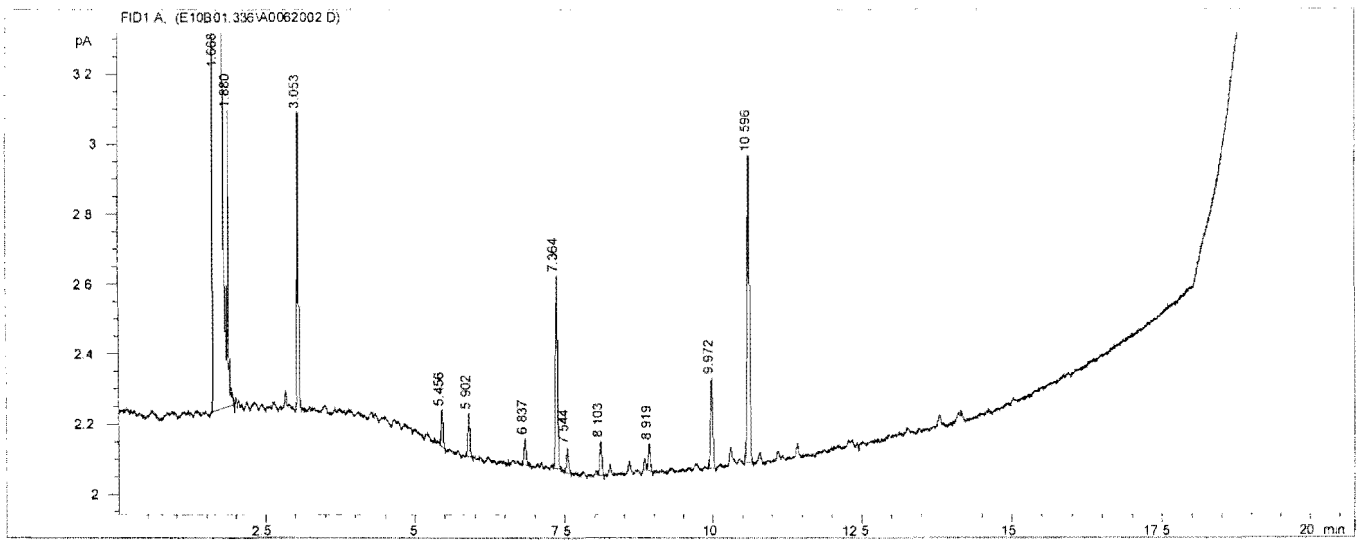


Volume: DATA File: E10B013.36A Samp Ctr: 6 ID Number: 2002
Type: Samp Bottle: 5 Method: MOORE6
Created: 11/1/2010 10:05:17 AM
Sample ID: 2R 11.01.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.668	3.382E+8	0.028	----	7.024	SOLVENT PEAK	----	< min rt	
1.880	1858	0.021	----	7.440		----	< min rt	
3.053	2860	0.027	1.260	9.737	unk 9.740	25.68	ECL deviates -0.003	
5.456	464	0.033	----	12.547		----		
5.902	555	0.036	1.012	12.930	Sum In Feature 1	4.00	ECL deviates 0.000	13:1 at 12-13
6.837	353	0.037	0.985	13.618	14:0 iso	2.48	ECL deviates 0.000	Reference -0.003
7.364	2667	0.038	0.973	13.999	14:0	18.48	ECL deviates -0.001	Reference -0.004
7.544	355	0.038	0.969	14.114	13:0 iso 3OH	2.45	ECL deviates 0.000	
8.103	450	0.034	0.960	14.474	14:0 DMA	3.08	ECL deviates 0.002	Reference 0.000
8.919	368	0.037	0.949	14.999	15:0	2.49	ECL deviates -0.001	Reference -0.002
9.972	1361	0.040	0.939	15.627	16:0 iso	9.10	ECL deviates 0.000	Reference -0.002
10.596	4845	0.043	0.934	16.000	16:0	32.25	ECL deviates 0.000	Reference -0.003
----	555	---	----	----	Summed Feature 1	4.00	13:1 at 12-13	14:0 aldehyde
----	-----	---	----	----		----	11:1 2OH	

ECL Deviation: 0.001 Reference ECL Shift: 0.003 Number Reference Peaks: 6
Total Response: 14278 Total Named: 13815
Percent Named: 96.75% Total Amount: 14040
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

*** No Matches found in MOORE6



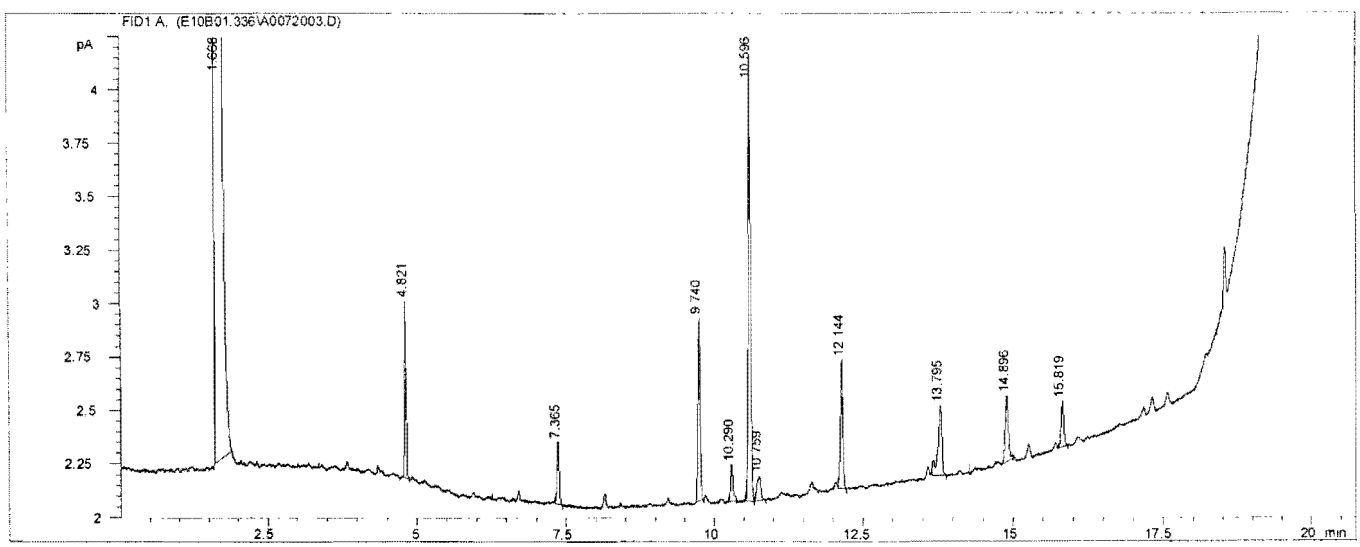
Volume: DATA File: E10B013.36A Samp Ctr: 7 ID Number: 2003
Type: Samp Bottle: 6 Method: MOORE6
Created: 11/1/2010 10:29:53 AM
Sample ID: 2S 11.01.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.668	3.356E+8	0.028	----	7.024	SOLVENT PEAK	----	< min rt	
4.821	3279	0.031	1.061	12.000	12:0	12.79	ECL deviates 0.000	Reference -0.001
7.365	1391	0.039	0.973	13.999	14:0	4.97	ECL deviates -0.001	Reference -0.003
9.740	4728	0.043	0.941	15.490	Sum In Feature 5	16.36	ECL deviates 0.002	14:0 3OH
10.290	1011	0.046	0.937	15.818	16:1 w7c	3.48	ECL deviates 0.000	
10.596	11717	0.044	0.934	16.000	16:0	40.25	ECL deviates 0.000	Reference -0.003
10.759	969	0.058	----	16.094		----		
12.144	3546	0.045	0.925	16.890	17:0 cyclopropane	12.06	ECL deviates 0.001	
13.795	2995	0.064	0.917	17.821	Sum In Feature 10	10.09	ECL deviates -0.003	18:1 w7c
14.896	2345	0.054	----	18.444		----		
15.819	1359	0.050	----	18.969		----		
----	4728	---	----	----	Summed Feature 5	16.36	15:0 DMA	14:0 3OH
----	2995	---	----	----	Summed Feature 10	10.09	18:1 w7c	unknown 17.834

ECL Deviation: 0.002 Reference ECL Shift: 0.003 Number Reference Peaks: 3
Total Response: 33339 Total Named: 28666
Percent Named: 85.98% Total Amount: 27205
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.150	Escherichia-coli

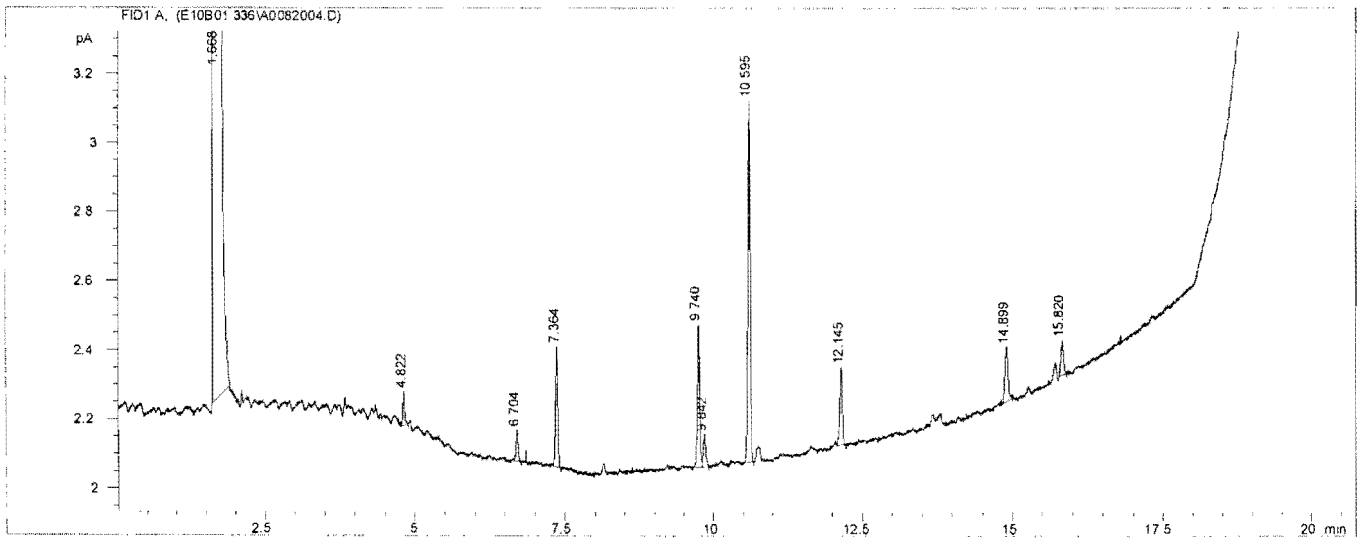


Volume: DATA File: E10B013.36A Samp Ctr: 8 ID Number: 2004
Type: Samp Bottle: 7 Method: MOORE6
Created: 11/1/2010 10:54:38 AM
Sample ID: 3S 11.01.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.668	3.383E+8	0.028	----	7.025	SOLVENT PEAK	----	< min rt	
4.822	429	0.036	1.061	12.001	12:0	4.12	ECL deviates 0.001	Reference -0.001
6.704	411	0.036	----	13.521		----		
7.364	1701	0.038	0.973	13.999	14:0	14.97	ECL deviates -0.001	Reference -0.004
9.740	2331	0.045	0.941	15.491	Sum In Feature 5	19.85	ECL deviates 0.003	14:0 3OH
9.842	593	0.044	----	15.552		----		
10.595	5887	0.044	0.934	16.001	16:0	49.77	ECL deviates 0.001	Reference -0.004
12.145	1348	0.045	0.925	16.891	17:0 cyclopropane	11.29	ECL deviates 0.002	
14.899	1194	0.053	----	18.447		----		
15.820	596	0.042	----	18.969		----		
----	2331	---	----	----	Summed Feature 5	19.85	15:0 DMA	14:0 3OH

ECL Deviation: 0.002 Reference ECL Shift: 0.003 Number Reference Peaks: 3
Total Response: 14489 Total Named: 11696
Percent Named: 80.72% Total Amount: 11052
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

*** No Matches found in MOORE6



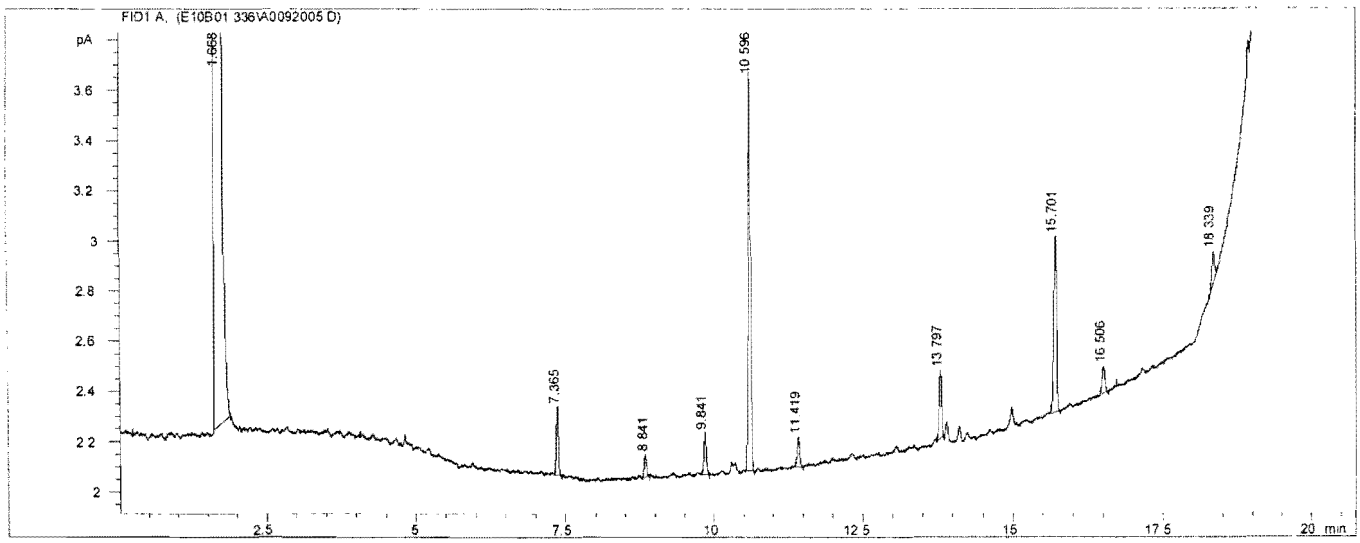
Volume: DATA File: E10B013.36A Samp Ctr: 9 ID Number: 2005
Type: Samp Bottle: 8 Method: MOORE6
Created: 11/1/2010 11:19:20 AM
Sample ID: 4S 11.01.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.668	3.407E+8	0.028	----	7.022	SOLVENT PEAK	----	< min rt	
7.365	1333	0.039	0.973	14.000	14:0	7.91	ECL deviates 0.000	Reference -0.003
8.841	466	0.042	0.950	14.950	16:0 aldehyde	2.70	ECL deviates -0.001	Reference -0.003
9.841	980	0.046	----	15.550		----		
10.596	8701	0.043	0.934	15.999	16:0	49.64	ECL deviates -0.001	Reference -0.003
11.419	701	0.044	0.929	16.472	16:0 DMA	3.98	ECL deviates 0.001	Reference -0.001
13.797	1432	0.042	0.917	17.822	Sum In Feature 10	8.02	ECL deviates -0.002	18:1 w7c
15.701	4362	0.048	0.904	18.903	19 cycloprop. 11,12	24.09	ECL deviates -0.001	Reference -0.005
16.506	667	0.046	0.897	19.365	19:0 cyclo 11,12 DMA	3.65	ECL deviates 0.001	Reference -0.004
18.339	877	0.050	----	20.418		----	> max rt	
----	1432	---	----	----	Summed Feature 10	8.02	18:1 w7c	unknown 17.834

ECL Deviation: 0.001 Reference ECL Shift: 0.003 Number Reference Peaks: 6
Total Response: 18642 Total Named: 17661
Percent Named: 94.74% Total Amount: 16378
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.332	Campylobacter-coli



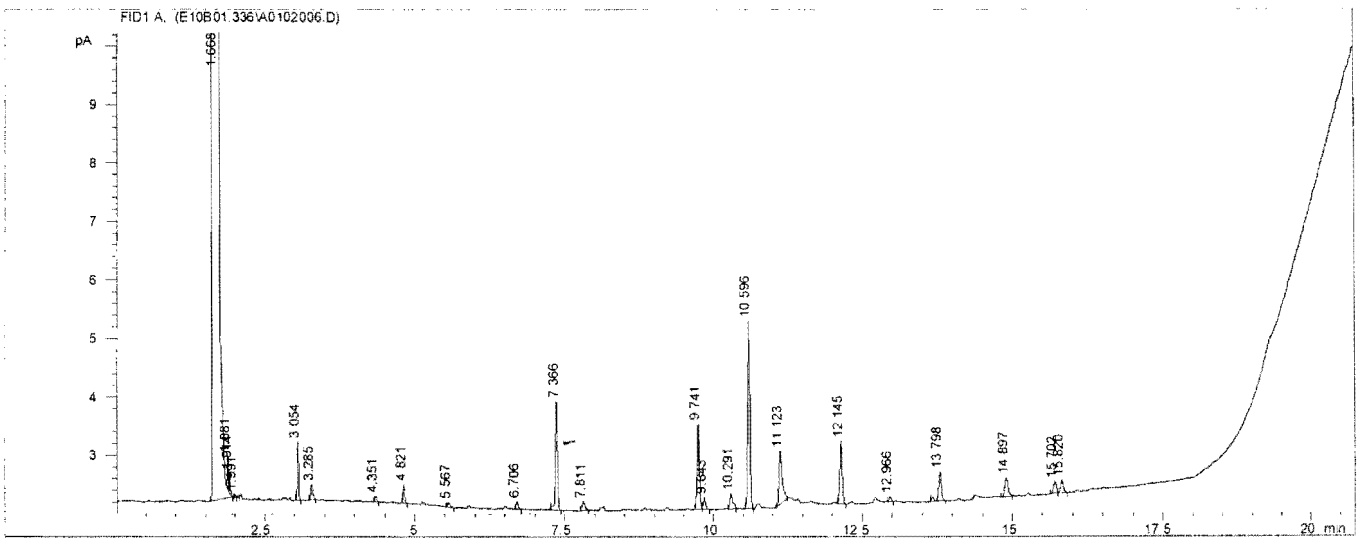
Volume: DATA File: E10B013.36A Samp Ctr: 10 ID Number: 2006
Type: Samp Bottle: 9 Method: MOORE6
Created: 11/1/2010 11:43:51 AM
Sample ID: 5S 11.01.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.668	3.33E+8	0.029	----	7.025	SOLVENT PEAK	----	< min rt	
1.881	1460	0.020	----	7.441		----	< min rt	
1.914	926	0.021	----	7.506		----	< min rt	
1.991	233	0.030	----	7.657		----	< min rt	
3.054	3343	0.027	1.260	9.738	unk 9.740	8.06	ECL deviates -0.002	
3.285	1226	0.035	----	10.139		----		
4.351	401	0.031	----	11.494		----		
4.821	1322	0.033	1.061	12.000	12:0	2.68	ECL deviates 0.000	Reference -0.001
5.567	499	0.045	----	12.642		----		
6.706	641	0.036	----	13.523		----		
7.366	9467	0.040	0.973	14.000	14:0	17.62	ECL deviates 0.000	Reference -0.003
7.811	963	0.048	0.965	14.287	14:1 w7c DMA	1.78	ECL deviates -0.004	
9.741	8153	0.044	0.941	15.490	Sum In Feature 5	14.68	ECL deviates 0.002	14:0 3OH
9.843	1157	0.043	----	15.552		----		
10.291	1899	0.053	0.937	15.818	16:1 w7c	3.40	ECL deviates 0.000	
10.596	17771	0.043	0.934	16.000	16:0	31.77	ECL deviates 0.000	Reference -0.003
11.123	7083	0.058	----	16.303		----		
12.145	6259	0.046	0.925	16.890	17:0 cyclopropane	11.08	ECL deviates 0.001	
12.966	427	0.035	0.921	17.355	17:0 cyclo DMA	0.75	ECL deviates 0.001	
13.798	3389	0.052	0.917	17.824	Sum In Feature 10	5.95	ECL deviates 0.000	18:1 w7c
14.897	2606	0.061	----	18.446		----		
15.702	1284	0.049	0.904	18.904	19 cycloprop. 11,12	2.22	ECL deviates 0.000	Reference -0.004
15.820	1410	0.050	----	18.971		----		
----	8153	---	----	----	Summed Feature 5	14.68	15:0 DMA	14:0 3OH
----	3389	---	----	----	Summed Feature 10	5.95	18:1 w7c	unknown 17.834

ECL Deviation: 0.002 Reference ECL Shift: 0.003 Number Reference Peaks: 4
Total Response: 69300 Total Named: 54278
Percent Named: 78.32% Total Amount: 52268
Profile Comment: Percent named is less than 85.00.

Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.129	Escherichia-coli

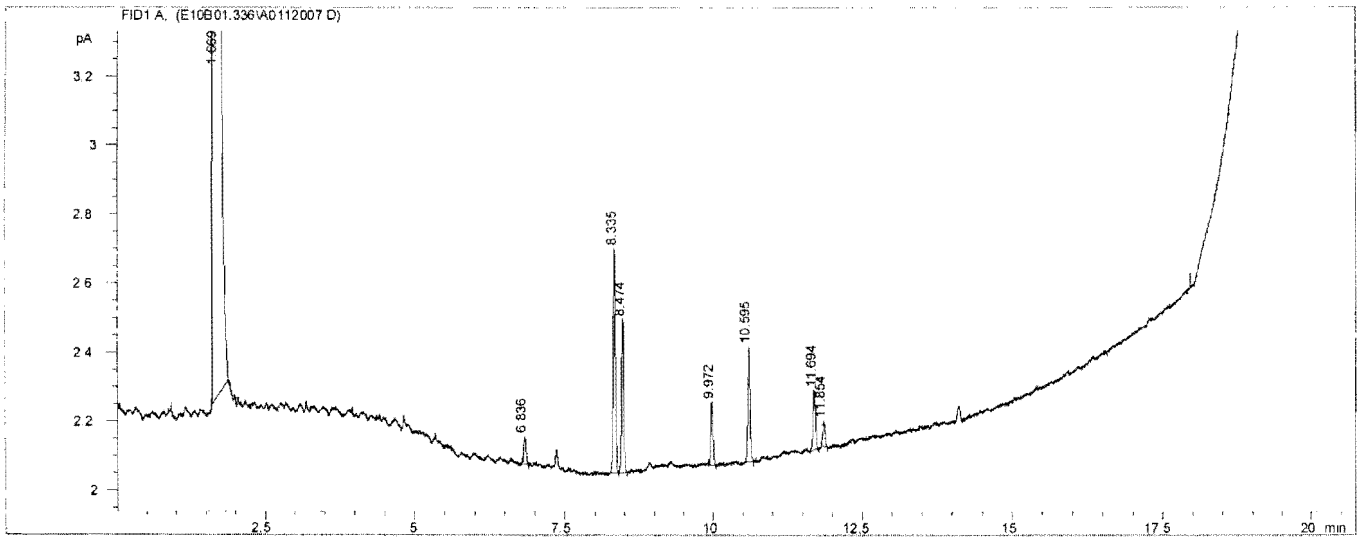

FID1 A, (E10B01336\A0102006.D)
pA
1.668
3.054
3.285
4.351
4.821
5.567
6.706
7.366
7.811
9.741
10.291
10.596
11.123
12.145
12.966
13.798
14.897
15.702
15.826
2.5
5
7.5
10
12.5
15
17.5
20 min

Volume: DATA File: E10B013.36A Samp Ctr: 11 ID Number: 2007
Type: Samp Bottle: 10 Method: MOORE6
Created: 11/1/2010 12:08:33 PM
Sample ID: 6S 11.01.10 AB

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent	Comment1	Comment2
1.669	3.378E+8	0.028	----	7.026	SOLVENT PEAK	----	< min rt	
6.836	397	0.038	0.985	13.618	14:0 iso	4.05	ECL deviates 0.000	Reference -0.003
8.335	3323	0.041	0.957	14.624	15:0 iso	32.90	ECL deviates 0.001	Reference -0.001
8.474	2283	0.040	0.955	14.714	15:0 anteiso	22.56	ECL deviates 0.000	Reference -0.002
9.972	962	0.042	0.939	15.627	16:0 iso	9.35	ECL deviates 0.000	Reference -0.002
10.595	1876	0.045	0.934	15.999	16:0	18.13	ECL deviates -0.001	Reference -0.003
11.694	959	0.045	0.928	16.630	17:0 iso	9.20	ECL deviates 0.000	Reference -0.003
11.854	397	0.042	0.927	16.722	17:0 anteiso	3.81	ECL deviates -0.001	

ECL Deviation: 0.001 Reference ECL Shift: 0.003 Number Reference Peaks: 6
Total Response: 10197 Total Named: 10197
Percent Named: 100.00% Total Amount: 9664
Profile Comment: Total response less than 50000.0. Concentrate and re-run.

*** No Matches found in MOORE6



Volume: DATA File: E10B013.36A Samp Ctr: 1 ID Number: 1
Type: Calib Bottle: 1 Method: MOORE6
Created: 11/1/2010 8:04:08 AM
Sample ID: Calibration Mix 11.01.10 AB

Profile Comment: Good peak matching. Peak position matching error (RMS) is 0.0018.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.997	MIDI Calibration Mix 1

Volume: DATA File: E10B013.36A Samp Ctr: 2 ID Number: 1
Type: Calib Bottle: 1 Method: MOORE6
Created: 11/1/2010 8:26:45 AM
Sample ID: Calibration Mix 11.01.10 AB

Profile Comment: Good peak matching. Peak position matching error (RMS) is 0.0018.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.997	MIDI Calibration Mix 1

Volume: DATA File: E10B013.36A Samp Ctr: 3 ID Number: 1999
Type: Blank Bottle: 2 Method: MOORE6
Created: 11/1/2010 8:51:22 AM
Sample ID: BLANK 11.01.10 AB

Profile Comment:
*** Library match not attempted

Volume: DATA File: E10B013.36A Samp Ctr: 4 ID Number: 2000
Type: Samp Bottle: 3 Method: MOORE6
Created: 11/1/2010 9:15:58 AM
Sample ID: 1R 11.01.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.158	Clostridium-botulinum-type B, proteolytic 2
	0.147	Leptotrichia-D37
	0.135	Clostridium-sporogenes

Volume: DATA File: E10B013.36A Samp Ctr: 5 ID Number: 2001
Type: Samp Bottle: 4 Method: MOORE6
Created: 11/1/2010 9:40:45 AM
Sample ID: 1S 11.01.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.440	Fusobacterium-rusii-GC subgroup C (4)
	0.373	Capnocytophaga-sputigena

Volume: DATA File: E10B013.36A Samp Ctr: 6 ID Number: 2002
Type: Samp Bottle: 5 Method: MOORE6
Created: 11/1/2010 10:05:17 AM
Sample ID: 2R 11.01.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
*** No Matches found in MOORE6

Volume: DATA File: E10B013.36A Samp Ctr: 7 ID Number: 2003
Type: Samp Bottle: 6 Method: MOORE6
Created: 11/1/2010 10:29:53 AM
Sample ID: 2S 11.01.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.150	Escherichia-coli

Volume: DATA File: E10B013.36A Samp Ctr: 8 ID Number: 2004
Type: Samp Bottle: 7 Method: MOORE6
Created: 11/1/2010 10:54:38 AM
Sample ID: 3S 11.01.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
*** No Matches found in MOORE6

Volume: DATA File: E10B013.36A Samp Ctr: 9 ID Number: 2005
Type: Samp Bottle: 8 Method: MOORE6
Created: 11/1/2010 11:19:20 AM
Sample ID: 4S 11.01.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.332	Campylobacter-coli

Volume: DATA File: E10B013.36A Samp Ctr: 10 ID Number: 2006
Type: Samp Bottle: 9 Method: MOORE6
Created: 11/1/2010 11:43:51 AM

Sample ID: 5S 11.01.10 AB

Profile Comment: Percent named is less than 85.00.
Matches:

Library	Sim Index	Entry Name
MOORE6 6.00	0.129	Escherichia-coli

Volume: DATA File: E10B013.36A Samp Ctr: 11 ID Number: 2007
Type: Samp Bottle: 10 Method: MOORE6
Created: 11/1/2010 12:08:33 PM
Sample ID: 6S 11.01.10 AB

Profile Comment: Total response less than 50000.0. Concentrate and re-run.
*** No Matches found in MOORE6

Ratio of Sample Radius to True Radius

November 10, 2010

The random variable Y is the radius of the circle that is some cross section of a sphere of radius r. Note that $Y \leq r$. The probability of $Y = y_i$ is equal to the probability of $Y = y_j$, that is

$$P(Y = y_i) = P(Y = y_j)$$

for $i \neq j$ where $y_i, y_j \in [0, r]$.

The arithmetic mean of Y, call it $\bar{y}$, is approximated by

$$\bar{y} \approx \frac{1}{n}\sum_{i=0}^{n} y_i.$$

From the exact value

$$\bar{y} = \lim_{n\to\infty} \frac{1}{n}\sum_{i=0}^{n} y_i,$$

it follows that

$$\begin{aligned}\bar{y} &= \frac{r}{r}\lim_{n\to\infty} \frac{1}{n}\sum_{i=0}^{n} y_i \\ &= \frac{1}{r}\lim_{n\to\infty} \frac{r}{n}\sum_{i=0}^{n} y_i \\ &= \frac{1}{r}\lim_{n\to\infty} \sum_{i=0}^{n} y_i \cdot \Delta y,\end{aligned}$$

where $\Delta y = \frac{r}{n}$. Furthermore,

$$\bar{y} = \frac{1}{r}\left(\frac{1}{4}A\right),$$

where the area of this circle $A = \pi r^2$, the largest cross sectional area. In particular,

$$\bar{y} = \frac{\pi r}{4}.$$

Now, the ratio of $\bar{y}$ to r,

$$p = \frac{\bar{y}}{r} = \frac{\pi}{4}.$$

This may be approximated by

$$p \approx 0.78539816339744830961566084581988$$