ANALYSES OF KOMBUCHA FERMENTS

By Michael R. Roussin

Based on:

Report on Growers: Analyses of Their Kombucha Ferments

By Michael Roussin

Internet Home Page http://www.kombucha-research.com

E-mail readers@kombucha-research.com

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This book is Dedicated to the Memory of Colleen Allen

MEDICAL DISCLAIMER

THIS INFORMATION IS PRESENTED BASED ON VARIOUS EXPERIMENTS AND ANALYSES OF KOMBUCHA. IF YOU HAVE ANY QUESTIONS ABOUT ANY OF THE COMPOUNDS IDENTIFIED IN KOMBUCHA, YOU SHOULD ASK YOUR HEALTH PROFESSIONAL. THE AUTHOR IS NOT A MEDICAL PROFESSIONAL. THE AUTHOR DOES NOT REPRESENT THAT KOMBUCHA CAN OR WILL PROVIDE ANY HEALTH BENEFITS, OR THAT ANY SAMPLE TAKEN FROM THE PUBLIC AT LARGE WILL PROVIDE AN EQUIVALENT ANALYSIS.

130 Applewood Drive
Fruita, Colorado 81521

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PREFACE:

When I started drinking Kombucha in late 1993, I never expected to form a research coalition to investigate this fine ferment. As I was reading the books by Frank, Fasching, and Tietz, I also never envisioned that I too would write a book about Kombucha. But, conflicting reports of the ferments' contents, along with a warning from the FDA, prompted me to take a closer look at what I was drinking. I believe our investigation of Kombucha was the most thorough, and most expensive, analyses of Kombucha ferments ever completed.

We were able to disprove several myths about Kombucha and also uncover several secrets about the Kombucha ferment. We were able to observe how the colony (the mushroom) is imprinted with information about what to create in each successive ferment. We also learned how to modify the behavior of the ferment.

During the 1995 to 1996 research period, several people were very encouraging about our research. I would like to mention the commercial growers who offered their ferments and other samples, including Ariana Estelle (Harmonic Harvest), Bev Ferguson (Kombucha Manna), Diane Minden, and Ed Laughlin (Natural Kombucha Farm). The at-home growers, Christine Muehling, Jack Barclay, Jim Bailey also contributed greatly to this research with additional colonies and ferments.

Finally, I must give special recognition to my dear friend Colleen Allen. Colleen's never faltering encouragement helped to keep me focused on the research, even when the results were not what I wanted. Colleen was the glue that held the Kombucha community together during the 1990's. Her passion for life and Kombucha was unparalleled. The next time you visit the Kombucha FAQ, know that it was through Colleen's seemingly endless effort that the information was compiled in one place. It was with her desire to share information about Kombucha that I began to change the Report on Growers into a book that is suitable for reading by those who are curious about Kombucha.

INTRODUCTION:

A great deal of positive testimonial and anecdotal tribute has been expressed regarding the regular consumption of Kombucha (Japanese, "kombu;" "cha,"), and its effects on physiological well-being. However, data relating to the components in Kombucha, the organisms producing those constituents, and reported health benefits derived from its consumption are primarily based on research performed more than 50 years ago. We are aware of the research conducted over the past 90 years and are not going to discount the research from 70 years ago, or even 40 years ago. We are also not going to reiterate it here. We believe that the research of the past two decades provides the most current and most reliable look at the chemistry and microbiology of this ferment As exemplified by Kombucha, the mix of metabolic products from any living culture containing a variety of yeasts and bacteria nourished by a varied mixture of nutrients, is quite complex. Previous research is the basis upon which we build our own understanding, and is by no means dismissed by the results of Information Resources' testing. However, our research is sufficiently strong to dispel some previously-held beliefs behind the reasons for the reported health benefits of Kombucha. To date, our research indicates important omissions in previously-reported research, and contradicts the presence of a particularly important purported component: glucuronic acid.

In an effort to differentiate between the culture (the fermented tea) and the colony ("zooglea" or "mushroom" or "polysaccharide/mucopolysaccharide colony", or "gelatinous cellulose mass"), **for the purposes of this book, we will identify the "ferment" as the culture and the zooglea ("mushroom"),("polysaccharide colony") as the colony.**

The analyses of the different growers were conducted under a variety of standard laboratory conditions. We analyzed samples from the tea and sugar solution, the innoculum (their starter tea), and from various stages of the fermentation process. This enabled us to determine if the constituents we observed were from the tea and sugar, or the fermentation processes.

Having seen so many samples from such a broad spectrum of viable Kombucha colonies, we hesitate to draw a broad-brush stereotype of Kombucha. Each ferment is unique. The expectations of effects of consumption of this ferment must also be ferment-dependent. Because each ferment is unique, we do not want to give the impression that your ferment is going to provide the constituents we have isolated in the ferments we have studied.

We have noted the effects of various types of stress on the colony. The bacteria and yeasts in a ferment appear to "remember" what environmental stresses have been imposed on them. If you grow a "great" ferment, the offspring colony will remember how to grow that ferment. If you grow vinegar, the resulting colony will remember that, too.

Because this is a dynamic ferment, it will produce what you tell it to produce. Based on the stresses that you present, it will adapt as best it can. What are the end results of the adaptation? It depends on the stress you employ. Acetic acid to gluconic acid ratios of 4:1 can be achieved. Gluconic acid to acetic acid ratios of 4:1 can also be achieved. Your ferment's environment and nutrients are key factors.

WHO WAS THE KOMBUCHA CONSUMER RESEARCH GROUP?

The Kombucha Consumer Research Group was formed as a research coalition in 1995. It consisted of three Utah businesses which shared time and financial resources to conduct and compile contemporary research on the topic of Kombucha. This coalition consisted of: Information Resources, LC; San Rafael Chemical Services, Inc.; and Earth Net Consulting, LC. The results of their study is available on the internet at *http://www.kombucha-research.com*.

Information Resources, LC, ("IRLC") was formed specifically to conduct Kombucha research and disseminate that information. San Rafael Chemical Services, Inc., ("SRCS") is a commercial chemistry laboratory licensed by the State of Utah. It routinely performs complex organic chemical analyses for a variety of food, vitamin, and pharmaceutical companies. The laboratory is equipped for a variety of chemical analyses using gas chromatography, high performance liquid chromatography (HPLC) and mass spectrometry (MS). Earth Net Consulting, LC, ("ENC") was a commercial microbiology laboratory licensed by the State of Utah for environmental microbiology.

Prior to commencing testing on these colonies and ferments, a series of tests were conducted to assure a high level of confidence in reported findings. These tests included:

Gas chromatography/positive electron ionization mass spectrometry.

< Reverse phase High Performance Liquid Chromatography ("HPLC") using detection by:
  - P Photodiode array ("PDAD") scanning UV
  - P Mass spectrometry interfaced to the Liquid Chromatograph using particle beam and also buffer assisted, filament mediated, positive thermospray chemical ionization

< HPLC size exclusion using PDAD

< HPLC cation exchange using PDAD

More than 300 HPLC/MS/PDAD analyses were conducted on Kombucha before the first grower's colony was analyzed.

Before we report our test findings, it seems appropriate to share a few observations regarding chromatography. Chromatography is a means of separating as completely as possible the components in a mixture so that they can be more easily identified and

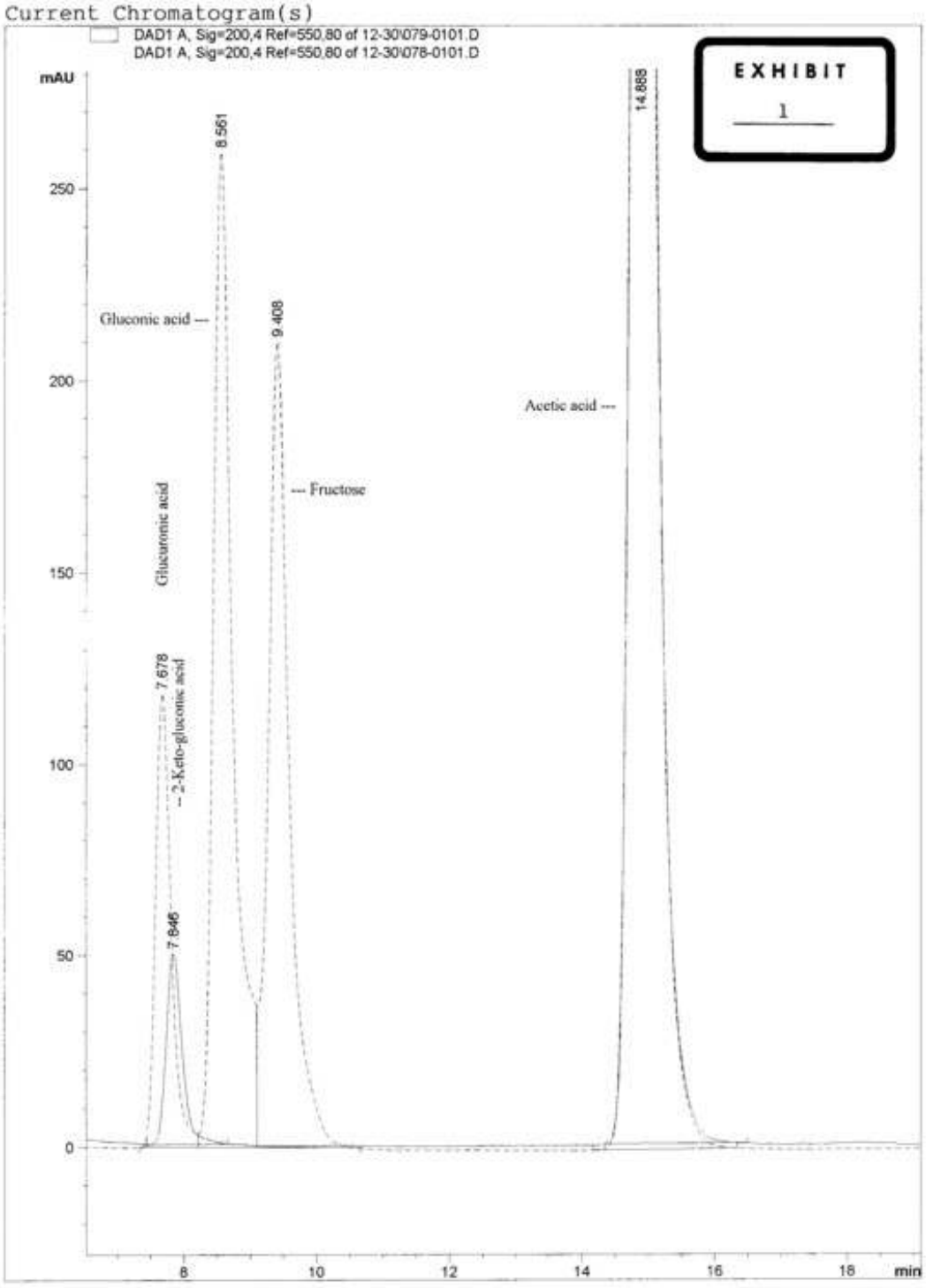
quantified. Results are based on two criteria. The first criterion is the closeness of a comparison between the time it takes unknown components to move through the separation media (for instance, through the cationic exchange chromatography column) in comparison to the time required for known reference materials ("standards") to flow through the same media. The second criterion is the similarity in the detector response between standards and suspected components or unknowns that are being examined. The more efficient the separation method and the more discriminating the detector, the higher the degree of confidence one has that accurate results are reported.

A variety of testing methods best define the chemical constituents of a complex solution like Kombucha. Mass spectrometry provides ion characteristics of chemicals, while size exclusion provides information about the elements' weight and size.

The utilization of different chromatography columns (stationary phase) and different organic solvents, modifiers, and buffers (mobile phase), and different detection methods (mass spectrometry, photodiode array-UV absorbency) provides a wealth of information about the chemical structure of the Kombucha ferment. For instance, the reverse phase column (C-18), using H_2O/acetonitrile for the mobile phase, provides the best separation and detection of the B vitamins in Kombucha. Cationic exchange gives the best separation and detection of the various sugar acids. A methylene chloride extraction and concentration to 100:1 gives a good separation and detection by gas chromatography of many of the acid esters, intermediates and other metabolites of the fermentation process.

To understand how reference materials are used, the first two chromatograms presented are the identified reference materials and a Kombucha ferment. The first are the reference materials obtained from chemical manufacturers (EXHIBIT 1), and the second is a Kombucha ferment (EXHIBIT 2). This is how a chemist determines which constituents are present in an organic mixture like Kombucha.

Current Chromatogram(s)



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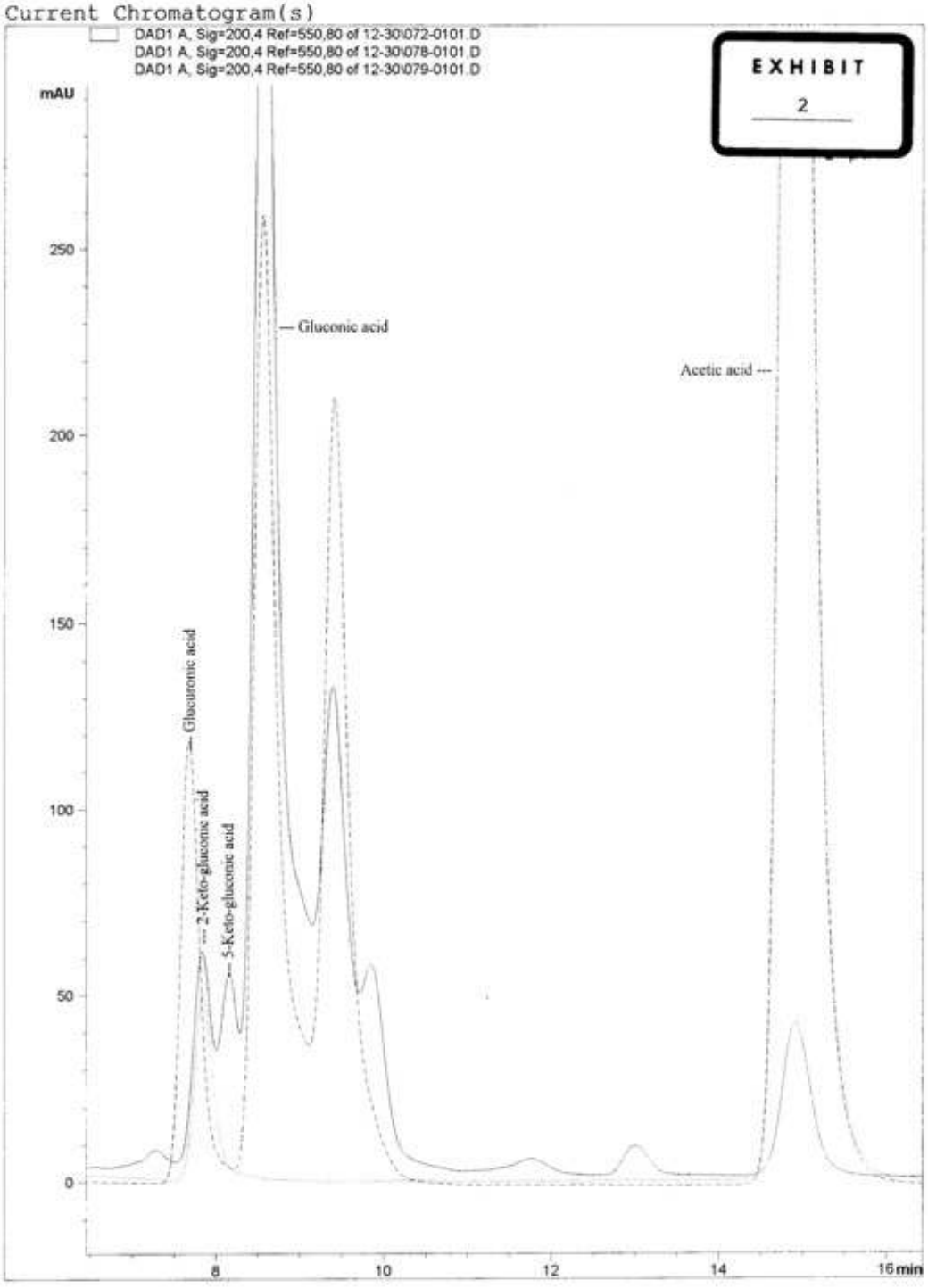

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BACKGROUND OF THE CHEMISTRY LAB:

With more than twenty years' experience in analytical chemistry and laboratory management/operation, Ralph Meibos the Director of the chemistry lab, is well-grounded in the precepts of scientific methodology. His laboratory, San Rafael Chemical Services, Inc., is certified in its procedures by the State of Utah, and was contracted by Information Resources, LC, to conduct the chemistry portion of this research effort.

The laboratory is equipped primarily for the separation and detection of organic constituents in complex mixtures. Because the lab routinely examines products for food supplement companies, vitamin companies, and pharmaceutical companies, it has the background and the instrument capability to address very efficiently the questions posed by this research.

EQUIPMENT:

Gas Chromatograph: Hewlett Packard Model 5890 II.

Column 1: 0.2 µm HP-5 30 x 0.21 mm fused silica capillary.

Column 2: 0.33 µm HP-1 12.5 x 0.21 mm fused silica capillary.

Detector: Hewlett Packard Model 5898A Mass Spectrometer positive electron ionization, scanning 45 to 650 amu.

Liquid Chromatograph: Hewlett-Packard Model 1090, Series II/L.

Column 1: Hypersil 10 x 0.21 cm 3 µm ODS, (reverse phase).

Column 2: Spherisorb 25 x 0.45 cm 5 µm ODS2, (reverse phase).

Column 3: Phenomenex-Biosep SEC-S2000 30 x 0.78 cm (size exclusion).

Column 4: Sarasep Car-H 30 x 0.78 cm (ion exchange).

Detector 1: Photodiode array, scanning from 190 to 600 nm; primary quantification at 200 and 210 nm.

Detector 2: Hewlett Packard Model 5898A Mass Spectrometer interfaced with:

A. Buffer-assisted filament mediated positive thermospray chemical ionization, scanning 102-700 amu

B. HP 59980B particle beam interface, electron impact, positive ionization .

ANALYSIS:

SCIENTIFIC STANDARDS:

The following scientific reference materials were used for standards in these analyses:

Acetoacetic acid, lot 44H5038, part A-8509, Sigma;
alpha-Ketoglutaric acid, lot 85H0461, part K-1750, Sigma;
L-Ascorbic acid (sodium salt), lot 07228EF, part 26,855-0, Aldrich;
d-Biotin, lot 42H0711, part B-4501, Sigma;
Cellulase, lot 74H0590, part C-8546, Sigma;
Chondroitinase ABC, lot 43H4054, part C-2905, Sigma;
Chondroitin sulfate A, lot 84H0127, part C-8529, Sigma;
cis-Aconitic acid, lot 85H5033, part A-3412, Sigma;
Citric acid, lot 83H1125, part C-0759, Sigma;
Cyanocobalamin (B-12), lot 14H0078, part V2876, Sigma;
Dihydroxyacetone, lot 105H0480, part D-7753, Sigma;
Folic acid dihydrate, lot 07903HF, part 23,587-3, Aldrich;
Formic acid lot 07113CN, part 25,136-4, Aldrich;
(-) Fructose, lot 83H0857, part F-0127, Sigma;
Fumaric acid, lot 104H3470, part F-2752, Sigma;
d-Galacturonic acid monohydrate, lot 74H07481, part G-2125, Sigma ;
Glacial acetic acid, lot 932937, part A490212, Fisher Scientific;
(+) Glucose, lot 34H0451, part G-8270, Sigma;
d-Gluconic acid lactone, lot 74H0888, part G-4750, Sigma;
d-Gluconic acid (sodium salt), lot 02824MX, part 18663-3, Aldrich;
d-Glucuronic acid lactone (Glucono-lactone), lot 104H0554, part G-8875, Sigma;
d-Glucuronic acid (Sodium Salt Monohydrate), lot 07726TY, part 21,949-5, Aldrich;
d-Glucuronic acid (Sodium Salt Monohydrate), lot 77209/1, part 20457-0250, ACROS Chemical;
(+) Glucosamine, lot 54H0700, part G4875, Sigma;
Glutaric acid, lot 15H3620, part G-4126, Sigma;
(-) Glyceric acid, lot 37F5021, part G-1144, Sigma;
Glycerol, lot 105H00251, part G-6279, Sigma; Heparin (Bovine) [Heparin Sodium Salt], lot 104H0754, part H-9399, Sigma;
Heparinase I, lot 75H3804, part H-2519, Sigma;
Hyaluronic acid, lot 14H04821, part H-0902, Sigma;
Hyaluronidase, lot 44H7065, part H-3506, Sigma;
d,l-Isocitric acid, lot 104H38101, part I-1252, Sigma;
Itaconic acid, lot 91H0771, part I-5877, Sigma;
2-Keto-d-gluconic acid (hemi calcium salt), lot 66172, part 100365, ICN Chemical Co.;

5-Keto-d-gluconic acid, lot 12H4011, K-4125, Sigma;
(+)l-Lactic Acid, lot 44H0047, part L-1750, Sigma;
Maleic acid, lot 124H5706, part M-0375, Sigma;
d,l-Malic acid, lot 35H1208, part M-0875, Sigma;
Malonic acid, lot 45H3407, part M-1750, Sigma;
l-Mannonic acid (gamma) lactone, lot 23H0596, part M-2261, Sigma;
Methylglyoxal, lot 75H2646, part M-0252, Sigma;
Mucin, Type II Crude, lot 14H0072, part M-2378, Sigma;
Mucic acid, lot 15H2626, part M-4778, Sigma;
Niacinamide, lot 122H0171, part N-3376, Sigma;
Nicotinic acid lot, 54H0979, part N-4126, Sigma;
Oxalic acid, lot 54H0280, part O-0376, Sigma;
Oxalacetic acid, lot 114H01162, part O-4126, Sigma;
d-Pantothenic acid, lot 32H0328, part P-2250, Sigma;
Paramino benzoic acid (PABA), lot 54C00801, part A-9878, Sigma;
6-Phosphogluconic acid, lot 104H3830, part P7752, Sigma
Propionic acid, lot 65H2504, part P-1386, Sigma;
Pyridoxal hydrochloride, lot 92H0528, part P-9130, Sigma;
Pyridoxamine, lot 81H0485, part P-9380, Sigma;
Pyruvic acid, lot 35H2514, part P-1656, Sigma;
1R, 3R, 4R, 5R, (-) Quinic acid, lot 01223HG, part 13,862-2, Aldrich;
Riboflavin, lot 43H1155, part R-4500, Sigma;
d-Saccharic acid (Glucaric Acid), lot 103H0693, part S-0125, Sigma;
d-Saccharic acid 1,4-lactone, lot 128F3887, Part S-0375, Sigma;
Sedoheptulose anhydride, lot 40H3792, part S-3375, Sigma;
Shikimic acid, lot 01029K2W, part S320-8, Aldrich;
Sucrose, lot 4480651, part S-9378, Sigma;
Succinic acid, lot 65H0006, part S-7501, Sigma;
Thiamin, lot 72H0102, part T-4625, Sigma;
Uridine 5'-diphosphoGlucuronic acid, lot 60H7225, part U-6751, Sigma;
(+) Usnic acid, lot 30H0120, part U-7876, Sigma;
d-Xylonic acid, calcium salt dihydrate, lot 01413EZ, part 39,373-8, Aldrich;

PHASE I:

The initial phase of this study occurred before we even knew that there was going to be a formalized study. Our original goal was to determine the concentrations of glucuronic, gluconic, and acetic acids in a two-week-old Kombucha ferment. A sample was taken to the laboratory in April, 1995, for quantification of gluconic, glucuronic, and acetic acids. The lab was also provided with highlighted excerpts in Guenther Frank's *Kombucha - Healthy Beverage and Natural Remedy from the Far East*; Rosina Fasching's *Tea Fungus Kombucha - The Natural Remedy and its Significance in Cases of Cancer and other Metabolic Diseases*; Alana Pascal's *Kombucha, What it is and What it's all About*; and Harald Tietze's *Kombucha The Miracle Fungus*; which explained the presence of these components in Kombucha.

The laboratory was NOT asked to prove or disprove the presence of these compounds; only to quantify them. This is an important distinction, since many molecules may have the same molecular weight (i.e., the monosaccharides glucose and fructose), but totally different structures. Methods for measuring what is known, as opposed to identifying what is unknown, are quite different.

Initial conditions were established for the separation of these target components, using reverse phase, high-performance liquid chromatography (HPLC). To optimize resolution and detection ability, chromatography columns with amine-bonded (NH3) groups and octadecyl (C18) bonded groups were employed, with various mixtures of aqueous buffers, modifiers, and the organic solvents acetonitrile and methanol. For the results that were reported to Information Resources, LC, the amine-bonded column was selected, as it had the capability to separate various acids from sugar components and the other constituents of the tea.

With these liquid chromatographic (LC) conditions, the Kombucha sample was analyzed using both Photodiode array (ultraviolet absorbency) and particle beam/electron impact positive ionization/mass spectrometry for compound detection and identification. This test clearly indicated the concentrations of the sugars, the caffeine, the polyphenolic components (tannins), and the acetic acid. In the region of the chromatographic arrays where gluconic and glucuronic acids were expected to be observed, a group of six or so incompletely resolved components were seen (UV at 208 nanometers).

Resolution between the closely-related compounds gluconic and glucuronic acids, however, was only partially achieved at this stage. Two of the incompletely resolved peaks eluting from the column were components closely matching the retention times of standard reference materials for gluconic and glucuronic acids. Their concentrations, however, were too low for detection by mass spectrometry. At this point in our research, as we expected to find both gluconic and glucuronic acids, and as the tests appeared to substantiate these findings, they were reported internally to Michael Roussin at IRLC to be present as such.

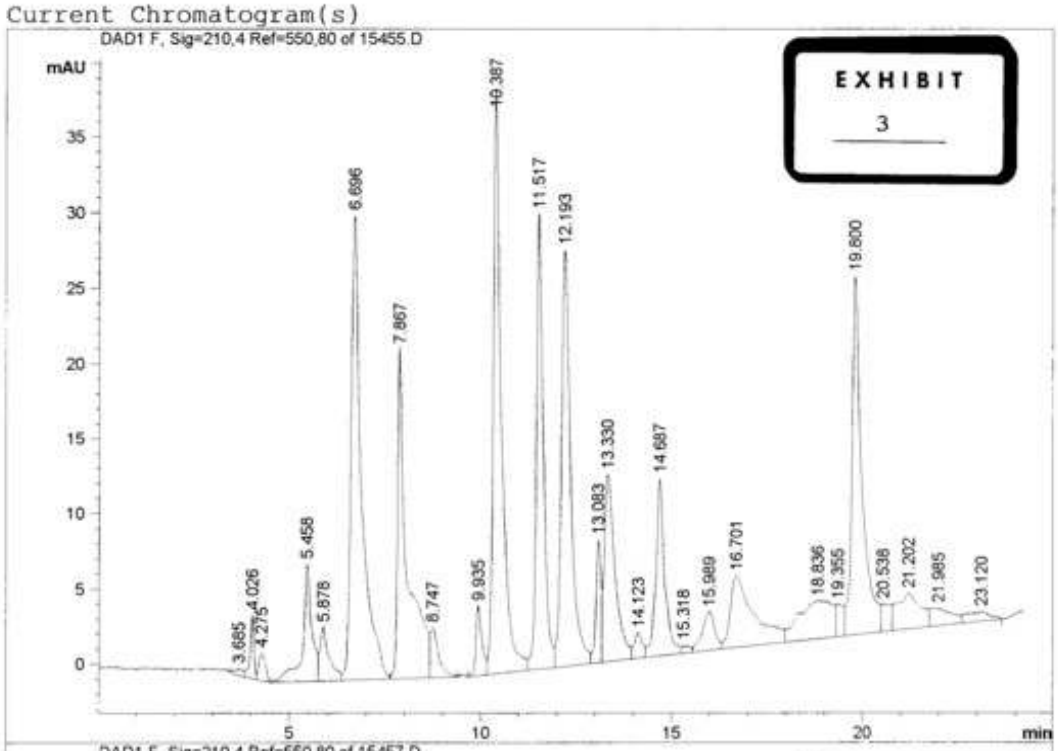
PHASE II:

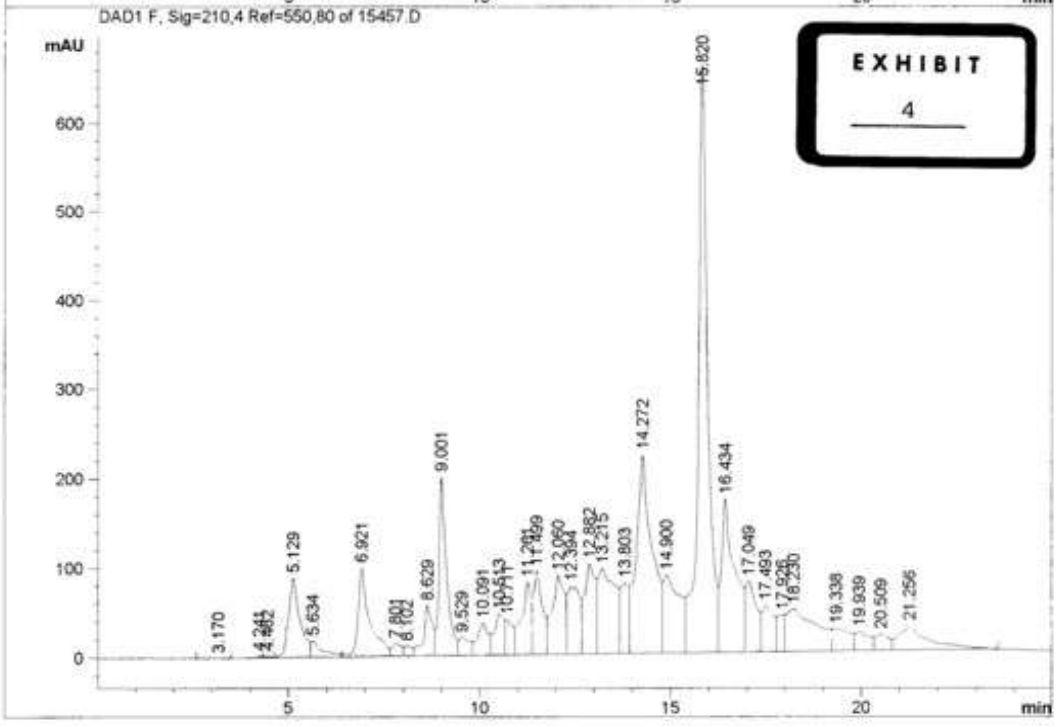
The next phase began two months later as an effort to establish analytical conditions for monitoring other nutritional components, in addition to gluconic and glucuronic acids. Two separate fermentation batches were begun at the laboratory. Each used 3.5 quarts of distilled water and one cup of sugar. They differed in that one used 5 green tea bags and the other used 5 black tea bags. At specific times after formulation, the nutrient content of the fermentations was determined. During this stage of testing, much more effort was made to optimize conditions for detection of the desired components.

It was not our intent during this phase to optimize conditions for each individual nutrient or vitamin for the lowest possible detectable level of monitoring. Rather, we wanted to screen for any particular vitamin which was present at a concentration that could be a basis for the reported physiological benefits of drinking Kombucha. For this particular set of analyses, the C18 reverse-phase chromatography column was chosen, using a dilute ammonium acetate/acetonitrile mobile phase. Constituents detectable under these conditions were: L-ascorbic acid, nicotinic acid, niacinamide, thiamin, pyridoxal, pyridoxine, folic acid, cyanocobalamin, riboflavin, paraminobenzoic acid, pantothenic acid, usnic acid, and biotin.

Our testing revealed that none of the constituents for which we were looking were found in appreciable concentrations in the Kombucha. We must note that the possible presence of pantothenic acid and biotin are inconclusive at this stage because of possible interference from other constituents in the formulations. We can report with a high degree of confidence that there were no detectable levels of usnic acid, a suspected component of considerable interest believed to be in Kombucha. Also, under these test conditions, the vitamin components which we were looking for were detectable in quantities ranging from 0.2 micrograms per milliliter to 5 micrograms per milliliter. We did see evidence for some of the above-listed vitamins in trace amounts. We believe that the concentration of these constituents is most probably dependent upon the biological activity of the ferment, the concentration of the yeast and bacterial cultures, and the extent of autolysis of the organisms by which these components would have been released into solution. EXHIBIT 3 shows the reference standards for the targeted vitamins and EXHIBIT 4 shows the results of an analysis of Kombucha under the same reverse phase conditions. As you can see from the contrasting chromatograms, only very small amounts of the targeted vitamins are present in Kombucha. The significant retention times are: Vitamin C = 5.458; Acetic acid = 6.696; Niacin = 7.867; Pantothenic acid = 9.935; Niacinamide = 10.387; Pyridoxal = 11.517; Pyradoxine = 12.193; Folic acid = 13.083; Pyradoxamine = 13.330; Biotin and B-12 = 14.687; Thiamin = 16.701; and Riboflavin =19.800.

Current Chromatogram(s)





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As traditionally accepted nutritional components, such as vitamins, did not appear to be sufficiently present to account for the reports of health benefits attributed to Kombucha, we decided to identify some of the other unknown constituents which did appear in the analyses. The caffeine component of most traditional Kombucha formulations was readily confirmed by mass spectrometry. However, the saccharides, sugar acids and possibly other related compounds were insufficiently resolved to produce isolated spectra for exact identification. There were sufficiently high ion responses generated by the most concentrated components, fructose and gluconic acid, to substantiate by mass spectrometry their presence in the Kombucha solution.

Several other components were detected by mass spectrometry, and elucidation of their spectra for identification will be discussed later. An additional aspect of this phase of research was the attempt to detect and identify any possible mucopolysaccharide which might be present in the Kombucha solution. Sets of analytical reference materials with which to test our samples were obtained. Analysis of Kombucha under these conditions indicated a total absence of polysaccharides or mucopolysaccharides in solution. As there was concern that the low pH of these samples may have skewed the test results, neutralized solutions were also analyzed. The same absence of high molecular weight, (greater that 2000 amu molecular weight) components, which would indicate the presence of polysaccharides or mucopolysaccharides was noted with the neutralized sample as well. It was, however, curious that after two days the neutralized Kombucha became somewhat gelatinous. Re-examination by size exclusion chromatography revealed indications of components indicative of polymerization. This may indicate the presence of the building blocks responsible for the formation of the mucopolysaccharides of the polysaccharide colony from the Kombucha solution. (Authors Note: This is an important finding, as many Kombucha drinkers have expressed increased joint flexibility and decreased joint pain. We can theorize from this “gelatinous” conversion of the Kombucha chemical constituents, that the building blocks are present in Kombucha for the body to be able to repair or replace joint cartilage and fluids.)

At that point in the research, we knew what wasn't in solution, and decided to concentrate on determining how the detected constituents were affected by different formulations, and how they might be involved in deriving health benefits from drinking Kombucha.

We established conditions to optimize isolation of the sugars, sugar acids, and the other metabolic by-products in order to get a more complete picture of the minor constituents as well as the major products of Kombucha fermentation. We needed to define protocols which would give the best over-all indication of the quality of Kombucha, in anticipation of the next phase of research designed to test multiple Kombucha samples from around the U.S. and the world. We could also then assess the effects of using different types of teas, sweeteners, and fermentation aids in formulation,

as well as evaluating various physical parameters during the formulation process. In retrospect, while the C18 bonded chromatography column and an acidic mobile phase provided the fullest picture of Kombucha's components, the resolution of acid components using that method was insufficient for positive identification. When using the amine column, the sugar and acid components were sufficiently separated from the non-acidic components, but uncertainty remained regarding closely-related sugar acid components, the identity of the other non-acidic components, and whether these constituents were involved directly in the fermentation process.

Using size exclusion chromatography, it was possible to rule out the presence of large polymeric, proteins or mucopolysaccharides in the Kombucha solution, but resolution by this means is insufficient to distinguish minor sugar acid components. With no indication that other nutritive components might be responsible for the claimed health benefits of Kombucha, we decided to focus on optimizing conditions for the isolation and detection of gluconic and glucuronic acids.

A chromatography column designed especially for this particular type of component was obtained. The column was a Sarasep CAR-H 300 x 7.8 mm, cation exchange column. The following instrumental conditions were established and used in the routine comparative testing that followed. Detection was by Photodiode array, scanning ultraviolet wave lengths of 200 to 600 nanometers. We incorporated fixed wave length monitoring at 200, 210, 220, 240, 260, and 280 nanometers. This provided a clear, overall indication of what acidic components were present, and at what concentrations. As stated previously, these conditions resolved the gluconic acid, and what appeared to be glucuronic acid, as well as many of the other constituents observed in the fermented Kombucha.

Using these conditions of ion-chromatography, at least 25 different components were seen in the majority of ripe Kombucha cultures. In an effort to identify constituents which had not been previously identified, a number of additional analytical reference standards were accumulated.

EXHIBIT 5 shows a chromatogram of black tea and sugar prior to inoculation with Kombucha. EXHIBIT 6 shows the Kombucha ferment that was grown with that tea and sugar solution. As you can see, what was simple becomes very complex.

Current Chromatogram(s)

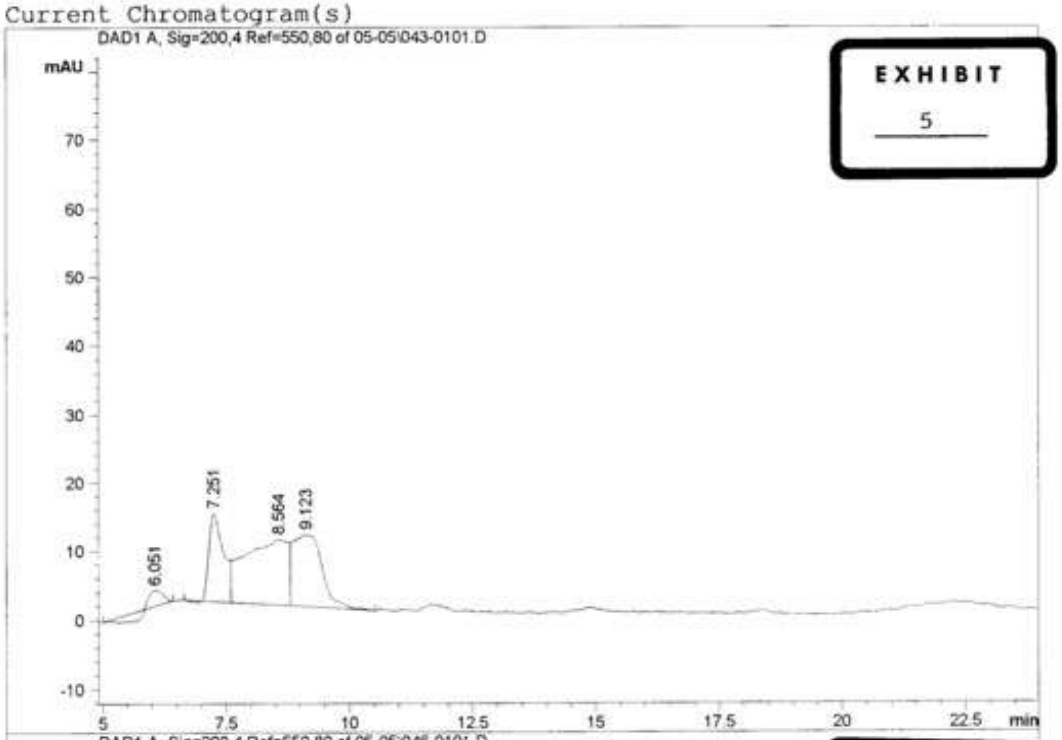

EXHIBIT 5

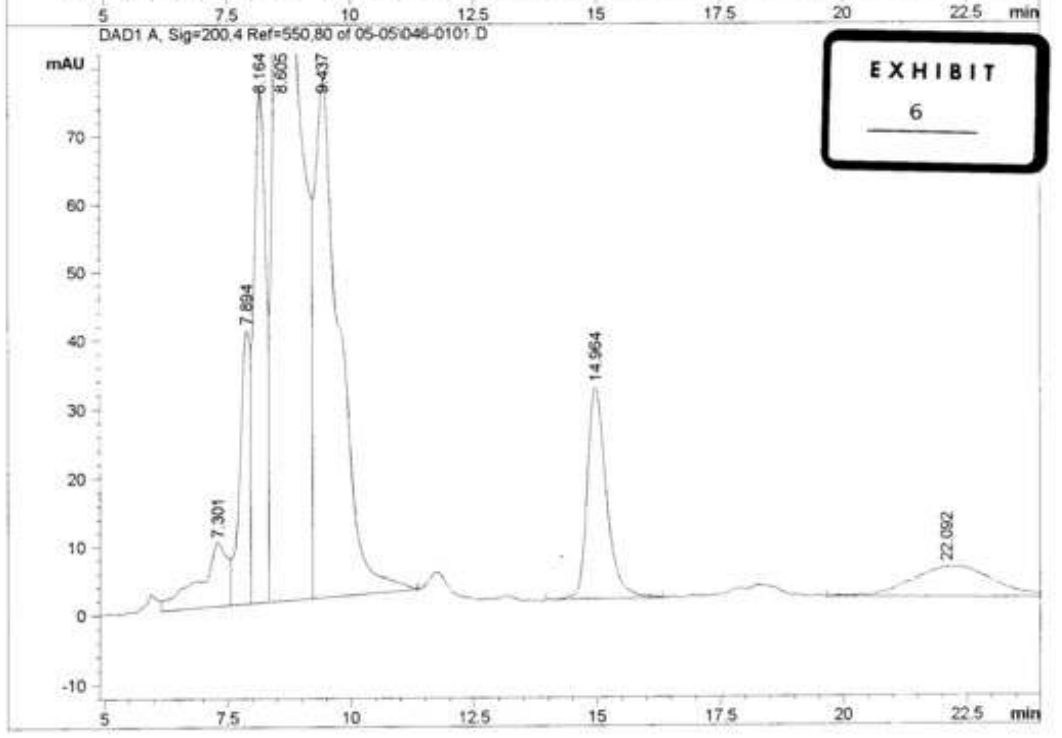

EXHIBIT 6

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So that you may better understand the different information from different testing conditions, EXHIBIT 7 shows the chromatogram of Kombucha using the amine-bonded reverse phase column, EXHIBIT 8 shows the chromatogram of a ferment using the size exclusion column and EXHIBIT 9 shows the chromatogram of a ferment using the cationic exchange column. As you can see from the different peaks that eluted from each column, each method provides a different picture of the Kombucha ferment.

Initial comparative work had involved testing the two solutions brewed in the laboratory using white sugar and either black tea or green tea at regular intervals. We had hoped to ascertain if the type of tea had an effect on the solution.

At least seven compounds were generated by the fermentation process. It was unclear at that point whether the variety of tea used affected aspects of the fermentation, such as the maturation rate of the beverage. We believe that variance of the inoculum (previously brewed Kombucha) and the added "mother" polysaccharide colony are the relevant factors, and whether or not one uses green tea or black tea is not a critical or limiting factor. We know from this set of analyses that fructose, gluconic acid and acetic acid are released into solution at varying ratios. In order to track the fermentation process the pH of the solution was measured on a daily basis, beginning on the second day following formulation. To help you visualize the activity in these ferments, a 100:1 extraction and concentration of green tea and sugar is shown in EXHIBIT 10. By contrast, a 100:1 extraction and concentration of the Kombucha fermented with that tea and sugar is shown in EXHIBIT 11.

The pH of the green tea decreased a bit more rapidly than that of the black tea; and in taste testing, it was interesting to note that although the pH of both solutions was approximately equivalent, the green tea was perceived as being much more tart than the black tea. Also, the green tea's polysaccharide colony grew to a much greater size and at a much faster rate.

It is understood that during the initial stage of fermentation, the sugar molecule sucrose is cleaved into fructose and glucose for the on-going maturation of Kombucha. The organisms producing gluconic acid from glucose were equally competitive in both solutions, and contributed to lowering the pH by this conversion.

To continue our comparative tests, we tested seven different Kombucha formulations, six of which were brewed with previous Kombucha as the inoculum and with colonies all from a similar source, all in one location. One of the batches tested in this stage was brewed in an isolated location without a parent polysaccharide colony like the other six; instead apple cider vinegar, rather than previously-brewed Kombucha, was used as the inoculum.

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Instrument : MS 5989X
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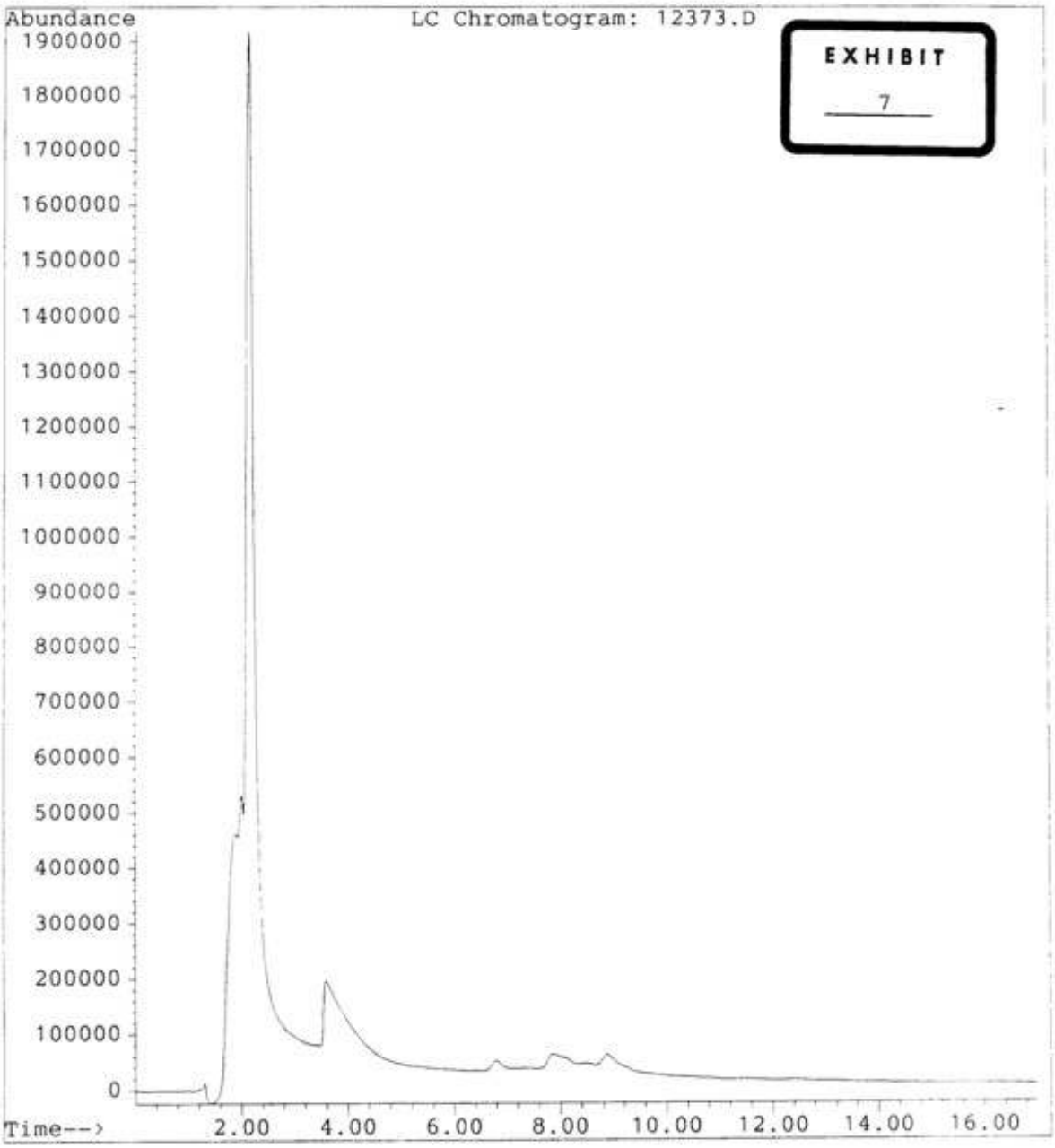

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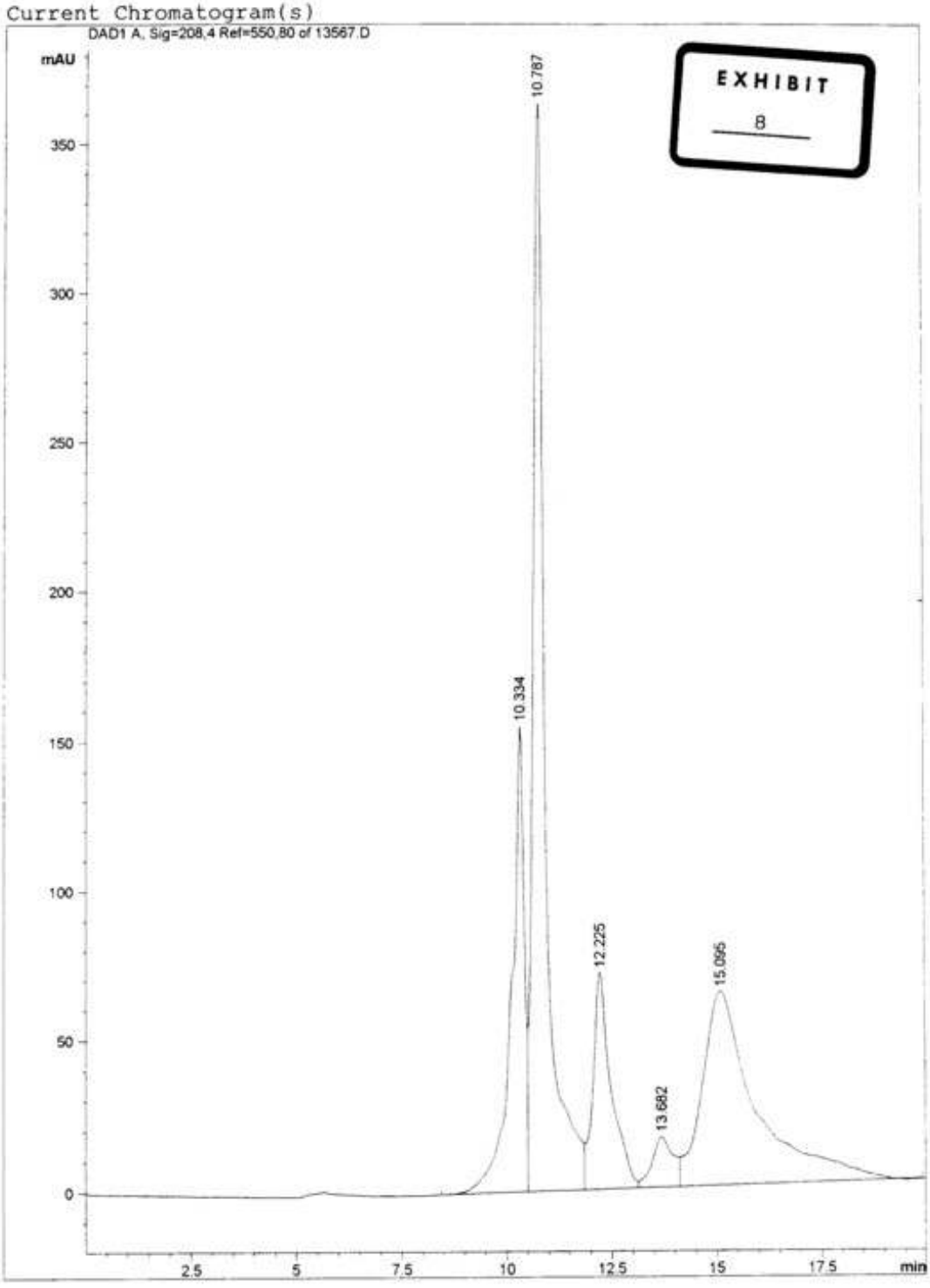

HP LC System Monday, August 05, 1996 7:20:55 PM by

EXHIBIT 9

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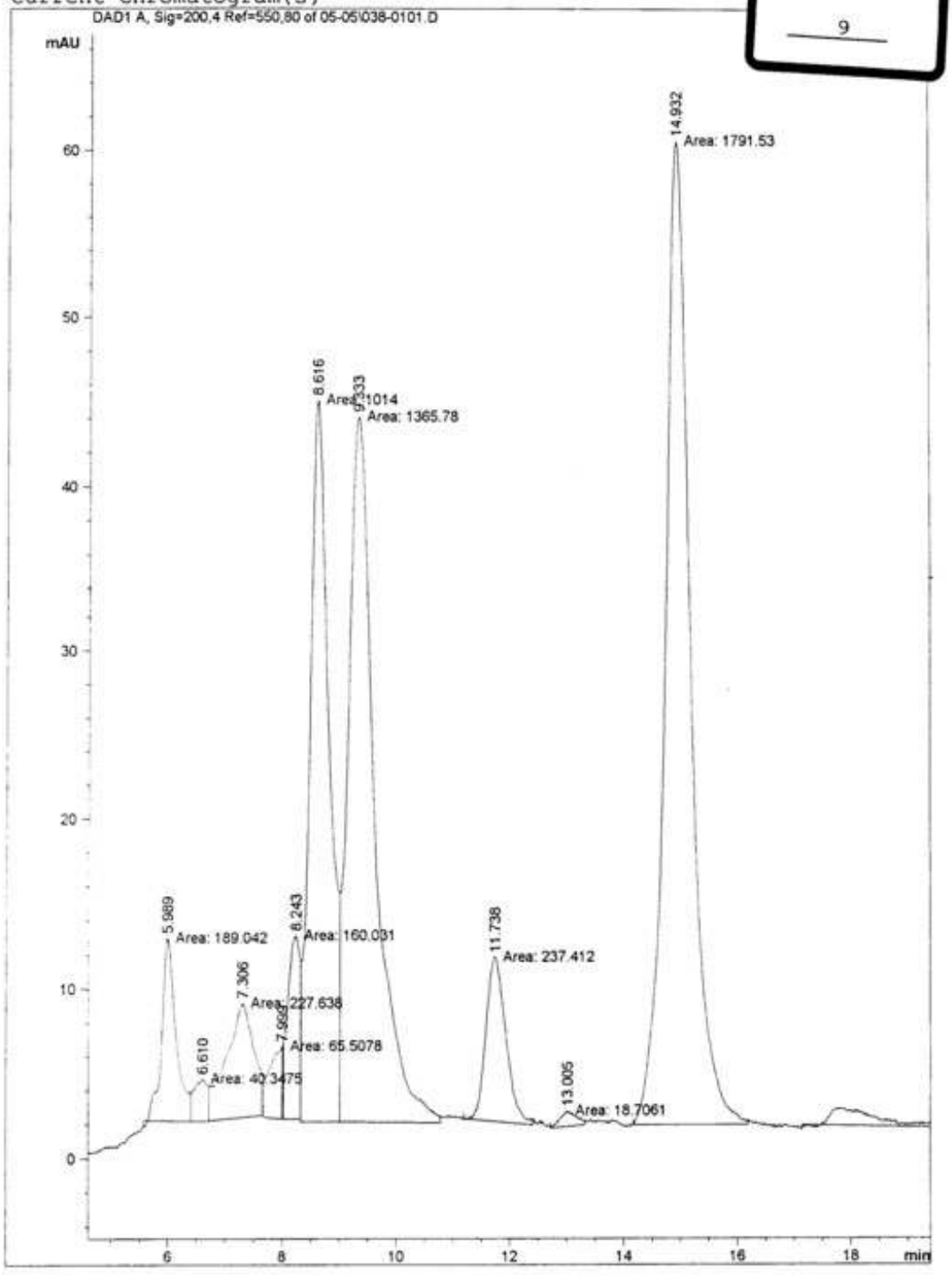

HP LC System Saturday, June 08, 1996 7:09:26 PM by

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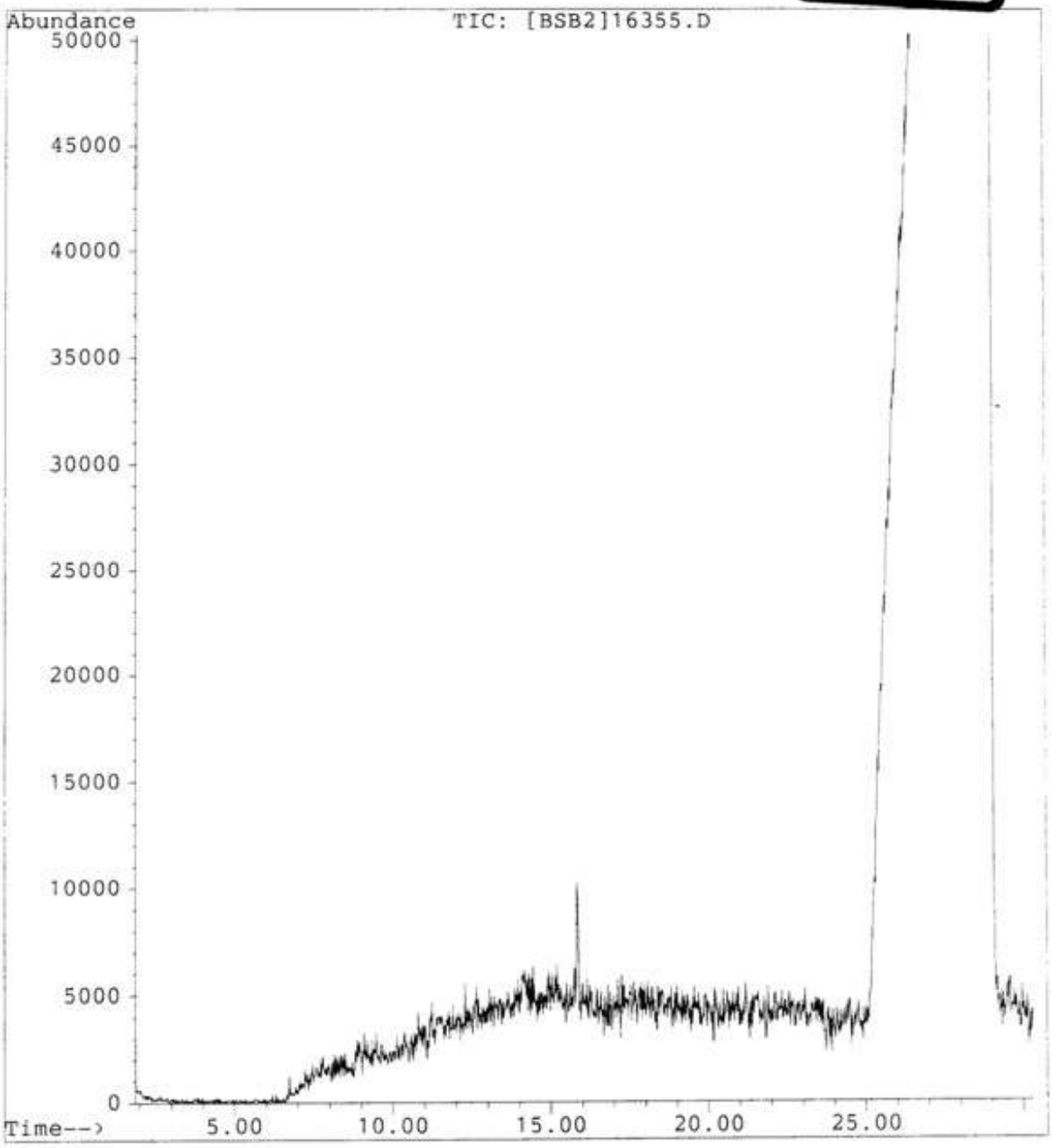
Sample Name: 188 ml/ml Tea Extract
Misc Info :
Vial Number: 1

EXHIBIT
10



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Acquired : 27 Jun 96 6:40 pm using AcqMethod GCEIPOS7
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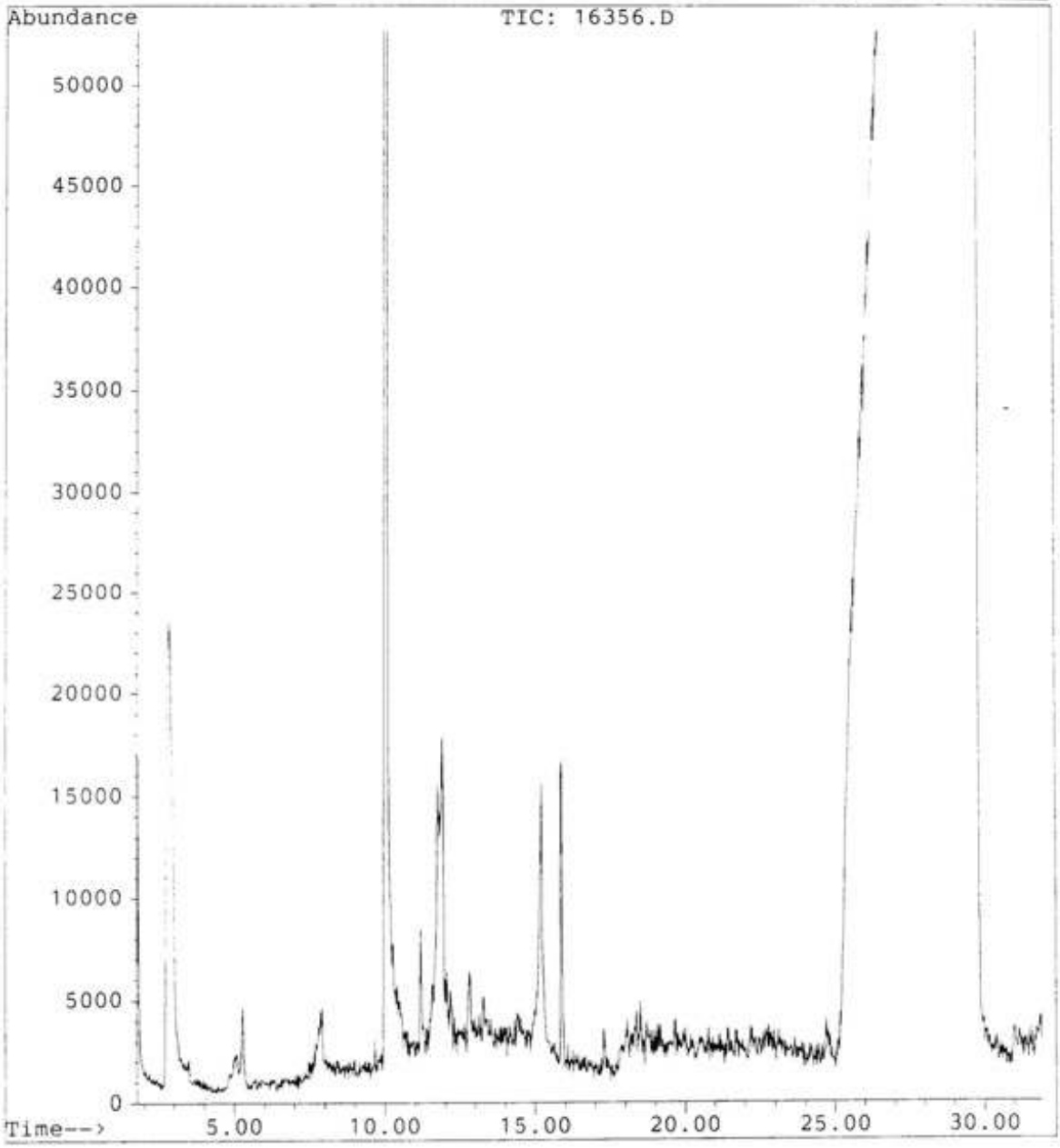
Sample Name: 188 ml/ml Kombucha Extract
Misc Info :
Vial Number: 1

EXHIBIT
11



As was seen in the previous analyses, at least seven components were produced in varying degrees by all of the formulations. At this stage of testing it became apparent that the overall concentration of the individual components wasn't the influential factor with regard to the taste of the individual ferments, but rather that the relative concentrations of key components were crucial in determining the palatability of the beverages. Subtle differences in the concentration ratios of particular components made the Kombucha either too bland, too tart, or what was considered to be well-balanced. We considered this an important initial observation from testing these seven different batches.

Table sugar (sucrose) and glucose were undetectable by ultraviolet absorption, and their concentrations in the solutions were not monitored at that stage. Fructose, which is produced by the cleavage of sucrose by yeasts, appears in its aldose form as opposed to its furanose form and produces a response at around 200 nanometers on a UV detector. The presence of fructose documents further the extracellular production of monosaccharides by the microorganisms in solution. Any direct relationship between this enzymatic process and the production of the polysaccharide colony, or for that matter, to the production of gluconic acid, acetic acid, ethanol or CO2, has yet to be established.

In each of the other formulations, varying concentrations of primarily gluconic acid, acetic acid, and fructose were produced in conjunction with several other smaller components. The concentration of the tannin constituents in the tea (tea flavanoids or catechins), as well as the caffeine, appear to remain constant in their concentrations in the solution, regardless of the age of the ferment. If they do function in the fermentation process, it is most likely as catalysts to other metabolic activities. (Author's Note: Several people have reported that caffeine is diminished during the fermentation of Kombucha. Our observation was that the caffeine and tea flavanoids remained constant throughout the fermentation process.)

The polysaccharide colony deserves comment at this stage. Polysaccharide colonies can be described as thin, thick, white, or in varying degrees of opacity, from translucent to dense white. It appears that there is no direct correlation between how thick the polysaccharide colony is or how robustly it grows, and the constituents that are produced. Of the seven Kombucha formulations, the component that appears to be highest in concentration, excluding one formulation in which brown sugar was used, is the peak representing gluconic acid.

Where the tasting notes indicated that there was a good blend of tartness and sweetness, the component concentration measurements consistently indicated that the gluconic acid was higher than the acetic acid level. However, this did not appear to impact the appearance of the polysaccharide colony as one of the good-tasting solutions had virtually no polysaccharide colony, and was thin and had holes, while another had a very thick white polysaccharide colony. In the one solution where acetic acid was at higher concentration, our notes indicated that it happened to be the thickest of all the colonies.

We have also noted the formation of a polysaccharide colony without the production of the above-mentioned acids. Conversely, we have never observed the production of the acids without formation of the polysaccharide colony.

The concentration of gluconic acid and acetic acid was fairly consistent in six of the seven ferments. The exception was a high concentration of acetic acid in the ferment brewed in separately from the rest of the samples with apple cider vinegar, which to no surprise, tasted like vinegar. For the other six ferments, the gluconic acid concentration was higher than the acetic acid and those ferments remained drinkable for a much longer period of time. At the point during fermentation in which acetic acid was about the same (or greater) as the gluconic acid, the ferments were noted as tasting acidic and not drinkable. This bears out the previous comment that the ratio of the two components, rather than their absolute concentrations, is what determines palatability.

We are at the point now where we can begin to define Kombucha. It appears that it is not merely a matter of the polysaccharide colony. The polysaccharide colony is a very basic component, but the mere presence of a polysaccharide colony does not mean you have Kombucha. You must also have acetic acid and gluconic acid, and the extent to which the acetic acid is balanced by gluconic acid and other sugar acid compounds, is a factor in the quality of the Kombucha.

In the Phase Two testing to determine differences between using green tea or black tea, it was found that the same group of compounds were generated by both fermentations. The green tea solution, however, generated components which would indicate a healthier concentration of yeast growth in the tea solution (acetic acid and CO2). At the same time these two samples were analyzed, the starting tea (previously fermented Kombucha) or the inoculum, and the sweetened teas (prior to adding the starter or polysaccharide colony) were also tested. By that process, we could determine precisely which components were originally present, and which were being formed in the fermentation process.

While initial testing was performed using reverse phase liquid chromatography (C18 and Amine bonded phases) all comparative testing from this point on will be performed using ion exchange HPLC. We've documented that the components present in which people seem to have the greatest interest, are best separated and quantified under these analysis conditions. Bear in mind, however, that under these conditions, we are unable to monitor sucrose, glucose or caffeine.

PHASE III:

The next phase of the comparative analyses that we undertook was a comparison of twelve separate formulations using green tea, black tea, de-caffeinated black tea, two types of fruit teas, sugar, glucose, honey and brown sugar, in different combinations. These formulations were analyzed at periodic intervals in the course of the ferment. This corresponded to a weekly monitoring schedule for one month's period of time.

The following solutions of Kombucha were formulated for this phase. Each ferment formulation consisted of 3.5 quarts of bottled spring water (except for a glucose-only batch which used distilled water), 5 tea bags, and one cup of a sweetener: either d-glucose, sucrose, light brown sugar, or honey. Number 1 was a previously-fermented batch of Kombucha which was designated as the starter solution for all of the other ferments.

Figure 1

SUMMARY TABLE:

ID	Water	Tea	Sugar	Starter
1.	Bot. Spring	Black/Green	White/Brown	For others
2.	Distilled	None	D-Glucose	None
3.	Bot. Spring	Black	D-Glucose	4 Oz.
4.	Bot. Spring	Black	White	4 Oz.
5.	Bot. Spring	Green	White	4 Oz.
6.	Bot. Spring	Raspberry	White	4 Oz.
7.	Bot. Spring	Blackberry	White	4 Oz.
8.	Bot. Spring	Decaf Black	White	4 Oz.
9.	Bot. Spring	Black	Brown	4 Oz.
10.	Bot. Spring	Black	Honey	4 Oz.
11.	Bot. Spring	Green	Honey	4 Oz.
12.	Balance of ID 1 placed in Tupperware pitcher.			

Before comparing concentrations of the known constituents being produced in each batch, further investigative work was performed to identify several unknown constituents and to re-confirm, under different chromatographic conditions, the presence of compounds previously identified. In this instance, we used ion exchange chromatography.

The results of this phase of testing are as follows. Three major constituents are detectable under these conditions, present at concentrations of a magnitude higher than other components produced by the fermentation. The concentration of each of these

components, and the ratio of these constituents to each other, is highly variable, depending on the vitality and age of the fermentation. The major components are, again, acetic acid and gluconic acid. Other possible components, which both have a similar retention time as gluconic acid include dehydro-ascorbic acid, and saccharic acid 1-4 lactone. That is not to say that other acids may not also be co-eluting with these that we have tested. The only component that the Kombucha may possibly contain that co-eluted with acetic acid from the compounds we analyzed is fumaric acid. (Author's Note: The possible presence of d-saccharic acid 1-4 lactone is a significant discovery. For years, it was reported that glucuronic acid was present in Kombucha. This reporting was based in part on the increase in glucuronides in the urine of Kombucha drinkers. Glucuronic acid is a major component of detoxification in the human body. It is produced in the liver and binds with toxic substances in the body. The resulting molecule is called a glucuronide, and these are excreted by the body as part of our waste products. An increase in the number of glucuronides either created or buttressed the belief that glucuronic acid was present in Kombucha. But, the opposite of glucuronic acid is glucuronidase. Glucuronidase cleaves the bond between glucuronic acid and the toxin to which it is bound. Glucuronidase does not bind to either the glucuronic acid or the toxin, so a single glucuronidase molecule and cleave the bonds of thousands of glucuronides. d-saccharic acid 1-4 lactone binds to glucuronidase and prevents it from breaking the bonds in the glucuronides. In other words, it is a glucuronidase inhibitor. The presence of an increased number of glucuronides in the urine of Kombucha drinkers is most likely the result of an inhibition of glucuronidase to break down those glucuronides. With the inhibition of glucuronidase, more bound glucuronic molecules are able to be excreted. This reduces the number of toxins in the human body, and the result is a healthier person.)

We noted that the retention time of ascorbic acid is fairly close to that for gluconic acid. However, under UV spectrum analysis (UV maximum at 245 to 265), ascorbic acid appears sufficiently different from gluconic acid so that we may safely eliminate the possibility of the presence of this compound in any appreciable concentrations being in the solution.

The third detectable major component is fructose, or a closely-related form of this monosaccharide, possibly undergoing some degree of oxidation. Malic acid and maleic acid have retention times similar to fructose and may be co-eluting with this component. However, by comparing the UV spectra of each of these components, the peak in Kombucha is most similar to fructose (UV absorbance at 200-205) versus the acids which absorb at higher wavelengths. All of these saccharide components and sugar acids have similar UV spectra.

The other constituents are classified in one of two categories. They will be referred to as either minor constituents or trace level constituents. There is good correspondence between reference materials (i.e., the "testing standards") and minor constituents for succinic acid, malonic acid, possibly xylonic acid, and, to a lesser degree,

oxalic acid. The presence of oxalic acid diminishes over the course of the fermentation. The chromatographic responses of those components are quite small, and indicate the presence of trace levels only. There are some constituents which appear in quite advanced Kombucha ferments that correspond to the reference materials for lactic acid and propionic acid. The chromatographic responses of those components are quite small, and indicate the presence of only trace levels. Again, these compounds appear in evaluating very advanced Kombucha culture samples (greater than 20 days in age).

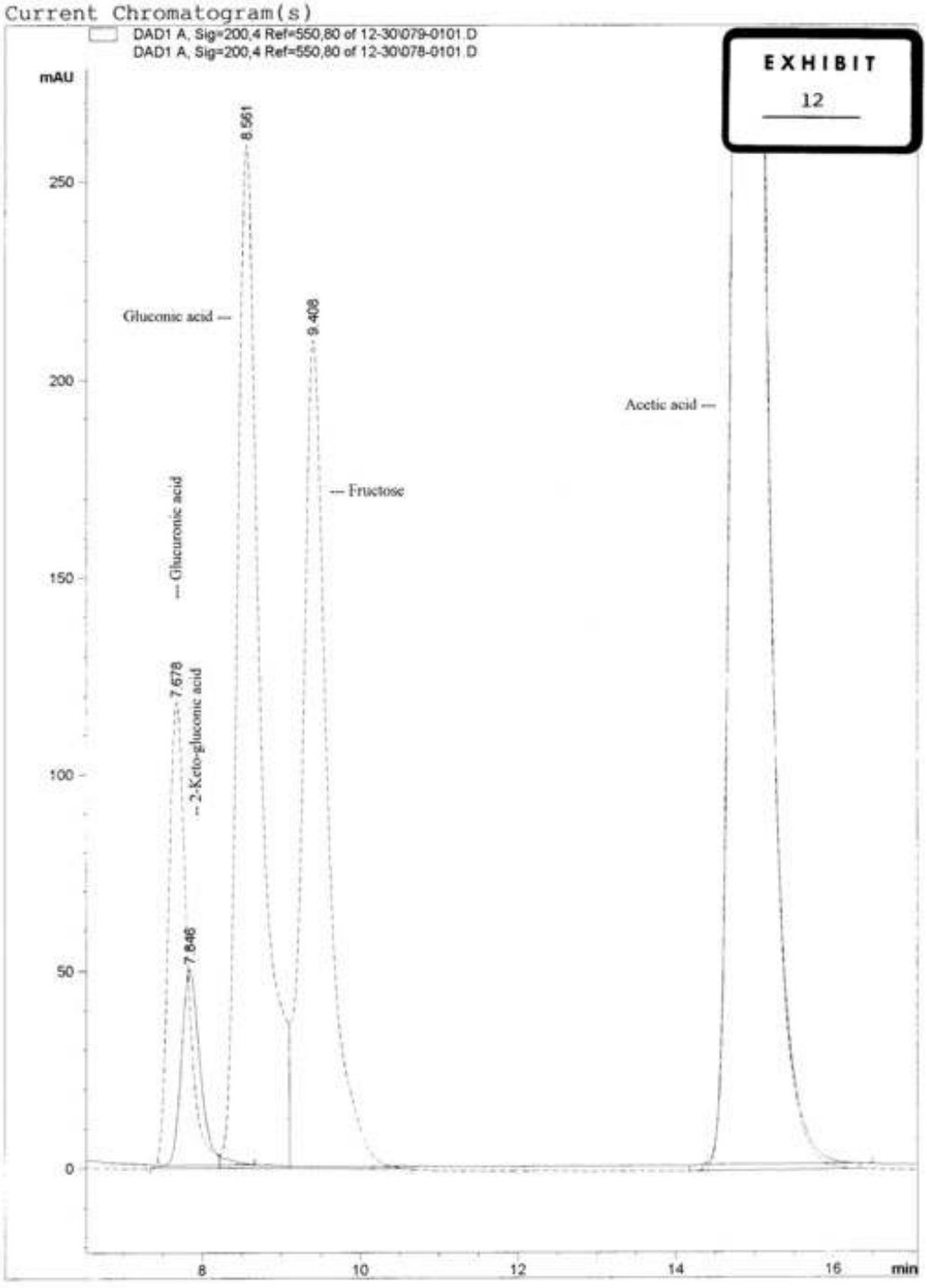
At that point in our investigation, three or four minor constituents had not been fully characterized. The important discovery to that point, however, was that there was a very poor correspondence between glucuronic acid and any constituent in Kombucha. A substantial minor constituent eluting from the chromatography column prior to gluconic acid was the component previously attributed by this retention time as being glucuronic acid, by our own research and previously by others. This peak, however, differed sufficiently in retention time in numerous sample analyses to question seriously our prior assignment of glucuronic acid. In order to alleviate the possibility that our reference standard from Aldrich may have been the reason for the lack of correspondence in our results, we ordered a second reference material of glucuronic acid from ACROS (a division of Fisher Scientific).

Additionally, we simultaneously ran references for the lactone forms of gluconic and glucuronic acid. Both glucuronic acid standards have the same retention times, and both were different from any other component in Kombucha. This was also the situation with glucurono-lactone, which has a retention time completely different from any of the peaks in Kombucha samples. The glucono-lactone, however, had the same retention time as gluconic acid. This may be due to the hydrolyzation of glucono-lactone under chromatographic conditions being used.

Since these peaks in question have been the reason for reports of glucuronic acid in Kombucha samples, our priorities at that point were to determine the identity of the peak, which, in some instances, is the fourth largest peak in Kombucha formulations. To accomplish this, a number of sugar acids and intermediary acids of the citric acid metabolic cycle were analyzed.

There is a very high probability that the constituent being labeled glucuronic acid is actually 2-keto-gluconic acid, or alpha-keto-gluconic acid. A chromatogram for the reference materials for glucuronic acid, 2-keto-gluconic acid, and gluconic acid is shown in EXHIBIT 12. This chromatogram is superimposed on a chromatogram of a Kombucha ferment sample in EXHIBIT 13. As you can see, the gluconic acid and 2-keto-gluconic acid match the peaks eluting in the ferment, while the glucuronic acid shows a small variation from the compounds found in the ferment. It is also probable that smaller quantities of 5-keto-gluconic acid, 2-keto-3-deoxy-gluconic acid, and 2,5-diketo-gluconic acid are also present in the solution.

Current Chromatogram(s)



HP LC System Wednesday, July 24, 1996 12:47:58 PM by

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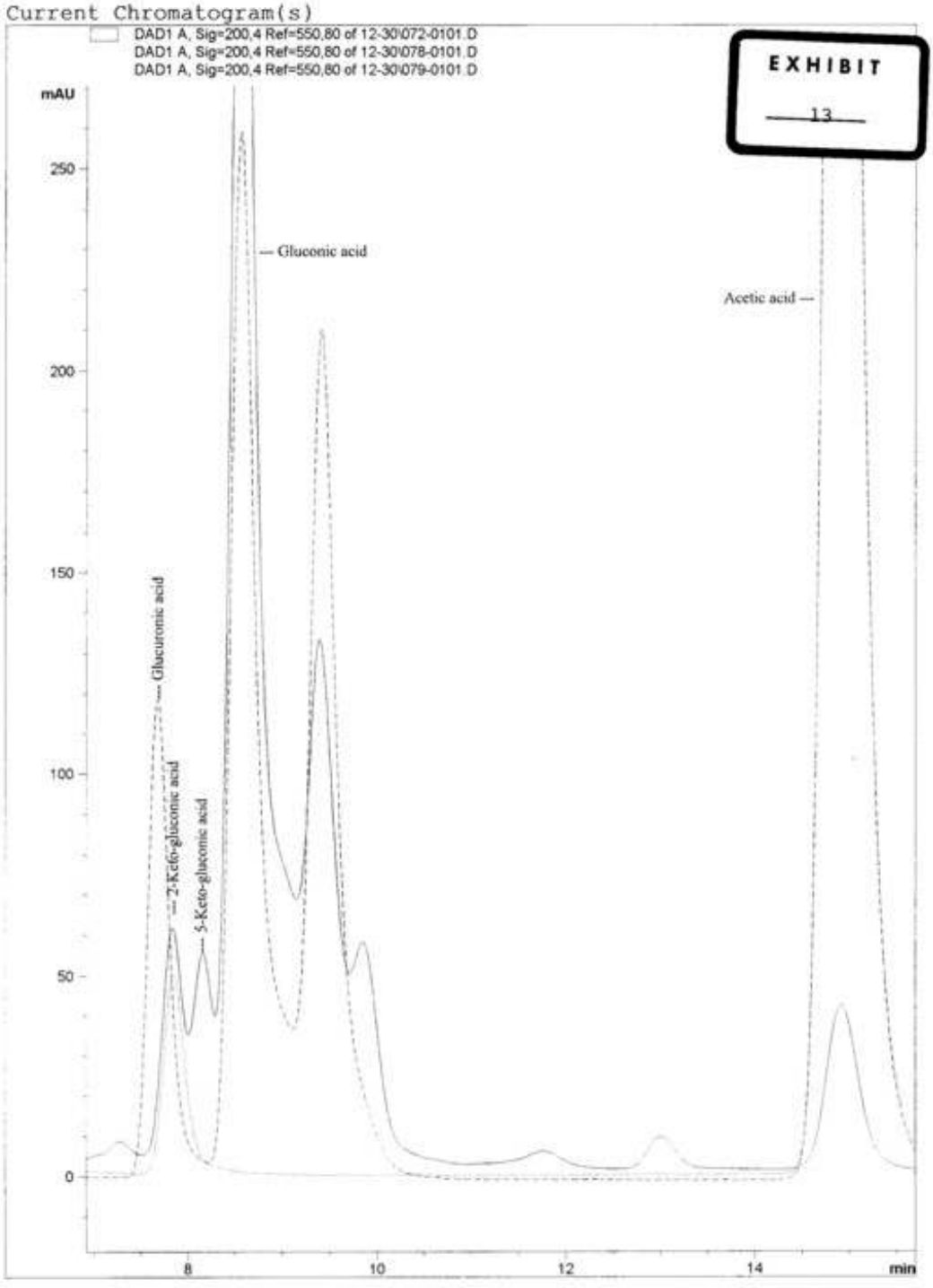

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During this time, a concerted effort was underway to determine the effect of using different constituents in the formulations. The following graphic representations show some of the interesting aspects of the 12 different solutions of Kombucha which were prepared. We did not prepare quantified peak chromatograms during this portion of our research, since we were more interested in the relative ratios of the sugar acids. Based on absorbancy unit data from those runs, the following chart represents approximate concentrations in mg/ml of the targeted sugars and acids.

Figure 1-a

CHEMICAL ANALYSES OF PHASE III FERMENTS:

Ferment	Keto-gluconics	Gluconic	Fructose	Succinic	Acetic
1	2.5	10	25	0.5	7.0
2	0.1	5	5	0.0	0.7
3	2.5	20	5	0.5	10.0
4	6.0	8	30	0.5	7.0
5	4.0	9	36	0.5	8.0
6	4.0	15	44	0.0	8.0
7	5.1	8	36	0.0	3.0
8	5.0	6	25	0.5	5.0
9*	5.2	4	23	0.0	7.0
10	3.1	7	34	0.5	5.0
11	3.2	8	35	0.5	4.0
12	1.2	9	18	0.0	20.0

(* contains 15 mg/ml of mannonic acid)

This particular set of analyses brought many important aspects of the fermentation process to light. First, with regard to the use of different teas, in almost every instance where black tea was used, there was a noticeably higher concentration of acetic acid produced, with a corresponding lower concentration of fructose. This was the case with both regular and decaffeinated black tea, regardless of the sugar source. Secondly, there was no distinguishable difference between components produced by the decaffeinated batch and the regular batch of black tea. The green tea and fruit tea batches had slightly higher gluconic acid concentrations than did the black teas. Both of the fruit teas had little, if any, succinic acid or alpha-keto-gluconic acid. Alpha-keto-gluconic acid may have been present, but was undetectable as it was obscured by other constituents, possibly fruit acids. The green tea and fruit tea batches both had slightly higher concentrations of fructose and gluconic acid than black tea, with the raspberry tea producing the highest levels of fructose and gluconic acid of all of the batches where white sugar was used.

The four sugar sources used were table sugar (sucrose), d-glucose, brown sugar and honey. The d-glucose was used in distilled water without tea or starter and a mature Kombucha polysaccharide colony. It was also used in black tea which included starter from batch one. Both batches produced very small quantities of acetic acid, with no

fructose and almost exclusively gluconic and alpha-keto-gluconic acids. The most interesting aspect of this portion of the experiment was the difference in the concentrations produced. The water formulation produced slightly less gluconic acid than the average amount produced by white sugar formulation. The use of glucose and black tea produced almost ten times the concentration of gluconic acid in the same time period, as did the glucose and water batch. (Author's Note: For years I produced my Kombucha with the offspring from the d-glucose and black tea batch. I often used green tea in future ferments and the result was always pleasant. During a recent remodeling accident, all of my hybrids were destroyed. I plan to grow a new hybrid using d-glucose and continue to make that ferment because of the high concentration of gluconic and keto-gluconic acids.)

One additional point that should be made regarding the glucose-only formulation was that although there was no apparent astringency to the taste (it was very bland), the pH was less than 3. It is obvious in the process by which the yeasts cleave the disaccharides that excess hydrogen ions are being released into solution. Either that, or the fructose is modified bacteriologically somehow and converted to some form of sugar acid which is contributing to the lowering of the pH.

It should be mentioned that several non-characteristic components appeared in the glucose and water batch, which most probably were acid esters that were being produced and showing up early in the chromatogram. Brown sugar produced a batch with a higher acetic acid concentration than did the standard black tea and white sugar batch; it had slightly less gluconic acid and slightly more alpha-keto-gluconic acid. A significant component not observed in either the white sugar or honey batch showed up quite early in the fermentation process of this brown sugar batch. Its concentration decreased over time, as is the case with fructose. This leads us to suppose that this peak is a saccharide component unique to brown sugar but still capable of being utilized by the organisms in the fermentation. From previous characterization of the saccharide components of brown sugar, we expect that this component is mannonic acid. Honey contributes free fructose and glucose to the newly formulated solution, which affects the timing of changes in the concentration of the components. But after 21 days, there is little difference seen in the type and concentration of fermentation products in honey batches versus white sugar batches.

The final batch, which was being stored in plastic, had an extremely high concentration of acetic acid — more than double that of the standard black tea/white sugar batch. This was not surprising, however, as this solution was three weeks older than any of the others. It did not show any indication of the formation or the occurrence in solution of components, other than those seen in fermentations being carried out in glass. No indication of any unique toxic products were observed under these conditions. (Author's Note: We were looking for chemical by-products from the break-down of the plastic tupperware container. It had been speculated that Kombucha might break-down the plastic and release toxins into the ferment. No such toxins were found in the Kombucha fermented in tupperware. We did subsequently note the presence of such

toxins in solutions that had been stored in plastic bags for an extended period of time. The lesson here is that “baggies” are not a suitable method for storing Kombucha tea or Kombucha colonies for more than a few days.)

Having HPLC conditions for separating the acid and sugar components of Kombucha, and also being able to detect them using mass spectrometry allowed us to perform a much broader analysis of Kombucha. At the expense of component resolution, by adjusting the HPLC conditions, we were able to produce conditions which were amenable to thermospray ionization. Mass spectral evidence (molecular ions) from sucrose, fructose, glucose, and gluconic acid were detected. In addition, ions characteristic of C6 aldaric acids or acid lactones were observed. This would be most consistent with mucic or saccharic acid, or saccharic acid lactone. Under these LC conditions, however, many of the major components elute from the column at the same time (co-elute). Although UV analyses is not amenable to finding some of the major constituents, it does allow us to segregate better the important individual components. Using mass spectrometry, we were able to confirm at least two separate sugar acid components, one with the ion characteristics of gluconic acid and another with the ion characteristics of saccharic acid. Under this particular set of conditions, it is also possible to detect and monitor caffeine.

Besides some peaks seen in the UV chromatograms, most probably representing polyphenolic (tannin) components of the teas which were not detectable by mass spectrometry, no other peaks of any consequence were observed.

Of particular interest to us in this study was glucuronic acid, which is purported to be the most valuable constituent in Kombucha. When glucuronic acid was not found, and the suspected peak actually co-eluted with 2-keto-gluconic acid, we began to focus on these other elements of the ferment. We soon isolated 5-keto-gluconic acid, and possibly 5-keto-dioxy-gluconic acid in some ferments. This led us to look for possible fructose lactones; and what we found was substantial evidence to support the presence of a potent exo-beta-glucuronidase inhibitor: d-saccharic acid 1,4 lactone.

The final stage of this portion of our testing, after defining acceptable protocols, involved analyzing Kombucha samples from a number of diverse U.S. and international sources. As indicated earlier, of the 25 or so components typically observed in analyses, not less than seven of those are direct products of the fermentation process. These primary organic constituents are: glucose, fructose, acetic acid, gluconic acid, 2-keto-gluconic acid, 5-keto-gluconic acid, and corresponding lactones. The quantity of sugar initially added to the brewing tea, what kind of sugar is added, what balance of yeast and bacteria exist, and the duration of time from the beginning of the ferment are all influential factors in what and how much is produced in the Kombucha solution.

ANALYSIS OF AT-HOME GROWERS

Before we present our findings on the various growers, we would like to remind our readers that many of the HPLC peaks early in the chromatograms co-elute; that is to say, multiple constituents may be occupying the same peak. We present these findings based on strong evidence. As of the date of this writing, and based on a large volume of evidence from a wide variety of samples, we present these findings of our analyses of the various growers.

We will begin our discussion with the NON-commercial growers. Since warnings surround the growing of Kombucha at home, we thought it important to examine ferments from home-growers that we did not personally know. We therefore accepted contributions from active home-growers that were supplying colonies to other home-growers. We asked each contributor to submit two separately packaged colonies; this allowed us to look for consistency and to be able to note any anomalies. We received colonies from Colleen Allen of British Columbia, Canada; Christine Meuhling of Maryland, USA; Jack Barclay of New Mexico, USA; and Jim Bailey of Oregon, USA. Additionally, we examined our own "at-home" ferments, a hybrid series we developed during the third phase of our testing. While all of the non-commercial home-growers have provided Kombucha colonies to others for free, none of them either grow the colonies commercially or offer them for sale. Prior to inoculating any of the ferments with the starter tea and colony, samples of the sugar/tea nutrient solution were taken, as well as samples from the starter tea. This allowed us to monitor what was present before the fermentation process and which compounds were actual products of the fermentation.

Colleen Allen

The first colonies were received from Colleen Allen in October, 1995. Colleen was an active member of the Kombucha mailing list and the author of the FAQ posted on the Internet at Bob Willian's Kombucha home page — http://w3.trib.com/~kombu/. Colleen also did the maintenance and moderating for the Kombucha mailing list.

Each colony, along with some Kombucha starter tea, arrived in taped-over, doubled, Ziplock bags, which were then enclosed in taped one gallon Ziplock bags. Both samples appeared healthy. Her sample number 6 was the size and shape consistent with growing in one gallon jars and represented her regular ferment. Her sample number 5 was from an experiment she had been conducting with frozen Kombucha tea, and had a size and shape consistent with using a one quart mason jar. The colony had been formed by adding Kombucha thawed from a two week storage in the freezer into a sugar/tea nutrient solution. The resulting colony and ferment were sent to us for analysis. We labeled these samples "A" and "B," respectively.

We prepared nutrient solutions for both colonies on November 4, 1995, following

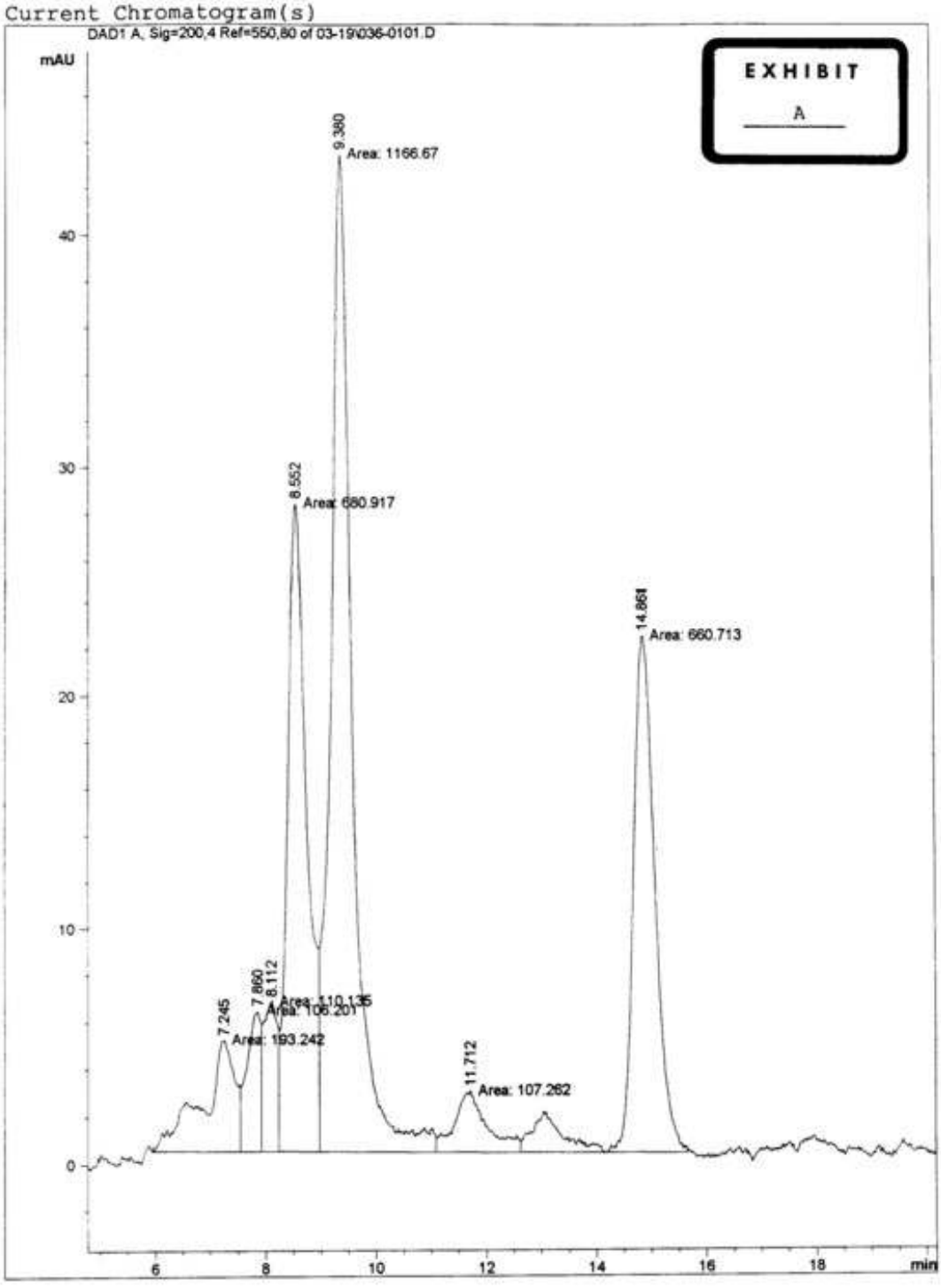
the instructions sent to us by Colleen Allen. We used 3 quarts plus 1 cup of bottled spring water, one cup of white sugar, and 5 Earl Gray tea bags for each ferment. The water was brought to a boil in a glass Visionware four quart casserole and the sugar added. We then boiled the sugar and water solution for five additional minutes. The container was removed from the heat, and the tea bags added and allowed to steep for 10 minutes. The tea bags were removed, and the nutrient solution was allowed to cool to room temperature in covered one gallon sun tea jars. We then added the entire contents of a sample bag (approximately 4 ounces of starter tea and the colony) to the jar of cooled nutrient solution.

Samples were taken on 11-4-95 (after inoculation), 11-14-95, 11-25-95, and 12-3-95. On 11-14-95 sample "A" was ¼" thick and dome-shaped from trapped CO2. Sample "B" was 3/8" thick, very smooth and showed a great deal of CO2 activity. Both ferments had a typical Kombucha flavor with the slight sweetness one associates with oil of bergamot from the Earl Gray tea. When samples were taken on 11-25-95, both samples had thickened to ½". "A" seemed to have more CO2, but both ferments were surprisingly pleasant to drink. Most ferments have a very strong vinegar flavor after 20 days and are not drinkable. We theorized that the oil of bergamot had slowed the production of acetic acid, perhaps because of its antibacterial qualities. When the 12-3-95 samples were taken, both ferments were still very drinkable, so much so, that we bottled the "B" ferment and placed it in the refrigerator. We consumed that over the next several days. The flavor of the "A" ferment was like very hard cider - too tart for my taste, but enjoyed by our chemist. The growth of the colonies seemed to accelerate after the 20 day samples were taken. When the colonies were harvested on 12-3-95, the "A" colony was 1" thick and the "B" colony was a robust 1 ½" thick.

An analysis of the two ferments from the samples taken on 11-14-95, is shown in the two chromatograms (EXHIBITs A and B) and revealed the following chemical compounds in the ferments:

	A mg/ml	B mg/ml
Glucosamine	0.00	0.00
Oxalic acid	0.00	0.55
Saccharic acid	0.97	0.49
Glucuronic acid	0.00	0.00
2-keto-gluconic acid	0.53	0.90
5-keto-gluconic acid	0.55	0.00
Gluconic acid	3.40	2.80
Fructose	20.00	30.00
Succinic acid	0.54	0.56
Itaconic acid	0.17	0.00
Acetic acid	3.30	2.70

Current Chromatogram(s)



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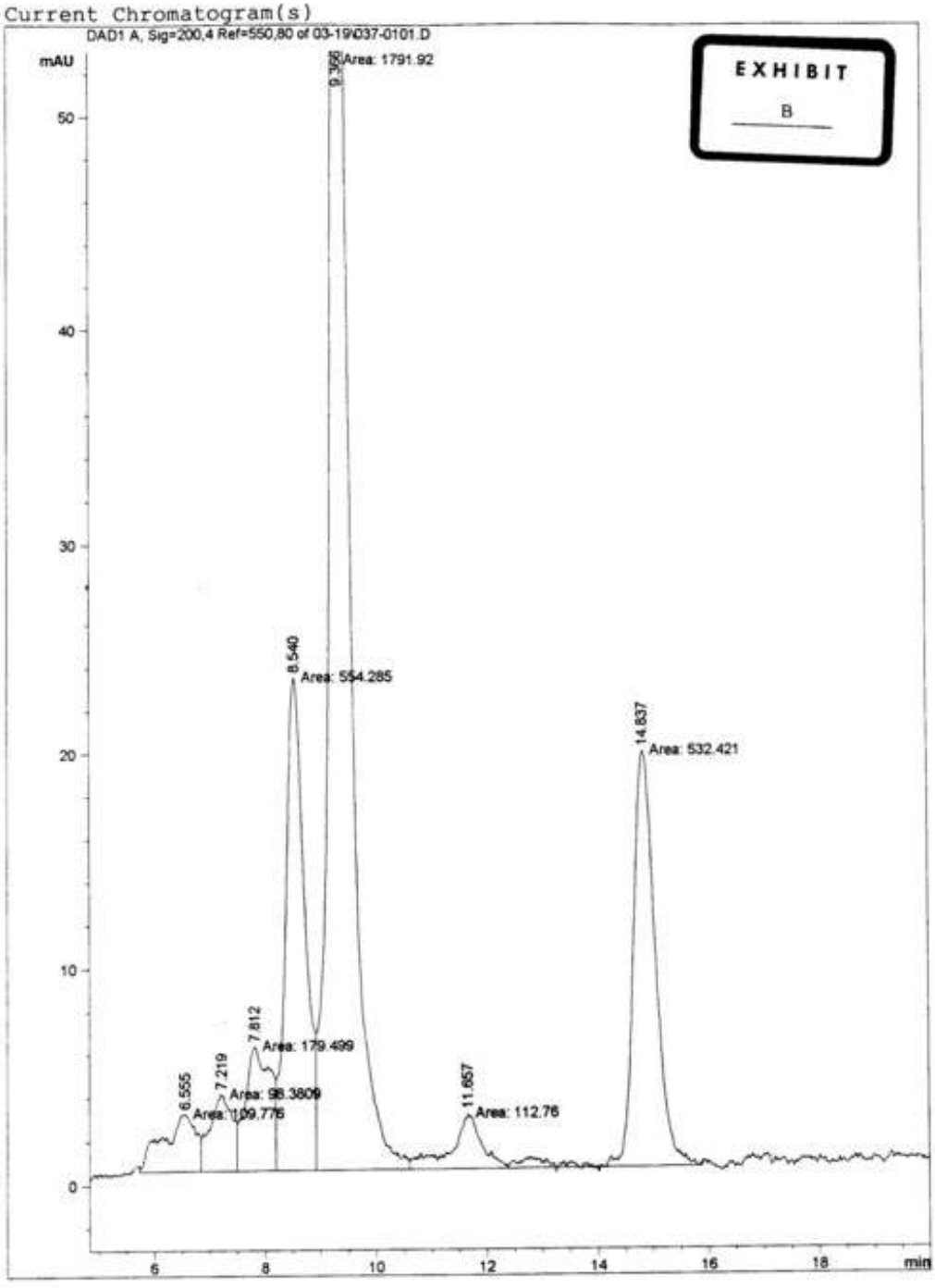

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Christine Muehling

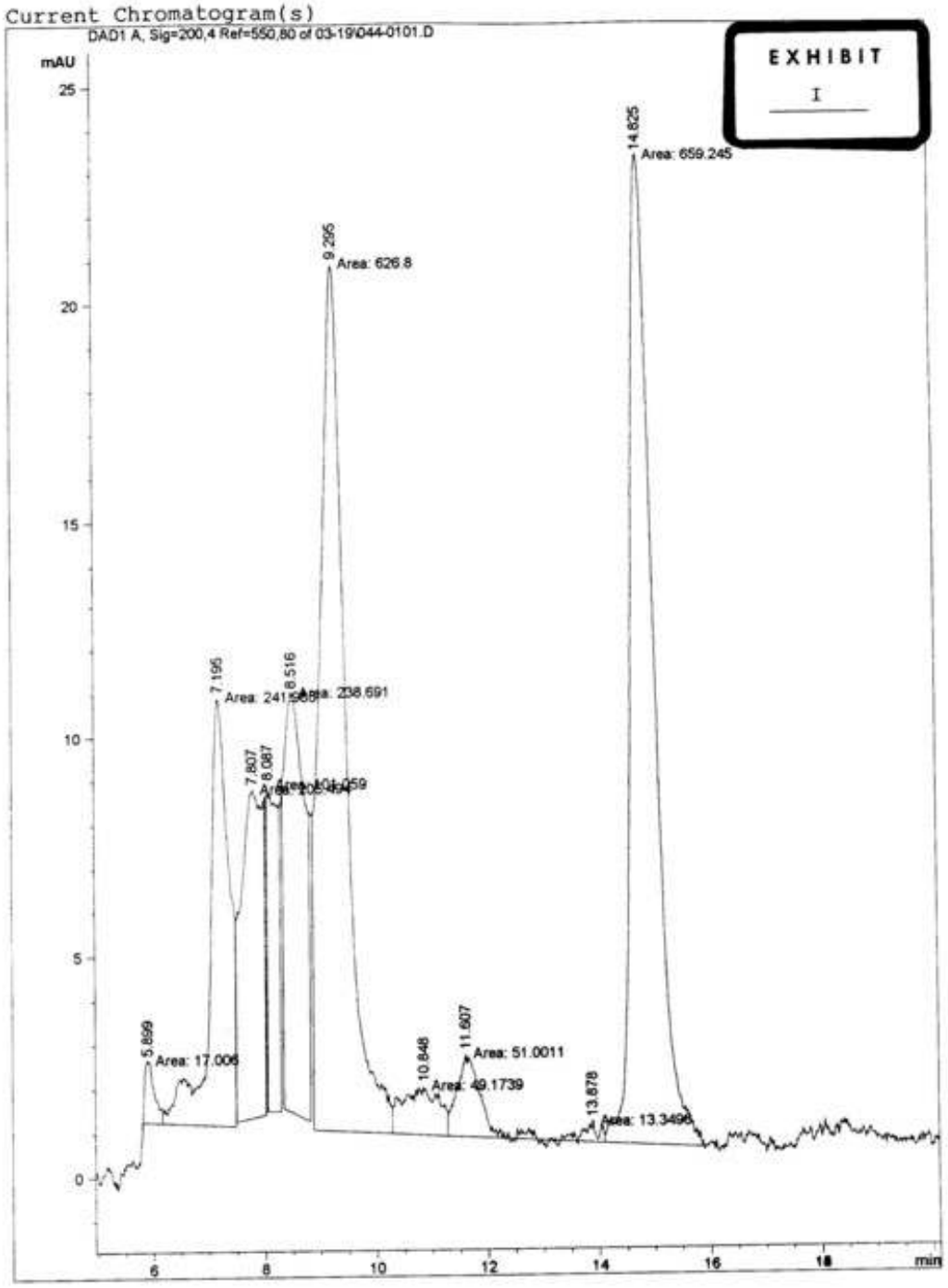
The next set of samples we received came from Christine Muehling on 11-3-95. Christine provides Kombucha to a large number of people on a daily basis. Because of the number of ferments she must deal with, she has opted to ferment her Kombucha in mason jars. Christine is active on CompuServe in both the alternative health section of the New Age Forum and the herbal section of the Health Forum.

Both samples were received in double Ziplock bags. The colonies were thin and about the size of small cookies. She ferments her Kombucha in 2 cup mason jars. The sugar and tea nutrient was prepared on 11-4-95 according to her instructions as follows: 3 quarts of bottled water, 1 1/3 cups of white sugar, 2 black tea bags and 3 green tea bags. The boiling of the sugar and steeping of the tea were the same as for Colleen Allen. Samples were taken on 11-4-95, 11-14-5, 11-25-95, and 12-3-95. We labeled her ferment A as our ferment "I" and her ferment B as our ferment "J". On 11-14-95, both colonies were fully formed and smooth with lots of CO2. Both ferments were still a bit sweet, probably due to the extra sugar. By 11-25-95, both colonies were 1" thick. The "I" ferment was much more drinkable, as the "J" ferment tasted like sweet vinegar. When the last samples were taken on 12-3-95, both colonies were about 1 ¼" thick and smelled and tasted like vinegar.

The analysis of the samples taken on 11-14-95 is shown in the two chromatograms (EXHIBITs I and J) and revealed the following chemical compounds in the ferments:

	I mg/ml	J mg/ml
Glucosamine	0.85	0.12
Oxalic acid	0.60	0.14
Saccharic acid	1.20	1.20
Glucuronic acid	0.00	0.00
2-keto-gluconic acid	1.00	0.00
5-keto-gluconic acid	0.51	1.30
Gluconic acid	1.20	1.20
Fructose	11.00	13.00
Succinic acid	0.26	0.32
Itaconic acid	0.07	0.00
Acetic acid	3.30	3.70

Current Chromatogram(s)



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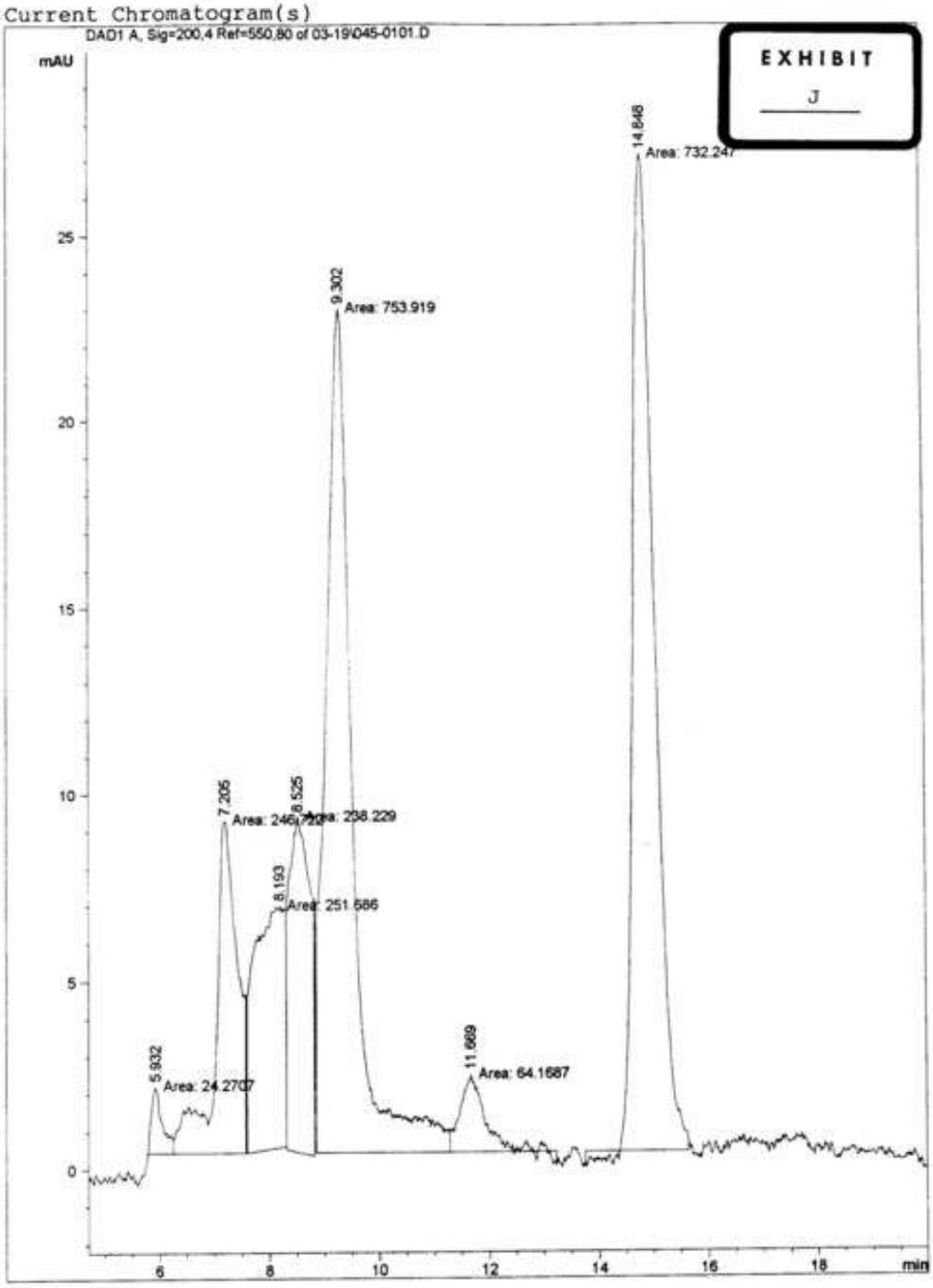


HP LC System Saturday, April 13, 1996 12:37:29 PM by REM

Jack Barclay

The next colonies and ferments we tested were received from Jack Barclay of New Mexico. Jack had been providing free colonies all around the country for many months. He is an active member of the Kombucha mailing list and a constant contributor on the topic of Kombucha.

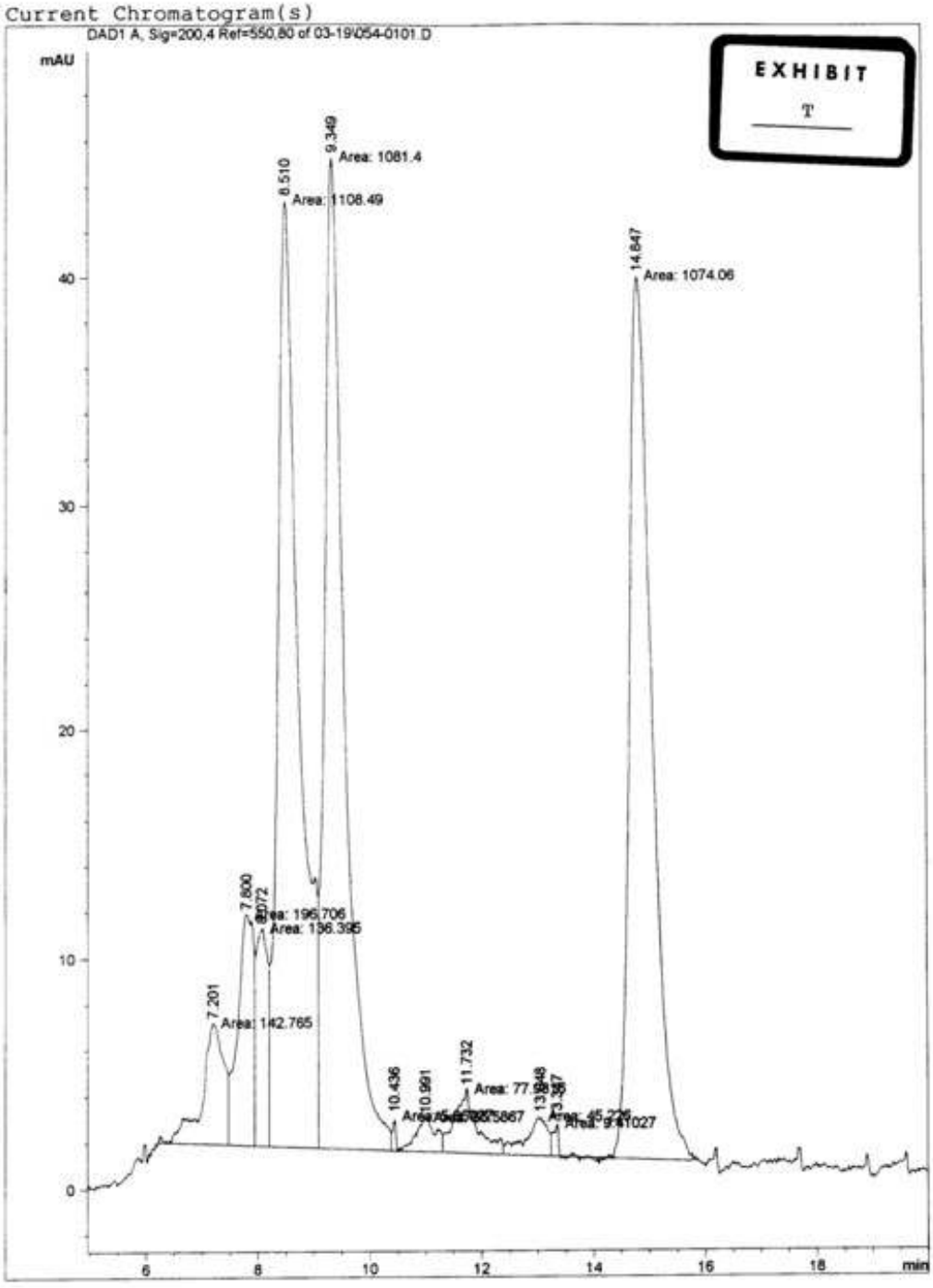
Jack's colonies were received on 11-20-95. They were packaged in double Ziplock bags inside of a coffee can with the lid taped on. This is a good packaging method. The packaging indicated it had been a 7 ½ day ferment. Both colonies appeared healthy and were the size of a large bowl or a squat fish bowl. The ferments were prepared in accordance with Jack's instructions as follows: 3 quarts of bottled water, 1 cup of white sugar, and 6 organic black tea bags. As with the other ferments, the water was brought to a boil, the sugar added and boiled for five minutes and then removed from the heat. The tea was added and allowed to steep for 10 minutes. The tea bags were then removed and the nutrient solution allowed to cool to room temperature in covered one gallon squat fish bowls. Samples were taken on 11-21-95, 12-3-95 and 12-15-95. His sample A became our ferment "T" and his sample B became our ferment "U".

When the samples were taken on 12-3-95, the "T" colony was about ¼" thick and the "U" colony was about 3/8" thick. Both looked very healthy. The "T" ferment had a mild vinegar aroma and a very good flavor. The "U" ferment had an aroma and flavor that was unusual to me. At 12-15-95, the "T" colony was ½" thick and beautiful. The flavor was very strong, but not yet vinegar. The "U" colony was ¾" thick and the flavor a lot milder than the "T" ferment.

An analysis of the ferment samples taken on 12-3-95 is shown in the two chromatograms (EXHIBITs T and U) and revealed the following chemical compounds:

	T mg/ml	U mg/ml
Glucosamine	0.00	0.00
Oxalic acid	0.00	0.47
Saccharic acid	0.71	0.98
Glucuronic acid	0.00	0.00
2-keto-gluconic acid	0.98	1.10
5-keto-gluconic acid	0.68	1.10
Gluconic acid	5.50	3.10
Fructose	17.82	12.31
Succinic acid	0.39	0.35
Itaconic acid	0.23	0.12
Acetic acid	5.40	4.67

Current Chromatogram(s)



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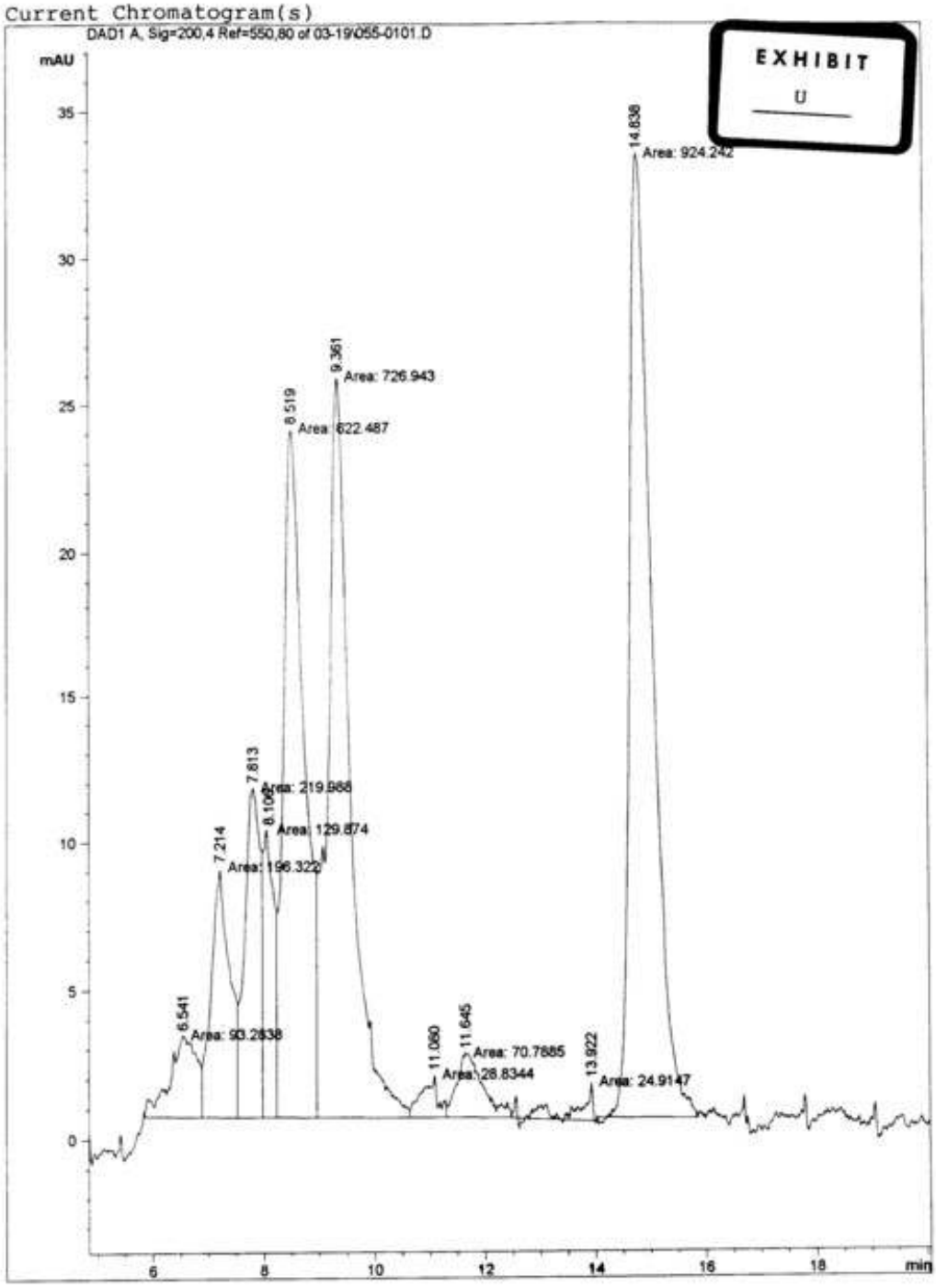


HP LC System Saturday, April 13, 1996 1:29:11 PM by REM

Jim Bailey - honey ferment

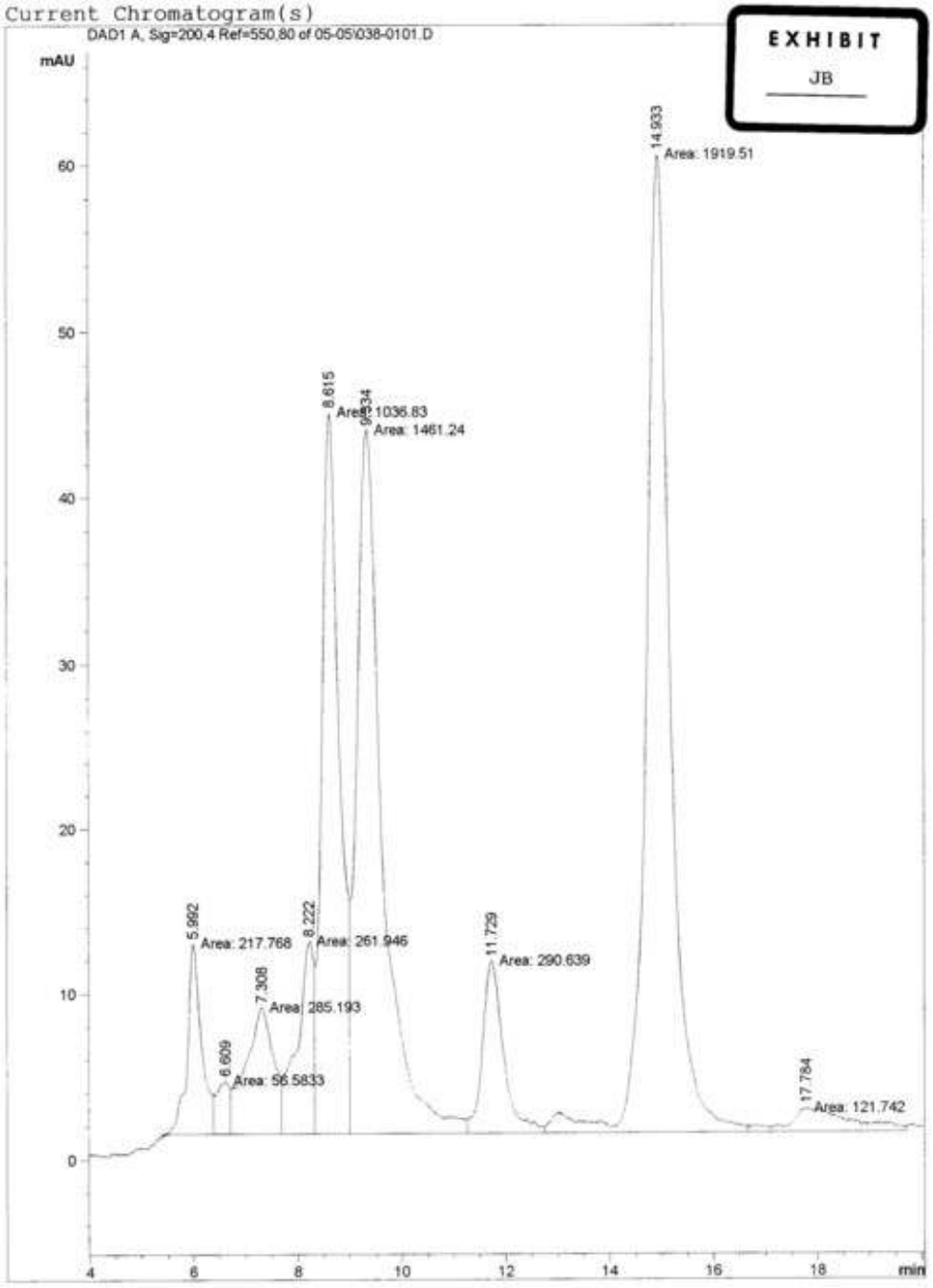
Many of us have heard the stories that growing with honey is going to kill the colony. It will become the vegetative yeast patty that is feared by every uninformed Kombucha drinker. Well, there is no vegetative yeast patty.

The next non-commercial sample received came from Jim Bailey of Oregon. This is a honey ferment. According to Jim, the ferment has been produced with honey for over two years. Jim sent along the honey for preparing the ferment. In addition to samples of the honey/tea solution and the starter tea, we also analyzed the honey. The colony was received in April, 1996 in a double Ziplock bag packaged inside of a coffee can. The bag had leaked a small amount into the can and a sample of that was also taken to test for contamination, but none was noted. The ferment was prepared in accordance with Jim's instructions, 3 quarts of bottled water, one cup of honey and five black tea bags. The water was brought to a boil and removed from the heat, then the honey was added and stirred to dissolve it into the water. The tea was added and allowed to steep for 10 minutes. The tea bags were removed and the nutrient solution was allowed to cool to room temperature in a covered one gallon sun tea jar.

We prepared the ferment on 4-13-96, and samples were taken on 4-20-96, 4-27-96 and 5-12-96. The ferment was identified as "JB" and the results of the analysis of 4-27-96 is shown in the chromatogram (EXHIBIT JB) and the following chemical compounds were revealed:

	JB mg/ml
Glucosamine	1.10
Oxalic acid	0.28
Saccharic acid	1.40
Glucuronic acid	0.00
2-keto-gluconic acid	0.00
5-keto-gluconic acid	1.32
Gluconic acid	5.24
Fructose	25.07
Succinic acid	1.59
Itaconic acid	0.00
Acetic acid	9.63

Current Chromatogram(s)



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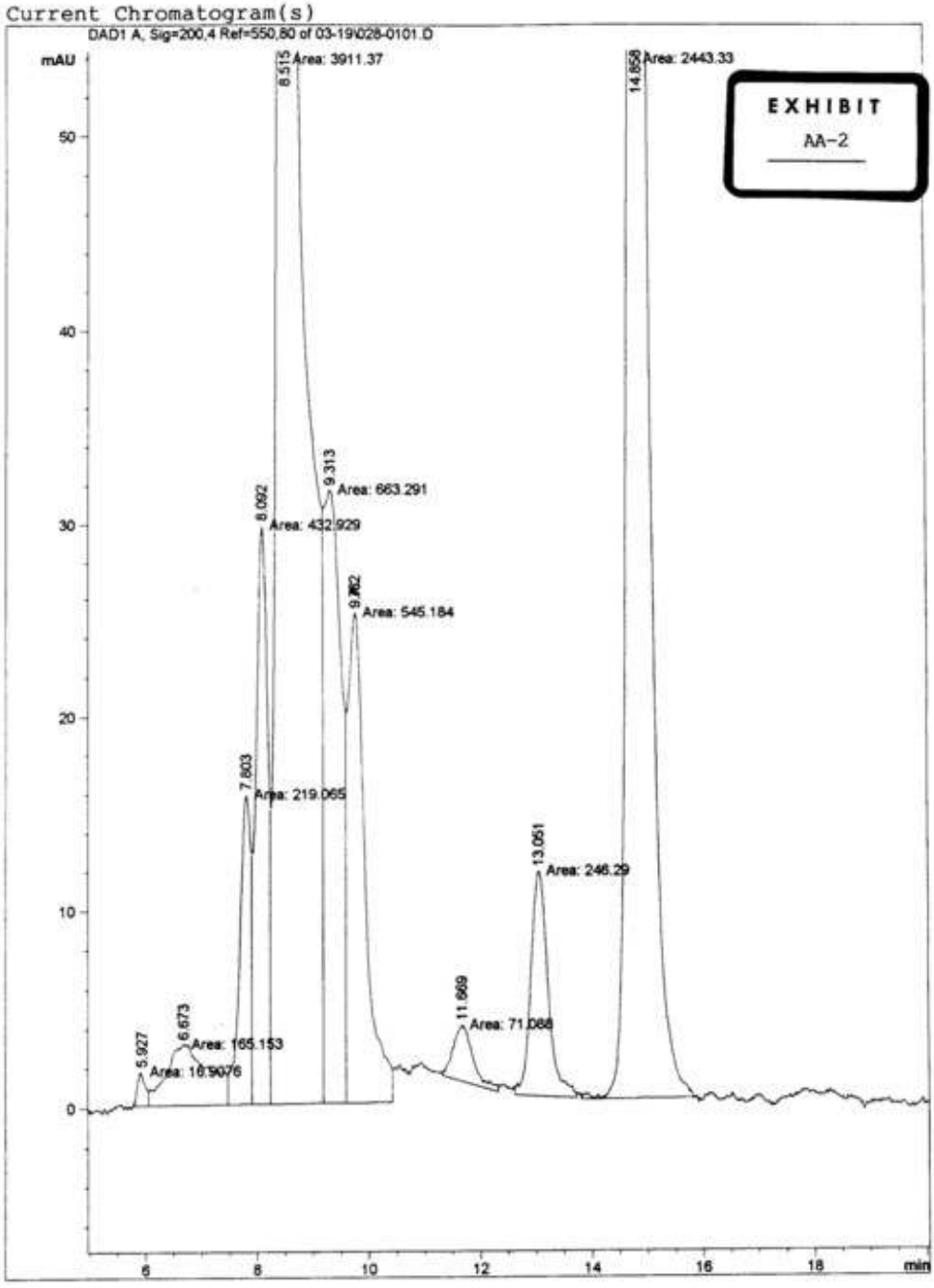
Michael Roussin - hybrid ferment

Here's the hybrid: The last at home ferment we are going to look at is mine. It is a hybrid we've labeled "AA". It is the result of one of the experiments we conducted back in 1995. The sample examined came from the fifth generation, and was taken on March 16, 1996. The ferment was prepared using 3 quarts of bottled water, 1 cup of white sugar and five regular-size green tea bags. Several bacteria and one yeast were bred out of this colony. This type of ferment had a very slow development, and was usually quite pleasant at about 20 to 30 days. They have never been ready for harvest in less than 20 days, although we have sampled it at 7 and 14 days for analytical purposes. They have a very mild flavor with very little acetic acid in ratio to the other sugar acids.

The analysis of the 3-16-96 sample is shown in the chromatogram (EXHIBIT AA-2) and showed the following compounds in solution:

	AA-2 mg/ml
Glucosamine	0.06
Oxalic acid	0.84
Saccharic acid	0.00
Glucuronic acid	0.00
2-keto-gluconic acid	1.10
5-keto-gluconic acid	2.20
Gluconic acid	20.06
Fructose	11.03
Succinic acid	0.36
Itaconic acid	0.12
Acetic acid	12.00

Current Chromatogram(s)



HP LC System Saturday, April 13, 1996 5:54:21 PM by REM

ANALYSIS OF COMMERCIAL GROWERS

We offered to perform analyses of ferments produced by commercial growers in exchange for publishing privileges. Several growers took us up on the offer; several others did not. The commercial growers who sent us colonies for testing are: Harmonic Harvest in Harwood, Texas; Diane Minden in Klamath Falls, Oregon; Avalon Springs in Atlanta, Georgia; Kombucha Manna in Croton, New York; and Natural Kombucha Farm in Grayson, Georgia. In addition, we purchased colonies from Kombucha Magic Mushroom Farm in New York, New York; Laurel Farms in Studio City, California; and Paper Ships in San Anselmo, California.

As with the non-commercial growers, we prepared each ferment according to the instructions we received with the colony. We analyzed samples of the sugar and tea mixture, the starter tea, and the ferment at various stages.

Harmonic Harvest — Ariana Estelle, Ph.D.

The first colonies to arrive came from Ariana Estelle, Ph.D., at Harmonic Harvest, PO Box 82, Harwood, Texas, 75632. Ariana is an active member of the Kombucha mailing list and is also the editor and publisher of the monthly Kombucha newsletter "Kombucha Konnection". She is also the author of Kombucha 101 A Kombucha Primer published by Pathways Press.

Ariana submitted three colonies for testing. Two are her regular ferment and the third is a ferment that she obtained from Gunther Frank. This was a blind study, so information on which colonies were hers and which came from Gunther Frank was not disclosed until after the findings were submitted to her. She labeled her ferments A, B, and C and these became our ferments "C," "D," and "E".

The three colonies were packaged in one quart "one zip" bags and placed inside a one gallon "one zip" bag. One of the ferments had leaked inside of the gallon bag, but there was no leakage from the larger bag. Each colony was about a one quarter cutting of what appeared to be gallon jar sized ferments. In accordance with her instructions, we prepared each ferment with three quarts of bottled water, one cup of white sugar and five black tea bags. The water was brought to a boil in a four quart Visionware casserole and the sugar was added and boiled for five additional minutes. The sugar water was removed from the heat and the tea bags added and allowed to steep for 10 minutes. The tea bags were removed, and the sugar and tea solution was allowed to cool to room temperature in a covered one gallon sun tea jar. On 11-3-95 when the sugar and tea solution was cool, the entire contents of one zip bag was added.

Samples were taken on 11-3-95, 11-14-95, 11-25-95 and 12-3-95. As is common with colony cuttings that float, two of the resulting offspring were not very uniform,

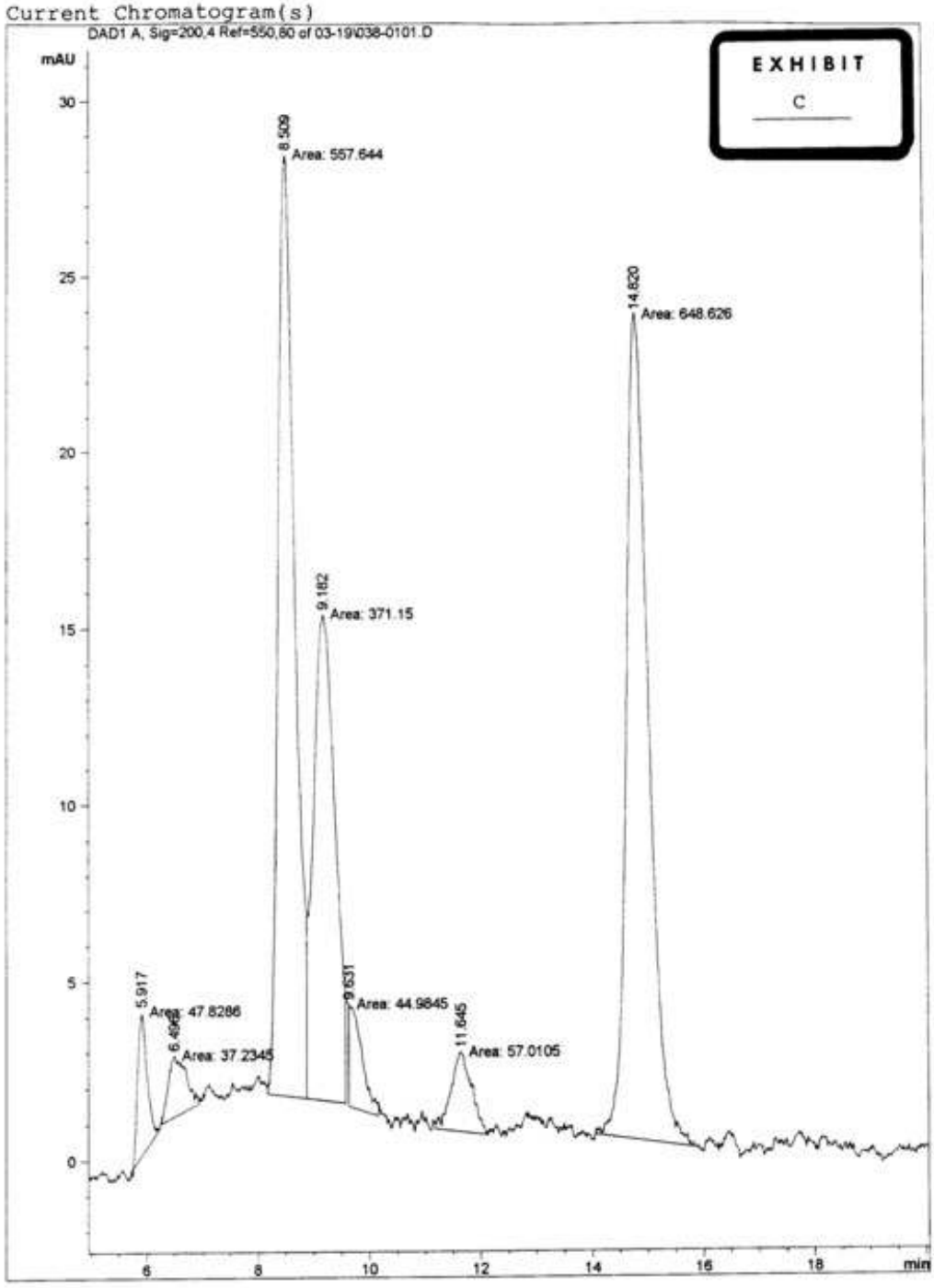
thicker in the areas that came in contact with the parent cutting. When the samples were taken on 11-14-95, the “C” colony was thin on one side and about 3/8” thick in the areas surrounding the parent cutting. It had a very good apple cider flavor. The “D” colony was almost identical in appearance and flavor. The “E” colony was fully formed, about 3/8” thick, and had a unique flavor, almost like pickle. It was very different, and difficult to describe.

When samples were taken on 11-25-95, the “C” colony was fully formed and 5/8” thick with two thin areas from trapped CO2. The aroma was neutral with lots of CO2 and the flavor was quite tart with a slight vinegar aftertaste. The “D” colony was ½” thick with a several small thin areas from trapped CO2. Like the C colony, this one had a lot of CO2. The aroma was of cider and the flavor was tart with a slight vinegar bite, but still quite drinkable. The “E” colony was 5/8” thick with visible strata. The aroma was bland but the flavor was quite good with a slight vinegar aftertaste. The CO2 was minimal, but present.

The analyses of the 11-14-95 samples revealed the following in their chromatograms (see EXHIBITs C, D, and E):

	C mg/ml	D mg/ml	E mg/ml
Glucosamine	0.24	0.23	0.00
Oxalic acid	0.19	0.31	0.18
Saccharic acid	0.00	0.25	0.00
Glucuronic acid	0.00	0.00	0.00
2-keto-gluconic acid	0.00	0.99	0.13
5-keto-gluconic acid	0.00	0.00	0.00
Gluconic acid	2.82	3.60	2.71
Fructose	6.30	24.02	25.09
Succinic acid	0.29	0.39	0.21
Itaconic acid	0.00	0.14	0.00
Acetic acid	3.20	4.61	3.83

Current Chromatogram(s)



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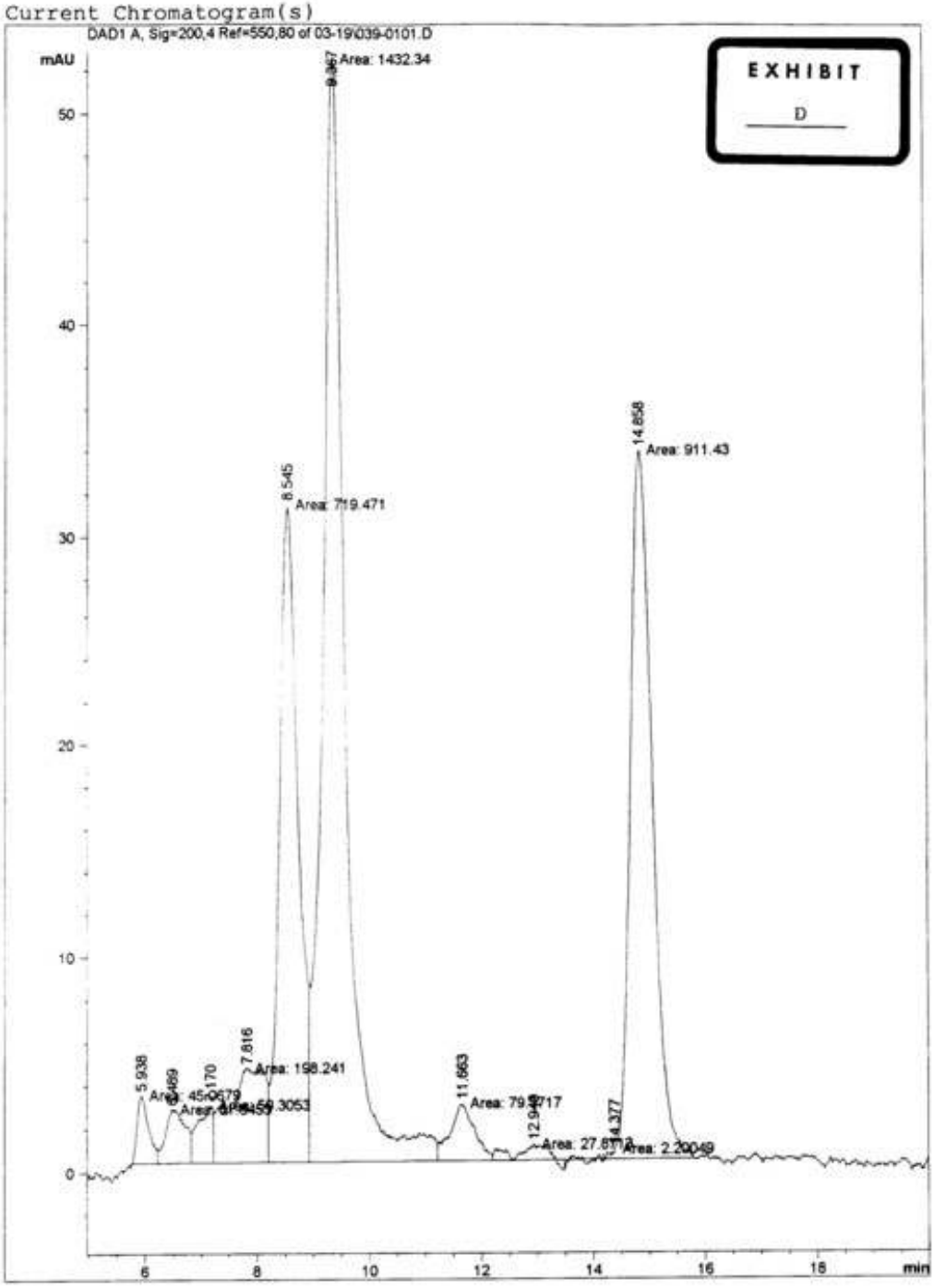

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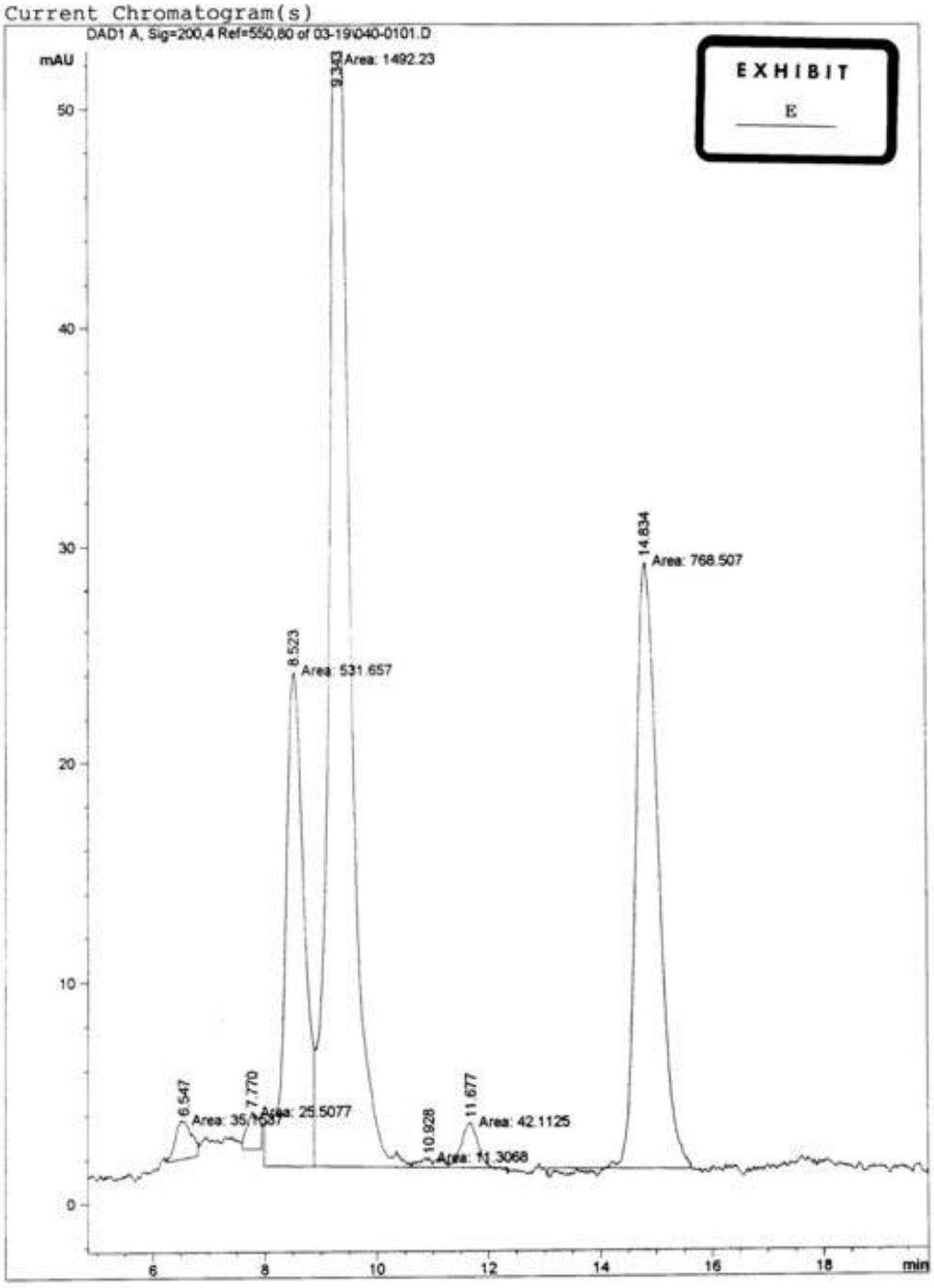

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Diane Minden

The next samples came from Diane Minden, Full Circle Press, 1121 Lincoln, Klamath falls, Oregon 97601. Diane is the author of Kombucha Health Drink of the Ages published by Full Circle Press. Diane was an occasional contributor to the Kombucha mailing list and maintained her own web site. Diane also submitted three colonies, again with one being from Gunther Frank as part of a blind study. The colonies were received on 11-31-96 and each appeared to be ½ of a one gallon colony. These colonies were received in a "seal-a-meal" bag packaged inside of a one quart Ziplock bag. This is an excellent packaging method since the sealed bag must actually be broken for any leakage to occur. She had labeled her samples A, B, and C, which became our "F," "G," and "H" respectively.

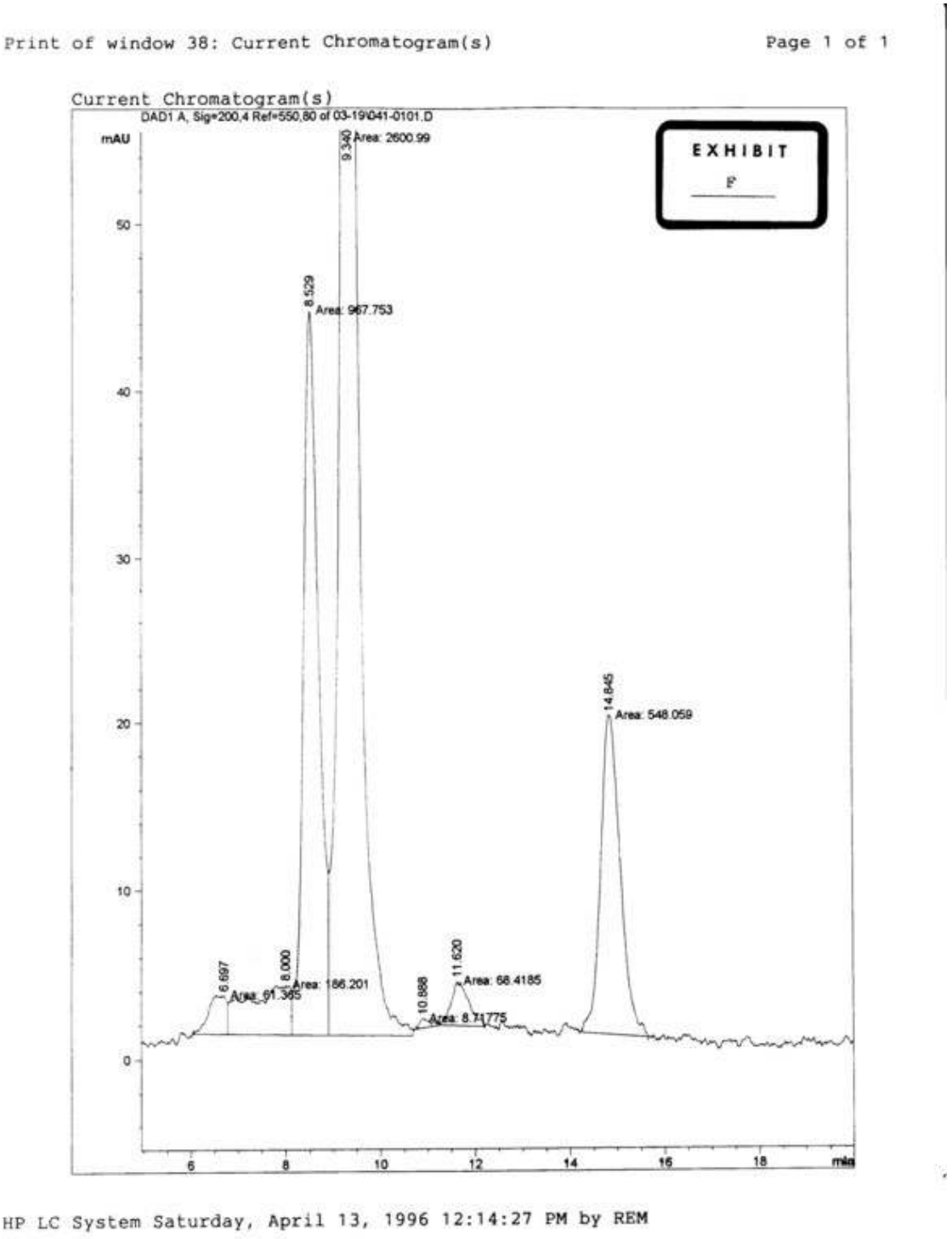
The ferments were prepared in accordance with her instructions using three quarts of bottled water, one cup of white sugar and five black tea bags. Preparation was by the standard method of bringing the water to a boil, adding the sugar and boiling for five minutes, removing the sugar water from the heat and adding the tea bags. The tea was allowed to steep for 10 minutes, and the tea bags were removed. The sugar and tea solution was allowed to cool to room temperature in covered one gallon sun tea jars. Samples were taken on 11-4-95, 11-14-95, 11-25-95 and 12-3-95.

When samples were taken on 11-14-95, all three colonies were fully formed and about 3/8" thick on the side where the parent made contact, and still quite thin on the area away from the parent. Colony "F" was still quite sweet while colony "G" was slightly tart. Colony "H" had a nice apple juice flavor. When samples were taken on 11-25-95, the "F" colony was about 3/8" with one large CO2 bubble. The ferment was cloudy, but there was good CO2 and a unique flavor, like sweet pickle vinegar. The "G" colony was 5/8" thick and looking very good with a light butterscotch color. The smell and flavor were both very sour, but not like vinegar, more like acidic apple juice. Plenty of CO2 was present in this ferment. The "H" colony was ¾" thick with visible strata observable on the colony. The smell was Kombucha and the flavor was very sour. This too had a lot of CO2.

The analyses of the 11-14-95 samples revealed the following in their chromatograms (see EXHIBITs F, G, and H):

	F mg/ml	G mg/ml	H mg/ml
Glucosamine	0.00	0.28	0.21
Oxalic acid	0.31	0.28	0.13
Saccharic acid	0.00	0.40	0.20
Glucuronic acid	0.00	0.00	0.00
5-keto-gluconic acid	0.93	0.00	0.00
2-keto-gluconic acid	0.00	0.89	0.84
Gluconic acid	4.81	3.30	3.81

Fructose	44.02	18.09	20.05
Succinic acid	0.34	0.40	0.27
Itaconic acid	0.00	0.13	0.22
Acetic acid	2.72	4.50	4.62



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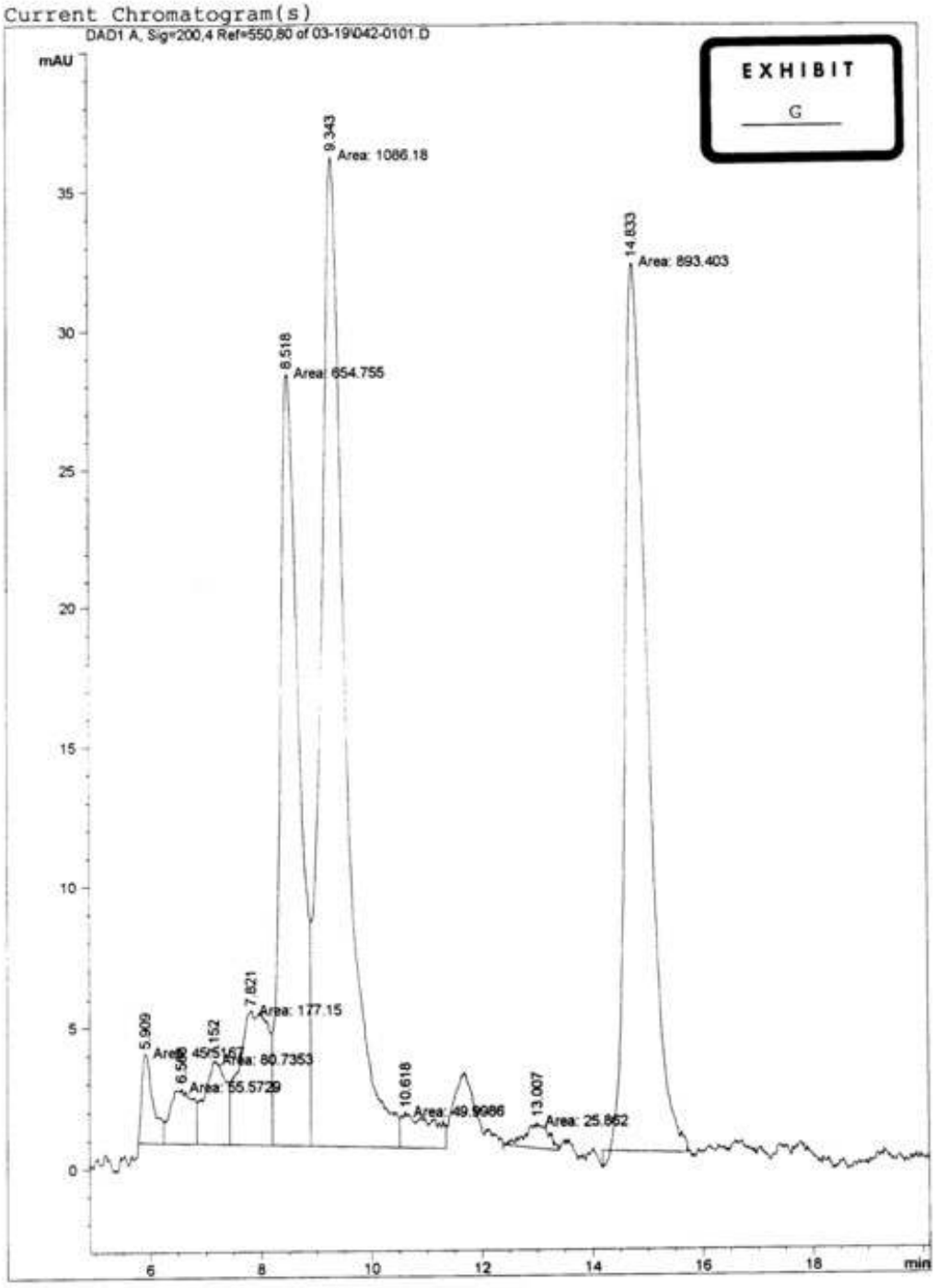

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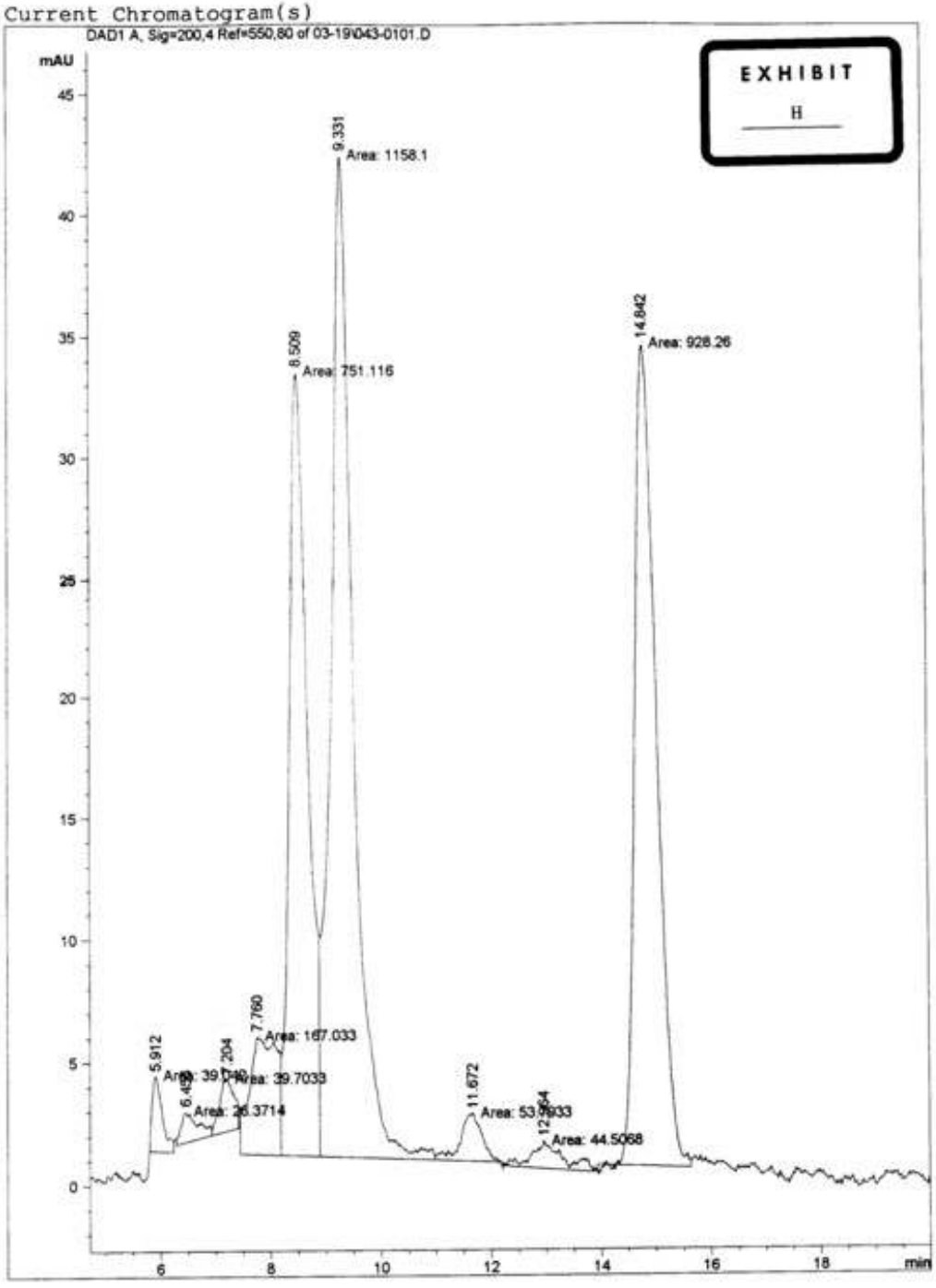

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Natural Kombucha Farms - Ed Laughlin

The next colonies we received came from Ed Laughlin at Natural Kombucha Farm, PO Box 686, Grayson, GA 30221. In addition to Kombucha colonies, they also produce Kombucha extract. The colonies that we received in November 1995 were fully formed and folded over in double Ziplock freezer bags. The colonies were of a size and appearance consistent with being fermented in a large bowl or squat one gallon fishbowl. We followed the instructions we received with the colonies, which allowed for either black tea or green tea. Because of this, we fermented one batch in green tea and the other batch in black tea. The colonies were labeled A and B. Ed also sent us a sample of their extract. We labeled the colonies "L" and "M," respectively and the extract "N".

The ferments were prepared on 11-4-95 using 3 quarts of bottled water, 4 tea bags and one cup of white sugar. The L ferment used black tea bags and the M ferment used green tea bags. The extract was sent to the lab unopened. Preparation was by the standard method of bringing the water to a boil, adding the sugar and boiling for five minutes, then removing the sugar and water from the heat. The tea bags were added and allowed to steep for 10 minutes. The tea bags were removed, and the sugar and tea solutions were allowed to cool to room temperature in covered one gallon squat fishbowls. When the sugar and tea solution was cool, we added the entire contents of one of the Ziplock bags.

Samples were taken on 11-4-95, 11-14-95, 11-25-95, and 12-3-95. When the 11-14-95 samples were taken, both colonies were fully formed and perfect. Both colonies were 3/8" thick and very light in color. The "L" ferment was still quite sweet and the "M" ferment tasted slightly sweet with a hint of mosel, almost like wine When samples were taken on 11-25-95, the colonies were both 5/8" thick. The "L" colony had visible strata which was not apparent in the "M" colony. The "L" colony had an excellent Kombucha aroma and flavor with abundant CO2. The "M" colony also had the traditional Kombucha flavor with just the slightest bite, like good hard cider. When the final samples were taken on 12-3-95, the colonies were both still 5/8" thick and the offspring were perfect duplicates of the parents. The "L" colony had a strong vinegar aroma, but the flavor was not nearly as sour as the aroma implied. The "M" colony had a very strong vinegar aftertaste.

The analyses of the 11-14-95 samples revealed the following in their chromatograms (see EXHIBITs L, M and N):

	L mg/ml	M mg/ml	N mg/ml
Glucosamine	0.00	0.00	0.10
Oxalic acid	0.00	0.22	0.11
Saccharic acid	0.82	0.73	0.00
Glucuronic acid	0.00	0.00	0.00
2-keto-gluconic acid	2.00	1.50	0.00
5-keto-gluconic acid	0.00	1.10	2.30

Gluconic acid	7.84	7.80	18.03
Fructose	21.06	21.03	9.07
Succinic acid	0.37	0.35	0.69
Itaconic acid	0.38	0.53	0.46
Acetic acid	2.10	1.70	12.10

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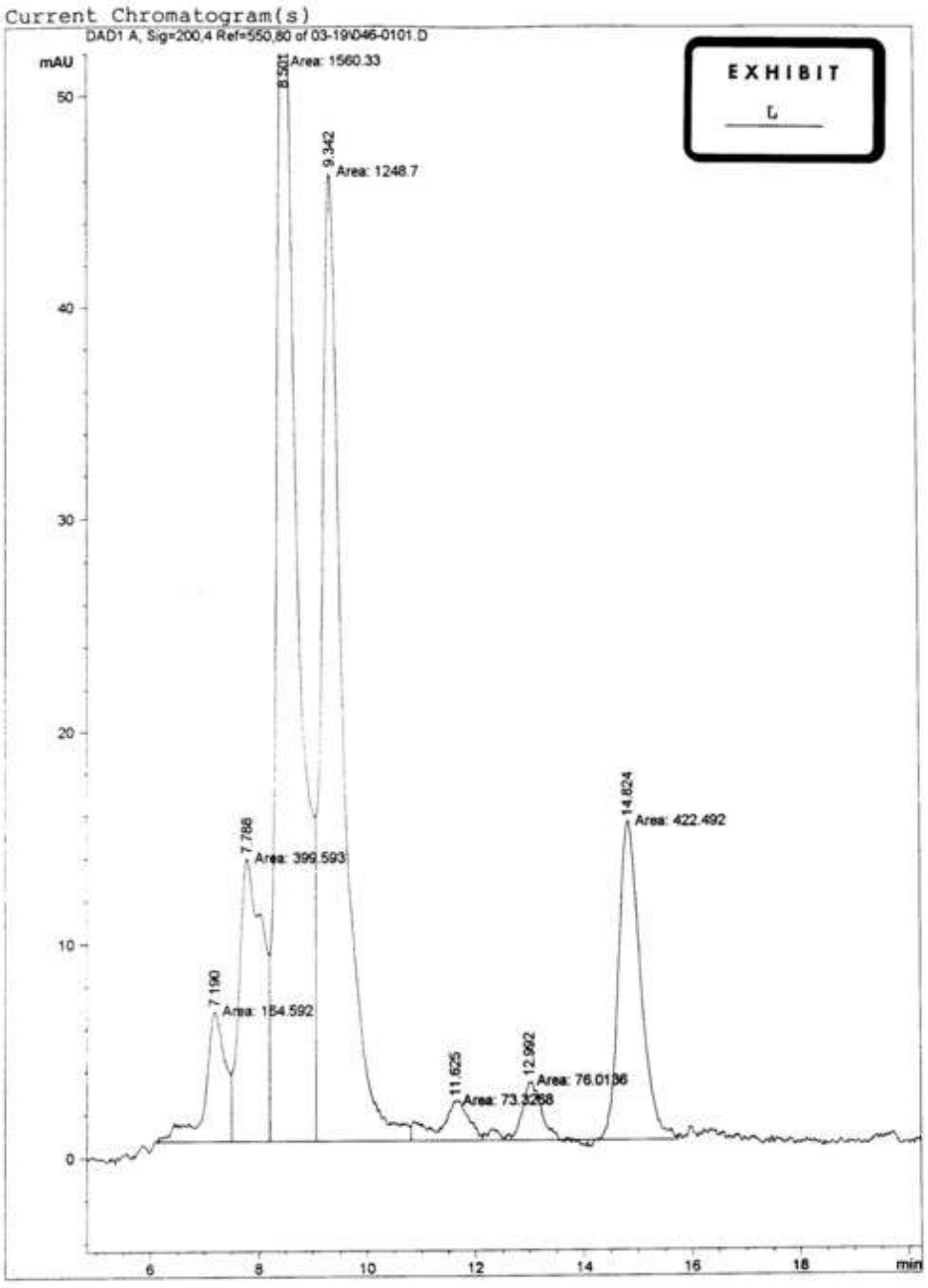

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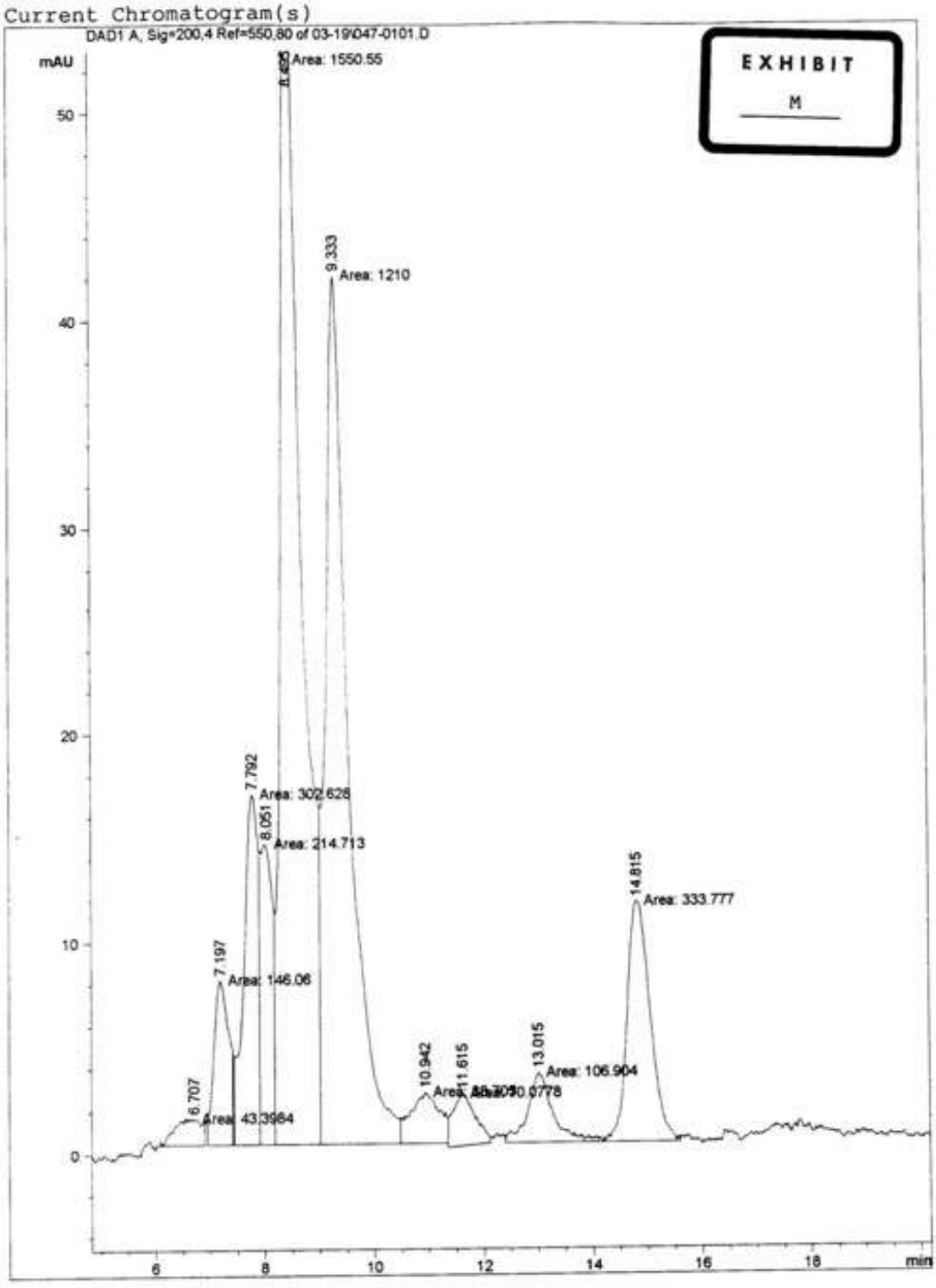

EXHIBIT M

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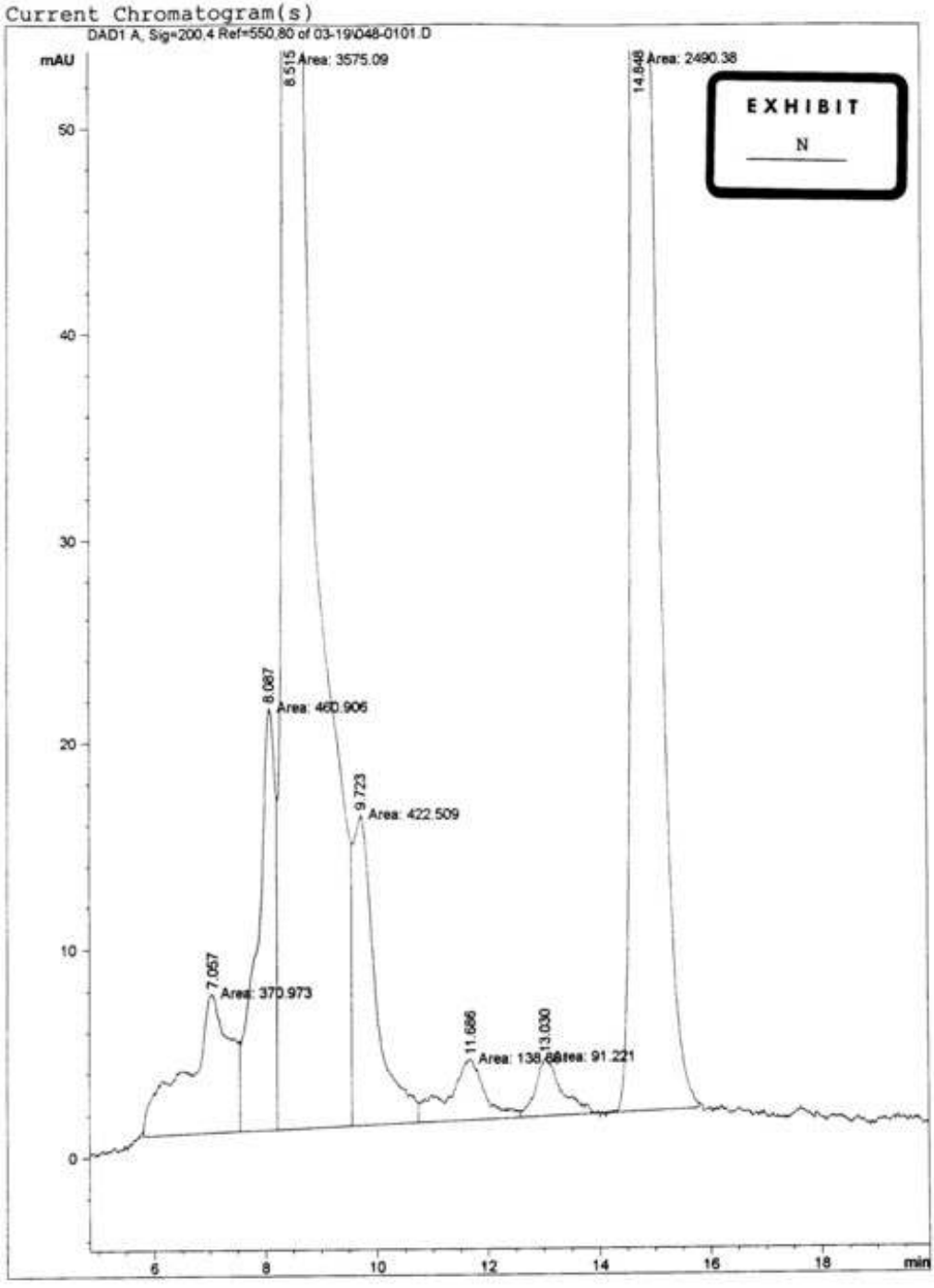

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Kombucha Manna — Beverly Ferguson

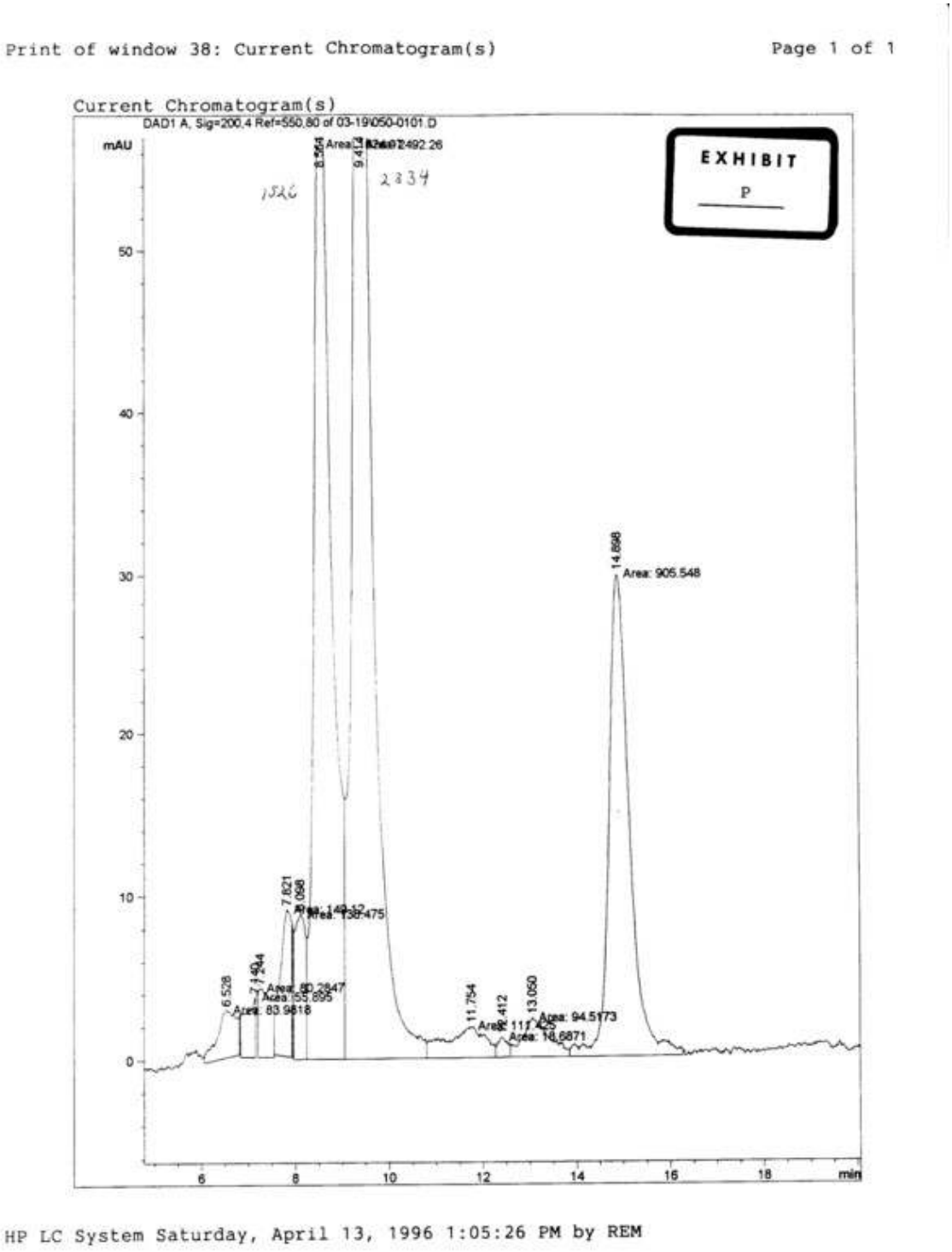
The next colonies received came from Beverly Ferguson at Kombucha Manna, Inc., 2 Alexander Lane, Croton, New York 10520. In addition to sending two colonies, Beverly also sent a bottle of Kombucha drops in alcohol. Kombucha Manna also prepares other Kombucha products, including hair and skin products and herbal Kombucha extracts. They have also begun to prepare Kombucha drops that are not stored in alcohol. Kombucha Manna, Inc. has a web site at URL *http:/users.bestweb.net/~om/kmi/*

She labeled her ferments A and B. We labeled them "P" and "Q" respectively. Additionally, we labeled the Kombucha drops R and sent them unopened to the lab. The colonies were packaged in one quart Ziplock bags inside of one gallon Ziplock bags. The colonies were consistent with the size and shape of growing in a large bowl or fishbowl. The colonies were thin like a tortilla with a light tan coloring. We prepared the ferments in accordance with the instructions we received with them. Unlike the usual fermentation method, this method calls for 4 quarts of bottled water, one and 2/3 cups of sugar, and 5 organic black tea bags. Kombucha Manna provided the organic tea bags for preparing the ferments. Each ferment was prepared by adding the sugar to one quart of water and bringing the water to a boil while stirring the sugar constantly. Once the water boiled, it was removed from the heat, and the tea added and allowed to steep for 15 minutes. The tea bags were removed and the sugar and three quarts of room temperature water were added. The sugar and tea solution was then transferred to a two gallon fishbowl, and the contents of one of the Ziplock bags were added to start the ferment.

Samples were taken on 11-19-95, 11-28-95, 12-3-95 and 12-15-95. When the 11-28-95 samples were taken, both colonies were ¼" thick and tasted quite sweet. When the 12-5-95 samples were taken, both colonies were still thin and dome shaped from trapped CO2. They had sort of a tanned leather appearance where the CO2 had lifted the new colony out of the nutrient solution. While the "P" colony still had a sweet flavor, the "Q" colony tasted like sweet vinegar. When the final samples were taken on 12-3-95, the "P" colony was 3/8" thick and still domed. The taste was sour like apple cider vinegar, but tolerable. The "Q" colony was ½" thick and smelled and tasted like vinegar.
The analyses of the 11-28-95 samples revealed the following in their chromatograms (see EXHIBITs P, Q and R):

	P mg/ml		Q mg/ml	R mg/ml
Glucosamine	0.00	0.00	0.36	
Oxalic acid	0.43	0.28	0.20	
Saccharic acid	0.40	0.25	0.12	
Glucuronic acid	0.00	0.00	0.00	
2-keto-gluconic acid	0.75	0.69	1.10	
5-keto-gluconic acid	0.69	0.34	0.46	
Gluconic acid	7.60	7.50	14.00	

Fructose	40.06	33.01	44.00
Succinic acid	0.56	0.45	0.34
Itaconic acid	0.47	0.31	0.68
Acetic acid	4.50	5.50	1.50



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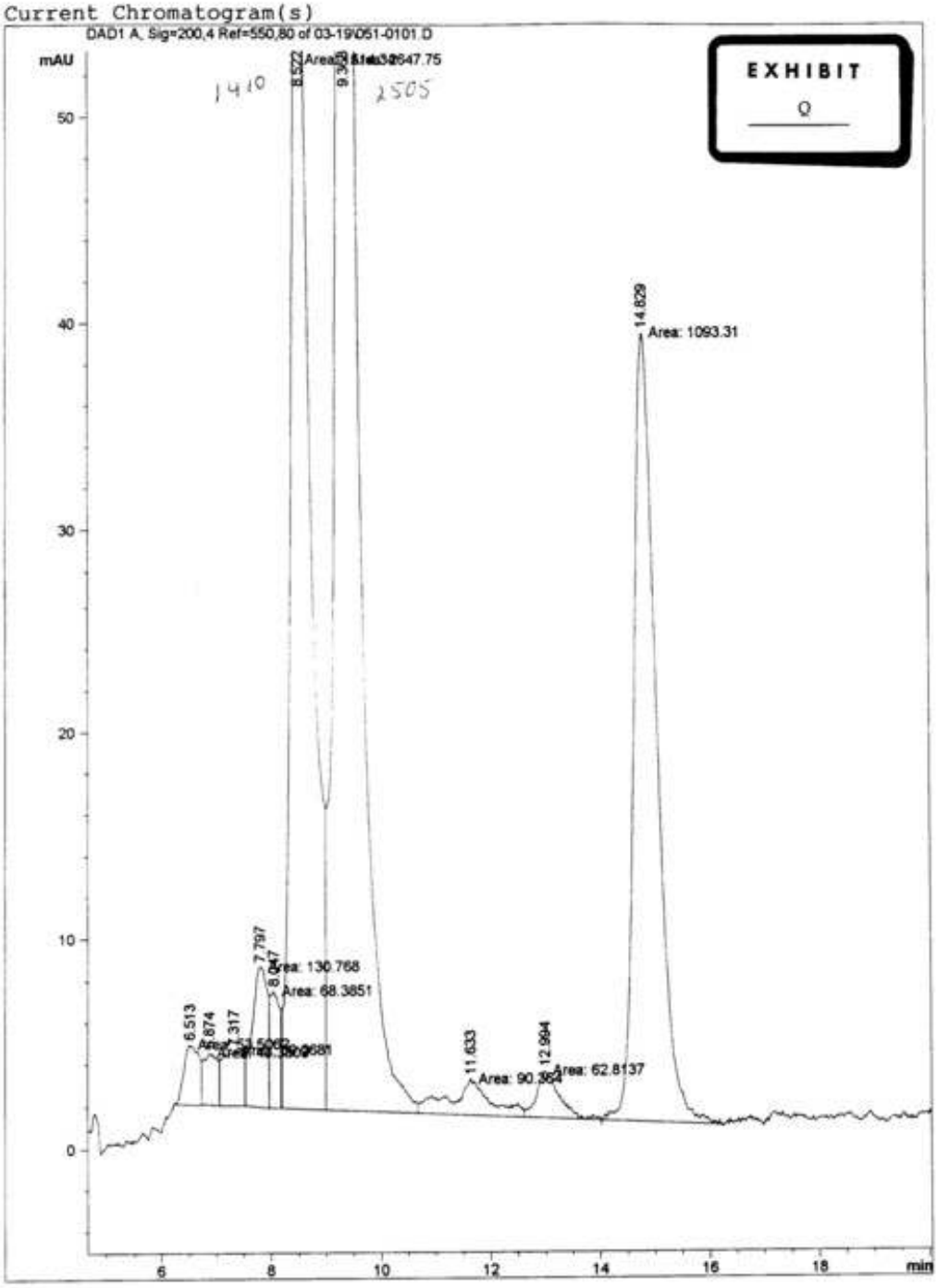

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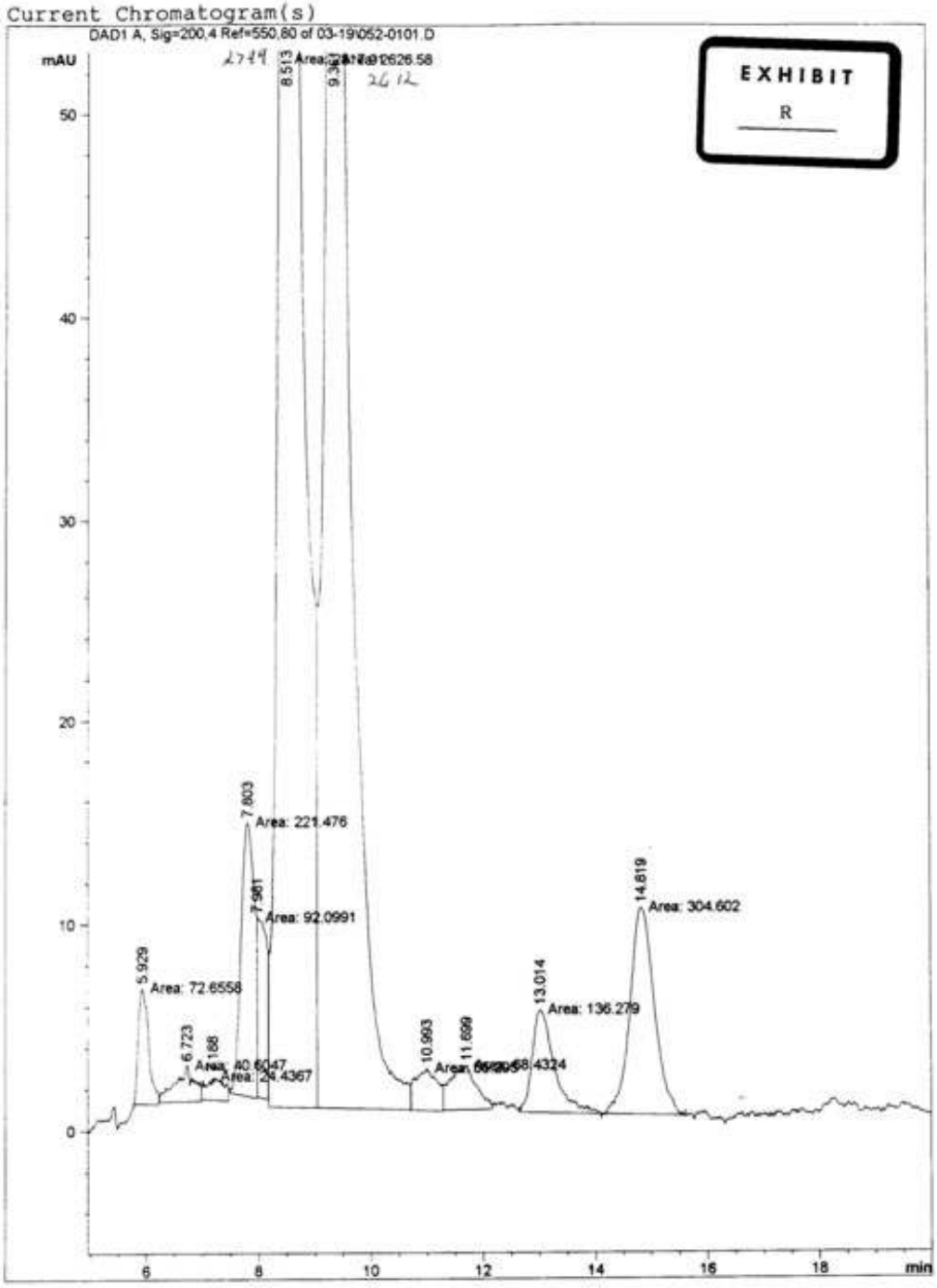

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Avalon Springs — Lisa Bijit

The next sample received came from Lisa Bijit of Avalon Springs, PO Box 250533, Atlanta, Georgia, 30325. Lisa was active on America on Line and on the Kombucha mailing list. She has compiled several papers on the topic and is also actively involved with the AIDS and cancer communities. Lisa is the author of "Everything You Need to Know About Kombucha" published by Kombucha Information Resources (not associated with Information Resources, L.C.). Avalon Springs and Kombucha Information Resources both maintain web sites.

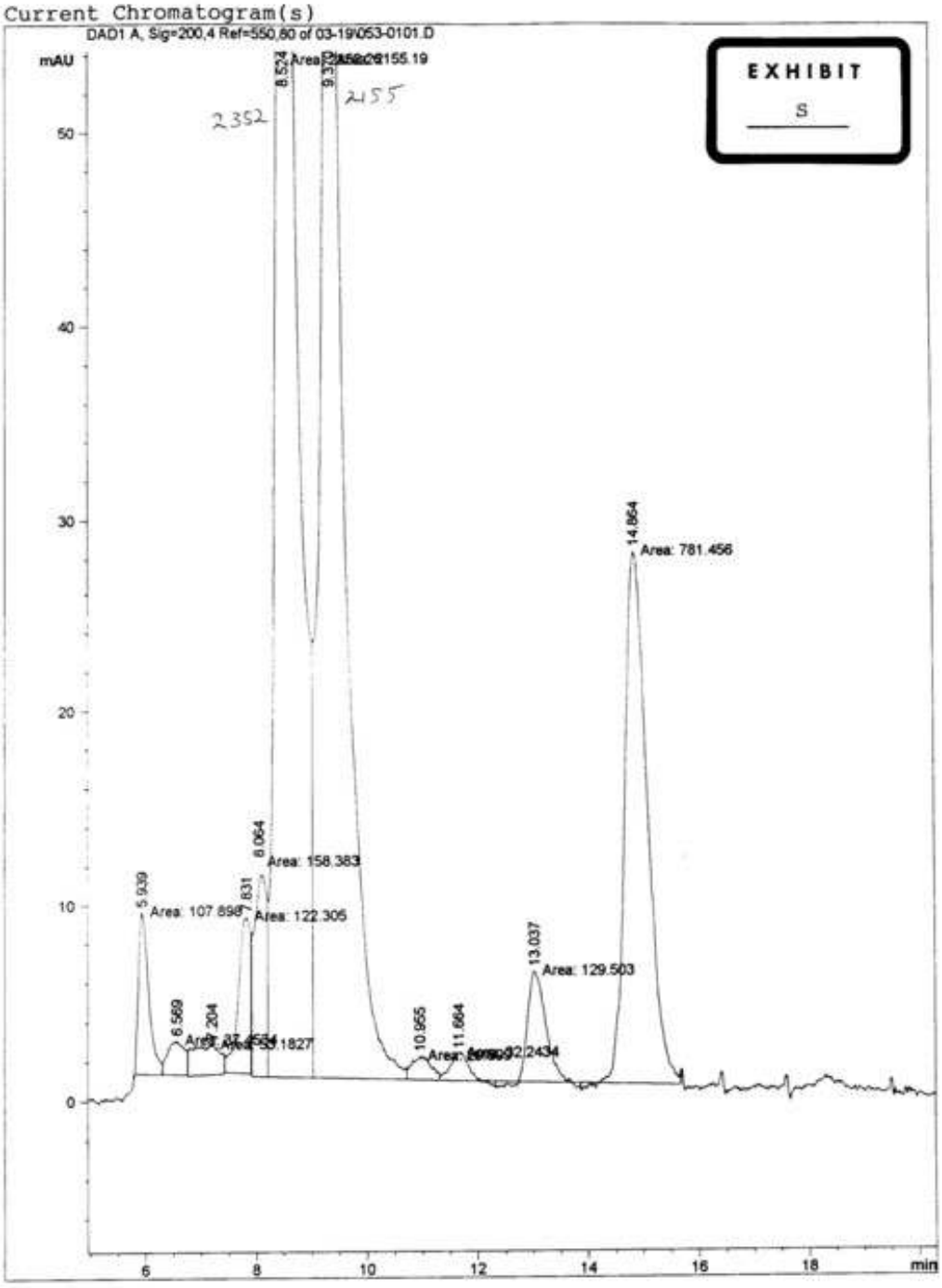
Unlike the other growers, Lisa only submitted one colony. The colony was received in a double Ziplock bag. It was of an odd shape, probably from being fermented in an oval fishbowl. The colony was labeled as our ferment "S" and prepared in accordance with the instructions which came with it. We used 3 quarts of bottled water, 1 cup of white sugar and 5 black tea bags. It was prepared with the standard method of boiling the sugar for five minutes, removing from the heat, and steeping the tea for 10 minutes. The sugar and tea solution was allowed to cool to room temperature in a covered one gallon squat fishbowl. When the nutrient solution was cool, we added the entire contents of the Ziplock bag to the fishbowl.

Samples were taken on 11-19-95, 11-28-95, 12-3-95, and 12-15-95. When the samples were taken on 11-28-95, the colony was 1/8" thick and the tea was still sweet. When the samples were taken on 12-3-95, the colony was about ¼" thick and while the aroma was sort of bland, the flavor was very good. It was a traditional Kombucha apple cider flavor. When the last sample was taken on 12-15-95, the colony was ½" thick and the ferment was quite sour, but still drinkable.

Analyses of the 11-14-95 samples revealed the following in their chromatograms (see EXHIBIT S):

	S mg/ml
Glucosamine	0.54
Oxalic acid	0.19
Saccharic acid	0.25
Glucuronic acid	0.00
2-keto-gluconic acid	0.61
5-keto-gluconic acid	0.79
Gluconic acid	12.08
Fructose	37.06
Succinic acid	0.16
Itaconic acid	0.65
Acetic acid	3.90

Current Chromatogram(s)



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Kombucha Magic Mushroom Farms

The first colony we purchased came from Kombucha Magic Mushroom Farms, PO Box 20717, Cherokee Station, New York, New York, 10021-0074. The package was shipped UPS ground to Morrison, Colorado, and forwarded by overnight delivery to Salt Lake City. The package contained the colony in a Ziplock bag and packaged in a tie box with a ribbon and a rose for decoration. The box also contained two plastic (HDPE) fermenting containers. The colony appeared healthy; however, the starter tea had congealed to an almost gelatin consistency.

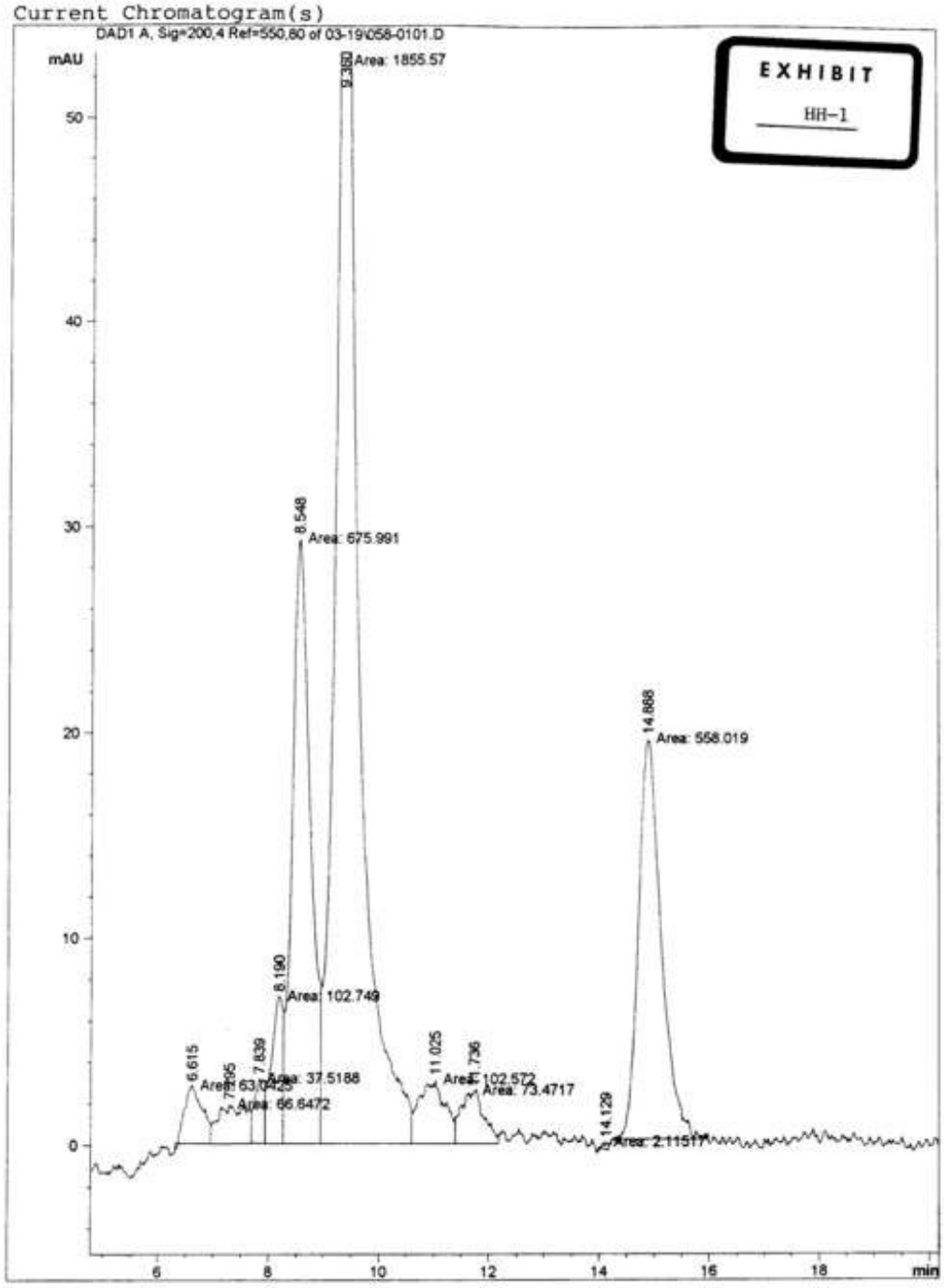
We received the colony and prepared the ferment on 12-2-95, and labeled it our sample W-1. We followed the instructions we received with the colony and prepared the ferment using three quarts of bottled water, one cup of white sugar, and five black tea bags. This ferment was prepared differently than any other. One quart of bottled water was brought to a boil in a four quart Visionware casserole, removed from the heat and the tea bags added. The tea was allowed to steep for 10 minutes and then the tea bags were removed and the sugar added. We transferred the sugar and tea solution to a plastic fermenting container and added two more quarts of room temperature bottled water. We waited until the sugar and tea solution was cool to the touch and added the starter tea and colony.

Samples were taken on 12-2-95, 12-9-95, and 12-16-95. We made a decision to break with the 30 day cycle on this ferment since they had optioned either black or green tea and we only had one colony. We also wanted to start a series of analyses on fermenting in plastic. On 12-16-95, we recycled this ferment into our "HH" and "II" samples using black tea for the "HH" ferment and green tea for the "II" ferment. We then cycled these ferments every seven days for 60 days to study the impact of fermenting in the plastic containers. No negative effects were noted. We will discuss the mass spectrometry of the plastic ferments later in this paper.

On 12-9-95, the colony was ¼" thick and fully formed, with numerous indications of CO2. The ferment smelled and tasted like Kombucha, although the chemist thought he detected a flavor of plastic. On 12-16-95, the colony was 3/8" thick and appeared to be very healthy. It tasted like good cider, but this one surprised me. I suppose that I had a preconceived notion of what Kombucha fermented in plastic would be like, and it was not the negative experience I expected. Samples of the black tea ferment after three generations were taken on 1-13-96 and after 5 generations on 2-4-96. Analyses of these two ferments revealed the following chemical compounds in solution (see EXHIBITs HH-1 and HH-3):

	HH-1 mg/ml	HH-3 mg/ml
Glucosamine	0.00	0.00
Oxalic acid	0.32	0.50
Saccharic acid	0.33	0.06
Glucuronic acid	0.00	0.00
2-keto-gluconic acid	0.19	0.47
5-keto-gluconic acid	0.51	0.23
Gluconic acid	3.40	3.35
Fructose	32.01	20.67
Succinic acid	0.37	0.40
Itaconic acid	0.00	0.00
Acetic acid	2.81	5.00

Current Chromatogram(s)



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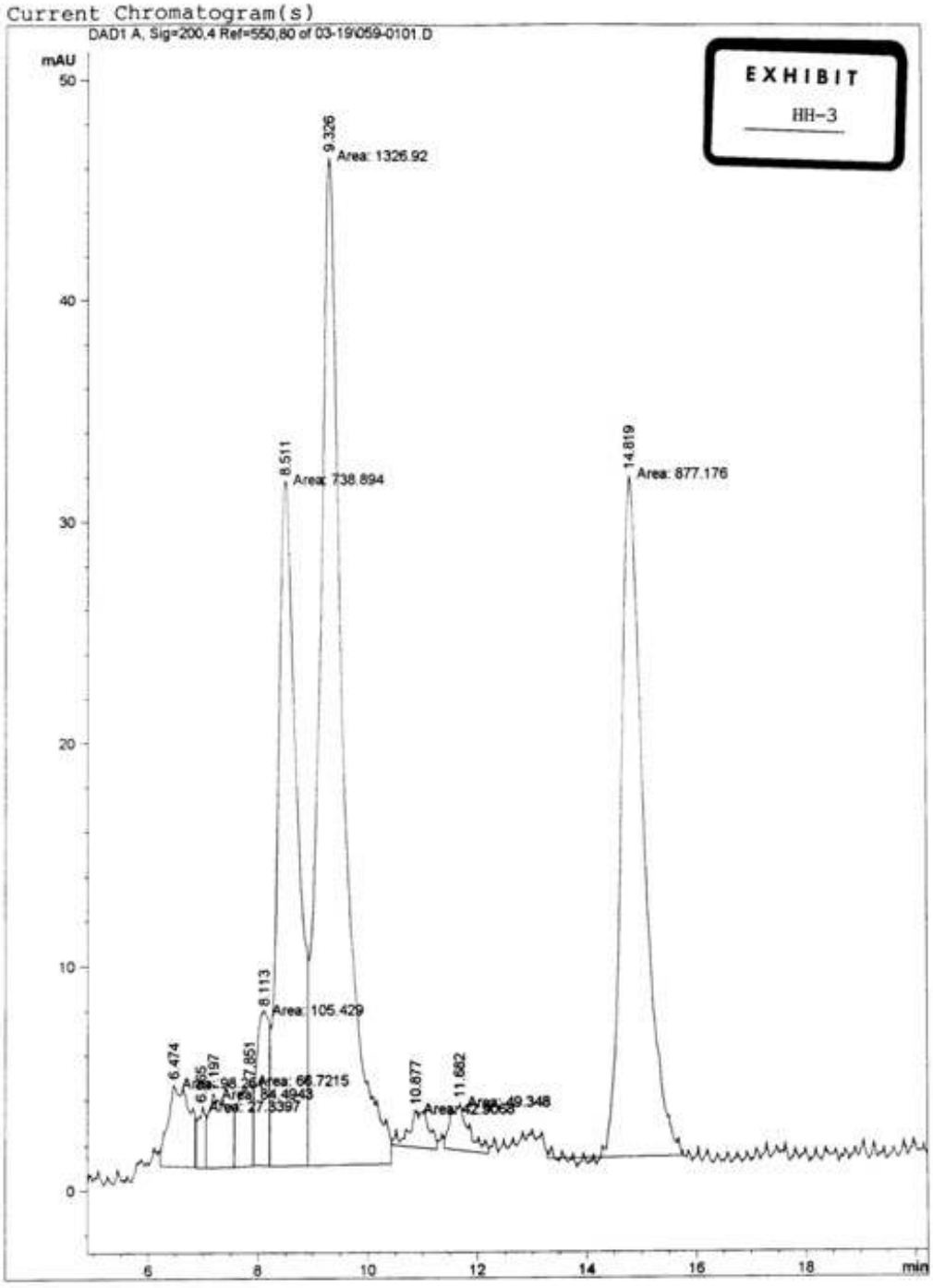

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Laural Farms

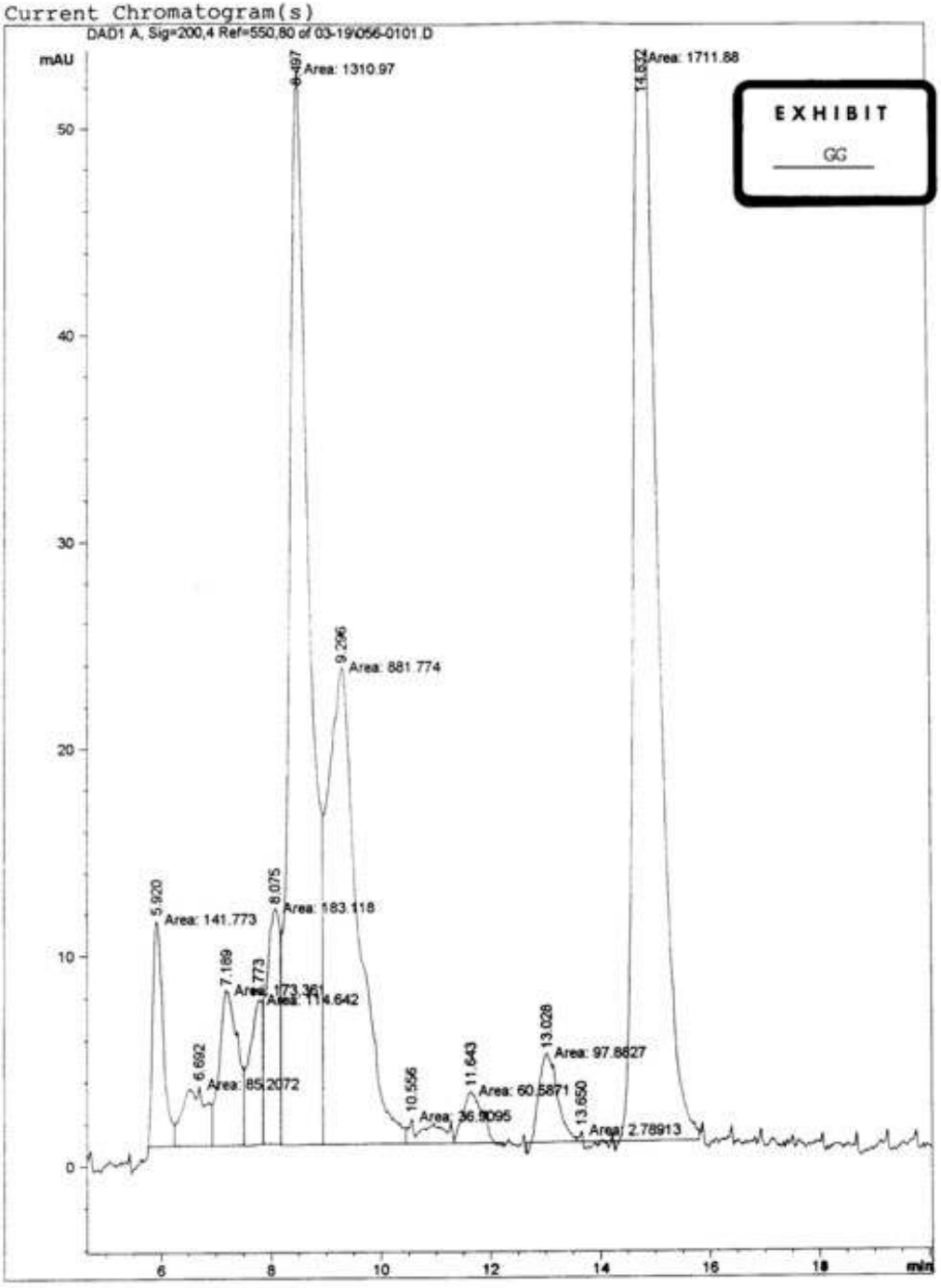
The next colony we purchased came from Laurel Farms, PO Box 7405, Studio City, California, 91614. The colony was shipped to Morrison, Colorado, and traveled the next day to Salt Lake City by car. It arrived on 12-22-95 and was labeled sample "GG". It was packaged in a Ziplock bag inside of a small white box with shredded paper, packaged inside of a priority mailing box with more shredded paper. The colony appeared healthy, and while larger than a gallon jar ferment, it was smaller than a one gallon fishbowl-sized colony.

The ferment was prepared on 12-23-95 in accordance with the instructions that came with the colony. We used three quarts of bottled water, one cup of white sugar and four black tea bags. The preparation was by the standard method: the sugar was added to boiling water and boiled for five minutes, removed from the heat and the tea bags added and allowed to steep for 10 minutes. This was allowed to cool for 20 minutes and was transferred to a one gallon squat fishbowl. We allowed it to cool to room temperature covered with a cotton cloth, and then the starter tea and colony from the Ziplock bag were added to the sugar and tea solution. Samples were taken on 12-23-95, 1-6-96, and 1-17-96. The colony was recycled on 1-17-96, and samples of the next generation were taken on 1-24-96 and 1-29-96. When samples were taken on 1-5-96, the colony was 3/8" thick and looked very healthy. It had a good typical Kombucha aroma and flavor like apple cider. When the samples were taken on 1-29-96, the colony was ½" thick and light colored. It had the typical vinegar flavor and aroma of a ferment that had gone too long. The ferment was harvested and restarted. A sample of the second generation was taken on 1-24-96: the second generation colony was ¼" thick and also looked very healthy. It had a good flavor and color.

Analyses of the samples taken on 1-5-96 and 1-24-96 revealed the following chemical compounds in solution (see EXHIBITs GG and GG-1):

	GG mg/ml	GG-1 mg/ml
Glucosamine	0.71	0.12
Oxalic acid	0.43	0.06
Saccharic acid	0.87	0.65
Glucuronic acid	0.00	0.00
2-keto-gluconic acid	0.87	1.80
5-keto-gluconic acid	0.92	1.80
Gluconic acid	6.64	7.21
Fructose	15.03	17.04
Succinic acid	0.30	0.30
Itaconic acid	0.49	0.24
Acetic acid	8.61	3.40

Current Chromatogram(s)



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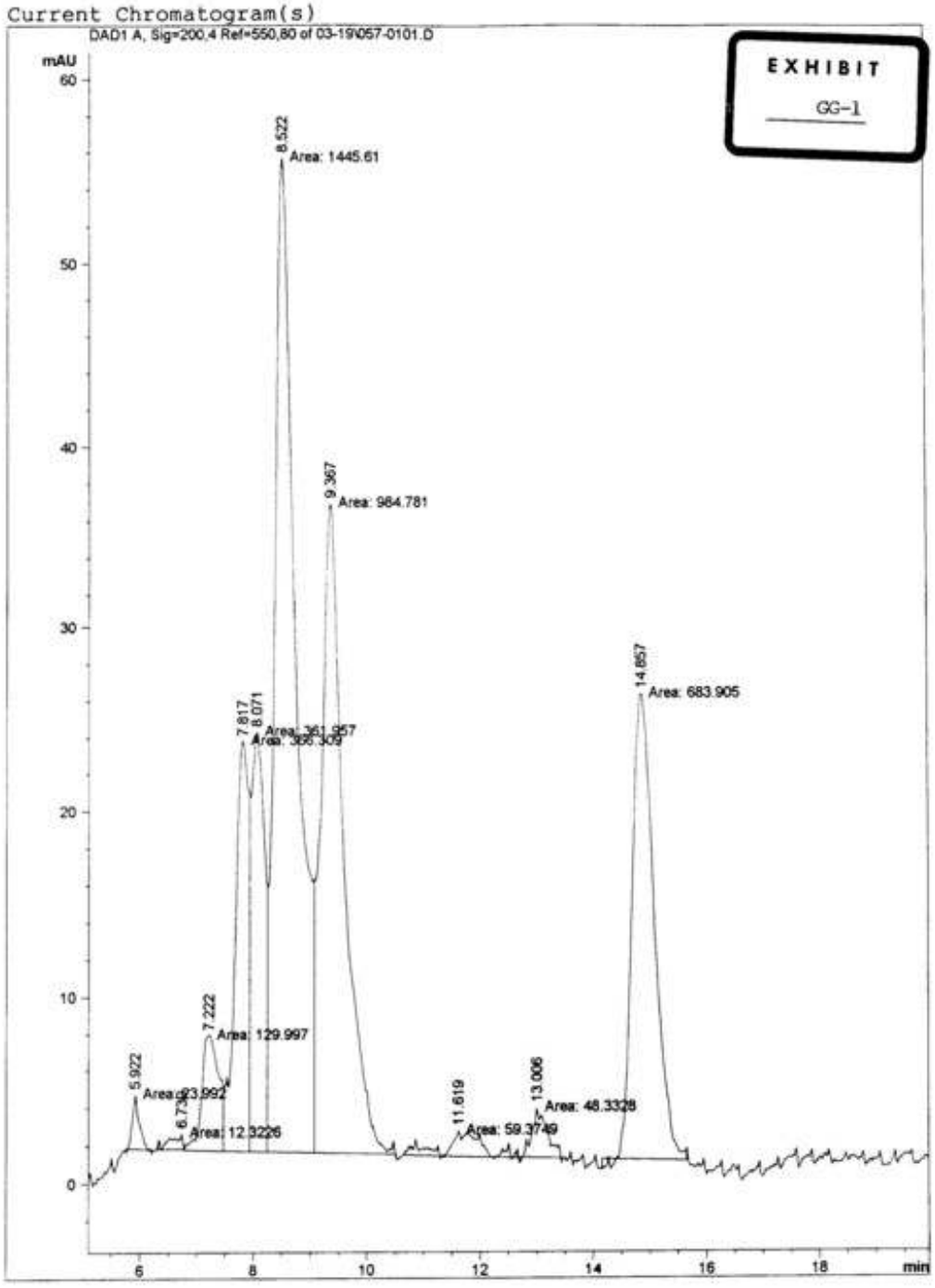

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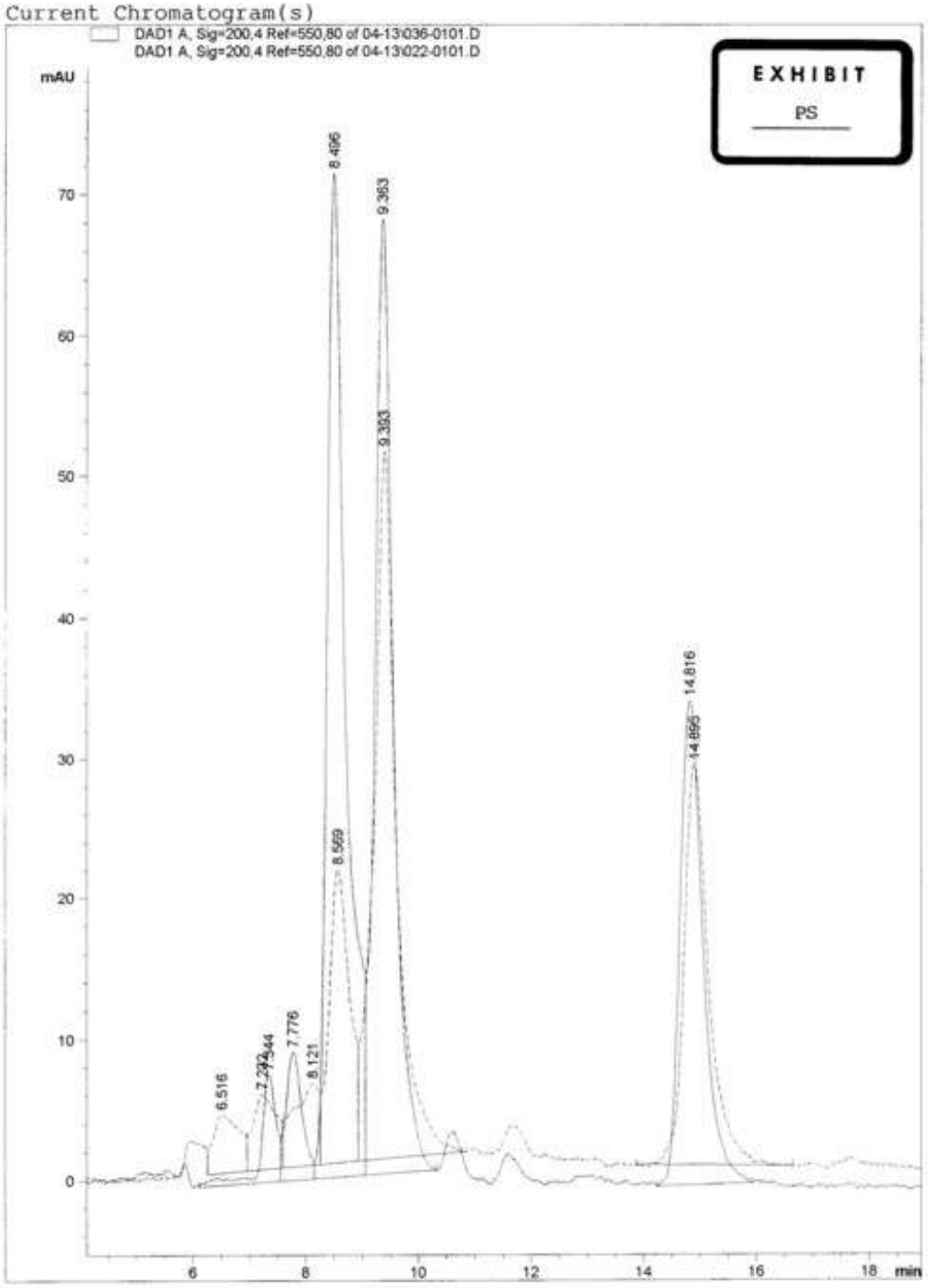
Paper Ships

The last colony we purchased came from Paper Ships, 630 San Anselmo Avenue, San Anselmo, California 94960. We ordered this colony only because it was the source of the colony examined by P. Blanc in his paper "Characterization of the Tea Fungus metabolites." The ferment was labeled "PS" and started on 3-13-96. Samples were taken on 3-24-96 and 4-12-96. At 3-24-96, the ferment already had a mild vinegar flavor, which then controlled the flavor of the ferment on 4-12-96.

The analyses of the 4-24-96 sample revealed the following in its chromatogram (see EXHIBIT PS):

	PS mg/ml
Glucosamine	0.29
Oxalic acid	0.55
Saccharic acid	0.54
2-keto-gluconic acid	0.36
5-keto-gluconic acid	0.20
Gluconic acid	2.21
Fructose	22.09
Succinic acid	0.30
Itaconic acid	0.00
Acetic acid	4.41

Current Chromatogram(s)



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Bottled Ferments and Capsules

ProNatura

The next commercial Kombucha products we obtained were from ProNatura. We analyzed both their bottled ferment and their Kombucha capsules. The labeling on the bottle of capsules stated that it contained not less than 5 percent glucuronic acid. We fully expected it to be there because of the labeling, but as with the bottled ferment, no glucuronic acid was found. We labeled these samples as “PNF” and “PNC” respectively. We also prepared a 100:1 extraction concentration of the bottled ferment.

Here are our findings on the analysis of the ferment and capsules:

	PNF mg/ml	PNC mg/ml
Glucosamine	0.21	5.20
Oxalic acid	0.40	0.00
Saccharic acid	0.58	0.00
Glucuronic acid	0.00	0.00
2-keto-gluconic acid	0.00	0.00
5-keto-gluconic acid	0.00	0.00
Gluconic acid	0.61	2.20
Fructose	22.09	22.00
Succinic acid	0.36	0.30
Itaconic acid	0.00	0.00
Acetic acid	1.93	4.40
Glucuronic Acid	0.00	0.00

The Chemistry of Mold Ferments

We noticed an interesting change in the chemistry of the Kombucha ferments contaminated by molds. For the moment, we shall ignore the ferment with *Mucor*, since it consisted only of fructose in distilled water with a Kombucha colony. That chemistry was expected to be unusual. The two that we're going to discuss are ferments with *Aspergillus niger* and *Penicillium notatum*. The chemistry of these ferments is also unusual, because of the biochemical changes brought about by the molds.

The first ferment we'll discuss is one infested by *Aspergillus niger*. The ferment was prepared on 11-16-95, using the traditional methods of three quarts of water, one cup of white sugar, and five green tea bags. The sugar and tea were allowed to stand at room temperature, uncovered, for about 30 hours. At that time, it was inoculated with 4 ounces of starter and the offspring colony from the glucose only batch (ferment ID 2 from the previous experiment). On 11-28-95, the first samples were taken. The ferment had visible mold growing on the surface. On 12-3-95, the mold had proliferated and there was a full colony of black, grey, and green molds on the surface. Another sample was taken for lab analysis. On 12-29-95 there was a full "carpet" of mold on the surface. It was then greyish black with white sections in it, and the green mold was losing ground to the black mold. There were only remnants of the green mold colony. It had raised itself partway up the side of the jar on one side by about an inch. You could see material hanging from the parent colony into the ferment, which appeared to be granules trapped in a spider's web, like morning dew.

By 1-18-96, the colony had climbed almost two inches up one side of the jar, dragging the colony off the surface as it went. When the last sample was taken on 1-26-96, a thin new colony was forming where the previous colony had been pulled off of the surface of the ferment. The chemistry for these ferments showed one thing in common, and at odds with regular Kombucha ferments, and that was the lack of acetic acid.

The *Aspergillus niger* ferment is shown in the following chromatogram (EXHIBIT V) and the results of our analyses are outlined below.

	V 12-29
Glucosamine	0.00
Oxalic acid	13.30
Saccharic acid	0.00
Glucuronic acid	0.00
2-keto-gluconic acid	4.05
5-keto-gluconic acid	1.92
Gluconic acid	47.01
Fructose	79.02

Succinic acid	1.60
Itaconic acid	0.74
Acetic acid	0.64

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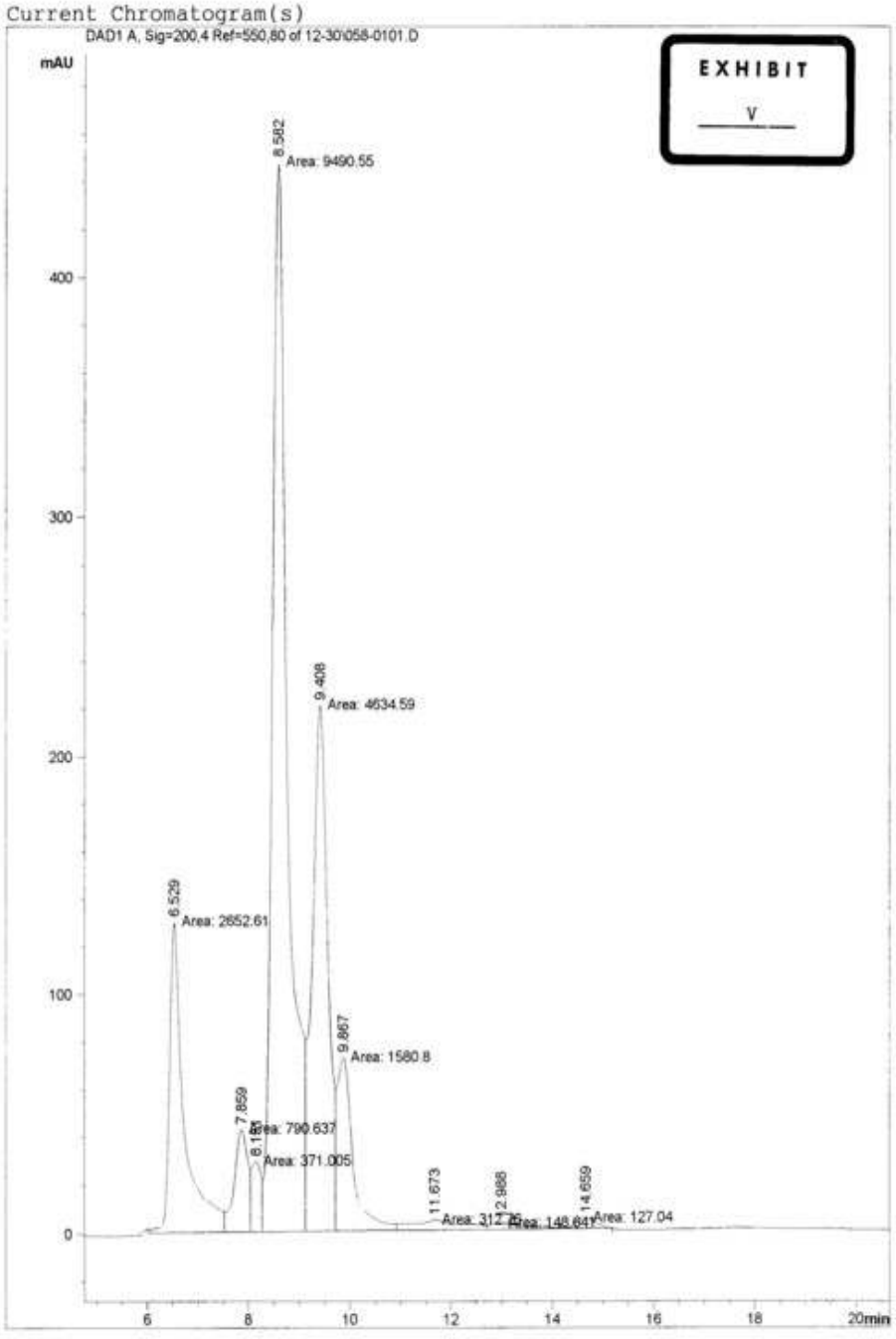

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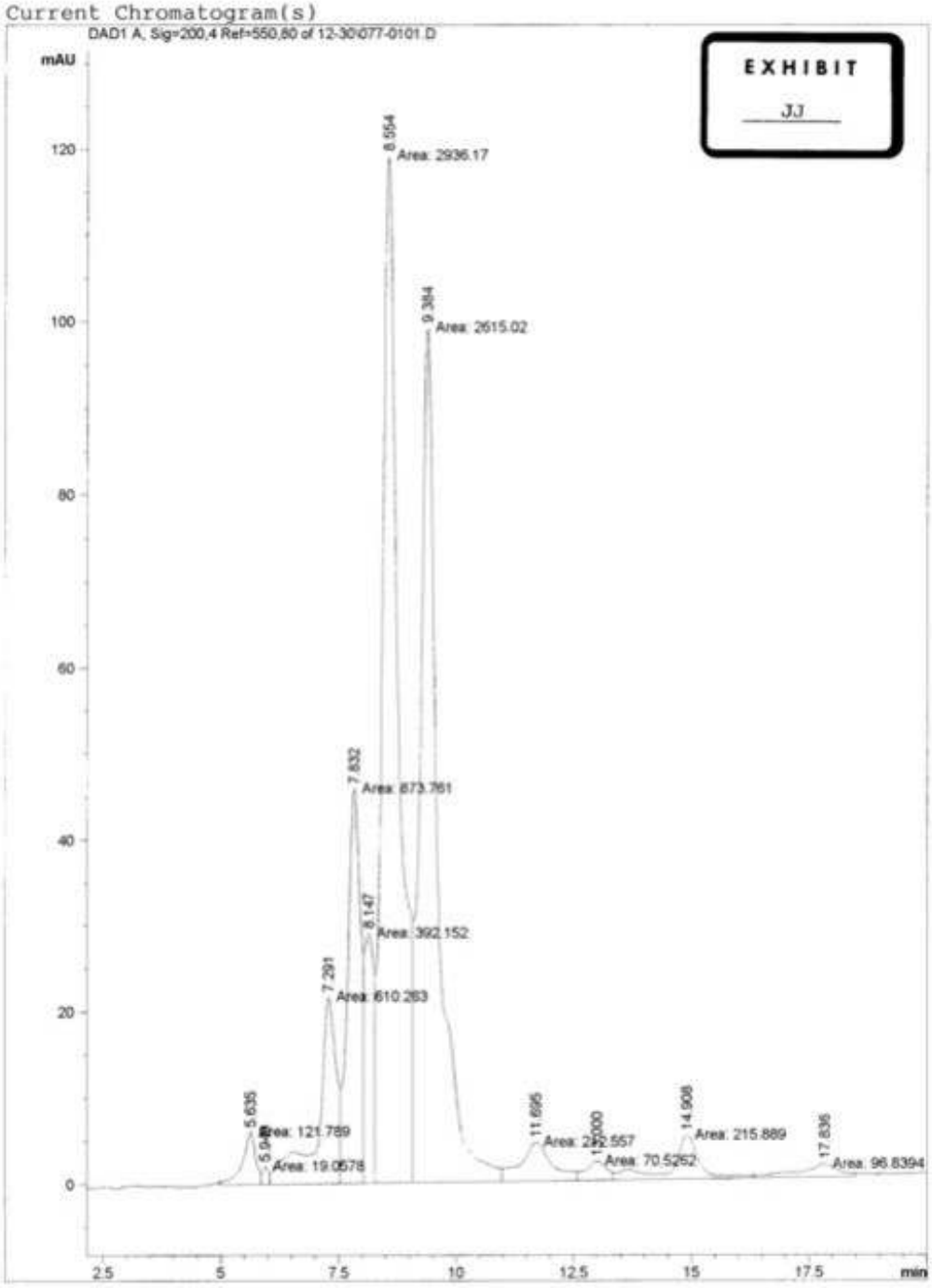
The ferment which produced the *Penicillium notatum* was prepared in a similar fashion, but using black tea, and stood only 12 hours before inoculation. This study was not an experiment, but an error in judgment. We hadn't planned to grow the mold; we just put it in close proximity (the same 11' x 12' room) to the mold we were already growing. We were attempting to restart the "B" colony to do some additional analysis of it. We have many different colonies from this grower that we've analyzed, and so we had a good point of reference for this part of the work. I brought this ferment in and set it on the opposite side of the room. We wanted to take a surface sample from the *Aspergillus* colony, and so I had removed the cotton cloth earlier that day, prior to assembling the "JJ" ferment.

The ferment was assembled on 12-23-95, and by 12-29-95 it had a full canopy of green mold, which was later identified as *Penicillium notatum.* I was disappointed at the loss of this ferment, but it was an opportunity to study a different mold. Samples were taken on 12-29-95, 1-5-96, 1-13-96, and 1-26-96. The analyses of this ferment showed some striking similarities with the *Aspergillus niger* ferment.

An analysis of the *Penicillum notatum* samples taken on 12-29-95 showed the following chemical structures in their chromatograms (EXHIBIT JJ).

	JJ 12-29-95
Glucosamine	0.00
Oxalic acid	1.95
Saccharic acid	3.10
Glucuronic acid	0.00
2-keto-gluconic acid	4.41
5-keto-gluconic acid	2.02
Gluconic acid	15.00
Fructose	44.16
Succinic acid	1.10
Itaconic acid	0.35
Acetic acid	1.12

Current Chromatogram(s)



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All of the previous analyses were conducted using ion chromatography and photodiode array UV analysis. Analyses with mass spectrometry of 100:1 extracted samples revealed many other components of the ferment. We looked at a wide variety of ferments, and selected what we thought was close to the typical ferment for an extraction analysis. We ran the standard cation exchange analyses for the tea and sugar solution and for the ferment after seven days, and then ran 100:1 extraction concentrations of the tea and sugar solution and the ferment. This allowed us to observe which components were in the sugar and tea solution, and which were components of the fermentation process.

The Chemistry of the Kombucha Ferment

Constituents vary from ferment to ferment, as we have already observed from the short list of ferments in this publication. The following is a list of the various acids, acid esters, and other compounds which we have isolated from Kombucha ferments. They are listed alpha-numerically, since what is abundant in one ferment may be totally absent from another ferment.

4-Acetamidophenol

Acetic acid

Acetoacetic acid

Benzoic acid, 2-amino-, 3-phenyl-2-propenyl ester

Benzonitrile, 4-hydroxy-

2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-

2,6-Bis(t-butyl)-4-(dimethylbenzyl) phenol

Butanoic acid, 3-methyl

1-Butanol, 3-methyl-

2-t-Butyl-4-(dimethylbenzyl) phenol

Caffeine

Citric acid

Cyanocobalamin (B-12)

Decanoic acid

D-Ribo-hexos, 2,6-dideoxy-3-0-methyl-

2,3-Dihydro-1-methylindene

2,5 Diketo-gluconic acid

Ethyl Acetate

Fructose

d-Gluconic acid

Glucose

Hexanoic acid

(1H)Imidazo[2,1-f]purine-2,4(3H,8H)-dione, 8-ethyl-1-methyl-7-phenyl-

Itaconic acid

2-Keto-gluconic acid

5-Keto-gluconic acid

2-Keto-3-deoxy-gluconic acid

Lactic acid

5-Methoxy-1-(3-methoxy-4-methylphenyl)-1,3,3,6-tetramethylindan

Niacinamide

Nicotinic acid

Pantothenic acid

6-Phospho gluconate

Octanoic acid

Oxalic acid

Phenol, 4-ethyl

Phenylethyl Alcohol

Propionic acid

Riboflavin

d-Saccharic acid (Glucaric acid)

d-Saccharic acid 1,4 lactone (Glucaro 1,4 lactone)

Succinic acid

Thiamin

d-Xylonic acid

Approximately 40 other acid esters in trace amounts.

We have also completed mass spectral analysis of Kombucha for UPD-Glucuronic acid and that compound was not present in Kombucha. As of this date (September 28, 1996), we have completed 887 separate HPLC/MS/PDAD or GC/MS analyses of the Kombucha ferment and colony, and have a reasonably high degree of confidence in our findings to date.

The Microbiology of the Kombucha Colony and Ferment

The microbiology of the Kombucha colony is as diverse as the various growers. Some colonies have only one yeast, some two, some three, some four, and it is not yet known how many yeasts the ferment will support. As for bacteria, it will support a broad spectrum as well – some with two, some with three, some with four, some with five, some with as many as ten species. Is ten the limit of bacteria that can be isolated in a single ferment? We don't think so. Since the process of fermenting Kombucha can best be described as selective spoilage, the choice of yeast and bacteria are important to the Kombucha ferment.

Investigation of the yeast in Kombucha over the past decade has been quite productive. Probably the most informative paper on this topic was "The yeast spectrum of the tea fungus Kombucha," Mayser P, Fromme Stephanie, Leitzmann C, Grunder K, (Mycoses 38 (1995) 38: 289-295). These investigators reported only on the yeasts in the Kombucha ferment/colony. They discovered that there were no less than seven possible yeast genera combinations. In their study they examined 34 colonies from around Germany and proved nine single yeast isolates (including *Candida albicans*), 19 double yeast isolates, four triple yeast isolates, and one quadruple yeast isolate. This is an excellent paper and an excellent study that I highly recommend for reading, even if your local library needs to order a copy for you. In their work, they isolated and identified *Brettanomyces, Zygosaccharomyces, Saccharomyces, Apiculatus, Candida Krusei, Candida kifir, and Candida albicans.*

Another impressive paper on the topic of the Kombucha colony is "Histochemical and anatomical observations upon the tea fungus," Anken, Ralf H. and Kappel, Thomas (Eur. Arch. Biol. 103: 219-222 (1992)). In their paper, they explained how the colony is formed in layers. "The distribution... was found to be in-homogenous due to an arrangement of the tea fungus' components in surprisingly distinctive horizontal layers." They found that the yeast and bacteria each have their own layers within the colony. While they are symbiotic in the ferment, they isolate themselves from each other in the colony construction. It is similar to a Kombucha condominium project. We can theorize that the purpose of the colony is to provide moisture and transportability to the various microorganisms. We have observed the formation of polymer compounds in samples of the testing standards. The formation of the colony may be as much a matter of chemistry as microbiology.

In discussing the "Iowa Incident" (the Food and Drug Administration ("FDA") press release warning, and the article in the Morbidity and Mortality Weekly Report), a woman in Iowa died from peritonitis associated with a perforated bowel (no link to Kombucha). Because she also fermented and consumed Kombucha, the FDA and Centers for Disease Control ("CDC") conducted an investigation into the Kombucha ferment. When the FDA and CDC did their analysis of the ferment they isolated four yeast. These were identified as *Saccharomyces cerevisiae, Pichia fermantans, Candida lambia* and

Candida validda, all of which are known to be non-pathogenic to the human organism. Neither the FDA nor the CDC conducted any sort of microbiological investigation for pathogenic organisms, other than the isolation of the yeast colonies. We know from their publications that they were not able to isolate any pathogenic bacteria, and now we have a better understanding of their research scope and methods (see government research documents produced under the Freedom of Information Act at Appendix B). Their investigation did not include any type of analyses of the colonies used by the Iowa woman for her ferments.

In our own studies of Kombucha, we have conducted numerous analyses for pathogenic bacteria. To date, we have isolated the following from visible molds growing on the surface of the ferment: *Aspergillus niger* (a black/gray mold, with typical mold characteristics of fuzzy/furry appearance, and growing mycelia [umbrellas or carpets] on the surface of ferments, with a beaded web of spores extending down into the ferment); *Penicillium notatum* (a green mold typically found growing on bread or rotten fruit); and *Mucor* (a yellow crystal-appearing mold, which was isolated only from a fructose ferment, without any tea or other organic additions except for a normal Kombucha colony).

We have analyzed every colony we received for the presence of *Candida albicans,* and we have never found it. We have run isolations for a variety of pathogens on numerous colonies, including isolations for *Staphylococcus aureus, Salmonella spp., Shigella spp., Pseudomonas, Escherichia coli* (total coliform group, including *Klebsiella), and* fecal *Streptococci.* None of these were isolated from any of the cultures or colonies we examined. Other pathogens were not screened due to either difficulty in culturing, or the remoteness of any possibility of finding them (i.e., *Clostridia,* which is anaerobic). Our overall observations are that it took each ferment only three or four days to drop from an initial pH of approximately 6.8 to one of 3.5 or lower. In these lower pH ranges, many pathogenic bacteria are unable to survive during the fermentation process.

Numerous factors affect the microbiology of the Kombucha ferment. We can consider the age of the colony and the culture, the holding time at room temperature prior to beginning analysis, differences in primary isolation, temperature, culture media, biochemical test methods, etc. One thing we are convinced of is that a number of combinations of microorganisms may effect similar looking colonies and similar tasting ferments (possibly with as few as two microorganisms), and the Kombucha products may vary greatly in content. Any alleged or potential benefits from its use may also very greatly, from ferment to ferment, especially if the consumer is compromised immunologically. Consumers with immunodepressive situations should always get a medical opinion concerning the reasonableness of consuming Kombucha, or in the alternative, should certainly inform their medical professional that they are consuming it.

There is a cliché that cleanliness is next to godliness. When fermenting Kombucha, cleanliness is of equal import. The major concern of everyone that performs an

independent analysis of this ferment is potential contamination from unclean or improper handling of the colony or the ferment.

Molds are generally the result of unclean fermenting containers, utensils, or hands. While it is possible to introduce airborne molds, the number of spores necessary to overtake a properly inoculated ferment is more than will generally be provided solely by airborne contamination. Still, if spore counts are high and the sugar and tea solution is allowed to sit for more than eight hours, contamination is a possibility. *Aspergillus* and *Penicillium* have been found to grow in sugar concentrations up to 67.5 percent. Acidification to a pH of 3.0 prevented the growth of either mold. These two molds, and *Mucor,* grow on walls, fermenting vessels, utensils, and counters and are kept to a minimum by adequate cleansing of the counters and fermenting tools. These molds produce glucosidase which attacks the glucose end of the sucrose molecule during fermentation.

The typical isolations of microorganisms found in the Kombucha samples we examined are *Acetobacter xylinum, Acetobacter xylinoides, Acetobacter ketogenum, Saccharomycodes ludwigii, Saccharomycodes apiculatus, Schizosaccharomyces pombe, Zygosaccharomyces,* and *Saccharomyces cerevisiae*.

The mainstay of Kombucha ferments in North America appear to be *Acetobacter xylinum, Zygosaccharomyces, Saccharomyces cerevisia.* Unlike the research in the Fromm paper, which showed *Brettanyomyces, Zygosaccharomyces* and *Saccharomyces* appearing in 56%, 29%, and 26% of the tested ferments respectively, we did not isolate *Brettanyomyces* from any of the ferments we examined. *Saccharomyces, Zygo-saccharomyces* and *Saccharomycodes* were the most common yeast in the ferments we examined. Of the genus of *Acetobacter, Acetobacter xylinum* was the most frequently isolated.

Saccharomyces cerevisiae is the leading species of its genus found in Kombucha. It reproduces by multi-polar budding or ascospore formation. It is employed in many food industries with special strains being used for the leavening of bread, as top yeasts for ale, and for the production of alcohol, glycerol, and invertase. The invertase production is of special interest in the fermenting of Kombucha, because invertase catalyzes the hydrolysis of sucrose into glucose and fructose. Invertase is a fructosidase which attacks the fructose end of the sucrose molecule, in contrast to the glucosidase of molds that attack the glucose end.

Zygosaccharomyces are still considered by some to be a subgenus of *Saccharomyces.* These yeasts are notable for their ability to grow in high concentrations of sugar, and they are involved in the spoilage of honey, syrups and molasses. They are also used in the fermentation of soy sauce.

Saccharomycodes are a lemon-shaped yeast which are considered objectionable in

wine fermentations because they give off-flavors, low yields of alcohol, and high yields of volatile acids.

Acetobacter xylinum is an acetic acid-producing bacteria that oxidizes ethyl alcohol to acetic acid and other oxidation products. It is not suitable for many commercial applications because of its excessive sliminess, which clogs vinegar generators.

During our investigation, two ferments that we considered to be opposites were examined. The first colony and ferment was very high in acetic acid, and the second colony and ferment was low in acetic acid, but very high in gluconic acid. . The colonies were placed in sealed Ziplock bags at room temperature for 30 days to observe which organisms could be isolated after such storage. The isolated organisms were identified by the Biolog Microsation™ System and confirmed by biochemical testing. The species of the organisms were determined by comparing their biochemical profile to those of known bacterial strains. The organisms tested from the high acetic acid ferment were morphologically and biochemically similar to the following: *Bacillus licheniformis, Bacillus megaterium, Bacillus amyloliquefaciens,* and *Rothia dentrocariosa*. The genus of *Bacillus* is generally associated with the soil and the grower of this particular ferment used well water.

From the second low acetic acid ferment, *Bacillus coagulans* was isolated.

We had not previously screened Kombucha for these types of organisms, for we had studied the ferment from the point of view of what microorganisms produced the ferment. It was surprising to find that these organisms were capable of surviving in the Kombucha ferment. So, what are these other organisms?

Bacillus licheniformis is a mild form of food poisoning and is frequently associated with cooked meats and vegetables that have been left at room temperature. According to Barbara M. Lund, *(The Lancet* Oct 20, 1990 v336 n8721 p982(5), the illnesses caused *by B. cereus, B. subtilis, B. licheniformis* and *C. perfringens* can easily be prevented by proper refrigeration. The major features of food-poisoning due to *B. licheniformis* in outbreaks recorded in the UK (24 episodes, [is greater than] 218 cases, 1975-86) were that: (a) the food vehicles most often involved were cooked meats and vegetables; (b) the median period of incubation was about 8 hours and the predominant symptom was diarrhea with vomiting in about half the cases (although the nausea, headaches, flushing, and sweating associated with *B. subtilis* food-poisoning were not characteristic of *B. licheniformis* food-poisoning). B. licheniformis has been excepted for use in commercial fermentation processes for enzymes, antibiotics and other specialty chemicals by the EPA (*TSCA Section 5(H)(4) Exemption for Bacillus licheniformis: History of safe commercial use*). *B. licheniformis* has been used in the fermentation industry for over a decade for production of proteases, amylases, antibiotics, or specialty chemicals. The ATCC Catalogue of Bacteria and Phages lists strains which are capable of producing alkaline proteases, a-amylases, penicillinase, pentosanases, bacitracin, proticin,

5'-inosinic acid and inosine, citric acid, and substituted L-tryptophan.

Rothia dentrocariosa is a common component of dental caries. According to Stuart J. Ruben, The Western Journal of Medicine,(Dec 1993 v159 n6 p690(2)) *Rothia* was first described as a genus in 1967 by Georg and Brown and shown in 1969 to be pathogenic but of low virulence.[1] This aerobic organism is gram-positive and varies in form from coccoid to filamentous to rod shaped. Branching is seen at times in the filamentous form, which resembles *Actinomyces, Corynebacterium*, and *Nocardia* species. It is a component of normal oral flora that can be recovered from dental caries and plaque. We can only surmise that somewhere in the history of this particular colony, someone tried to take a bite out of it. The *Bacillus* found the environment suitable, and survives generation to generation.

Bacillus amyloliquefaciens is employed in fermentation processes. Its presence in Kombucha is not totally surprising.

Bacillus coagulans is aciduric and produces a low pH (4.0 to 5.0) in media containing utilizable carbohydrates. Spoilage of acid foods, such as canned tomatoes, is usually cased by *Bacillus coagulans. B. coagulans* is a flat sour bacteria that can produce considerable amounts of lactic acid from sugar. We note here that we found little or no lactic acid in most ferments. A bottled ferment from Temple City Kombucha of Culver City, California was the only ferment we examined with any appreciable concentration of lactic acid.

DISCUSSION

Introduction

Over a period of 16 months, we learned a lot about Kombucha and the Kombucha ferment. We think its important to bear in mind that regardless of how confident we are in our findings or how much we have discovered or determined, there is still a large amount of information regarding Kombucha that is not yet known, and may not be known for decades. Our analyses of Kombucha were multifaceted, and the results are intended to provide as broad an understanding of the ferment as possible. We have made substantial progress in identifying some individual constituents. To the date of this paper (September 30, 1996), we have positively identified several previously unknown or undocumented components. What follows is a discussion on how our study and the components we have found might help clarify the actual health benefits derived from Kombucha. There is certainly a great deal going on in this ferment.

For several decades, the health benefits associated with Kombucha have been attributed to the presence of glucuronic acid in solution. This was reported as recently as 1996 by P. Blanc of France (see “Characterization of the tea fungus metabolites,”

Biotechnol. Lett., 1996 18/2 (139-142). Conjugation with glucuronic acid is one of the major detoxification mechanisms in mammalian species for a variety of foreign and endogenous compounds. This process is catalyzed by endoplasmic reticulum-bound UDP-glucuronosyltransferases (see "characterization of beta-glucosidase and beta-glucuronidase of alkalotolerent intestinal bacteria," Biol. Pharm. Bull, 1994 Mar; 17(3): 423-6). When it became evident that what was previously identified as glucuronic acid by our research was instead 2-keto-gluconic acid, our investigation focused on determining what was present in solution that could account for Kombucha's claimed improvements to the consumer's health.

The Kombucha Colony

Quite frankly, it seems that since carbohydrate metabolism has been studied for more than two hundred years, there should be a wealth of information, and all of it conveniently available. However, the criteria for tests are always constructed from the standpoint of one organism and one substrate. Kombucha consists of several organisms with a variety of substrates and is not the kind of experiment used in research studies.

The metabolism of carbohydrates is described by Morgan in Convergent Pathways of Sugar Catabolism in Bacteria and that of extracellular metabolic processes by Midgley and Dawes in "The Regulation of Transport of glucose and methyl alpha-glucoside in *Pseudomonas aeruginosa,*" (Journal of Biochemistry 132:141-154). Extracellular polysaccharide production is frequently encountered in microorganisms, including *Acetobacter xylinum* (see William H. McNeel, Biosynthetic Polysaccharides). The production of extracellular heteropolysaccharides probably has a survival function for the bacteria. The water-holding power of some polysaccharides is remarkable; for it enables the bacteria producing the polysaccharide to maintain moisture in their immediate environment, even after prolonged exposure to low humidities. In summary of these articles, some processes occur within the cells of the microorganisms, while others take place in the ferment and outside the cells of the organisms responsible for those portions of the fermentation.

In 1886, Brown reported that *Acetobacter xylinum* produced tough membranes of cellulose when grown in a suitable nutrient solution. Because we have observed polymerization in synthetic Kombucha reference materials, we can assume that the production of the Kombucha colony includes chemical reactions in association with the microorganisms' metabolic by-products. This information is further investigated by Fontana *et. al.* "Nature of plant stimulators in the production of Acetobacter xylinum ("tea fungus") biofilm used in skin therapy" (Appl Biochem Biotechno 1991 Spring; 28-29: 341-51). We find this paper interesting and agree with many of their findings, althought we disagree on their xanthiane theory and suggest further study of the polyphenolic components in Kombucha.

Since d-glucose is the most abundant carbohydrate in the biosphere, it is not surprising that the ability to catabolize this sugar is prevalent in the bacterial world. Metabolic enzymes described in the Entner-Doudoroff pathway are activated during growth on glycogen, as is the enzyme for the extracellular oxidation of d-glucose to d-gluconate (Hylemon and Phibbs, 1972). Two distinct reaction sequences for the oxidation of d-glucose, to eventually produce 6-phosphogluconate, have been identified (Midgley and Dawes, 1973; Lessie and Phibbs, 1984). The first acts directly on extracellular d-glucose with glucose dehydrogenase and in some cases gluconate dehydrogenase. Membrane-associated, pyridine nucleotide-independent enzymes acting in periplasmic space form gluconate and 2-ketogluconate, respectively. *Acetobacter xylinum* is apparently devoid of phosphofructokinase activity (Benziman, 1969). However, *A. xylinum* contains an enzyme, fructose-6-phosphate phosphoketolase, that cleaves fructose-6-phosphate in the presence of inorganic orthophosphate to give aceylphosphate and erothrose-4-phosphate (Schramm and Racker, 1957). One aspect of the microbial metabolism of the Kombucha fermentation that is clear from our testing, is the presence of fructose in solution: the concentration is quite batch and time dependent.

Physiological Benefits of Detected Components

By activating or stimulating certain enzyme systems, the body's equilibrium is driven in different directions. The constituents of Kombucha that we are looking at have the possibility to either activate or deactivate immune system components as well as hormonal or enzyme system components or interactions. For instance, a component that stimulates or inhibits monoamine oxidase will definitely affect one's mental capacities. The enzyme monamine oxidase plays a key role in the regulation of certain physiological amines in the human body. Inhibitors of monamine oxidase have a potential as antidepressive drugs since they increase both noradrenaline and serotonin levels in the brain. Recent investigations point towards xantohnes and flavonoids as a major source of antidepressive activity since they have potent monamine oxidase inhibitory properties (Phytochemistry of Plants Used in Traditional Medicine). The stimulation of the phase two liver enzymes, the glucuronidase and heprinase, will likely have a pronounced effect on membrane transport of toxic and nutritive type constituents. If, on the other hand, you deactivate these enzymes, as would be the case with the saccharic acid 1,4 lactone, you'll have the reverse effect. The body's systems deal with these particular compounds in Kombucha from an evolutionary standpoint, i.e., as components that can be utilized directly, or added too, or broken down. Their presence could possibly, in the right concentrations, in the right places, and at the right times, be able to effectively deal with improving a particular aspect of abnormal metabolism that is occurring. The potential ability of some of these constituents to stimulate the autoimmune system deserves serious consideration and study. The process by which interleuken, natural killer cells, leukotrines and various phagocytolytic activities or toxins are removed from circulation by the body could certainly be something to which Kombucha is contributing.

Each one of these individual constituents, or some yet to be determined, could act independently on any number of physiological systems. Any combination of any of the hundreds of different compounds might be the key to what occurs when Kombucha is consumed. Until those various individual compounds or combinations of compounds can be administered in a controlled environment in clinical studies, we will not really know what physiological effects Kombucha actually has upon the human organism. But that certainly does not prevent us from speculating or hypothesizing on the possible interactions based upon studies that have been done.

At the outset, we know that Kombucha contains living bacteria and yeasts. It contains, for the most part, plant extracts derived from tea, herbs, fruit, or combinations of those organic products. It contains sugars and it contains reaction products, both chemical reaction products and the microorganisms' metabolic by-products, and water. Our study of the microorganisms at this stage are limited to helping us understand what is in solution and providing guidance in isolating some additional compounds in Kombucha. The decision to isolate the keto-gluconic acids was based on the isolation of bacertia that are known to produce those acids.

The plant extract content of the Kombucha ferment is, in our opinion, under-estimated. We do not know how many Kombucha consumers were regular tea drinkers before they began fermenting their own Kombucha – there certainly may be some physiological improvements, simply from consuming the tea in which the Kombucha is fermented. That is an issue that should be studied in the future. Much research is being done relating to the bittering principle of certain herbs, caffeine itself and the different polyflavanoid constituents, and the tannins and the condensed tannins and their effects on the body (see "Stimulation of granulocytic cell iodination by tannins and related compounds"). Those particular aspects of Kombucha may very well be of physiological importance.

As well, there are far too many instances where placebo effects have altered a person's perception to rule out their role in the consumption of Kombucha. This phenomenon is just not limited to the consumption of Kombucha. There's really nothing that we can do from the point of science to tell them that they're wrong. Nor can we tell them that they are right. Getting to a point where we can substantiate placebo perceptions is going to be difficult, if not impossible.

Entner and Doudoroff identified a new biochemical pathway in the bacteria for the oxidative metabolism of glucose, possibly involving the intermediary of the 6-phosphate of 2-keto-3-deoxy-gluconic acid (Dioxalanones as synthetic intermediates, Part 6.). In the Reichstein process for production of L-ascorbic acid, 2,5-keto-d-gluconic acid is an important intermediary ("Microbial processes for ascorbic acid biosynthesis: A review" Enzyme Microb. Technol. 1990 12/5 (322-329). D-gluconic, 5-keto-d-gluconic and 2-keto-d-gluconic acids are produced by biochemical and catalytic oxidation of glucose. After conversion of glucose to gluconic acid, some bacteria carry the oxidation

further to yield keto-gluconic acids ("Determination of D-gluconic, 5-keto-D-gluconic, 2-keto-D-gluconic and 2,5-diketo-D-gluconic acids by high-performance liquid chromatography," Chromatogr. 1984 Vol. 312 (211-219).

The ability of ascorbic acid precursors, 2-keto-gluconic acid and 5-keto-gluconic acid, in some Kombucha ferments to act as free radical scavengers, is not documented and deserves further study. In chemistry, the natural progression is hydroxy-gluconic, because it is easier to make than the keto-gluconic, which is easier to make than the 2,5-diketo-gluconic. We find all of these compounds in various Kombucha ferments, so we assume that when the ferment is capable of catabolizing the glucose through these steps, it is capable of continuing the process. It is reasonable to assume that since this is a live ferment, that these processes may continue even after consumption by the human organism.

People using Kombucha have reported higher levels of energy. This perception could conceivably be attributed to the presence of fructose in solution. It is our opinion that these effects are not soley the result of individual constituents acting independently, but rather the synergistic effect of the metabolic by-products of the fermentation. Plant extract materials are almost certainly producing some benefits ("Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants," Arch. Biochem. Biophys, 1995 322/2 (339-346): catechins, epicatechins, epicatechin gallate, epigallocatechin, gallic acid, caffeic acid, benzoic acid, and the balance of the breakdown products and precursors of the aromatic, amino acids, and the flavanols. Also the citric, shikimic quinic acids that show up in fruit are all probably interacting as well in the metabolism of the microorganisms that are in solution.

So picking the right tea and not being afraid to experiment to see what's going to happen is something we think should be encouraged. The "traditional" insistence on five black tea bags or five green tea bags, or only that combination is too limiting. In our opinion, there's too much room for improvement to stick with the convention at this stage. But, for the average at-home grower, convention offers the assurance of a safe ferment.

Saccharide-Mediated Transport Circulation Via Conjugates

The interaction of the sugars is straight forward, unless the ferment has something like molasses; in regular Kombucha we have sucrose, glucose, and fructose. There are at least 30 to 40 different saccharide constituents that could be used as nutrient sources by the organisms found in Kombucha. We focused our study on ferments prepared with white sugar, brown sugar, and honey, as these are the nutrients most often used by the daily at-home Kombucha grower and consumer.

The sugars themselves and the sugar acids may be facilitating in transport of

nutrients into the system through the stomach and the intestines. They may also mediate transport into the bloodstream, the liver, the kidneys and other organs as well. In doing so, they may be facilitating the removal of toxic materials and the incorporation of beneficial compounds. It may be as simple as improving the transport through the cells and allowing the body's systems to deal with other nutrients more effectively.

It is known that various sugars facilitate the transport across cell membranes. The gluconic acids, keto-gluconic acids, and the acetic acid might perform that basic stimulus, or make the physiological processes more efficient. It is important to keep in mind that the body is designed to act as efficiently as possible, and if you're providing it with the needed constituents and activators, then it's going to do a better job. I think for the most part, trying to optimize our physiology through drinking Kombucha is the result for which we are striving.

Injesting Kombucha in sufficient quantities would certainly have an effect on blood sugar levels. Fructose is easily metabolized by the body, and it might be the constituent that is providing the additional energy. The glucose should not be overlooked in solution either, and it might also be a source of quick energy.

Connective Tissue Building, Maintenance, and Repair

The next area to try and understand is what chemical reactions are going to be possible within the Kombucha ferment. We observed that a synthetic mix of chemicals for standard analysis of Kombucha formed its own polymerization. We are not yet ready to speculate on what chemical reactions brought about that polymerization. We do know that in any kind of a mixture, the chemistry is going to try and reach an equilibrium and a lowest possible state of energy for inertial equivalents. The chemistry of Kombucha is going to conform to chemical laws, and other physical laws of nature, with the entropy of low energy and trying to become stable. So if you have a component in there which has a potential for binding with another, they're going to bind if it produces a lower energy state. It is apparant that the low-energy stabilization of the chemistry is what is occurring the formation of the polysaccharide/mucopolysaccharide colony. It is not strictly a function of the enzymatic activity of the microorganisms in solution.

When we look at some of the acids that are being produced, the citric acid cycle intermediates, pyruvic acid, hydroxy-citric acid, possibly even the itaconic or the succinic acids, not enough is known about their interactions to state conclusively at this stage exactly what is going on. There have been studies that indicate that these particular types of constituents are effective in the body's ability to perform anabolic activities, such as muscle building. People now spike their protein drinks with hydroxy-citric acid and alpha-hydroxy-butyric acid, and these particular types of constituents have been linked to the production of lean muscle mass. These types of compounds are certainly found as well in Kombucha.

When the body is deprived of vitamin C, its connective tissues break down. Working with the flavanoids and the vitamin C precursors, we're setting up conditions which would make connective tissue repair an aspect that could be coming from Kombucha. If your enzymes are stimulated, or if your enzymes have the substrate necessary to produce hydroxy-proline from proline, then you're going to go right on to the polymerization of hydroxy-proline into the formation of connective tissue (tendons, ligaments, cartilage, etc.). So, that is certainly something to also consider when discussing this area of Kombucha consumption.

We know that the keto-gluconic acids are the precursors of ascorbic acid, and theorize that these compounds may also take part in maintaining cell membrane integrity as well as neutralizing free radicals and their negative impact on health. This type of interaction could well provide the benefits that are so often recounted in anecdotal descriptions of the ferment's effects on physiology. We then extended this theory of interaction based on the possible presence of saccharic acid 1,4, lactone. The consumption of moderate amounts of these compounds help in the relief from arthritis, gout, asthma, and other ailments which degrade connective tissue. The disruption of heparin, hyaluronic acid, mucoitin sulfate, and glucuronic acid conjugates by glucuronidase enzymes is greatly reduced by saccharic acid 1,4, lactone. These, along with the presence of phenethyl alcohol and the ability of gluconic acid to bind with heavy metals and thus be excreted in the urine, provide a different picture of the cause and effect relationships of the constituents of the Kombucha ferment.

Lowering of Physiological pH

If you drink Kombucha, then one of the things you know is that it has a lower pH than your stomach, so you are going to lower the pH of your stomach if you drink it. The acidification of your upper gastrointestinal tract may have particular beneficial effects, including aiding in digestion. One hears about a glass of wine helping digestion; and this may also be the situation with Kombucha. Also, Kombucha may possibly be harmful to bacteria that don't belong there, like *pylori,* which is being associated with stomach ulcers. Kombucha consumption has also been shown to lower blood pH, often creating a hostile environment for pathogenic and carcinogenic organisms.

Antimicrobial, Antibacterial, Antiviral Activities

We know how Kombucha can affect certain pathogenic bacteria. With regard to its antibacterial and antiviral activities, what exactly is occuring with those constituents from the sugar metabolism or from the tea in the blood stream, is something that should be more seriously examined. We know the polyphenols and esterified gallic acid have antimicrobial capabilities ("Antimicrobial activity of tea extracts on cariogenic bacterium (*Streptococcus mutans*)," Food Hyg. Soc. Jpn., 1996 37/2 (104-108). Phenethyl alcohol,

itaconic acid and acetic acid are all known for their antimicrobial abilities and all three are found in a typical Kombucha ferment.

Analgesia - Acetamidophenol

The presence of the acetominophen component, acetamidophenol, in solution may certainly have analgesic effects. This may be especially true when considered in the context of the bioactive Kombucha ferment with its phenolic compounds ("Phenolic anticyclooxygenase agents in antiinflammatory and analgesic therapy"). We have already discussed mediated transport across cell membranes, and that may certainly be a consideration with acetamidophenol as well.

Glucuronidase Inhibition by Saccharic acid 1,4 lactone

The possible presence of saccharic acid 1,4 lactone in solution provides some other possible explanations for claims of improved health by Kombucha drinkers. Briefly, I will share a synopsis of four studies from our bibliography:

In analyses of colon cancer patients, it was found that carcinogenicity is due to the induction of some enzymes, such as beta-glucuronidase. Beta-glucosidase and beta-glucuronidase of the intestinal bacteria are associated with the conversion of a pro-carcinogen into a carcinogen. Beta-glucosidase hydrolyzes natural glycosides, and beta-glucuronidase acts on glucuronic acid conjugates of endogenous and exogenous compounds, bilirubin and benzo[a]pyrene. The beta-glucuronidase was inhibited by saccharic acid 1,4 lactone.

Among tobacco-specific nitrosamines, NNK is the most potent carcinogen known. It was found that NNK can be isolated in the urine as a glucuronide metabolite. Again, beta-glucuronidase broke down the glucuronide, but was inhibited by saccharic acid 1,4-lactone.

In their study of tumor cell-mediated destruction, Nakajima *et. al.* discovered that heparin sulfate was degraded by heparinase, an endo-beta-glucuronidase. B-16 melanoma heparinase is highly active against various heparin sulfate molecules. Higher concentrations of saccharic acid 1,4 lactone markedly inhibited HS degradation, suggesting that HS-degrading endoglycosidases (heparinase) may be sensitive to high saccharic acid 1,4 lactone concentrations.

Beta-glucuronidase catalyzes the hydrolysis of terminally linked beta-glucuronic acid in mucopolysaccharides and other complex carbohydrates. Saccharic acid 1,4 lactone showed powerful inhibition, and is effective in micro quantities. The glucuronidation of bile acids appears to be a major detoxification mechanism of bile acids in humans. This is of biological significance since bile acids have been shown to play a role in the

pathogenesis of disease. Again, the hydrolysis of the glucuronides by beta-glucuronidase was inhibited by saccharic acid 1,4 lactone.

These complex studies relate to us that glucuronidation of both endo- (internal) and exo- (external) toxins in the body is important to the prevention of disease. Once a glucuronic acid molecule binds to toxic molecule, they are both slated for elimination from the blood and excretion from the body. The binding of these molecules is in a one to one ratio to form a glucuronide. The beta-glucuronidase enzyme does not bind with either, but rather, cleaves the bond between the molecules in the glucuronide. So while a glucuronic acid molecule can only remove one toxic molecule, a single glucuronidase enzyme can cleave the bonds of thousands of glucuronides releasing the previously bound toxins back into the body. Saccharic acid 1,4-lactone inhibits the cleaving of that bond by glucuronidase. It also inhibits the breakdown of connective tissues by heparinase. In short, while saccharic acid 1,4 lactone is not glucuronic acid or mucoitin sulphate, it helps both be more effective within the body.

The commercial production and uses of gluconic acid are explained by George Ward, including the industrial significance of gluconic acids' ability to form soluble complexes with heavy metals in Production of Gluconic Acid, Glucose Oxidase, Fructose, and Sorbose. This is significant in light of reports of increased levels of heavy metals in the urine of new Kombucha drinkers.

Topical Application of the Kombucha Colony

The preparation of bacterial cellulose pellicles, which are applied as a biotechnological tool in the treatment of skin burns and other dermal injuries, were investigated by Fontana *et. al*. The use of *Acetobacter xylinum* pellicles for the treatment of second and third degree skin burns and for aid in the healing of skin grafts is discussed in the Fontana paper. This provides evidence concerning Kombucha's ability to relieve the pain of sunburn victims as well as anecdotal evidence of Kombucha's positive effects with other dermal problems.

Toxicity and Warnings about the Kombucha Ferment

The possibility of hepatotoxicity as a side effect to Kombucha consumption was raised by Drs. Perron, Paterson and Yanofsky in their letter to the editor of the Annals of Emergency Medicine. In our investigation, we were unable to find anything that would cause damage to the liver or cause liver enzyme levels to move out of balance, with the possible exception of the presence of acetamidophenol. The presence of acetamidophenol is in such minor quantities however, that we believe only persons consuming large amounts (more than 16 ounces per day) could possibly be at risk, and even then we doubt that the levels are sufficient to bring about a response by the liver.

Much has been made about the growing of Kombucha by at-home growers. The possibility of contamination by molds has been raised by both the noted mycologist Paul Staments, and by the United States Department of Health and Human Services, through both the Food and Drug Administration ("FDA") and the Centers for Disease Control ("CDC"). These U.S. Government agencies published their combined findings in the Morbidity and Mortality Weekly Report ("MMWR") on December 8, 1995. The FDA had previously issued a press release warning against the at-home brewing of Kombucha (FDA Talk Paper of March 23, 1995). In their press release (Talk Paper) they cited: T95-15 Brad Stone March 23, 1995 (202) 205-4144

"FDA CAUTIONS CONSUMERS ON "KOMBUCHA MUSHROOM TEA"

FDA has been receiving inquiries about "Kombucha mushroom tea" -- a product which has been mentioned in media reports lately for many uses, from inducing a general state of well-being to treating diseases, such as AIDS and cancer. FDA has not approved this product as a treatment for any medical condition.

...

The product contains considerable quantities of acids commonly found in some foods, such as vinegar, and smaller quantities of ethyl alcohol. Because the acid could leach harmful quantities of lead and other toxic elements from certain types of containers -- some ceramic and painted containers and lead crystal -- such containers should not be used for storing Kombucha tea.

The unconventional nature of the process used to make Kombucha tea has led to questions as to whether the product could become contaminated with potentially harmful microorganisms, such as the mold Aspergillus. Such contamination could produce serious adverse effects in immune-compromised individuals.

FDA studies have found no evidence of contamination in Kombucha products fermented under sterile conditions. FDA's and state of California's inspections of the facilities of a major Kombucha tea supplier also found that its product was being manufactured under sanitary conditions.

However, the agency still has concerns that home-brewed versions of this tea manufactured under non-sterile conditions may be prone to microbiological contamination. FDA will continue to monitor the situation, and encourages consumers to consult with appropriate health professionals for the treatment of serious diseases."

While Aspergillus is not in and of itself generally of any harm, some strains could cause serious illness, or worse. People should know that if they grow any visible molds, the ferment should be discarded.

Although ferments that we've examined for pathogens and microbiological constituents have shown nothing that would cause such caution, we haven't seen them all. The paper by Mayser, Fromme, Leitzmann, and Grunder (Mycoses 38, 289-295 (1995)) found *Candida albicans*. While we've never found *Candida albicans*, they state that they did. While no death has ever been proven to be the result of consuming Kombucha, it might be because they were able to link such a death to the consumption of a source of contamination. I'm not aware of any such incident, but I certainly can't rule that out. High pH pathogens are not likely to be found in this ferment.

The December 8, 1995 MMWR stated in part: "Samples of the mushrooms and samples of the tea consumed by both case-patients were sent to FDA for analysis. Microbiology analysis of the tea and mushrooms identified several species of yeast and bacteria, including *Saccharomyces cerevisiae* and *Candida validda*. No known human pathogens or toxin-producing organisms were identified. (Editorial Note): FDA has evaluated the practices of the commercial producers of the Kombucha mushroom and has found no pathogenic organisms or hygiene violations (5). However, because the tea is produced under varying conditions in individual homes, contamination with pathogenic organisms, such as Aspergillus [a visible black mold] is possible. When prepared as directed, the pH of the tea decreases to 1.8 in 24 hours. ***(Author's note: our findings were much closer to a pH of 3.8 after 24 hours***) Although this level of acidity should prevent the survival of most potentially contaminating organisms, tea drinkers have reported molds growing on the Kombucha (CDC, unpublished data). Because of the acidity of the Kombucha tea, it should not be prepared or stored in containers made from materials, such as ceramic or lead crystal, which both contain toxic elements that can leach into the tea."

From the documents produced to us by the FDA and the CDC through a request under the Freedom of Information Act, they isolated four yeasts in two different laboratories. It is clear from their documentation that these analyses were of the same ferment, which we had assumed was from the ferment of the Iowa woman that died. In the first samples tested, they isolated *Saccharomyces cervesea* and *Candida valida*. From the second series of tests, they isolated *Pichia fermentans* and *Candida lambia*. We have included portions of those documents at the back of this paper for your review (Appendix B). Nothing in the documents supplied by the FDA and the CDC indicated any type of bacterial culturing or any other pathogenic screening. Further, all studies by the FDA and CDC were concluded before the death of the Iowa woman. The government concluded their analyses of Kombucha in March 1995. Lila Williamson (the "Iowa woman") died the following month, in April 1995. No analysis of her ferment was ever conducted.

Conclusion

The research methods we have employed to date for the analyses of this ferment are not the only possible methods. Different chromatography columns to interface with mass spectrometry for better resolution of other components are already planned, that will give us an even broader picture of the Kombucha ferment. While we have compiled a great deal of data on the various compounds we have already isolated from Kombucha, it is not totally understood at this point which of these compounds or combinations of these compounds provide any health benefits. But to date, we are only seeing a part of the entire picture.

We know that phenethyl alcohol, as well as acetic acid and the polyphenolic compounds, have been reported as having antibacterial capabilities (J. Pharm 21, 681 (1969). The abundance of gluconic acid could also be a source of detoxification for the human body. Additionally, many of the compounds are precursors or intermediates of other known components, such as ascorbic acid. It is quite possible that these metabolites are further oxidized or hydrolyzed in the body. What we can say with confidence, is that nothing in our investigation revealed anything in a healthy Kombucha ferment that would constitute a risk to a healthy person.

The study of Kombucha needs to be an on-going research project. This paper provides information about conventional Kombucha ferments examined from 1995 through the summer of 1996. We may update this document as additional information becomes available. Finally, we hope to publish our findings on Kombucha hybrids, catalyzed Kombucha ferments, Kombucha extracts, and exotic Kombucha ferments some time in the future. We hope you found this e-book informative.

You may E-mail your comments to *readers@kombucha-research.com*.

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Thank you for reading this book. I would still like to write a book on the exotic ferments and catalyzed ferments and if this book is well received I will try to do that in the future. In the meantime, thank you.

Michael R. Roussin
Fruita, Colorado
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