Ulcerative Colitis: A Review of its Causes and Can a Fermented Food Help?

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Approval

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Dedication

I dedicate this thesis to my older brother Ronald. He has inspired and supported me immensely throughout grad school studies and is the reason behind my research. I am thankful beyond words for your constant faith in me. I also dedicate this thesis to my father Dominique, who has always loved me and encouraged me unconditionally. Lastly this thesis is dedicated to my close friends and family that showed up for me when I felt I could not show up for myself. My love and appreciation for you all cannot be quantified.

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Abstract

Ulcerative colitis (UC) is an inflammatory bowel disorder that starts in the rectum and can work its way to the colon. It is treated in many ways, those being medication, and other therapies such as immunosuppressants, corticosteroids, amino salicylates, biological agents, and diet. One of the main contributors to UC is the lack of diversity of the gut microbiome. Fermented foods like yogurt and kefir can help maintain a healthy gut microbiome if they are probiotic. With the addition of fermented foods to one's diet, remission and comfort can occur because of the increase in diversity of the probiotics specifically *Saccharomyces* and *Lactobacillus*. These are identifiable bacteria in kefir that are not present in those battling UC. Kefir is a probiotic fermented food, that has the possibility to help those diagnosed with UC and contains bacteria that can positively affect the gut biome and can be store bought or homemade. In this thesis, strawberry kefir was made using an at home method and went through an array of tests in comparison to store-bought kefir. Those tests consisted of sensory and microbial plating of pathogenic and non-pathogenic bacteria. The beneficial bacteria that were tested for were *Lactobacillus* and *Saccharomyces*. *Escherichia coli*, *Coliforms, Listeria* and *Salmonella* were the pathogenic bacteria tested. The homemade strawberry kefir was shown to have beneficial bacteria, and food safe. While the number of sensory evaluators was limited, this preliminary study demonstrated that further research could be done with the addition of other probiotics to widen the scope of what a consumer can enjoy.

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Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease that affects the rectum and colon. The pathogenesis of this disease is multifactorial. One cause may be an imbalance in the microbiota. Gut microbiota contains specific microorganisms that live explicitly in the digestive tract. Causes can also be genetics, epithelial barrier defects, dysregulated immune responses, and environmental factors [1]. Patients with UC show signs of mucosal inflammation that starts in the rectum and can extend to segments of the colon [2,3]. Dysbiosis, the disruption of the normal intestinal microbiota, has been shown to also be a contributing factor to UC [2,3].

The human gut and its parts are key to overall human health. It can affect mental health as well as health in other parts of the body [4]. UC can become severe and if untreated, deadly. Diseases like irritable bowel syndrome, colon cancer, Crohn’s disease, and inflammatory bowel disease are also diseases of the gut that can easily worsen [1]. If left untreated UC can transform into Crohn’s disease or colonic cancer and be debilitating [1]. Diet is one of the most important things that humans can control when it comes to their gut health.

Millions of people are affected by UC worldwide, it is considered a global public health challenge [2]. This disease typically affects adults ages 30-40 years of age of any sex, but that margin is increasing over time [2]. There are different results of UC per country, due to their specific environment, which makes environmental factors, as well as biological factors important to study.

The intestine alone, is comprised of hundreds of microbial species and there is an estimate of over 40 trillion bacteria cells in the colon of an adult human [10]. The gut biome of someone with UC has a less diverse microbial community compared to someone without UC [2]. The key to a healthy biome is the complex environment of bacteria, short chain fatty acids, and a strong immune response. Currently research shows a variety of microorganisms lacking from the human gut can cause UC. UC thrives when there is a change in diversity in the biome.

Successful treatments for UC include immunosuppressants, corticosteroids, amino salicylates, biological agents, and diet. There is some research that shows fecal microbiota transplant (FMT) is a highly effective alternative treatment [3]. The role of the immune system will be discussed in dept in the next section.

It is predicted that a fermented food would aid in the improvement of UC is a probiotic that has been fermented [4]. For fermented foods to be helpful to gut health, they should be full of vitamin K_2 and butyric acid. These two factors are shown to contribute to a healthy microbiome by activating precursor materials that detoxify sulfate-induce colitis and protect the integrity of the intestinal epithelium [3]. Detoxifying sulfate-induced colitis and protecting the intestinal epithelium reduces inflammation and promotes immune response.

Milk products cannot be considered fermented unless the milk in the product is inoculated with a starter culture that transforms parts of lactose to lactic acid in the milk. For this reason, a high-fat fermented dairy product that has the possibility to protect against *Helicobacter pylori* infection can be developed that is desirable for a person with UC to consume [4]. Examples of these products are yogurts, ymer, kefir, cultured buttermilks, filmjölk, cultured cream and koumiss. Yogurt is the most common of these products currently in the United States. Per the Food and Drug Administration (FDA) yogurt contains no less than 3.25 percent milkfat, 8.25 percent milk solids not fat, and has an acidity of no less than 0.7 percent expressed as lactic acid, or a pH of 4.6 or lower. Kefir has a diverse microbial make up of lactic acid bacteria, acetic acid bacteria and yeasts [7]. Lactic acid bacteria defined by the World Gastroenterology Organisation (WGO), is a functional classification of nonpathogenic, nontoxigenic, gram-positive, fermented bacteria that are associated with the production of lactic acid from carbohydrates, making them useful for food fermentation [10].

The objective of this proposed research is to better understand what microorganisms cause UC through review of the literature and how those organisms can be added to the diet via consumption of a fermented food. There are two specific aims: 1). To complete a thorough literature review to examine and coordinate what microorganisms are lacking in the human gut that cause UC, and current treatments, and 2). to identify a fermented food that contains a microorganism that can holistically aid in the improvement of UC that can be homemade. The kefir prepared in this project showed overall positive sensory characteristics and a beneficial microbiology evaluation. Further it was also determined that the product will be shelf stable for 2 weeks, long enough for consumers to reap the benefits. This research provides preliminary evidence for further investigation into homemade food products that may improve the effects of UC. This research was not to provide a cure for the disease, but to simply open doors for additional research on the disease and how food can affect it.

Literature Review

UC Diagnosis

UC is diagnosed by the presence of bloody stool and diarrhea as well as erythema, loss of normal vascular pattern, granularity, erosions friability and ulcerations can only be diagnosed by a colonoscopy and stool assessments [1]. A colonoscopy with a biopsy of cells of someone with UC will show colonic cells, mucous barrier, and epithelial barrier defects [1]. A small percentage, about 8-14% of patients with UC have a family history of inflammatory disease, and a personal history of gastroenteritis [1]. Environmental factors, including diet and overall health are a significant variable in the development of UC. For example, cigarette smokers are at higher risk to have UC than non-smokers [1]. Those that take oral contraceptives, hormone replacement therapy and non-steroidal anti-inflammatory drugs have also been linked to an increased risk of UC [1].

UC Treatments

While the current treatments of UC suppress symptoms, there is no cure for the disease. Researched treatments of the disease included in this study, but are not limited to, are immunosuppressants, corticosteroids, amino salicylates (5-ASA), biological drugs, and diet. Anti-tumor necrosis factor-alpha (Anti-TNF-α) drugs are immunosuppressant which assist in decreasing inflammation and aid in repairing mucosal barrier function [2]. Corticosteroids are anti-inflammatory steroids [2]. 5-ASA drugs are widely used in treatment because they also anti-inflammatory, 5-ASA, can maintain mild to moderate disease management and remission in combination with or without corticosteroids [2]. Those who do not improve after 5-ASA treatments may need to switch to rectal or oral corticosteroids. Young patients that who have extensive colitis, deep ulcerations and that have undergone multiple steroid treatments benefit from thiopurine drugs or biologics that are anti-TNF-α [1]. Moderate to severe disease management is done with thiopurine drugs or biologics, sometimes in combination. These drugs are also anti-TNF-α. This treatment is recommended for those with steroid-dependent moderate to severe UC [1].

Studies show that fecal microbiota transplantation (FMT) is affective to treat people with UC as well [2,3]. FMTs move fecal bacteria from a donor to a recipient the process described by Shen et al (2018), of collection of feces is done the day of transplant by homogenizing the feces with a saline and water solution. Since there are no standard criteria for screening, donors are usually family members or healthy volunteers [3]. FMTs can reduce bowel permeability by increasing the production of the short chain fatty acid, butyrate, that helps maintain the epithelial barrier [3]. FMTs can also restore immune dysbiosis by inhibiting the secretion of proinflammatory cytokines. This process helps restore the dysbiosis of the microbiota creating a healthy biome, but there is not enough research for it to be efficient [3]. FMT in combination with probiotics is a promising treatment but needs further research, identification of the specific microbiota improves the dysbiosis in UC has not been established.

Microbial Environment

The gut biome of someone with UC has a less diverse microbial community compared to someone without UC [2]. Short chain fatty acids, carbohydrates, vitamins, and gases contribute to the complex microbial environment of the gut. A disturbance of any of these, can contribute to the cause of diseases like UC [2,3]. Someone without UC typically has a gut microbiota comprised of 400-500 bacteria species [2]. 90% of the human microbiota belongs to the four major phyla, which are *Firmicutes, Bacteroidetes, Proteobacteria (*i.e. *Helicobacter),* and *Actinobacteria* [2]. *Bacteroidetes* and *Firmicutes* account for 90% of 90% of those microbes. People that have UC have fewer of the dominant bacteria, *Firmicutes* and *Bacteroidetes* [10]. These two bacterial families were observed to be decreased, while *Proteobacteria* and *Actinobacteria* numbers were increased in patients with gut health issues [3]. Specifically, butyrate-producing firmicutes were decreased in those with active UC, while *Faecalibacterium prausnitzii* was increased in the remission of patients battling the disease [3]. *F. prausnitzii* is a probiotic bacterium that produces anti-inflammatory proteins to inhibit the nuclear factor κB pathway (NF-κB) pathway in intestinal epithelial cells [2]. The immune cells impacted by UC are T-cells, in particular T-helper-2 lineage, *Bacteroides*, can improve and regulate their function by a.) regulating the NF-κB pathway, (which regulates inflammatory responses,) b.) regulating the secretion of TNF-α (protein used by the immune system for cell signaling) and c.) regulating Interleukin 13 (IL-13 promotes production of T-helper-2 cells) [2].

A singular cause of UC has not been established; research suggests that dysbiosis can be a cause of intestinal inflammation because it causes many other diseases that are similar, like Crohn's disease. The reason why it's uncertain if dysbiosis causes UC is because there is a less evidence of dysbiosis in those with UC compared to Crohn's disease. However, the presence dysbiosis is enough to hypothesize that it contributes to causing the disease [1].

H. pylori infection in the stomach leads to gut diseases, and can be controlled by foods that are fermented and probiotic. The foods that have the best anti-*H. pylori* effects are fermented, probiotic dairy products [4]. Research also demonstrates that when yogurt was consumed, one serving per week or more, it has protected against *H. pylori* infection [4]. Strains of lactic acid bacteria in fermented milk and kefir also protected against *H. pylori* infection as well as reduced side effects from the infection. *H. pylori* is not specific to UC, it is more of a precursor to gastric cancer. However, this shows the benefits and healing properties of consuming foods.

Research has shown that when fermented foods are consumed raw, they provide fresh microbes to the gastrointestinal tract [4]. The microbes, made up of bacteria, yeast and molds in fermented foods are what provide these health benefits. Two of the microbes that fermented foods may bring to the digestive tract are *Lactobacillus* and *Saccharomyces* [4]. Probiotics are live microorganisms that have been shown to help relieve antibiotic gut symptoms like diarrhea, food allergies and cancer [4]. WGO defines probiotics as “a live microorganism that, when administered in adequate amounts, confer a health benefit.” Probiotics typically promote anti-inflammatory factors that help the intestinal mucosal barrier and immune system function by the NF-κB pathway [3].

Probiotic Background

According to the WGO scientist Elie Metchnikoff discovered that lactic acid bacteria offered health benefits that helped with longevity [10]. He developed a diet of fermented milk with a bacterium he named “Bulgarian bacillus” a name for lactic acid bacteria, which started other early developments of this concept [10]. Henry Tissier isolated a *Bifidobacterium* from one breast-fed infant, with goals to treat infants with diarrhea. His research hypothesized that the *Bifidobacterium* would displace the bacteria causing the diarrhea [10]. In Japan, Dr. Mioru Shirota isolated a *Lactobacillus casei* strain from healthy people and successfully treated people with diarrhea. After these discoveries, probiotic products with *Bifidobacterium* and *L. casei* strains have been on the market since 1935 [10]. *Lactobacillus*, and *Bifidobacterium* are the most common probiotics, while *Saccharomyces*, some *E. coli*, and some *Bacillus* strains are also used [10].

Probiotics are defined by the genus, species, subspecies and an alphanumeric designation. Recommendations for probiotic consumption depend on the specific strains and claimed benefits. *Lactobacillus* is one probiotic genus that is well-studied and provides many health benefits; however, the dose needed varies and depends on the strain and product as well. Over the counter products typically range between 1-10 billion CFU/dose [10]. However, there are effective probiotics at lower doses [10]. The purpose of probiotics is to support digestive health, thus foods containing *Lactobacillus* and other probiotics must contain an adequate amount. Knowing that an unhealthy gut has fewer beneficial microbes and an unbalanced microbiota, there is not enough research that fully defines the exact composition of a healthy human microbiota [10]. According to the WGO the recommended dose of probiotics needed to induce remission of UC in an adult is the consumption of 1800 billion bacteria twice daily, containing a mixture of *Lactobacillus* species strains, *Bifidobacterium* and *Streptococcus*. To maintain remission, *E. coli* Nissle 1917, should also be taken twice daily at a 5×10^{10} CFU/dosage.

Probiotics decrease cytokines like TNF-α and IL-1β and increase IL-10, which are anti-inflammatory factors that make them helpful to gut health. Those mechanisms maintain the balance of normal intestinal microbiota (good, health enhancing microbes), enhancing intestinal mucosa barrier function, promoting immune tolerance of the intestinal mucosa, interfering with intestinal inflammatory response, and inhibiting intestinal epithelial cell apoptosis [3]. One study showed that the addition of *Lactobacillus* in combination with usual treatments for UC reduced relapse [1]. Another showed that the administration of *Saccharomyces boulardii* with mesalazine (a 5-ASA drug) to patients with active to moderate UC also resulted in clinical remission [1]. From these studies, it can be inferred that multiple probiotics can promote remission and reduce relapse of UC in addition to current treatments. Certain probiotics have been found to be safe and effective as conventional therapy in achieving a higher responses and remission rate in mild to moderately active UC in adults and children [10].

Clinical Studies

A double-blind study was done with Japanese patients with UC to determine if fermented milk produced remission of UC [5]. A diagnosis of UC was confirmed by clinical, endoscopic, and historical findings. The age groups of these patients were 20-70 years of age and they had worsening symptoms such as bloody stool for a week or longer, started 5-ASA treatment, escalation of cytapheretic and immunosuppressants up to the day of the enrollment of the study [5]. Out of 195 patients some were given fermented milk, a placebo, or *Lactobacillus acidophilus* each day for 48 weeks and observed for relapse-free survival for 3 consecutive days. Relapse between the two groups of those treated were not statically significant. There were adverse effects of this study that caused safety concerns, those events were avascular necrosis of bilateral femoral head (placebo group), surgical removal of granuloma in the throat (fermented milk group) and pulmonary thromboembolism (from the placebo group) [5]. The research of this study showed that probiotics modulate gut microbiota and have positive effects on the immune system and may be used to treat UC. In a randomized study by Ishiawa et al, treating patients with UC with B. *beve* (bifidobacteria) strain Yakult (probiotic yogurt beverage) powder and gagalacto-oigosaccharides was shown that the clinical status of UC that was reported after a years' time was improved but not compared to control groups [5]. This shows that an additional of the fermented milk product plus current therapies would be best versus completely replacing current treatments.

Food probiotics can protect remission but may not induce it [5]. Kefir is a fermented probiotic dairy product that has shown to improve patients' quality of life with Inflammatory

Bowel Disease (IBD) in the short term [6]. Kefir can help maintain remission of UC but not necessarily produce it; however, it is a good food to research more due to its positive affect on UC [6]. In another study 45 patients with IBDs were given 400mL/day of kefir to patients for 4 weeks day and night, and stool *Lactobacillus* content was quantified [6]. The aim was to discover if kefir effects the quality of life of patients with IBDs. Abdominal pain, bloating, stool frequency, stool consistency and feeling good scores were recorded. These are all symptoms of IBDs, and UC is a type of IBD therefore some of these symptoms present themselves in both diseases. The kefir in this study had a count of 5×10^7 CFU/mL count of lactic acid bacteria and 2.1×104 CFU/mL yeast [6]. The *Lactobacillus* bacterial load of feces of all of the subjects in the treatment group was between 10^4 and 10^9 CFU/g [6]. These initial and final measurements were statically significant in those with UC and Crohn's disease. The conclusions of this study demonstrated that kefir may improve the patient's quality of life in the short term. From the conclusions there are positive effects but only temporary.

Kefir Background

Kefir recipes can be complex or simple. Complexity depends on the type of milk used and if there are added microbes. A review by Farag et al. (2020) evaluated the many variations of kefir and compared the benefits of each variety [7]. It was noted that the chemical compositions of kefir depend on the starter grains as well as its geographical origin, temperature, conditions of fermentation and type of volume of milk that is used [7]. Cow milk kefir, like the one in this thesis, is rich in protein, fat, and lactose, making it one of the best variations.

The smell and flavor of kefir are linked to the volatile and non-volatile compounds produced during fermentation by lipolysis, glycolysis, and proteolysis. Kefir should have a yeasty flavor, acidic taste, and an overall sharp acid flavor. These flavors are produced by carbon dioxide from the yeast flora during fermentation. Free fatty acids that are produced from lipolysis of the milk in kefir create the taste and aroma. Interestingly, fermented milk encourages lipolysis, resulting in 5 to 10 times more free fatty acids than non-fermented milk [7]. Creating the kefir flavor profile.

Other health benefits associated with kefir are the anti-stress properties, immune-modulation, cholesterol-lowering, anti-allergenic, anti-asthmatic, anti-microbial, anti-cancerous, and gastrointestinal benefits [7]. These benefits are derived from the peptides, amino acids, vitamins, ethanol, and CO_2 generated during fermentation. At an industrial level of production, a back-slopping technique is done to increase beverage volume. Fresh milk is pasteurized, incubated with kefir grains at 25˚C for 18 hours, the grains are separated, and the product is cooled and stored at 4˚C for consumption [7]. The disadvantages to producing kefir is keeping the microflora of the grain diverse, milk type, and storage conditions. Some limitations for industrial production are sensory, physicochemical properties and the quality due to mass production [7]. Therefore, making kefir at a smaller scale, homemade can improve these disadvantages.

Lactobacillus and *Saccharomyces* are dominant in kefir, compared to mostly lactic acid bacteria in yogurts or other fermented milk products. This is because kefir is a mesophilic fermented food. Mesophilic fermentation consists of started cultures that are comprised of lactic acid bacteria and lactose/non-lactose fermenting yeasts [8]. These bacteria and yeasts give it its probiotic properties that make it a good food for gut health.

When it comes to sensory analysis of kefir, the fermentation is equally as important as the type of milk used. The mesophilic cultures of kefir that contribute to final taste, texture and flavor are from the lactic acid bacteria. Those cultures are divided into groups of acidifiers and aroma producers [8]. *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* ferment lactose acidifying the milk and producing exopolysaccharides that affect the viscosity and texture [8]. *Lactococcus lactis* subsp. *lactis biovar* deacetylates and *Leuconoctoc* sp. metabolism contribute to the odor and flavor of the product. Diacetly is another compound that adds to the flavor of kefir, is characterized as producing a "buttery" flavor. Acetaldehyde gives a fruity flavor, and CO_2 provides a "sparkly" flavor [8]. In the study done by Paucean *et al.* three kefirs were produced with different mesophilic cultures, one added brewer's yeast, the other added kefir's yeast and the last one adds kefir's yeast and brewer's yeast. The goal of this study was specific to evaluate sensory attributes of different yeasts added to kefir and helped provide guidance to this thesis.

For sensory of this study a hedonic scale, common in sensory analysis, as well as a triangle test, score test, and sensory profiling test were done at day 1, day 7 and day 14. Conclusions made from each test showed that kefir types that were different from the traditional kefir was more preferred and appreciated. The consensus of all the kefir was that they were all most enjoyable within 10 days of storage.

In separate study by Rosa *et al.* (2016), hypertensive rats that consumed kefir daily were studied for ten weeks [7]. It was concluded that kefir produces extracellular vesicles that ease the TNF-induced inflammation in intestinal cells by inhibiting inflammatory cytokine production. This led to a 42% reduction of TNF-α/IL-10 and a 50% reduction of pro-inflammatory IL-6 level ratio [7]. This encourages more studies on animals or human subjects' findings to be more conclusive. Monitoring the human gut after consumption of different probiotics in kefir products would also provide a wider understanding of this research.

Methods

Kefir Preparation

Twenty 7oz. jars and lids were washed and sanitized with Saniguard per manufacturer's directions. The jars were left to air dry. The kefir was made according to the Cheesemaking.com (Figure 1). The jars were left to air dry. 2% milk was heated to 86˚F for approximately 12 minutes then transferred into a sanitized stainless-steel bowl. Kefir grains (from Cheesemaking.com) were added, and hydrated for 1 minute. After hydration, the kefir grains were mixed well into the milk. Once well mixed, the milk mixture was poured into the 7oz. jars and left to ferment at room temperature for 46 hours (Figures 2 and 3). The final recipe is located in Appendix 3.

Microbial Testing

After fermentation the kefir was stored in the refrigerator. The two batches of kefir were made one week after apart. In each batch, 5 jars were used for microbial testing and the other 10 jars were retained for sensory analysis. The kefir was plated on YPD agar, MRS agar, oxford agar, *E. coli*/Coliform plates and *Salmonella* plates (Table 1).

Type of Media	Bacteria Cultivation/Enumeration	Good (+) or Bad (-) in Kefir
YPD agar	Cultivation of s*accharomyces cerevisiae*	+
MRS agar	Cultivation of *Lactobacillus*	+
E.coli/Coliform petrifilm	Enumeration of *E. coli* and *Coliforms*	-
Oxford agar	Cultivation of *Listeria*	-
Salmonella petrifilm	Enumeration of *Salmonella*	-

The YPD, MRS, Oxford, and *Salmonella* medias were prepared per manufactures' instructions. The Salmonella media procedure is shown in Figure 5. Homemade kefir was serially diluted with a sterile distilled water and plated on day 0 of shelf life (Figure 4). Day 0 is the day after fermentation is complete. The dilution plated were 10^{-4} on *E. coli*/*Coliform* petrifilm plates, YPD plates, MRS plates, and Oxford media. The microbial plating method was adjusted to include a longer shelf life, a smaller dilution and to include *salmonella* testing.

Each batch of homemade kefir was tested on a weekly basis: 7 days after fermentation, 14 days after fermentation, 21 days after fermentation and 28 days of fermentation after the addition of strawberries. The dilution plated was changed to 10^{-5} *for E. coli/*Coliform plates, and 10^{-6} for YPD, MRS, and Oxford media plates.

Batch 1 kefir was tested for *Salmonella* at the 21-day mark and after freeze-dried strawberries were added, shown in Figure 6. Batch 2 kefir was tested for *Salmonella* at days 14 and 21.

Figure 6. Strawberry Flavored Kefir

Institutional Review Board (IRB) Process

In order to do sensory testing an IRB application with a Collaborative Instituional Training Initiative (CITI PROGRAM) certificate had to be filled out and approved by Mount Mary's IRB committee. The application included an in-dept explanation of what the risks and gains from this research would be and how participants' information would be kept confidential. That application had to be approved before data could be collected. Along with an in-depth description of this study, the sensory ballot and informed consent form was attached.

Kefir Sensory Testing

Sensory testing was done to evaluate flavor profiles and other sensory attributes. The sensory testing layout is shown in Figure 7. Once the research was IRB approved, and the kefir was produced and deemed safe for consumption, an email was sent to food science students asking for their participation in a community sensory tasting. Four students were in attendance and evaluated a store-bought strawberry flavored kefir to the homemade kefir side by side. The students completed an informed consent form, a prescreen survey for eligibility, and began tasting.

The tasting panel questionnaire consisted of 14 questions: 10 related to sensory attributes, 3 for background and 1 for preference. The sensory attribute questions asked included an acceptable mouth feel, after taste, overall flavor, intensity of sparkly flavor, sourness, yeastiness, bitter flavor, level of creaminess, and overall acceptability and preference (Appendix 2).

Results

Observations

Over time the homemade kefir showed clear signs of separation. After fermentation there was separation in the kefir as well as smell changes. In Figures 8.1-8.4 the progression of the stored kefir is shown. Figure 8.1 looks more homogenized than Figure 8.3. 8.2 had about half an inch of separation, while 8.3 had about an inch. In Figure 8.4 there was little to no separation at all. 7 days after fermentation the kefir was seemingly homogenized, 14 days after fermentation there is a clear increase of carbon dioxide which caused the lid to open up. By day 21 there was clear separation of liquid in the kefir. By day 28 after fermentation, freeze dried strawberries were added that help with the texture of the kefir. The dried strawberries absorbed some of the extra liquid making the kefir smooth and creamy as it was day 7 of fermentation.

Microbial Testing

There were positive results from microbial testing. Plates showed a strong presence of *Saccharomyces* on each YPD plate and *Lactobacillus* on each MRS plate up until week 3 of testing. By week 3 of testing, there was clear degradation of live *Saccharomyces* and *Lactobacillus* organisms which is normal after a product is stored and chilled. Those results are expressed on Table 1 and Table 2. There was a single contamination during micro testing that resembled in spore of Bacillus on two YPD agar plates, this is likely from an air contaminant as it is confirmed to have active bacilli, resulting from plating technique.

Throughout the entire testing process each batch was negative for *E. coli* and coliforms as shown in Figures 10.1 and 10.2. Batches were negative for *Salmonella* as well after 3 weeks of storage, presented in Figures 11.1 and 11.2. The plates contained no reportable bacteria, therefore the were empty.

Below in Tables 2 and 3 are results from overall microbial testing. Week 1 of testing for batch 1 produced TNTC results for *Saccharomyces* and *Lactobacillus*, and negative results for *Listeria*, *E. coli*/coliform. Week 2 results were too numerous to count (TNTC) for *Saccharomyces*, an average of 190.8 × 10^6 CFU/mL for *Lactobacillus* and negative results for *Listeria*, and *E. coli*/coliform. Week 3 had an average of 106 × 10^6 CFU/mL of *Saccharomyces*, 17.4 × 10^6 CFU/mL of *Lactobacillus*, and had negative results for *Listeria*, *E. coli*/coliform, and *Salmonella*. Week 4 with the strawberry addition was only tested for pathogenic bacteria, and resulted in negative results for *Listeria*, *E. coli*/coliform, and *Salmonella*.

Batch 1

Test	**Week 1 (3-7-22)**	**Week 2 (3-14-22)**	**Week 3 (3-21-22)**	**Strawberry (week 4) (3-28-22)**	**Initial (day 0)**
Saccharomyces	TNTC*	TNTC	106 × 10^6 CFU/mL	X	TNTC
Lactobacillus	TNTC	190.8 × 10^6 CFU/mL	17.4 × 10^6 CFU/mL	X	TNTC
Listeria	Neg	Neg	Neg	Neg	Neg
E. coli	Neg	Neg	Neg	Neg	Neg
Coliforms	Neg	Neg	Neg	Neg	Neg
Salmonella	X#	X	Neg	Neg	X
Notes	Samples 2 & 4 had air contaminations on YPD plates			Only did pathogenic testing	
Observations		Looks curdled, chunky. smells like sour cream	Lots of separation, sweet cream smell, white	Little to no separation, sweet smell, pinkish	

*TNTC= Too numerous to count, >300 colonies

#X= Did not test

Week 1 of testing for batch 2 produced TNTC results for *Saccharomyces* and *Lactobacillus*, and negative results for *Listeria*, *E. coli/*coliforms. Week 2 results were TNTC for *Saccharomyces*, an average of 168.2 × 10^6 CFU/mL for *Lactobacillus* and negative results for *Listeria*, *E. coli/*coliforms and *Salmonella*. Week 3 had an average of 6 × 10^6 CFU/mL of *Saccharomyces*, 30× 10^6 CFU/mL of *Lactobacillus*, and had negative results for *Listeria*, *E. coli/*coliforms, and *Salmonella*.

Batch 2

Test	**Week 1 (3-14-22)**	**Week 2 (3-21-22)**	**Week 3 (3-28-22)**
Saccharomyces	TNTC*	TNTC	6 × 10^6 CFU/mL
Lactobacillus	TNTC	168.2 × 10^6 CFU/mL	30 × 10^6 CFU/mL
Listeria	Neg	Neg	Neg
E. coli	Neg	Neg	Neg
Coliforms	Neg	Neg	Neg
Salmonella	X#	Neg	Neg
Notes		S4 had air contamination on YPD plate	
Observations	Creamy pure white	Some separation, sour cream smell, pure white	Visual separation, cream cheese smell, white

*TNTC= Too numerous to count, >300 colonies

#X= Did not test

Sensory Testing

The sensory results of the homemade kefir were positive. The hedonic scale that was used went from 9-like very much to 1-dislike very much. From left to right in Figures 12.1 and 12.2, the strawberry flavor intensity was higher in the store bought over the homemade. The sparkly flavor intensity was higher in the homemade kefir. The sour flavor intensity was higher in the homemade kefir while the yeasty flavor was preferred in the store bought. The bitter flavor intensity scored evenly between the two kefirs. Creaminess had a better score from the store-bought kefir. Mouthfeel and after taste had the better score with the homemade kefir. The overall preferred kefir was the homemade one as shown in Figure 12.3. The overall preferred kefir was the homemade kefir (Appendix 1).

Discussion:

My older brother Ronald was diagnosed with UC 5 years ago, when he was 24 with no prior health issues or family history, and this is how this study was born. I was determined to research a food or way that he would feel comfort and rekindle his love of food again. In my research I found that probiotic foods are good for digestive health, and could be good for my brother. He is self-sufficient but not the best cook, so I had to find something that would help his gut that he could make at home.

Kefir ended up being the food I decided to research. I could not recommend this food to him without the proper research. Microbial testing on the homemade kefir ensured it would be safe for consumers, and it actually had the healthful probiotic bacteria, at significant amounts. The grains from cheesemaking.com lists *S. lactis, L. cremoris L. diacelilactis* and *L. acidophilus* as ingredients, so therefore it was expected that there would be probiotic bacteria present. I am confident in the results of pathogenic bacteria because of the microbiological results, however testing with a control is ideal to confirm there is no presence of the harmful bacteria. There were not any signs of spoilage, such as off smell and discoloration even after 28 days after fermentation. I do not assume when someone makes this at home it will produce the exact same microbial results, because the homemade kefir in this study was made and tested in a microbiology lab, and not in someone's home.

The store-bought kefir states to have 12 live and active cultures with 25-30 billion CFUs per 8oz. serving at the time of manufacturing. There was not a plate count done on the store-bought kefir but it could be done with future permissions and research. It was not deemed appropriate at the time because the manufacturer supplies the bacterial count amount. The homemade kefir was calculated to have at least 2 live cultures around more than 6.3×10^{10} CFUs per 7oz. jar. According to the WGO, someone with UC should consume 1800 billion CFUs of bacteria twice daily to positively contribute to remission [10]. The homemade kefir has sufficient numbers of live *Lactobacillus* and *Saccharomyces* to accomplish that recommendation. This result was not expected. I knew there would be some bacterial counts but not as high because the recipe was so easy to make. These results may not be conclusive because more replications of testing should be done at a smaller dilution to have more accurate counts of bacteria.

Limitations were expected in this research, due to the type of data collection that was used. A limitation that presented itself was the little turn out of sensory panelists. The goal number of panelists was fifteen students in the food science program at Mount Mary University. Unfortunately, only four students were available; however, being that they were food science students well-trained in the methods of sensory analysis, it was reasonable to believe their feedback was very useful. Also, these students were chosen because they would appreciate the necessity for data collection and could provide what they have learned in their course work to evaluate products effectively.

Though the sample size of the panelist was too small for the statistics to be significant, the scores are still interesting to evaluate. According to the prescreen survey the panelist admitted they do not consume kefir and would rarely to never make it at home. The results from those that do consume kefir often or have experience making it at home might be different; however, it can not be assumes to be any more conclusive.

Final comments made after the sensory tasting by a few panelist were, “I am a texture person so I do not like chunks that much", "I like seeing the real strawberries", "I like that the chunky one is not super sweet", "The strawberries are my favorite part, those are really good", and " I like the smoother one for a morning snack because it is sweeter, and the chunky one for maybe an afternoon snack". The smooth kefir was the store-bought kefir and the “chunky” one was the homemade kefir. These comments indicate a liking for the homemade kefir which was similar to the store-bought kefir.

The original flavor for this study was going to be vanilla, but strawberries were chosen instead because I wanted it to have stronger identifiable flavor. Freeze-dried strawberries were also chosen because have very little moisture content so I was not concerned with bacterial contamination as much as I would be with fresh fruit. I also figured it would help with natural separation in the kefir as shown in Figure 8.2 and 8.3, which it did. The texture of the freeze-dried strawberries was also liked, which were added 3-days before the kefir was tasted.

Recommended future developments of this research would be to add more probiotics initially, and to add a more diverse species like *Bifidobacterium* and *Streptococcus* since they are named by the WGO to help encourage remission. To do this at home, someone would have to order these species online. Other future developments on a larger scale would be to determine a longer shelf life study, and a development of plant-based kefir, using plant milks instead of dairy milks, for those that do not consume dairy.

This research is significant to the food science community because it helps confirm by reviewed literature, that added probiotics to food really make a difference to consumer health by increasing diversity in the gut microbiota. The food industry is constantly changing and having new research provides some insight into how to develop new and innovative foods further, especially if the foods have healing properties and can help someone like my brother, who enjoyed the homemade kefir.

I recommend people who consume kefir, and have UC to try making it at home. The results from sensory show strong results that it is better than, if not close enough to, the store-bought kefir. Which is important because if the product does not have good sensory results it would not be consumed, therefore the benefits of it would not be received. Also, the homemade recipe yields more than a singular bottle of kefir from the grocery store. The store-bought kefir comes in 32oz. bottles while this recipe yields 140 oz. The store-bought kefir is cheaper in price and requires no additional work, therefore may be cost efficient, but knowing all the ingredients in a homemade food product is worth the financial difference.

Conclusion:

UC is an inflammatory bowel disease that causes inflammation from the rectum to the colon and can be deadly. This disease has no official cause and is treated by medication, diet, and surgery. However, there is some hope in treating this disease, or assisting in remission, through healthy food consumption in addition to proper medication. Fermented probiotic foods may help in leading to remission. Kefir is a fermented food that contains the proper probiotics that can help with remission of UC. Those probiotics are *Lactobacillus* and *Saccharomyces*. Kefir can be store bought or made at home. The kefir in this research was developed with the purpose of providing high levels of *Lactobacillus* and *Saccharomyces* for people with UC.

The aims of this research were 1). To complete a thorough literature review to determine what microorganisms are lacking in the human gut that cause UC, and 2). to identify a fermented food that contains a microorganism that can holistically aid in the improvement of UC that can be homemade. Two microorganisms that are lacking in the human gut were *Lactobacillus* and *Saccharomyces* and the fermented food that contained these microorganisms is kefir.

In this study, a recipe from cheesemaking.com was adapted to produce a better recipe for strawberry kefir that had better sensory results than store-bought kefir. The final recipe of homemade kefir is as follows; wash and sanitize jars and all other utensils and let them air dry. For sanitizer you can make a bleach solution with 5mL bleach to 1L of water, or use a food safe sanitizing solution. Heat 1 gal of 2% milk to 86˚F. Once milk has reached that temperature pour it in a bowl with kefir grains and let them hydrate for 2 minutes. After hydration mix milk and kefir grains well and set aside at room temperature for 46 hours to ferment. After fermentation add 2 ½ cups of freeze-dried strawberries and place in refrigerator to cool. After cooling the kefir is ready to eat. 1 gallon of milk makes 17 ½ cups of kefir. The kefir should last at least two weeks if stored properly if the refrigerator. See Appendix 3 for kefir recipe card.

Results were more positive than negative, showing that making a kefir at home with beneficial bacteria is possible. This study is not recommending the consumption of kefir to replace current treatments. Kefir is food that has the possibilities to provide some relief to those with UC which is important, because of the growing population of those being affected by the disease. Research about benefits of kefir should continue and help bridge gap between UC and its current treatments.

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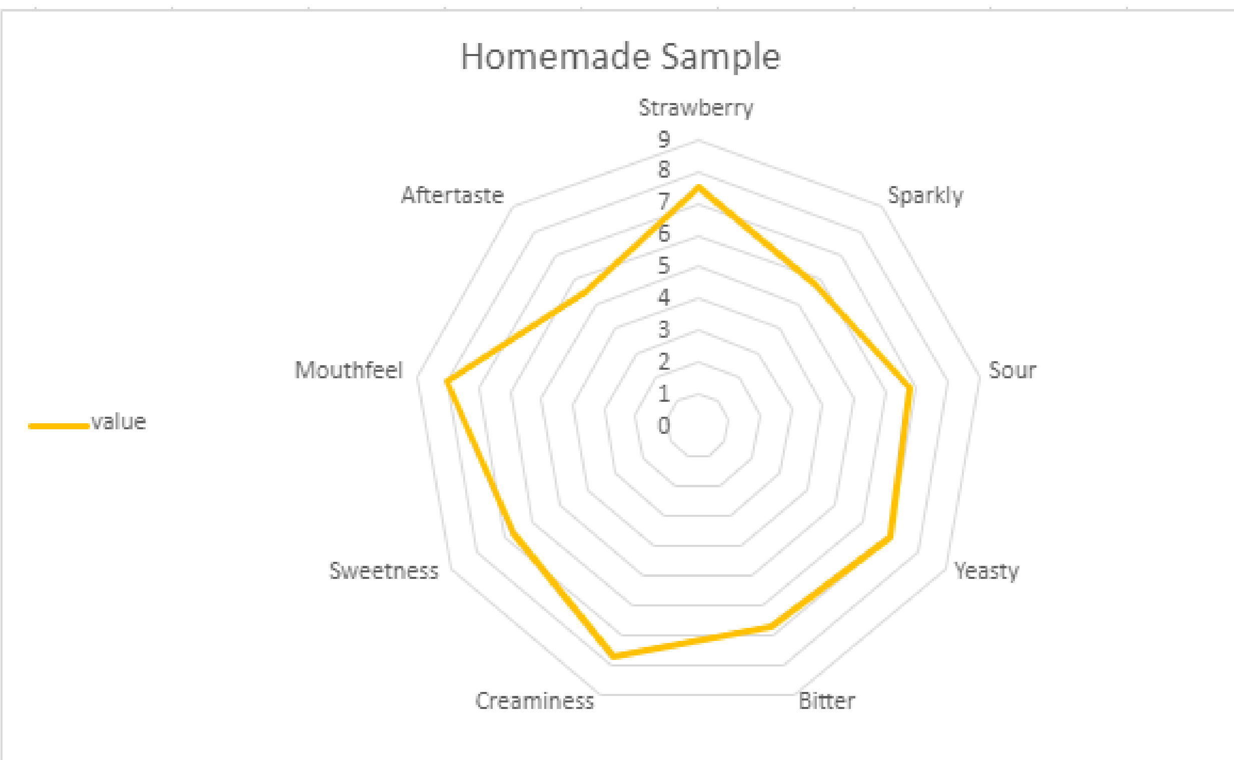
Appendix 1


Store-bought Sample
Strawberry
Sparkly
Sour
Yeasty
Bitter
Creaminess
Sweetness
Mouthfeel
Aftertaste
9
8
7
6
5
4
3
2
1
0
Value


Homemade Sample
Strawberry
Sparkly
Sour
Yeasty
Bitter
Creaminess
Sweetness
Mouthfeel
Aftertaste
9
8
7
6
5
4
3
2
1
0
value

Appendix 2

In this sensory tasting you will be given two samples to taste and evaluate. Before beginning the tasting, cleanse your palate by taking a sip of D.I. water. Evaluate one sample at a time. Once you are ready to move on to the next sample, cleanse your palate by taking another sip of water. Be sure to answer all the questions for one sample before moving on to the next. Flavor definitions are located at the bottom of page 2.

Please circle response:

1. How often do you consume Kefir?

Daily, three times a week, once a week, several times a month, never

2. To your knowledge, how often do you consume fermented, probiotic foods?

Daily, three times a week, once a week, several times a month, never

3. How likely are you to make Kefir at home?

Likely, somewhat likely, neither likely or not likely, rarely, not likely at all

Sample #:

1. How do you feel about the intensity of strawberry flavor?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

2. How do you feel about the intensity of sparkly flavor?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

3. How do you feel about the intensity of sour flavor?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

4. How do you feel about the intensity of yeasty flavor?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

5. How do you feel about the intensity of bitter flavor?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

6. How do you feel about the level of creaminess?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

7. How do you feel about the level of sweetness?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

8. How much do you like or dislike the mouthfeel of the sample?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

9. How much do you like or dislike the aftertaste of the sample?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

10. How do you fell about the overall flavor of the sample?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

Sample #:

1. How do you feel about the intensity of strawberry flavor?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

2. How do you feel about the intensity of sparkly flavor?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

3. How do you feel about the intensity of sour flavor?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

4. How do you feel about the intensity of yeasty flavor?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

5. How do you feel about the intensity of bitter flavor?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

6. How do you feel about the level of creaminess?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

7. How do you feel about the level of sweetness?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

8. How much do you like or dislike the mouthfeel of the sample?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

9. How much do you like or dislike the aftertaste of the sample?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

10. How do you feel about the overall flavor of the sample?

Like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely

Answer in a few sentences:

Which of the two samples do you prefer? Why?

Flavor Definitions:

Sparkly: fizzy, carbonated

Sour: Having an acidic taste; fermented

Yeasty: tasting of or like yeast

Bitter: Sharp pungent taste; not sweet

Appendix 3

Ronald's Recipe

Wash and sanitize jars and all other utensils and let them air dry.

For sanitizer you can make a bleach solution with 5mL bleach to 1L of water, or use a food safe sanitizing solution.

Heat 1 gal of 2% milk to 86˚F

Once milk has reached that temperature pour it in a bowl with kefir grains and let them hydrate for 2 minutes.

After hydration mix milk and kefir grains well and set aside at room temperature for 46 hours to ferment.

After fermentation add 2 ½ cups of freeze-dried strawberries and place in refrigerator to cool.

After cooling the kefir is ready to eat. 1 gallon of milk makes 17 ½ cups of kefir. The kefir should last at least two weeks if stored properly if the refrigerator.