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Investigating the Potential of Almond Hulls as a Feedstock for Fermented Cattle Feed

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Investigating the Potential of Almond Hulls as a Feedstock for Fermented Cattle Feed

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Abstract

California produces 2.55 billion pounds of almond kernels annually, and along with that 4.03 billion pounds of byproduct almond hulls are generated. Almond hulls have high sugar contents and are mainly used as livestock bedding and feed at present. The almond industry is seeking ways to reduce the environmental impact of conventional almond production by reducing dust generation and achieving zero waste. Off-ground harvesting is an emerging harvesting strategy that generates significantly less dust; however, off-ground harvesting produces a stream of wet hulls that can easily spoil if they are not processed properly. Wet almond hulls are an ideal feedstock for fermentation into high quality, probiotic-rich animal feed which can help the almond industry meet its zero-waste goal and increase revenue for almond farmers.

Fermented animal feed is an alternative to the use of growth-promoting antibiotics in livestock and has already been recognized as an effective tool to improve gastrointestinal health and improve productivity in swine and poultry. It is possible that similar benefits can be observed in cattle fed fermented feed. Additionally, studies have shown that probiotic supplementation and alternative feeding strategies can reduce ruminant methane generation, the largest source of anthropogenic methane emissions in the US. The goal of this study is to assess the potential of using almond hulls as a feedstock for the production of a fermented cattle feed which can potentially reduce the environmental impact of the almond industry while concurrently increasing the value of almond hulls, improving cattle digestion, and reducing enteric methane emissions.

In this study, California almond hulls of Nonpareil, Monterey, Independence, and Fritz varieties from a hulling facility were characterized for their chemical composition. The hulls had high sugar contents ranging from 31.8% to 42.2% by weight on a dry-basis (db) and phenolic compound content ranging from 3.4% to 7.6%, db. On- and off-ground harvested hulls of

Independence, Monterey, and Fritz varieties were also characterized and compared. Off-ground harvested hulls had an average moisture content 3.5 times higher than on-ground harvested hulls of the same variety. Hulls were low in protein and fat at an average of 5.7% and 3.7% db, respectively and an average of 24.1% acid detergent fiber and 33.3% neutral detergent fiber.

The hulls were characterized for the quantity of contaminants vs. hulls and on average, hull samples contained 88.9% hulls and 10.1% contaminants. Sieving and terminal velocity analysis were conducted to investigate methods for separating hulls from contaminants. The results showed that sieving separation was an effective method for separating hulls from contaminants.

Solid-state, inoculated fermentation trials were conducted using *Saccharomyces cerevisiae* and *Lactobacillus plantarum* as inoculums. The effect of inoculum type, fermentation duration, inoculum amount, hull variety, particle size, and fermentation temperature were investigated through several fermentation experiments. It was found that fermentations using *S. cerevisiae* produced higher amounts of ethanol and acetic acid and caused an increase in pH, and fermentations using *L. plantarum* produced higher amounts of lactic acid and caused a drop in pH. Hull variety had a large impact on the characteristics of the fermented hulls under the conditions tested. 14-day fermentation durations produced similar results as 30-day fermentation durations in the conditions studied. Additionally, in-vitro digestion results indicated that almond hulls fermented with *S. cerevisiae* for 14 days reduced enteric CH_4 production by 96% over 72 hours digestion at a 20% inclusion rate in a cattle diet.

The nutritional composition of almond hulls, especially their high sugar content, makes them an ideal feedstock for the creation of fermented cattle feed. Almond hulls fermented with *S. cerevisiae* and *L. Plantarum* produce desirable characteristics for fermented feed including a pH below 4.5, high concentrations of lactic acid, and low concentrations of acetic acid. In-vitro

digestion tests suggest that fermented feed is potentially an effective strategy to reduce enteric CH_4 production.

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Chapter 1 – Introduction

1.1 Background

California is the world's leading almond producer, producing 2.55 billion pounds of almond kernels annually (Almond Board of California, 2020). Standard almond harvesting practice involves on-ground drying which generates large amounts of dust when the almonds are collected (Chen et al., 2021a). Additionally, for every pound of almond kernels produced, 1.58 pounds of byproduct almond hulls are produced, amounting to an annual production of 4.03 billion pounds of almond hulls. At present, almond hulls are used as cattle feed and livestock bedding (Almond Board of California, 2020).

An alternative harvesting method involving off-ground harvesting, wet-hulling, and supplemental drying of only the almond kernels shows promise as an effective method for reducing dust generation during harvest in addition to lowering drying time and energy consumption (Chen et al., 2021a). However, the off-ground harvested almond hulls have high moisture contents and need to be quickly dried or otherwise processed to preserve their quality and ensure stability (Chen et al., 2021c).

Simultaneously, there is interest from the California almond industry to create higher-value products from almond byproducts, including hulls (Almond Board of California, 2021). Almond hulls have the potential to be used for different valorization routes which could offer greater price points for the industry due to their high composition of fiber, sugars, and antioxidant compounds (Salgado-Ramos et al., 2022). Additionally, almond acreage is expanding in California while dairies are simultaneously consolidating and moving out of state, further increasing the need to find new and more profitable uses for hulls and shells (Almond Board of California, 2021).

Almond hulls are a good feedstock for due to their high content of sugar (25 to 33% dry basis) (Offeman et al., 2014). Additionally, off-ground harvested almond hulls have high-moisture contents that can be used as a fermentation feedstock without the need for drying (Chen & Pan, 2022). Fermented feed is a new development in the livestock industry that has shown benefits in swine and poultry such as improved performance, nutrient digestion, and immune responses (Sugiharto & Samir, 2018; Missotten et al., 2015).

Less research has been conducted on feeding cattle fermented feed, but studies have shown that alcohol-fermented feed can bring nutritional benefits to beef and dairy cattle and that probiotic supplementation increases diet digestibility and enhances performance parameters in dairy animals (Li et al., 2012; Lin et al., 2004). Feeding yeast to cattle has also been shown to reduce enteric methane emissions (Cottle et al., 2011; Vallejo-Hernandez et al., 2018; Eun et al., 2003). Additionally, cattle are already consuming almond hulls in low amounts (Swanson et al., 2021). Fermenting the hulls into a high-value, probiotic-rich feed product could increase the number of hulls fed to cattle and potentially bring additional benefits such as reduced methane generation, improved feed utilization rates, and better productivity.

Increased adoption of off-ground harvesting and the use of byproduct hulls to generate fermented cattle feed would help the Almond Board of California achieve two of its four 2025 Almond Orchard Goals: 1) achieve zero waste in orchards by putting everything grown to optimal use, and 2) reduce dust generation during harvest by 50% (Almond Board of California, 2019). It would also allow almond producers to generate more revenue from the almond production process by raising the value of almond hulls. A fermented almond hull feed may also be nutritionally beneficial and reduce methane emissions from cattle.

The goal of this project is to investigate the potential of using California almond hulls to create a stable, high quality, and nutritious fermented cattle feed. Conventionally harvested hulls as well as off-ground harvested hulls that are wet-hulled prior to drying will be investigated as fermentation feedstock. Various fermentation parameters will be explored to elucidate their effect on the quality and composition of the feed.

1.2 Objectives

The objectives of this study are to investigate the feasibility of using conventionally harvested and off-ground harvested California almond hulls as a fermentation feedstock for the production of a fermented cattle feed that will raise the economic value of byproduct almond hulls. The specific objectives of this research were:

1. Determine the physical and chemical properties of almond hulls and study methods for separating hulls from contaminants (shells, kernels, woody biomass).
2. Develop an effective fermentation process for producing fermented feed from almond hulls and investigate the effects of different fermentation parameters on the characteristics of the fermented feed.

Chapter 2 – Literature Review

2.1 Almonds

2.1.1 California Almond Industry

Almonds are stone fruits that grow on trees annually between mid-February and July in California. California produces 78% of the world's almond supply and is the largest almond-producing state in the United States. In 2020, almonds were California's second most valuable commodity, valued at $6.09 billion. (Almond Board of California, 2020). Additionally, almond production can be expected to increase dramatically when the nonbearing orchards come into production in the next five to ten years (DePeters et al., 2020). The most widely grown almond variety in California by acreage is Nonpareil, making up about 40% of California's annual almond production. Other top varieties include Monterey, Independence, Carmel, Fritz, Butte, and Padre (USDA NASS, 2021).

2.1.2 Conventional Almond Harvesting

The almond harvest occurs once per year in California from August through October. In the early fall, the almond hull splits open and exposes the almond shell, allowing it to be dried by the sun. When the hulls open fully, mechanical tree shakers shake the trunks of each tree to knock the fruits to the ground. Next, a mechanical sweeper sweeps the fruits into rows where they sun-dry for up to two weeks before being collected by a harvesting machine using a vacuum (Sumner et al., 2014; Almond Board of California, 2021a). The process of shaking, sweeping, and vacuuming generates large amounts of dust in the air which causes air pollution and impacts the health of millions of people (Chen, et al., 2021c). The collected, in-shell nuts are taken to a processing facility where the fruits pass through rollers to separate the almond's hull and shell from the kernel. From here, the kernels are further processed and sold to consumers, whereas the

hulls are used as livestock bedding and dairy feed (Sumner et al., 2014; Almond Board of California, 2021a).

2.1.3 Almond Industry Sustainability Goals

In recent years, the environmental impact of conventional almond harvesting and processing has become a topic of interest and concern. In 2018, the Almond Board of California declared the following four goals that they aim to achieve by 2025 as a commitment to continuous improvement (Almond Board of California, 2019):

1) Reduce the amount of water used to produce almonds by 20%
2) Achieve zero waste in orchards by putting everything grown to optimal use
3) Increase adoption of environmentally friendly pest management tools by 25%
4) Reduce dust generation during harvest by 50%

The Almond Board of California has provided $89 million to researchers since 1973 to investigate novel methods for improving farming practices, minimizing environmental impacts, utilizing orchard biomass, and ensuring food quality and safety in the California almond industry (Almond Board of California, 2021b).

2.1.4 Off-ground Harvesting

Off-ground harvesting is an alternative almond harvesting method that uses a catch frame method to collect almond fruits directly from the tree and skip the on-ground drying and collection steps. The Almond Board of California is currently researching off-ground harvesting technology because of its potential to mitigate dust generation as well as reduce insect damage and microbial contamination due to the almonds not contacting the orchard soil (Chen et al., 2021c; Chen et al., 2021a), Other benefits of off-ground harvesting include cleaner fruits delivered to processors, flexibility in irrigation scheduling, and less orchard floor management (West Coast Nut, 2021). In

Italy, the Tenias Machinery Company have developed and piloted an over-the-row shake and catch almond harvester that combines elements of a grape and a Californian-style trunk shaking head to harvest almond fruits directly from the tree (Brown, 2014).

Because off-ground harvested almonds usually have much higher initial moisture contents than conventionally harvested almonds, there is a need for efficient drying methods that can handle the large volume of production in the short harvest season to ensure product quality and safety. (Chen & Pan, 2022). However, the high moisture content of the almonds means artificial drying is not economically favorable when compared to conventional harvesting (Chen et al., 2021c). Wet dehulling and pre-sorting of the off ground harvested almonds, methods that have been implemented in the walnut and pistachio industries, can improve drying efficiency and reduce energy consumption significantly (Chen & Pan, 2022). In a study on hot air column drying of off-ground harvested almonds, more than 60% drying energy consumed was used to dry the hulls and only about 20% of the total energy was used to dry the kernels (Chen et al., 2021c). Wet dehulling and hot air column drying of hulled, in-shell almonds has been shown to reduce drying time by 75% and resulted in 78.6% lower specific energy consumption compared to drying in-hull off-ground harvested almonds (Chen et al., 2021a). Additionally, it was found that loose hulls and hulls from in-hull almonds make up around 60% of the weight in the dryer, so separating the loose hulls and de-hulling the in-hull almonds before the drying will also reduce the space and cost needed for handling, transportation, and drying (Chen et al., 2021b). The Tenias harvester uses rubber rotors in the field to wet-hull almonds without damaging the in-shell almond (Brown, 2014).

2.1.5 Almond Hulls

Almond hulls are the outer leathery hull of the almond fruit that are produced as a byproduct of kernel production. Almond hulls are anatomically similar to the fleshy portion of a peach and they protect the almond shell and kernel inside (DePeters et al., 2020). Almond hulls tend to contain substantial quantities of sugars (~30%) and soluble fiber (~17%) (Fuquay et al., 2011). They are also rich in natural antioxidants such as tannins and phenolics (Chen & Pan, 2021).

Almond harvest field weight distribution is approximately 31% kernel, 20% shell, and 49% hull. In the 2019/20 crop year, California almond farmers produced 2.55 billion pounds of almond kernels and 4.03 billion pounds of almond hulls (Almond Board of California, 2020). Almond hulls are currently used for dairy feed (90%) and bedding and mulching (10%) (Almond Board of California, 2020). The downsizing of the dairy industry in California has lowered the need for almond hulls as dairy feedstuff and bedding. However, the almond industry continues to grow, resulting in an excess of hulls (Hart et al., 2020). Alternative uses for conventionally harvested almond hulls need to be identified to turn them into value-added products.

The emerging harvesting method know as off-ground harvesting produces almond hulls that not only have high sugar contents but also have high-moisture contents which allows them to be used for fermentation without the need of drying (Chen et al., 2021c; Chen & Pan, 2021). Both on- and off-ground harvested almond hulls are an ideal raw material for producing fermented animal feed of high economic and nutritional value. Increased adoption of off-ground harvesting and the use of the byproduct wet hulls to generate a marketable fermented animal feed would help the Almond Board of California achieve its 2025 Almond Orchard Goals and enable almond growers to generate more revenue from the almond production process. Additionally, conventionally harvested hulls could be included in the production process by increasing their

moisture content, thus creating a more profitable use of on-ground harvested hulls while the industry transitions to off-ground harvesting.

2.2 Fermented Animal Feed

2.2.1 Classification of Fermented Feed

Fermentation is a dynamic process involving microorganisms, substrates, and environmental conditions to convert complex materials into simpler compounds. Fermentation can be spontaneous where it occurs through the action of indigenous microflora present on the substrate or inoculated by the addition of external bacteria and/or yeasts (Missotten et al., 2015). Fermented feed is a new development in the industry in which the fermentation process is employed to produce functional feeds that have the potential to improve health, production, and performance of the animals consuming it (Sugiharto & Samir, 2018). Fermented feed has a low pH, and contains high concentrations of lactic acid, several volatile fatty acids (VFAs) and large numbers of lactobacilli (Van Winsen et al., 2001). The quality of a fermented feed is based on interactions between the micro-organisms present (bacteria, fungi and yeasts, coliforms), the fermentation parameters (time, temperature, feed:water ratio), and substrate composition (carbohydrates, fibers, proteins, amino acids, vitamins) (Missotten et al., 2015).

2.2.2 Usage of Fermented Feed

2.2.2.1 Swine

Over the last 20 years fermented feed has been successfully incorporated into swine production in Europe, where it is now recognized as an effective tool to improve gastrointestinal health and reduce the use of antibiotics in swine. The European Union's 2006 ban on the use of antibiotics as antimicrobial growth promoters for swine partially spurred this development

(Missotten et al., 2015). When ingested by pigs, fermented feed increases in the concentration of lactic acid bacteria in the stomach and small intestine and increases the number of yeast cells in the gastrointestinal tract which helps inhibit enteropathogens such as Salmonella spp. and E. Coli (Missotten et al., 2015). Fermentation also causes a reduction of pH which inhibits pathogenic organisms from developing in the gastrointestinal tract and the feed itself (Canibe & Jensen, 2012). In multiple studies, pigs fed a fermented diet have shown improved performance and nutrient digestion (Liu et al., 2022; Huang et al., 2020; Yuan et al., 2017). Fermented feed is now accepted as one of the most effective feeding strategies to replace the use of antibiotic growth promotors in swine (Missotten et al., 2015).

2.2.2.2 Chickens

Preliminary studies have also shown that fermented feeds help to maintain healthy gastrointestinal ecosystems in chickens, owing to key characteristics such as low pH, high numbers of lactobacilli, high concentrations of lactic and acetic acids, and low enterobacteria numbers (Sugiharto & Samir, 2018). The lactic acid bacteria and low pH in fermented broiler chicken feed has been suggested to inhibit the growth of bacteria such as *Salmonella typhimurium* and *Escherichia coli* in the chicken diet (Niba et al., 2009). The fermented feed has also been shown to decrease mortality rates, positively affect immune responses, and increase the weight of immune organs in broiler chickens. Improved feed conversion ratio and increased weight gain were observed in broiler chickens fed fermented feed (Sugiharto & Samir, 2018).

2.2.2.3 Cattle

Scientific studies on fermented cattle feed are scarce, but there has been some interest in alcohol fermented cattle feed. It has been shown that the addition of ethanol to beef cattle diets improves both feed efficiency and meat quality. Alcohol is known to be absorbed through the

rumen wall and about 20% is converted to acetate and other volatile fatty acids (VFAs) by rumen microorganisms (Li et al., 2012). In the rumen, alcohol can also be synthesized by fungi and bacteria (Matthews et al., 2019). The use of alcohol-fermented feeds has been shown to increase the marbling score of Korean native steers (Ho et al., 2020). Another study observed that production of VFAs in the rumen was affected by supplementation of alcohol-fermented feed and that alcohol-fermented feed increased the body weight gain of Korean native steers via decreased protein degradation and increased fat synthesis (Lin et al., 2004). Alcohol fermented feed has also been shown to reduce cholesterol concentration in milk, thus improving human consumer health without negatively affecting feed intake or milk production of the lactating cows (Li et al., 2012).

2.2.3 Fermentation Feedstock

A byproduct or coproduct feedstuff is a substrate that can be utilized as livestock feed but is not the primary product derived from processing (Mathis et al., 2012). The use of agroindustiral byproduct feedstuffs to produce fermented feed is recommended because these substrates are abundant, readily available, possess suitable nutrient composition, have less competition for human consumption, and can aid microbial development during fermentation. Rapeseed meal, canola meal, cottonseed meal, palm kernel cake, lupin flour, and cassava pulp have all been the focus of fermented animal feed studies (Olukomaiya et al., 2019).

2.2.4 Fermentation Methods

There are several fermentation methods that can be utilized to produce fermented animal feed. Solid state fermentation (SSF) involves microorganisms growing on solid materials under controlled conditions in the absence of a free-flowing liquid. SSF is used to produce fermented dry feed (FDF) which can be added to feed mixes or produced in a powder form. SSF is low cost, generates little wastewater, and requires minimal technology, making it an easy process to conduct

on farms. Other advantages of SSF include high productivities and extended stability of products, lower energy requirements, less wastewater, and less solid waste disposal (Holker & Jurgen, 2005). Disadvantages of SSF include difficulties scaling and controlling the process, varying consistency in the final product, loss of some feed nutritional components, and decreased palatability (Sugiharto & Samir, 2018; Olukomaiya et al., 2019). SSF is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content (Subramaniyam & Vimala, 2012).

Fermented feed can also be produced by submerged fermentation (SmF). In SmF, a dry feed substrate is mixed with water or another liquid to generate a fermented liquid feed (FLF). In SmF, substrates are utilized quite rapidly; hence there is a constant need for supplemental substrate and nutrient additions. This fermentation technique is best suited for microorganisms such as bacteria that require high moisture content. An advantage of this technique is that purification of products is easier (Subramaniyam & Vimala, 2012). Liquid animal feed production creates opportunities for the recycling of liquid co-products from the human food industry (Sugiharto & Samir, 2018). Additionally, wet feeding in hot climates has been shown to improve feed intake and growth rates in poultry; however, liquid feeds have the potential to serve as potent reservoirs of enteropathogens unless steps are taken to prevent their introduction and proliferation during storage and feeding (Niba et al., 2009). Also, SmF is generally a more expensive process than SSF due to higher use of substrate and water and more complex processing equipment (Holker & Jurgen, 2005).

SSF has received the most interest for fermented livestock feed production as this method generates higher yields and better product characteristics than SmF (Sugiharto & Samir, 2018). Another possible feed production method involves fermenting only the carbohydrate-rich cereal

components of the diet and combining them with the protein-rich components just before feeding (Niba et al., 2009).

2.2.6 Fermented Feed from Almond Hulls

Almond hulls are a suitable substrate for the production of fermented cattle feed because their high sugar content can be quickly fermented into organic acids. Almond hulls are composed of soluble sugars (21-25%), cellulose (9-16%), hemicellulose (7-10%), lignin (4-15%), pectin (4-6%), fat (1-2%), ash (6-13%), and sugar alcohols such as inositol (2-2.5%) and sorbitol (3-5%) on a dry weight basis (Holtman et al., 2014). Additionally, almond hulls naturally contain several phenolic compounds that have antioxidant properties such as chlorogenic acid, catechin, and protocatechuic acid which can help preserve the fatty acids produced during the fermentation process (Kahlaoui et al., 2019). Previous studies were successful in using almond hull extracts to produce lactic acid and edible fungi via fermentation (Thomas et al., 2019; Zhang et al., 2020).

2.2.7 Desired Characteristics of Fermented Feed

Levels of at least 75 mmol/L or 6.8 g/kg WB of lactic acid have been shown to prevent growth of enterobacteria including Salmonella spp. in fermented liquid pig feed (Beal et al.,2002). This level of lactic acid has the additional benefit of improving feed intake, daily body weight gain, and feed efficiency (Roth & Kirchgessner, 1998). In a study on fermented liquid feed for pigs in which a dry pig feed was mixed with water in a 1:2.5 ratio and was stored in a tank at 20°C for 4 days, it was found that the pH at feeding needs to be below 4.5 in order to eliminate enteric pathogens, such as E. coli and Salmonella spp. Feed costs represent nearly 50% of the total milk production cost, so it is economically important for dairy farmers to maximize feed intake and improve efficiency of feed use (Grant, 1990). Although there are naturally occurring yeasts and bacteria present on almond hulls that could be used for spontaneous fermentation, spontaneous

fermentation can result in acetic acid concentrations above 30 mmol/L or 1.8 g/kg WB, which negatively affects the palatability of fermented liquid pig feed (Brooks et al., 2003). Additionally, propionic acid at average levels of 1.0 g/kg DM have been shown to decrease silage dry matter intake of cattle (Krizsan & Randy, 2007).

2.3 Ruminant Methane Emissions

2.3.1 Ruminant Digestion

Cattle are ruminant animals. They have four stomach compartments and do not completely chew their food when they eat. Instead, they partially chew it and store it in their largest stomach compartment (the rumen) where it is regurgitated and chewed until it passes through the next three compartments (the reticulum, the omasum and the abomasum). Ruminant animals produce methane gas (CH_4) via their digestion process, and they emit this CH_4 to the atmosphere through belches and flatulence (Boadi et al., 2004).

The process of ruminant CH_4 generation is as follows: bacteria, protozoa, and fungi in the rumen hydrolyze the proteins, starch, and plant cell wall polymers contained in the feed into amino acids and sugars. These products are then fermented into volatile fatty acids (VFAs), hydrogen gas (H_2) and carbon dioxide (CO_2). The majority of the VFAs produced by cattle are acetate, propionate, and butyrate, which are utilized by the animal as their main energy source. H_2 is produced as a byproduct of acetate and butyrate synthesis, with the majority being generated by the acetic acid fermentation pathway. This H_2 is then utilized by microorganisms called methanogens which reduce CO_2 to CH_4 via a process called methanogenesis (Boadi et al., 2004). CH_4 has no nutritional value to cattle, so its production represents a loss of dietary energy to the animal; cattle typically lose 2%–15% of their ingested energy as eructated CH_4 (Reynolds et al., 2010).

2.3.2 Dairy Cattle Diet

The creation of dairy cattle feed involves the sciences of nutrition, biochemistry, and microbiology combined with animal husbandry to create a complete, healthy cattle diet. Diet has a direct effect on dairy cattle performance which is measured in terms of growth and milk production. Carbohydrates, amino acids, fatty acids, minerals, vitamins, and water are all required by the lactating dairy cow to enable the mammary gland to produce milk. Carbohydrates including forages, roughages, grains, and sugars comprise up to 70% of the diet and are the primary source of calories. Fat recommendations for cattle feed typically do not exceed 8% of the total dry matter. Cattle do not have a protein requirement, but they do require specific amounts of amino acids to produce enzymes, milk proteins, immunoglobulins, muscles, and various organs and tissues throughout their body. Through proper nutrition and management, the dairy heifers can produce to their maximum genetic potential and provide the most economic benefit to farmers (Erickson & Kalscheur, 2020).

2.3.3. Cattle and Almond Hulls

Almond hulls as a dairy feed have a nutritional value equal to mid-grade alfalfa hay and provide dietary fiber and highly fermentable carbohydrates to cattle in the form of sucrose, fructose, glucose, inositol, and sorbitol (UC Davis, 2014; DePeters et al., 2020). Almond hulls are considered a "pseudo forage" and a "pseudo concentrate" in cattle diets, but the highly fermentable sugars make them a better replacement for concentrates instead of forages in a lactating cow diet. They are moderate on energy and digestibility levels and have similar characteristics to an overly ripe fruit that has lost some of its quality due to age (California Feed and Grain Association, 2016). Almond hulls have comparable energy values to corn silage but are much more cost-effective for

use in cattle diets. Recently, almond hulls have been on the market for $13/wet ton ($155/ton DM), whereas corn silage values are $80-$85/ton cured ($250/ton DM) (Oliveria, 2021).

Some cows are already consuming almond hulls in small amounts of about 5% of their diet; a survey conducted in California in 2012 found that of the 104 cattle diets sampled, 39 contained almond hulls with an average of 1.45 kg/day (3.2 lb/day) per cow being fed (Swanson et al., 2021). A more recent survey found that the feeding amount had increased to approximately 2.3 kg (5 pounds) per lactating cow daily (DePeters et al., 2020). With an estimated 1.75 million dairy cows in California in 2017, even if every cow consumed almond hulls at this inclusion rate, there would still be a surplus of about 800 million pounds of almond hulls annually (DePeters et al., 2020; Swanson et al., 2021). Research results have shown that hulls can be included in dairy rations at levels as high as 20% of diet dry matter with little impact on milk production, and that increasing amounts of almond hulls in the diet up to 20% could lead to improved digestibility and milk fat percentage (Swanson et al., 2021). Another study found that hulls can be fed up to 25% of the total mixed ration with no negative effects on milk production or feed intake (Aguilar et al., 1984). Fermenting the hulls could potentially make them easier for cattle to digest and therefore increase the amount of total almond hulls that can be included in their feed rations. Almond hulls' sugar content can also add palatability to a dairy cattle ration (Oliveria, 2022).

2.3.4 Ruminant Methane Mitigation Strategies

27% of CH_4 emissions in the United States are attributed to enteric fermentation (EPA, 2022). CH_4 is a potent greenhouse gas; per unit of mass, the impact of CH_4 on climate change is 86 times greater than CO_2 over a 20-year period and 28 times greater than CO_2 over a 100-year period. Ruminant CH_4 emissions are part of the biogenic carbon cycle, so any reduction in ruminant CH_4 emissions effectively "pulls" carbon from the atmosphere an induces a cooling effect

(UC Davis CLEAR Center, 2020). Much research has been done in recent years on reducing CH_4 emissions from ruminant animals because of this positive environmental effect that it can induce. Current CH_4 reduction strategies include but are not limited to: using antibiotics, promoting viruses/bacteriophages, using feed additives such as fats and oils, nitrate salts, and dicarboxylic acids, defaunation, vaccination against methanogens, inoculating with acetogenic species, feeding highly digestible feed components favoring 'propionate fermentations', modifying rumen conditions, improving animal productivity, alternative grazing strategies, and genetic modification through selective breeding (Cottle et al., 2011).

The majority of enteric CH_4 mitigation strategies currently being studied are mechanistically geared toward enhancing the ratio of propionate: acetate produced during rumen fermentation because this lowers the amount of free H_2 available to be reduced into CH_4 (Boadi et al., 2004). Researchers have discovered several ways to increase the propionate: acetate ratio in ruminant animals. Ruminants can be fed feed additives such as monensin, a naturally occurring polyether ionophore antibiotic, which results in reduction of acetate formation and associated hydrogen production by inhibiting the release of hydrogen from formate (Grainger et al., 2008). Defaunation, or the elimination of protozoa from the rumen, has been shown to produce a higher propionate: acetate ratio in total rumen VFA and a higher microbial protein outflow from the rumen. (Cottle et al., 2011). Diets high in rapidly fermentable grains will more rapidly lower rumen pH, which has been found to kill protozoa, removing one of the major habitats of methanogens (Martin et al., 2010). Feeding more forage-based diets high in cellulose, hemicellulose, and lignin favor production of acetate and butyrate, whereas starch-based diets favor propionate production (Johnson & Johnson, 1995).

Probiotic supplementation is another way to increase the propionate: acetate ratio and reduce CH_4 generation. In one study, feeding live probiotic yeast of the strain *S. cerevisiae* to lactating dairy cows reduced their CH_4 emissions 4% (Tristant & Moran, 2015). It has been found that yeast cultures reduce CH_4 production in ruminants by increasing butyrate or propionate production, reducing protozoan numbers, and improving animal productivity (Cottle et al., 2011, Eun et al., 2003). Probiotic supplementation has also been proven to improve feed utilization rate, milk yield and component profiles, and dry matter intake in cattle (Xu et al., 2017). Several studies have found that feeding live yeast cultures to cattle improves fiber digestion, milk yield, and milk protein percentage (Schlabitz et al., 2022; Rossow et al., 2018).

2.3.5 California Methane Emissions Targets

California's 1.7 million cows and 1,250 dairies account for 19% of all U.S. milk production and draw $1.8 billion in annual export value. At the same time, 7% of California's GHG emissions are attributed to agriculture, with 70% of those emissions being CH_4 emissions (Hooker, 2022). In 2016, Senate Bill (SB) 1383 was passed, establishing both a statewide CH_4 emissions target to 40% below 2013 levels by 2030 and an equivalent target for the dairy and livestock sector (Lara, Chapter 395, Statutes of 2016). So far, the state has depended on incentives grants to lower emissions in the dairy industry, primarily through anaerobic digester projects that capture manure emissions. California's existing digesters will remove about two million tons of emissions each year—amounting to about 22% of the CH_4 reductions needed to meet the 2030 target. A 2022 analysis by the California Air Resources Board (CARB) predicts that with current practices, the dairy and livestock sector will achieve just over half of the CH_4 reductions needed to meet the 2030 target. CARB recommends that the state focuses more on enteric CH_4 mitigation strategies if it wants to meet the 2030 CH_4 emissions reduction target (CARB, 2022).

Chapter 3 – Almond Hull Characterization and Separation

3.1 Introduction

Although hulling facilities are able separate a majority of the non-hull components from hulls, it is not possible with current methods to remove all shells, sticks, and other contaminants of short length from the hull stream (DePeters et al., 2020). Whereas almond hulls are a good quality feed ingredient, almond shells and woody biomass add no nutritional value and decrease digestibility because these materials have high fiber (32.5%) and lignin (32.83%) contents (Li et al., 2018). In order to create a high-quality, nutritious, and consistent feed product from almond hulls, a reliable, high purity stream of almond hulls is needed. Therefore, the amount and type of contaminants in current almond hull streams from hulling facilities needs to be quantified and characterized and a method for separating the debris components from the hulls to achieve desired feed-quality purity levels is needed. Additionally, the physical and chemical attributes of the hulls need to be clearly understood. The results of this research will inform the selection of fermentation conditions to be tested in Chapter 4.

3.2 Objectives

The objectives of this research were to: (1) Determine the physical and chemical properties of almond hulls; and (2) Study methods for separating hulls from contaminants (shells, kernels, woody biomass).

3.3 Materials and Methods

3.3.1 Hull Samples

Almond hull samples of Nonpareil, Monterey, and Independence varieties were collected directly after hulling from West Valley Hulling Co. in Firebaugh, CA during the 2020 harvest season. These almond hulls were harvested using conventional industry methods including on-

ground drying. The almond hulls were stored in plastic-lined metal bins at ambient conditions. The obtained samples contained mostly hulls but also contained shells, kernels, twigs, and immature kernels.

Figure 1: A sample of Monterey hulls from West Valley Hulling Co.

Almond hulls were also collected directly during the 2021 harvesting season from various local orchards, listed the Appendix. Independence, Monterey, Nonpareil, and Fritz hulls were collected from both on-ground and off-ground harvesting operations. The almond hulls were stored in a freezer at 0°C.

3.3.2 Bulk Density

The almond hull samples from the huller were first characterized for bulk density using ASTME Standard 1109-19. The bulk density is the ratio of the mass sample to its total volume when almond components are stacked in bulk. Specifically, a sample of known mass was poured and loosely filled into a container with known volume, and any excess amount was removed. The bulk density was then calculated using the equation

$$\rho_b = \frac{W_{component,i}}{V}$$

where ρ_b is the bulk density in kg/m^3, $W_{component,i}$ is the mass of the almond samples in kg, and V is the volume of the container in m^3 (ASTME Standard 1109-19). A Mettler Toledo scale and a 5-gallon food-grade bucket were used. Bulk density was measured in quadruplicate.

3.3.3 Distribution of Almond Components

The almonds hull samples from the huller were sorted to determine the composition and percent of hulls in each variety. A sample of 500 g of each variety was sorted manually into the following categories: hulls, shells, kernels, in-shell, twigs, and immature kernels. Sorting was performed in quadruplicate.

3.3.4 Sieving

The almond hull samples from the huller were characterized for separation efficiency and particle size distribution using ASTM standard sieves ranging from 12.5 to 4.75 mm. ASTM Standard E828-81 was used for particle size distribution analysis. Starting with the sieve having the largest opening, an almond hull sample of known weight was added to the sieve and was hand-shaken with vertical as well as horizontal motion to allow particles to pass through the openings until no more material passed. The particles that passed through the first sieve were passed through successively smaller screens until no more material passed through the screen. The sieving analysis was performed in duplicate, and 130 g of each almond hull sample was used for each trial.

3.3.5 Terminal Velocity

Terminal velocity measurements for the hull samples were accomplished using the method described in the article of Khir et al. (2014). The terminal velocity of individual nuts was measured using a cylindrical air column in which a single particle was suspended. The column was made of transparent plastic with a 100 cm height and a 10 cm diameter. A centrifugal fan (Dayton Electric Mfg. Co., Chicago, Ill.) produced a vertical flow of air upward in the column. The airflow was

distributed uniformly in the column by the use of three layers of mesh screen. This grid also straightened the airflow and reduced turbulence. The air speed was controlled with a variable speed drive that regulated the motor (Dayton Electric Mfg. Co.). For each test, an individual almond particle was inserted into the column. Terminal velocity was considered to be the velocity at which the particle was suspended in the air. The air velocity was measured with a digital anemometer (Control Co., Friendswood, Tex.) with a sensitivity of 0.1 m/s. Three replicate measurements were taken for each almond particle. The relationship between Air Velocity (y) and VFD Frequency (x) were related using a linear calibration curve $y = 0.0067x$.

3.3.6 Solids Content

Methods 2540B and 2540E from "Standard Methods for the Examination of Water and Wastewater" were used to characterize total solids, moisture content, volatile solids and fixed solids for each almond hull component and sieve size from the particle size characterization. Solids measurements were also taken for all 8 almond varieties and harvest types collected during the 2021 harvest season. "Total solids" is the term applied to the material residue left in the vessel after evaporation of a sample and its subsequent drying an oven at 105°C until a constant weight is obtained or until the weight change is less than 4% of the previous weighing or 0.5 mg, whatever is less. The weight of water loss from drying is called "moisture content". "Fixed solids" is the term applied to the residue of total solids after heating at 550°C until a constant weight is obtained or until the weight change is less than 4% of the previous weighing or 0.5 mg, whatever is less. The weight loss from ignition is called "volatile solids" (American Public Health Association, 2017). 200-500 mg of each sample was used for these tests. Solids analysis was performed in quadruplicate.

3.3.7 Sugars and Phenolic Compounds

The content of total reducing sugars in the samples was measured using the Dinitro Salicyclic Acid method as described in Miller, 1959. This method tests for the presence of free carbonyl groups which indicate reducing sugars. This involves the oxidation of the aldehyde functional group present to the corresponding acid while 3,5-dinitrosalicyclic acid is simultaneously reduced to 3-amino-5-nitrosalicylic acid under alkaline conditions which creates a color change that can be quantified by visible-light spectrophotometry (American Chemical Society, 1959). Sugars were extracted from the almond hulls using distilled water as the solvent at a loading of 7 g hull/100 mL water in a water bath at 80°C for 1.5 hours. The samples were mixed every 30 minutes. After extraction, 1 mL of 3,5-dinitrosalicyclic acid was added to 0.1 mL of each extract sample. The samples were mixed and heated for 10 minutes in a boiling water bath and then cooled under running tap water adjusted to ambient temperature. Afterwards, the absorbance of each sample was read on a spectrophotometer at a wavelength of 540 nm. Glucose was used as the reference standard for this test.

Total phenolic compounds were measured using the Folin-Ciocalteu method as described in the article of Lowry et al. (1951). Folin–Ciocâlteu reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric in vitro assay of phenolic and polyphenolic antioxidants. These compounds react with the Folin-Ciocalteu reagent to form a blue complex that can be quantified by visible-light spectrophotometry (Lowry et al., 1951). Phenolic compounds were extracted from the almond hulls using a 50% ethanol/50% water mixture as the solvent at a loading of 7 g hull/100 mL water in a water bath at 60°C for 1.5 hours. The samples were mixed every 30 minutes. After extraction, 2.5 mL of 10x diluted Folin-Ciocalteu reagent were added to 0.5 mL of each extract sample. The samples were mixed and left to stabilize for 5 minutes. Then

2 mL of 7.5% m/v Na_2CO_3 were added to each sample and the samples were mixed and heated in a 45°C water bath for 15 minutes. Afterwards, the absorbance of each sample was read on a spectrophotometer at a wavelength of 765 nm. Tannic acid was used as the reference standard for this test.

3.3.8 Chemical Composition

Finally, the almond hull samples from the 2021 harvest year were characterized for moisture content, organic matter, crude protein, acid detergent fiber, natural detergent fiber, carbohydrates, and fats using wet chemistry and dietary cation-anion difference (DCAD) analysis by Denele Analytical Labs in Woodland, California. Wet chemistry uses established laboratory tests performed on samples in the liquid phase to quantify precise levels of protein, fiber, fat, and minerals by isolating those substances in their dry form. DCAD is the interrelationship of positively charged minerals (cations) and negatively charged minerals (anions) on animal performance. DCAD is often used when formulating diets for dairy cows.

3.4 Results and Discussion

3.4.1 Bulk Density

Results of the bulk density measurements are displayed in Table 1 below. Bulk density is very important in determining the capacity of drying systems for feedstuffs (Chen et al., 2021b). The bulk density Nonpareil, Monterey, and Independence almonds hull samples from the huller ranged from 186 to 237 kg/m^3 for the three varieties with Monterey hulls having the lowest bulk density and Nonpareil hulls having the highest bulk density. These results were in accordance with the findings reported by Chen et al., 2021b.

Table 1: Bulk Density of Almond Hull Samples from Huller

	Bulk Density (kg/m^3)
Nonpareil	237 ± 17
Monterey	186 ± 3
Independence	204 ± 7

3.4.2 Distribution of Almond Components

Results of the hull purity measurements are displayed in Figure 2 below. On average, the hull samples from the almond huller contained 88.9% hulls and 10.1% contaminants. Nonpareil hulls contained the highest content of hulls by weight (94.1%) whereas Monterey hulls had the highest contamination rate (23.4%). Detailed data can be found in the Appendix.

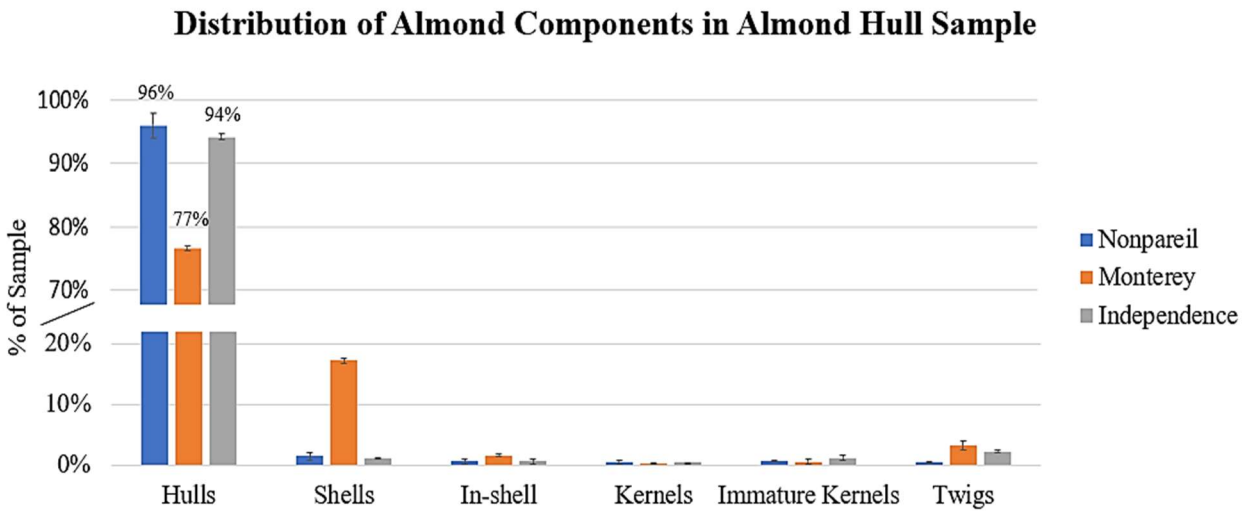


Figure 2: Distribution of Almond Components in Almond Hull Sample

3.4.3 Sieving

The results of the sieving analysis are displayed in Table 2, and a more detailed breakdown for each hull variety can be found in the Appendix. 100% of the almond hulls from each of the three varieties were able to be captured by a 4.75 mm screen. It is recommended that a 7.925 mm screen is used to sort hull samples for feed production as this screen level was able to produce an

average of 95% hulls for all three varieties sampled. Additionally, using an estimate of 12.96% crude fiber for almond hulls and 44.36% crude fiber for almond hull contaminants per DePeters et al., 2020, this level of purity also meets the State of California's feed law for almond hulls which states that almond hulls that are sold as by-product feed must contain less than 15% crude fiber; if they contain more than 15% crude fiber, they are classified as "hull and shell" (CDFA, 2013).

Table 2: Particle Size Distribution of Almond Hulls

	Total Hulls Captured (%)			
Particle Size (mm)	Nonpareil	Monterey	Independence	Average
≥ 12.5	72.7 ± 5.3	49.9 ± 3.3	67.7 ± 7.9	63.4 ± 10.1
≥ 9.5, < 12.5	91.0 ± 4.3	79.2 ± 1.7	91.1 ± 4.4	87.1 ± 6.1
≥ 7.925, < 9.5	95.8 ± 2.7	90.1 ± 1.0	97.3 ± 1.6	94.4 ± 3.3
≥ 4.75, < 7.925	99.5 ± 0.3	97.8 ± 0.9	100.0 ± 0.0	99.1 ± 0.8
< 4.75	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

3.4.5 Terminal Velocity

Terminal velocity was measured to determine if air separation is an effective way to separate almond hulls from the other almond components in the hull mixtures from the huller. Average terminal velocity measurements for each particle type are shown in Figure 3 below. Detailed data can be found in the Appendix. Twigs had the highest terminal velocity at an average of 9.4 m/s for the three varieties tested and shells had the lowest terminal velocity at an average of 5.3 m/s for the three varieties tested. As there were non-hull components for both almond varieties that had terminal velocities both higher and lower than that of the hull, it is not recommended that air separation be used to sort almond hulls from their other components.

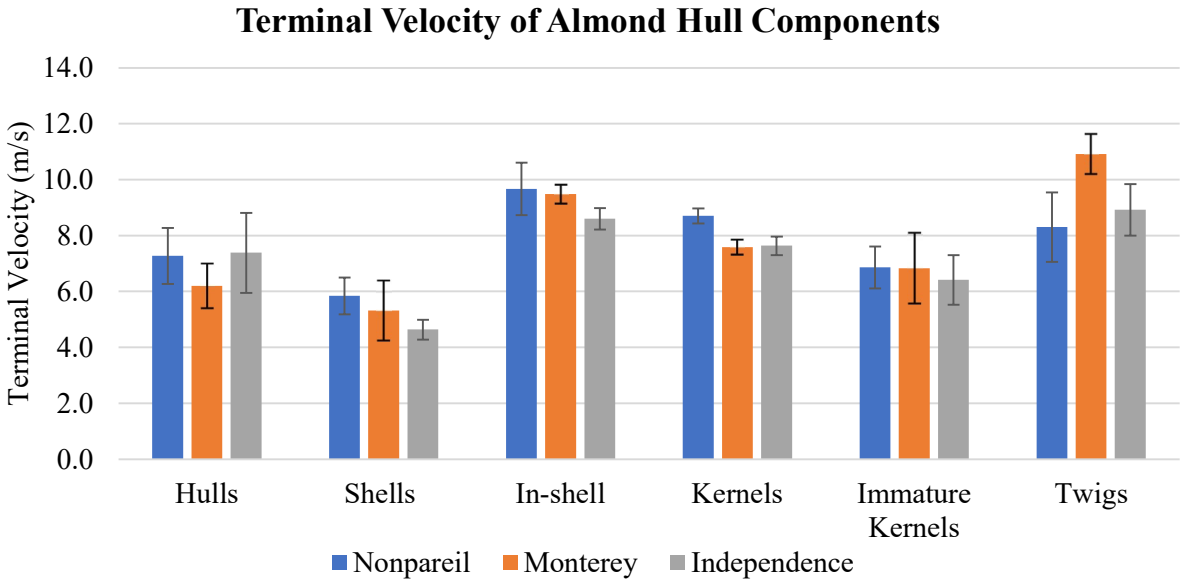


Figure 3: Terminal Velocity Measurements of Almond Hull Sample Components

3.4.6 Solids Content

Solids measurements were taken for each component (hull, twig, shell, etc.) at each of the sieve levels used previously for all three almond varieties from the 2020 season. Table 3 shows average solids values for each component across all five-sieve levels for all three varieties of almonds from the 2020 season (Nonpareil, Monterey. Independence). For the 2021 season hulls, solids measurements were only performed for hulls. The results are displayed in Table 4. On-ground harvested hulls from the 2020 had an average moisture content of 16.0% and on-ground harvested hulls from the 2021 harvest year had an average moisture content of 15.6%. For the 2021 varieties, off-ground harvested hulls had an average moisture content 3.50 times higher than on-ground harvested hulls of the same variety. Volatile and fixed solids for on- and off-ground hulls of the same variety were all within +/- 2% of each other.

Table 3: Solids Measurements for Almond Hull Sample Components, 2020 Harvest Year

	Moisture Content (%)	Total Solids (%)	Volatile Solids (%)	Fixed Solids (%)
Hulls	16.0 ± 2.1	84.0 ± 2.1	74.6 ± 3.4	25.4 ± 3.4
Shells	7.4 ± 0.9	86.6 ± 0.9	89.3 ± 1.8	10.7 ± 1.8
In-shell	6.2 ± 0.8	93.8 ± 0.6	92.0 ± 0.7	8.0 ± 0.7
Kernels	5.7 ± 1.1	92.7 ± 1.7	89.7 ± 0.9	10.3 ± 0.9
Immature Kernels	10.6 ± 1.3	89.6 ± 0.2	85.6 ± 2.0	14.4 ± 2.0
Twigs	8.7 ± 0.8	85.7 ± 0.8	82.9 ± 2.3	17.1 ± 2.3

Table 4: Solids Measurements for 2021 Harvest Year Almond Hulls

Variety/On or Off Ground Harvest		Moisture Content (%)	Total Solids (%)	Volatile Solids (%)	Fixed Solids (%)
Nonpareil	On	8.1 ± 2.8	91.9 ± 2.8	93.2 ± 0.5	6.8 ± 0.5
	Off	25.0 ± 1.9	75.0 ± 1.9	93.4 ± 1.0	6.6 ± 1.0
Monterey	On	23.6 ± 2.5	76.4 ± 2.5	89.2 ± 2.1	10.8 ± 2.1
	Off	52.9 ± 2.5	47.1 ± 2.5	91.3 ± 1.8	8.7 ± 1.8
Independence	On	22.5 ± 2.6	77.5 ± 2.6	91.5 ± 1.0	8.5 ± 1.0
	Off	67.8 ± 1.3	32.2 ± 1.3	89.4 ± 0.6	10.6 ± 0.6
Fritz	On	11.2 ± 1.9	88.8 ± 1.9	90.7 ± 0.5	9.3 ± 0.5
	Off	45.7 ± 5.2	54.3 ± 5.2	90.2 ± 1.0	9.8 ± 1.0

3.4.6 Sugars and Phenolic Compounds

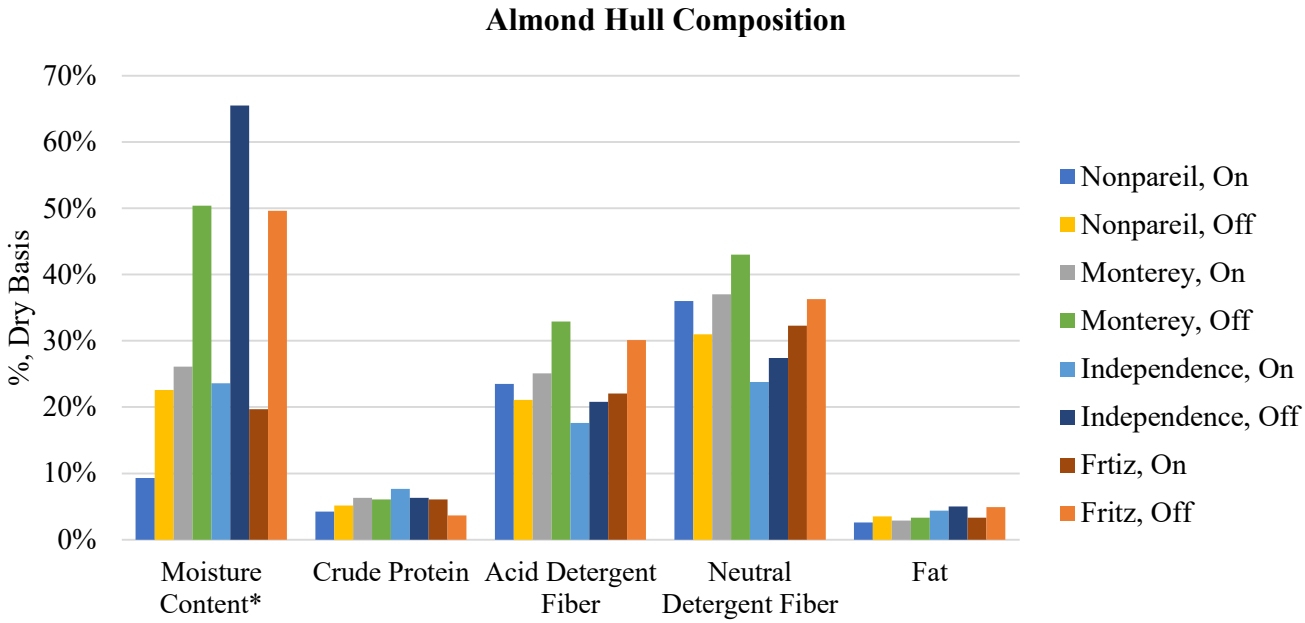
Results of the sugar and phenolic compound content measurements are shown in Table 5 below. Independence hulls had the highest sugar content (42.2%) and lowest phenolic compound content (3.4%), Fritz hulls had the highest phenolic compound content (7.6%), and Monterey hulls had the lowest sugar content (33.1%).

Table 5: Sugar and Phenolic Compound Content of Almond Hulls

Variety	Sugar content (% d.b.)	Phenolic compounds content (tannic acid eqv, % d.b.)
Nonpareil	31.8 ± 3.5	5.5 ± 0.9
Monterey	33.1 ± 1.4	6.8 ± 0.4
Independence	42.2 ± 4.1	3.4 ± 1.2
Fritz	41.7 ± 4.4	7.6 ± 0.9

3.4.7 Chemical Composition

Abbreviated results from the chemical analysis are shown in Figure 4 below. A complete data set of the parameters measured can be found in the Appendix. All values are reported on a 100% dry basis except for moisture content, which is reported "as received". Solids values were consistent with values measured in Section 3.4.4. On average, the hulls contained 5.9% crude protein, 23.5% acid detergent fiber, 33.0% neutral detergent fiber, and 3.6% fat. These values are in the range of 12 previous almond hull studies as summarized in the review paper by DePeters et al., 2020. The low fat value is in-range of the fat value recommended for cattle diets (Erickson & Kalscheur, 2020).



**Reported "as received" rather than % d.b.*

Figure 4: Chemical Composition of Almond Hulls

Chapter 4 – Almond Hull Fermentation

4.1 Introduction

Almond hulls are a suitable feedstock for the creation of fermented dairy cattle feed due to their high sugar, fiber, antioxidant, and moisture contents, and their abundance and availability as a byproduct feedstuff from the almond industry (Salgado-Ramos et al., 2022, Chen & Pan, 2022). Little research has been done on fermenting almond hulls for feed purposes, so the effect of different fermentation conditions on the hull feedstock needs to be investigated. Fermentation parameters such as time, temperature, particle size, almond hull variety, and inoculum type and amount will produce different results in the final product (Missotten et al., 2015).

Although there are naturally occurring yeasts and bacteria present on almond hulls that could be used for spontaneous fermentation, this is not a reliable method to obtain a safe feed product because unpredictable variations in the fermentation pattern can occur (Beal et al., 2002). Spontaneous fermentation can also result in higher concentrations of both acetic acid and biogenic amines which adversely affects palatability in fermented pig feed (Brooks et al., 2003). Therefore, a controlled, inoculated fermentation will be investigated in this study.

In multiple studies, supplementation of cattle feed with the yeast strain *S. cerevisiae* brought improvements such as improved fiber digestibility, increased milk yield, and decreased loss of body condition (Schlabitz et al., 2022; Bach et al., 2007). Therefore, inoculation of the almond hulls with *S. cerevisiae* will be investigated. Additionally, lactic acid has been shown to bring nutritional benefits to pigs and chickens fed fermented feed (Missotten et al., 2013; Roth & Kirchgessner, 1998). Lactic acid also causes a drop in pH which is important for preservation and stability in feed (Missotten et al., 2015). Therefore, a bacteria inoculation will also be investigated.

L. Plantarum was chosen as the bacteria inoculum to be investigated in this study because it is widely used in fermentation.

In a study that used a mathematical-empirical approach to estimate the cardinal growth temperature parameters of 27 different yeast strains, *S. cerevisiae* was the yeast best adapted to grow at high temperatures within the *Saccharomyces* genus. The study found that the highest optimal growth temperature (the temperature at which the specific growth rate equals its optimal value) for *S. cerevisiae* was 32.3°C and the maximal temperature (the temperature above which no more growth occurs) was 45.5°C (Salvado et al., 2011). For *L. Plantarum,* the optimal fermentation temperature was found to be 35°C and the maximal temperature was found to be 40°C (Zhou et al, 2015; Matejcekova et al., 2016). Fermentation temperatures within this range will be studied. Room temperature fermentation will also be investigated to see if supplemental heating can be avoided in the scaled-up process.

4.2 Objectives

The objectives of this research were to: (1) Develop an effective fermentation process for producing fermented feed from almond hulls; (2) Investigate the effects of different fermentation parameters on the characteristics of the fermented feed.

4.3 Materials and Methods

Almond hulls of the variety Monterey, Independence, Nonpareil, and Fritz that were collected from California almond orchards in Summer/Fall 2021 were used for this part of the research study (see Table for orchard locations). Both on-ground and off-ground harvested almonds of each variety were collected. The almonds were stored in a freezer and were thawed to room temperature prior to inoculation. These hulls were characterized for their physical and chemical properties in the analysis described in Chapter 2.

Fermentation experiments were conducted using yeast, bacteria, and a mixture of yeast and bacteria as inoculum treatments. The yeast inoculum utilized was *S. cerevisiae* or brewer's yeast, sourced from Red Star, and the bacteria inoculum utilized was *L. Plantarum*, sourced from Creative Enzymes. "Control" fermentations were also carried out simultaneously with the same conditions but with no added inoculum. Vacuum bags were used as flexible containers for the hulls during the experiments to simulate silage bags. Air was removed from the bags and the bags were sealed using a vacuum sealer.

4.3.1 5- and 30-Day Fermentations

Two fermentation trials, one with a duration of 5 days and one with a duration of 30 days, were carried out at a temperature of 40°C. Independence off-ground harvested hulls were used for these experiments because they had the highest moisture and sugar contents of the hulls obtained (67.8% and 42.4%, respectively). The hulls were ground to a fine consistency using a Ninja blender. For each trial, 100 g of almond hulls were utilized. An inoculum rate of 2 g inoculum/kg wet hull was used (i.e., 0.2 g of inoculum was used in each 100 g bag). Four treatments were tested, each in duplicate. The treatments were (1) yeast, (2) bacteria, (3) a mixture of bacteria and yeast (mixed), and (4) an uninoculated sample (control). After being inoculated, the hulls were placed into bags and vacuum sealed. The bags were then incubated at 40°C for 5 days in a Fischer Isotemp Refrigerated Incubator model 11-679-25C.

4.3.3 Bacteria vs. Yeast, Fermentation Duration

Using the results observed from the 5- and 30-day fermentations, a much larger third experiment was designed. The goal of this experiment was to understand the effect of both fermentation duration and inoculum rate on the final product. This experiment included six different sub-experiments. The variables included 2 inoculum types (bacteria or yeast), 4 inoculum

rates (0 g/kg (control), 2 g/kg, 5 g/kg, and 20 g/kg wet hulls), and 3 fermentation times (5, 14, or 30 days). Each utilized 20 g of ground Independence off-ground harvested hulls. The fermentation temperature for all trials was 40°C, and all trials were performed in duplicate.

4.3.4 Hull Variety, Particle Size, Fermentation Temperature

Finally, a final experiment was conducted to understand the effect of hull variety, particle size, and fermentation temperature on the almond hulls. This experiment included four different sub-experiments. The variables included 3 varieties of off-ground harvested almond hulls (Independence, Monterey, and Fritz), 2 particle sizes (whole and ground), and 2 fermentation temperatures (ambient (25°C) and 40°C). Each trial utilized 20 g of almond hulls inoculated with yeast at a rate of 5 g yeast/ kg wet hulls and fermented for 14 days. The ground hulls were ground to a fine consistency using a Ninja blender and the unground hulls were used without adjusting their particle size. Each hull variety had a different initial moisture content, so moisture content for all hull varieties was adjusted to 67.8%. All trials were performed in duplicate. The particle size, inoculum type, inoculum rate, and fermentation time for all four sub-experiments were chosen because these conditions produced the most desirable and statistically significant results in the previous experiment.

A "control" experiment with no supplementary inoculum was conducted alongside the four sub-experiments. The same three varieties of almond hulls (Independence, Monterey, and Fritz) were utilized. All trials in the control experiment utilized ground hulls fermented at 40°C for 14 days, but this time no additional yeast was added as an inoculum. The control experiment was performed in duplicate.

For all experiments, raw, unfermented versions of the almond hulls used in fermentation were analyzed for comparison to the fermented hulls. These hulls are labeled as "unfermented" in the sections below.

4.3.5 Methodology

The volume of gas produced during the 5-day fermentation trial was measured using the water displacement method. In this method, a watertight container was filled to the top with water, and this container was placed into another larger container. Each bag was inserted into the water until it was fully submerged. The volume of the water that overflowed from the first container into the second container was measured using a graduated cylinder. This volume minus the volume of an empty bag was taken to be the amount of gas inside of the fermentation bag. Volume measurements were taken in duplicate.

Method 2540B and 2540E from "Standard Methods for the Examination of Water and Wastewater" was used to characterize total solids, moisture content, volatile solids, and fixed solids for the fermented feeds. "Total solids" is the term applied to the material residue left in the vessel after evaporation of a sample and its subsequent drying an oven at 105°C until a constant weight is obtained or until the weight change is less than 4% of the previous weighing or 0.5 mg, whatever is less. The weight of water loss from drying is called "moisture content". "Fixed solids" is the term applied to the residue of total solids after heating at 550°C until a constant weight is obtained or until the weight change is less than 4% of the previous weighing or 0.5 mg, whatever is less. The weight loss from ignition is called "volatile solids" (American Public Health Association, 2017). 200-500 mg of each sample was used for these tests. Solids analysis was performed in quadruplicate.

The pH of the unfermented and fermented almond hulls was measured according to a methodology from the University of Kentucky College of Agriculture, Food and Environment using a Mettler Toledo FiveGo F2 pH/mV Meter for Solid and Semi-Solid Samples. The pH probe was calibrated before use according to the manufacturer's instructions. After calibration, the pH electrode was rinsed with distilled water and blotted dry. The rinsed electrode was placed in the sample deep enough so that the electrode is immersed in the sample. The pH meter was left to stabilize, and the stabilized pH value was recorded (Vijayakumar & Adedeji, 2017). pH measurements were performed in triplicate.

High-performance liquid chromatography was used to analyze lactic acid and volatile fatty acids (VFAs) concentration in the unfermented and fermented almond hulls using a UFLC Shimadzu HPLC. Samples were diluted 20x in mili-Q water and filtered using 0.2 μm filters. A stock solution containing lactic acid, acetic acid, propionic acid, iso-butyric acid, butyric acid, valeric acid, and iso-valeric acid was used to create standards ranging from 125 to 10,000 ppm. The mobile phase was 5mM sulfuric acid and the column used was a BioRad Aminex HPX-87H column. Analysis was performed in quadruplicate.

Gas chromatography was used to analyze the amount of ethanol present in unfermented and fermented almond hulls using a Gas Chromatograph. Samples were acidified to a pH of < 2 using 30% ortho-phosphoric acid. Samples were also diluted 20x in mili-Q water and filtered using 0.2 μm filters. A stock solution containing diluted ethanol was used to create standards ranging from 100 to 2,000 mg/L. Hydrogen and helium were used as carrier gases. Analysis was performed in quadruplicate.

One-way analysis of variance (ANOVA) is a statistical method that was used to determine whether there are any statistically significant differences between the means of three or more

independent (unrelated) groups. One-way ANOVA compares the averages between groups and determines whether any of those means are statistically significantly different from each other. Specifically, it tests the null hypothesis:

$$H_0: \mu_1 = \mu_2 = \mu_3 = \cdots = \mu_k$$

where μ = group average and k = number of groups. If, however, the one-way ANOVA returns a statistically significant result, we accept the alternative hypothesis. which is that there are at least two group means that are statistically significantly different from each other. This means that the observed differences among the sample averages could not reasonably be due to random chance alone. All detailed ANOVA analysis can be found in the Appendix.

In-vitro rumen digestion tests were performed by the Hess Lab in the UC Davis Animal Science Department. An artificial (in-vitro) rumen system was inoculated with rumen content and the fermented almond hulls were examined for their effect on CH_4 and CO_2 production when added at various inclusion rates to a regular dairy cow diet. Gas production was measured in 24-hour intervals of in-vitro rumen incubation and analyzed for its composition using a gas chromatograph. Each fermentation product was tested in quadruplicates for its effect on gas production in the rumen system.

Finally, the almond hull samples were characterized for moisture content, organic matter, crude protein, acid detergent fiber, natural detergent fiber, carbohydrates, and fats using wet chemistry and dietary cation-anion difference (DCAD) analysis by Denele Analytical Labs in Woodland, California. Wet chemistry uses established laboratory tests performed on samples in the liquid phase to quantify precise levels of protein, fiber, fat, and minerals by isolating those substances in their dry form. DCAD is the interrelationship of positively charged minerals (cations) and negatively charged minerals (anions) on animal performance. DCAD is often used

when formulating diets for dairy cows. Chemical analysis was performed on both the ground unfermented hulls of Independence, Monterey, and Fritz varieties as well as the same varieties of ground hulls fermented at 40°C for 14 days.

4.4 Results and Discussion

4.4.1 5- and 30-Day Fermentations

The volume of gas produced by each treatment over the 5-day fermentation duration is displayed in Table 6. Both the yeast treatment and the mixture of yeast and bacteria (mixed treatment) had a higher concentration of CH_4 and CO_2. There was no significant difference between the yeast and the mixed treatments. Low amounts of CH_4 and CO_2 were produced from the bacteria treatment and control. The production of CO_2 may be due to that fact that it is a byproduct of the fermentation of sugars, presented in the hulls, by yeast. The production of CH_4 might be attributed to the endogenous methanogenic archaea presented in the hulls prior to the fermentation.

Table 6: Gas Composition for 5-Day Fermented Hulls

	CH_4 (mL/kg dry hulls)	**CO_2 (mL/kg dry hulls)**	**Total Gas (mL/kg dry hulls)**
Control	0.03	46.75	46.77
Yeast	0.12	234.97	235.08
Mixed	0.11	250.70	250.81
Bacteria	0.01	29.02	29.03

The change in pH over the 5 day fermentation is shown in Table 7 below. The unfermented hulls had an initial pH of 4.63. The mixed treatment had the largest pH increase from 4.63 to 4.77, and the bacteria treatment had the largest pH drop from 4.63 to 4.24.

Table 7: pH Values for 5-Day Fermented Hulls

	Initial pH	Final pH (5 days)
Yeast	4.63 ± 0.04	4.77 ± 0.03
Bacteria	4.63 ± 0.04	4.24 ± 0.05
Mixed	4.63 ± 0.04	4.87 ± 0.02
Control	4.63 ± 0.04	4.56 ± 0.08

Organic acid and ethanol concentrations for the 5-day fermented feed are displayed in Figure 5. The yeast and the mixed treatments produced higher acetic acid concentrations than the other treatments. The bacteria treatment had the highest concentration of lactic acid, followed by the mixed treatment. The mixed treatment produced the most ethanol at 39,155 ppm and the yeast treatment produced the second most ethanol at 35,648 ppm. Propionic acid levels were low, at concentrations between 0 and 1,864 ppm.

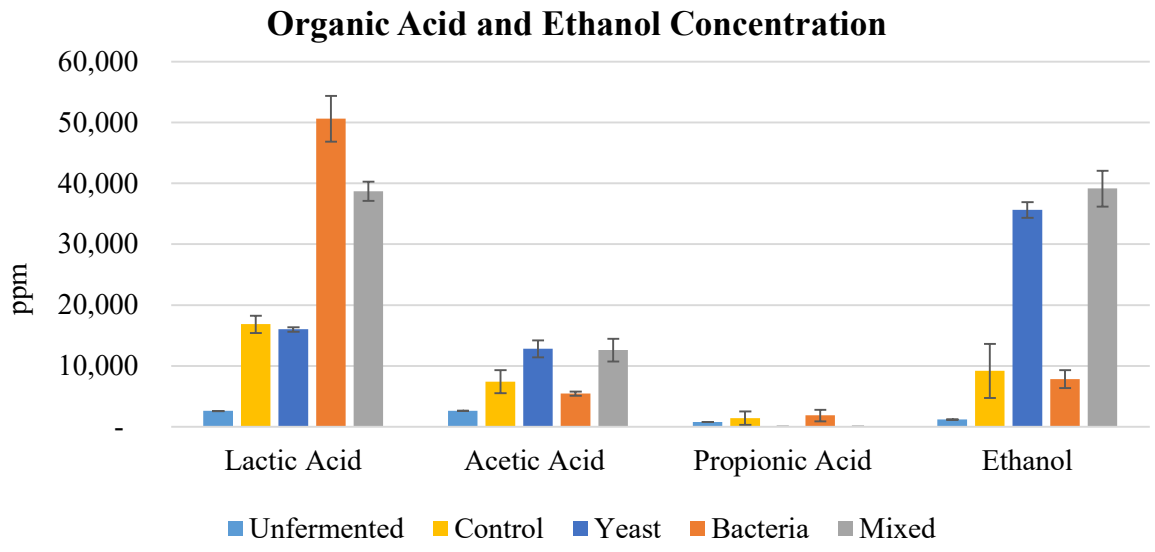


Figure 5: Organic Acid and Ethanol Concentration of 5-day Fermented Hulls

A 30-day fermentation using the same conditions as the 5-day fermentation was conducted to observe the effect of fermentation duration on the hulls. The change in pH for each 30-day

fermentation trial can be found in Table 8 below. The yeast treatment had the highest final pH at 4.92, and the bacteria treatment had the lowest final pH at 4.35.

Table 8: pH Values for 30-Day Fermented Hulls

	Initial pH	Final pH (30 days)
Yeast	4.63 ± 0.04	4.92 ± 0.09
Bacteria	4.63 ± 0.04	4.35 ± 0.03
Mixed	4.63 ± 0.04	4.78 ± 0.05
Control	4.63 ± 0.04	4.48 ± 0.02

The organic acid and ethanol contents of the 30-day fermented feed are displayed in Figure 6. As before, bacteria treatment had the highest concentration of lactic acid. The control, yeast, and mixed treatments produced similar levels of acetic acid ranging from 16,678 to 20,412 ppm. Propionic acid levels remained low except for the control treatment, which had an increase in propionic acid content from 1,426 to 6,682 ppm. The yeast treatment produced the most ethanol.

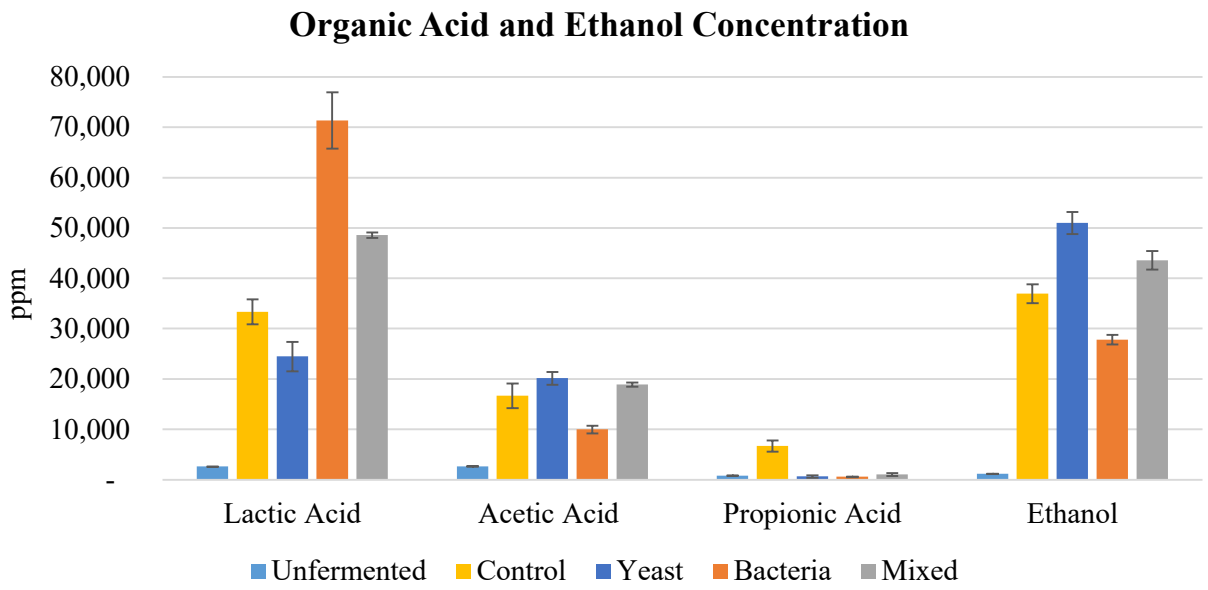


Figure 6: Organic Acid and Ethanol Concentration of 30-day Fermented Hulls

In-vitro rumen digestions tests for the 5-day fermented almond hulls over 24 hours at 5% and 20% inclusion rates (IR) to a regular dairy cow diet showed that CO_2 production increased when almond hulls were added that had been fermented in the presence of both yeast and bacteria,

suggesting that this pretreatment might render a fermentation product that stimulates overall microbial growth in the rumen system. A negative correlation between the inclusion rate of fermented almond hulls and CH_4 production was observed (48.77 ml CH_4/g DM with 5% IR and 33.25 ml CH_4/g DM with 20% IR). It is therefore possible that the microbial driven fermentation process by itself might result in measurable reduction of CH_4 in the rumen system.

This hypothesis was investigated with a follow up in-vitro rumen digestion test in which 30-day fermented and unfermented hulls (without additional microbes added) were evaluated over 72 hours at 5% and 20% IR. Results suggest that adding 20% of almond hulls that had been fermented over 30 days reduced enteric CH_4 production. Reduction of CH_4 production was observed after 24 hours for almond hulls that were fermented in the presence of yeast or a mixture of bacteria and yeast. Almond hulls that were fermented in the absence any additional microbes (control treatment) also reduced CH_4 production but only at 72 hours, which supports findings from the initial test. The production of CH_4 overtime increased slightly (~6%) with almond hulls that were fermented in the absence of yeast or yeast and bacteria, whereas the overall amount of CH_4 declined by >97% when almond hulls fermented in the presence of yeast or yeast and bacteria were added to the in-vitro rumen system. CO_2 production remained the same

4.4.3 Bacteria vs. Yeast, Fermentation Duration

Results of the third experiment which investigated bacteria vs. yeast inoculums and fermentation duration are displayed in Figures 7 to 16. Charts are divided to show results from the bacteria-only treatments and yeast-only treatments separately. The pH for all bacteria treatments dropped below the pH of the control treatment for all fermentation durations. There was an increase in pH observed between days 5 and 14 for the bacteria treatments, but there was a drop in

pH at day 30. The pH for the yeast treatments increased steadily above the control pH for all treatments, with an inoculum rate of 2g yeast/wet hulls having the highest final pH at 4.95.

Using a one-way analysis of variance (ANOVA) technique on the 14-day fermented hulls at an inoculum rate of 5 g inoculum/kg hulls, it was found that the final pH of the yeast and bacteria treatments are significantly different from the pH of the unfermented hulls, meaning that the observed differences among the sample averages could not reasonably be due to random chance alone. Additionally, the pH of the yeast and bacteria treatments are significantly different from the pH of the control treatment.

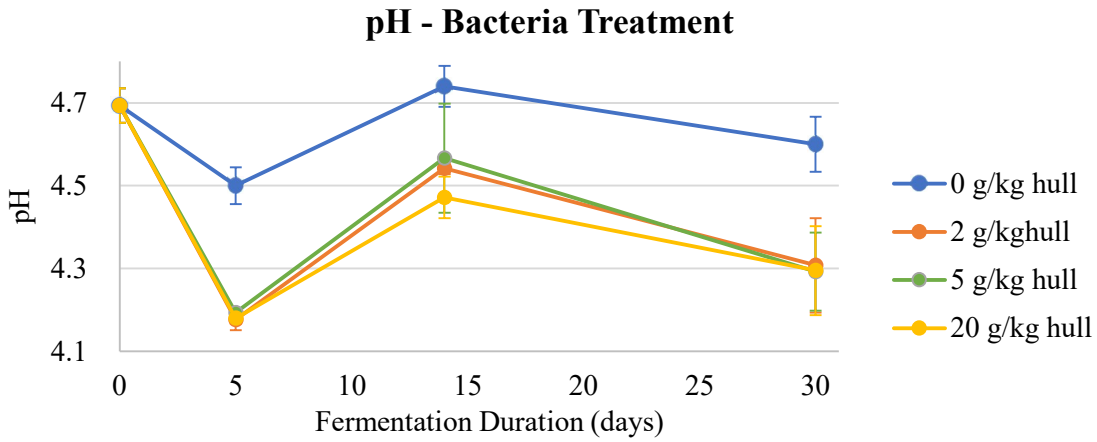


Figure 7: pH Change of Almond Hulls, Bacteria Treatments

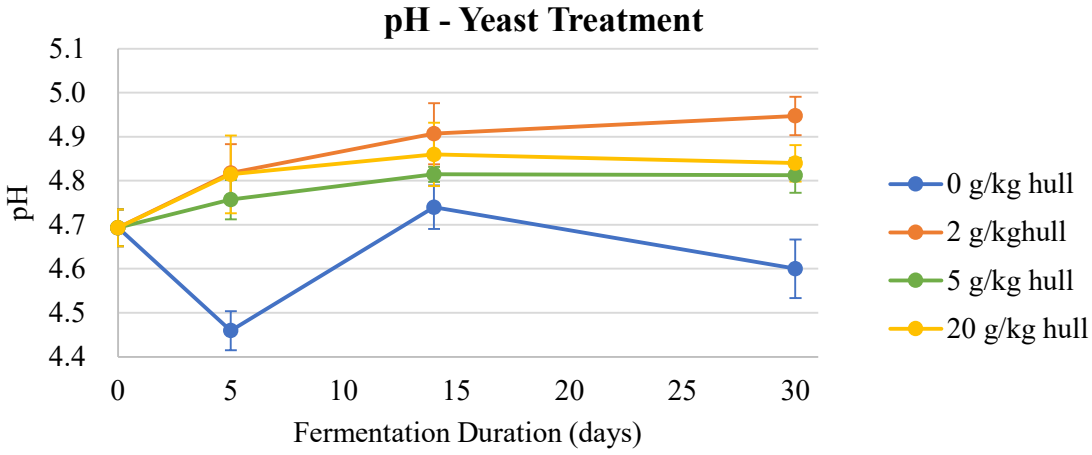


Figure 8: pH Change of Almond Hulls, Yeast Treatments

Lactic acid concentrations for the bacteria treatments increased for all inoculum levels studied, with 20 g bacteria/kg wet hulls having the highest lactic acid content after 30 days. There was a steady increase in lactic acid concentration for all the yeast treatments from day 0 to 14, but after day 14 there was a slight drop in lactic acid concentration. Overall, the yeast treatments produced more lactic acid than the control treatment after 30 days of fermentation.

Using a one-way ANOVA technique on the 14-day fermented hulls at an inoculum rate of 5 g inoculum/kg hulls, it was found that the lactic acid levels produced by the yeast and bacteria treatments are significantly different from the lactic acid levels of the unfermented hulls. However, when comparing the lactic acid concentrations of the yeast and bacteria treatments to that of the control treatment, the result is not statistically significant.

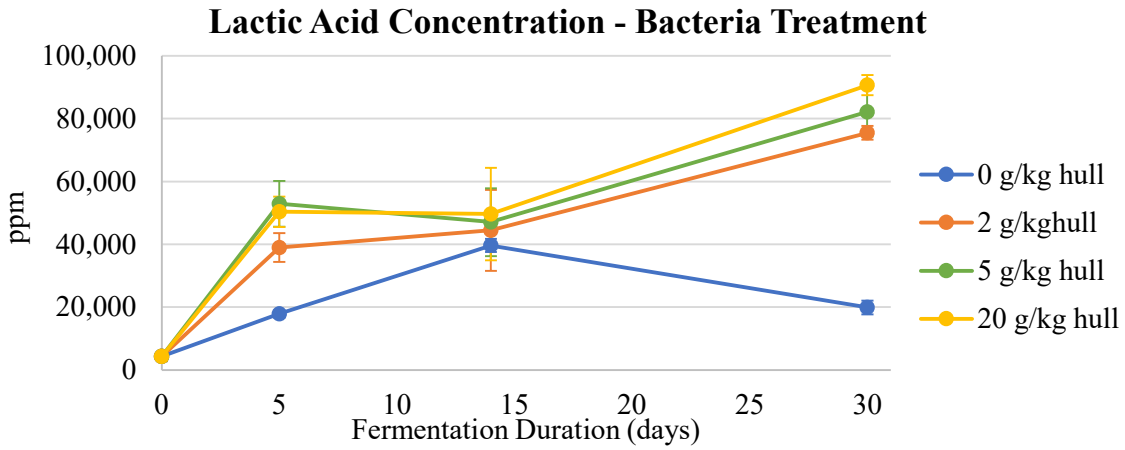


Figure 9: Lactic Acid Concentration - Bacteria Treatment

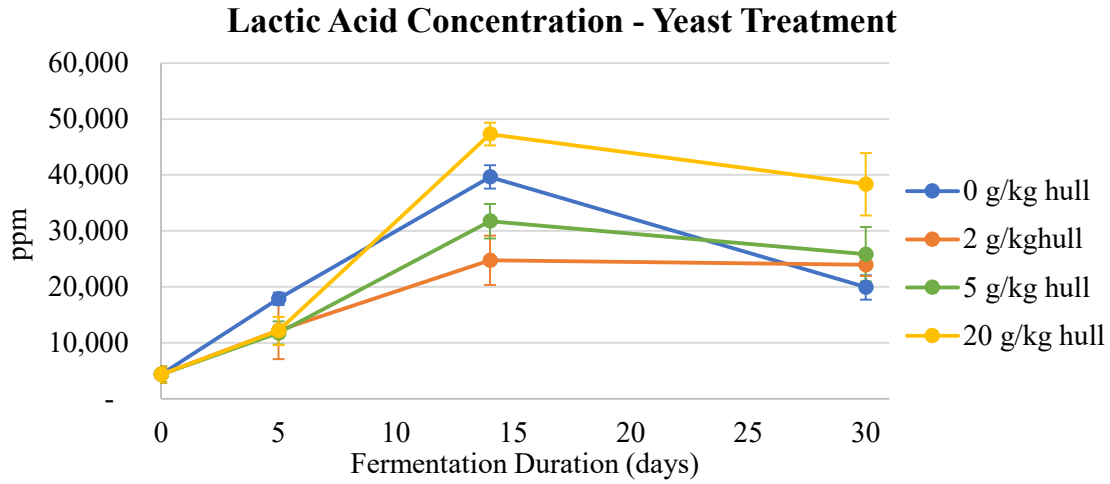


Figure 10: Lactic Acid Concentration - Yeast Treatment

Acetic acid concentrations increased steadily between days 5 and 30 for all of the bacteria treatments, but the bacteria treatments produced less acetic acid than the control treatment for each fermentation duration tested. There was a steady increase in acetic acid concentration in the yeast treatments, with 20 g yeast/kg wet hulls producing the most at 26,233 ppm at 30 days. For all treatments studied, acetic acid levels increased after day 0 but leveled out by day 14.

Using a one-way ANOVA technique on the 14-day fermented hulls at an inoculum rate of 5 g inoculum/kg hulls, it was found that the final acetic acid concentrations of the yeast and bacteria treatments are significantly different from the acetic acid concentration of the unfermented hulls. However, when comparing the acetic acid concentrations of the yeast and bacteria treatments to that of the control treatment, the result is not statistically significant.

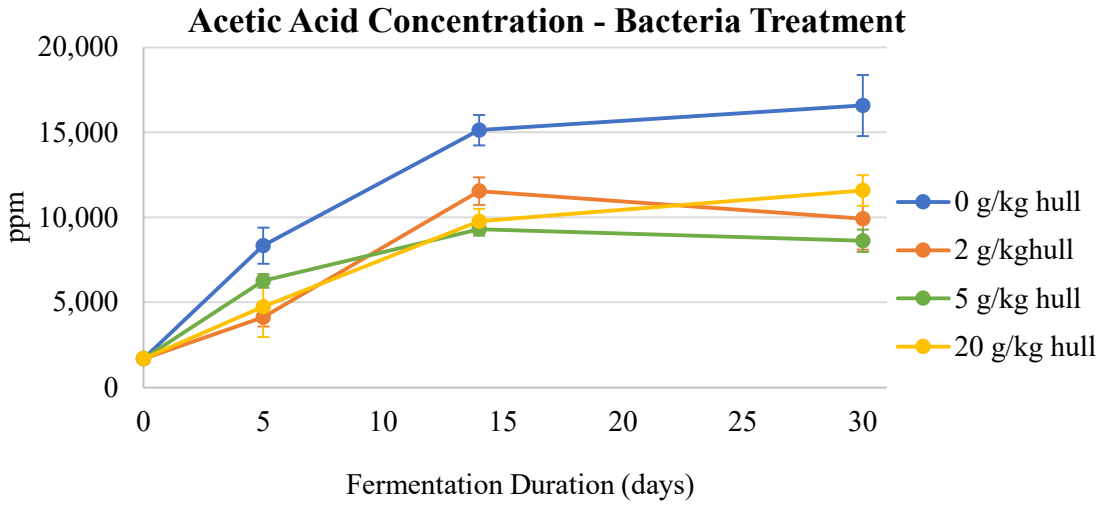


Figure 11: Acetic Acid Concentration - Bacteria Treatment

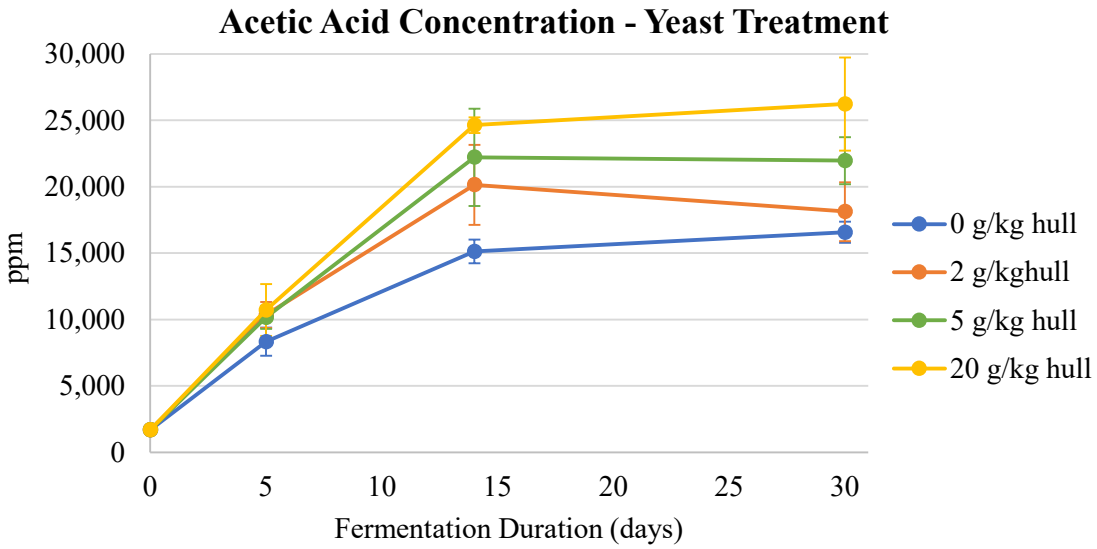


Figure 12: Acetic Acid Concentration - Yeast Treatment

Propionic acid levels stayed low for all treatments tested, with the 20 g bacteria/kg wet hulls producing the most propionic acid at day 5. The bacteria treatments had a spike in propionic acid concentration at day 5 and then decreased over 14 and 30 days. The yeast treatments had an initial decrease in propionic acid at day 5, then increased to a maximum during day 14.

Using a one-way ANOVA technique on the 14-day fermented hulls at an inoculum rate of 5 g inoculum/kg hulls, it was found that the propionic acid concentrations of the yeast and bacteria

treatments are significantly different from the propionic acid concentration of the unfermented hulls. Additionally, the propionic acid concentrations of the yeast and bacteria treatments are significantly different from that of the control treatment.

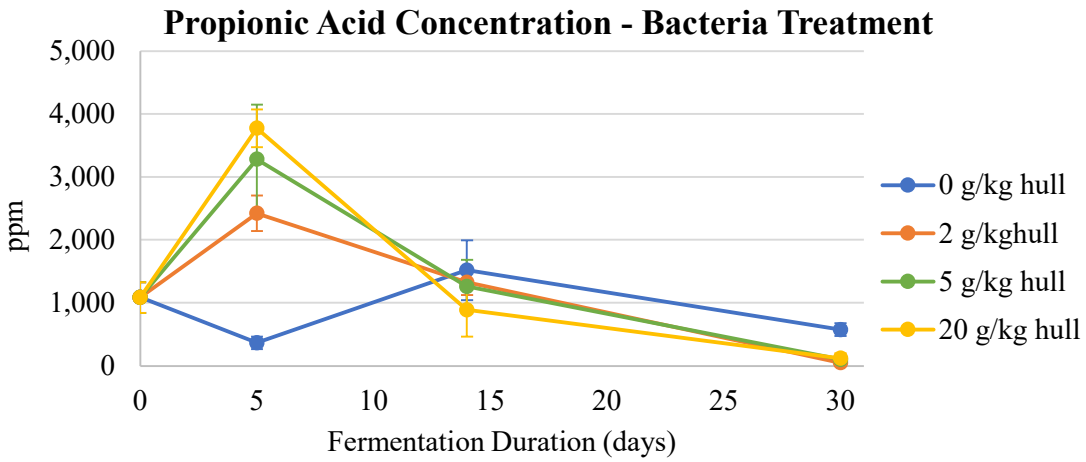


Figure 13: Propionic Acid Concentration - Bacteria Treatment

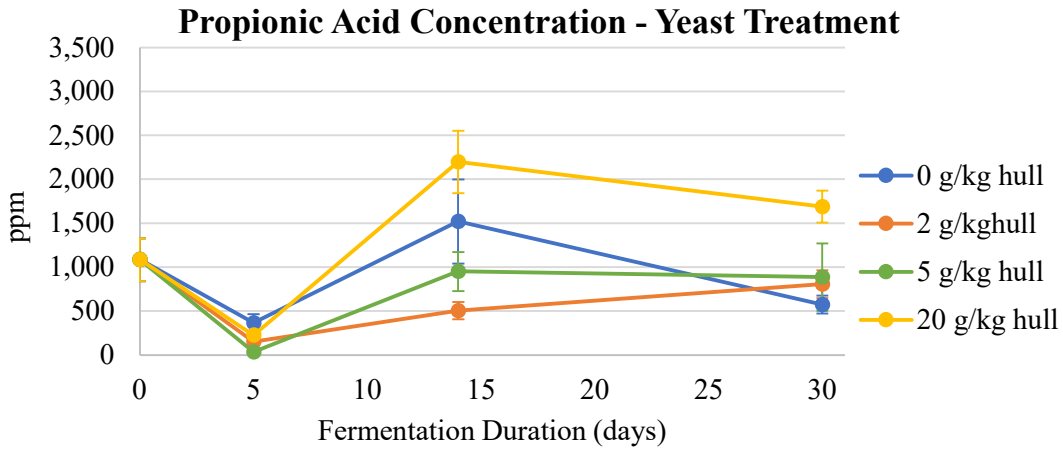


Figure 14: Propionic Acid Concentration - Yeast Treatment

Like acetic acid, ethanol levels for all treatments increased steadily over 5 and 14 days and stayed steady between 14 and 30 days. The concentration of ethanol in the bacteria treatments was below that of the control treatment for all inoculum levels, and ethanol concentrations for the yeast treatments was above that of the control treatment for all inoculum levels.

Using a one-way ANOVA technique on the 14-day fermented hulls at an inoculum rate of 5 g inoculum/kg hulls, it was found that the ethanol concentrations of the yeast and bacteria treatments are significantly different from the ethanol concentration of the unfermented hulls. Additionally, the ethanol concentrations of the yeast and bacteria treatments are significantly different from that of the control treatment.

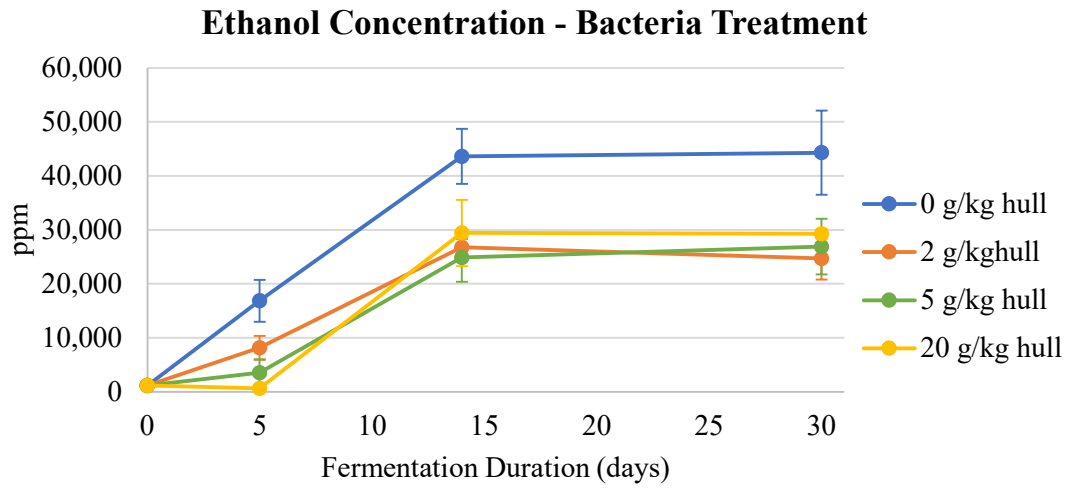


Figure 15: Ethanol Concentration - Bacteria Treatment

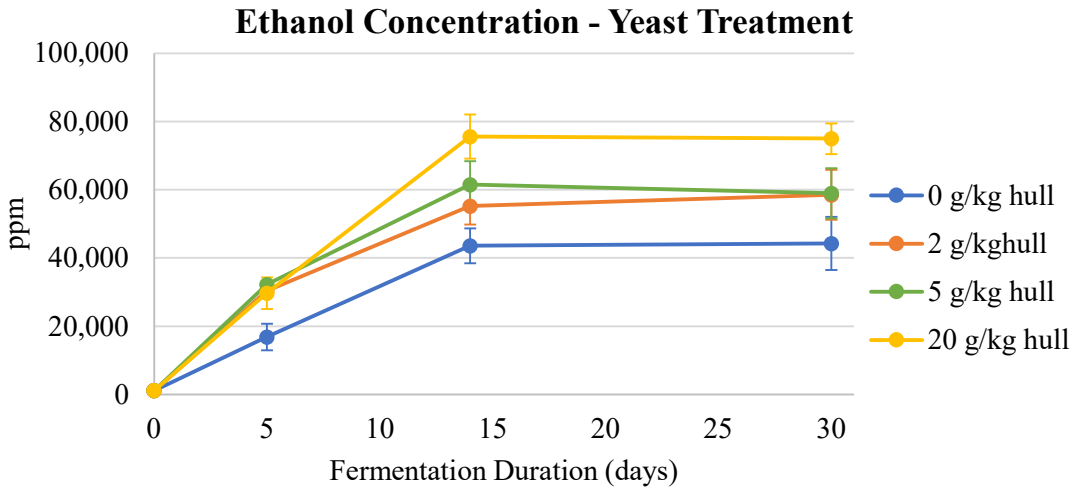


Figure 16: Ethanol Concentration - Yeast Treatment

4.4.4 Hull Variety, Particle Size, Fermentation Temperature

Results of the fourth experiment investigating hull variety, particle size, and fermentation temperature are displayed below in Figures 17 to 21. Overall, it appears that hull variety has a large impact on the characteristics of the fermented hulls under the variety of different fermentation conditions tested.

The Independence, Monterey, and Fritz hulls had different initial pH values of 4.63, 4.02, and 4.86, respectively. There did not appear to be any trend between pH change and particle size or temperature, as results varied greatly between hull varieties with the same fermentation conditions.

Using a one-way ANOVA technique on the hulls that were ground and fermented at 40°C, it was found that the sample averages of the pH of the three different varieties (Independence, Monterey, Fritz) are significantly different from each other, meaning that the observed differences among the sample averages could not reasonably be due to random chance alone.

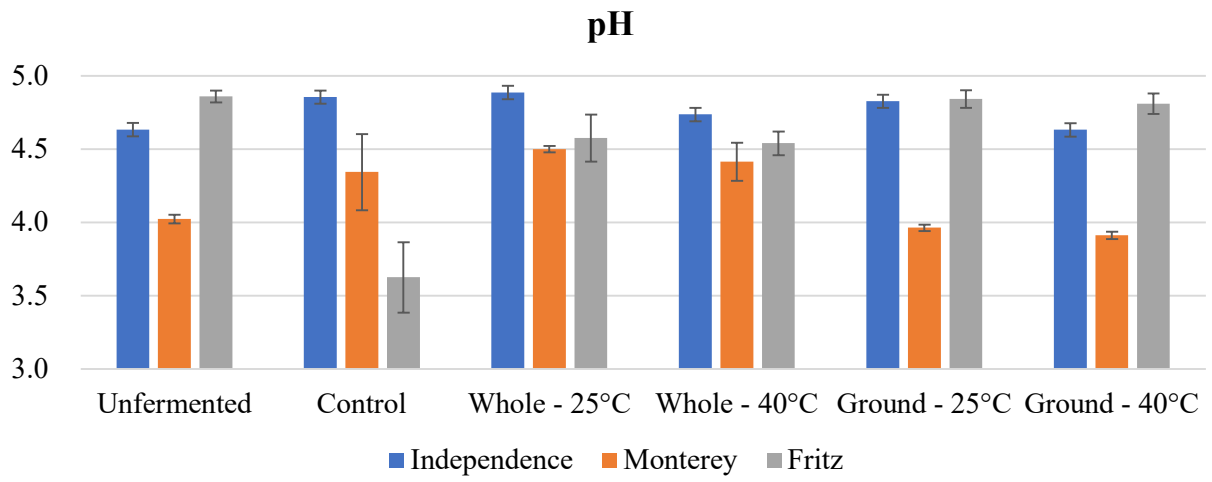


Figure 17: pH Change of Almond Hulls

Lactic acid concentrations are displayed in Figure 18 below. Grinding the hulls created more lactic acid than the whole hulls for both fermentation temperatures tested. Ground Fritz hulls fermented at 40°C generated the most lactic acid, at 93,690 ppm.

Using a one-way ANOVA technique on the hulls that were ground and fermented at 40°C, it was found that the sample averages of the lactic acid concentration of the three different almond varieties tested are significantly different from each other.

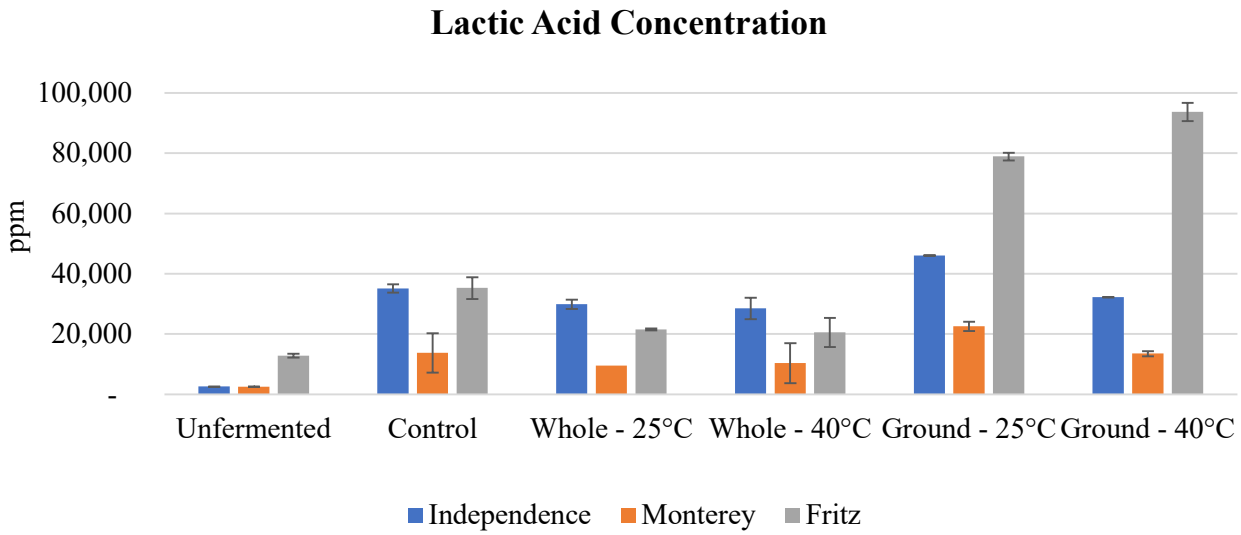


Figure 18: Lactic Acid Concentration of Almond Hulls

Acetic acid concentrations at 40°C were higher than the 25°C treatments with the same hull variety and particle size. Using a one-way ANOVA technique on the hulls that were ground and fermented at 40°C, it was found that the sample averages of the acetic acid concentration of the three different almond varieties tested are significantly different from each other.

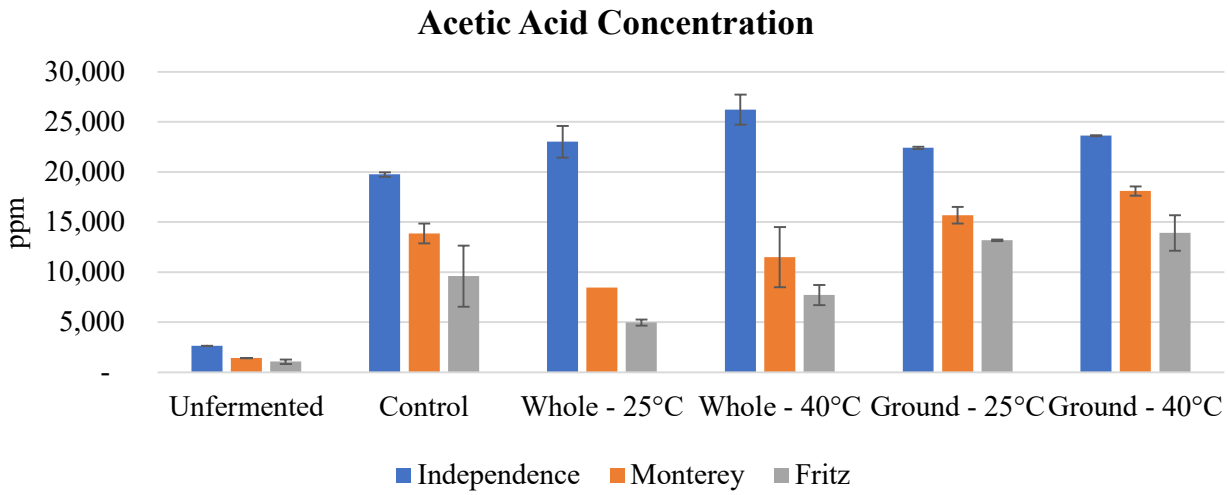


Figure 19: Acetic Acid Concentration of Almond Hulls

Propionic acid concentrations are shown in Figure 20 below. For both ground and unground hulls of all varieties, more propionic acid was produced with a 25°C fermentation temperature than a 40°C fermentation temperature. Using a one-way ANOVA technique on the hulls that were ground and fermented at 40°C, it was found that the sample averages of the propionic acid concentration of the three different almond varieties tested are not significantly different from each other and therefore the result is not statistically significant.

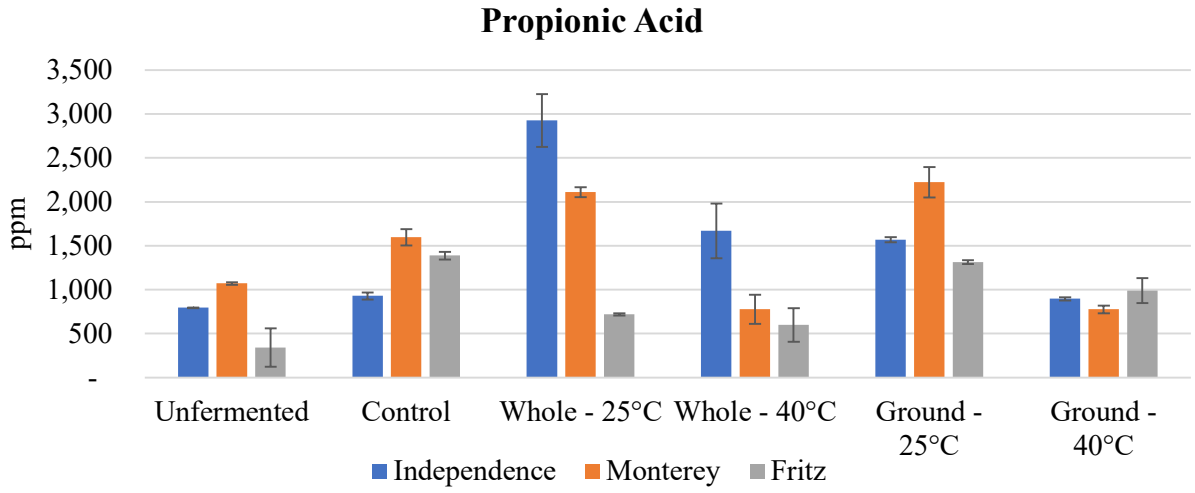


Figure 20: Propionic Acid Concentration of Almond Hulls

Ethanol concentrations at 25°C were higher than the 40°C treatments with the same hull variety and particle size. This is because more sugars were consumed during the 25°C fermentations than during the 40°C, which was confirmed by measuring total reducing sugars before and after each fermentation. Using a one-way ANOVA technique on the hulls that were ground and fermented at 40°C, it was found that the sample averages of the ethanol concentration of the three different almond varieties tested are significantly different from each other.

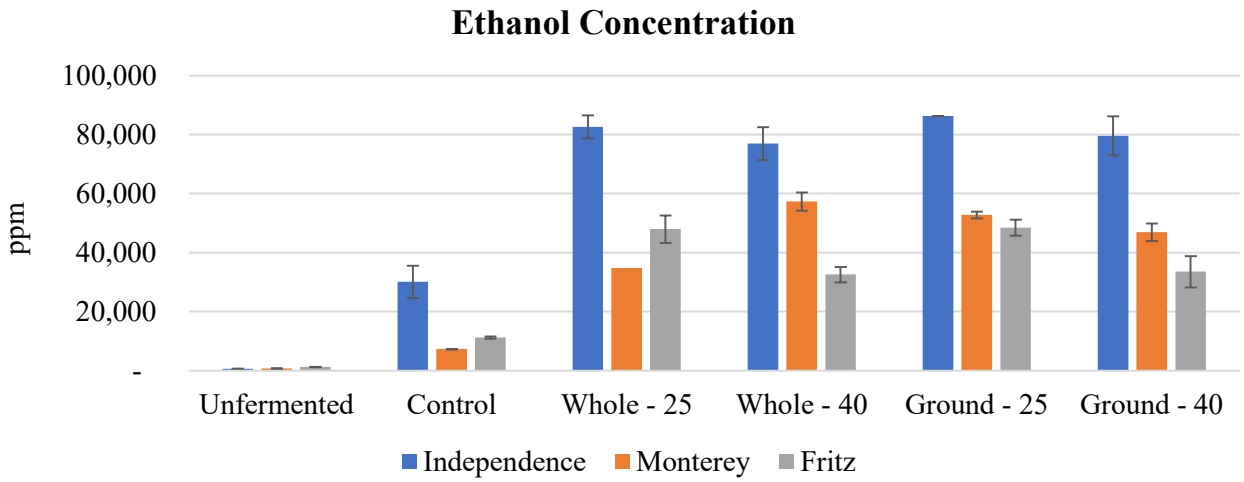


Figure 21: Ethanol Concentration of Almond Hulls

Results from the chemical analysis comparing the unfermented hulls to the hulls ground and fermented at 40°C for 14 days are shown in Table 9 below. All values are reported on a 100% dry basis except for moisture content, which is reported “as received”.

Table 9: Chemical Composition of Fermented and Unfermented Almond Hulls

	Independence, Unferm	Independence, 40 C/14 day	Monterey, Unferm	Monterey, 40C/14 day	Fritz, Unferm	Fritz, 40C/14	Unit
Moisture*	65.5	82.7	50.4	78.0	49.6	82.7	%
Dry Matter*	34.5	17.3	49.6	22.0	50.4	17.6	%
Crude Protein	6.3	10.0	6.1	6.8	3.6	17.3	%
Acid Detergent Fiber	20.8	34.1	32.9	36.2	30.1	7.4	%
Neutral Detergent Fiber	27.4	43.2	43.0	43.4	36.3	38.6	%
Fat	5.0	0.0	3.3	3.2	4.9	2.7	%
Calcium	0.4	0.8	0.2	0.2	0.4	0.6	%
Chloride	0.3	0.5	0.1	0.1	0.2	0.4	%
Magnesium	0.2	0.3	0.1	0.0	0.2	0.2	%
Phosphorus	0.1	0.3	0.1	0.2	0.1	0.0	%
Potassium	5.2	6.2	3.5	2.0	4.3	8.9	%
Sodium	0.0	0.1	0.0	0.0	0.0	0.1	%
Sulfur	0.1	0.2	0.0	0.3	0.1	2.1	%
Boron	100	183	131	295	284	621	ppm
Copper	11	19	2	8	8	52	ppm
Iron	585	415	263	138	625	1104	ppm
Manganese	27	29	21	20	49	170	ppm
Zinc	26	118	11	152	67	1014	ppm
DCAD	1242	1334	852	285	953	932	meq/ KG

**Reported "as received" rather than % d.b.*

Another 72-hour in-vitro rumen digestion test was performed using the control and ground Independence hulls fermented at 40°C from this experiment. Obtained results suggest that fermenting almond hulls with yeast is an efficient strategy to reduce enteric CH_4 production. Whereas reduction of enteric CH_4 production with hulls that were fermented in the absence of yeast becomes statistically relevant after 48 hours, hulls that were fermented in the presence of

yeast reduce CH_4 production within the first 24 hours. Importantly, when taken into consideration the reduction of CH_4 over the entire duration of in-vitro rumen digestion, only almond hulls fermented in the presence of yeast were capable of triggering a significant (~96%) reduction of enteric CH_4.

It has been shown in previous studies on enteric CH_4 reduction that yeast culture products increase dry matter digestion and propionic acid production and decrease acetic acid production and protein degradation (Miller-Webster et al.., 2004). As an increase in the propionate:acetate ratio is accepted as one of the main mechanisms to reduce enteric CH_4 production, it is likely that this change in VFA composition is what caused the success of the almond hulls fermented with yeast cultures in lowering enteric CH_4 production the in-vitro tests conducted in this study, however, the specific mode of action is still unknown (Boadi et al., 2004). Another study found that brewer's yeast culture enhanced the activity of bacteria that convert H_2 to acetate and decreased CH_4 output by 25% (Eun et al., 2003). The outcome of the control treatment is unpredictable due to its use of spontaneous fermentation, which explains the variation in enteric CH_4 production observed when supplementing those hulls in-vitro (Brooks et al., 2003). Bacterial inoculants have been used in silage to enhance quality and palatability, and stimulation of lactic acid utilizing bacteria has been theorized to reduce lactic acid and create a more stable ruminal environment, but there is little record of bacterial inoculants reducing enteric CH_4 production (Boadi et al., 2004). This information aligns with the results found in this study which shows that almond hulls fermented with yeast-containing inoculums produced the largest reduction in enteric CH_4 when compared to hulls fermented with bacteria, a mixture of bacteria and yeast, and no inoculant (control).

Chapter 5 – Conclusions

Almond hulls are a suitable feedstock for the creation of fermented dairy cattle feed due to their high sugar, fiber, antioxidant, and moisture contents, their low protein and fat contents, and their abundance and availability as a byproduct feedstuff from the almond industry.

Almond hulls have high sugar contents ranging from 31.8% to 42.2% by weight on a dry-basis (db) and phenolic compound contents ranged from 3.4% to 7.6%, db. On average, hull samples from hulling facilities contained 88.9% hulls and 10.1% contaminants. It is recommended that sieving separation be utilized to sort hulls. Off-ground harvested hulls had an average moisture content 3.5 times higher than on-ground harvested hulls of the same variety. Hulls were low in protein and fat at an average of 5.7% and 3.7% db, respectively and an average of 24.1% acid detergent fiber and 33.3% neutral detergent fiber. Almond hulls fermented with *S. cerevisiae* and *L. Plantarum* bacteria produce desirable characteristics for fermented feed including a pH below 4.5, high concentrations of lactic acid, and low concentrations of acetic acid. In-vitro digestion tests suggest that fermented feed is potentially an efficient strategy to reduce enteric CH_4 production. It was found that fermentations using yeast inoculums produce higher amounts of ethanol and acetic acid and cause an increase in pH, and inoculums containing bacteria produce higher amounts of lactic acid and cause a drop in pH. Hull variety has a large impact on the characteristics of the fermented hulls under the fermentation conditions tested, as all parameters tested were significantly different from each other between varieties. 14-day fermentation durations produced similar ethanol, acetic acid, and lactic acid concentrations as 30-day fermentation durations. Additionally, in-vitro digestion results indicated that almond hulls fermented with *S. cerevisiae* for 14 days reduced enteric CH_4 production by 96% over 72 hours digestion at a 20% inclusion rate in a cattle diet.

Appendix

Table 10: Terminal Velocity Measurements for Almond Hull Samples Components

	Terminal Velocity (m/s)		
	Nonpareil	**Monterey**	**Independence**
Hulls	7.3 ± 1.00	6.2 ± 0.79	7.4 ± 1.43
Twigs	8.3 ± 1.24	10.9 ± 0.70	8.9 ± 0.92
Shells	5.8 ± 0.66	5.3 ± 1.07	4.6 ± 0.36
Immature Kernels	6.9 ± 0.75	6.8 ± 1.25	6.4 ± 0.89
Kernels	8.7 ± 0.27	7.6 ± 0.25	7.6 ± 0.33
Shell + Kernel	9.7 ± 0.94	9.5 ± 0.31	8.6 ± 0.38

Table 11: Solids Measurements for Nonpareil Hulls and Contaminants

	Nonpareil			
	Moisture Content (%)	**Total Solids (%)**	**Volatile Solids (%)**	**Fixed Solids (%)**
Hulls	16.9 ± 3.2	83.1 ± 3.2	75.0 ± 6.0	25.0 ± 6.0
Twigs	8.2 ± 1.0	75.0 ± 1.0	78.4 ± 4.9	21.6 ± 4.9
Shells	7.1 ± 0.9	75.0 ± 0.9	87.1 ± 3.7	12.9 ± 3.7
Immature Kernels	11.5 ± 0.1	89.0 ± 0.1	82.9 ± 0.6	17.1 ± 0.6
Kernels	4.0 ± 1.8	93.0 ± 1.8	87.6 ± 0.3	12.4 ± 0.3
Shell + Kernel	6.3 ± 0.9	93.7 ± 0.9	95.1 ± 0.5	4.9 ± 0.5

Table 12: Solids Measurements for Monterey Hulls and Contaminants

	Monterey			
	Moisture Content (%)	**Total Solids (%)**	**Volatile Solids (%)**	**Fixed Solids (%)**
Hulls	13.8 ± 2.4	86.2 ± 2.4	78.5 ± 2.6	21.5 ± 2.6
Twigs	9.2 ± 0.9	90.8 ± 0.9	86.5 ± 1.5	13.5 ± 1.5
Shells	7.4 ± 0.7	92.6 ± 0.7	91.0 ± 0.5	9.0 ± 0.5
Immature Kernels	9.1 ± 0.4	90.9 ± 0.4	92.0 ± 4.2	8.0 ± 4.2
Kernels	4.8 ± 1.5	95.2 ± 1.5	91.7 ± 0.3	8.3 ± 0.3
Shell + Kernel	5.2 ± 0.9	94.8 ± 0.9	92.0 ± 0.8	8.0 ± 0.8

Table 13: Solids Measurements for Independence Hulls and Contaminants

	Independence			
	Moisture Content	**Total Solids**	**Volatile Solids**	**Fixed Solids**
Hulls	17.4 ± 0.8	82.6 ± 0.8	70.1 ± 1.5	29.9 ± 1.5
Twigs	8.7 ± 0.6	91.3 ± 0.6	83.9 ± 0.3	16.1 ± 0.3
Shells	7.7 ± 1.0	92.3 ± 1.0	90.0 ± 1.1	10.0 ± 1.1
Immature Kernels	11.0 ± 3.5	89.0 ± 0.2	82.1 ± 1.1	17.9 ± 1.1
Kernels	8.3 ± 0.1	89.8 ± 1.9	89.7 ± 2.2	10.3 ± 2.2
Shell + Kernel	7.0 ± 0.5	93.0 ± 0.0	88.9 ± 0.9	11.1 ± 0.9

Table 14: Location of Almond Hull Collection Locations (2021 harvest)

Almond Variety	**Harvest Type**	**Orchard Location**	**Collection Date**
Independence	On-Ground	Vacaville, CA	8/27/2021
	Off-Ground	Vacaville, CA	8/27/2021
Monterey	On-Ground	Woodland, CA	9/1/2021
	Off-Ground	Oakdale, CA	9/3/2021
Nonpareil	On-Ground	Arbuckle, CA	9/15/2021
	Off-Ground	Arbuckle, CA	9/15/2021
Fritz	On-Ground	Arbuckle, CA	9/15/2021
	Off-Ground	Arbuckle, CA	9/15/2021

Table 15: Chemical Composition of Almond Hulls

	Nonpareil On-Ground	**Nonpareil Off-Ground**	**Monterey On-Ground**	**Monterey Off-Ground**	
Moisture*	9.3	22.6	26.1	50.4	%
Dry Matter*	90.7	77.4	73.9	49.6	%
Crude Protein	4.2	5.2	6.3	6.1	%
Acid Detergent Fiber	23.5	21.1	25.1	32.9	%
Neutral Detergent Fiber	36.0	31.0	37.0	43.0	%
Fat	2.6	3.5	2.9	3.3	%
Calcium	0.2	0.2	0.3	0.2	%
Chloride	0.1	0.1	0.2	0.1	%
Magnesium	0.1	0.1	0.1	0.1	%
Phosphorus	0.1	0.0	0.1	0.1	%
Potassium	3.4	3.1	3.9	3.5	%

Sodium	0.0	0.0	0.0	0.0	%
Sulfur	0.0	0.0	0.0	0.0	%
Aluminum	205	442	355	190	ppm
Boron	40	173	309	131	ppm
Copper	3	3	7	2	ppm
Iron	481	636	1068	263	ppm
Manganese	13	23	27	21	ppm
Zinc	9	16	16	11	ppm
DCAD	828	749	927	852	meq/KG

**Reported "as received" rather than % d.b.*

	Independence On-Ground	**Independence Off-Ground**	**Frtiz On-Ground**	**Fritz Off-Ground**	
Moisture*	23.6	65.5	19.7	49.6	%
Dry Matter*	76.4	34.5	80.3	50.4	%
Crude Protein	7.7	6.3	6.1	3.6	%
Acid Detergent Fiber	17.6	20.8	22.1	30.1	%
Neutral Detergent Fiber	23.8	27.4	32.3	36.3	%
Fat	4.4	5.0	3.3	4.9	%
Calcium	0.5	0.4	0.3	0.4	%
Chloride	0.3	0.3	0.2	0.2	%
Magnesium	0.2	0.2	0.1	0.2	%
Phosphorus	0.1	0.1	0.1	0.1	%
Potassium	4.0	5.2	3.8	4.3	%
Sodium	0.1	0.0	0.0	0.0	%
Sulfur	0.0	0.1	0.0	0.1	%
Aluminum	269	439	not reported	not reported	ppm
Boron	76	100	142	284	ppm
Copper	8	11	6	8	ppm
Iron	401	585	650	625	ppm
Manganese	19	27	20	49	ppm
Zinc	17	26	14	67	ppm
DCAD	937	1242	897	953	meq/KG

**Reported "as received" rather than % d.b.*

	Monterey 40C/14 day	Independence 40C/14 day	Fritz 40C/14 day	
Moisture*	78.0	82.7	82.7	%
Dry Matter*	22.0	17.3	17.6	%
Crude Protein	6.8	10.0	17.3	%
Acid Detergent Fiber	36.2	34.1	7.4	%
Neutral Detergent Fiber	43.4	43.2	38.6	%
Fat	3.2	0.0	2.7	%
Calcium	0.2	0.8	0.6	%
Chloride	0.1	0.5	0.4	%
Magnesium	0.0	0.3	0.2	%
Phosphorus	0.2	0.3	0.0	%
Potassium	2.0	6.2	8.9	%
Sodium	0.0	0.1	0.1	%
Sulfur	0.3	0.2	2.1	%
Boron	295	183	621	ppm
Copper	8	19	52	ppm
Iron	138	415	1104	ppm
Manganese	20	29	170	ppm
Zinc	152	118	1014	ppm
DCAD	285	1334	932	meq/KG

**Reported "as received" rather than % d.b.*

Table 16: One-way ANOVA Results for pH, Experiment 3

Groups	*Count*	*Sum*	*Average*	*Variance*		
Yeast	6	28.89	4.815	0.00035		
Bacteria	6	27.06	4.51	0.01056		
Control	4	18.94	4.735	0.0023		
Unfermented	3	13.9	4.633333	0.000233		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.300494	3	0.100165	24.26599	5.25E-06	3.287382
Within Groups	0.061917	15	0.004128			
Total	0.362411	18				

Table 17: One-way ANOVA Results for Lactic Acid Concentration, Experiment 3

Groups	*Count*	*Sum*	*Average*	*Variance*		
Yeast	4	127070.4	31767.608	227884935		
Bacteria	4	189381.5	47345.387	70820851.		
Control	4	158704.6	39676.163	194124800		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	485372623.7	2	242686311.	1.47730062	0	4.25649
Within Groups	1478491763	9	164276862			
Total	1963864387	11				

Table 18: One-way ANOVA Results for Acetic Acid Concentration, Experiment 3

Groups	*Count*	*Sum*	*Average*	*Variance*		
Yeast	4	60633.1	15158.28	7238353		
Bacteria	4	69259.69	17314.92	14391436		
Control	4	60697.39	15174.35	8528602		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12311268	2	6155634	0.61233	0.563211	4.256495
Within Groups	90475174	9	10052797			
Total	1.03E+08	11				

Table 19: One-way ANOVA Results for Propionic Acid Concentration, Experiment 3

Groups	*Count*	*Sum*	*Average*	*Variance*		
Yeast	5	4758.249	951.6498	446837.9		
Bacteria	5	18913.99	3782.798	2296459		
Control	5	7516.152	1503.23	142071		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	22526787	2	11263394	11.71087	0.001512	3.885294
Within Groups	11541471	12	961789.3			
Total	34068258	14				

Table 20: One-way ANOVA Results for Ethanol Concentration, Experiment 3

Groups	*Count*	*Sum*	*Average*	*Variance*		
Yeast	4	246038.6	61509.64	3.78E+08		
Bacteria	4	139456.8	34864.21	26785760		
Control	4	174468.8	43617.21	79291892		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.48E+09	2	7.38E+08	4.574277	0.042589	4.256495
Within Groups	1.45E+09	9	1.61E+08			
Total	2.93E+09	11				

Table 21: One-way ANOVA Results for pH, Experiment 4

Groups	*Count*	*Sum*	*Average*	*Variance*		
Independence	3	13.9	4.633333	0.000233		
Monterey	3	12.07	4.023333	0.001233		
Fritz	6	29.01	4.835	0.00263		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.328283	2	0.664142	371.644	2.24E-09	4.256495
Within Groups	0.016083	9	0.001787			
Total	1.344367	11				

Table 22: One-way ANOVA Results for Lactic Acid Concentration, Experiment 4

Groups	*Count*	*Sum*	*Average*	*Variance*		
Independence	4	131028.76	32757.1909	3378877.78		
Monterey	4	54053.081	13513.2704	950117.214		
Fritz	4	374760.7	93690.178	5203529.0		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	140153253	2	70076626	2205.39575	0	4.2564
Within Groups	28597572	9	3177508.0			
Total	14043922932	11				

Table 23: One-way ANOVA Results for Acetic Acid Concentration, Experiment 4

Groups	*Count*	*Sum*	*Average*	*Variance*		
Independence	4	9454.458	2363.614	2946.731		
Monterey	4	72434.43	18108.61	296142.9		
Fritz	4	55685.77	13921.44	4168001		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.32E+08	2	2.66E+08	178.6489	5.71E-08	4.256495
Within Groups	13401273	9	1489030			
Total	5.45E+08	11				

Table 24: One-way ANOVA Results for Propionic Acid Concentration, Experiment 4

Groups	*Count*	*Sum*	*Average*	*Variance*		
Independence	4	3392.264	848.0659	6153.239		
Monterey	4	2997	749.25	12198.25		
Fritz	4	3856	964	132727.3		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	92430.48	2	46215.24	0.917705	0.433798	4.256495
Within Groups	453236.5	9	50359.61			
Total	545666.9	11				

Table 25: One-way ANOVA Results for Ethanol Concentration, Experiment 4

Groups	*Count*	*Sum*	*Average*	*Variance*		
Independence	2	348491	174245.5	1.09E+09		
Monterey	2	134644.6	67322.29	502643.5		
Fritz	2	134052.8	67026.4	1.28E+09		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.53E+10	2	7.64E+09	9.5413	0.049117	9.62094
Within Groups	2.37E+09	3	7.89E+08			
Total	1.77E+10	5				

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