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OPTIMIZATION OF TEFF FERMENTATION BY *LACTOBACILLUS*
CORYNIFORMIS STARTER CULTURE AND INVESTIGATION OF SORGHUM
AND BUCKWHEAT AS SUBSTITUTE COMPONENTS IN INJERA BATTER

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ABSTRACT

Injera, a flatbread indigenous to Ethiopia, is traditionally made from teff flour fermented in water. The organisms responsible for the process are native to the flour, and the fermentation is traditionally initiated by backslopping a portion of the previous batch. This staple food is typically made in relatively small batches, and scaling up for commercial production offers the potential to produce injera at reduced price to the consumer, which may benefit Ethiopia socially and economically. This two-phase study aims first to optimize the fermentation of teff by using *Lactobacillus coryniformis* as a starter culture. A bioreactor was used to ferment 2.5 L batches of ivory teff, water, and starter culture with full agitation at 25, 28, 31, 34, 37, 40, and 43°C. Batches with and without starter culture and agitation were performed at ambient temperature. During fermentation, the pH of the batter was monitored for up to 72 h. Kinetic parameters (lag duration λ and maximum acidification rate μ_m) relevant to the modified Gompertz model were determined to characterize the change in pH. The time to reach pH 3.8 was used to assess the time to complete the fermentation. Acidification to pH 3.8 is accomplished in the least time (5.9 h) when using *L. coryniformis* starter culture, 300 rpm impeller speed for agitation, and temperature control at 37°C. The second phase of the project assessed how flour composition influences fermentation kinetic parameters. Inoculated injera batters were fermented at 37°C under agitation, with the flour composition varying between teff, sorghum, buckwheat, and their mixtures. Teff and a teff-sorghum composite fermented in the least time. A higher fraction of buckwheat in the batter arrested the fermentation at a higher pH. The results of this study are used to make recommendations for scaling up the teff fermentation process.

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Chapter 1

Introduction

Injera, the Staple Bread of Ethiopia

Injera is a large, pancake-like bread made of fermented cereals such as teff and sorghum. The bread is made in thin, flat, round loaves of about 50 to 60 cm in diameter. In Ethiopia, injera is a staple food that functions as the serving medium, food, and eating utensil. Ethiopians eat injera with wot, which is a meat, vegetable, or legume stew (Steinkraus, 1996). During a typical Ethiopian meal, family members sit around a tray covered in injera, onto which wot is ladled. More injera is served rolled up and sliced crosswise, such that pieces may be broken off and used to pick up food during the meal. Flat, thin, pliable, and porous, the bread is ideal for soaking up sauces and holding food.

Teff (*Eragrostis tef*) is a pseudo-cereal grass native to the Ethiopian highlands of Africa. While it is virtually unknown as a food crop elsewhere in the world (Stewart and Asnake 1962), teff is responsible for over two thirds of human nutrition in Ethiopia (Stallknecht, 1993). Teff straw is used as an animal feed, and the grain is milled into flour and primarily used to make injera. Adults in Ethiopia consume two to three injera per day, and the typical diet of most people consists of 92% injera and 8% wot (Steinkraus, 1996).

Injera comes in several varieties, although teff injera is both the most preferred and most common type, especially in the highlands. Barley, wheat, sorghum, maize, and millet are also used to make injera. Teff is the most popular grain in Ethiopia in terms of hectares planted, but it is an expensive crop due to high labor costs and low yields. However, while teff may be more expensive to cultivate, it is convenient to mill. Milling teff requires less work than milling other

grains. While other grains require steps such as winnowing and dehusking, teff needs only be washed and dried before milling. Teff injera is preferred because it can be stored for up to three days and still retain the desired softness and pliability. By contrast, wheat injera becomes sticky after baking, maize injera dries out and develops cracks within hours, and sorghum injera dries out after one to two days. Of the teff varieties, white teff is more expensive than red teff because it makes more desirable injera (Steinkraus, 1996).

A natural fermentation process is essential to injera production, providing flavor and visual appeal. Microorganisms present during the incubation period produce a batter that is noticeably acidic. In addition, the fermentation produces gas, which during the baking process forms bubbles that produce “eyes” on the top surface of the injera. While the bottom surface is flat, the top surface of the injera is pockmarked with eyes, which Ethiopians view as a desirable characteristic.

Injera Preparation

In Ethiopia, injera batter is made by mixing teff flour and water in a bohaka, a container made of clay, metal, or wood. To this mixture is added irsho, a clear yellowish fluid leftover from the previous fermentation batch. The three ingredients are mixed thoroughly, and the thin, watery mixture is incubated at room temperature for 12-72 hours, depending on the desired flavor of the injera. Ethiopians use batter that was only fermented for 12 to 24 hours to prepare aflegna injera, which is characterized by its thickness, sweet flavor, odor, and brownish red bottom. Traditional injera is fermented for 48 to 72 hours, and undesirably sour komtata injera is made from overly fermented batter (typically attributed to the labors of unskilled women, often newly-wed) (Stewart and Getachew, 1962).

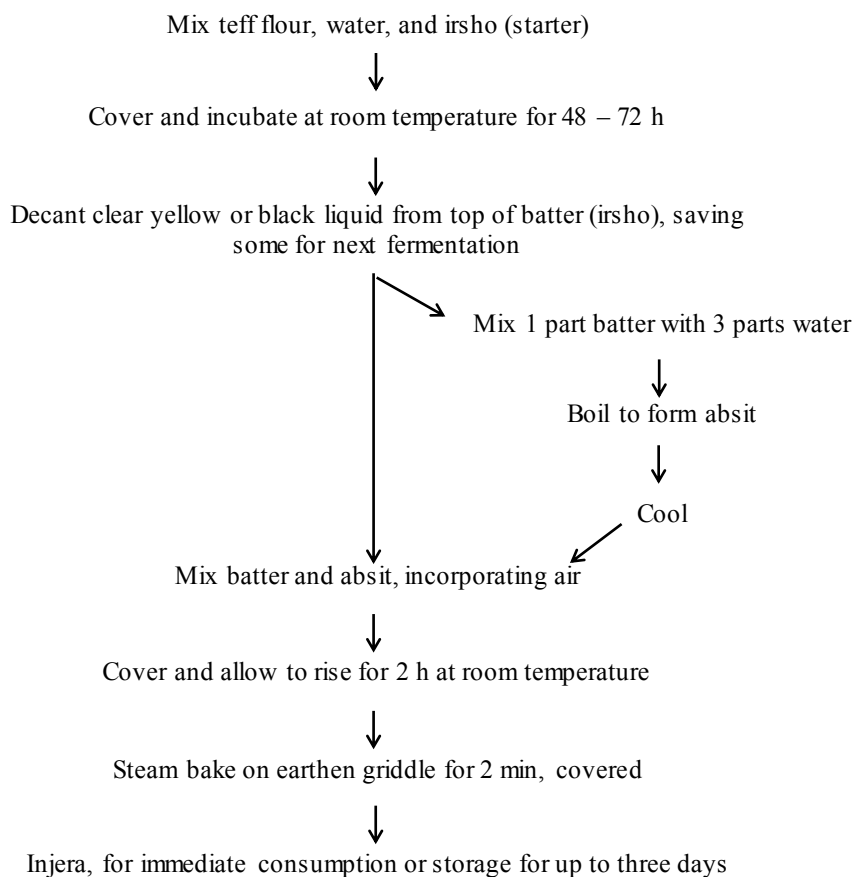


Figure 1-1: Flow diagram describing the preparation of teff injera.

Over the course of the fermentation, the teff settles on the bottom of the bohaka, leaving a yellowish or blackish liquid on top. A portion of this irsho is saved for the next batch, and the rest is poured off (Stewart and Getachew, 1962). About 10% of the fermented paste is mixed with three parts of water and boiled. This boiled batter is called absit, and it is added back to the batter in the bohaka to initiate a second fermentation that lasts 1.5 to 2 hours (Steinkraus 1996). Adding absit enhances gas formation and causes the paste to rise (Gashe, 1985). Adding absit is critical to developing the desired texture and consistency, as injera made without absit tends to be powdery and have fewer of the “eyes” which are so prized by Ethiopian consumers. It is

important to note that teff, millet, and corn are the only grains that require absit during the process of making injera (Steinkraus, 1996).

To bake the injera, a thin layer of batter is poured over the surface of an earthen pan or griddle. Once bubbles form on the surface, the griddle is covered and the injera steam cooks for about 2 minutes (Steinkraus, 1996). The injera is removed from the griddle and stored in a mesob, a flat-bottomed cylindrical container made of grass stems (Stewart and Getachew, 1962). A flow diagram describing the injera-making process is provided in Figure 1-1.

Injera-making traditionally involves the mixing of teff, water, and irsho; however, the exact proportion of each ingredient varies in the literature. Gashe (1985) mixed teff flour and water in a 1 : 1.6 ratio (unspecified as to weight or volume basis). Steinkraus reports an ingredients ratio of 1 kg teff flour, 2 kg water, and 0.16 kg irsho (1996). Heckner (2011) used a volume ratio of 1 part teff flour, 2 parts water, and 0.25 parts irsho.

Fermentation of Injera Batter

Source of Microorganisms

There are three main sources for the microorganisms involved in the fermentation: the teff flour itself, the irsho, and the fermentation vessel. Teff flour likely contains a wide variety of soil and fecal microorganisms as a result of the threshing process. After harvest, bundles of teff are placed on the ground in an area cleared of animal manure. Cattle are driven across the bundles, and the repeated stomping action of hooves separates the seeds from the shaft (Steinkraus, 1996). Due to the exposure of the teff to fecal matter and soil, threshing is a likely source of microorganisms found in teff flour. A microbiological analysis found that Ethiopian

teff flour contains 10^8 CFU/g of aerobic mesophilic bacteria, and 71% of isolates were gram positive bacteria (Ashenafi, 1994).

Irsho is traditionally added to initiate the fermentation. This fluid contains riboflavin (Stewart and Getachew, 1962) and is rich with microorganisms. Ashenafi (1994) sampled irsho from various households, finding that while the pH was consistently about 3.5, the titratable acidity varied considerably between households. Microbiological analysis of the irsho found yeasts to be the most prevalent organism in the irsho, which agrees with Gashe's (1985) observation that yeasts become the dominant microflora toward the end of teff fermentation. The microorganisms found in the irsho are most likely the same organisms found at the end of the fermentation process. Ashenafi's (1994) analysis found that *Candida milleri* was the only yeast present in all households irsho samples, and that of the isolated yeasts, only *C. milleri* and *Kluyveromyces marxianus* are capable of gas production. Because Enterobacteriaceae and lactic acid bacteria counts from irsho were low (<10 and <100 CFU/ml, respectively), Ashenafi suggests that different yeasts in the irsho of different households were responsible for the variations in injera flavor between households. A more recent analysis of two American injera operations found *Lactobacillus* concentrations between 10^7 and 10^9 in the irsho (Heckner, 2011). Of these, *Lactobacillus coryniformis* was the most prevalent, while the study also identified *L. plantarum*, *L. farciminus*, and *L. diolivorans*.

The third source of fermentation organisms is the fermentation vessel, or bohaka. Traditionally, neither this vessel nor the mixing utensils are ever thoroughly washed between batches. Some fermented batter is left on the bottom and sides of the bohaka when the new batch is mixed (with irsho from that previous batch as well). Ethiopian housewives claim that a new bohaka would not ferment properly, and that in order for a successful fermentation to occur, the container should be previously used for at least three batches, and irsho must be added (Stewart and Getachew, 1962). This practice may have some grounds in superstition, but it is apparent

that some form of inoculation, either irsho and/or fermented batter left in the bohaka, is needed to initiate a successful fermentation.

Responsible Organisms and Succession of Flora

Several studies have examined the microbiological flora in fermenting teff, but the exact succession of organisms and the effects of these microorganisms on the batter require further investigation. Stewart and Getachew were the first to investigate the fermentation process associated with injera, postulating that the fermentation was driven by yeasts. These researchers isolated yeasts and bacteria from fermented teff, inoculated teff batter with the isolates. They found that while all samples fermented to some degree, only the sample inoculated with yeast produced the desired fermentation, and the sample inoculated with both yeast and bacteria produced injera with the best flavor (1962). It is reasonable to conclude that both the yeast and bacteria have important roles in fermenting teff for injera production.

Gashe studied the progression of microbial flora during the wild fermentation of teff (no starter culture, irsho, or back slopping of any kind is mentioned), determining that lactic acid bacteria were responsible for the acidic flavor and pH development. Members of *Enterobacteriaceae* decrease the pH from 6.6 to 5.8 over the first 18 h, after which they are succeeded by *Streptococcus faecalis* and *Leuconostoc mesenteroides*. *Pediococcus cerevisiae*, *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Lactobacillus plantarum* appear in significant numbers (10^6 CFU/g) at 30 h (pH 4.7) and dominate the fermentation after 42 h. Yeasts become the most prevalent flora by hour 60, after which there is little change in pH, but high counts of lactic acid bacteria remain until the termination of fermentation at 72 h (1985).

Further research is required to verify the progression of microflora in wild fermentations and in fermentations initiated by irsho. Variations in the microorganisms intrinsic to irsho and

teff flour may cause considerable variation between the fermented products (Stewart and Getachew, 1962; Ashenafi, 1994; Moroni et al, 2010; Heckner, 2011); therefore it is likely that the fermentation processes are unique. Furthermore, the type of flour may influence and direct the growth of lactic acid bacteria in sourdoughs (de Vuyst et al, 2009). Therefore, it likely that injera made from different types of teff or from different flours altogether (e.g. sorghum or buckwheat) may experience slightly different fermentations. Moroni et al identified *Lactobacillus pontis* as the dominant species in teff sourdoughs which were spontaneously initiated and stabilized by backslopping; however, in buckwheat sourdoughs, *Lactobacillus plantarum* was the dominant species (2010).

A review of literature and previous work shows little consensus regarding the microorganism responsible for the pH decrease in teff injera batter. Especially in spontaneous fermentations, the dominating organism varies depending on the source of the flour, the species of flour, and possibly the fermentation conditions. However, it is apparent that the decrease in pH is caused by lactic acid bacteria, with the exact species varying with the above mentioned variables.

Impact of Fermentation on Chemical Attributes

In addition to providing acidity, microbiological metabolism affects the chemical makeup of the fermenting teff batter. Analysis of fermented teff by light and electron microscopy determined that the initially angular appearance of starch granules develop an eroded appearance, most likely due to enzymatic degradation during fermentation. However, bran and embryo fragments, cell walls, and protein bodies were not noticeably affected by fermentation (or baking). During fermentation, microorganisms were observed to produce strands of fibrillar

material that bound flour particles together (Parker et al, 1989). It is likely that this fibrillar material is exo-polysaccharide produced by lactic acid bacteria.

Impact of Temperature on Fermentation

The fermentation process could be expedited by determining optimum temperature conditions for the responsible microorganisms. In the highlands of Ethiopia (8000 feet altitude), the fermentation process occurs at ambient temperatures, for example between 17 and 25°C (Steinkraus, 1996) or between 20 and 23°C (Gashe, 1985). Temperature has been found to impact the pH profiles of spontaneous teff fermentations and teff fermentations initiated by backslopping. Teff flour batter fermented by backslopping at 35°C reached a pH of 4 in less than 10 hours of fermentation, while teff batter fermented by backslopping at room temperature reached a pH of 4 in 20 hours. In addition, compared to teff fermented at room temperature, teff fermented at 35 C exhibited a steeper slope as part of the pH profile (Yigzaw et al, 2004).

Flour Composition

While injera is primarily made from teff, several other flours and blends of flours have been used. Choice of flour may depend on availability, price, and nutritional value. Typically, sorghum, millet, and corn flours are used as alternatives to teff when making injera. Teff flour is effectively gluten-free (Moroni et al, 2010). If blending several flours for injera-making, it may be desirable to complement teff flour with other gluten-free flours such as sorghum or buckwheat (Figure 1-2).

Sorghum is the second most preferred flour for injera preparation in Ethiopia. Teff injera is preferred because it can be stored for 3 days without losing pliability (Steinkraus, 1996). Due

to the significantly lower cost of sorghum flour, sorghum injera is much less expensive. However, sorghum injera tends to develop an undesirably firm and friable texture within 48 h of storage. Selection of sorghum cultivar impacts the textural characteristics of sorghum injera after storage (Yetneberk et al, 2004). More promisingly, injera made from composite flours of sorghum and teff experienced improved storage quality compared to sorghum injera (Yetneberk et al, 2005).

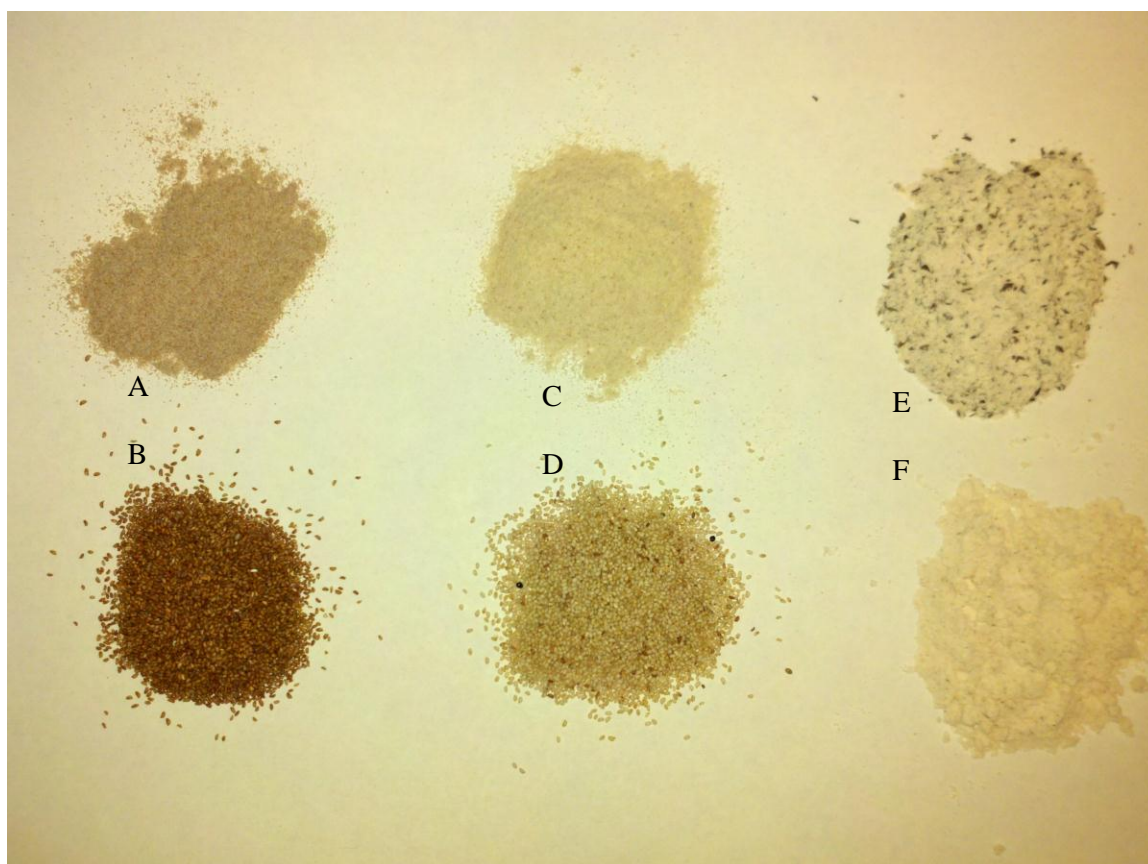


Figure 1-2: Raw materials for injera making: brown teff flour (A), brown teff (B), ivory teff flour (C), ivory teff (D), buckwheat flour (E), and sweet sorghum flour (F).

Use of Starter Cultures

Currently, injera batter fermentation is initiated by organisms intrinsic to the flour. The process of backslopping, or initiating subsequent fermentations by adding a small portion of a previous successful fermentation, is an ancient process that has been used for centuries.

Sourdough fermentation, which is quite similar to the fermentation of injera batter, is traditionally performed by a similar backslopping process once a stable culture is obtained. On commercial scale, modern sourdough fermentations may be initiated by a starter culture. A successful starter culture elicits the desired acidity and flavor, thrives in the environmental conditions, and out-competes its microbial competitors (De Vuyst et al, 2009). If properly selected, the starter culture allows for the production of a consistent sourdough. The use of starter cultures may benefit commercial-scale injera production as well.

Moroni et al (2009) investigated the ability of commercial starter cultures to ferment teff and buckwheat sourdoughs. The competitiveness of each strain in the starter culture depended on the flour species and the temperature of fermentation. Starter strains of lactic acid bacteria such as *L. helveticus* and *L. paracasei* did not persist in teff or buckwheat sourdoughs. Starter strains of *L. plantarum* dominated teff fermentation at 25°C but not 35°C. Starter yeasts were completely out-competed by autochthonous strains. The dominance of native microorganisms over commercial starter cultures indicates that adaptability to both substrate and environmental conditions must be considered during starter selection.

Fermentation by *Lactobacillus coryniformis*

A recent unpublished study by a Penn State researcher examined two injera samples from teff injera batters prepared in-house and in an American Ethiopian restaurant. In both samples,

Lactobacillus coryniformis was the dominant bacteria present in the irsho (Heckner, 2011). No other studies have mentioned this organism in the context of injera batter fermentation. However, *L. coryniformis* was identified among the lactic acid producers in other fermented plant products (Battcock and Azam-Ali, 1998). *L. coryniformis* subsp. *coryniformis* has been frequently isolated from kocho (Nigatu, 2000). Kocho is a fermented bread-like food made from the grated pulp of the ensete (false banana) plant, which is consumed by approximately one sixth of Ethiopians as an alternative to injera (Steinkraus, 1996). Hancioglu and Karapinar (1996) identified *L. coryniformis* in boza, a traditional Turkish fermented beverage made from cooked maize, wheat, and rice flours.

Bustos et al (2003) used *L. coryniformis* to produce D-lactic acid using corn steep liquor as a substrate. The fermentations in the Bustos study were performed at 37°C, although it is unknown if this temperature was chosen by simple convention or because the temperature fosters optimum growth. Favorable conditions for D-lactic acid production from glucose by *L. coryniformis* subsp. *torquens* were 37°C and pH 6 (Yanez et al, 2003). One strain of *L. coryniformis* (CECT 5711) was found to have considerable probiotic effects in a human clinical study, particularly with regulating the immune system (Olivares et al, 2006). Lara-Villoslada et al (2007) demonstrated that that same strain is nonpathogenic and safe for human consumption. *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 produces a broad-spectrum antifungal compound that is active against several common molds and weakly active against certain yeasts (Magnusson and Schnurer, 2001). Martin et al (2005) isolated *L. coryniformis* CECT 5711 from a goat's milk cheese and characterized the strain, determining that it produces a broad spectrum antimicrobial in addition to exhibiting probiotic properties. If utilized in the food industry, the antifungal properties of *L. coryniformis* may extend the shelf life of foods fermented by this organism. However, to the author's knowledge, the role of this microorganism in food fermentations has not been studied.

Experimental Objectives and Relevance of Study

Injera is typically made by individual households in the Ethiopian highlands, but city inhabitants may purchase injera from a local baker. There is interest in scaling up the production of injera. An Ethiopian entrepreneur is currently working with Penn State Engineering and Food Science faculty and students to develop technology germane to the construction of Ethiopia's first injera-manufacturing plant. Past engineering senior design projects have focused on developing inexpensive equipment for depositing batter on a conveyor belt and baking injera on a continuous basis. The independent project that is the subject of this thesis is focused on optimizing the fermentation of injera batter and providing recommendations for scale up. The primary goal is to reduce the fermentation time from 72 h to a time frame that is more appropriate for an industrial food production operation. This goal is accomplished by utilizing a starter culture and by determining the fermentation conditions (temperature, agitation speed) that minimize the time to reduce the pH of the batter. A second goal is to investigate the impact of varying the flour composition on fermentations operated at the optimum conditions. Teff, buckwheat, and sorghum flours (and their mixtures) were assessed to determine if composition affects fermentation time. Teff is an expensive ingredient, so cutting the teff with buckwheat or sorghum offers an opportunity to reduce production costs, assuming the product is favorably received by the market. The outcomes of this study are used to make recommendations for the scale-up of injera batter fermentation.

Chapter 2

Materials and Methods

Preparation of Frozen Stock Cultures

Lactobacillus coryniformis was identified and isolated from injera batter *irsho* by Heckner and Miller and preserved at -80°C for later use. Frozen sample was thawed, and 0.1 ml was added to 9 ml MRS broth (Difco, Sparks, MD) and incubated anaerobically for 24 h at 37°C. One loopful was plated onto MRS agar and incubated anaerobically at 37°C for 24 h to check for purity. One single colony from the plate was used to inoculate 9 ml MRS broth. After 24 h incubation at 37°C, 500 µl vortexed turbid broth and 500 µl sterile 20% glycerol were added to each of 10 sterile cryovials (Nalgene, location). After 15 min, cultures were frozen at -80°C.

Starter Culture Preparation

MRS broth was prepared and dispensed in 9 ml volumes into test tubes, which were capped and autoclaved. MRS broth tubes were refrigerated for storage. Prior to inoculation, MRS broth tubes were warmed to ambient temperature (20°C). Inoculation protocol varied slightly between the temperature optimization phase and the flour composition optimization phase of the project. During optimization phase, 7 tubes containing 9 ml sterile MRS broth were inoculated with 1.0 ml *L. coryniformis* previously grown in MRS broth for 24 h. These 7 inoculated tubes, plus one additional inoculated tube for culture propagation, were incubated anaerobically for 24 h ± 6 h at 37°C before being used to inoculate the teff batter. The 7 tubes (70 ml total volume) were vortexed and optical density was measured if turbidity appeared

anomalous. The remaining tube was used to propagate the culture by transferring 1.0 ml to a sterile tube of 9 ml MRS broth. During flour mix optimization phase, 8 tubes containing 9 ml sterile MRS broth were inoculated with 0.1 ml *L. coryniformis* previously grown in MRS broth for 24 h. These 8 inoculated tubes, plus one additional inoculated tube for culture propagation, were incubated anaerobically for $24 \text{ h} \pm 6 \text{ h}$ at 37°C . The 8 tubes (72.8 ml total volume) were vortexed and optical density was measured if turbidity appeared anomalous. The remaining tube was used to propagate the culture by transferring 0.1 ml to a sterile tube of 9 ml MRS broth. This minor adjustment was made to the procedure when it was noted that using 0.1 ml to inoculate the broth resulted in a higher final turbidity than when using a 1.0 ml inoculum (Table 2-1).

Table 2-1: Turbidity of MRS broth when inoculated with 0.1 and 1.0 ml *L. coryniformis*. Using a smaller inoculum volume resulted in a lower initial turbidity but a higher final turbidity compared to using a 1.0 ml inoculum. For this experiment, $n = 4$.

Inoculum volume ml	Initial Turbidity		Turbidity, 20 h incubation	
	Mean	SD	Mean	SD
1	0.38	0.0082	1.12	0.036
0.1	0.063	0.015	1.18	0.01

Bioreactor Setup

A Bioflow 3000 fermentation unit (New Brunswick, Edison, NJ) was connected to a computer running the BioCommand Plus (New Brunswick, Edison, NJ) software package. A 2.5 L fermentation vessel was used with all head plate ports permanently closed except for the inoculation port and the pH port. One propeller-type impeller was attached at the bottom of the drive shaft, and a second was attached 5 cm above. Water jacket hoses were connected to allow temperature regulation of the fermentation vessel, and cold water hoses were connected to the condenser to prevent moisture from escaping. A sterile air filter (Pall, East Hills, NY) was placed

at the outlet of the condenser. The pressure of the water supply to the fermentation unit was controlled to 18 psi by a backpressure regulator. The fermentation unit was set to fermentation mode. Figure 2-1 shows the bioreactor setup.

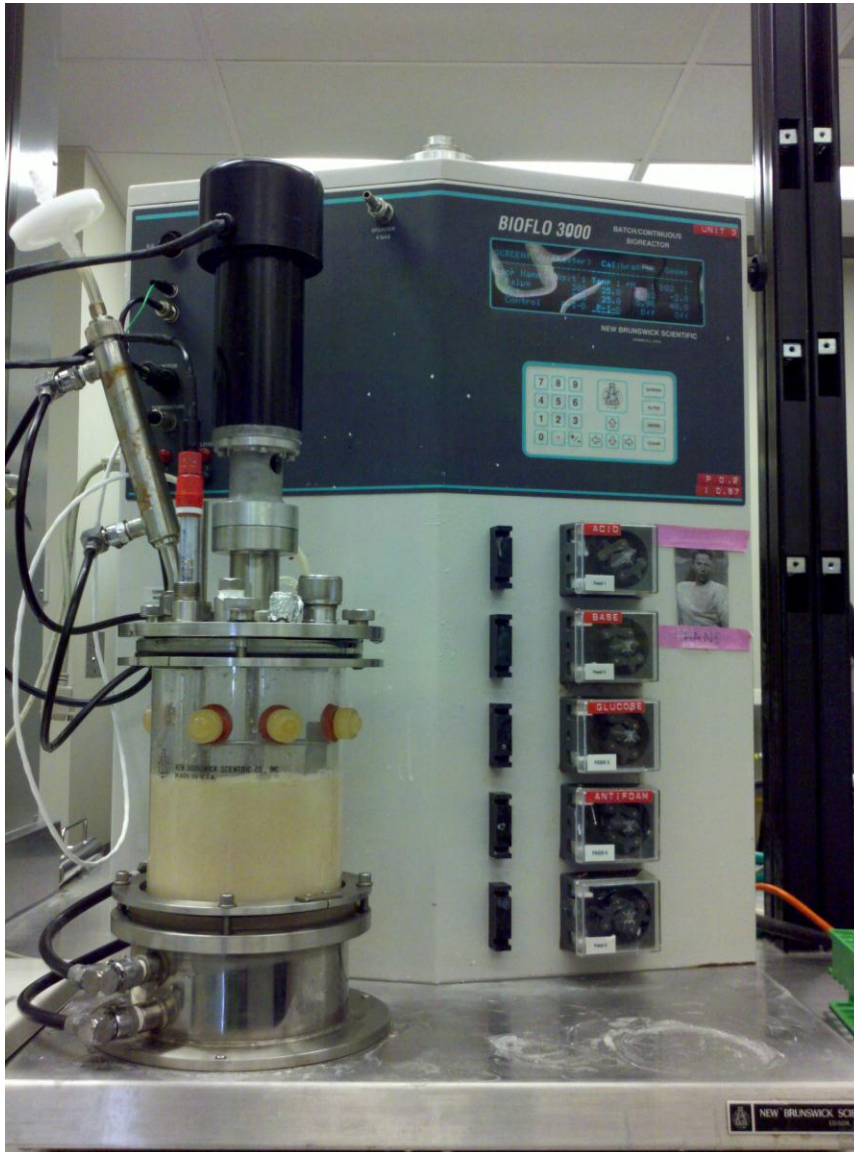


Figure 2-1: Setup of the operating bioreactor.

General Batch Preparation

The fermentation vessel was cleaned with deionized water, autoclaved at 121°C, 15 psig for 25 minutes, and cooled to ambient temperature. A pH probe (VWR, location) was sanitized with 70% ethanol prior to calibration with pH 4.0 and 7.0 standard buffer solutions. The pH probe was rinsed with deionized water prior to insertion through the pH port. Injera batter was prepared by gradually combining 541 g teff flour and 1.63 L deionized water (this equates to approximately 1 part flour to 2.25 parts liquid (v:v) when the 70 ml inoculum is included) in a sanitized flask. If a batch was to contain no starter culture, 1.7 L deionized water was used. The pH electrode was inserted only after the injera batter was added via the inoculation port. The BioCommand software was set to record pH, temperature, and agitation speed every 6 minutes. Temperature and agitation control were set on the fermentation unit. Time for the batch began when controls are initiated and starter culture is added. The pH was monitored electronically for up to 72 h fermentation time. Fermented batter was packaged in plastic freezer bags and frozen for later use by project collaborators as test batter for validating the baking process.

Design of Experiment for Optimization of Teff Fermentation

Injera batter was made from 100% ivory teff flour and deionized water. The inoculum consisted of 70-72 ml *L. coryniformis* grown in MRS broth for 24 h \pm 6 h at 37°C. Agitation speed of 300 rpm was selected based on trial and error experimentation (Table 2-2). With full agitation and 70-72 ml starter culture, two batches each were run while holding the temperature constant at 25, 28, 31, 34, 37, 40, and 43°C. Two control batches were operated at 300 rpm and 37°C, using 72 ml sterile MRS instead of starter culture. These two control batches were acidified to pH 5.8 with lactic acid. Still (non-agitated) fermentations cannot be temperature-

controlled simply using the water jacket, so 3 batches were uncontrolled (0 rpm, ambient temperature) but inoculated with 70 ml starter culture. A spontaneous (un-inoculated), still fermentation was conducted with no temperature control, no agitation, and no starter culture. A spontaneous, agitated fermentation was conducted at an agitation speed of 300 rpm, without temperature control, and without starter culture. Table 2-3 summarizes the batches in the teff fermentation optimization phase of the project.

Table 2-2: Determination of ideal agitation level by varying impeller speed until agitation prevents settling of flour particles.

ImpellerSpeed	Observations
0 RPM	No agitation. Visible settling of flour particles
100 RPM	Some turbulence in lower 1/2 of vessel, visible settling in unmixed top half of batter
200 RPM	Visible turbulence in lower 3/4 of vessel, top portion is not well mixed, visible settling of particles in the upper layer below surface
300 RPM	Visible turbulence, thorough mixing with no settling

Table 2-3: Design of teff optimization experiment.

Batch Number #	Temperature °C	Agitation rpm	Starter Volume mL
2	25	300	70
19	25	300	72
3	28	300	70
18	28	300	72
8	31	300	70
17	31	300	72
4	34	300	70
14	34	300	72
10	37	300	70
1	37	300	71
12	40	300	70
13	43	300	70
15	37	300	0
16	37	300	0
6	ambient	300	0
7	ambient	0	0
9	ambient	0	70
5	ambient	0	70
11	ambient	0	70

Design of Experiment for Flour Analysis

The composition of the flour portion of the injera batter was varied according to Table 2-4. All batches contain 541 g flour, 1.63 L deionized water, and 72 ml *L. coryniformis* starter culture previously incubated for 24 h \pm 6 h in MRS broth. Fermentations were operated at 37°C with agitation of 300 rpm.

Table 2-4: Design of experiment for flour composition analysis.

Batter Composition	Temperature °C	Agitation rpm	Starter Volume ml
100% Teff	37	300	72
100% Teff	37	300	72
100% Buckwheat	37	300	72
100% Buckwheat	37	300	72
100% Sorghum	37	300	72
100% Sorghum	37	300	72
50% Teff, 50% Buckwheat	37	300	72
50% Teff, 50% Buckwheat	37	300	72
50% Teff, 50% Sorghum	37	300	72
50% Teff, 50% Sorghum	37	300	72
33% Teff, 33% Buckwheat, 33% Sorghum	37	300	72

Chapter 3

Results and Discussion

Extraction of Fermentation Parameters

Raw data was exported from BioCommand software to Microsoft Excel (manufacturer, location), which was used to extract key parameters, which include lag phase duration (λ), maximum hourly rate of pH decline (μ), the change in pH over 22 h ($\Delta\text{pH}_{\text{max}}$), and the time to reach pH 3.8. The time to reach pH 3.6 and 3.5 was also determined. The absolute value of the minimum value of dpH/dt is μ_m , and dpH/dt was evaluated using the central difference method starting at $n = 5$:

$$\left(\frac{\text{dpH}}{\text{dt}}\right)_n = \frac{\text{pH}_{n+5} - \text{pH}_{n-5}}{t_{n+5} - t_{n-5}}$$

Lag phase duration (λ) was calculated by plotting ΔpH against time, where ΔpH is the difference between the maximum batter pH and the pH of the batter at time t . The value of λ is the t axis intercept of the best-fit tangent line through the inflection point. In theory, the value of μ_m may alternately be calculated as the slope of this same line. Sample calculations are shown in Appendix 1. These parameters are suitable inputs in the modified Gompertz equation (Zwietering et al, 1990):

$$y = A \exp \left\{ -\exp \left[\frac{\mu_m e}{A} (\lambda - t) + 1 \right] \right\}$$

The equation from Zwietering models a sigmoidal curve in which the dependent variable increases. While this equation was originally modified to model biomass concentration, it has also been used to model acidification during sourdough fermentations (Gaggiano et al, 2006). By

simply graphing pH against time, the dependent variable experiences a sigmoidal decrease over time. However, the data plot assumes the correct form when considering ΔpH (maximum pH minus current pH) as the dependent variable. Using the parameters relevant to this study, the modified Gompertz equation can be expressed as follows:

$$\Delta pH = \Delta pH_{max} \exp \left\{ -\exp \left[\frac{\mu_m e}{\Delta pH_{max}} (\lambda - t) + 1 \right] \right\}$$

The parameter ΔpH_{max} is theoretically the difference between the initial pH and the pH during the stationary phase. The modified Gompertz model will not account for pH changes beyond the time point associated with ΔpH_{max} . Because most fermentations in this study were operated for 22 to 24 h, ΔpH_{max} is defined here as the difference between the initial pH and the pH after 22 h of fermentation. Some fermentations were run far beyond 24 h, so for these cases the model is only useful for predicting the first 22 h. However, the purpose of including this model is to provide a context for the extracted parameters λ and μ_m ; at this stage the model will not be used to predict pH changes. While application of the model itself is not a focus of this study, the parameters relevant to the model were used to characterize the fermentation pH data and make comparisons between the outcomes of fermentations operated under different conditions.

In addition, each fermentation was characterized by the time required to achieve a defined pH value. Since there exist several varieties of injera based on acidity, it is practical to consider several final pH setpoints. In Gauche's study (1985), a pH of 3.8 was achieved after 72 h. Ashenafi (1994) measured irsho samples to have a pH of 3.5 with relative consistency. For this study, the times to achieve pH 3.8, 3.6, and 3.5 were recorded for each batch. As the exact final pH desired by Ethiopians is unknown, it is desirable to record multiple pH endpoints. However, for purposes of discussion, the time to reach pH 3.8 was considered the most relevant of these values.

Spontaneous Fermentations of Teff

Spontaneous fermentation refers to pH development achieved without inoculating the batch with starter culture. Several batches were prepared without starter cultures and allowed to ferment for 72 h via the action of microorganisms intrinsic to the flour. These fermentations were conducted at ambient temperatures with no temperature control, simulating the commercial scenario in which temperature control may be too expensive to perform. Two ambient temperature, spontaneous fermentations were prepared, one performed under 300 rpm of agitation and one performed without agitation (Figure 3-1). The pH time curves for both wild fermentations follow a similar pattern characterized by a lag in pH development, a slight decrease in pH, another lag in pH development, a more dramatic decrease in pH, and a final stationary phase. These biphasic pH time curves reflect the production of lactic acid, which is growth-associated. The curves suggest that a succession of organisms drives the wild fermentation of teff. Gashe (1985) studied the succession of organisms in a wild teff fermentation. The pH v. time plot generated from the Gashe data displays similar characteristics to both wild fermentations performed in this study. While both batches from this study started at approximately pH 6.2, the agitated batch achieved a final pH of 3.57 while the non-agitated batch only acidified to pH 3.96. In addition to achieving a lower final pH, the agitated fermentation experienced shorter lag periods. The Gashe pH curve appears to more closely simulate the 300 rpm curve instead of the non-agitated curve. This discrepancy is most likely due to the potentially high variability of wild fermentations. It is also possible that the Ethiopian teff flour used by Gashe contains different microorganisms than the Idaho teff flour used in this study, resulting in different fermentations. It is worth noting that the temperatures recorded for the agitated fermentation were approximately 5°C greater than the temperatures recorded for the non-agitated fermentation. As the room temperature did not change dramatically, the elevated batter

temperature in the agitated batch is likely caused by frictional heating as the impellers shear through the batter.

In order to optimize the fermentation of injera batter, the characteristics of the pH decline must be understood and controlled. Based on this brief assessment of wild fermentations, it is apparent that without the use of any form of starter culture, the pH decline is notably unpredictable. In all three spontaneous fermentations shown in Figure 3-1, the pH decline curve is a composite of two sigmoidal decreases in pH. The complex nature of these wild fermentations would be difficult to optimize industrially. Therefore it is sensible to use a starter culture to avoid the biphasic pH decline and to decrease the total acidification time.

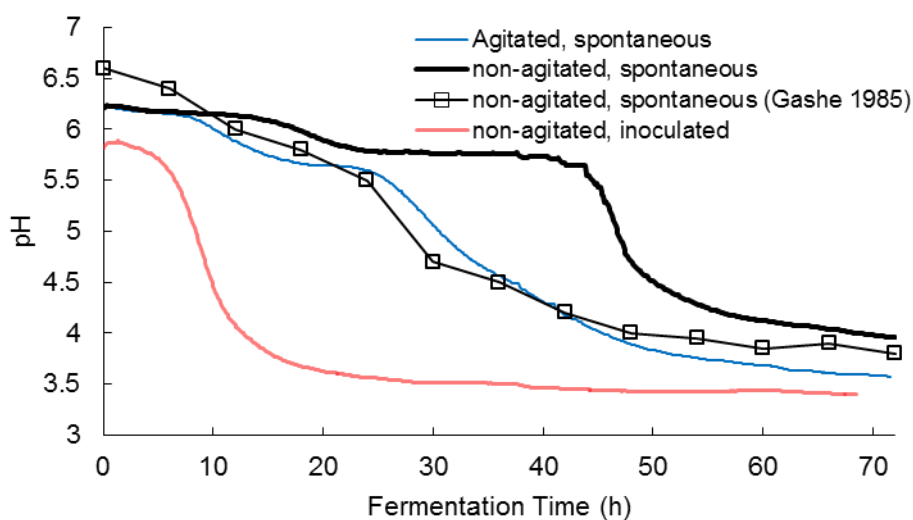


Figure 3-1: pH profiles for teff fermentations at ambient temperatures. Batter temperature of the non-agitated, spontaneous batch ranged from 20-23°C, while batter temperature of the agitated (300 rpm), spontaneous batch ranged from 25-28°C. Also shown is published pH data from the fermentation of Ethiopian teff flour (at 20-23°C) without addition of irsho or starter of any kind (Gashe, 1985). These spontaneous fermentations are contrasted with the non-agitated fermentation inoculated with *L. coryniformis*.

Non-agitated, ambient temperature fermentations using starter culture

Two teff batter fermentations were operated at ambient temperatures with no agitation. For starter culture, these batches were inoculated with *L. coryniformis*, which was previously incubated for $24 \text{ h} \pm 6 \text{ h}$ in MRS broth. While the ambient temperature ranged from 20°C to 23°C , batter temperatures during these uncontrolled fermentations were recorded in the range of 25 to 27°C . It is possible that the elevated batter temperature occurred due to heat production by microbial metabolism.

The pH profile for an ambient temperature, non-agitated, inoculated fermentation is displayed in Figure 3-1. Unlike the spontaneous fermentations, which exhibit biphasic pH profiles, the fermentations involve a lag phase leading into a dramatic decrease in pH, which levels off into a stationary phase. For both fermentations, the kinetics of ΔpH can be described by the modified Gompertz model. The lag phase duration (λ) was 5.55 h and 7.75 h. The maximum rate of pH development (μ_m) was 0.35 h^{-1} and 0.41 h^{-1} . There are significant advantages to using the *L. coryniformis* starter culture to initiate fermentation rather than using spontaneous fermentation. Using the starter culture produces a simple pH profile which, unlike the spontaneous fermentations, can be described using a well-established model. More practically, initiating the fermentation with the starter culture allowed the fermentation to be completed much faster, reaching pH 3.8 in 15.1 h and 17.5 h. By contrast, the spontaneous fermentation operated under identical conditions failed to reach pH 3.8, leveling off around pH 4.0 after approximately 65 h. Similarly, the ambient spontaneous teff fermentation in Gashe's (1985) study achieved pH 4.0 at 48 h and pH 3.8 at 72 h.

Yigzaw et al found that at ambient conditions, spontaneous teff batter fermentations reached pH 4.0 within 40 h, while fermentations initiated by backslopping achieved pH 4.0 within 20 h (2004). In the current study, at ambient temperature without agitation, teff batter

inoculated with *L. coryniformis* achieved a lower pH (3.8) in less time (15.1 – 17.5 h) when compared to Yigzaw's fermentation initiated by backslopping, which achieved pH 4.0 in slightly less than 20 h (2004). Comparing the two studies, it is apparent that batches inoculated by *L. coryniformis* starter culture achieve a similar pH profile and appear to acidify the batter in less time than batches inoculated with irsho. However, while these are separate studies performed under similar conditions, the inoculum volume and concentration probably do differ. In the Yigzaw study, backslopping was performed by using 5% of the previous batch. In the present study, the *L. coryniformis* inoculum was 2.8% (v/v) of the batter. Irsho contains *Lactobacilli* on the level of 10^7 to 10^9 CFU/ml (Heckner, 2011), and it is likely that the *L. coryniformis* inoculum in MRS exhibited a cell concentration on the level of 10^9 CFU/g. Therefore, both methods of inoculation likely involved adding similar cell loads, but adding a *L. coryniformis* monoculture reduced the pH in less time.

It was observed that these inoculated, non-agitated fermentations produced unique characteristics in the finished teff batter. While the unfermented batter was thin and watery, the batter fermented under these conditions was thick and ropey. The non-agitated batter acidified by wild fermentation did not demonstrate these properties, suggesting that the starter culture may be responsible for the thickness in the inoculated batter. The thick, ropey texture is most likely due to the production of exopolysaccharides (EPS) by the starter culture. In agitated fermentations using starter culture, the batters did not demonstrate these properties. However, allowing finished batter to settle for several hours after ceasing agitation led to the development of some ropey character. It is possible that the shear stress of agitation disrupts the EPS in some manner but does not inhibit EPS formation.

Optimum conditions for teff fermentation

A series of 12 batches examined the impact of temperature on the acidification kinetics of teff batter fermentations under agitated conditions. These batches were inoculated with a *L. coryniformis* starter culture previously grown in MRS broth for $24 \text{ h} \pm 6 \text{ h}$. All batches discussed in this section were agitated at a rate of 300 rpm. Table 3-1 provides a summary of the fermentation outcomes parameters, including λ , μ_{nb} , and $\Delta\text{pH}_{\text{max}}$, pH after 22 h, time to reach pH 3.8, time to reach pH 3.6, and time to reach pH 3.5. The initial pH of teff batter inoculated with $70 \pm 2 \text{ ml}$ *L. coryniformis* was 5.83 with a coefficient of variation of 1.69%.

When fermenting teff flour with *L. coryniformis* starter culture, the optimum conditions are 300 rpm and 37°C . At 37°C , the fermentation experiences the shortest lag period (λ values of 1.36 h and 2.33 h) and the steepest decrease in pH (μ_{m} values of 0.68 h^{-1} and 0.66 h^{-1}). At these conditions, the pH was reduced to 3.8 in the least time (within 5.7 h and 6.5 h). It also took less time to reach pH 3.6 (8.0 h and 8.3 h) and 3.5 (10.9 h and 10.6 h) at these conditions. In total, four runs were performed under these conditions, and the mean values for each parameter are summarized in Table 3-2.

Table 3-1: Results of teff fermentation temperature optimization. All batches were inoculated with 70 ± 2 ml *L. coryniformis* starter culture and agitation level was 300 rpm.

Temperature °C	λ h	μ 1/h	pH at 22h	Δ pHmax	t pH = 3.8 h	t pH = 3.6 h	t pH=3.5 h
25	4.13	0.38	3.60	2.29	14.1	21.5	31.2
25	3.50	0.33	3.59	2.30	13.6	21.4	28.7
28	3.02	0.51	3.51	2.56	10.6	15.6	22.1
28	3.94	0.45	3.49	2.34	11.3	15.8	20.7
31	3.83	0.49	3.43	2.35	10.0	13.2	16.8
31	4.28	0.52	3.45	2.36	10.4	13.7	18.5
34	3.30	0.56	3.43	2.38	8.6	11.3	14.5
34	2.31	0.59	3.41	2.36	7.1	9.9	13.1
37	1.36	0.68	3.40	2.49	5.7	8.0	10.9
37	2.33	0.66	3.38	2.43	6.5	8.3	10.6
40	7.73	0.73	4.16	1.56	--	--	--
43	7.02	0.70	3.93	1.76	--	--	--

Table 3-2: Summary of teff fermentation parameters at optimum conditions (n = 4). All batches were inoculated with 70 ± 2 ml *L. coryniformis* starter culture, agitation level was 300 rpm, and temperature was 37°C.

Parameter	Mean	%CV
λ	1.39	27.7
μ	0.67	2.25
Δ pH max	2.42	2.22
pH at 22h	3.39	0.28
time pH = 3.8	5.9	7.32
time pH = 3.6	8.1	2.75
time pH = 3.5	10.8	1.31

Introducing agitation to starter-initiated teff fermentations decreases the fermentation time, as defined by the time to reach pH 3.8. Compared to the ambient temperature, non-agitated, starter-initiated fermentation, the 25°C, agitated, starter-initiated fermentation reached pH 3.8 in 13.6-14.1 h as opposed to 15.1-17.5 h.

As temperature increases, the fermentation times become shorter, as measured by the time required to decrease the pH to 3.8 (Figure 3-2). Beyond 37°C, the fermentation process failed to reach pH 3.8 within 22 h of fermentation time.

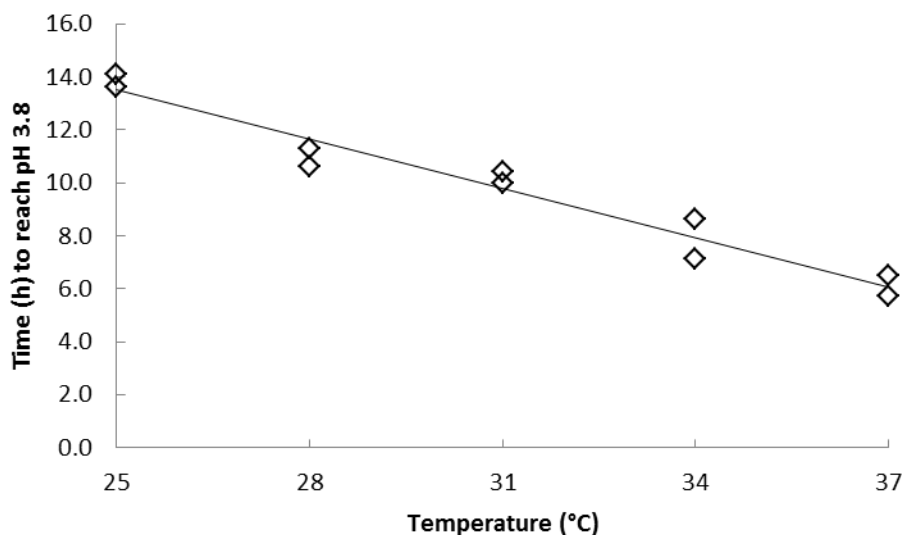


Figure 3-2: Effect of temperature on the time to reach pH 3.8 during fermentations initiated by *L. coryniformis* starter culture and operated with agitation of 300 rpm. A coefficient of determination of 0.9524 was obtained for the correlation.

With increasing temperature, μ_m increases. Figure 3-3 shows the strong correlation between temperature and μ_m , the maximum rate of pH change per hour. Batches performed at higher temperatures experienced sharper declines in pH. This trend agrees with the observations of Yigzaw et al (2004), who found that teff fermentations performed at 35°C exhibited steeper pH drops than teff fermentations performed at room temperature. There was considerable variation in the lag parameter, λ . No correlation found between temperature and λ . However, unusually high values of λ indicate irregular fermentations.

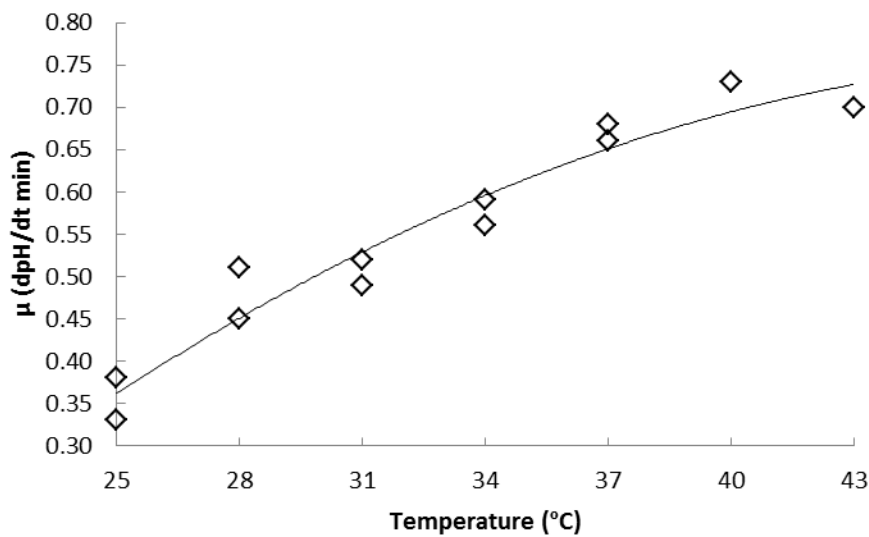


Figure 3-3: Effect of temperature on μ_m during fermentations initiated by *L. coryniformis* starter culture and operated with agitation of 300 rpm. A coefficient of determination of 0.9406 was obtained for the correlation.

Batches controlled at 40°C and 43°C were not duplicated. The batters fermented under these conditions were characterized by foul odors inconsistent with typical injera batter. It was deemed inappropriate to utilize more resources to replicate injera batters that would be quite unacceptable to consumers. Both the 40°C and 43°C batches experienced abnormally long lag phases (λ values of 7.73 h and 7.02 h respectively) during which the pH actually increased noticeably (Figure 3-4). When the pH did drop, it did so quite rapidly (μ_m values of 0.73 and 0.70 respectively). It is likely that these high temperatures are harmful to the *L. coryniformis* starter culture, as the high temperature pH profiles are quite different from the pH profiles at lower temperatures. Two control batches were performed to assess the impact of the starter culture on the fermentation process (Appendix 1). The control batches subjected teff to 37°C and 300 rpm. A sterile “inoculum” of 70 ± 2 ml sterile MRS broth was added, and the batter was acidified to the typical start pH of 5.8 using lactic acid. The pH profile for the un-inoculated control batch

exhibits the same characteristics as the pH profiles for the inoculated batches operated at 40°C and 43°C, suggesting that the starter cultures did not function at the high temperatures.

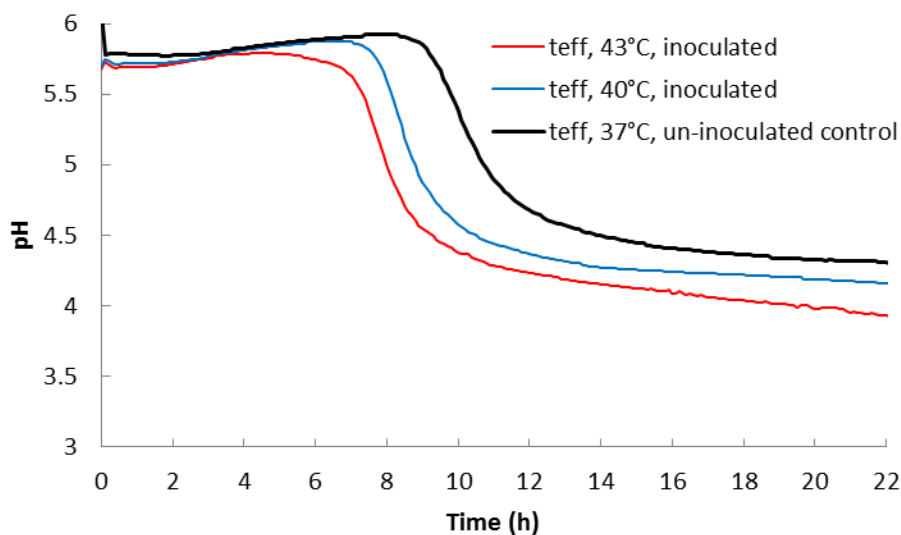


Figure 3-4: Irregular pH profiles for teff fermentations operated at high temperatures (40°C and 43°C) and without starter culture (control). Agitation for all three fermentations is 300 rpm. Control batch contains sterile MRS broth instead of cultured broth, and batter is acidified with lactic acid to the starting pH of 5.8.

Analysis of fermentation of teff, sorghum, and buckwheat (and their mixtures)

Teff, buckwheat, and sorghum flours were fermented using *L. coryniformis* starter culture. The fermentations were performed under the optimum conditions determined for teff (300 rpm agitation, 37°C). The fermentations were monitored continuously for 22h. Regardless of flour composition, each fermentation produced a pH profile consistent with the modified Gompertz equation. The fermentation parameters, including λ , μ_m , and $\Delta\text{pH}_{\text{max}}$, pH at 22 h, and time to reach pH 3.8 were calculated and summarized in Table 3.3. There is no apparent correlation between the flour composition and the parameter λ , the lag phase duration. There is

minimal correlation between flour composition and μ_m , although it is noteworthy that 100% sorghum flour experienced slightly lower values of μ_m . Yet while the pH of the sorghum fermentations declined at a slightly slower rate (0.46 h^{-1} and 0.53 h^{-1}), a pH of 3.8 was still achieved within 6.2 to 7.2 h, which is 5% - 22% longer than the average optimum teff fermentation.

Table 3-3: Fermentation parameter results of flour composition optimization. All batches were inoculated with $70 \pm 2 \text{ ml}$ *L. coryniformis* starter culture, agitated at 300 rpm, and maintained at 37°C .

Dough Composition	λ h	μ 1/h	ΔpHmax	pH at 22h	t pH = 3.8 h
100% Teff	1.77	0.65	2.36	3.39	5.9
100% Teff	1.35	0.68	2.41	3.4	5.5
100% Sorghum	2.21	0.46	2.15	3.44	7.2
100% Sorghum	1.45	0.53	2.23	3.45	6.2
100% Buckwheat	2.13	0.62	1.96	3.86	--
100% Buckwheat	1.39	0.72	2.02	3.83	--
50% Teff, 50% Sorghum	2.09	0.59	2.29	3.38	6.7
50% Teff, 50% Sorghum	1.22	0.68	2.41	3.33	5.1
50% Teff, 50% Buckwheat	4.03	0.66	2.2	3.56	8.5
50% Teff, 50% Buckwheat	2.13	0.65	2.18	3.58	6.7
33% Teff, 33% Buckwheat, 33% Sorghum	2.31	0.58	2.24	3.42	6.9

The E-Chip software package was used to fit a linear surface regression to the data. A contour plot was generated showing the response of fermentation time (time to reach pH 3.8) to the flour composition (Figure 3-5). The 100% buckwheat fermentations did not achieve pH 3.8 within 22 h. For the purposes of plotting, these buckwheat fermentations were said to reach pH 3.8 at a time of 22 h. Increasing the buckwheat content of the flour mixture dramatically increases the time required to acidify the batter to pH 3.8. To minimize fermentation time, the flour should not contain buckwheat. Batters made from mostly or entirely teff flour minimize the fermentation time.

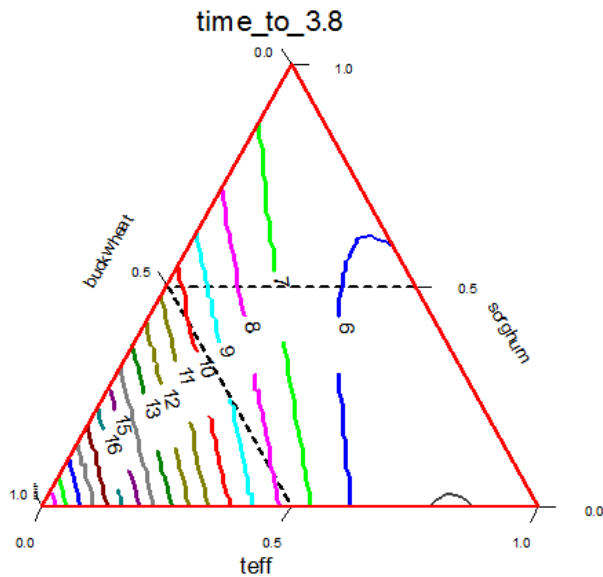


Figure 3-5: Contour plot showing the effect of flour composition on the time to reach pH 3.8 during fermentations initiated by *L. coryniformis* starter culture and operated at 37°C with agitation of 300 rpm.

A similar response surface was generated to illustrate the relation of pH at 22 h to the flour composition (Figure 3-6). The pH at 22 h is directly related to $\Delta\text{pH}_{\text{max}}$, which is the difference between the pH at 0 h and 22 h (the initial pH was 5.74 with a 1.37% coefficient of variation). The lowest final pH was achieved in batters composed of 100% teff or a mixture of teff and sorghum.

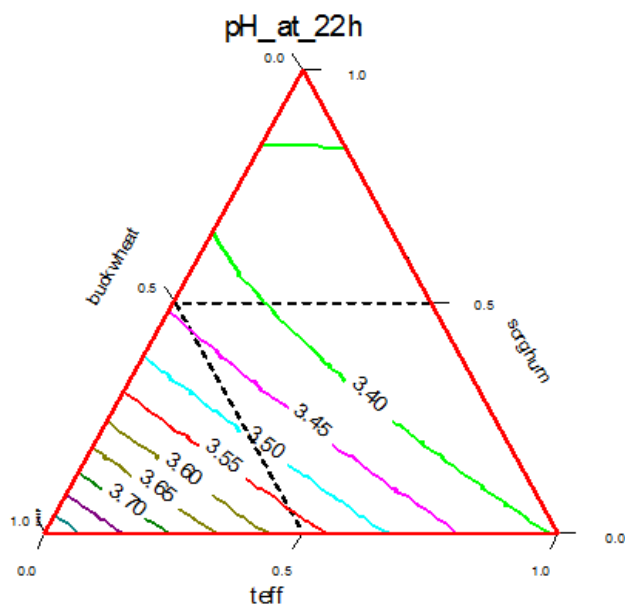


Figure 3-6: Contour plot showing the effect of flour composition on the pH after 22h (which is related to $\Delta\text{pH}_{\text{max}}$). Fermentations were initiated by *L. coryniformis* starter culture and operated at 37°C with agitation of 300 rpm.

Based on the flour composition optimization, it appears that while batters made from 100% teff ferment in the least time, composing teff and sorghum flours does not vastly hinder the fermentation. By contrast, composing teff with buckwheat flour dramatically increases the fermentation time as the buckwheat content increases. The pH profiles of fermentations involving buckwheat are shown in Figure 3-7. During the first five hours, all three pH profiles exhibit remarkably similar characteristics. However, after 5 h the 100% buckwheat fermentation appears to abruptly stop. The 50% buckwheat-50% teff batter fermented to a final pH less than the 100% buckwheat but greater than the 100% teff batter. The buckwheat flour may contain higher concentrations of polyphenolic compounds, which perhaps have an inhibitory effect on the fermentation at low pH. However, since the first 5 h of fermentation were roughly identical, it is more likely that the starter culture exhausted the fermentable substrate in the 100% buckwheat flour. It is important to note that this study used a dark variety of buckwheat and a light variety

of teff (and a light variety of sorghum). Fermentations involving lighter varieties of buckwheat may not display these pH profile characteristics.

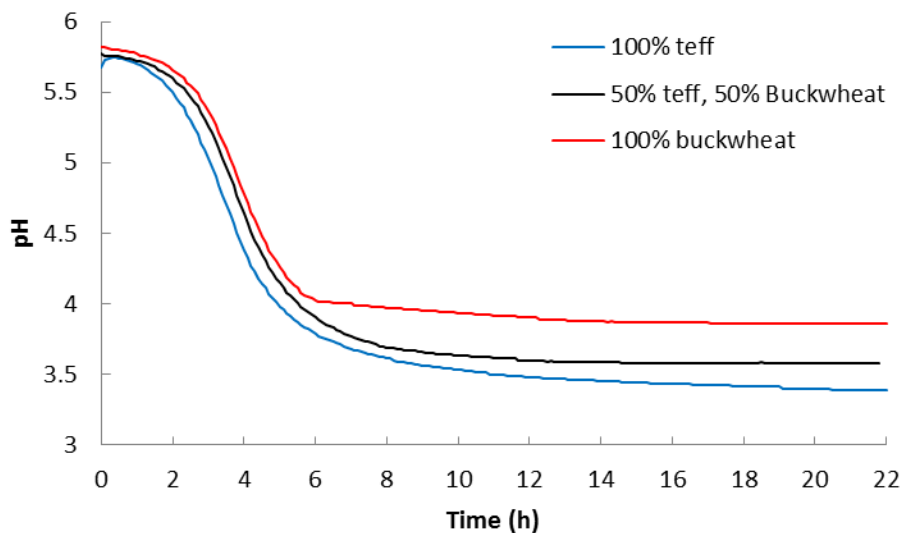


Figure 3-7: pH profiles for fermentations of 100% buckwheat flour, 50% buckwheat-50% teff flour, and 100% teff flour. Fermentations were initiated by *L. coryniformis* starter culture and operated at 37°C with agitation of 300 rpm.

Possible effects of starter culture growth phase

The lag phase parameter λ varies considerably more than any other process parameter. The value of λ does not appear to be influenced by the process conditions. However, a cursory glance of Table 3-3 reveals that between identical batch pairs, the batch with the smaller λ achieves pH 3.8 in less time. Indeed, it makes kinetic sense that batches reach the pH endpoint sooner if the microorganisms experience a shorter lag phase (as long as μ_m is similar). The lag phase for pH development is likely related to the lag phase for the starter culture. The lag period for the culture occurs because the organism must transition from its current growth phase and potentially change metabolic pathways to adjust to the different substrate. The age of the starter culture can be assessed based on the degree of settling that occurred in the test tube. Cultures

incubated for significantly less than 24 h tended to be fully turbid and relatively unsettled, while cultures incubated for 24 h or more were characterized by a thicker layer of biomass settled on the bottom. Older cultures that were relatively settled were potentially in stationary phase, while younger cultures that were relatively unsettled (but still approximately the same optical density when vortexed) were potentially still in exponential growth. It was noted that batches initiated by younger starter culture (incubated for less than 24 h and exhibiting the previously mentioned traits) experienced lower values of λ . It stands to reason that starter cultures in the exponential growth phase would experience a shorter lag phase when transferred to the injera batter, even though some lag phase would undoubtedly be required as the organism adjusts to the substrate.

Chapter 4

Conclusion and Recommendations

Preparing the Ethiopian staple bread known as injera involves a primary fermentation that traditionally lasts 30-72 h. For commercial production of injera, it is desirable to develop the bread's characteristic acidic flavor in a significantly reduced time frame. This study utilized a *L. coryniformis* strain previously isolated from irsho to drive the fermentation. In addition to pioneering the use of this unique starter culture, this study optimized the fermentation conditions, including temperature and agitation, to minimize the time required to acidify teff injera batter to a pH of 3.8. Using optimum conditions of 300 rpm agitation speed, temperature control at 37°C, and a starter inoculum volume of 70 ± 2 ml, the pH was reduced to 3.8 within an average of 5.9 h.

The Δ pH profiles were analyzed to extract parameters (λ , μ_{m} , and $\Delta\text{pH}_{\text{max}}$) relevant to the modified Gompertz equation. While λ could not be correlated with any parameters, μ_{m} was found to increase as fermentation temperature increases.

Ambient, spontaneous teff fermentations experienced the most irregular pH profiles. Introducing the starter culture simplified the pH profile and decreased the fermentation time to 15.1-17.5 h for ambient, non-agitated teff fermentations. Under similar conditions, the starter-initiated fermentation achieved the desired pH endpoint in less time than reported in the literature for traditional, backslopped teff fermentations. Therefore it is recommended that commercial-scale teff fermentations be initiated by starter culture.

Operating starter-initiated teff fermentations under agitation further decreases the fermentation time. Controlling the temperature allows further minimization of the fermentation time. As would be expected from Arrhenius kinetics, fermentation time decreases with

increasing temperature, reaching a minimum at 37°C. At temperatures 40°C and 43°C, the pH profile changes dramatically and resembles the un-inoculated control, suggesting that the starter culture was unable to drive the fermentation at these conditions.

The *L. coryniformis* used as a starter culture in this study was grown in MRS broth. While this growth media was appropriate within the context of laboratory experiments, it will be necessary to develop a growth medium suitable for commercial food production. Ideally, a suitable growth medium would be inexpensive and provide the nutrition required for *L. coryniformis* to grow rapidly. The nutrient profile should be similar to that of teff batter in order to minimize the lag period as the microorganism adjusts to the new environment. Perhaps the growth medium could be derived from teff flour.

Further microbiological research could explore the persistence of *L. coryniformis* during fermentation. As this organism was previously isolated from irsho (Heckner, 2011), it is likely that the organism is responsible for the acidification of the batter. However, there exists the possibility that metabolites produced by the starter culture served as a substrate for another organism.

The flour optimization found that while pure teff flour ferments in the least time, cutting the teff with some sorghum does not dramatically impact the fermentation time or pH profile. Sorghum flour is one of the more common alternatives to teff for injera making. Sorghum injera is relatively common in parts of Ethiopia, and sorghum flour is less expensive than teff. Therefore, when scaling up teff fermentation, using a mixture of teff and sorghum allows for a degree of cost savings. In order to determine the flour composition for commercial production, the properties of the finished injera should be evaluated. The shelf life of the finished product should also be considered. Teff injera has the longest shelf life, followed by sorghum, so compositing the two flours should produce a product with a reasonable shelf life.

Sensory evaluation is the most important factor that should be considered during scale-up. While fermentation time and cost may be minimized with certain temperature, agitation, and starter culture usage, the finished injera must be desirable to the customer. When considering any recommendations to decrease fermentation time or cost, it is critical to assess the impact that these process modifications will have on the product. For example, the shorter fermentation process may affect the quality of the flavor. The long fermentation period typical of traditional injera production may allow for flavor development beyond the simple acid flavor.

To scale up the fermentation of injera batter to the commercial level, it is important to consider production capacity. The size and number of fermentation vessels will depend on the desired daily production of finished injera. For commercial fermentation, several smaller vessels should be used instead of one large vessel to lessen the impact of a failed fermentation.

Based on this study, the fermentation can be accomplished in 6 h if agitation and temperature controls are utilized. The agitation speed will need to be optimized for the industrial fermentation vessel, and the vessel design could be optimized to minimize the agitation speed required to maintain a well-mixed state. This study used a bioreactor to ferment teff. Scaling up the vessel and agitation does not necessarily imply utilizing an industrial bioreactor. An ideal design might involve a wide cylindrical vessel with scraper flaps that rotate on the bottom surface to lift settling teff back into suspension.

A well-mixed state is desirable for temperature control. In absence of agitation, temperature control becomes difficult, as the settled teff becomes a barrier to heat transfer. Depending on the heating control feedback, the slow response of the teff batter to heating may result in overheating and gelatinization of a layer adjacent to the hot steel. This layer presents a barrier to heat transfer as well. Attempting temperature control without agitation is not recommended at this time.

Fermentation is only the first stage in the injera making process. After fermentation, the irsho is typically removed, as excess water will make the batter too thin. If the fermentation uses agitation, a new step will be required to separate the fermented teff from the unwanted extra water. Simply allowing the batter to settle and pouring off the irsho may require more time than the entire fermentation process. Evaporation may result in undesirable over gelatinization of starch. Therefore, a filtration step may be the best option.

Chapter 5

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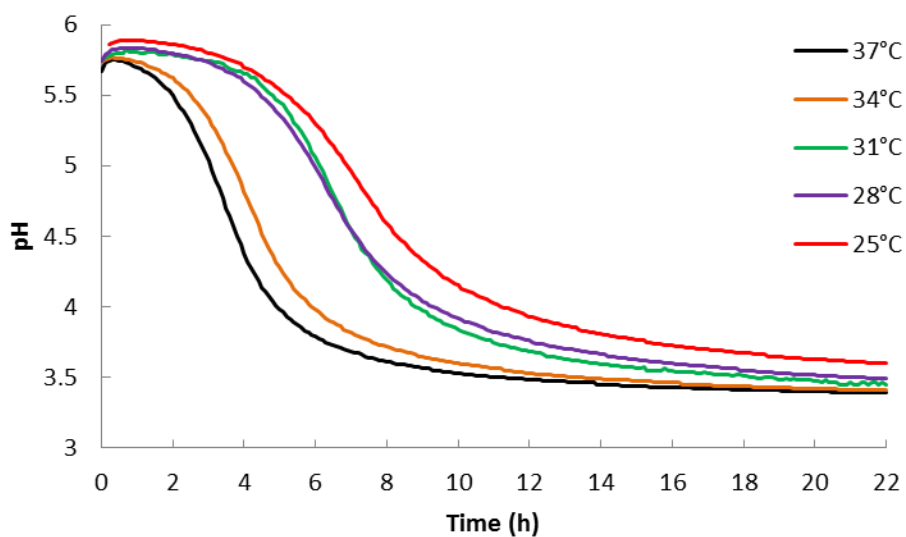
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Appendices

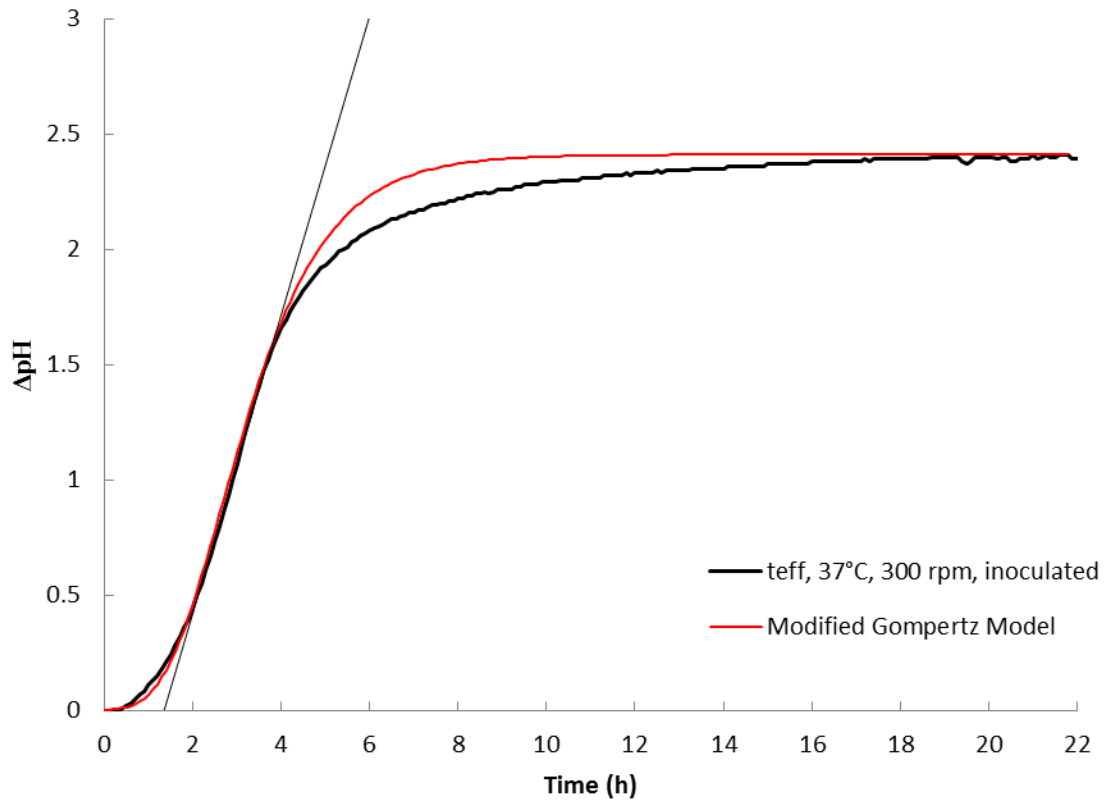
Additional Figures and Tables

Appendix 1: Fermentation parameter results of ambient, non-agitated fermentations of teff using *L. coryniformis* starter. Also shown are results of control fermentations (*) operated at optimal conditions (37°C, 300 rpm), with 70 ± 2 ml sterile MRS broth (no starter culture) and acidified to pH 5.8 with lactic acid.

Temperature °C	Agitation rpm	λ h	μ 1/h	pH at 22h	Δ pHmax	t pH = 3.8 h	t pH = 3.6 h	t pH=3.5 h
ambient (20-26°C)	0	5.55	0.35	3.59	2.28	15.1	21.3	35
ambient (23-27°C)	0	7.75	0.41	3.63	2.21	17.5	23.4	31.3
*37	300	9.27	0.57	4.31	1.48	--	--	--
*37	300	9.27	0.59	4.34	1.47	--	--	--



Appendix 2: pH profiles for teff batter fermentations operated at various constant temperatures. All batches were initiated by *L. coryniformis* starter culture and operated with agitation of 300 rpm.



Appendix 3: ΔpH (initial pH – pH) profile for teff batter fermented under optimum conditions (300 rpm, 37°C) using *L. coryniformis* starter culture. μ_m is the maximum value of $|\text{dpH}/\text{dt}|$ or the slope of the linear regression through the steepest part of the ΔpH curve. λ is the time axis intercept of the trend line. $\Delta\text{pH}_{\text{max}}$ is the maximum value of the ΔpH curve, or the pH at 22 h. These parameters are relevant to the modified Gompertz model, which fits the data especially well early in the fermentation process.

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- Worked on an independent project under the mentorship of Dr. Tina Jeoh
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