Creation of Riboflavin Enhanced Probiotic Apple Juice

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Objective and Background

Goal: To create a riboflavin-enriched probiotic beverage from apple juice fermented with *Lactobacillus plantarum*

Objectives:
- Create a new probiotic beverage that has additional nutritional values
- Determine fermentation conditions that give optimal riboflavin concentration in the juice

Background:
- Fermented food and drinks are growing in popularity
- Consumer trends in the food industry are focused on well-being, naturalness, convenience and innovation

Ethical, Global, and Societal Considerations

Ethical:
- Quality of the Product
- Safety of the Product
- Packaging and Branding

Global:
- Global Market Demands
- Cultural Differences
- Legal and Regulatory Barriers
- Global Access
- Economic Feasibility

Societal:
- Recyclability

Impact

Public Health Impact
- Polyphenols in Apple Juice
- Women's Health
- Riboflavin Deficiency
- Mental Health

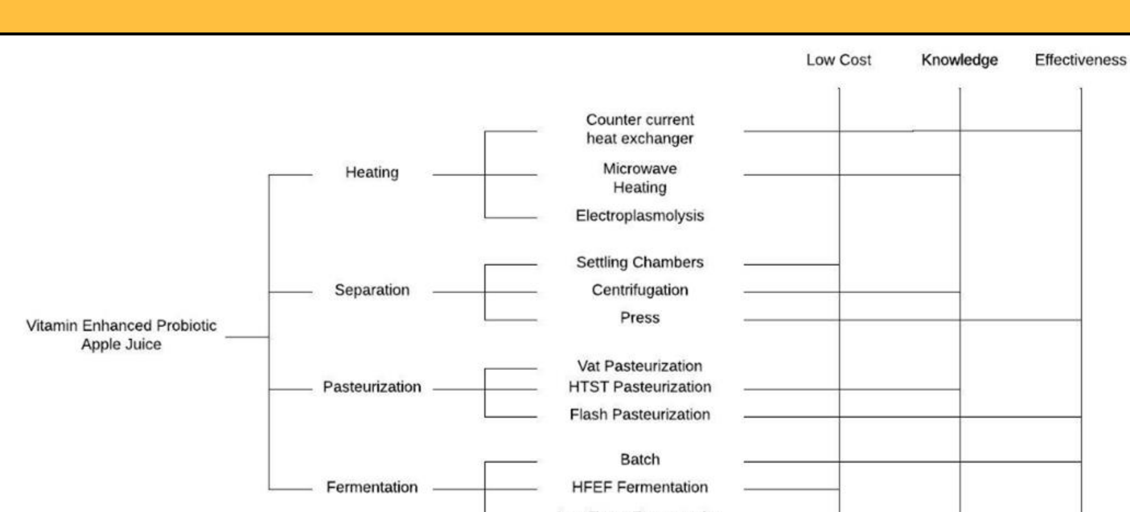
Market Analysis

- Projected $200,000,000 dollar market for probiotic consumable goods
- In 2018 , there was a 2% growth rate
- Apple Juice is part of the largest growth share, with 88.8%

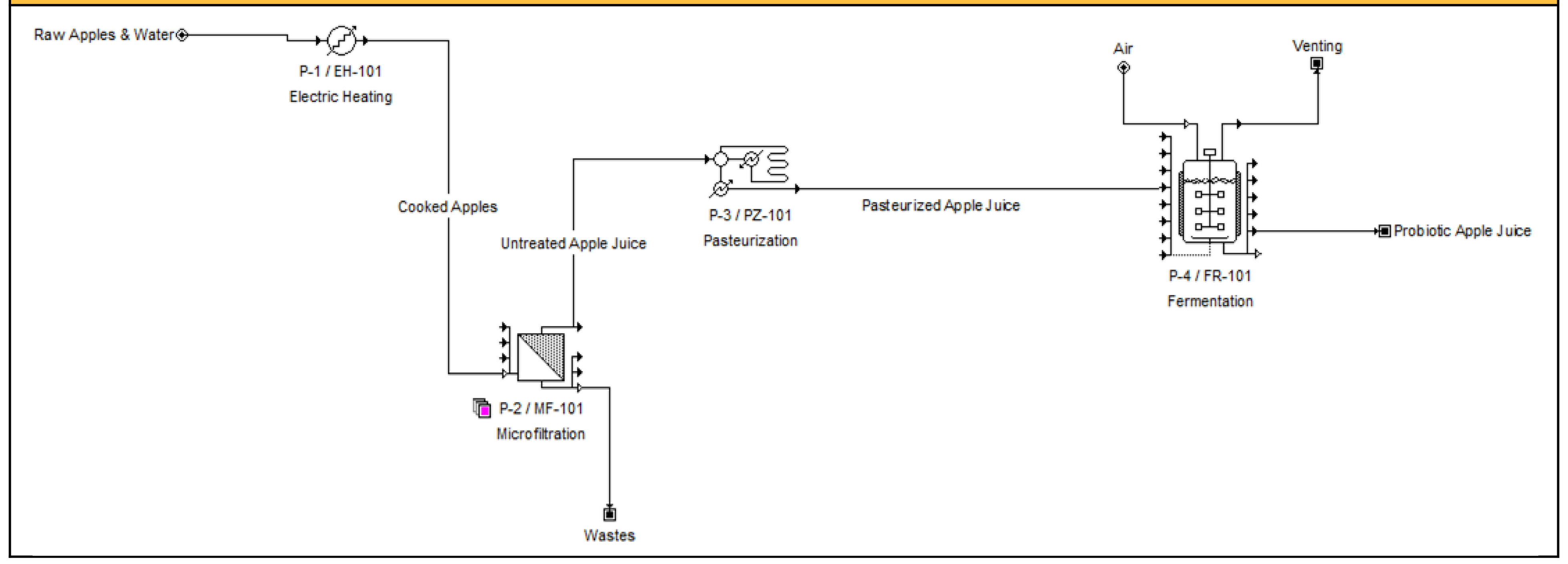
Unit Operations

Unit Operation	Optimization Variable
Heating & Pasteurization	Exit Temperature of the Utility Fluid & Heat Exchanger Area
Separation	Belt Speed
Fermentation	Tank Size

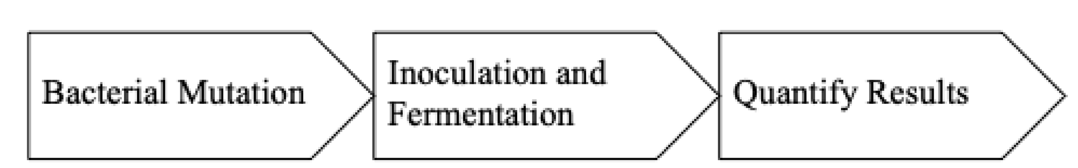
Evaluations of Alternatives



Process Flow



Experiment



Bacterial Mutation

1. *L. plantarum* was grown in Riboflavin Assay Medium (RAM) and transferred into fresh medium every day for two weeks.
2. After the 12th sequential transfer, liquid bacterial culture was transferred to an agar plate containing 50 mg roseoflavin/L and incubated at 37˚C for 48 hours
3. Colonies were picked from the plate containing 50 mg roseoflavin/L and transferred to another plate containing 100 mg roseoflavin/L. The plate was incubated at 37˚C for 48 hours.
4. Step 3 was repeated one more time. Colonies were picked and transferred to another plate containing 100 mg roseoflavin/L. Again, the bacteria were kept at 37˚C for 48 hours.
5. Colonies from the last 100 mg roseoflavin/L plate were picked and added to 25 mL RAM. The bacterial culture was incubated at 37˚C for 24 or 48 hours, before being inoculated into the apple juice.

Experimental Design

Four variables were tested in the creation of the apple juice:

1. Fermentation Time: Amount of time the bacteria were allowed to ferment in the apple juice
2. Volume of Inoculum: Amount of bacterial culture added to the apple juice
3. Age of Liquid Culture: Amount of time the bacteria were allowed to grow in Riboflavin Assay Medium before being inoculated into the apple juice
1. Fermentation Temperature: The temperature that the fermentations occurred at

Variables	Values
Fermentation time	1. 24 hours 2. 48 hours 3. 72 hours
Volume of inoculum	1. 200 uL/200 mL apple juice (0.001% v/v) 2. 2000 uL/200 mL apple juice (0.01% v/v)
Age of liquid culture	1. 24 hours 2. 48 hours
Fermentation temperature	1. Room Temperature 2. 37°C

Results

Fermentation conditions that result in higher riboflavin concentration in the juice:
- Volume of inoculum: 2000 uL / 200 mL juice
- Age of liquid culture: 48 hour culture
- Bacterial strains: The mutant strains produce more riboflavin at 48 hours of fermentation, but the wild type strains produce more at 72 hours of fermentation
- Fermentation temperature: room temperature
- Fermentation Time: The riboflavin concentration increases over fermentation time for some samples but decreases over time for some others.

Hypothesis: Bacteria might have used the riboflavin during the fermentation process. They were both producing and consuming riboflavin.

Combination of fermentation conditions that produced the highest riboflavin concentration (1260 ug/L): mutants, 24 hours culture, 37°C, 2000 uL culture / 200 mL juice

Riboflavin concentration in normal apple juice: 525 ug/L

Economic Analysis

The plant will produce 202,699 kg product/ year. The product will be sold at $4.00/ 16 fl oz. This gives a sale rate of $1,789,836.65/ year.

Manufacturing Costs:		
	Direct Production Costs:	$924,056.36
	Fixed Charges (including dep):	$83,022.21
General Expenses:		$256,375.53
Total Capital Investment:		$1,082,599.10
Total Product Cost:		$1,281,877.68
Cash Flow:		$445,407.63
Return of Investment:		42.73%

Assessment and Future Recommendations

Assessment: The use of mutated *L. plantarum* was able to increase the amount of riboflavin in the apple juice. The combination of fermentation conditions that produced the highest riboflavin concentration was: mutant strains, 24 hours culture, 37°C, 2000 uL culture / 200 mL juice. These fermentation conditions result in a probiotic apple juice that has a riboflavin concentration that is 2.4 times higher than that in normal apple juice.

Future Recommendations:
- Optimize HPLC method to accurately measure the riboflavin concentration in the juice
- Measure the concentration of samples at 24 hours of fermentation, and have more replicates to verify under which fermentation conditions do the bacteria consume more riboflavin than producing it
- Test more variables to optimize fermentation conditions.
- Develop other novel vitamin bio-enriched foods using the same biotechnology strategy

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