

Fermentation of coffee beans with common brewing/winemaking yeast strains for novel flavor
properties

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ABSTRACT

Fermentation is a critical step in the production of coffee when following standard wet processing. Wet processing is one of the most common methods used to remove the mucilage layer from coffee cherries. During this step, the de-pulped coffee cherries undergo a fermentation with native yeast that modifies the aroma and flavor profile of the resultant coffee. This study aimed to ferment green coffee beans using commercial yeast strains used to produce specific flavor properties in beer and wine making, and subsequently evaluate the aroma and flavor of the coffee with human sensory panelists. Five *Saccharomyces cerevisiae* strains were used: Belgian Ale, *Toluraspora delbrueckii*, Sourvisiae, 71 B, and Tropical IPA, alongside one non-inoculated control sample. The green coffee beans underwent a controlled wet fermentation for 72 hours. Results showed that flavor profiles varied broadly by yeast strain, suggesting that producing novel flavors in coffee through fermentation is possible. However, higher liking scores were still reported for the control sample compared to the fermented samples. *Toluraspora Delbrueckii* resulted in coffee with highly fruity notes, and all strains were more floral than the control, while the sample fermented with Sourvisiae yeast common in the brewing of sour ales resulted in coffee that was both perceived as sour and had the lowest pH, likely due to the degree of lactic acid this strain is designed to produce. Further, there were significant color differences between the samples. In conclusion, fermenting green coffee beans with brewing and winemaking yeast strains strongly impacted the flavor and aroma of the resultant coffee, however optimizing the strains selected for fermentation may yield flavor profiles more suitable for coffee.

BIOGRAPHICAL SKETCH

Natalia Calderon graduated from Universidad del Valle de Guatemala with a Bachelor's Degree in Nutrition in 2020. She worked in clinical nutrition for two and a half years and then started her professional Master's Degree in Food Science at Cornell University in August 2022 and graduated in May 2023.

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1. Introduction

Coffee is one of the most important beverages worldwide. Coffee plantations are in over 50 developing countries (De Melo *et al.* 2014), allowing coffee to play an important economic role worldwide. In December 2022, the worldwide exportation of coffee was 10.88 million bags, of which one bag consists, of 60 kg with an average price of 157.73 US cents/ lb (International Coffee Organization, 2022). The biggest coffee producers in the world include Brazil, Vietnam, Colombia, and Indonesia (Voora *et al.* 2019). Coffee is of various species; the two most common are *Coffee canefora* (Robusta) and *Coffee arabica* (Arabica) (Ruta and Farcasanu, 2021 & Konieczka *et al.*2020). The altitude and temperature where coffee is grown, and its harvesting method also can affect the sensory profiles of the resultant coffee (Konieczka *et al.*2020 & Pereira *et al.* 2021). Robusta coffee has a bitter taste and more prominent mouthfeel, where in contrast, Arabica is more acidic and has a higher presence of aromatics (Abubakar *et al.* 2020).

The coffee cherry has different layers. The outer layer is the pericarp, which has three layers: the exocarp, known as the pulp; the mesocarp, mucilage; and the endocarp, the parchment. After the pericarp, the perisperm is found, and, lastly, the coffee seed is called the endosperm (Ruta and Farcasanu, 2021 & Klingel *et al.* 2020). A crucial element in coffee production is the coffee cherry's natural fermentation which occurs when the mucilage is degraded (Ruta and Farcasanu, 2021). This step permits the development of flavor and aroma compounds that make the product unique, when removing the mucilage from the coffee parchment (Haile and Hee, 2019). After the coffee fruit has been harvested, it needs to be processed. Commonly there are three coffee processing methods: wet, dry, and semi-dry. The dry method, which is the oldest, involves drying and fermenting the ripe coffee cherries in the sun. After ripening, the beans are washed and laid

on the ground for between 10 to 25 days until they reach the desired humidity of 11-12% (Ruta and Farahani, 2021). The fruit ferments and produces a sweet, smooth, and complex body during this time. This method is widely used in Arabica, where it is estimated that 95% of production utilizes this process to obtain high-quality coffee (Silva *et al.* 2008). In the semi-dry method, the coffee cherry is de-pulped, separating the mesocarp and the mucilage from the rest of the fruit, and is left in the sun until reaching the same desired humidity (Ruta and Farcasanu, 2021). Lastly, in the wet process, the pulp is removed from the cherry, then submerged in water for 24-72 hours then dried (De Melo *et al.* 2014).

During wet fermentation, mucilage degradation is caused by microorganisms that produce enzymes (Pereira *et al.* 2020), such as protease and cellulose, that degrade the layer. In this process microbial metabolites and organic compounds are produced that migrate inside the bean modifying the aroma and taste of the final product (Elhalis *et al.* 2021). These compounds include ketones, alcohols, aldehydes, acids, esters, and furans (Elhalis *et al.* 2021 & Reis *et al.* 2014). The uncontrolled natural coffee fermentation occurring in the coffee cherry is caused by bacteria such as *Lactobacillus*, *Bacillus*, *Arthrobacter*, *Acinetobacter*, and *Escherichia* (Elhalis *et al.* 2021 & Reis *et al.* 2014), and yeasts such as *Saccharomyces cerevices*, *Saccharomyces bayanus*, *Cryptococcus*, *P.anomala*, *Pichia kluyveri*, and *Hanseniaspora uvarum* (Elhalis *et al.* 2021 & Reis *et al.* 2015).

Recently coffee producers have discussed using starter cultures to enhance, accelerate, and control the fermentation of single or multiple strains (Reis *et al.* 2014; Batista *et al.* 2020). Depending on the starter culture, the acidity of the coffee may be affected, for example starter cultures such as *Erwinia*, *Klebsiella*, *Aerobacter*, *Escherichia*, and *Bacillus* can reduce the pH from 5.5-6.0 to 3.5-4.0 with a cup of coffee with higher acidity, caused by these microorganisms

producing lactic and acetic acid (De Melo *et al.* 2020). Even though coffee fermentation may have an impact on the coffee quality, microorganisms can also impact quality negatively. In this case, coffee with an unacceptable aroma profile derived from producing chemical compounds such as propionic, butyric, and short-chain fatty acids can occur (Haile and Hee, 2019). Mostly in wet fermentation, time and temperature are key factors that determine the development of the aroma and flavor profile (Lee *et al.* 2015).

In this study, we investigated the flavor and aroma modification of green coffee beans by fermenting with *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*, which are two common starter cultures in the beer and wine industry, through sensory analysis of the final product.

2. Materials and Methods

2.1. Fermentation

Green coffee beans were used for fermentation due to the seasonality of coffee cherries. We sourced from Primos Coffee Co. green beans (*Coffea arabica*) grown in Nicaragua. In order to mimic the matrix of fresh coffee cherry fermentation, 0.48 grams of glucose was supplemented based on the relative ratio of fermentations reported in a previous study (Jiyuan *et al.* 2021). Fourteen yeast and bacteria strains were selected for the preliminary trial of the study (see appendix table 1). Five yeast strains were selected for the main trial based on the aroma and flavor profile of the result coffee as well as commercial availability (see Table 1). Strains used in this main trial included 2 wine strains, one *Saccharomyces cerevisiae*, and one *Torulaspora delbrueckii* strain. A bioengineered *Saccharomyces cerevisiae* strain Sourvisiae was also selected due to its ability to produce lactic acid during fermentation. Two beer *Saccharomyces cerevisiae* strains were also employed, for a Belgian Ale and a Tropical IPA. Through preliminary trials, we noticed that

adding excess water to the fermentation appeared to trigger seed germination, which then produced an undesirable aroma in the result coffee, which was consistent with what was suggested in the previous literature (Haile and Hee, 2019). A protocol of using a yeast slurry with minimal water and a high yeast cell concentration (70 ml of water per 71g of coffee beans with initial cell count at inoculation of 5.94×10^{11} CFU/ml) was applied to all fermentations. Each fermentation was conducted in triplicates. A set of triplicates that did not go through fermentation was included as negative control. The samples were fermented for 72 hours under anaerobic conditions.

Strain commercial			Common fermentation
number	Organism (Genus species)	Manufacturer	product
Belgian Ale	<i>Saccharomyces cerevisiae</i>	Omega yeast	Beer
Tropical IPA	<i>Saccharomyces cerevisiae</i>	Omega yeast	Beer
Biodiva	<i>Torulasporea delbrueckii</i>	Lallemand	Wine
71B	<i>Saccharomyces cerevisiae</i>	LALVIN	Wine
Bioengineered			
Sourvisiae	<i>Saccharomyces cerevisiae</i>	MASCOMA	Beer
Control	None	N/A	N/A

Table 1. Commercial yeasts selected for the fermentation.

2.2. Coffee Drying, Roasting, Grinding, and Extraction

The fermented coffee beans plus control were dried using a commercial dehydrator for 4 ½ hours at 113°F to reach the original weight of the green coffee beans (71 g), followed by roasting at low-temperature setting (390°F) for 7 minutes using a coffee roaster (FreshRoast SR500 Automatic Bean Roaster, China). After roasting, the beans were ground by adjusting them in a fine coarse level (Approximate 800 µm particle size) on a coffee grinder (Cuisinart DBM-8P1 Supreme Grind Automatic Burr Mill, China) (Fibrianto *et al.* 2018). Finally, the hot brew coffee was prepared using a standard drip coffee maker (CM1070B Black+Decker, China), measuring 88 grams of ground coffee beans for 2000 ml of water and 1700 ml of brew coffee.

2.3. pH and Titratable Acidity

The pH was measured with a calibrated Oakton 510 (Vernon, IL, USA) pH meter. The electrode was submerged into every sample and, after the measurement, was rinsed. The pH electrode was submerged into 10 ml of coffee and titrated with NaOH 0.1 M until a pH of 8.0 was achieved. Volume of NaOH was recorded to calculate the Chlorogenic acid content.

2.4. Color measurement

The color of the brewed coffee was measured with a Hunterlab ColorQuest XE (Reston, VA, USA) calibrated using white and black standards. The readings were expressed with CIE LAB values for L*, a*, and b*. L measures black and white, in which values from 0 to 50 indicate dark color and values higher than 50 a lighter color. a* measurements positives indicate redness and negative greenness. In the case of b* values, positive indicates yellowness and blueness negative values. Every sample triplicate was measured three times, with nine measurements per sample.

2.5. Sensory Analysis

The Cornell University Institutional Review Board approved all human study procedures for Human Participants. A total of 98 panelists were prescreened for being familiar with black coffee without any additional additives, and all gave informed consent. Panelists were told they would participate in a sensory test involving coffee, but didn't know the purpose of the research. The sessions took approximately 20 minutes. The questionnaire was designed using the sensory software RedJade (IL, USA). Every panelist, after participating, received a gift card for \$5.00 for their time. The samples were delivered monadically so the temperature was consistent. Each sample was evaluated for overall liking, overall appearance, and aroma liking with a 9-point scale, and aromas of coffee, nutty, fruity, floral, sweet-potato, and tastes of sweetness, acidity, and

bitterness were evaluated using a generalized labeled magnitude scale (gmLs). Attributes were determined in a bench test of samples before the main test. Panelists were trained on how to use the scale before starting the test. The six samples were kept warm in insulated carafes until they were served. The samples were coded using three digit blinding codes, with sample order counterbalanced. Panelists were instructed that after tasting and smelling each sample, they should clean their palettes using water. At the end of the test, they were asked demographic questions and coffee-related habits.

2.6. Statistical Analysis

The coffee attributes (Coffee-like, nutty, floral, fruity, sweet potato, sweetness, acidity, and bitterness) and the pH of the six samples were analyzed with one-way repeated ANOVA and post hoc Tukey's test. The program used for analyzing the data was GraphPad Prism 5.0 (CA, USA). For the analysis, the results of the colorimeter were interpreted by a Kruskal-Wallis test, with post- hoc Dunn's test using XLSTAT (Paris, France).

3. Results and discussion

3.1 pH measurement and Chlorogenic Acid content

The pH of the coffee was measured after brewing (Figure 1), with significant differences ($p < 0.05$) between the pH of the samples. The fermented coffee samples actually increased pH, excluding *Sourvisiae*, a lactic acid producer (Mascoma LLC, 2019). These results differ from the literature, which states that fermented coffee beans decrease their pH during the fermentation process (Wang *et al.* 2020). During this process, polysaccharides break down into sugars, producing different compounds that modify acidity, such as alcohols and acids

(Carvalho *et al.* 2023). The coffee fermented with 71B was numerically the highest pH. The non-inoculated sample was the second lowest pH, being similar to results reported in the literature for Nicaraguan coffee (Moon *et al.* 2009).

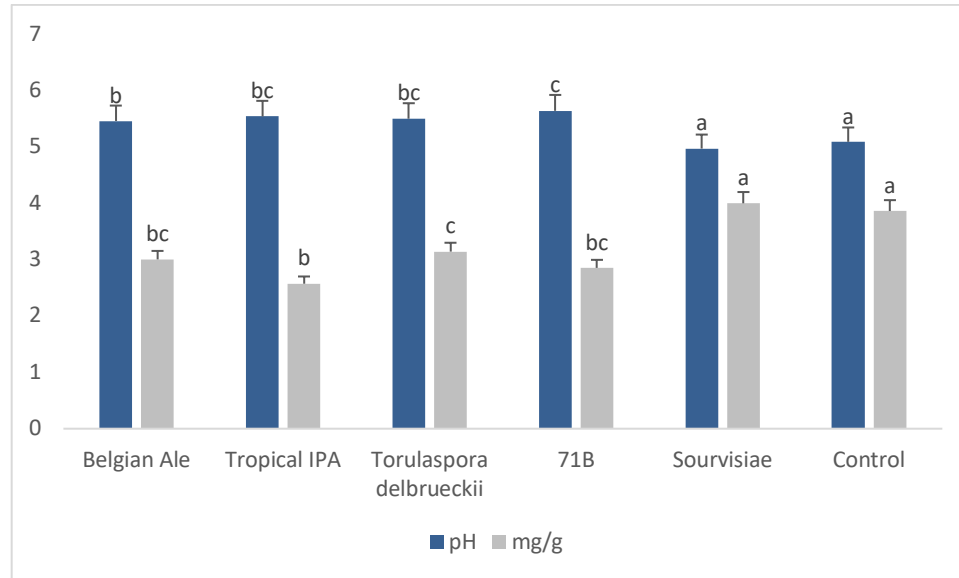


Figure 1. Samples pH and mg Chlorogenic Acid/g roasted Coffee beans.

The Chlorogenic acid (mg Chlorogenic acid/ g roasted coffee) differed from the Nicaraguan Chlorogenic Acid content reported in the literature (Moon *et al.* 2009). However, this content varies depending on the type of roasting done. If the roasting is darker, the Chlorogenic Acid level is less than in a light roast. In this study, the samples were subjected to a darker roast which may have resulted in a lower concentration. In decreasing order the Chlorogenic acid content was Sourvisiae>Control>Torulaspora delbrueckii>Belgian Ale >71 B> Tropical IPA. On the other hand, the pH decreasing order was 71B> Tropical IPA> Torulaspora delbrueckii> Belgian Ale> Control> Sourvisiae. At a higher pH value, generally samples had a

lower Chlorogenic Acid level, such as in the Belgian Ale, sample which had a pH of 5.46 (See figure 1), and its Chlorogenic acid concentration was 3.00mg/g, compared with *Sourvisiae*, which had a final pH of 4.97 and a Chlorogenic content of 3.99 mg/g (see figure 1).

3.2. Colorimeter data

A statistical difference ($p < 0.05$) between the color of the brewed coffee was apparent. The sample with the highest *L value was Belgian Ale coffee. Fermenting the coffee beans increased the *L value, excluding *Sourvisiae*, which had a lower result (20.79), indicating that the coffee sample had a darker color. In the case of the *a value, the parameter increased, indicating that the fermented coffee was redder. The *b value of the control sample, excluding *Sourvisiae*, was lower than the fermented coffee samples (38.61). This result indicates that fermented coffee were a more yellow color compared to the control. The fermented coffee samples' color can be compared to light-roasted coffee. In a study by Yeager et al. (2022), they determined that on average, the *L values for light-roasted coffee were between 37.52-36.42, the *a value that corresponds to light-roasted was between 25.91-24.35, and the *b value was between 22.05-24.10 (Yeager et al. 2022). Only the *b value was completely different from the results obtained in this study, indicating that the fermented coffee has a more yellow color.

The color difference could also be attributed to the fact that the experiment was performed with green coffee beans instead of coffee cherries, so when the bean was submerged in water, some phenolic compounds, such as Chlorogenic Acid, could have been extracted from the bean (Farah & Marino, 2006). This could in turn affect the Maillard reaction that happens during roasting, in which Chlorogenic Acid plays a role in the development of Melanoids (Starowicz & Zielinski, 2019).

Sample	L	SD	a	SD	b	SD
p Value	<0.0001		<0.0001		<0.0001	
Belgian Ale	39.91 ^{bc}	2.29	18.84 ^c	0.91	56.01 ^{ab}	0.81
Torulaspora delbrueckii	37.73 ^{bc}	5.26	20.69 ^{bc}	2.31	55.14 ^{ab}	2.32
Sourvisiae	20.79 ^a	0.8	19.00 ^b	0.49	33.69 ^{ab}	1.03
Control	22.77 ^a	6.45	29.62 ^a	3.29	38.61 ^c	10.81
71B	31.15 ^{ab}	1.51	22.72 ^{ab}	0.98	50.08 ^{bc}	1.5
Tropical IPA	43.7 ^c	4.54	16.53 ^{bc}	1.84	55.15 ^a	0.64

Table 2. $L^*a^*b^*$ values of the fermented and unfermented coffees. The numbers with different letters indicate a significant difference ($p < 0.05$) between samples.

3.3. Sensory attributes

3.3.1. Aroma and flavor attributes

Saccharomyces cerevisiae and *Torulaspora delbrueckii* are both commonly used in the beer and wine industry for fermentation and different strains are used to provide flavors and aroma. *Saccharomyces cerevisiae* produces a wide variety of alcohols and ester groups, modifying the beverage's flavor (Humia *et al.* 2019). One of the descriptors develop by the fermentation with this strain is a floral aroma. *Torulaspora delbrueckii* also has been characterized for producing floral and fruity aromas (Romero-Rodriguez *et al.* 2020). Even though both yeasts have been characterized as fruity, a study reported that *Saccharomyces cerevisiae* is more fruity than *Torulaspora delbrueckii* (Pereira *et al.* 2021). The aroma and flavor attributes significantly differed

with fermentation ($p < 0.05$). The sample with the highest score in the coffee-like attribute was unsurprisingly the control (the untreated sample). Each of the fermented samples were rated lower than 15 points on this scale. This significant difference between the attributes indicates that fermenting the coffee beans with *Saccharomyces cerevisiae* and *Torulaspota delbrueckii* modified the coffee-like nature of the product, in exchange for other sensory notes. *Saccharomyces cerevisiae* samples were more fruity, floral, nutty, and sweet than the control. These descriptors were also found by Wang *et al.* (2020) in fermented green coffee beans within the same yeast. The fruity, floral, nutty, and sweet attributes are developed in the final product due to the flavor compounds generated during fermentation (Wang *et al.* 2020, De Melo *et al.* 2015). *Toluraspora delbrueckii* was the sample that obtained the highest floral and sweetness rates, which may be related to the second highest pH, followed by 71B, which obtained the highest level. Belgian Ale, 71 B, and *Toluraspora* were mentioned as having a chocolate attribute. This result is in concord with Pereira *et al.* (2021) who determined that *Saccharomyces cerevisiae* and *Torulaspota delbrueckii* can be characterized with a chocolate descriptor (Pereira *et al.* 2021).

In terms of the perceived acidity of the samples, they performed similarly, excluding *Sourvisiae*, which produces lactic acid (Mascoma LLC, 2019). The control received the second-highest acidity score. These results correlates with the pH (Figure 1) where the Control and *Sourvisiae* were statistically similar, obtaining the lower pH ratings. The untreated sample and *Sourvisiae* were also considered more bitter. Also, people may confuse the acidity of the cup of coffee with bitterness. This happens because the compounds generated during the fermentation that give distinctive fruity, floral, and nutty attributes reduce the bitterness (Wei *et al.* 2015). The floral attribute in coffee may be an interesting characteristic to increase the coffee market by giving options to tea consumers.

Even though the nutty attribute has been recognized in *Saccharomyces cerevisiae*, and *Torulaspora delbrueckii* fermentations (Wang *et al.* 2020), the nutty attribute was the only note that did not present a significant difference, meaning that the six samples yielded a similar score for this attribute, thus panelists perceived the control sample with the same nutty aroma as all others. Finally, the descriptor corresponding to the Sweet- potato aroma was more predominant in the Sourvisiae sample, and the Tropical IPA sample. This aroma is a volatile compound generated by fermentation (Pereira *et al.* 2020). For further research, it would be interesting to test samples with Gas Chromatography to determine the more predominant compounds and record how they correlate with the attributes evaluated during the sensory test.

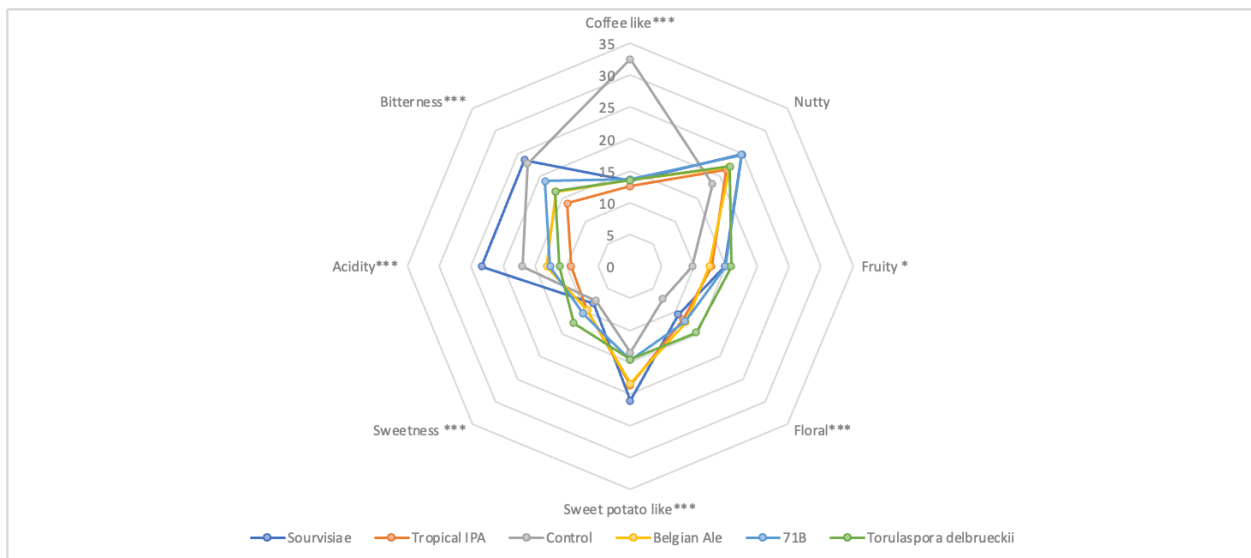


Figure 2. Effects of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* of flavor profiles. The attributes were evaluated on a scale from 0-100. Stars indicate the level of significance.

3.3.2 Hedonic scores for overall liking

A statistical difference was observed between the hedonic scores of overall aroma and appearance liking ($p < 0.05$). Previous work suggested that coffee beans fermented with *P. fermentans* with sucrose supplementation were less accepted than the aroma and taste of the control sample (De Melo *et al.* 2015). In the study, the non-inoculated control sample obtained the highest overall aroma and appearance scores, being more liked than the fermented ones. The fermented samples in our test performed similarly, excluding *Sourvisiae*, which was the less liked in the three attributes, probably due to the acidity of the coffee. Our results suggest that better strains are needed more suited to coffee fermentation, however providing new sensory notes in coffee through fermentation may be an affected way to manipulate flavor in a way that would not impact labeling or ingredient lists.

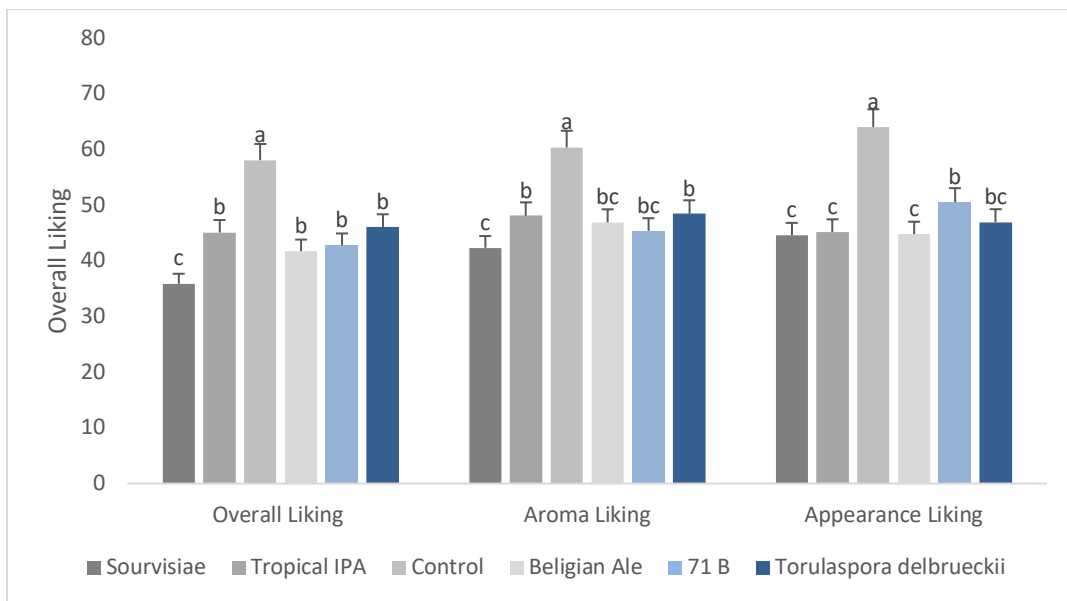


Figure 3. Hedonic scores for overall, aroma and appearance.

Conclusion

In conclusion, fermenting green coffee beans with *Saccharomyces cerevisiae* and *Torulaspota delbrueckii* significantly modified the aroma and flavor of the final cup of coffee, validating yeast fermentation as a promising approach to produce flavored coffees without the use of additional ingredients that consumers may not wish to see on labels. The fermentation developed a floral and fruity aroma, and reduced the acidity and bitterness of the product, excluding Sourvisiae, however the fermented samples were liked less than the regular black coffee. For further research, yeast strains specifically designed to produce flavors more appropriate to coffee may be beneficial, and the fermentation process may need to be optimized to result in higher acceptability of the coffee.

APENDIX

PGY collection number	Strain comercial number	Organism	Manufacturer	Common fermentation product	Aroma trial descriptors
OYL- 024	Beligian Ale	Saccharomyces cerevisiae	Omega yeast	Beer	Hazelnut, medium flavor intensity
OYL- 021	Hefeweizen	Saccharomyces cerevisiae	Omega yeast	Beer	Medium plus, flavor intensity, toast it walnut
OYL- 400	Bananza	Saccharomyces cerevisiae	Omega yeast	Beer	Medium aroma intensity, woody
OYL- 402	Cosmic Punch	Saccharomyces cerevisiae	Omega yeast	Beer	Medium plus aroma intensity, hazelnut Medium plus, brown sugar, Chinese medicine, cough syrup
OYL- 600	L.Plantarum	Saccharomyces cerevisiae	Omega yeast	Beer	
OYL- 200	Tropical IPA	Saccharomyces cerevisiae	Omega yeast	Beer	High aroma intensity, coconut, fruity
OYL- 205	Fantame	Saccharomyces cerevisiae	Omega yeast	Beer	Medium aroma intensity, rubber
PGY 329	Biodiva	Torulasporea delbrueckii	Lallemand	Wine	Medium minus, peanuts
PGY 327	Metschnikowia pulcherrima	Metschnikowia pulcherrima	Lallemand	Wine	Medium flavor intensity, brown sugar
PGY 71	71B	Saccharomyces Cerevisiae	LALVIN	Wine	Medium plus aroma intensity, toasted oak
PGY 539	Sourvisiae	Bioengineered Saccharomyces cerevisiae	MASCOMA	Beer	Medium plus aroma intensity, brown sugar, dried red fruit.
PGY 83	EC1118	Saccharomyces bayanus	LALVIN	Wine	Medium plus aroma intensity, toast coconut

Table 1. Commercial wine and beer yeasts selected for the preliminary trail.

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