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Strawberry tree fruit fermentation using the probiotic *Saccharomyces boulardii*

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INTRODUCTION & AIM

The strawberry tree (*Arbutus unedo*) is an evergreen shrub or small tree native to the Mediterranean region also found in other areas characterized by hot summers and mild, rainy winters. Various parts of this plant have been used traditionally in folk medicine to treat a wide range of ailments. The fruit, although edible, has a bland, slightly sweet and tart flavor and is mainly processed for jams or alcoholic beverages.

To enhance its nutritional and functional value, we investigated the fermentation of *A. unedo* fruits using the probiotic yeast *Saccharomyces cerevisiae var. boulardii*, which has been widely used for decades in the prevention and treatment of gastrointestinal disorders, including diarrhea.

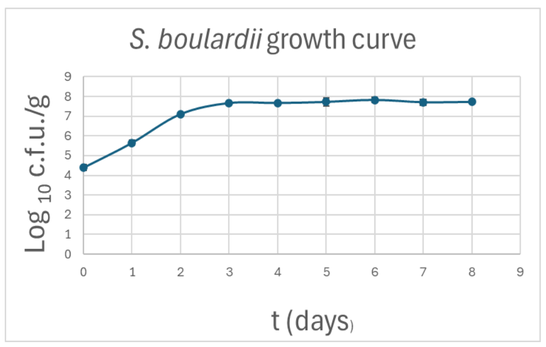
Following fermentation, the product was separated by centrifugation into two fractions: the supernatant, representing a juice enriched with fruit metabolites and yeast-derived bioactive compounds, and the precipitate, containing the remaining fruit pulp along with viable *S. boulardii* cells. Both fractions were analyzed separately to evaluate their potential as functional food products.

METHODS

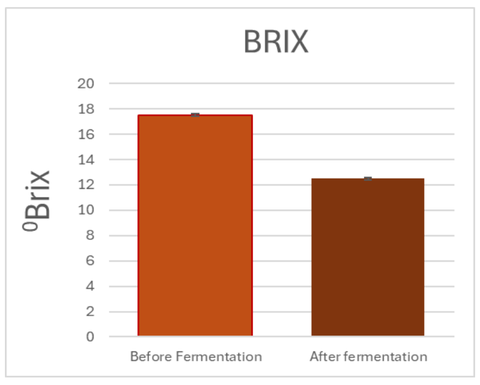
S. cerevisiae var. boulardii was isolated from a pharmaceutical product (Lamberts) in Greece. The strawberry tree fruits were collected when the level of ripeness was suitable for eating. Then they were freeze-dried, pulverized and sieved. The resulting powder was homogenized, moistened with sterile deionized water in a ratio of 1:4, pasteurized at 80 ^{o}C for 20 min and inoculated with *S. boulardii (*2.5x10^4 cfu/g of fruit).

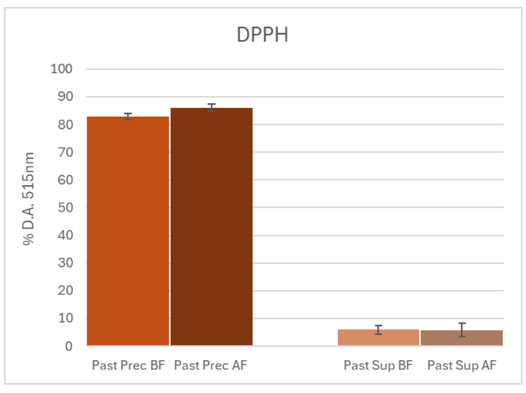
The plate count method on Chlopamphenicol Rose Bengal Agar was used for yeast enumeration. The pH was assessed during the fermentation. ^{o}Brix was measured using refractometer. Color parameters (L*, a*, b* and Δ.E.) were measured spectrophotometrically (CM-5, Konica Minolta, Osaka, Japan). Total Phenol Content (TPC) was determined using the Folin-Ciocalteu assay and antioxidant activity was assessed by the DPPH• assay. Phenolic compounds were analyzed using a Dionex UltiMate 3000 Rapid Separation UHPLC system (Thermo Fisher Scientific, Bremen, Germany) coupled with an Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a Heated Electrospray Ionization source (HESI-II). External standard quantification was performed based on a series of different standard concentrations of phenolic compounds ranging from 0.5 ng/mL to 5000 μg/mL). All measurements were performed in triplicates and results are expressed as mean ± standard deviation. Statistical analysis was carried out using t-test or ANOVA, with pairwise multiple comparisons by Tukey test for parametric data, or Mann-Whitney rank sum test for non-parametric data.

RESULTS & DISCUSSION

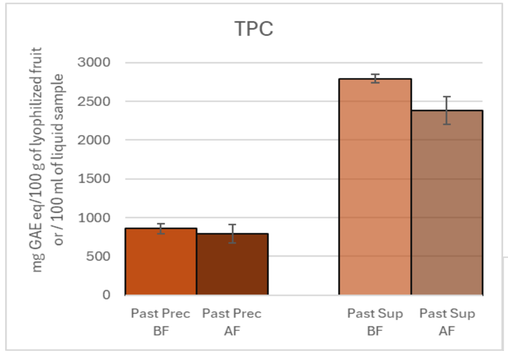


- ✓ During fermentation, *S. boulardii* reached 1,3*10^7 cells/g of fruit within two days and remained between 4.7-6.8*10^7 cells/g throughout the study.
- ✓ ^{o}Brix decreased significantly by 28.8% after fermentation indicating significant sugar consumption, though 12,5 ^{o}Brix remained, suggesting longer fermentation time might be sustainable.
- ✓ pH was maintained relatively stable at low levels (3.5-3.6)

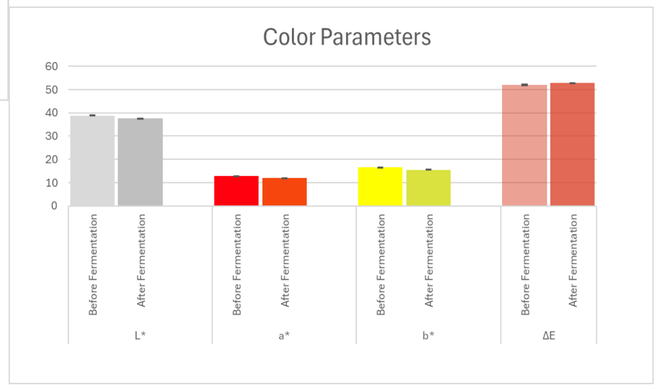




- ✓ DPPH radical scavenging activity was significantly higher in precipitates compared to supernatants. Fermentation did not significantly alter DPPH• scavenging activity in either fraction.



- ✓ Conversely, TPC was signifficantly higher in supernatants than in precipitates. After the fermentation, TPC remained unchanged in precipitates but decreased signifficantly in supernatants.



- ✓ All color parameters (L*, a*, b*) slightly but significantly decreased after fermentation, indicating a shift towards a darker, less red and less yellow juice. ΔE values were high, showing strong coloration and slightly increased after fermentation, suggesting enhanced color intensity.

These changes may be attributed to increased turbidity due to fermentation, anthocyanin degradation and oxidation of polyphenols and carotenoids.

PHENOLIC COMPOUNDS (mg/100 g of lyophilized sample)			BEFORE FERMENTATION mean ± st dev	AFTER FERMENTATION mean ± st dev
PHENOLIC ACIDS	HYDROXYBENZOIC ACIDS	Gallic acid	7,31±0,57	8,85±0,61
		Protocatechuic acid	4,83±1,34	4,15±0,79
		p-Hydroxybenzoic acid	0,56±0,32	0,28±0,04
		Syringic acid	0,07±0,05	0,18±0,00
		Vanillic acid	0,52±0,29	0,69±0,09
		Homovanillic acid (HVA)	0,13±0,12	0,23±0,09
	HYDROXYCINNAMIC ACIDS & DERIVATIVES	Caffeic acid	0,01±0,00	0,02±0,01
		p-Coumaric acid	0,14±0,02	0,18±0,02
		Ferulic acid	0,06±0,00	0,07±0,01
		Sinapic acid	0,05±0,01	0,07±0,01
		trans-Coutaric acid	0,22±0,01	0,22±0,02
		trans-Chlorogenic acid	0,15±0,03	0,14±0,01
FLAVONOIDS	FLAVANOLS	Catechin	16,42±0,03	12,77±1,38
		Epicatechin	0,49±0,17	0,32±0,01
		Epigallocatechin gallate (EGCG)	0,43±0,01	0,44±0,10
		Epicatechin gallate (ECG)	1,09±0,11	1,16±0,22
		Procyanidin B2	0,62±0,11	0,52±0,12
	FLAVONOLS	Quercetin	1,51±0,30	1,69±0,26
		Myricetin	0,96±0,43	0,79±0,03
		Kaempferol	0,00±0,00	0,00±0,00
		Isorhamnetin	0,01±0,00	0,01±0,00
	FLAVONOL GLYCOSIDES	Rutin	0,83±0,34	0,65±0,14
		Isoquercetin	5,34±1,73	3,70±0,77
		Astragalin	4,70±1,73	3,64±0,80
	FLAVANONES	Naringenin	0,03±0,01	0,08±0,02
	FLAVONES	Luteolin	0,22±0,00	0,21±0,00
	ANTHOCYANES	Kuromanin	18,04±0,71	14,59±1,34
		Myrtillin	1,53±0,11	1,22±0,15
STILBENES		Trans-resveratrol	0,03±0,01	0,08±0,02
		Piceid (Polydatin)	0,25±0,05	0,22±0,02
OTHER PHENOLIC COMPOUNDS		Vanillin	0,19±0,03	0,09±0,02

- ✓ 31 different phenolic compounds were identified in the precipitate.
- ✓ Individual phenolic compounds did not differ significantly before and after fermentation.

CONCLUSION

- ✓ *S. boulardii* can grow on pasteurized strawberry tree fruits substrate, effectively ferment it, maintaining a satisfactory viable cell level and significantly reducing ^{o}Brix.
- ✓ DPPH radical scavenging activity was high in precipitates and remained unaffected by fermentation, while individual phenolic compounds showed no significant changes, providing thus a promising basis for developing a novel symbiotic product, containing *S. boulardii* probiotic strain and a rich in polyphenols substrate.
- ✓ The high TPC content of the supernatant highlights the potential of fermented fruit juice as a biofunctional food.

FUTURE WORK / REFERENCES

- ✓ Detailed analysis of phenolic compounds in supernatants to determine whether the increased TPC reflects higher concentrations of specific phenols of particular interest.
- ✓ Further investigating the fermentation conditions (e.g. non-heated vs.sterilized fruit, varied fermentation temperatures) and exploring the effect of substrate enrichment with *A. unedo* leaves.
- ✓ Evaluating the antimicrobial potential of both fractions.
- ✓ Assessment of probiotic functionality of *S. boulardii* in precipitates.

REFERENCES

Ramires, F. A., et al. (2024). Novel fermentation strategies of strawberry tree *Arbutus unedo* fruits to obtain high nutritional value products. *International Journal of Molecular Sciences,* 25(2), p.684. Available at: https://doi.org/10.3390/ijms25020684