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OPTIMIZATION OF THE CONDITIONS FOR MICROBIAL FERMENTATION OF BLACK CHOKEBERRY (*Aronia melanocarpa* L.) POMACE

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INTRODUCTION

The increasing global fruits processing has generated substantial amounts of agro-industrial by-products, raising both environmental and economic concerns. Among these by-products, berry pomace represents one of the most abundant residues generated during berry processing. Interest in the valorisation of fruit processing by-products as sources of bioactive compounds has increased significantly in recent years. Chokeberry pomace possesses considerable bioactive potential and associated health-promoting properties, making it a promising raw material for functional food applications. Among various techniques, microbial fermentation has emerged as a low-toxicity, cost-effective, and environmentally sustainable method for modifying the composition of chokeberry pomace by increasing the content of key compounds that contribute to its biological activity.

METHODOLOGY

The full factorial experiment design with two or three levels was used to study the effect of microorganism's type (lactic acid bacteria *Lactiplantibacillus plantarum*, yeast *Candida utilis*, and their combination), the addition of glucose as a carbon source, the addition of yeast extract as a nitrogen source, and the addition of pectinolytic enzyme on the analytical results, primarily the content of bioactive compounds (TPC and TAC) and antioxidant activity (FRAP and ABTS assays). Moreover, some physicochemical parameters of the samples were also determinated, including pH value, total acidity, chromatic parameters according to the CIELab system (L, a, b, C, h). Preparation of chokeberry fermentation: 5 g of freeze-dried and ground chokeberry pomace was mixed with 100 mL of sterile distilled water in a flask. Depending on the experimental design, 1.5 % glucose (as a carbon source), 2 % yeast extract (as a nitrogen source), and/or 0.1 % pectinolytic enzyme were added to specific flasks. The inoculum was added at a concentration of 1 % v/v. Fermentation was carried out at 32 °C for *Lactobacillus plantarum* and 30 °C for *Candida utilis*, and samples for analysis were taken after 24 and 48 hours of fermentation.

RESULTS

Table 1. The influence of fermentation conditions on the analyzed parameters (pH, total acidity, TPC, TAC, ABTS and FRAP)

	pH	TOTAL ACIDITY (g/L)	TPC (mg/L)	TAC (mg/L)	ABTS (mM)	FRAP (mM)
MICROORGAN.	p<0.001*	p=0.034*	p<0.001*	p<0.001*	p=0.542	p=0.227
LAB	3.76±0.02^{ab}	4.21 ± 0.44^{ab}	897.16 ± 32.83^{b}	159.89 ± 12.56^{b}	7.11 ± 0.25^{a}	7.12 ± 0.23^{a}
YEAST	4.10 ± 0.07^{b}	2.28 ± 0.11^{a}	767.71 ± 25.27^{a}	110.99 ± 5.65^{a}	7.89 ± 0.37^{a}	7.61 ± 0.26^{a}
LAB+YEAST	3.70 ± 0.02^{a}	4.55 ± 0.50^{b}	774.98 ± 15.37^{a}	101.57 ± 5.32^{a}	8.29 ± 0.36^{a}	7.37 ± 0.16^{a}
CARBON	p=0.567	p=0.692	p=0.250	p=0.703	p=0.269	p=0.295
0	3.86 ± 0.04^{a}	3.07 ± 0.39^{a}	793.58 ± 21.36^{a}	122.31 ± 7.87^{a}	7.55 ± 0.31^{a}	7.23 ± 0.18^{a}
1	3.84 ± 0.04^{a}	3.22 ± 0.40^{a}	832.98 ± 23.14^{a}	125.99 ± 7.85^{a}	7.98 ± 0.24^{a}	7.50 ± 0.17^{a}
NITROGEN	p<0.001*	p<0.001*	p<0.001*	p=0.277	p=0.554	p=0.477
0	3.71 ± 0.02^{a}	1.90 ± 0.07^{a}	742.96 ± 20.57^{a}	127.04 ± 7.02^{a}	7.65 ± 0.30^{a}	7.24 ± 0.20^{a}
1	4.00 ± 0.05^{b}	5.45 ± 0.32^{b}	883.60 ± 19.37^{b}	121.26 ± 8.60^{a}	7.88 ± 0.25^{a}	7.50 ± 0.16^{a}
ENZYME	p<0.001*	p=0.212	p=0.991	p=0.123	p=0.120	p=0.524
0	3.90 ± 0.04^{b}	3.52 ± 0.36^{a}	827.78 ± 25.91^{a}	135.58 ± 9.16^{a}	7.46 ± 0.21^{a}	7.45 ± 0.19^{a}
1	3.80 ± 0.04^{a}	3.84 ± 0.33^{a}	798.79 ± 18.13^{a}	112.72 ± 5.85^{a}	8.06 ± 0.32^{a}	7.29 ± 0.17^{a}
TIME	p<0.001*	p<0.001*	p=0.595	p<0.001*	p<0.001*	p<0.001*
24	3.91 ± 0.04^{b}	3.15 ± 0.28^{a}	833.03 ± 22.64^{a}	159.59 ± 7.53^{b}	8.75 ± 0.26^{b}	8.15 ± 0.13^{b}
48	3.80 ± 0.05^{a}	4.21 ± 0.39^{b}	793.53 ± 21.90^{a}	88.72 ± 3.67^{a}	6.77 ± 0.25^{a}	6.59 ± 0.15^{a}
grand mean	3.85	3.68	813.28	124.15	7.76	7.37

Table 2. The influence of fermentation conditions on chromatic parameters (L*, a*, b*, C* and h*)

	L*	a*	b*	C*	h*
MICROORGANISM	p=0.014*	p<0.001*	p=0.215	p=0.013*	p=0.620
LAB	39.79 ± 1.63^{a}	59.70 ± 0.69^{b}	43.95 ± 1.76^{a}	74.45 ± 1.43^{b}	0.62 ± 0.02^{a}
YEAST	44.12 ± 1.60^{ab}	53.95 ± 1.33^{a}	39.72 ± 1.89^{a}	67.63 ± 1.59^{a}	0.63 ± 0.03^{a}
LAB+YEAST	45.90 ± 1.52^{b}	57.11 ± 0.94^{ab}	40.53 ± 2.13^{a}	70.52 ± 1.79^{ab}	0.61 ± 0.02^{a}
CARBON	p=0.792	p=0.573	p=0.578	p=0.514	p=0.573
0	43.59 ± 1.35^{a}	56.57 ± 0.89^{a}	40.73 ± 1.67^{a}	70.22 ± 1.44^{a}	0.61 ± 0.02^{a}
1	42.95 ± 1.33^{a}	57.28 ± 0.89^{a}	42.07 ± 1.50^{a}	71.52 ± 1.28^{a}	0.63 ± 0.02^{a}
NITROGEN	p<0.001*	p=0.054	p<0.001*	p<0.001*	p<0.001*
0	45.97 ± 1.47^{b}	57.95 ± 0.85^{a}	34.18 ± 1.53^{a}	67.59 ± 1.46^{a}	0.52 ± 0.01^{a}
1	40.57 ± 1.06^{a}	55.89 ± 0.92^{a}	48.62 ± 0.71^{b}	74.15 ± 1.07^{b}	0.72 ± 0.01^{b}
ENZYME	p=0.809	p=0.809	p=0.889	p=0.884	p=0.437
0	42.69 ± 1.38^{a}	56.29 ± 1.02^{a}	41.60 ± 1.53^{a}	70.43 ± 1.43^{a}	0.63 ± 0.02^{a}
1	43.85 ± 1.29^{a}	57.55 ± 0.75^{a}	41.20 ± 1.65^{a}	71.31 ± 1.30^{a}	0.61 ± 0.02^{a}
TIME	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p=0.012*
24	35.63 ± 0.51^{a}	60.49 ± 0.45^{b}	47.17 ± 0.88^{b}	76.85 ± 0.68^{b}	0.66 ± 0.01^{b}
48	50.91 ± 0.92^{b}	53.35 ± 0.93^{a}	35.64 ± 1.70^{a}	64.88 ± 1.32^{a}	0.58 ± 0.02^{a}
mean	43.27	56.92	41.40	70.87	0.62

LAB=lactic acid bacteria; TPC=total phenolic content, TAC=total anthocynin content
0=no addition; 1=addition

The type of microorganism, addition of nitrogen source and pectinolytic enzyme showed a statistically significant influence on the pH value of the samples, while the concentration of total acids was statistically significantly influenced by the type of microorganism, addition of nitrogen source and fermentation time. The type of microorganism, addition of nitrogen source and fermentation time showed a statistically significant influence TPC, while the type of microorganism and fermentation time has a statistically significant influence on TAC; the fermentation time showed a statistically significant effect on the antioxidant activity (ABTS AND FRAP) of the samples.

The type of microorganism, the addition of a nitrogen source, and the fermentation time showed a statistically significant effect on sample lightness (L*) and color saturation (C*); the type of microorganism and fermentation time showed a statistically significant effect on the red/green color component (a*); and the addition of a nitrogen source and fermentation time showed a statistically significant effect on the yellow/blue color component (b*) and the hue angle (h*).

CONCLUSIONS

- ✓ Based on the research results, the lactic acid bacterium *Lactiplantibacillus plantarum* was selected as the optimal microorganism for the fermentation of chokeberry pomace, with the addition of 2 % yeast extract as a supplementary nitrogen source and a fermentation duration of 24 hours as the chosen fermentation conditions. This treatment produced the highest increase in TPC, TAC and antioxidant activity.
- ✓ The microbial fermentation with the selected microorganism could significantly affect the levels of bioactive compounds. This fermentation-based approach proved as an effective strategy for enhancing the value of chokeberry pomace.

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