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PHYSICOCHEMICAL EFFECTS AND SENSORY CHARACTERISTICS OF POMERAC (MALAY APPLE; SYZYGIUM MALACCENSE) WINE: EFFECTS OF VARYING PECTOLASE CONCENTRATIONS

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ABSTRACT

Malay apple fruits are susceptible to bruising and degradation with short shelf life. The Malay apple fruit can be economically converted into functional food and consumed as wine. Overcoming challenges of competitive market quality standards and consumer approval must be achieved by producing wines with optimal sensory and physicochemical characteristics during fermentation. The objective was to identify optimal pectolase enzyme concentration that produce, the most superior physicochemical characteristics and consumer acceptability during fermentation. Malay apple wine was made after secondary fermentations of 21 days of ripe fruits using 4 labeled treatments (T_1 –T_4) different pectolase concentrations 0.0%, 0.3%, 0.6%, 0.9% respectively and the physicochemical characteristics of the treatments were measured. Hedonic testing, focus group evaluation, quality appraisal of the wines and microbiological stability tests were performed. All results were computed statistically using the MINITAB Statistical Software Package. Acidity, Flavor, Finish, Hue, Chroma and Turbidity were superior in theT_2 treatments while the T_4 treatment scored higher in Aroma and Bouquet, Balance, Body, Overall Acceptability and Alcohol Content. All treatments T_1 – T_4 were microbiologically stable and contained < 10 cfu.mL^{-1} of total aerobes, yeast, molds and lactic acid bacteria and were microbiologically stable. It was concluded that treatments T_2 and T_4 (0.3% and 0.9%) produced dry wines, with the best physicochemical and hedonistic characteristics of the Malay apple wine.

Keywords: *Syzygium malaccense*, Malay apple, Fermentation, Pectolase, Physicochemical, Pomerac, Hedonistic evaluation, Sensory quality.

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Contribution/ Originality

This study contributes to the existing literature, the optimization of the pectolase enzyme concentration required for efficient fermentation. The efficient conversion of the highly perishable Malay apple fruit into quality wine with excellent physicochemical properties, long shelf life and preserved nutritional benefits is also desirable, as a commercial product.

1. INTRODUCTION

The *Syzygium malaccense* (L.) Merr. & Perry or Malay apple is native to Indo Malaysia, Polynesia, Micronesia, Melanesia and Southeast Asia. The Malay apple is a glossy red, fleshy, ovoid berry 3 – 7 cm long, that contains one or pair of sub-globose seeds to hemispherical seeds with dimensions 1.6 – 2 cm [1]. The mature Malay apple trees grow to approximately 16m and limit economic viability by enduring numerous problems from cumbersome harvesting and quality constraints to wastage of ripe fruit by high perishability, pest control and enzymatic browning, during storage. Fruit quality is usually compromised and difficult to preserve, to be economically viable. The Malay apple does not readily meet market standards for export, due to its fragility and short shelf life, which can be from 4 – 6 days at an ambient temperature of 28°C [2]. Optimal physicochemical characteristics contribute to the wine quality and to the success of the wine as viable commercial product. These parameters influence olfactory and gustatory wine properties with aromatic intensity and related to presence of tannins, pH and alcoholic content that comprise the measure of quality and acceptability [3]. The yeast diversity and composition contribute significantly to the wine sensory characteristics whose species characterized by a specific metabolic activity, determines concentrations of flavour compounds in the final product [4]. Product survival depends on optimised fermentation procedures and parameters that satisfy these quality requirements. Anthocyanins exist in plants to protect it from free-radical oxidation. The pomerac fruit contains anthocyanins, located in the red outermost skin. Anthocyanins contribute little to wine taste, but are closely associated with colour via complex chemical interactions [5]. Anthocyanins are well- known powerful antioxidants with positive health benefits [6] and add to the importance of pomerac wine as a functional food. Market penetration is difficult due to the high variability in fruit quality produced by Caribbean countries [7]. Converting the Malay apple fruits into wine, ensure that fruits that are susceptible to degradation by short shelf life, can be utilized economically. The Malay apple wine must therefore meet market quality standards and consumer approval, to ensure market penetration and product survival.

The objective of this study was to identify the optimal pectolase enzyme concentration that produces the most favorable physicochemical and hedonistic quality characteristics of the Malay apple wine, upon fermentation. The optimal pectolase concentration producing the best sensory acceptability is being elicited and sought to produce wines for functional food market penetration.

2. MATERIAL AND METHODS

2.1. Fermentation Procedure

Mature, ripe, medium-dark red pomerac fruits were selected, deseeded and washed in a solution containing 200 ppm of sodium hypochlorite. The fruit was blended into a must composed of 30% fruit, using (Oster Blender Model No. 889 16R, New Hartford, Connecticut, USA) for 3 min. 50 ppm of sodium metabisulphite and crystallized sucrose was added to increase the brix from 4° to 22°. Pectolase enzyme was added to the must in containers designated (T_1, T_2, T_3 and T_4), corresponding to added percentage pectolase enzyme concentration 0%, 0.3%, 0.6% and 0.9% respectively. All pectolase enzyme treatments were performed in duplicate. The must was incubated for 24 hrs at ambient temperature, pasteurized at 80°C for 20 mins and cooled to 37°C. Red Star (Milwaukee, USA) Active Dry Wine Yeast and yeast nutrient were added to the must and pasteurized at 80°C, for 10 mins. 2.8g of yeast and then 6 mls of the yeast solution were added to each of the containers. 4.15mls of yeast nutrient were added to each container and underwent primary fermentation for 7 days. The must was filtered and clarified using bentonite. The wine filtrate underwent secondary fermentation for 3 weeks. The wine was then racked, treated with 100 ppm sodium metabisulphite and bottled.

2.2. Focus Group Evaluation

The wines ($T_1 - T_4$) were presented to 12 trained panelists for a focus group evaluation. The evaluation was performed to identify and explore specific wine sensory characteristics [8] and determine consumers' reactions to the product [9].

2.3. Ranking and Hedonic Testing

Ranking and hedonic tests were performed by fifty one panelists. The ranking and hedonic population consisted of 60% and 30% adult females and males respectively. Each panelist was presented with a questionnaire and 4 coded wine categories. Hedonic testing criteria of the wines were evaluated using parameters of appearance, aroma, flavor, body/mouth feel, acidity, sweetness and overall acceptability. The evaluation was scored on a 9-point category scale [10].

2.4. Quality Appraisal of Wines

Sensory quality testing was performed by 12 panelists trained in the identification of critical sensory attributes of wines. The two wines with highest overall acceptability scores were chosen from hedonic testing panel results (i.e., T_2 and T_4 wines) and presented to panelists for sensory quality testing. Quality attributes and attitudes were evaluated by the Likert Scale (5-Excellent; 4-Very Good; 3-Good; 2-Fair and 1-Poor) for parameters of Appearance, Colour, Body, Flavour and Aroma.

2.5. Physicochemical Analysis

The colour of raw fruit and pomerac wines was determined using a Minolta Portable Tristimulus Colourimeter (Model No. Cr-200b, Minolta Co., Japan) after being calibrated with a Minolta calibration plate CR-a 43. The total soluble solids (TSS) were determined by an Atago

Refractometer (Model No. 4E, 1/50%, Brizo Vee Gee Scientific, Inc., Kirkland, Washington, USA).The pH of raw fruit, and wines was measured on an Orion pH meter (Model 520, Orion Research Inc., Boston, USA).The total titratable acidity TTA, was determined by titrimetric method No. 947.05, AOAC 1999 [11]. The tannin concentration was measured @ 760nm absorbance spectrophotometric range.

2.6. Microbiological Analysis

Microorganisms were cultivated using potato dextrose agar (PDA, Oxoid Basingstoke, England), Tomato Juice Agar (TJA, Oxoid), Plate Count Agar (PCA, Oxoid), Eosine Methylene Blue Agar (EMBA, Oxoid), Mannitol Salt Agar (MSA, Oxoid) and Lee's Agar (LA, Laboratory made) for lactic acid bacteria/Escherichia Coli, Staphylococci and Streptococcus Thermophilus, yeasts and molds, and total mesophilic aerobic counts.

PCA plates were incubated at 308°C for 24h, TJA and PDA plates at 288°C for 24 h, and LA, EMB and MSA plates at 35_/378°C for 48 h . LA plates were incubated in anaerobic jars (GASPAKTM). 10g of wine sample was serially diluted and transferred sterile pour-plated petri dishes. Microbial colony counts were tallied and recorded as colony forming units (cfu/ml)

2.7. Statistical Analysis

Sensory data from hedonic testing of wines with T_1 – T_4 wines and sensory quality evaluation of T_2 and T_4 wines using (Minitab 13, release 2000, Minitab Inc, State College, Enterprise Drive, Pa, USA). Significant differences between means were separated by the Least Significant Difference (LSD) at 0.05% level of significance.

3. RESULTS AND DISCUSSION

Table-1. Physicochemical Attributes of the Fermented Wines

Parameter	T_1 (0.0%)	T_2 (0.3%).	T_3 (0.6%)	T_4 (0.9%)	LSD	P - Value
Colour before	119.34±0.658	104.60±14.82	69.11± 8.425	87.16±0.121	21.5	0*
Colour After	30.82±2.143	65.64±10.31	35.97±6.888	35.97±6.888	70.32	0.033*
pH	3.47	3.495	3.355	3.385		
Turbidity/(NTU)	7.6	7.3	5	24		
TTA/% Citric Acid	0.39087±0.003	0.9613±0.003	0.50897±0.003	0.4617±0.003	0.009	0
Brix (°)	4.7	4.2	4.5	4.7		
Alcohol v/v%	8.0	10.0	12.0	14.0		
Tannin g/100ml	3.47± 0.00	3.495±0.005	3.355±0.005	3.385± 0.005		

n.s- not significant at P < 0.05; * Significant at P < 0.05;** Significant at P < 0.01
LSD – Least Significant Difference @ 0.05% level significance

Table-2. The Hedonic Testing Scores

Sensory Attributes	Before	After	P - Value
Colour	7.020 ± 0.237	7.196 ± 0.2115	0.22*
Clarity	7.392 ± 0.2344	7.039 ± 0.222	0.04*
Aroma	6.667 ± 0.235	6.784 ± 0.233	0.497**
Flavor	5.863 ± 0.30035	6.5098 ± 0.263	0.008*
Sweetness	5.314 ± 0.32	5.922 ± 0.315	0.01*
Overall Acceptability	6.1373 ± 0.288	6.3922 ± 0.266	0.124*

n.s- not significant at P < 0.05; * Significant at P < 0.05;** Significant at P < 0.01; n=51

Table-3. Hedonic Scores for Pomerac Wines

Sensory Attributes	0.0%	0.3%.	0.6%	0.9%	LSD	P - Value
Appearance / Colour	1.8033 ± 0.193	1.000± 0.175	0.333 ± 0.225	1.0833 ± 0.229	0.58	0.04*
Aroma and Bouquet	3.000 ± 0.348	3.167± 0.474	3.250 ± 0.494	3.500 ± 0.485	ns	0.86
Acidity	5.000 ± 0.151	0.8333± 0.112	0.5833 ± 0.149	0.75 ± 0.131	0.36	0.306
Balance	0.4167 ± 0.149	0.9167± 0.0833	0.5833 ± 0.149	1.1667 ± 0.112	ns	0.001
Body	0.3333 ± 0.284	0.6667± 0.142	0.5000 ± 0.151	0.8332 ±0.112	ns	0.077*
Flavor	1.3333 ± 0.142	1.8333± 0.167	1.4167 ± 0.260	1.6667 ±0.188	ns	0.404*
Finish	0.4167 ± 0.149	0.9167± 0.149	0.6667 ± 0.142	0.1667 ±0.207	0.47	0.015*
Overall Acceptability	0.7500 ± 0.179	1.0833± 0.229	0.8333 ± 0.207	1.500 ± 0.151	0.55	0.04*

n.s. - not significant at $P < 0.05$; * Significant at $P < 0.05$; ** Significant at $P < 0.01$ n=12
LSD – Least Significant Difference @ 0.05% level significance

Table-4. Colour parameters before and after fermentation

Treatment	L		a		b		δe		Chroma		Hue	
Fresh Fruit	44.467	-	33.6	-	16.267	-	64.167	-	37.343	-	25.89	-
Blended Fruit	44.467	-	13.667	-	1.467	-	48.833	-	13.747	-	6.09	-
0.0%	34.367	22.167±0.145	-1.333	1.0667±0.067	2.367	0.6333±0.033	62.467	74.767±0.145	2.367	1.2433±0.050	119.34	30.82±2.413
0.3%	33.677	23.167±0.033	-0.633	0.5667±0.133	1.300	0.8333±0.185	63.233	73.773±0.033	1.662	1.1567±0.0833	104.60	65.64±10.31
0.6%	32.267	22.2±0.058	0.633	0.7333±0.133	-1.533	0.6000±0.058	64.767	74.773±0.088	1.690	0.9633±0.081	69.11	35.97±6.888
0.9%	31.633	21.90±0.058	0.800	0.600±0.115	0.8	1.0±0.115	65.367	74.033±0.066	2.067	1.1900±0.042	87.16	35.97±6.888
LSD	2.66	0.28	4.65	-	2.61	-	12.17	0.91	4.5	-	21.5	70.32
P -Value	0*	0*	0*	0.055 n.s.	0*	0.119**	0*	0*	0*	0.076 n.s	0*	0.033

LSD – Least Significant Difference @ 0.05% level significance
n.s- not significant at $P < 0.05$; * Significant at $P < 0.05$;** Significant at $P < 0.01$

4. DISCUSSION

There was a successive 2% linear increase in alcohol content with each 0.3% increase of pectolase enzyme treatment. The Total Titratable Acidity (TTA) was statistically significant at the 95% confidence interval with the T_2 treatment being an outlier. Anthocyanins are also very unstable in weakly acidic or neutral solution where they rapidly disappear [12]. A pH 3.5 stabilized a light pink colour in the T_2 treatment, whereas a high turbidity 24 NTU affected the overall appearance of T_4 treatment.

4.1. Colour

The values for a and Chroma were insignificant at the 95% confidence interval and b at the 99 % confidence interval. δe and Hue were significantly different at the 95% confidence interval. There was a significant difference in hue with the T_2 treatment after fermentation. The T_2 a desirable clear light plush pink colour was comparable to a rosé wine. Anthocyanins are unstable and easily degraded by heat light and pH [13]. Controlling these factors during production would conserve the colour in the final product.Additional ascorbic acid and citric acid are traditionally used to optimize pH to usually around pH 3 - 3.5, to achieve optimum colour via anthocyanin stability. Anthocyanins become stable due to trance formation of the flavylium cation structure in acidic aqueous solution [12]. Optimization of colour can definitely influence the product attractiveness in the wine as a commercial product. A mechanism proposed for the anthocyanins stabilization of food is metal ion complexation, complex formation of anthocyanins with flavanoid pigments (co-pigmentation) [14]. Self association [15], [16] and intermolecular type stacking of the anthocyanin nucleus and acyl groups (acyl co-pigmentation) [17-19] are also possible.

4.2. Sensory Quality

The sensory evaluation show a significant difference at the 95% significance level ($p = 0.04$). Appearance and colour Least Significant Difference (LSD) showed that with compared with the control treatment T_1 (0.0%), the other treatments differed significantly. T_3 (0.6%) varied significantly with T_1 (0.0%), T_2 (0.3%), and T_4 (0.9%), in the appearance and colour. Aroma and bouquet values showed no significant difference at the 95% significance level. There is some statistical difference in acidity between the control treatment (T_1) and the other treatments which established T_1 as an outlier. However, there was no significant difference in acidity between the T_2, T_3 and T_4 treatments. Balance and body show significant differences at the 95% level, but no significant difference between flavor, finish and overall acceptability. There is a statistical difference of overall acceptability in T_2 and T_4. A difference in finish exists between T_1 and T_2, the T_4 and the T_2 and T_3 treatments, but no significant difference between T_1 and T_4. However, the value for finish in T_2 is significantly greater than the T_1, T_3, and T_4. The overall acceptability was due to the body and lack of acidity/ astringency in the wine. The T_2 and T_4 treatments produced superior scores in the hedonic testing.

4.3. Microbiological Quality

All treatments $T_1 - T_4$ were microbiologically stable and contained < 10 cfu.mL^{-1} of total aerobes, yeast, molds and lactic acid bacteria.

5. CONCLUSION

Acidity, Flavor, Finish, Hue, Chroma and Turbidity were superior in theT_2 treatments, while T_4 scored higher in Aroma and Bouquet, Balance, Body, Overall Acceptability and Alcohol Content. Differences in hedonic Acceptance in hedonic testing and Colour and Chroma values were insignificant and the wines were microbiologically stable. Pectolase enzyme treatments T_2 and T_4 (0.3% and 0.9%) produce the most optimal physicochemical and sensory characteristics for the Malay apple (Sygzgium Malaccense) wine.

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