Process Optimization and Evaluation of Functional Properties of Wine from Adlai (*Coix lacryma-jobi*) and Taro (*Colocasia esculenta* Linn.)

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Abstract

A process for the production of wine from *adlai* and taro was established using Response Surface Methodology. This study aimed to determine the optimum conditions for the production of wine using *adlai* and taro as substrates and to evaluate the functional properties of the wine. Central Composite Design was employed with taro level, water level, and fermentation time as the independent variables. Response surface regression analyses was also used to determine the effects of the independent variables on the wine. Increasing taro levels enhanced the aroma of the wine that subsequently increased the general acceptability of the product. Physicochemical properties were not affected by different taro and water levels, and fermentation time except pH. Moreover, increasing taro level and the interaction of water and fermentation time result to higher pH of wine. The optimum region for *adlai*-taro is at 220-450 grams taro and 1,300-1,490 mL water for five days. At any of these levels, wine with the most acceptable quality can be produced. *Adlai*-taro wine contained reducing sugars: fructose, and inulin. It exhibited free radical scavenging activity of 312.60 µmolTE/100g. This suggested that the product has added functionality. This study provides information on the potential of *adlai* and taro as substrate for wine-making. The presence of fructose and inulin in the wine has demonstrated its prebiotic potential.

Keywords: Plackett-Burman screening, Central Composite design, Jobs tears, fermented beverage, taro, antioxidant

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Introduction

Adlai (Coix lacryma-jobi.) is a tall, grain-bearing plant found in the tropics. Also known as Jobs tears, *adlai* comes from the grass family *Poaceae* to which wheat, corn, and rice also belong. Although not very popular, the crop has long been cultivated and grown in the Philippines particularly in the highlands as staple food. In some parts of Asia, particularly in India, adlai is pounded, threshed, and winnowed as cereal. The pounded *adlai* is sometimes mixed with water or turned into a sweet dish by frying and coating it with sugar (Duke, 1983). It is also boiled and eaten like rice. In addition, *Adlai* grains are used in soup and broths (Jansen, 2006). Due to its starchy nature, it can be a potential substrate for wine.

It is widely known that various sugary and starchy materials have been used for wine production. The major sugars present in wine from fruits particularly grapes are largely glucose and fructose (Amerine et al., 1965). Traditional rice wine contains mostly glucose, as well as sucrose, maltose, and fructose (Kim et al., 2013). Previous studies have also demonstrated that wine from starchy materials is more superior in quality than wine from fruits. Rice wine contains large amount of protein, sugar, and vitamin B2. *Adlai* is more nutritious than rice and wheat since it contains more fat and protein (Burnette, 2012). Thus, it can be a more suitable substitute for rice in winemaking. In this study, in order to further enhance the nutritional qualities of wine from *adlai*, purple variety of taro was incorporated. In a study by Tan and Roa (2012), it was found that different types of good sugars, particularly fructose and inulin, are present in natural sweetener

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from taro processed through fermentation which makes it ideal for diabetic people and as potential prebiotic sweetener. Inulin and oligofructose are considered as functional food ingredients since they affect the physiological and biochemical processes in humans resulting in better health and reduction in the risk of many diseases (Kaur and Gupta, 2002). By incorporating taro in the process, it is possible that these types of good sugars will also be produced in wine. This study aimed to determine the optimum conditions for the production of wine using adlai and taro as substrates and to evaluate the sensory, physicochemical, and functional properties of the wine.

Methodology

Procurement of Raw Materials

Adlai (*Gulian* and *Tapol* varieties) were procured from DA-NOMIARC, Dalwangan, Malaybalay City, Bukidnon while taro (VG-9) was obtained from the experimental fields of the Philippine Root Crop Research and Training Center of the Visayas State University (VSU). Bubod starter was procured from Baguio.

Variable Screening

Seven input variables (taro level, water level, *adlai* variety, cooking time, amount of starter, fermentation time, and mixing frequency) were subjected to screening using the Plackett-Burman Design (7 variables with 8 runs) to determine their main effects on the quality of the wine and to isolate significantly independent variables. The collected wine was evaluated, in terms of acceptability of color, aroma, sweetness, sourness, alcohol strength,h and general acceptability. The results were then subjected to Placket-Burman screening analysis wherein the main effects of the independent variables were identified.

Optimization Experiment

The three variables identified from variable screening were optimized. Optimization was carried out using 3^3 fractional factorial design (Central Composite Design) with 15 treatments for experimental combinations. One-kilogram capacity fermentors were used for the set-up. The wine was processed by initially boiling the desired levels of shredded taro (VG-9), adlai, and water in a pot for 15 minutes. The cooked mixture was then spread in a sterilized cloth to facilitate cooling. Well-pulverized bubod starter (5% w/w) was inoculated into the cooled mixture and loaded in a fermentor, lined with stainless screen, and with an amber glass container serving as catcher for the dripping fermented liquid. The stainless container was kept covered with sterile manila papers to prevent insects and other foreign bodies from entering the set-up. The mixture was allowed to ferment for the desired number of days (5, 7 and 9 days) and the juice was collected, pasteurized, and analyzed for its sensory, physicochemical, and functional properties.

Sensory Evaluation

Sensory evaluation was done following the Incomplete Block Design (IBD) by Cochran and Cox (1957) with five replications per block. The wine was evaluated for its color, aroma, sweetness, sourness, alcohol strength, and general acceptability.

Evaluation of Physicochemical and Functional Properties

Total soluble solids content (TSS) was obtained using a hand-held refractometer (American Optica^{TM}) and pH using a Beckman Coulte^{TM} pH meter. The total titratable acidity was determined by titration method using 0.1 N NaOH and alcohol content by distillation method. Sugars present were characterized qualitatively following methods for Benedicts, Fehlings, Barfoeds, and Seliwanoffs Tests described by Sadasivam and Balasubramanian (1985). Fructose and inulin content of the treatments were determined using the method by Ashwell (1957). Free radical scavenging activity was determined using the DPPH radical scavenging assay.

Statistical Analysis

The response surface regression (RSREG) analysis was employed using the Statistical Analytical Software (SAS 1998) in the analyses of the sensory and physicochemical properties of the wine formulations. The Statistica Version 8 software was used for the graphical presentation of the response surface and contour plots to illustrate the effects of the different variables.

Results and Discussion

Variable Screening

The summary of the effects of the seven identified process variables on the acceptability of wine is presented in Table 1. Statistical analyses showed that color acceptability increases as level of taro substitution is increased. Higher levels of water level and *adlai* variety would result to decrease in aroma acceptability as indicated by its negative significant effect. Sweetness was significantly affected by all variables except mixing frequency. Taro level, *adlai* variety, cooking time, and fermentation time significantly affected the sourness acceptability of the wine. Alcohol strength was also affected by *adlai* variety and cooking time. In addition, general acceptability of the wine was affected by taro and water level and *adlai* variety. Positive effects mean that the acceptability rating of the different sensory attributes increases as the level of the variable is increased; while negative effect indicated a decrease in the acceptability rating of the product as level of the variable is increased.

The summary of effects for the physicochemical attributes of wine is presented in Table 2. The yield of wine was significantly affected by the water level and fermentation time, together with preference towards higher levels as shown by its positive effect. Moreover, higher values of taro level, water level, and fermentation time resulted in decrease on the total soluble solids (TSS) of wine and into an increase on its total titratable acidity (TTA). On the other hand, taro and water level decreases pH of wine when levels of these variables are increased. Finally, increasing taro level and fermentation time result to higher alcohol content.

Based on their significant effects on sensory and physicochemical properties of wine, as a result of variable screening, three variables were chosen and subjected to optimization experiment. The top three variables with significant effect on the sensory and physicochemical properties of wine were taro level (150, 300, and 450 grams), water level (1300, 1500, and 1700 mL) and fermentation time (5, 7, and 9 days). Levels of other variables that were not considered were held constant based on their effects on acceptability.

Optimization

The different sensory attributes of the wine were evaluated to determine the effect of the variables on the acceptability of the product. Analysis of variance of the acceptability scores of the different sensory parameters revealed that color, sweetness, sourness, and alcohol strength were significantly affected by lumped linear effects (Table 3). In addition, higher taro level result to increased color acceptability of wine that is attributed to the purplish color of taro flesh. Aroma acceptability is significantly influenced by taro level as indicated in its parameter estimates. Increasing taro level would result to increased aroma acceptability. According to Clarke and Bakker (2004), aroma release and perception are influenced by the reactions involving interaction or binding of polyphenols with aroma compounds in wine. Tan and Roa (2012) reported that taro wine produced using purple taro (VG-9) and black glutinous rice contained high phytonutrient contents (polyphenols, flavonoids and anthocyanidin). For general acceptability, the trend is significant at linear regression. Increasing taro levels enhanced the aroma of the wine that subsequently increased the general acceptability for the product. The interaction of water and taro levels also significantly affected the general acceptability of the wine.

Optimum Formulation

Contour plots were generated using predicted models for consumer acceptance ratings on the sensory attributes tested. These gave an idea as to what levels of taro, water, and fermentation time would result into wine with desirable acceptability level. In determining the optimum conditions, contour plots were generated for two independent variables at a time with the third variable held constant. The regions of acceptability for each sensory attribute were then superimposed. A minimum acceptability value of 6.75 that cor-

Table 1. Summary of Effects for the Different Sensory Attributes of Wine

Variables	Color	Aroma	Sweetness	Sourness	Alcohol Strength	General Acceptability
Mean/Interc	6.848**	5.871**	5.719**	5.438**	6.063**	5.915**
Taro level	0.804**	0.366^{ns}	-1.027**	-0.554*	-0.375ns	-0.580**
Water level	-0.286^{ns}	-0.688**	-0.955**	-0.375^{ns}	-0.143^{ns}	-0.723**
Adlai variety	0.036^{ns}	-0.991**	-0.741**	-0.839**	-0.536*	-0.920**
Cooking time	0.250^{ns}	0.152^{ns}	0.634**	0.661**	0.500*	0.580**
Starter	0.268^{ns}	-0.045^{ns}	0.509*	0.125^{ns}	0.161^{ns}	0.223^{ns}
Fermentation time	-0.357^{ns}	-0.045^{ns}	-0.509*	-0.589*	-0.250^{ns}	-0.366^{ns}
Mixing	0.268^{ns}	-0.295^{ns}	-0.259^{ns}	-0.054	-0.089^{ns}	-0.027^{ns}

* – significant ($p < 0.05$); ** – highly significant ($p < 0.01$); ns – not significant

Table 2. Summary of Effects on the Physicochemical Attributes of Wine

Variables	Yield	TSS	pH	TTA	Alcohol content
Mean/Interc	949.063**	14.975**	3.729**	1.413**	10.796**
Taro level	19.375 ^{ns}	-8.050**	-0.390**	0.435**	1.453*
Water level	114.375*	-3.850**	-0.273**	0.240*	0.880 ^{ns}
Adlai variety	-28.125 ^{ns}	-1.300 ^{ns}	-0.063 ^{ns}	0.110 ^{ns}	-0.163 ^{ns}
Cooking time	104.375 ^{ns}	0.300 ^{ns}	-0.040 ^{ns}	-0.005 ^{ns}	0.088 ^{ns}
Starter	-40.625 ^{ns}	0.200 ^{ns}	0.075 ^{ns}	-0.155 ^{ns}	-0.570 ^{ns}
Fermentation time	136.875*	-2.350*	-0.115 ^{ns}	0.270*	2.950**
Mixing	11.875 ^{ns}	0.600 ^{ns}	0.053 ^{ns}	-0.160 ^{ns}	-0.155

* – significant ($p < 0.05$); ** – highly significant ($p < 0.01$); ns – not significant

Table 3. Summary of F-ratios for the Sensory Attributes of the Adlai-Taro Wine

	Color	Aroma	Sweetness	Sourness	Alcohol	General Acc.
Linear	23.41**	5.88**	7.55**	3.30*	1.25 ^{ns}	3.72*
Quadratic	2.02 ^{ns}	1.41 ^{ns}	1.41 ^{ns}	1.83 ^{ns}	0.75 ^{ns}	1.45 ^{ns}
Cross product	0.12 ^{ns}	0.56 ^{ns}	1.33 ^{ns}	0.52 ^{ns}	0.59 ^{ns}	1.34 ^{ns}
Total Regress	8.52**	2.62**	3.43**	1.89 ^{ns}	0.86 ^{ns}	2.17*

* – significant ($p < 0.05$); ** – highly significant ($p < 0.01$); ns – not significant

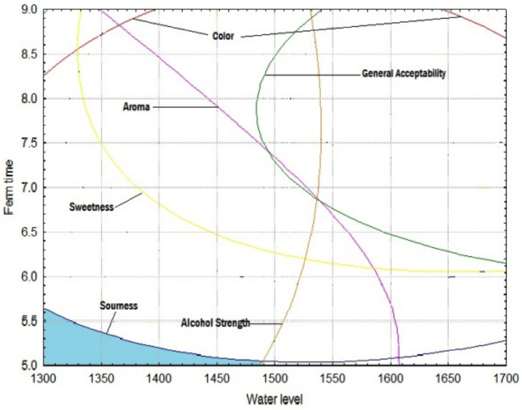

Figure 1. Superimposed contour plot showing optimum region at constant taro level

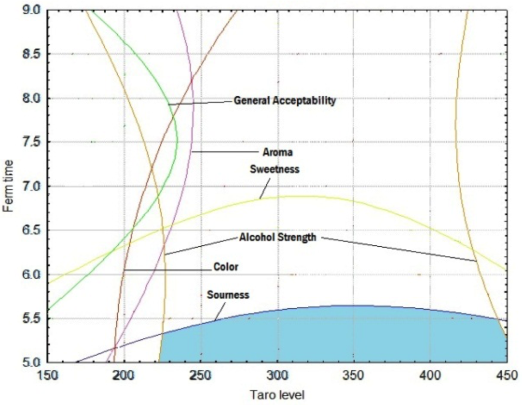

Figure 2. Superimposed contour plot showing optimum region at constant water level

responds to like slightly to like moderately in the 9-point Hedonic scale was set as the cut-off score for the acceptability. The graphs with shaded region represent values for consumer acceptance for a particular attribute of the product. Contour plots show that at constant taro level (Figure 1), 1300 mL to 1490 mL of water and a fermentation time of 5 to 5.6 days will satisfy the shaded region with sourness and alcohol strength being the limiting factors.

At constant water level, a combination of taro level from 220 to 450 grams and fermentation time from 5 to 5.6 days filled the shaded region but was also limited by sourness and alcohol strength (Figure 2).

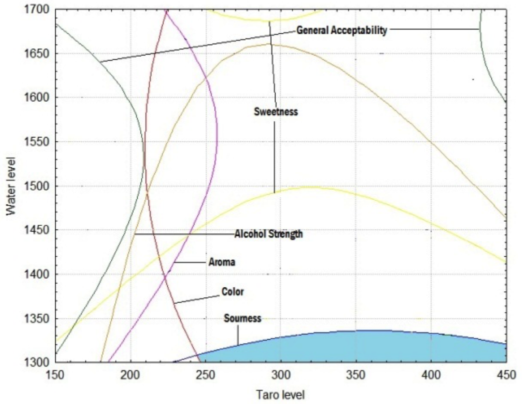

Figure 3. Superimposed contour plot showing optimum region at constant fermentation time

When fermentation time was held constant, a combination of taro level from 250 to 450 grams and water level from 1300 to 1330 mL satisfied the shaded region with limitation caused by the sourness and color attributes (Figure 3). The three integrated plots when combined shows that treatment with 450 grams taro, 1300 mL water, and 5 days fermentation time falls within the optimum range.

Evaluation of Physicochemical and Functional Properties

Analysis of variance for physicochemical attributes of *adlai*-taro wine is summarized in Table 4. Results showed that yield, total soluble solids, total titratable acidity, and alcohol content of wine were significantly affected by lumped linear effects. Furthermore, pH was shown to be significantly affected by both linear and cross-product regression. Parameter estimates revealed that pH is significantly affected by taro level, fermentation time, and the interaction of water and taro levels. Decreasing taro level would result into higher pH of wine; similarly, shorter fermentation time would also result into higher pH that would generally produce less sour-tasting wine.

The presence of the types of sugar found in the different treatments of the wine was also tested qualitatively. All treatments of wine tested positive for Benedicts, Fehlings, Barfoeds, and Seliwanoffs test. This outcome indicated that one or more of the reducing sugars (glu-

Table 4. Summary of ANOVA F-ratios for the Physicochemical Properties of the Adlai-Taro Wine

	Yield	TSS	pH	TTA	Alcohol content
Linear	50.18**	11.83**	27.65**	16.84**	6.23**
Quadratic	1.92 ^{ns}	0.54 ^{ns}	2.75 ^{ns}	0.39 ^{ns}	0.70 ^{ns}
Cross product	0.91 ^{ns}	0.67 ^{ns}	5.07**	1.73 ^{ns}	1.51 ^{ns}
Total Regression	17.67**	4.35**	11.82**	6.32**	2.81*

* – significant ($p < 0.05$); ** – highly significant ($p < 0.01$); ns – not significant

cose, fructose, glyceraldehydes, lactose, arabinose, maltose, etc.), as well as, monosaccharides are present in the wine. Quantitative analyses further revealed that fructose (ranging from 137.92-521.87 µg/mL) and inulin expressed in terms of fructose concentration (ranging from 78.34-741.56 µg/mL) are present in *adlai*-taro wine. Fructose is a commonly consumed simple sugar with sweetness approximately 1.7 times higher than sucrose and 2.3 times higher than glucose and is known to have the lowest GI of any of the sugars (Truswell, 1992). Given this fact, fructose is being promoted as the preferred sugar for diabetics (Uusitupa, 1994). Roberfroid (2002) identified nutritional compounds that met his definition of prebiotics. These include inulin-type prebiotics (inulin, oligofructose, and fructooligosaccharides) and galactooligosaccharides (GOS). Inulin is a non-digestible oligosaccharide that has a potential to increase healthy intestinal microflora when digested in the large intestine (http://www.prebiotic.ca/inulin.html). Reducing sugars and inulin nowadays are being considered not only for their sweetness but also for being a potential functional ingredient. The free radical scavenging activity (FRSA) of the optimum treatment was analyzed and was found to possess an FRSA of 312.60 µmolTE/100g. Furthermore, taro corm was reported to contain flavonoids (Prajapati et al., 2011). Flavonoids are phenolic substances that act as antioxidants by reducing free radical formation and scavenging free radicals (Pietta, 2000). Taro when processed into wine was found to contain high phytonutrient and antioxidant activity (Tan and Roa, 2012).

Conclusions

Increasing taro level enhanced the aroma of the wine that subsequently increased the general acceptability of the product. Meanwhile, decreasing taro level would result in higher pH of wine. Similarly, shorter fermentation time would result to higher pH that would also generally produce less sour-tasting wine. Wine from the combination of *adlai* and taro can be processed optimally at 450 grams taro, 1,300 mL water and 5 days. Using these combinations, an acceptability score of $\geq$6.75 can be achieved. *Adlai*-taro wine contained monosaccharides and reducing sugars and further analyses revealed that fructose and inulin are present. The presence of reducing sugars, fructose, and inulin suggests that the product may have added functionality. Moreover, the optimum treatment for wine from adlai and taro was also found to exhibit antioxidant property with a free radical scavenging activity of 312.60 µmolTE/100g.

This study provided information on the potential of *adlai* as substrate for wine-making. The presence of fructose and inulin in wine has demonstrated its potential prebiotic effect. Future studies may focus on increasing inulin content of *adlai*-taro wine and on nutritional and toxicological analyses to further establish its nutritional benefits.

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