



**MICROBIAL DYNAMICS
AND PHYSIOCHEMICAL
EVALUATION OF
CARROT WINE**

PRODUCED WITH *Saccharomyces cerevisiae*

***T. V. BALOGU^{1,2}, M. T. IKEGWU², Y. UMAR¹, C. B. AKPADOLU¹, AND K. C. AKPADOLU¹,**

¹Department of Microbiology, Ibrahim Badamasi Babangida University, Lapai Nigeria ²Department of Food Science and Technology, Nnamdi Azikiwe University, Awka

Abstract

Microbial dynamics and physiochemical evaluation of carrot wine produced with *Saccharomyces cerevisiae* were assessed. Fresh ripe and health carrot (6kg) were sequentially processed (washed, preheated, blended and sieved) into juice and fermented for 60 days with *Saccharomyces cerevisiae*. Airtight glass jars composed of juice (2000g), distilled water (2000mL) and sugar (200g) at controlled temperature (20 -25 °C) was used for fermentation. Wines were clarified (siphoning), aged (45 days) and pasteurized (50°C – 60°C) to stop fermentation. Proximate analysis, microbial dynamics, physiochemical and wine qualities were assessed. Result showed that juice extraction process reconstitute nutritional composition of

carrot, such that moisture, ash and total carbohydrates increased, while others (fat, crude fiber and crude protein) decreased. A trendy

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progressive microbial dynamic model of $Y = -0.195x^2 + 1.822x + 4.566$ with coefficient ($R^2 = 0.907$) was observed. Fermentation significantly decreased pH and increased total acidity. Observed wine qualities include alcoholic content (7.88 - 9.19%v/v), attenuation (121% - 142%)

and calories (0%). Clarification and ageing have diminishing effect on alcohol content. Carrot wine was judged as physically appealing moderate alcoholic beverage, with smooth consistent taste (authors' opinion), and could be modeled with microbial dynamics.

INTRODUCTION

Wines other than grape wine are classified as fruit and vegetable wines. Alcoholic beverage of fermented carrots is among the vegetable and fruit wines. Across African, Asian and Latin American countries, there are many locally fermented alcoholic wines brewed from popular fruit and vegetables such as banana, dates pineapples and grape (Swami et al. 2014), and unpopular among them is carrot. Carrot (*Daucus carota*) is one of the edible root vegetables classified as tubers; with prehistory from central Asia region. Popular bright orange varieties (used in this study) are among others (purple, red, white, and dark) colour varieties (Siemonsma, 1994) composed of high level of β -carotene (pre-vitamin A) and carbohydrates (sugars), vitamin B complex and minerals (Bystrická et al. 2015). Preheating of carrot to tenderness improve the natural sweetness and nutritional value (Snodgrass. 2004). Cooked or raw carrot vegetable have varying medicinal purposes (Carlos and Dias 2014) and as well reduces the risk of oxidative diseases due to high antioxidants (Shukla et al. 2014; Kumari et al., 2014;).

Most winery processes are sequentially in three operational stages such as before fermentation, during fermentation and after fermentation. Typically, fermentation stage of wine production, yeasts convert sugar to carbondioxide gas and alcohol (Swami et al. 2014) within varying time duration in control or uncontrolled chamber. Optimization of wine processes is mostly targeted at controlling the fermentation environments of physiochemical (pH, temperature, salt, chambers etc), redox (O_2 , H_2 etc) microbial (yeast-bacterial kinetic), nutrition, time and organoleptic parameters (Keller, 2010). Proliferation of some species of yeast (*Zygosaccharomyces* and *Brettanomyces*) in wines, sometimes leads to failed fermentation (Loureiro, et al. 2003), due to synthesis of array of volatile

phenolic metabolites. These metabolites poison the yeast and add odd flavours to the wine.

Saccharomyces cerevisiae is most popular yeast used in bakery and winery industries; it is single celled eukaryotic budding yeast (White *et al.*, 2010) initially isolated from the surface of grapes. *Saccharomyces cerevisiae* used as primary fermenter due to their ability to produce CO₂ and alcohol in aerobic or microaerobic conditions by metabolizing sugar. For predictability of fermentation, sufficient primary fermenters are initially added to repress the wild yeast (Vaughan-Martini *et al.*, 1995; Gonzalez *et al.*, 2001). Varying fermentative and physiological properties of different strains of *S. cerevisiae*, necessitate the selection of appropriate strain to achieved required impact on finished wine (Swami *et al.*, 2014; Dunn *et al.*, 2005).

Materials and Methods

Sample Collection/preparation

A total of 6kg of fresh ripe and healthy carrot vegetables were obtained from community markets within Lapai, LGA Niger state, Nigeria. Samples (bright yellow carrots) were transported to the laboratory in clean plastic bags (< 4°C), then sorted, washed, weighed and stored in refrigerator prior fermentation processing.

Preparation of Inoculum Starter Culture

S. cerevisiae pure colonies were obtained from repository, Microbiology laboratory, Ibrahim Badamasi Babangida University, and verified using potato dexterosus broth (PDB) in 250ml Erlenmeyer conical flask at room temperature incubation for 24hrs with relevant biochemical assay. Verified Isolates were primed for 3 to 6hrs in PDB media and yeast cell concentration (mean of triplicate counts) was determined by direct counting method using hemacytometer (Levy, USA). The stock solutions were reconstituted to cell count of ~10⁶cfu/mL and stored (4 °C) as described by Balogu *et al.*, (2016).

Fermentation Protocol

A total 6kg of three sets(2000g) of fresh carrots root vegetable were separately chopped (~2cm³), steamed for 10 mins, minced with industrial

blender for 20mins to achieve fine slurry. The slurries were sieved in muslin bag to obtain the carrot juice. Each juice (500 mL), distilled water (2000 mL) and sugar (200g) were discharged in a clean glass jar (steam sterilized at 15 psi and 121°C for 15 minutes) and allowed to cool to room temperature. The glass jars were sealed with stoppers (fitted with gas-valve), agitated and allowed to stand for 24 hr. The 24 h old mixture was degassed (mild sulfur gas) before 200mL of starter culture ($\sim 10^6$ cfu/mL) was added. Culture fermented for 60 days at 25°C - 30 °C, with agitation and degassing every 48 h. Wine was clarified by siphoning the supernatant of wine sediments into a clean steam sterilized long neck round bottom glass bottle sealed with gas-valved stopper. Citric acid (to limit spoilage by bacteria) added to the clarified wine and allowed to continued fermentation for 7 weeks (46days) and pasteurized (50°C – 60°C) to stop fermentation.

Specific Gravity

50ml of each sample were discharged into volumetric flasks at 20°C and hydrometer was used to determine specific gravity (appropriate correction factor was factored in with temperature variations). The alcohol content (%), apparent attenuation and calories were determined (Balogu and Towobola 2017).

Proximate composition and Physiochemical analysis parameter

Nutritional composition of fresh carrot root vegetable was analysed and selected physiochemical parameters such as pH, temperature and titratable acidity were assessed. Temperature and pH were determined using standard methods. (AOAC, 1999, Balogu and Towobola 2017).

Microbial Analysis

Microbial assays (isolation, characterization and enumeration) was conducted on samples collected at 15 days intervals of fermentation using PDA (yeast), Nutrient Agar (bacteria) and relevant biochemical tests in accordance with the methods of Cowan and Steel (2004).

Statistical Analysis

Data generated were subjected to ANOVA, Duncan's Multiple Range Test and Chi – square using SPSS software version 20 of 2014.

Results and Discussion

Proximate composition of Carrot (juice and whole) showed that moisture were 89.25% and 78.06%, ash(1.42% and 1.26%), fat (0.91% and 1.83%) crude fiber (1.24 % and 13.36%), crude protein (1.12 % and 2.44%) and total carbohydrates were 6.06% and 3.05% respectively (Table 1).

Composition of whole carrot were significantly ($P < 0.05$) different from the carrot juice. The discarded carrot chaff after juice extraction accounts for these discrepancies. This was obvious in the values of crude fiber (major constituent of the chaff) between the whole carrot and the juices. High moisture component decreases shelf life of beverages due to enhanced microbial proliferation. Invariably, whole carrot drinks are likely to have longer shelf life than carrot juice drinks. Olalude et al. (2015), observed similar composition of carrot juices and expressed related opinion on the correlation of moisture and microbial growth. Also higher carbohydrate composition in juice than whole carrot would further enhance the rate of microbial spoilage.

Microbial profile increased steadily from $6 \log_{10}$ cfu/mL to $8 \log_{10}$ cfu/mL within 60 days fermentation. Within the five interval sampling, the microbial load were $7.95 \log_{10}$ cfu/mL, $7.86 \log_{10}$ cfu/mL, $8.75 \log_{10}$ cfu/mL, $8.84 \log_{10}$ cfu/mL observed at day 15, 30, 45 and 60 respectively. Dynamic microbial model of $Y = -0.195x^2 + 1.822x + 4.566$ with $R^2 = 0.907$ was observed (Fig 1). Application of models in winery processes would optimize the process and minimize production cost. The coefficient ($R^2 = 0.907$) indicated more than 90% optimization of microbial profile within 60 days fermentation. This means that the conditionings of intrinsic and extrinsic factors are responsible for the optimization.

Among the physiochemical properties, pH significantly decrease from 7.47 to 5.21 and TTA increased significantly from 1.20 to 2.12, while temperature was not significantly ($P < 0.05$) altered after fermentation. (Table 2). Since

temperature predetermines microbial activities, the relative stability in temperature (24.7 ± 0.02) attributes to the trendy typical microbial climax observed in this study.

Matunda (2015) and Idise, (2012) collaborated that alcoholic beverage of pineapples are optimized at temperature range of 20 - 30°C. The observed change in pH depicts that carrot wines are acidic alcoholic beverages though, less acidic than pineapple produced under similar parameters. Goswami and Ray (2011) optimized alcoholic yield of grape wine at pH 5.0 - 5.5, validating this study's average pH (5.21). It is not surprising that fermentation increased TTA by more than 56%, confirming that fermentation unlocked most of the acidic antioxidants (organic acids) abundant in carrots. This strengthens the finding of Grooper et al., (2012), that most vegetable fruits rich in antioxidants are acidic (at pH 1-5).

Alcoholic strengths of carrot wine were evaluated using the specific gravity (SG) of the samples. Within 15 and 60 days interval, SG were 0.9800 and 0.9900, Alcoholic content (9.19 and 7.88), attenuation (142% and 121%) respectively. However, carrot wine has no calories after 60 days fermentation (Table 3). Making carrot wine a good option for those that are calorie and weight sensitive. Alcoholic content decreased by 14% within 46 days of ageing, due to utilization of ethanol after depletion of chapitized sugar. Reduction in percentage attenuation (rate of sugar conversion to ethanol) of aging wine, further strengths this argument.

Conclusion

Carrot wine processed with *Saccharomyces cerevisiae* would yield a moderate alcohol and low calories wine. Carrot wine is physically appealing (Fig. 2), consistent smooth feeling (authors' opinion only) and could be modeled with microbial dynamics.

Conflict of Interest

The authors declare no conflict of interest.

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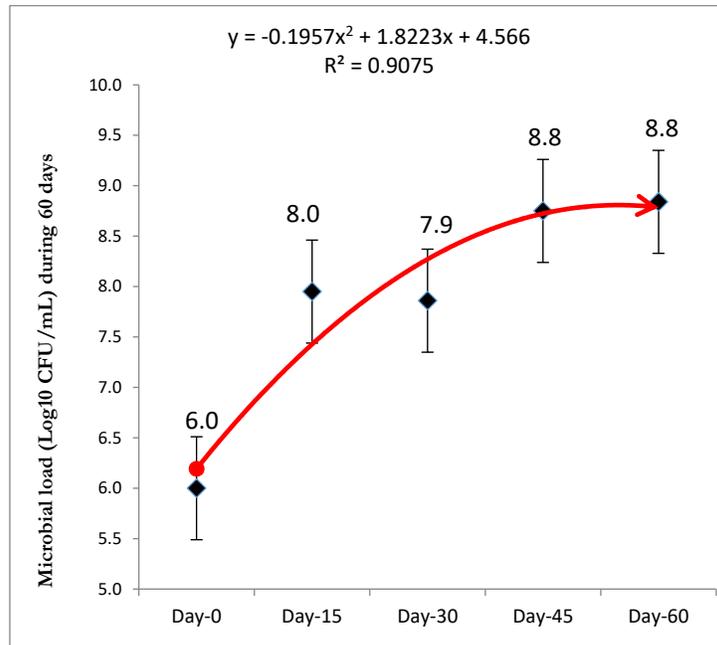


Figure 1: Microbial load dynamic of carrot wine fermented for 60 days.

NB: ◆ = mean plot of triplicate values \perp = standard error of mean plot



Figure. 2: Bottled carrot wine after 60 days fermentation

Adjudged as physically appealing and smooth taste feel.

Table 1: Proximate analysis of carrot (*Daucus carota*) juice

Component	Carrot (%)	
	Whole	Juice
Moisture	78.06	89.25
Ash	1.26	1.42
Crude fiber	13.36	1.24
Crude protein	2.44	1.12
Fat	1.83	0.91
Total carbohydrate	3.05	6.06

Table 2: Physicochemical properties of carrot juice and wine after 60 fermentation

Parameter	Fresh carrot juice (0 day)	Fermented carrot wine (60days)
Temperature (°C)	24.6 ± 0.17	24.7 ± 0.02
pH	7.47 ± 0.16	5.21± 0.02
TTA (%v/v)	1.20 ± 0.016	2.12 ± 0.0721

Table 3: Alcoholic content of Carrot Wine

Duration	Specific gravity	Alcoholic (%v/v)	Calories (/355ml)	Attenuation (%)
15 days	0.9800	9.19	0.0	142
60 days	0.9900	7.88	0.0	121

Initial SG = 1.050