EFFECT OF FERMENTATION ON SENSORY, NUTRITIONAL AND ANTIOXIDANT PROPERTIES OF MIXTURES OF AQUEOUS EXTRACTS OF *HIBISCUS SABDARIFFA* (ZOBO) AND *RAPHIA HOOKERI* (RAFFIA) WINE

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This study investigated the effect of fermentation on the nutritional, antioxidant and sensory properties of aqueous extracts of *Hibiscus sabdariffa* (popularly called Zobo in Nigeria) and *Raphia hookeri* (raffia) wine mixed in various proportions: P_{100} - Z_0, P_{80} -Z_{20}, P_{60} -Z_{40}, P_{40} -Z_{60}, P_{20} -Z_{80} and P_0 -Z_{100}. Glucose, soluble protein, vitamin C content, antioxidant activity and sensory properties (colour and taste) were evaluated in fresh mixture preparations, and in formulations fermented at room temperature for 72 h (day 3). Glucose was depleted significantly ($p < 0.05$) as fermentation progressed, the order of decrease was: P_{100} - Z_0 (97.59%) > P_{80} -Z_{20} (95.90%) > P_{60} - Z_{40} (95.17%) > P_0 - Z_{100} (90.94%) >P_{20} -Z_{80} (71.30%) > P_{40} -Z_{60} (66.42%). Soluble protein concentration also decreased with fermentation, the highest and lowest % decrease was observed for the P_{100} - Z_0 (25.97%) and P_{60} - Z_{40} (5.35%) respectively. The highest DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity was detected in the P_{80} - Z_{20} (72.20%) mix. Sensory evaluations confirmed the best combination was the P_{80}-Z_{20} raffia wine/zobo extract mixture, this maintained an appealing red colour, sweetness of palm wine and the contributory antioxidant properties from zobo extracts optimized.

Key words: palm wine, *Hibiscus sabdariffa, zobo*

INTRODUCTION

Raphia hookeri wine or Raphia wine, also known by the generic name of palm wine is produced by fermentation of the sap obtained from cut/incised Raphia palm trees. In Nigeria, palm wine is mostly obtained from raphia palm (*Raphia hookeri*) and oil palm (*Elaeis guineensis*) (Morah and Robinson, 2015). Whether from raphia palm or oil palm, palm wine is a popular local brew consumed by many across Africa that is mildly alcoholic, sweet in taste and gradually increases in alcoholic content upon fermentation (Chandrasekhar et al., 2012; Ogbonna et al., 2013; Morah and Robinson, 2015).

The economic, medicinal and social importance of palm wine has been the focus of a number of studies. Economically, palm wine tapping is a major occupational engagement for rural inhabitants in Nigeria, which can undergo prolonged fermentation to produce vinegar, distilled to obtain spirit or gin, and is a rich source of bacteria and yeasts which can be used as inoculum in fermentation processes (Chandrasekhar et al., 2012).

In terms of nutritional value, palm wine is a rich source of nutrients; the fresh palm sap is composed of sugars (sucrose, glucose and fructose), proteins, titrable organic acids, alcohol, vitamins (ascorbic acid, thiamine, riboflavin etc.), mineral elements and water (Obahiagbon, 2009; Chandrasekhar et al., 2012; Ogbonna et al., 2013; Kigigha et al., 2016). The most noticeable effects of prolonged fermentation (> 24 h) according to reports include: depletion of sugars, increase in alcoholic content, increase in acidity due to production of acetic acid, decrease in pH and loss in organoleptic properties, in particular, taste and flavour (Morah and Robinson, 2015).

Fermented palm wine is characterized by a slappy mouth feel, off flavour and loss in sweetness (Morah and Robison, 2015); several bacteria and yeast species inherent in palm wine have been

implicated for the fermentative processes (Chandrasekhar et al., 2012; Ogbonna et al., 2013; Kigigha et al., 2016). The aqueous extract of the dried reddish-brown petals (calyces) of *Hibiscus sabdariffa* is popularly called zobo in Nigeria. The plant which is commonly known as roselle is native to India and Malaysia where it is commonly cultivated, and is now found in many tropical countries of both hemispheres (Ogundapo et al., 2014).

Hibiscus sabdariffa is a dicotyledonous plant belonging to the family, Malvaceae (Oguntona, 1998). In Nigeria, it is grown commonly in the middle belt regions like Plateau, Nasarawa and Benue states and south western states like Ondo and Osun (Ogundapo et al., 2014). Roselle plant is rich in phytochemicals, especially anthocyanins, glycosides, alkaloids, tannins, polyphenols and saponins (Ogundapo et al., 2014). Accordingly, the plant has been demonstrated as possessing antihypertensive, antiseptic, stringent diuretic and puragative activities; which has been used as remedy for cancer, abscesses, cough, debility, dysuria, laxative, scurvy and fever (Olawale, 2011).

Depending on individual preferences, the extract is normally sweetened to taste with sugar, honey or pineapple juice, and sometimes flavoured with spices like ginger; other flavourings used include lime juice or artificial flavours like strawberry, vanilla etc. (Ogundapo et al., 2014). The acclaimed medicinal benefits and antioxidant potential of zobo drink has been the focus of several studies which have attributed these unique properties to the high content of polyphenols, in particular, the group anthocyanins (Philip et al., 2010; Sini et al., 2011; Ijeomah et al., 2012; Okereke et al., 2015).

Due to the varied applications, including its use as an alcoholic beverage in different ceremonies in Nigeria and other parts of Africa and as a stimulator or activator of breast milk in nursing mothers (Chandrasekhar et al., 2012; Kigigha et al., 2016), palm wine is a very important plant exudate in many African societies.

The present study therefore aimed at evaluating the effects of fermentation on the sensory, nutritional and antioxidant properties of mixtures of aqueous extract of *H. sabdariffa* (zobo drink) and raphia palm wine. The rationale was to obtain a beverage which had the sweet taste of palm wine preserved and antioxidant properties optimized as determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity.

MATERIALS AND METHODS

Source and preparation of samples

The Raphia palm wine was purchased fresh from a local palm wine tapper; stored in clean sterile containers and refrigerated at about 4°C. *H. sabdariffa* calyces were purchased from Abraka market, Delta State, Nigeria. Roselle calyces (1471 g) were steeped into 6 L of water (80°C for 30 min), and filtered using a clean sieve cloth (25% w/v) (Oboh and Okhai, 2012; Okoro, 2007).

Experimental design

Six (6) different palm wine and zobo blends (samples) were formulated within 24 h of wine purchase into sterile sample containers, and labeled as follows:

P_{100}-Z_0 = 100 ml palm wine mixed with 0 ml zobo extract
P_{80}-Z_{20} = 80 ml palm wine mixed with 20 ml zobo extract
P_{60}-Z_{40} = 60 ml palm wine mixed with 40 ml zobo extract
P_{40}-Z_{60} = 40 ml palm wine mixed with 60 ml zobo extract
P_{20}-Z_{80} = 20 ml palm wine mixed with 80 ml zobo extract
P_0-Z_{100} = 0 ml palm wine mixed with 100 ml zobo extract.

Samples were prepared in triplicates, and immediately sub-samples were collected for chemical/biochemical assays to determine concentrations for fresh unfermented samples (that is, Day 0). The remaining samples were left to ferment for 72 h (Day 3) at room temperature prior to analyses. Holes were bored on the cover of the sample containers to prevent bursting of containers due to accumulation of carbon (vi) oxide, a by-product of fermentation.

Chemical analyses

Glucose concentration was determined using the Randox glucose kit (Lothar, 1998) which is

based on the oxidase reaction which allows glucose determination after enzymatic oxidation in the presence of glucose oxidase. Procedures followed was as described by kit manufacturer. Briefly, 2000 µl of glucose reagent was added to 20 µl of sample. Standard contained only the glucose reagent, 2000 µl, a blank was also prepared which contained 20 µl of water. Resulting mixtures were incubated for 10 min at 37°C. Absorbance of standard ($A_{standard}$) and the sample (A_{sample}) were read against the reagent blank within 60 min at 546 nm. The concentration of glucose in sample was obtained using the relationship:

$$\text{Glucose concentration} = [A_{sample} \div A_{standard}] \times \text{Conc. of standard (100 mg ml}^{-1}\text{)}$$

Soluble protein content was determined by the method previously described by Ezeonu et al. (2014). 3 ml of Biuret's reagent was added to 2 ml of sample in a test tube, the content was thoroughly mixed and tubes kept at 37°C for 10 min, within this period colour (purple) development was observed. Absorbance of the colour complex was measured at 540 nm. Adjustment were carried out using the reagent blank (5 ml of biuret). The concentration of protein in the sample was determined with reference to standard Bovine Serum Albumin (BSA), and using the expression:

$$\text{Concentration of protein} = [A_{sample} \div A_{standard}] \times 30$$

Ascorbic acid (Vitamin C) concentration was determined by an iodometric titeration (Hartmann et al., 2008). 20 ml of sample was pipetted and mixed with 500 µl of 1% starch solution, and then titrated with iodine solution until end point (first sign of blue colour that remains after at least 20 s of swirling). The final volume was recorded, and the titeration repeated three times. Difference in volume was calculated as: D_v = Vf-Vi to give the volume of iodine solution used. 0.05% w/v that is, 0.05 g $100ml^{-1}$ of standard vitamin C solution was prepared and titeration performed immediately following similar procedure described earlier to prevent air oxidation of the ascorbic acid.

$$\text{Concentration of vitamin C in sample} = [\text{Titre value of sample} \div \text{Titre value of standard}] \times 0.05\%$$

Assay for DPPH radical scavenging activity

The DPPH radical scavenging activities of the samples were determined by the method of Blois (1958). 1 ml of methanol solution containing 0.2 ml of each of the samples was mixed with 1 ml of 0.15 mM DPPH solution (dissolved in methanol that is, 0.0059 g $100ml^{-1}$). The reaction mixture was then incubated for 30 min in the dark at room temperature. The control contained all reagents without the sample, while methanol was used as a blank. All measurements were performed in triplicates. DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm and expressed as the inhibition percentage of free radicals by the sample after calculation using the formula:

$$\%\ \text{DPPH scavenging activity or DPPH inhibition} = [A_c - A_s \div A_s] \times 100\%$$

Where A_c = Absorbance of control (DPPH), and A_s = Absorbance of sample

Sensory evaluation

Sensory evaluation of the palmwine-zobo blended samples was carried out for taste and colour as described by Obahiagbon et al. (2012).

Statistical analysis

The results were expressed as mean ± standard deviation. All the data were analysed using the SPSS statistical software (version 21). Mean concentrations or mean % inhibition were compared using a one-way Analysis of variance (ANOVA). Means that differed significantly were separated using least significant difference (LSD) multiple range test. Significant differences were accepted at $p < 0.05$.

RESULTS AND DISCUSSION

In an attempt to investigate the effect of fermentation on blends of raphia wine-zobo drink, concentrations of glucose, soluble protein and

Vitamin C were determined; in addition to assessment of the sensory and antioxidant properties based on the DPPH method of palm wine-zobo drink mixtures before and after fermentation.

Determination of glucose, protein and ascorbic acid content

The result for glucose determination is presented in Table 1. Generally, there was decrease in glucose concentrations upon fermentation for 72 h. The highest decrease was observed in the P_{100}-Z_0 blend (97.59 %), while the lowest decrease was in the P_{40}-Z_{60} blend (66.42 %).

At day 0 (that is, fresh samples) there was no significant difference in glucose concentrations in blended mixtures; however, compared to the control (100% palm wine sample, P_{100}-Z_0), glucose concentration was significantly higher ($p < 0.05$). The detected high glucose content corroborates with previous studies that have reported that the second predominant sugar in fresh palm sap is glucose (Chandrasekhar et al., 2012; Ogbonna et al., 2013; Santiago-Urbina et al., 2013; Ulonoeme et al., 2014).

Furthermore, Philip et al. (2010) reported that *H. sabdariffa* (zobo drink) is low in sugar. This

Table 1. Glucose concentration in pure (100%) and mixed samples of *R. hookeri* (raffia) wine and *H. sabdariffa* (zobo) aqueous extract (mg 100ml^{-1} of sample). Results expressed as Mean ± S.D. Values not sharing a common alphabet (a - b) differed significantly (P < 0.05).

Sample	Day 0	72h fermentation	Decrease in glucose (%)
P_{100}-Z_0	308.3±34.2^a	7.42±1.95^b	97.59
P_{80}-Z_{20}	218.7±4.67^a	8.97±0.64^b	95.90
P_{60}-Z_{40}	136.5±34.2^a	6.59±0.00^b	95.17
P_{40}-Z_{60}	153.0±32.2^a	51.38±2.72^{a,b}	66.42
P_{20}-Z_{80}	181.9±80.0^a	52.20±3.89^{a,b}	71.30
P_0-Z_{100}	130.3±0.78^a	11.81±6.60^b	90.94

low sugar content coupled with a diluting effect could have resulted to the observed decrease in the glucose content of fresh palm wine-zobo mixtures.

Nevertheless, the addition of zobo drink did not significantly alter the sweetness of fresh palm wine. Therefore, mixtures of fresh palm wine and zobo drink could easily serve as a beverage drink retaining the popularly loved sweetness of palm wine and packed full with antioxidants from zobo. The major effect of fermentation on glucose levels was a depletion in glucose content across all samples; the order of decrease was: P_{100} - Z_0 (97.59%) > P_{80} -Z_{20} (95.90%) > P_{60} - Z_{40} (95.17%) > P_0 - Z_{100} (90.94%) >P_{20} -Z_{80} (71.30%) > P_{40} -Z_{60} (66.42%). This is in line with previous studies that have identified decreases in the levels of sugars among others as observable changes in palm wine upon fermentation (Onwuakor and Ukaegbu-Obi, 2014; Morah and Robinson, 2015).

Interestingly, the P_{40}-Z_{60} blend had the least decrease in glucose content (66.42%) after fermentation for 72 h; closely followed by the P_{20}-Z_{80} blend (71.30%). Increasing the concentration of zobo in the mixtures appeared to reduce the rate at which fermentative microorganisms utilized the glucose and possibly other sugars. Olaleye (2007), Fullerton et al. (2011) and Puro et al. (2014) in separate studies have reported the antibacterial activities of aqueous, methanolic and ethanolic extracts of roselle against a wide genera of bacteria; including *Staphylococcus*, *Bacillus*, *Clostridium*, *Klebsiella* and *Pseudomonas* species. This bactericidal action may have reduced available microbial population thus slowing down the rate of fermentation. There are no reports to indicate that roselle plant extracts have any antifungal properties.

For fresh samples, soluble protein concentrations increased significantly ($p < 0.05$) with addition of zobo drink for the P_{40}-Z_{60} and P_{20}-Z_{80} blends (Table 2). There was significant decrease in protein content in samples P_{80}-Z_{20}, P_{40}-Z_{60} and P_{20}-Z_{80} ($p < 0.05$) after fermentation. Protein concentration in sample P_{60}-Z_{40} before and after fermentation did not differ significantly ($p > 0.05$). The highest decrease was observed in the P_{100}-Z_0 mix (25.97%); fermentation significantly decreased

Table 2. Soluble protein concentration in pure (100%) and mixed samples of *R. hookeri* (raffia) wine and *H. sabdariffa* (zobo) aqueous extract (mg $100ml^{-1}$ of sample). Results expressed as Mean ± S.D. Values not sharing a common alphabet (a - b) differed significantly ($P < 0.05$).

Sample	Day 0	72h fermentation	Decrease in protein content (%)
P_{100}-Z_0	37.82±0.06[a]	28.00±1.40[b]	25.97
P_{80}-Z_{20}	39.92±0.30[a]	33.47±0.07[a,b]	16.50
P_{60}-Z_{40}	39.60±1.56[a]	37.48±0.10[a]	5.35
P_{40}-Z_{60}	50.02±0.75[a]	40.00±0.78[a,b]	20.03
P_{20}-Z_{80}	47.80 ±0.34[a]	40.11±1.46[a,b]	16.09
P_0-Z_{100}	45.49±1.36[a]	42.27±0.92[a]	7.08

protein content ($p < 0.05$) in the 100% palm wine sample. The sample with the least decrease was the P_{60}-Z_{40} blend; mean soluble protein concentration decreased from 39.60±1.56 mg $100ml^{-1}$ to 37.48±0.01 mg $100ml^{-1}$ corresponding to only 5.35% decrease.

Compared to crude protein content values reported in studies such as Obahiagbon and Oviasogie, (2007), Ogbonna et al. (2013) and as reviewed by Chandrasekhar et al. (2012), the mean soluble protein concentration for fresh palm wine reported in this study was high (37.82±0.06 mg $100ml^{-1}$ of sample). Differences could be attributed to particularly high synthesis in the strain of Raphia palm from which exudate was obtained and method of analysis.

Nevertheless, studies have indicated that the Raphia palm sap and hence palm wine have low protein content compared to *H. sabdariffa* which is rich in protein (Halimatul et al., 2007; Obahiagbon and Oviasogie, 2007; Obahiagbon et al., 2012; Ogbonna et al., 2013). Fermentation decreased soluble protein content in 4 out of 6 samples (Table 2); this is contrary to the report by Singaravadivel et al. (2012) and Nwafor and Akpomie (2014), who observed an increase in protein content during storage of palm wine and zobo drink respectively.

Ascorbic acid concentration in mg $100ml^{-1}$ of sample is shown in Table 3; generally, higher concentrations were detected in fresh samples and increased with increasing amount of zobo drink added.

This was not unexpected since both *H. sabdariffa* and Raphia palm have high ascorbic acid

Table 3. Ascorbic acid concentration in pure (100%) and mixed samples of *R. hookeri* (raffia) wine and *H. sabdariffa* (zobo) aqueous extract (mg $100ml^{-1}$ of sample). Results expressed as Mean ± S.D. Values not sharing a common alphabet (a - b) differed significantly ($P < 0.05$).

Sample	Ascorbic acid concentration (mg $100ml^{-1}$ of sample)		
	Day 0	72h fermentation	Decrease (%)
P_{100}-Z_0	70.00±10.0[b]	40.00±0.00[b]	42.86
P_{80}-Z_{20}	80.00±10.0[a]	80.00±0.00[a]	0.00
P_{60}-Z_{40}	90.00±0.00[a]	89.00±10.0[a]	1.11
P_{40}-Z_{60}	130.0±20.0[a]	130.0±0.00[a]	0.00
P_{20}-Z_{80}	170.0±10.0[a]	140.0±40.0[a]	17.65
P_0-Z_{100}	190.0±0.00[a]	160.0±20.0[a]	15.79

(Vitamin C) content (Ijeomah et al., 2012; Oboh and Okhai, 2012; Chandrasekhar et al., 2012). The most striking observation is the lack of change in mean concentrations for samples P_{80}-Z_{20} and P_{40}-Z_{60} after fermentation for 72 h. In 100% palm wine samples, there was a significant decrease in ascorbic acid concentration on fermentation ($p < 0.05$).

However, for the various blends and 100% zobo samples, either there was no change or decrease in concentration was not significant ($p >$

0.05). As mentioned earlier, this may be attributed to reduction in fermentation rate due to the antibacterial potential of *H. sabdariffa*.

Antioxidant content as determined by DPPH (2, 2- diphenyl-1-picrylhydrizyl) method

The values obtained for antioxidant content based on % DPPH inhibition for fresh and fermented samples are presented in Figure 1. The values displayed by fresh samples followed the trend: P_{80}-Z_{20} > P_{40}-Z_{60} > P_{60}-Z_{40} > P_{20}-Z_{80} > P_0-Z_{100} > P_{100}-Z_0. After fermentation for 72 h, the trend became: P_{20}-Z_{80} > P_0-Z_{100} > P_{60}-Z_{40} > P_{40}-Z_{60} > P_{100}-Z_0 > P_{80}-Z_{20}.

By contrast, the blend with the highest activity prior to fermentation became the least with

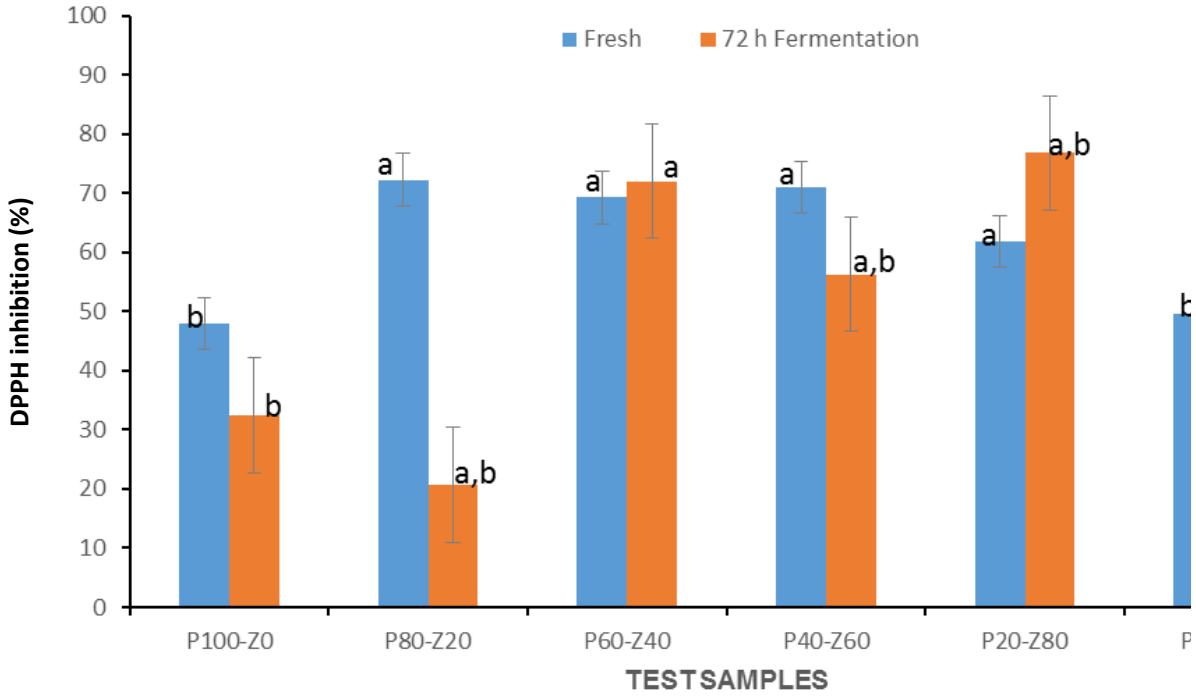

Figure 1. DPPH radical scavenging activity of pure (100%) and mixed samples of *R. hookeri* (raffia) wine and *H. sabdariffa* (zobo) aqueous extract (mg 100ml^{-1} of sample). Results expressed as Mean ± S.D. Values not sharing a common alphabet (a - b) differed significantly (P < 0.05).

free radical scavenging activity against DPPH. Aside from high vitamin C content, fresh palm wine has not been reported as having any of the phytochemicals previously documented for roselle calyces of *H. sabdariffa* used in the preparation of zobo drink (Ijeomah et al., 2012). Both fresh 100% palm wine and fresh 100% zobo drink exhibited antioxidant activities in scavenging DPPH radical (47.95 ± 2.19% and 49.50 ± 0.42% respectively) and no significant difference ($p < 0.05$) was observed.

Addition of zobo to palm wine in various proportions significantly increased the antioxidant activities of fresh samples to varying levels ($p < 0.05$); while fermentation (72 h) either increased or decreased antioxidant content in blended samples. Like palm wine, zobo drink is subject to microbial fermentation and many studies have reported changes in organoleptic properties, increase in acidity and loss of nutritional value as some of the deleterious effects (Ogiehor et al., 2008; Braide et al., 2012). However, the benefits of controlled fermentation during zobo drink preparation have been explored; Ogiehor et al. (2008) recorded gradual increases in protein, carbohydrate, vitamin C and total soluble solids (TSS) contents in zobo drink for a storage period of 6-9 days.

In another study, Nwafor and Akpomie (2014) reported that though fermentation decreased pH and increased titrable acids, there was an increase in

protein, carbohydrate and vitamin C contents over a 48 h period, and fermentation by *S. cerevisiae* was recommended as best for preservation of aroma, visual appearance, taste and overall acceptability. For sensory evaluation based on aroma, taste, colour and overall acceptability the P_{80}-Z_{20} mixture scored higher since it had an appealing red colour, with the sweet taste of palm wine preserved (data not shown).

Conclusion

Fermentation by inherent micro flora had an effect on the sensory, nutritional and antioxidant properties of fresh palm wine (100%) / zobo drink (100%), and blends of the two beverages mixed in different proportions.

Generally, attributes such as protein, vitamin C and antioxidant contents were increased in fresh samples upon addition of zobo drink. The observed effect of fermentation over a period of 72 h was dependent on the food parameter being measured and whether samples were blended or fresh.

Overall, glucose and protein contents were decreased, vitamin C content was either decreased or unchanged and DPPH radical scavenging activity increased depending on the amount of zobo added. The decision as to the best combination of raphia wine and zobo extract is not a clear cut one since blends were optimized differently for the parameters studied.

Based on antioxidant properties and sensory evaluation, we recommend the P_{80}-Z_{20} raffia wine/zobo extract mixture, this blend had an appealing red colour, retained the sweet taste of palm wine, and DPPH radical scavenging activity was optimized; it is advised that this be consumed fresh to avoid sour test and depletion of nutrients associated with prolonged fermentation. In terms of estimated cost of formulation, it is also relatively cheaper compared to the fresh 100% palm wine.

Conflicts of interest

The authors have not declared any conflict of interests.

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