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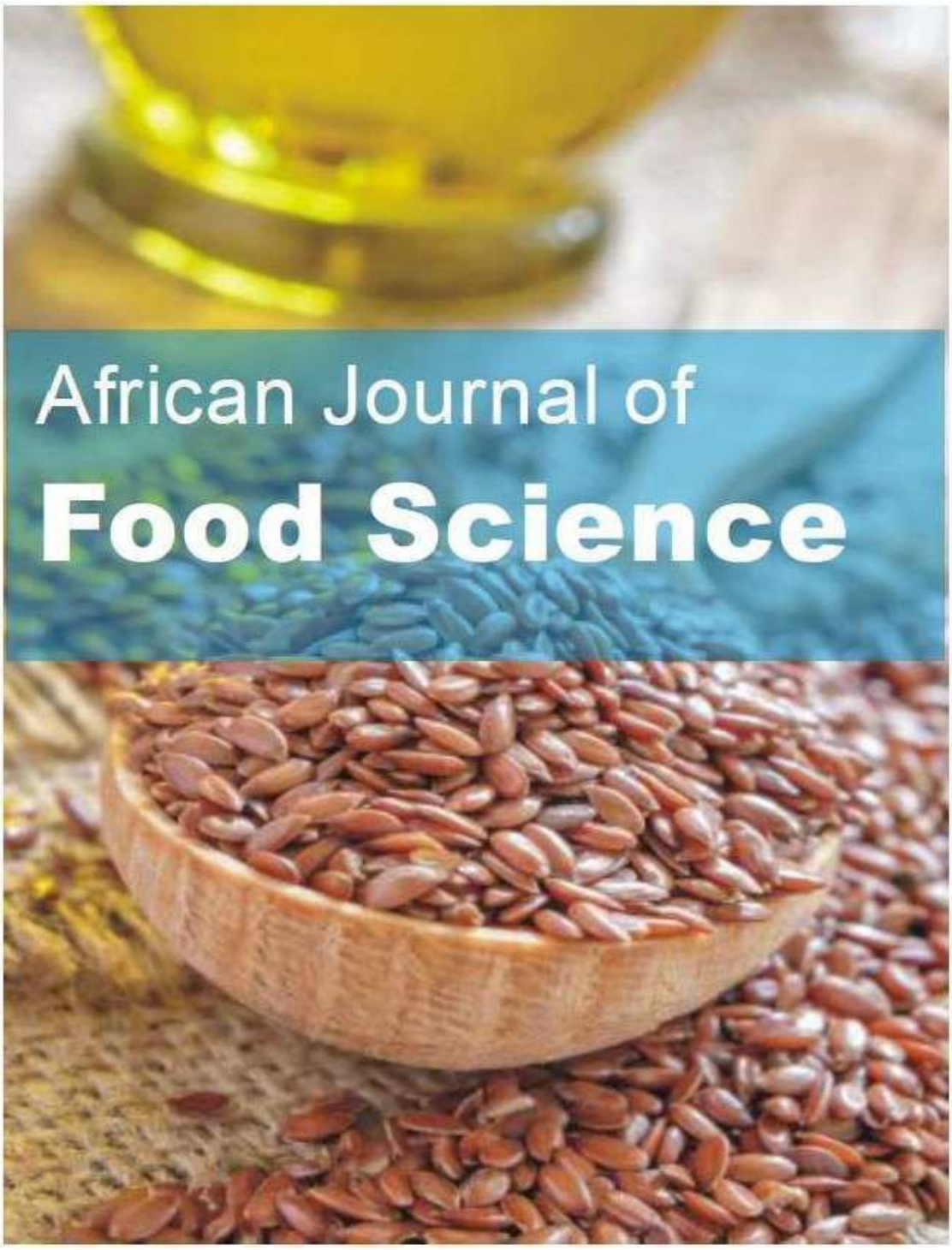

# African Journal of Food Science

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# Table of Content

**Fruit consumption and storage practices among rural households in Chamwino district, Dodoma, Tanzania** 130

Mwanaisha Assim Ngawembela, Akwilina Wendelin Mwanri and Lucy Mlipano Chove

**Studying the ethylic fermentation process of the mucilage juice of cacao by Saccharomyces cerevisiae yeast** 141

Philippe Diakabana, Michel Elenga, Okil Derda Goma, Jean Paul Latran Ossoko, Arnaud Wenceslas Geoffroy Tamba Sompila, Claudia Bassoumba and Léa Nkounkou

**Quantification of ethanol and identification of other chemical constituents in homemade morula beer using gas chromatography-mass spectrometry (GC-MS)** 148

Mmaabo Tsenang, Tshepo Pheko-Ofitlhile, Janes Mokgadi, Wellington Masamba and Gothatamang Norma Phokedi

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African Journal of Food Science

*Full Length Research Paper*

# Fruit consumption and storage practices among rural households in Chamwino district, Dodoma, Tanzania

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**Fruit consumption is still a challenge in many parts of Africa, and hence micronutrient deficiency continues to be a serious problem in the continent. This study was conducted between December 2017 and May 2018 in Chinoje and Mzula villages in Chamwino district, Dodoma to assess availability of fruit, consumption, storage practices and nutrient content. People responsible for food preparation were interviewed from 345 randomly selected households by using semi-structured and food frequency questionnaires. Multiple logistic regression model was used to determine the relationship between frequency of fruit consumption and household socio-economic features by using SPSS. Laboratory analysis was conducted to determine nutrient content of baobab, which was the most consumed fruit. Analysis of Variance (ANOVA) was used to determine if significant variations existed in the nutritional quality of baobab fruit by using SAS. Only 35% of the households consumed fruit daily, while the majority consumed fruit from one to three days in a week. Monthly income, household size and headship significantly affected fruit consumption at $p<0.05$. Most of the baobab fruits were stored in polypropylene sacks (77.4%), followed by plastic buckets (3.3%) and others as shelled fruit (18.4%). Significant losses in Vitamin C and total carotenoids were observed in baobab fruits that were stored in sacks. Storage of baobab fruit in plastic bucket is recommended for quality maintenance of nutrients.**

**Key words:** Fruit availability, consumption, micronutrients, food frequency, storage.

## INTRODUCTION

Most of the developing countries face serious malnutrition problems. Vitamins and mineral deficiencies are high in Tanzania; whereby about 34 and 58% of children below five years are vitamin A and iron deficient, respectively (MoHCDGEC et al., 2016). Most rural diets are dominated by starchy and legume staples with little diversity leading to insufficient intake of micronutrients (Mbwana et al., 2016). More than 80% of people in low and middle income countries consume small amount of fruit that is below the WHO minimum recommended amount of 400 g

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per day (Frank et al., 2019). Generally, Tanzania's fruit and vegetable consumption is lower than the recommended servings per day with higher consumption in urban compared to the rural consumers (Van der Maden et al., 2021).

Fruit is an important components of a healthy diet due to their low energy density, while rich in vitamins, minerals, fibres, and phytochemicals (Wallace et al., 2020; Del Río-Celestino and Font, 2020; Ahmed et al., 2022a). The burden of micronutrient deficiencies in the developing world particularly in populations with low intake of animal protein foods is intensified by low fruit intake (Frank et al., 2019). Evidence shows that fruit intake is associated with reduced risk for several non-communicable and chronic diseases (Yahia et al., 2017; Ahmed et al., 2022b). Approximately 2.7 million (2.8%) deaths per annum worldwide are linked to inadequate intake of fruit (WHO, 2022). The WHO ranks low fruit consumption as the sixth main risk factor for mortality in the world. Food consumption, especially fruit can be influenced by various factors including knowledge, attitude, social cultural factors, socio-economic factors, seasonal variation, level of production, processing and storage technologies (Amini et al., 2021; Basarir et al., 2022; Wallace et al., 2020).

Fruit are highly perishable, facing up to 50% postharvest losses in developing countries, particularly in Tanzania (Mujuka et al., 2020; Baltazari et al., 2020; Nadeem et al., 2022). Fruit storage is among the important post-harvest treatments towards reducing fruit losses thus improving their availability (Baltazari et al., 2020; Xylia et al., 2022; Al Shaibani et al., 2022).

Researches that have been conducted in Tanzania and other parts of the world, report about the nutritional and health importance of fruit and promote production and proper post-harvest handling so as to improve their availability (Van der Maden et al., 2021; Amao, 2018; Makule et al., 2022; Etefa et al., 2022). However, information on fruit consumption, rural household storage practices and the effectiveness in maintaining their nutritional quality is limited. Therefore, this study is aimed at assessing the frequency of fruit consumption, identifying storage practices and their effect on nutritional quality of fruits in rural Dodoma.

## METHODOLOGY

### Description of the study area

Chamwino is one of the six districts in Dodoma region. It is located in the central plateau of Tanzania extending between latitude 4° and 8° South and between 32° and 37° East. According to 2012 National Population Census, Chamwino district council has about 330 543 people of which 171 661 are females and 158 882 are males. The district has 5 divisions, 28 wards and 77 villages (Figure 1). The district has a dry savannah type of climate characterized by a long dry season starting late April to early December, and a short rain season starting from December to mid-April (Mutabazi, 2013; URT, 2013).

### Study design and sampling procedure

The cross sectional study was conducted from December 2017 to May 2018. Multistage sampling techniques were used for the study whereby, two villages, Mzula and Chinoje from Chamwino district in Dodoma region, were randomly selected. Dodoma region was purposively selected because it is dominated by semi-arid climate which can affect fruit production and hence consumption. In addition, it is one of the regions with high prevalence of micronutrient deficiency such as iron deficiency (48%) (MoHCDGEC et al., 2016).

A sample of 172 and 173 households were selected from Chinoje and Mzula villages, respectively. Households were randomly selected with inclusion criteria of having a mother/caregiver who consented to participate in the study. Respondents were mothers/caregivers from the sampled households who were responsible for food preparation in the households.

### Data collection

Interviewer administered questionnaire was used to collect demographic and socioeconomic information. Information about types of fruit available and consumed, factors influencing fruit consumption and storage practices were collected through face to face interview by using semi structured questionnaire. Data on frequency of fruit consumption was collected by using Food Frequency Questionnaire (FFQ).

### Sampling of baobab fruit for laboratory analysis

Baobab fruits were the only sampled fruits for laboratory analysis since they were the most consumed fruits in both villages. Using completely randomized design (CRD) with $2 \times 3 \times 2$ factorial arrangement, sample size was determined by treatment combination and their replication. When two locations, three storage practices and four replicates were combined, 24 samples were obtained. The 24 samples were then multiplied into two storage times making a total of 48 samples for laboratory analysis. A total of 24 samples were collected at the beginning of the study, and the rest were collected after six months of storage. These samples were put in labelled polyethylene bags, packed in cool boxes and transported to Sokoine University of Agriculture (SUA) at the Department of Food Science and Agro-processing Laboratory for nutritional analysis.

### Laboratory analysis

Fruit samples were analysed for moisture content, vitamin C and total carotenoid contents. Moisture content in the fruit samples was determined by oven drying method according to AOAC Method 934.01 (AOAC, 2007). Ascorbic acid was determined by using 2, 6-Dichloro-indophenol Titrimetric Method according to AOAC Method 967.22, 45.1.15 (AOAC, 2007). Total carotenoid was determined by spectrophotometric methods at 450 nm according to AOAC method 941.15 (45.1.03) (AOAC, 2007).

### Ethical clearance and consideration

Ethical clearance was granted by the Ministry of Health, Community Development, Gender, Elderly and Children (MoHDEC) through the National Institute for Medical Research in Tanzania with reference number NIMR/HQ/R.8a/Vol. IX/2226. The permit and introduction letter was provided by the Sokoine University of Agriculture and respective region, district and villages authorities. Participants were

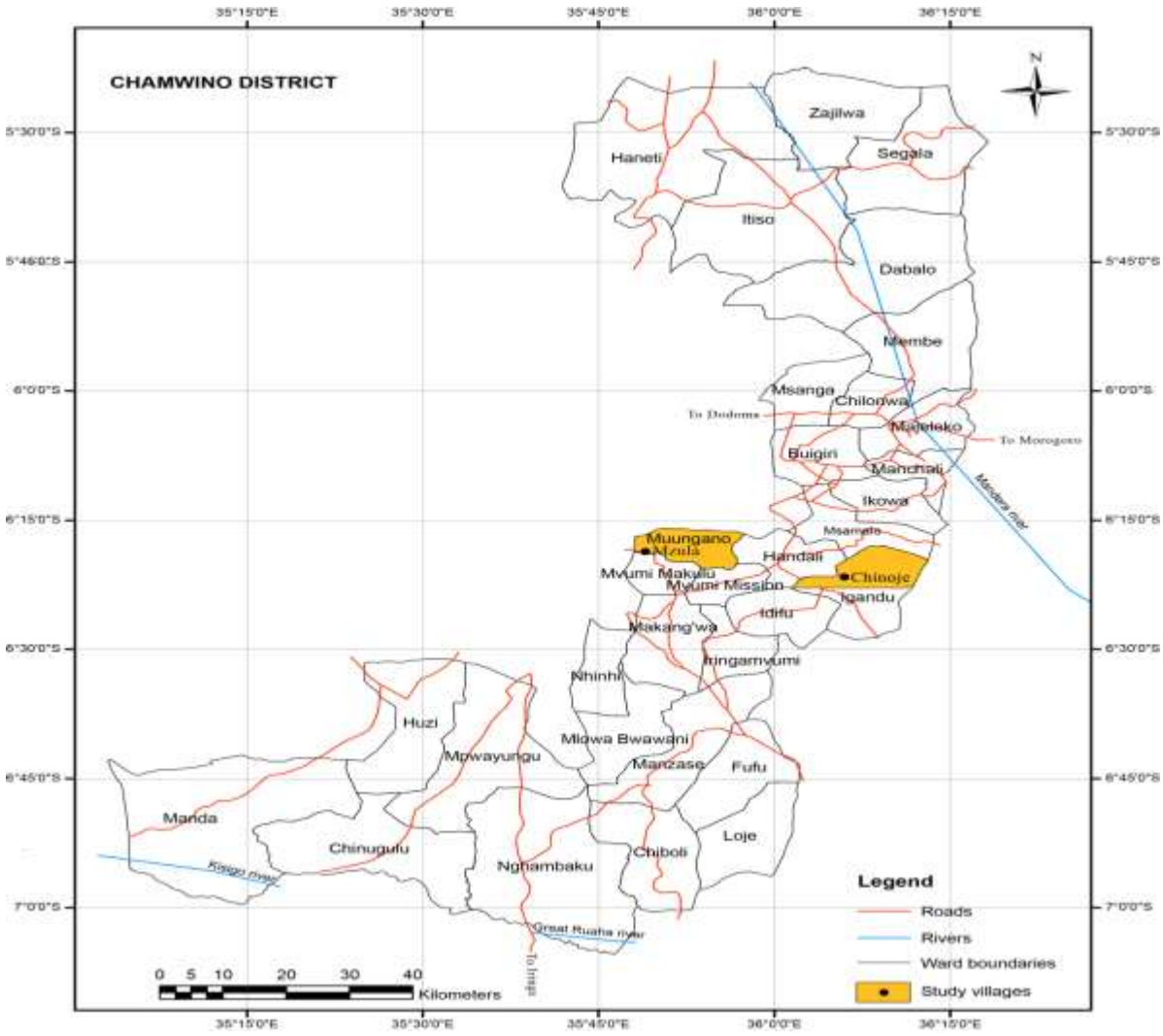


**Figure 1.** Map of Chamwino District showing the study areas.
Source: Mutabazi (2016).

provided with detailed information about the study and were requested to give their consent to participate in the study.

### Data analysis

This was done by using Statistical Package for the Social Sciences (SPSS) software version 21. Descriptive statistics (frequency) was used for presenting the household socio-economic characteristics, levels of fruit consumption as well as fruit storage practices identified in the study area. Logistic regression model was applied to establish relationship between fruit consumption frequency and socio-economic factors. Daily fruit consumption was the dependent variable while household social, demographic and economic features were independent variables.

Laboratory data was analysed using Statistical Analysis System (SAS) version 9.2. Means and standard error were used to present nutritional quality of fruit. Analysis of Variance was done to determine the significant variations at ($p<0.05$) in the nutritional quality due to location, storage practice and time and their interaction effect. Means were separated by Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

### Socio-economic and demographic characteristics of the respondents

About 65 and 10% of the respondents were monogamous and polygamous couples, respectively. Almost all respondents were farmers, 56.5% had primary education and 56.2% could read and write. Majority of households were male headed (Table 1). The average monthly household income was Tanzanian Shillings (TZS) 75000.

### Availability of fruit

Both exotic and indigenous fruits were available in the study area. These included mangoes, oranges, pawpaw, watermelons, pineapples, bananas, baobab, *Grewia*

**Table 1.** Socio-economic and demographic characteristics of rural respondents in Chamwino District

| Variable | Category | n | % |
|---|---|---|---|
| Status of the respondent | Mother | 290 | 84.1 |
| | Caregiver | 55 | 15.9 |
| Sex of the household head | Male | 257 | 74.5 |
| | Female | 88 | 25.5 |
| Marital status of mother or caregiver | Monogamous | 223 | 64.7 |
| | Polygamous | 34 | 9.9 |
| | Widowed | 38 | 11 |
| | Single | 50 | 14.5 |
| Literacy level of mother or caregiver | Not able to read and write | 151 | 43.8 |
| | Can read and write | 194 | 56.2 |
| Educational level of mother or caregiver | No formal education | 141 | 40.9 |
| | Adult education | 8 | 2.3 |
| | Primary school | 195 | 56.5 |
| | Post-secondary | 1 | 0.3 |
| Household size | 1-3 | 25 | 7.2 |
| | 4-5 | 133 | 38.6 |
| | >6 | 187 | 54.2 |
| Main Source of income | Farmer | 343 | 99.4 |
| | Self employed | 2 | 0.6 |
| Monthly income (TZS) | Low income (< 200,000) | 330 | 95.6% |
| | Low middle income (200,000 - 800,000) | 15 | 4.4% |

Source: Authors

**Table 2.** Source of fruits available in Mzula and Chinoje households.

| Fruits | Own production | | Purchase | | Gift/food aid | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| Papaya | 21 | 6.1 | 321 | 93 | 3 | 0.9 |
| Watermelon | 325 | 94.2 | 15 | 4.3 | 5 | 1.5 |
| Oranges | 0 | 0 | 345 | 100 | 0 | 0 |
| Avocado | 0 | 0 | 345 | 100 | 0 | 0 |
| Grapes | 1 | 0.3 | 344 | 99.7 | 0 | 0 |
| Mangoes | 9 | 2.6 | 333 | 96.5 | 3 | 0.9 |
| Banana | 0 | 0 | 345 | 100 | 0 | 0 |
| Pineapples | 0 | 0 | 345 | 100 | 0 | 0 |
| Dates | 0 | 0 | 345 | 100 | 0 | 0 |

Source: Authors

*bicolor* (donkey berries), *Grewia fallax* and *Grewia platyclada,* most of which were available on season. Most fruits were obtained through purchase, except for watermelon which was produced by more than 90% of the households (Table 2).

Fruits available in the study area did not differ from other parts of Tanzania (Aluko et al., 2016; Match Maker Associates, 2017; Tairo, 2021). Unlike other fruits, baobab fruits were available throughout the year and could also be stored for longer periods of time. Several other fruits were obtained through purchase in the village retail/periodic markets and also from vendors. Watermelons were produced by 94% of households in both villages, while a few households produced papaya

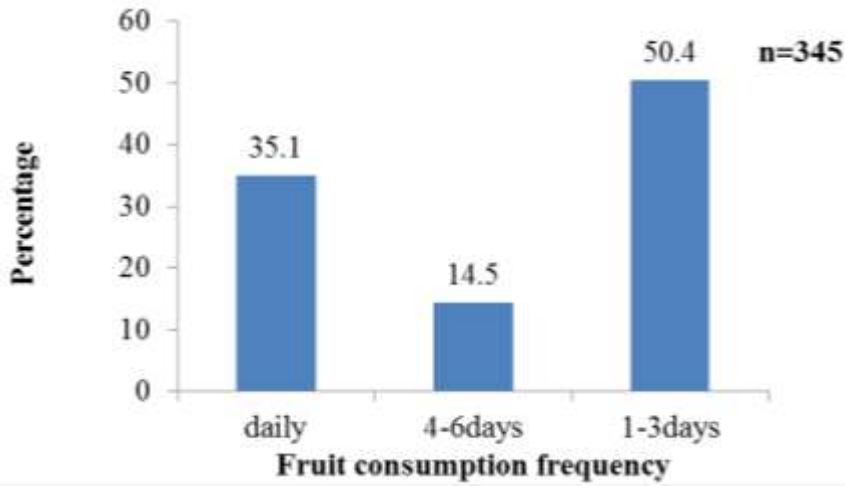


**Figure 2.** Weekly household fruit consumption.
Source: Authors

**Table 3.** Households fruit consumption by types and frequency (N = 345).

| Fruit | Everyday in the week | | 4-6 days/week | | 2-3 days/week | | Rarely | | Never | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % | n | % |
| Papaya | 4 | 1.2 | 25 | 7.2 | 38 | 11 | 129 | 37.4 | 149 | 43.2 |
| Oranges | 9 | 2.6 | 34 | 9.9 | 28 | 8.1 | 124 | 35.9 | 150 | 43.5 |
| Avocado | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0.6 | 343 | 99.4 |
| Grapes | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1.2 | 341 | 98.8 |
| Mangoes | 12 | 3.5 | 2 | 0.6 | 57 | 16.5 | 194 | 56.2 | 80 | 23.2 |
| Banana | 9 | 2.6 | 11 | 3.2 | 61 | 17.7 | 113 | 32.8 | 151 | 43.8 |
| Pineapples | 0 | 0 | 0 | 0 | 0 | 0 | 29 | 8.4 | 316 | 91.6 |
| Dates | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.3 | 344 | 99.7 |
| Tamarind | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 9.6 | 312 | 90.4 |
| *Grewia bicolor (Mtafuta)* | 11 | 3.2 | 5 | 1.4 | 28 | 8.1 | 67 | 19.4 | 234 | 67.8 |
| *Grewia fallax (Ngwelu)* | 11 | 3.2 | 4 | 1.2 | 35 | 10.1 | 58 | 16.8 | 237 | 68.7 |
| *Grewia platyclada (Pelemehe)* | 10 | 2.9 | 16 | 4.6 | 36 | 10.4 | 111 | 32.2 | 172 | 49.9 |
| Baobab | 75 | 21.7 | 69 | 20 | 179 | 51.9 | 22 | 6.4 | 0 | 0 |

Source Authors

and mangoes. Low production of fruits in the villages could be attributed to households' preferences for satisfying basic energy needs instead of micronutrients. As a result more effort was put on production of starchy staples and legumes such as maize, millet, beans and peas. Limited knowledge and awareness regarding the role of fruits on alleviation of micronutrients deficiency could be the reason for low fruit production. These observations were also reported by other researchers (Mbwana et al*.,* 2016; Kissoly et al., 2018).

## Consumption of fruit

Majority of the households consumed at least one fruit once (rarely) to about 2-3 days in a week while 35.1% consumed fruits daily (Figure 2). Daily consumption of some fruit such as baobab, bananas and mangoes was practiced by only 21.7, 2.6 and 3.5% households, respectively (Table 3).

Low daily consumption of mangoes, papaya, oranges, and bananas was due to low purchasing power. This has been reported in Tanzania and other parts of Africa (Kinabo et al., 2016; Msambichaka et al., 2018; Kabwama et al., 2019; Xaba and Dlamini, 2021). Baobab fruits were the most consumed fruits by all households in both villages. This is because they were available throughout the year, even during off season. Moreover, diversification into various products such as spices, sugar substitutes and/or flavours to dishes such as porridge

**Table 4.** Factors affecting fruit consumption.

| Characteristics | n | % | Crude OR (95%CI) | *P*-Value | Adjusted OR* (95%CI) | *P*-Value |
|---|---|---|---|---|---|---|
| Monthly income | 345 | 100 | 1.2 (1.11-1.57) | 0.016 | 1.6 (1.19-2.92) | 0.004 |
| Household size | 345 | 100 | 0.3 (0.20-0.76) | 0.023 | 0.5 (0.37-0.63) | 0.035 |
| **Education level** | | | | | | |
| Formal education | 204 | 59.1 | 1.5 (0.32-2.86) | 0.85 | 1.4 (0.2-2.87) | 0.27 |
| No formal education | 141 | 40.9 | 0 | | 0 | 0 |
| **Sex of HH** | | | | | | |
| Female | 88 | 25.5 | 1.6 (1.30-2.52) | 0.036 | 2.1 (1.66-4.96) | 0.040 |
| Male | 257 | 74.5 | 0 | | 0 | 0 |
| **Awareness** | | | | | | |
| Aware | 322 | 93.3 | 1.2 (0.54-3.31) | 0.56 | 1.1 (0.27-3.82) | 0.864 |
| Not aware | 23 | 6.7 | 0 | | 0 | 0 |

*Multivariate analysis of all the variables with backward exclusion.
Source: Authors

have all contributed to their increased consumption. This was also reported in Kilifi and Kitui counties in Kenya (Wanjeri et al., 2020). They are also used as medicine to relieve the symptoms of stomach upset. Medicinal uses of indigenous fruits by rural residents in Africa such as treatment of diarrhoea and skin diseases were reported by Maroyi (2019), Lisao et al. (2017) and Pfukwa et al. (2020).

## Factors affecting fruit consumption

Table 4 shows factors affecting fruit consumption. Monthly income, household size and headship significantly influenced daily fruit consumption. Neither the education level of a mother or caregiver nor knowledge on the benefits of fruit to health had any significant influence on daily fruit consumption.

The income of consumers may determine and/or affect what they are able to purchase or consume. In the current study, an increase in household income significantly contributed to the increased fruit consumption. This suggests that, low income households allocate their budget to staple foods such as maize, sorghum and some pulses in order to fulfil their basic energy needs and to avoid hunger while higher income earners tend to have increased demand for other foods including fruits and vegetables. Studies in Tanzania and other African countries have reported on the significant positive influence of income status on increased fruit consumption (Mayen et al., 2016; Miller et al., 2016; Xaba and Dlamini, 2021).

Household size had a negative influence on daily fruit consumption. Daily intake of fruit decreased as household size increased. As stated previously, the priority of most poor households is to fulfil basic energy requirements. Fruits are not considered as a priority when resources are limited, especially where large families are involved. Similar findings reported by Plataroti (2016).

Daily fruit consumption increased more in female headed households than in the male headed households. This is an indication of difference in budget allocation to fruit between male and female headed households in the study area. These results are consistent with Asli (2020) and Plataroti (2016) who reported significant differences between male and female headed households with the later consuming more fruits than the former. These researchers stipulated that the quality of diets could be improved when females have full control over household resources.

Knowledge about fruit consumption did not show any significant impact on fruit consumption. Participation in nutrition training and awareness programs can contribute to new knowledge, leading to change in consumer behaviour. However, behavioural change is influenced several other factors including experience and culture. A study by Wagner et al. (2016) reported an improvement in the consumption frequency of antioxidant-rich fruits and vegetables among overweight and obese adults as a result of nutrition education. Nutritional education intervention was also reported to have improved fruits and vegetables preference among school children in West Texas, USA (Saha et al., 2020).

Education level of mothers/caregivers did not significantly influence fruit consumption in the households. The level of literacy may influence individuals to seek knowledge about healthy diets and lifestyles hence leading to increased consumption of healthier foods such as fruits. However, as previously stated, culture and experience play a significant role in behavioural change is irrespective of whether the individuals are educated

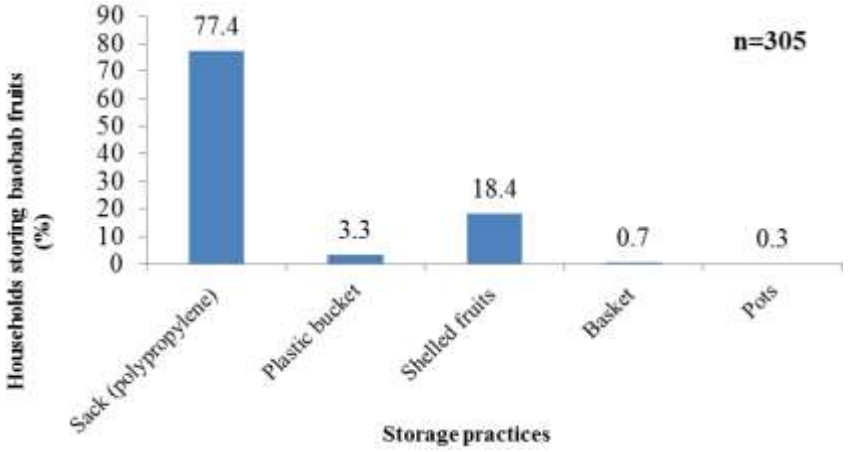


**Figure 3.** Baobab fruit storage practices in the village households.
Source: Authors

or not. These results are contrary to Msambichaka et al. (2018) who reported significantly higher fruit intake by educated people than the non-formally educated people in semi-urban Tanzania. A study by Kebede et al. (2022) in Addis Ababa Ethiopia, found that increased intake of plant foods including fruits was related to the education level of the mother/caregiver.

## Baobab fruit storage practices

Baobab fruits were the most stored fruit in the households. They were stored in polypropylene sacks (Figure 3). Though the sacks were used for both shelled and un-shelled baobab fruits, it was more common for unshelled fruits. Storing of unshelled fruit allowed maximum use of the space in the sacks. A study by Eldoom et al. (2014) reported the use of polyethylene bags for storage of baobab fruit in Sudan. Normally, the shelled fruit were kept in polypropylene sacks and some were spread on the ground and roofs. Storage of fruit in their shells maintained colour and prevented fruit contamination by dust. Storage of shelled (whole) fruit has also been observed in other parts of East Africa (Wanjeri et al., 2020). Plastic containers were also used for storage of unshelled fruit. Baobab fruits and their products such as pulp powder were packed in plastic labelled containers for trading to be used as food (Darr et al., 2020). Baskets were normally used to keep shelled fruit whereas pots were used to keep unshelled fruit. The use of baskets and pots in baobab fruit storage has also been reported by Dandago et al. (2016).

## Baobab fruit storage time

Baobab fruit were stored for about one week to ten months with the majority of households storing them for up to six months (Figure 4).

## Quality deterioration observed during baobab fruit storage

Pests and insect infestation were the most important factors responsible for quality deterioration of baobab fruits during storage (Table 5).

Rats and termites were responsible for the observed infestation in baobab fruit both in the sacks as well as in shells. Poor strength of sacks and baobab shells provided easy entry and growth of pests and insects which could contaminate stored fruit by larvae, exuviae, and excreta (Vukajlović et al., 2018). Insect infested fruits lose their quality and appearance leading to their rejection (Adedeji et al., 2020). In some parts of Africa and Asia pest infestation of stored and non-stored fruit was also reported (Ansari et al., 2019; Nnzeru et al., 2021).

The colour of baobab fruit changed from pale yellow, to white and finally to red which was not acceptable by some consumers. There was no significant change in colour of baobab milk nectar stored from 1 to 30 days under different conditions (Chadare et al., 2017). Ndiaye et al. (2022) reported the stability of yellow colour after three months storage of baobab seed oil at temperature ranging from 20 to 45°C.

Mould growth was revealed by green and dark green colour of the unshelled fruits as reported by Patil and Kukade (2020) and Rawat (2015). Change of fruit colour to green and dark green which signified mould growth was not considered as a serious problem by respondents who considered it a normal colour change. This implies lack of awareness about the occurrence of moulds in stored fruit which are known to produce mycotoxins thus

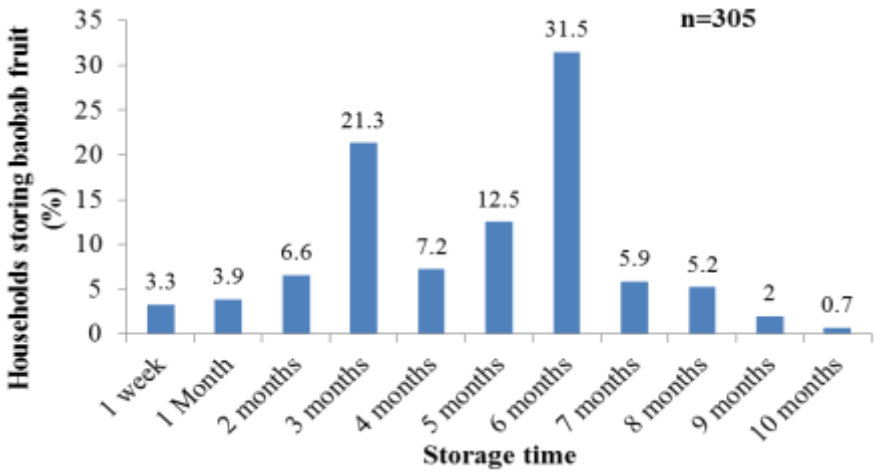


**Figure 4.** Storage time of baobab fruit in the village households.
Source: Authors

**Table 5.** Problems observed during baobab fruit storage.

| Problem | n | % | Solution | n | % |
|---|---|---|---|---|---|
| Mould growth | 22 | 18.6 | Discarding fruits | 7 | 7.5 |
| Pests and Insect infestation | 87 | 73.7 | Putting sacks on platforms | 40 | 43 |
| | | | Changing sacks | 15 | 16.1 |
| | | | Discarding fruits | 10 | 10.8 |
| | | | Sorting out insects | 21 | 22.6 |
| Colour change | 9 | 7.6 | None | | |
| Total | 118 | 100 | | 93 | 100 |

Source: Authors

endangering the health of consumers. The presence of moulds in baobab as well as other dried fruit has been reported (Abbas et al., 2019; James et al., 2022).

## Effect of storage practice, location, and storage time on the nutritional quality of baobab fruit

Moisture content decreased in all samples after six months of storage. It was significantly lower in baobab fruit stored in plastic and shells. Regardless of the location, baobab fruit stored in sacks had the lowest vitamin C content whereby more than 50% was lost after six months of storage. Similarly, total carotenoid decreased significantly after six months of storage regardless of the storage practice and location ($p<0.05$) (Table 6).

After six months of storage, more than 30% of vitamin C was retained in the baobab fruits stored in plastic buckets and shells compared to those stored in sacks. This could be due to better barrier features of these materials against atmospheric oxygen which could oxidize the vitamin resulting in its loss. These findings are similar to those reported by Dandago et al. (2016) who reported maximum losses of vitamin C in unshelled fruit stored in baskets which exposed them to atmospheric oxygen.

There was more than 60% loss of carotenoid in stored samples for six months regardless of the storage practice. These losses could be due to instability nature of this vitamin which increased with storage time. A study done in Romania by Pop et al. (2016) reported 63% losses of carotenoids in dried apricots during storage in polythene bags for six months at room temperature. These results are in agreement with the current study.

## Conclusions

Although several types of fruits were available in the study area, fruit consumption was generally low. About half of the households reported to have consumed fruit from one to three days a week. Fruit intake among households also depended on their socio-economic characteristics. Although education level of the mother

**Table 6.** Moisture (%), vitamin C (mg/100 g) and total carotenoids (µg/100 g) contents of Baobab fruit during harvesting and after 6 months of storage time*.

| Storage location time | Moisture (%) | Vitamin C (mg/100 g) | Total carotenoid (µg/100 g) |
|---|---|---|---|
| MzulaSackTime1 | 11.22±0.49[a] | 259.52±19.92[a] | 3.35±0.75[a] |
| MzulaSackTime2 | 10.06±0.82[a] | 66.17±30.81[d] | 0.96±0.062[b] |
| MzulaShellTime1 | 9.78±0.47[a] | 220.327±5.98[b] | 2.97±0.684[a] |
| MzulaShellTime2 | 6.55±0.4[b] | 72.09±13.383[d] | 1.25±0.11[b] |
| MzulaPlasticTime1 | 10.61±0.64[a] | 234.98±24.48[c] | 3.02±0.684[a] |
| MzulaPlasticTime2 | 7.35±0.66[b] | 120.74±18.91[ac] | 1.08±0.044[b] |
| ChinojeSackTime1 | 10.71±0.15[a] | 264.8±18.59[a] | 3.01±0.52[a] |
| ChinojeSackTime2 | 9.35±1.46[a] | 35.55±14.96[f] | 0.92±0.101[b] |
| ChinojeShellTime1 | 9.73±0.13[a] | 217.99±5.396[b] | 3.67±0.52[a] |
| ChinojeShellTime2 | 6.62±0.75[b] | 64.06±26.04[d] | 1.24±0.172[b] |
| ChinojePlasticTime1 | 10.23±0.65[a] | 240.73±14.96[c] | 2.93±0.13[a] |
| ChinojePlasticTime2 | 7.01±0.02[b] | 94.84±31.63[e] | 1.123±0.04[b] |

*Time 1 refers to harvesting time and time 2 refers to 6 months after harvesting. **Means with different superscript on the same column are significantly different following separation by Duncan's Multiple Range Test (DMR) at P<0.05.
Source: Authors

was not associated with increased fruit consumption, the female headed households consumed more fruits than the male headed households. In addition, households that had fewer members and higher income consumed significantly more fruits than the low income earners. Most households stored baobab fruit. Plastic containers were the best storage practises in maintaining the quality of baobab. In contrast, baobab fruits stored in sacks resulted in significant nutrient loss. There is a need to create awareness to consumers about the importance of fruit in the diet and also to promote home fruit gardens in order to increase fruit consumption. More research should be conducted to develop innovative and cost-effective technologies to prevent postharvest losses of fruit.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Studying the ethylic fermentation process of the mucilage juice of cacao by *Saccharomyces cerevisiae* yeast

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**The aim of the study was to optimize the ethylic fermentation process of the mucilage juice of cacao by *Saccharomyces cerevisiae* yeast. The juice fermentation process was effected by inoculating *S. cerevisiae* on 0.2 g of activated yeast/liter of juice for essay E1, and on 0.5 g of activated yeast/L for essay E2. Results reveal that increasing the pH value of the process effected in the acidic zone raised the rate of the essay inoculated with 0.5 g of yeast/liter than that inoculated with 0.2 g/L yeast/liter of juice. The amounts of the soluble extract transformed and yield of the transformed soluble extract were greater (90.60%) for E1 compared to those of E2 (84.41%). The efficacy of yeast utilized in the transformed soluble extract/100 g of the mucilage juice of cacao/gram was higher for essay E1 (81.95) compared to that of essay E2 (30.54).**

**Key words:** Mucilage juice of cacao, ethylic fermentation, rate of yeast, soluble extract, yield of transformation, efficacy.

## INTRODUCTION

Cacao is the third foodstuff that is highly merchandized in the whole world. Having three million tons of cacao per year, African continent has become the world leader in cacao production. Cacao tree (*Theobroma cacao* L) is one of the most important plants in the tropical agro-forester. This tree known as cacao tree belongs to the family of Sterculiaceae, Theobroma kind. It is esteemed that in plantation it must keep pace from twenty-five to thirty years. Theobroma kind has 22 species of which *T. cacao* is the sole one that is cultivated commercially.

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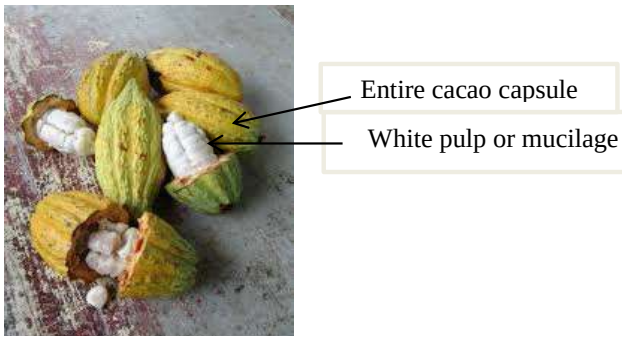


**Photo 1.** Entire cocoa capsules and by cutting transversal showing surrounded seeds of the white pulp or mucilage.
Source: Authors

Indeed, it produces seeds used to prepare chocolate or to extract cacao butter (Zhang et al., 2011).

Cacao tree is a bush cane of 5 to 7 m height on average. Its fruits, cocoa capsules (Photo 1), are berries that are grossly lengthened like American football.

Cacao capsules contain numerous seeds (between 25 and 75) which are regrouped by splice and named cacao beans rich in starch, fats, and alkaloids. A bean of cacao, obtained after undergoing different stages of post-harvest treatment, weighs about 1 g. It is enveloped with resistant thin dandruff named husk. Every mulberry seed is surrounded by a pulp named mucilage. It is white, aqueous, and sweetened. The post-harvest treatments of cacao depend on its over-production.

During the stage of indenting, a great quantity of pulp is obtained, which ferments after some time (Hamdouche, 2015). At this stage, more than 300 million liters of mucilage juice, commonly called water of cacao, produced every year are abandoned in the fields. Yet, it is a good drink that is appreciated by agricultural workers and their children.

Due to its high level of sugar, the mucilage juice of cacao is very fermentable and could serve as raw material for the production of ethanol (Stewart, 2016), vinegar, and other derivative products.

In Congo, except for the production of beans for exportation, the mucilage juice and cacao capsules that are not mature enough are not used. They are considered as industrial wastes.

The principal objective of this work was to contribute to the valorization of the mucilage juice of cacao via fermentation, notably to determine the physico-chemical and technological parameters relative to the process of fermenting the mucilage juice of cacao.

## MATERIALS AND METHODS

### Raw material

The raw material used for the study is cacao fruit. It was obtained from cacao tree from the fields located at *Pokola* village in the Administrative Department of Sangha, situated at 642 km of Brazzaville. A total volume of the reaction mixture of 6 L constituted the mucilage juice of cacao at 17% Brix of soluble extract standardized with sugarcane.

This mucilage juice of cacao fruit was standardized on ground, in the month of August 2022. It was pasteurized at 85°C for 20 min, and then placed in bottles of 1 L. It was immediately corked and sent to Brazzaville for it to undergo the process of ethylic fermentation in the laboratory of Food Microbiology of the National Institute of Engineer Sciences Research, Innovation and Technology.

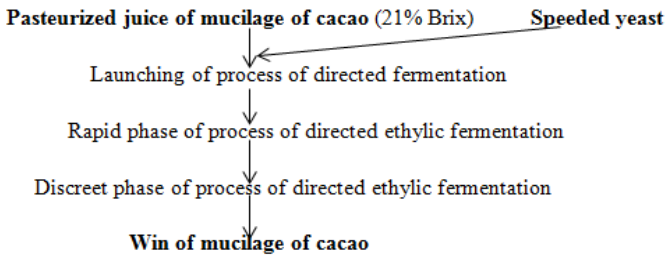


**Figure 1.** Diagram of process of ethylic fermentation of juice of mucilage of cacao into wine of mucilage of cacao.
Source: Authors

### Biological yeast

The biological yeast used for the seeding of the samples of mucilage juice to direct the fermentation process was an industrial strain commercialized at Brazzaville under Saf-instant label (S.I. Lesafre, France).

This reactional medium is separately sown with activated yeast via ethylic fermentation; notably 0.2 g of yeast/L was used for essay E1 and 0.5 g of yeast/liter was for essay E2, to optimally seed the biological yeast (Diakabana et al., 2016).

### Operatory material

The operatory material with a total voluminal capacity of 1.5 L was used for the experimentation; it is a cylinder-conical fermenter that allows one to take the samples for analysis, and also allows the evacuation of fermentation gas.

The combined volume of the reaction mixture for each one of the two tested essays done in triplicate was 1 L.

### Methods

The ethylic fermentation process of the mucilage juice of cacao was in two phases: one, consumption of soluble extract spontaneously and the other is discreet phase (Figure 1).

### Activation of the yeast

Before the mucilage juice of cacao was seeded, 0.2 g of *Saccharomyces cerevisiae* yeast and 0.5 g of yeast were first activated in 10 mL of the mucilage juice of cacao, for 30 min. They were incubated at ambient temperature. This was the mother-suspension. The cell density of the inoculum was then evaluated based on Heineken method adapted by Diakabana et al. (2013):

(1) 0.1 mL of the mother-suspension of the yeast was taken and

diluted in 0.9 mL of sterilized distilled water;
(2) after this diluted suspension was homogenized, a drop was taken and then deposited on Malassez cell. Then, it was covered with a special slide and observed with a microscope with objective X40. The rectangular (Faurie, 2019) cell of Malassez was squared;
(3) the number of the yeast cells was counted in each one of the 5 zones A, B, C, D, and E;
(4) the number of yeast was determined as follows: Number of yeast cells = (A + B + C +D + E) × k; with k = 0.5 × $10^6$ cells/mL (coefficient given by Heineken).

### Seeding the mucilage juice of cacao

For the two samples tested for triple times, ethylic fermentation was done using the active yeast (initial biomass) as follows: 13.4 × $10^6$ cells/mL of cacao for essay E1 and 33.5 × $10^6$ cells/mL for essay E2.

### Determination of the technological parameters

The determination of the soluble extract was done with two methods: using refractometer and pycnometer. In the two tested cases, the work was started via preliminary cooking, filtration, and homogenization.

### Using refract meter

In measuring the soluble extract using refractometer, the value obtained was expressed in % Brix at 20°C.

### Using pycnometer

The method of pycnometer described by EBC and adapted by Diakabana et al. (2013) was employed (Dowdy and Mosher, 2022) to determine the soluble extract and the ethanol content of the mucilage of cacao.

In a balloon of 500 mL, 100 ± 0.1 g was weighed and filtered inside the mucilage. After collecting 85 mL of the distillate, this quantity (100 ± 0.1 g) was poured into the distilled water and weighed. After homogenization, the density of the distillate was determined using a Gay-Lussac pycnometer at 20°C.

After measuring the density, the alcohometeric titer was evaluated based on the table of conversion of Goldiner and Klemann (De Clerck, 1963).

This technique permits us to follow the evolution of the ethylic fermentation of the mucilage juice of cacao, notably the yield of the transformed sugars (% in weight) and the coefficient of yield relative to the conversion of soluble extract into ethanol (% in weight) (Diakabana et al., 2013):

$$Rdt = \frac{St}{S0} \times 100;$$

where St = quantity of soluble extract transformed and So = initial quantity of soluble extract.

The formula described by Leveau and Bouix, adapted by Diakabana et al. (2013) was used to calculate the coefficient of yield relative to the conversion of soluble extract into ethanol (% in weight):

$$Y_{Et/St} = \frac{Et}{St} \times 100;$$

Where Et = Global quantity of formed ethanol and St = transformed soluble extract.

### Calculation of daily consumption of soluble extract

Daily consumption of the soluble extract was calculated in the linear zone using the kinetic curve of the process as follows:

Ei-Ef;

Where Ei= initial value and Ef=following value.

### Determining the efficacy of the yeast used

The efficacy of the yeast used in the process of ethylic fermentation was determined by the relation of the quantity of transformed soluble extract St/quantity of yeast used.

### Statistical analysis

For the analysis of the technological parameters relative to the process of fermentation, the method based on the law of bell of Gauss-Laplace (Larrieu, 1988) was used to determine the repeatability of measures of analysis and operations. The average values of pH, soluble extract in the mucilage juice, speed of the fermentation process of the tests done three times, standard deviation, and confidence intervals were determined for a coefficient of variation inferior or equal to 0.1%. The average efficacy of the yeast used the yield of relative transformation of soluble extract, and the coefficient of relative yield to the conversion of soluble extract into ethanol were appreciated for a coefficient of variation inferior to or equal to 0.1%.

## RESULTS AND DISCUSSION

The physico-chemical and technological parameters relative to the fermentation process of the mucilage-juice of cocoa were examined.

### Evolution of the consumption of soluble extract and the pH during the fermentation process of the mucilage juice of cacao

The evolution of the consumption of the soluble extract and the pH during the fermentation process of the mucilage juice of cacao is visualized by the profile of the soluble extract, the rise of the pH, and technological parameters of every essay tested.

### Evolution of soluble extract

During the ethylic fermentation process, the consumption profile of the soluble extract of the mucilage juice of cacao by *S. cerevisiae* decreases (Table 1).

Starting from the initial value of the soluble extract evaluated at 21% Brix, essay $E_1$ (with 0.2 g of yeast/liter) went down more rapidly (3.35% Brix/day), during the 4

**Table 1.** Evolution of the soluble extract during the progress of the process of ethylic fermentation of the must of cacao in function of the rate of seeding of *S. cerevisiae* yeast employed.

| Period (day) | Essay 1 (E1) | | Essay 2 (E2) | |
|---|---|---|---|---|
| | Soluble extract (% Brix) | Consumed Soluble extract by day (% Brix/day) | Soluble extract (% Brix) | Consumed Soluble extract by day (% Brix/day) |
| 0 | 21 | **0** | 21 | 0 |
| 1 | 17.1 | 3.9 | 18.3 | 2.7 |
| 2 | 13.5 | 3.6 | 14.7 | 3.6 |
| 3 | 10.2 | 3.3 | 11.4 | 3.3 |
| 4 | 7.6 | 2.6 | 8.3 | 3.1 |
| 5 | 7.4 | 0.2 | 7.8 | 0.5 |
| 6 | 7 | 0.4 | 7.5 | 0.3 |
| Average value between 0 and 4 days | / | 3.35 | / | 3.175 |

E1: Seeding with 0.2 g of yeast/liter of must (13.4 × $10^6$cells/mL of must); E2: with 0.5 g/liter (33.5 × $10^6$cells/mL); Daily consumption of soluble extract calculated into linear zone (between 0 and 4 days) of the progress of the process.
Source: Authors

**Table 2.** Evolution of the pH during the progress of the process of ethylic fermentation of the must of cacao in function of the rate of seeding of *S. cerevisiae* yeast employed.

| Period (day) | Essay 1 (E1) | Essay 2 (E2) |
|---|---|---|
| | pH | pH |
| 0 | 3.34 | 3.34 |
| 1 | 3.41 | 3.44 |
| 2 | 3.5 | 3.51 |
| 3 | 3.53 | 3.57 |
| 4 | 3.55 | 3.61 |
| 5 | 3.56 | 3.65 |
| 6 | 3.57 | 3.67 |

E1: Seeding with 0.2 g of yeast/L of must (13.4 × $10^6$cells/mL of must); E2: with 0.5 g/L (33.5 × $10^6$cells/mL).
Source: Authors

first days, until at lower degree of consumption of soluble extract estimated at 7.6% Brix compared to essay $E_2$ (with 0.5 g of yeast/liter) which went down less rapidly (3.175% Brix/day) until at 8.3% Brix. During the 2 last days of incubation, the consumption of the soluble extract was very slow, discrete until at day 6, respectively where we have 7% Brix for E1 and 7.5% Brix for E2 till the end of the incubation.

## Evolution of pH

The evolution of the ethylic fermentation process of the juice of cacao by *S. cerevisiae* was noted by a progressive augmentation of pH (Table 2).

Starting from the initial pH value of 3.34 of the juice during the fermentation process, the profile of the pH ascended in the two cases tested.

For $E_1$ (with the smallest level of inoculation), the pH profile was low and stopped at the value of 3.57 compared to that of essay $E_2$ (with the more high level of inoculation) which stopped at pH –3.67 at the end of the incubation period of 6 days.

## Evaluation of the technological parameters

The technological parameters, notably the speed of the fermentation process, the quantity of soluble extract transformed, the quantity of residual soluble extract, and the quantity of ethanol formed for–essay E1 tested are rationally valued (Table 3).

The quantity of residual soluble extract at the end of the fermentation process was estimated at 1.70 g/100 g of cacao juice for essay E1 which was effected by seeding the mucilage juice with 0.2 g of yeast/liter (13.4 ×

**Table 3.** Evaluation of the technological parameters of the process of ethyl fermentation of the must for each essay tested; E1 (essay with 0.2 g of yeast/L) and E2 (essay with 0.5 g of yeast/L).

| Essay tested | Evaluation of the technological parameters of the process of ethylic fermentation | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial quantity of soluble extract (g/100 g of juice) | Quantity of residual soluble extract (g/100 g of juice) | Quantity of transformed soluble extract (g/100 g of juice) | Efficacy of use of yeast (g of transformed soluble extract/100 g of juice/g of yeast used) | Rdt (%) | Finale quantity of formed ethanol (g/100 g of juice) | $Y_{Et/S}$ (g of formed ethanol/g of transformed soluble extract) % weight |
| E1 | 18.09 | 1.70 | 16.39 | 81.95 | 90.60 | 4.86 | 0.29 |
| E2 | 18.09 | 2.82 | 15.27 | 30.54 | 84.41 | ND | ND |

Rdt: Yield of relative transformation to conversion of soluble extract; $Y_{Et/S:}$ coefficient of relative yield at the conversion of soluble extract into ethanol; E1: seeding with 0.2 g of yeast/L of must ($13.4 \times 10^6$cells/mL of must); E2: with 0.5 g/L ($33.5 \times 10^6$cells/mL). ND: no determined.
Source: Authors

$10^6$cells/mL of must); essay E2 was obtained by seeding 0.5 g of yeast/liter *(*$33.5 \times 10^6$cellules/mL*)* with 2.82 g/100 g of cacao juice.

## Appreciation of the performance in the ethylic fermentation process of the essays tested

To know the performance of the essays tested with the initial soluble extract of the mucilage juice of cacao, the efficacy of the yeast and the yield of the transformed soluble extract are presented in Table 3.

For the two essays tested (E1 and E2), the initial quantity of the soluble extract was estimated at 18.09 g/100 g of the mucilage juice. Yield of the transformed soluble extract was evaluated at 90.60% for essay E1 (with 0.2 g of yeast/liter of mucilage juice) and 84.41% for essay E2 (with 0.5 gram of yeast/liter of mucilage juice). The amount of the transformed soluble extract during the process was evaluated at 16.39 g/100 g of juice for E1 and 15.27 g/100 g of juice for E2. In the same sense, the efficacy of the yeast tested was evaluated at 81.95 g of the transformed soluble extract/100 g of juice/gram of yeast used for E1, and 30.54 g of the transformed soluble extract/100 g of juice/gram of yeast used for E2.

Concerning essay E1 (done with 0.2 g of yeast/liter of the mucilage juice), the final quantity of the ethanol formed was estimated at 4.86 g/100 g of the mucilage juice, which corresponded coefficient of converting the soluble extract into 0.29 g ethanol formed.

The mucilage juice of cacao fruit, the by-product of cacao fruit treatment containing organic and inorganic substances can inflict damages on the floral and microbial eco-systems in the environment where the activity takes place (Spevacek and Ritchson, 2020). Improving the ethylic fermentation process of mucilage juice of cacao could produce wine of commercial quality. It brought an added value to the industrial treatment of cacao fruit in preservation environment as revealed by Ngampika et al. (2022) on valorization of cacao capsules and Vu et al. (2018) on valorization of banana peels.

The process of ethylic fermentation could enhance the mucilage juice of cacao fruit to produce ethanol (Stewart and Speers, 2019). This process being gas production favours auto-agitation of liquid medium as the fermentation progresses by employing an appropriate fermenter, which is at the center of the installation of fermentation (Smith, 2021).

The interest to use *S. cerevisiae* in the ethylic fermentation process of the mucilage juice of cacao was proved after deducing that *Saccharomyces* is implicated in the traditional process of elaborating many African fermented foods (Flibert et al., 2016; Diakabana et al., 2019).

As the ethylic fermentation process progressed, the soluble extract diminished rapidly from 21% Brix from 0 to 4 days, revealing the degradation of the fermentable soluble extract by *S. cerevisiae* yeast (Diakabana et al., 2007). This value of the soluble extract content was stabilized at about 7 to 7.5% Brix from day two to the last fourth day of the fermentation. This indicates the soluble extract was not degraded by *S. cerevisiae* and constituted the fraction of the residual soluble extract (Diakabana et al., 2013).

The fermentation of the mucilage juice started as soon as the initial biomass of the yeast was realized with concentration of $13.4 \times 10^6$ cells/mL of cacao juice (0.2 g of dry yeast/liter) for essay E1 and $33.5 \times 10^6$ cells/mL (0.5 g of dry yeast/L) for essay E2 (Diakabana et al., 2019). In relation to that, this directed process was carried out for 6

days (incubation of the two cases tested). The average consumption speed of the soluble extract for essay E1 which was effected by seeding cacao juice with 0.2 g of yeast/liter ($13.4 \times 10^6$ cells/mL of juice) was higher (3.35% Brix/day) compared to essay E2 (3.175% Brix/day) which was realized by seeding 0.5 g of yeast/liter ($33.5 \times 10^6$ cellules/mL). That signifies high yeast rate ($33.5 \times 10^6$ cellules/mL). Saturated concentration did not favour well the transformation of the fermentable soluble extract of the mucilage juice of cacao as obtained by Diakabana et al. (2019) in the fermentation of ginger juice.

The fermentation of the soluble extract for essay E2 was precociously stopped by using a seeding rate relatively high ($33.5 \times 10^6$ cells/mL). This was done because the yeast culture had accomplished its metabolic role, which was notably negative effect of high dose of ethanol on yeast (Maskell, 2016; Castro and Bryant, 2021; Cisilotto et al., 2021). It was associated with the flocculation of the population of yeast (Speers, 2016) in the saturated concentration of inoculum ($33.5 \times 10^6$ cells/mL) in relation to essay E1 which was effected with a feeble yeast ($13.4 \times 10^6$ cells/mL of must). This precocious stop of the fermentation explains how higher quantity of residual soluble extract was obtained for essay E2 compared to essay E1.

The increased profile of the pH of the two essays tested and developed in the zone of acidic pH guaranteed the character of the food (Nout et al., 2003; Bousmaha et al., 2009; Tchibozo et al., 2012).

The augmentation of the pH was relative to the production of ethanol progressively, which became the exhausted source of assimilable nitrogen in the mucilage juice during the ethylic fermentation by *S. cerevisiae* (Akin, 2008). That is made clear by the feeble content of ammoniacal nitrogen estimated at 0.03% relative to composition of the dried beans of cacao despite the importance of the nitrogen protein estimated at 10.0%.

The feeble coefficient of yield relative to the conversion of the soluble extract into ethanol for essay E1 (yeasting with 0.2 g of yeast/liter) is probably caused by the Crabtree effect, notably the respiration of ethanol by *S. cerevisiae* and the high content of soluble extract during the long hours of the fermentative process (Engasser, 1988).

## Conclusion

This work allows us to understand that the optimal value of the rate of yeast of the mucilage juice is 0.2 g of yeast/liter. The notable presence of residual soluble extract in the two cases indicates the soluble extract is not fermented by *S. cerevisiae* in the mucilage juice of cacao.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Quantification of ethanol and identification of other chemical constituents in homemade morula beer using gas chromatography-mass spectrometry (GC-MS)

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**Morula (*Sclerocarya birrea*) beer is a seasonal homemade alcoholic beverage made from morula fruits pulp. Unlike the commercial morula alcoholic beverage, the alcohol content of homemade morula beer is not known. The major challenges with homemade alcoholic beverages arise from batch-to-batch differences in product quality and safety due to the variability of raw materials and lack of quality control. Consequently, the alcohol content of alcoholic beverages made by the same individual may differ. In this study, a validated liquid-liquid extraction gas chromatography-mass spectrometry (GC-MS) method is used for the quantification of ethanol and identification of volatiles in homemade morula alcoholic beverage. The analysed samples contained ethanol with concentrations ranging from 0.5 to 5.8% v/v depending on the number of days the sample was fermented. Other volatile compounds that were identified were acetic acid, propanol, propanal, butanal, 3-methyl-1-butanol; 2-methyl-1-butanol, butanoic acid, iso-amyl acetate, butanoic acid ethyl ester, benzyl alcohol, phenyl ethyl alcohol, lactic acid, humulene, decanoic acid ethyl ester and 2-methyl-1-hexadecanoic acid ethyl ester. The volatile compounds were found to decrease over time. These results provides a validated method for the characterisation of homemade alcoholic beverages and information on the quality of morula beer.**

**Key words:** Morula beer, home-made alcoholic beverage, gas chromatography-mass spectrometry (GC-MS), standardisation.

## INTRODUCTION

Many wild plant food resources are often eaten across Africa (Kamanula et al., 2022). They are a source of

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vitamins, minerals, amino acids, and trace metals (Mdziniso et al., 2016; Bonić et al., 2013). One such type of plant whose fruits have been part of a supplemental diet in many Southern African countries is Morula (*Sclerocarya birrea*). Morula fruits have been known and consumed for a very long time (Sinthumule and Mzamani, 2019). It occurs naturally in various types of woodland, on sandy soil or occasionally sandy loam though some plant it in their homesteads and farms worldwide (Jinga et al., 2022). The tree itself is used for firewood as well as to make housing/fencing poles and wooden utensils (Tapiwa, 2019). Fruits are used for food, whereas leaves, branches, bark, and roots are used for traditional medicine (Sinthumule and Mzamani, 2019; Mojeremane and Tshwenyego, 2004). The fruit is processed to make jams, candies, and juices, and the kernel is extracted for the oil which is used in the manufacturing of a range of cosmetics (Manyeula et al., 2021; Hilou et al., 2017). Morula fruit juice has higher ascorbic acid than orange juice and the oil extracted is rich in antioxidants and oleic acid which prevent diseases such as cancer and heart disease (Murye et al., 2018; Galvão et al., 2020; Mashau et al., 2022).

A widely known commercial morula-based liqueur, known as 'Amarula cream' with an ethanol content of 17% v/v is produced, bottled, and marketed across the world by Cape Distell Pty Ltd. in Stellenbosch (South Africa) (Sinthumule and Mzamani, 2019). Furthermore, homemade fruit 'beer' called morula beer is common among rural communities. Unlike the commercial type, the alcohol content of the home-made beer' is not known. Morula beer is consumed between December and April every year as this is the time when the fruit ripens. The recipe for making morula beer is adopted from the traditional fermentation recipe of making wines but without the aging process. To make morula beer, the ripe fruits are collected, and then the skins are removed. The pulp, pips, and juice are then mixed with water and mashed thoroughly until the liquid thickens. The stones and then removed from the juice. The juice is then left to ferment for a few days (Shackleton et al., 2012). When fermentation is complete, the mixture is filtered, and the alcoholic beverage is ready for consumption. This recipe is passed from generation to generation and has remained the same.

The production and selling of homemade alcoholic beverages are not regulated in Botswana. The fermentation process in traditional home brews is not regulated and as such the amount of alcohol in these drinks may continue to rise even after filtration and during storage (Tulashie et al., 2017). Traditionally fermented alcoholic beverages contain a lot of volatile organic compounds arising from various chemical reactions taking place during the fermentation process or from raw materials used with ethanol as the main psychoactive component (Mashau et al*., 2022)*. While morula beer has been consumed for a very long time in Botswana, research on the safety, quantification of ethanol and identification of other chemical constituents of morula beer has never been done. Previous studies have been done on the fermentation process and alcohol content of khadi, a local homemade alcoholic brew (Mapitse et al., 2015; Motlhanka et al., 2020). It is worth noting that in these reports the methods were not validated, and some were costly (Isaac-Lam, 2016). Therefore, this research reports a validated method for the quantification of ethanol and identification of other volatile components in morula alcoholic beverages using GC-MS.

## MATERIALS AND METHOD

A validated Liquid–Liquid Extraction method for the quantitative analysis of ethanol in the different types of homebrewed alcoholic beverages of Botswana using the Gas Chromatography Flame Ionization Detector method was adopted and used for the purpose of this study (Tsenang et al., 2022).

### Chemicals and reagents

Ethanol (99.9%) and Ethyl acetate (99.9%) were purchased from Merck Chemicals (Pty) Ltd (Germiston, South Africa). Sodium chloride (AR) was purchased from Rochelle Chemicals (Johannesburg, South Africa).

### Sampling

500 ml of each fermented morula beer samples (1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B, 5A, and 5B), were collected from local brewers in five different villages in Central District near Palapye. The collected samples were collected from five different drinking sites. Figure 1 shows the villages where samples were collected. The samples were collected in glass bottles and transported in cooler boxes containing ice. Upon arrival at the laboratory, they were extracted and kept at 4°C for an average of 3 days.

### Preparation of calibration standards

Nine ethanol standard solutions were prepared by mixing 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 ml of ethanol with the required volumes of ethyl acetate in separate volumetric flasks to achieve a final solution volume of 10 ml. The resultant concentration of the prepared solutions was 2.5, 5.0, 7.5, 10, 20, 30, 40, 50 and 60% v/v. Three replicate injections of each solution were performed, and their response was recorded.

### Liquid-liquid extraction of samples

The samples were extracted by dissolving 1.5 ml of homemade morula beer sample in 0.5 ml of each ethyl acetate. NaCl (0.5 g) was added to each solution to enhance phase separation. Following this, the solutions were vortexed for 1 min and centrifuged for 10 mins at 10000 rpm. The organic layer was removed and transferred into GC vials.

### Gas chromatography conditions

The ethanol content in the samples was determined using an

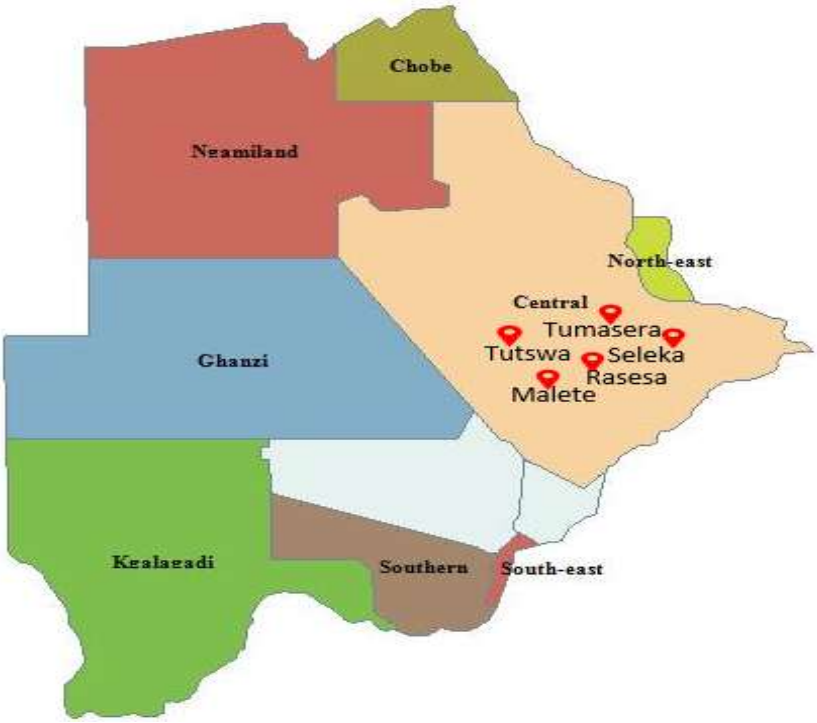


**Figure 1.** Map of Botswana showing the different sampling sites in the central district.
Source: Tsenang et al., 2022

Agilent 6890N capillary gas chromatograph connected to an Agilent G5977 mass spectrometer and a PAL 3 auto-sampler. The NIST library was used for the identification of compounds (NIST, Mass-spectrometry Data Center, 2017). Separation was achieved on a standard mid-polar DBALC1 capillary column, (30 m length, 0.32 mm i.d, and 0.25 µm film thickness). Sample injections were made in a split mode using a general-purpose split/split-less liner packed with glass wool. The GC oven temperature program was started at 35°C and held for 2.5 min, then increased to 90°C at a rate of 10°C $min^{-1}$ and held for 4 min, and then ramped to 220°C at a rate of 10°C resulting in a total run time of 23 min. Extracted sample volumes of 1 µL were injected into the instrument at a split ratio of 50:1 using helium as a carrier gas. The flow rate of the helium was set at a constant flow of 0.5 ml min -1. The injector and Mass transfer line temperature settings were 220°C and 280°C, respectively.

## RESULTS AND DISCUSSION

The results obtained (Figure 2), revealed that the ethanol concentration of samples ranged between 0.5 and 5.8% v/v. The results are comparable to those obtained by Motlhanka and coworkers during the analysis of microbes of Khadi (a homemade alcoholic beverages made from *Grewia flava* fruits) who found ethanol concentrations to range from 4-8% v/v (Motlhanka et al., 2020). The concentration of ethanol increased with an increase in the number of fermentation days. The lowest concentration was found in sample 3A which was collected during the day it was prepared while the highest ethanol content was observed in sample 1B which was collected 2 days after it was prepared. The difference in ethanol concentration in samples 2B and 3B which had fermented for the same number of days (1 day) before sampling may be due to different conditions and utensils used. While some just fermented the fruit juice, others added a previously fermented morula beer to the freshly prepared beer (back slopping) leading to the difference in the amount of ethanol. In general, the ethanol content of the fermented homemade morula beer (2.5-5.8% v/v) was similar to those found in most commercial alcoholic beers (2.92-15.66 % v/v) (Destanoğlu and Ateş, 2019; Galvão et al., 2020; Sawadogo-Lingani et al., 2021). After a successful quantification of ethanol in the samples, they were analysed for the presence of other volatile components. Apart from ethanol and carbon dioxide, the fermentation process also produces a broad range of secondary metabolites (Pinho et al., 2006). While these substances are only produced at very low concentrations, they are responsible for the complex aromas and taste of fermented beverages (Leão et al., 2018; Bettenhausen et al., 2018). The major higher alcohols found in morula beer were n-propanol, 2-methy-l-propan-1-ol, 3-methylbutan-1-ol, and the aromatic alcohols β-phenyl-ethanol and benzyl alcohol (Figure 3). Most of these compounds are common to those previously reported for other alcoholic beverages

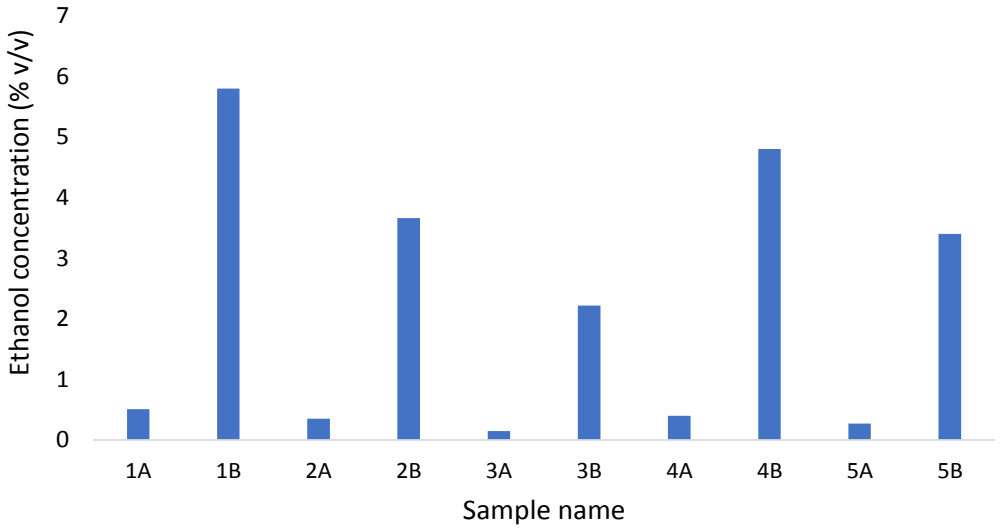


**Figure 2.** Concentration of ethanol in morula beer samples [ 1,2, 3,4,5: Sampling sites, A: 1-day old samples, B: 2-day old sample].
Source: Authors

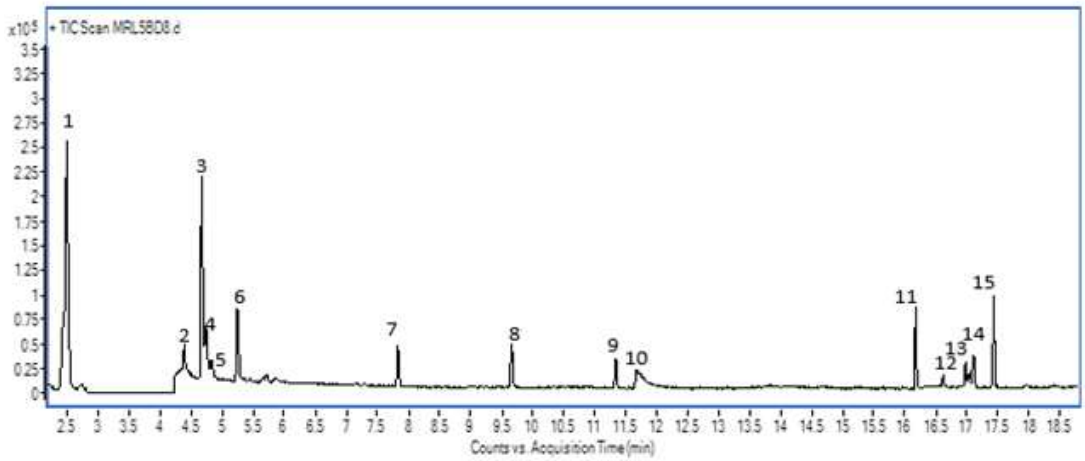


**Figure 3.** GCMS chromatogram of a 2-day-old morula beer. 1: ethanol, 2: acetic acid, 3: propanol, 4: propanal, 5: butanal, 6:3-methyl-1-butanol; 2-methyl-1-butanol,7: butanoic acid,8: isoamyl acetate,9: butanoic acid ethyl ester,10: benzyl alcohol, 11: phenyl ethyl alcohol, 12: lactic acid, 13: humulene, 14: decanoic acid ethyl ester, 15: 2-methyl-1-hexadecanoic acid ethyl ester.
Source: NIST, Mass-spectrometry Data Center, 2017.

(Mapitse et al., 2015, Destanoğlu and Ateş, 2019; Bettenhausen et al., 2018). The chemical composition of these homemade alcoholic beverages have different impacts on the aroma and flavor as high concentrations of higher alcohols can lead to a strong, pungent smell and taste, whereas reduced amounts impart desirable characteristics (Lu et al., 2020). During yeast metabolism, higher alcohols are formed as by-products of amino acid synthesis from pyruvate through the anabolic pathway or they could be produced through amino acid catabolism (Zhu et al., 2021). Two aldehydes (propanal and butanal) were found in morula beer. Aldehydes are formed from the oxidation of primary alcohols (Sinthumule and Mzamani, 2019). Esters present in morula beer were iso-amyl acetate, butanoic acid ethyl ester, decanoic acid ethyl ester, and 2-methyl-1-hexadecanoic acid ethyl ester. During alcoholic fermentation, several esters can be produced due to yeast metabolism (Torres-Guardado et al., 2022). Esters are some of the compounds found in beer and therefore impact greatly on its aroma. In optimal quantities, they can give a pleasant, full-bodied character to beer aroma, but in large amounts, they give beer aroma an overly fruity quality, which is disliked

**Table 1.** Difference in the relative abundances of volatile compounds based on the number of fermentation days.

| Volatile compounds | Relative abundances of volatile components (peak area) | | | | |
|---|---|---|---|---|---|
| | Day 0 (Fresh) | Day 1 | Day 2 | Day 3 | Day 4 |
| ethanol | 201 | 9545 | 20598 | 15125 | 11021 |
| acetic acid | 5003 | 4523 | 2548 | 1547 | 514 |
| 1-propanol | ND | 1578 | 2485 | 2045 | 1457 |
| 3- methyl butanol | ND | 2514 | 6654 | 4214 | 2141 |
| propanal | ND | 1986 | 2801 | 3651 | 2958 |
| butanal | ND | 2541 | ND | 3024 | 2541 |
| butanoic acid | ND | 912 | 3510 | 4510 | 4501 |
| iso-amyl acetate | ND | 1484 | 3307 | 2599 | 2841 |
| butanoic acid ethyl ester | ND | 1042 | 6585 | 5874 | 5584 |
| benzyl ethanol | ND | 4521 | 4856 | 1457 | ND |
| phenyl ethyl alcohol | 2156 | 5865 | 10254 | 9847 | 5140 |
| lactic acid | 7548 | 5142 | 4215 | 2145 | 658 |
| humulene | 3584 | 3216 | 3201 | 3120 | 2914 |
| decanoic acid ethyl ester | ND | 1040 | 5247 | 5547 | 5915 |
| 2-methyl-1-hexadecanoic acid ethyl ester | ND | 4244 | 9855 | 10240 | 10954 |

ND: Not detected.
Source: Authors

by most consumers (Mapitse et al., 2015). Acetic acid and lactic acid were also found in morula beer. Organic acids are formed from the oxidation of alcohols and acidic hydrolysis of esters while fatty acids form from the oxidation of aldehydes (Bettenhausen et al., 2018). Humulene was also found in morula beer. It is a biogenic volatile compound present in some plants, and it is a naturally occurring monocyclic sesquiterpene (Hilou et al., 2017). Humulene and its reaction products in the brewing process of beer give many beers their hoppy aroma (Galvão et al., 2020). It is an anti-bacterial, anti-inflammatory, and appetite suppressant and is also believed to kill cancer cells (Manyeula et al., 2021). Most of the volatile compounds detected in morula beer have also been reported by Destanoğlu and Ateş (2019) in homemade and commercial beers.

Different compounds identified in the morula beer sample on Day 0 (fresh) and how they varied over the 4 days are shown in Table 1. The volatile compounds in this study were not quantified, therefore the discussion was made in terms of the peak area (which is directly proportional to concentration) of volatile compounds that are present in morula beer relative to the peak areas obtained at Day 0. It was observed that the most important volatile compounds increased in amount up to Day 2 and eventually decreased gradually. In the fresh sample, only ethanol, acetic acid, lactic acid, and humulene were detected. Ethanol amount increased up to day 2 of fermentation but eventually decreased in a 3- and 4-day-old sample. The same trend was observed in other alcohols (n-propanol, 2-methy-l-propan-1-ol, 3-methylbutan-1-ol, phenyl ethyl alcohol, and benzyl alcohol) from day 1 to day 2. Acetic and lactic acid concentrations also decreased. The decrease in the amounts may be a result of the oxidation of alcohols and organic acids to form esters and aldehydes whose concentration increased from day 1 to day 3. The concentration of aldehydes decreased after day 3 and this might be due to the formation of fatty acids (for example, butanoic acid) whose concentration increased up to day 4 of fermentation. The amount of humulene dropped from day 1 but at a lower rate compared to other volatiles. The high amounts of aldehydes on days 3 and 4 could lead to off-flavour (soapy/cardboard) reported by consumers as the beer ages (Tulashie et al., 2017). It can be concluded that day 2 is an important day during morula beer shelf life because, after day 2, the amounts of important compounds like alcohols started to drop while unwanted compounds like aldehydes increase.

## Conclusions

A simple, rapid, cheap, and highly sensitive method that requires the use of a minimal amount of solvent was used for the analysis of homemade morula alcoholic beverages. This method can be used for routine analysis of alcohol and other volatile substances in homemade alcoholic beverages to provide reliable quantitative and qualitative data. The results show that the quality of homemade alcoholic beverages is variable and unpredictable, both quantitatively and qualitatively due to the lack of specifications and the absence of

routine scientific quality control. Ethanol was quantified in morula beer and ranged from 0.5 to 5.8% v/v in all samples, which is lower than that of the commercial Amarula cream liquor (17% v/v). This work revealed the presence of fifteen other volatile compounds which included alcohols, aldehydes, and esters. The results revealed a general decrease in the amount of volatile compounds over the fermentation period. The amount of some compounds (especially alcohols and esters) that contribute to important beer flavors was maximum around Day 2 of brewing before dropping significantly. No harmful substances were detected in Morula beer.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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