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ARTICLE

# Use of Bitter Leaf (*Vernonia amygdalina*) Extract and Pasteurization Aim at Improving the Sensory Quality and Shelf-life of *Mpedli*, a Traditional Opaque Sorghum White Beer from Northern Cameroon

**Koge[1] James Ronald Bayoï[1*] Bakari Daoudou[1] Yonas Vandi[1] Bruno Foundikou[1] Roger Darman Djoulde[2] François-Xavier Etoa[3]**

1. Department of Biological Sciences, Faculty of Science, University of Maroua, Maroua, P.O. Box 814, Cameroon
2. Department of Agriculture, Livestock and by-products, National Advanced School of Engineering of Maroua, University of Maroua, Maroua, P.O. Box 46, Cameroon
3. Department of Microbiology, Faculty of Science, University of Yaounde I, Yaounde, P.O. Box 812, Cameroon

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ABSTRACT

The purpose of this study was to evaluate the sensory property and shelf life of the processed *mpedli* beer using aqueous leaves extract of *Vernonia amygdalina* (VA) and heat treatment. The white sorghum beer was made at the laboratory scale using home-made procedure (Control). Following filtration, the beer was blended with an aqueous leaf extract (1/10, v/v) of VA (BUB). Pasteurization (60 °C/30 min) was performed on a portion of the VA blended sample (BPB). The sensory parameters and shelf life of the three samples were evaluated at room temperature during a month storage. The sensory characteristics of blended and non-supplemented *mpedli* beer differed significantly ($p < 0.05$). During storage, the colour, bitterness, aroma, odour, viscosity, texture, and overall acceptability of the processed samples were improved. Bitterness ($r = 0.898$; $p < 0.01$) and odour ($r = 0.930$; $p < 0.01$) were both highly correlated with the acceptance of the processed beer. The non-supplemented samples had the highest sensory scores 48 hours after preparation, while the relevant sensory ratings in processed BUB and BPB samples were recorded from the 12$^{th}$ to the 21$^{st}$ and 27$^{th}$ day of storage, respectively. The findings suggest that combining pasteurization with addition of aqueous leaf extract of VA may help small-scale brewers for improving the sensory quality and extending the shelf life of *mpedli*. According to the findings, the use of bitter leaf could be proposed as an alternative hop in the local brewing industry and may increase incomes of producers of local sorghum beer.

*Corresponding Author:
James Ronald Bayoï,
Department of Biological Sciences, Faculty of Science, University of Maroua, Maroua, P.O. Box 814, Cameroon;
*Email: jabar982002@gmail.com*

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## 1. Introduction

Food processing is becoming a recurring activity in developing countries. Brewing is among the most recurrent food transformation technology. Despite the high importance of directly consuming cereals, it is estimated that the latter is greatly processed for the production of alcoholic [1,2] and non-alcoholic [3] drinks than fruits. Most of the brewed drinks surrounding the market are processed by highly specialised industries but the African population have their own approach, which is still rudimentary and less documented. Beer production dates from prehistoric era and it is the most consumed beverage worldwide [4]. In west and central Africa, beer production is highly undertaken by women but most final products are only destined for men consumption [5]. In the northern part of Cameroon, the white sorghum is used in the production of an indigenous beer called *mpedli*, also known as white kapsiki beer. *Mpedli* beer, like many other cereal-based beverages, is regarded as good source of minerals and probiotic required for the metabolism of living cells [6]. The production of this local northern beer is a source of income and employment for many young ladies in northern Cameroon, which is considered as the least developed and poorest region of the country [7,8]. However, due to the poor hygienic quality of this traditional beer, unpreserved *mpedli* has a very short shelf-life that cannot exceed 48 hours at room temperature [9]. To overcome the short-term shelf life of this indigenous beer, proper preservation techniques like refrigeration, irradiation, heat treatment or the use of plant extract as natural food supplement are required. However, due to financial constraints and increased production costs, some techniques involving thermal treatment or irradiation may be difficult to apply for *mpedli* producers [10]. As a result, the import of plant extracts appears to be a viable option for addressing shelf life and quality issues of the local sorghum-based beers [11].

Besides being alcoholic, beer is highly valued for its bitterness. Hops are responsible for the bitter taste [12]. Brewers influenced the sensory quality, stability, and health safety of beers in the ancient brewing system by adding medicinal and edible plants that contained active principles responsible for bitterness or flavouring of the beer. In the 21$^{st}$ century, a resilient approach is being put in place to encourage the consumption of natural or less processed products, including herbal beers, depending on the region [13]. Indeed, due to their antimicrobial activities against certain pathogens, antioxidative properties, and essential oils, plants have been used as beer additives for flavouring and, more importantly, as food preservatives since ancient times [14].

*Vernonia amygdalina* is a perineal edible vegetable common to tropical Africa [15]. It is commonly known as *Ndolé* or bitter leaf in Cameroonian local markets. The leaves of this plant are considered as nutraceutical because they are used as both food and medicine [16]. The medicinal properties of this plant are related to the presence of bioactive compounds [17] but its bitterness is a key component, which serves as a hop in local beer production [18]. As such, the addition of aqueous extract of *Vernonia amygdalina* (VA) may result in *mpedli* beer to have a longer shelf life because of its bacterial and antifungal effects [19]. However, because the indigenous beers are served in an actively fermenting state with no pasteurization stage, VA extract coupled to the pasteurization could improve both the shelf-life and nutritional value of the *mpedli* beer because the plant contains various relevant nutrients (vitamins, minerals, carbohydrates, free amino acids, and fat) [20]. This combined preservation technique may also be an effective alternative to the synthetic beer preservatives, which can be harmful to consumers' health [21]. Given consumer interest in less processed or natural food products, a newly developed *mpedli* beer containing *Vernonia amygdalina* as a natural beer additive may also appeal to health-conscious consumers [22]. Therefore, the purpose of this study was to assess the efficacy of adding VA aqueous leaf extract with or without pasteurization on sensory quality and shelf-life of *mpedli* beer. This study was undertaken because there has been insufficient attention paid to the use of indigenous bitter vegetables as local hops in the Cameroonian brewing industry.

## 2. Materials and Methods

### 2.1 Plant Materials

White sorghum grains, *Sorghum bicolor* (madjeri) and the leaves of *Vernonia amygdalina* were the main plant materials used in this study (Figure 1). The white sorghum was purchased from IRAD Maroua while the mature fresh leaves of *Vernonia amygdalina* were manually harvested from Bouha village, Mayo-Tsanaga Division, Far North Region, Cameroon. Both plant materials were authenticated by botanical experts in the Department of Biological Sciences, University of Maroua, Cameroon. The collected leaves were sorted and shed dry until a constant weight was obtained. The dried leaves were ground, sieved, and the powder obtained was introduced into air free sealed plastic bottles for further use.

### 2.2 Preparation of Aqueous Extract of *Vernonia amygdalina* Leaf

The powdered material was extracted with distilled water (1:50 w/v). The mixture was stirred at 500 runs/min for 6 hours and the preparation was allowed to stand for 18 hours before being filtered through Whattman n° 2. The filtrate was refrigerated at 4 °C until further use.

### 2.3 White Sorghum-based "*Mpedli*" Beer Production and Treatment Process

The method previously described by Bayoï et al. [9] and recently re-used by Bayoï and Etoa [23] was used to produce *mpedli* beer. The sixth of the sorted sorghum grain was malted, while the remaining five sixth were milled into non-malted flour. The flour was soaked in water for about 10 h after which the whole content was cooked to a dough called "*fufu*". After cooling at room temperature, the "*fufu*" was kneaded and the malted flour was sequentially added to obtain the wort which was covered and allowed to ferment for 48 hours at room temperature. The resulting alcoholic fermented white sorghum beverage was filtered through muslin material and partitioned into three aliquots. Two of them served as test samples were adjuncted with the bitter leaves aqueous extract (1/10, v/v). One of the blended aliquots was pasteurized at 60 °C for 30 minutes [24]. The remaining aliquot was used as control and left without VA extract blending. Both assay and control samples were labelled and stored for a month at room temperature. The three-treatment groups of samples as shown in Figure 1 were labelled as follows:

- Control non-supplemented *mpedli* beer (CMB);
- Blended unpasteurized *mpedli* beer (BUB);
- Blended and pasteurized *mpedli* beer (BPB).

### 2.4 Sensory Analysis and Shelf-life Estimation

To evaluate the sensory attributes of *mpedli* beer samples during storage, a descriptive test with a 9-point-hedonic scale system, ranging from dislike extremely = 1 to extremely like = 9, was used [25]. After the preliminary training section, a panel of sixteen members (male and female, ages 19 to 32 years) was assembled, and ten of them were retained. The members were recruited among the university community (students and staff) and the experienced consumers of the fermented alcoholic beverage. Thirty millilitre (30 mL) of the concoction was randomly served to the panelists in transparent coded cups to assess the sensory attributes. Every morning between 7 a.m. and 8 a.m., the analysis was carried out in the Food and Microbiology laboratory, unit of IRAD Maroua, Cameroon. The sensory attributes such as colour/appearance, odour, taste (bitterness, acidity, and alcoholic), aroma, texture, viscosity, and overall acceptability were appreciated and scored by the panelists. After being graded, the tested sample was sipped and the tongue was immediately rinsed with mineral water before the next sample was evaluated. The sensory attributes were followed by the same panel over a four-week period, and the shelf life was estimated by testing the samples after 0, 2, 4, 6, 8, 12, 15, 18, 21, 24, and 27 days of storage at room temperature.

### 2.5 Statistical Analysis

The experiments were repeated three times, and the results were presented as mean ± standard deviation. The mean values were analysed using one-way ANOVA, and the HSD Turkey test was used to discriminate pairs of mean values that were significantly different at probability level of $p < 0.05$. Moreover, radar plots were created to reveal close similarities between the samples, and principal component analysis (PCA) was used to assess correlation


**(a)** **(b)** **(c)**

**Figure 1.** Dried bitter leaf (a); white sorghum grains (b); blended and non-Supplemented beer samples (c)

between the sensory attributes and the association between the beer samples during the storage. To perform univariate and multivariate statistical analysis, STATGRAPHICS and XLSTAT software were used, respectively.

## 3. Results

### 3.1 Sensory Properties of Beer Samples during Storage

#### 3.1.1 Colour, Odour and Aroma

The colour, aroma and odour scores of *mpedli* beer samples during storage are compiled in Table 1. There were not significant changes ($p>0.05$) in terms of colour of treated beer samples throughout the storage at room temperature. However, odour and aroma attributes significantly varied ($p<0.05$) for both unprocessed and processed samples. Independently to the storage time, there were significant differences between treated and untreated control beer samples in terms of colour ($p = 0.0000$), odour ($p = 0.0223$) and aroma ($p = 0.0019$). The scores were ranged between 4.60±2.10 and 7.30±1.76; 2.90±1.79 and 6.90±1.66; and 3.20±2.20 and 6.60±0.84, for the sensory attributes colour, odour and aroma respectively. The colour of BUB (unpasteurized blended with VA aqueous leaf extract) and BPB (blended and pasteurized) samples was highly graded on the 12$^{th}$ (7.20±0.91) and 21$^{st}$ (7.10±1.44) day of storage respectively, while the CMB control sample was most appreciated on the zeroth day (7.30±1.76), when freshly prepared. Odour (4.70±1.70 and 6.90±1.66) and aroma (4.10±1.72 and 5.90±1.37) of the BPB samples increased with storage time from the 0$^{th}$ to 12$^{th}$ day of storage. BPB samples were the most graded in terms of odour (5.58±0.75) whereas BUB ones were the most appreciated in regards to aroma (5.55±0.68) compared to CMB control samples (4.57±1.10 and 4.59±0.80, respectively).

#### 3.1.2 Taste Descriptors

Table 2 presents taste sub attributes grouped as taste descriptors into bitterness, acidic and alcoholic of *mpedli* beer. Changes of taste attributes of BPB samples were not significant ($p>0.05$) while those of BUB and CMB samples varied significantly ($p<0.05$) during storage time. According to the processing method, bitterness and alcohol taste were significantly different ($p = 0.0008$ and $p = 0.0116$, respectively), while acidic taste was not significant ($p = 0.0702$) between the beer samples. Bitter taste of BUB and BPB was most appreciated on the 8$^{th}$ and 27$^{th}$ day of storage (6.60±1.34 and 6.30±1.63, respectively). Alcoholic taste of BPB samples received the best grades after day 8 and day 27 of storage (6.7±0.82 and 6.80±1.31, respectively). BUB samples were well scored in terms of alcoholic taste on the 8$^{th}$ day of storage (6.90±0.73). However, the CMB samples registered their greatest scores for bitterness (5.80±5.86 and 6.60±1.17, respectively) and alcoholic taste (6.60±1.34 and 6.40±1.64, respectively) on both the 0$^{th}$ and 2$^{nd}$ day of storage. The acidity of BUB and BPB *mpedli* beer was most appreciated on the sixth (6.40±0.84) and eighteenth (6.10±2.02) day of storage, while CMB samples recorded the highest scores on the 0$^{th}$ and 2$^{nd}$ day of storage (6.10±1.66 and 6.10±1.72, respectively). During storage, BUB samples were globally rated

**Table 1.** Mean scores for colour, odour and aroma attributes of *mpedli* beer samples during storage at room temperature

| Storage time (days) | Colour | | | Odour | | | Aroma | | |
|---|---|---|---|---|---|---|---|---|---|
| | CMB | BUB | BPB | CMB | BUB | BPB | CMB | BUB | BPB |
| 0 | 7.30±1.76$^{a}$ | 6.70±1.63$^{a}$ | 4.60±2.10$^{a}$ | 5.30±1.56$^{ab}$ | 5.10±1.20$^{ab}$ | 4.70±1.70$^{a}$ | 5.70±1.63$^{ab}$ | 5.60±1.83$^{ab}$ | 4.10±1.72$^{a}$ |
| 2 | 6.40±1.42$^{a}$ | 6.80±1.22$^{a}$ | 4.60±1.64$^{a}$ | 6.50±0.70$^{a}$ | 6.30±1.25$^{ab}$ | 5.00±1.63$^{ab}$ | 6.00±1.24$^{a}$ | 6.60±0.84$^{a}$ | 4.00±1.88$^{a}$ |
| 4 | 5.80±2.14$^{a}$ | 7.10±0.73$^{a}$ | 4.90±1.59$^{a}$ | 4.90±2.02$^{ab}$ | 6.50±1.08$^{b}$ | 5.00±2.05$^{ab}$ | 4.80±1.81$^{ab}$ | 6.00±0.94$^{ab}$ | 5.30±1.63$^{a}$ |
| 6 | 5.90±1.85$^{a}$ | 7.00±0.94$^{a}$ | 5.80±2.20$^{a}$ | 5.10±2.13$^{ab}$ | 6.00±1.63$^{ab}$ | 6.10±0.87$^{ab}$ | 6.00±2.16$^{a}$ | 6.00±1.41$^{ab}$ | 5.50±1.43$^{a}$ |
| 8 | 5.80±1.54$^{a}$ | 7.20±0.78$^{a}$ | 6.20±0.78$^{a}$ | 4.90±1.96$^{ab}$ | 6.40±0.96$^{ab}$ | 6.10±0.87$^{ab}$ | 4.30±1.82$^{ab}$ | 6.00±0.94$^{ab}$ | 5.60±1.26$^{a}$ |
| 12 | 5.20±2.01$^{a}$ | 7.20±0.91$^{a}$ | 5.90±1.79$^{a}$ | 2.90±1.79$^{b}$ | 6.40±0.69$^{ab}$ | 6.90±1.66$^{ab}$ | 3.20±2.20$^{b}$ | 6.20±1.13$^{ab}$ | 5.90±1.37$^{a}$ |
| 15 | 5.00±1.26$^{a}$ | 6.30±1.41$^{a}$ | 6.10±1.96$^{a}$ | 3.50±2.32$^{b}$ | 4.10±1.91$^{a}$ | 5.80±1.98$^{a}$ | 4.10±2.02$^{ab}$ | 5.80±1.03$^{ab}$ | 4.60±1.17$^{a}$ |
| 18 | 6.20±1.34$^{a}$ | 6.30±1.41$^{a}$ | 5.70±1.82$^{a}$ | 5.80±1.31$^{ab}$ | 4.60±1.77$^{ab}$ | 4.70±1.76$^{ab}$ | 3.80±1.98$^{ab}$ | 4.40±1.47$^{b}$ | 5.30±1.15$^{a}$ |
| 21 | 6.00±1.49$^{a}$ | 6.80±1.13$^{a}$ | 7.10±1.44$^{a}$ | 3.70±2.54$^{ab}$ | 5.00±1.88$^{ab}$ | 5.60±1.95$^{ab}$ | 4.80±1.75$^{ab}$ | 4.90±1.52$^{ab}$ | 5.40±1.34$^{a}$ |
| 24 | 5.40±1.34$^{a}$ | 6.30±1.33$^{a}$ | 6.60±1.77$^{a}$ | 3.60±2.45$^{ab}$ | 4.80±1.93$^{ab}$ | 5.30±1.94$^{ab}$ | 4.70±1.76$^{ab}$ | 5.70±1.41$^{ab}$ | 5.50±1.50$^{a}$ |
| 27 | 5.90±2.02$^{a}$ | 7.00±0.94$^{a}$ | 6.60±1.64$^{a}$ | 4.10±2.37$^{ab}$ | 5.80±2.14$^{ab}$ | 6.20±0.63$^{b}$ | 4.80±1.13$^{ab}$ | 5.50±1.50$^{ab}$ | 5.90±1.52$^{a}$ |
| Mean score | 5.90±0.62 | 6.79±0.35 | 5.83±0.83 | 4.57±1.10 | 5.55±0.85 | 5.58±0.75 | 4.59±0.80 | 5.55±0.68 | 5.19±0.66 |
| p-value | 0.0000* | | | 0.0223* | | | 0.0019* | | |

CMB: Control non-supplemented *mpedli* beer; BUB: Blended unpasteurized *mpedli* beer; BPB: Blended and pasteurized *mpedli* beer. For each sensory attribute, mean values in the column not followed by the same superscript lowercase letter (s) were different at $p < 0.05$ by storage time. (*) P-value lower than 0.05 indicating significant difference by processing method.

**Table 2.** Taste sub attributes of *mpedli* beer samples during storage

| Storage time (days) | Bitterness | | | Alcoholic | | | Acidic | | |
|---|---|---|---|---|---|---|---|---|---|
| | CMB | BUB | BPB | CMB | BUB | BPB | CMB | BUB | BPB |
| 0 | 5.80±5.8$^{6b}$c | 5.80±1.87$^{abc}$ | 5.10±2.07$^{a}$ | 6.60±1.34$^{a}$ | 5.70±1.41$^{abc}$ | 5.50±1.26$^{a}$ | 6.10±1.66$^{b}$ | 5.50±2.01$^{abc}$ | 5.10±2.02$^{a}$ |
| 2 | 6.60±1.17$^{c}$ | 6.60±1.57$^{c}$ | 4.60±1.83$^{a}$ | 6.40±1.64$^{ab}$ | 6.90±1.59$^{c}$ | 5.50±2.17$^{a}$ | 6.10±1.72$^{b}$ | 6.30±1.05$^{bc}$ | 4.80±1.87$^{a}$ |
| 4 | 5.50±1.90$^{abc}$ | 6.60±1.34$^{c}$ | 5.30±2.00$^{a}$ | 5.00±2.30$^{ab}$ | 6.40±1.07$^{bc}$ | 5.70±2.16$^{a}$ | 4.40±1.71$^{ab}$ | 6.10±1.28$^{bc}$ | 5.40±2.06$^{a}$ |
| 6 | 4.40±1.95$^{abc}$ | 6.50±0.70$^{bc}$ | 6.00±1.63$^{a}$ | 5.30±2.3$^{ab}$ | 5.80±1.13$^{abc}$ | 6.20±1.54$^{a}$ | 4.60±2.54$^{ab}$ | 6.40±0.84$^{c}$ | 5.80±1.47$^{a}$ |
| 8 | 4.80±2.25$^{abc}$ | 6.60±1.34$^{c}$ | 6.00±0.94$^{a}$ | 4.80±2.20$^{ab}$ | 6.90±0.73$^{bc}$ | 6.70±0.82$^{a}$ | 4.20±2.61$^{ab}$ | 5.80±1.68$^{abc}$ | 5.40±1.89$^{a}$ |
| 12 | 2.80±1.03$^{a}$ | 6.10±1.44$^{abc}$ | 5.70±1.56$^{a}$ | 3.60±1.95$^{b}$ | 6.50±0.84$^{c}$ | 7.00±1.05$^{a}$ | 2.90±2.28$^{a}$ | 5.20±1.39$^{abc}$ | 5.10±1.10$^{a}$ |
| 15 | 3.40±2.01$^{ab}$ | 4.30±1.94$^{a}$ | 5.70±1.15$^{a}$ | 3.60±2.11$^{b}$ | 4.50±2.22$^{ab}$ | 6.10±1.10$^{a}$ | 2.50±1.64$^{a}$ | 3.60±1.57$^{ab}$ | 4.10±1.10$^{a}$ |
| 18 | 3.30±2.26$^{ab}$ | 4.40±1.17$^{ab}$ | 6.20±1.87$^{a}$ | 4.40±1.26$^{ab}$ | 4.10±1.28$^{a}$ | 6.30±1.05$^{a}$ | 3.10±1.28$^{ab}$ | 3.30±1.41$^{a}$ | 6.10±2.02$^{a}$ |
| 21 | 3.90±2.23$^{abc}$ | 5.90±1.52$^{abc}$ | 5.90±1.79$^{a}$ | 4.80±2.25$^{ab}$ | 4.20±1.98$^{ab}$ | 5.40±1.71$^{a}$ | 3.40±2.22$^{ab}$ | 3.80±2.85$^{abc}$ | 4.20±2.09$^{a}$ |
| 24 | 3.80±2.30$^{abc}$ | 5.90±1.72$^{abc}$ | 6.50±1.50$^{a}$ | 4.80±1.68$^{ab}$ | 5.70±1.82$^{abc}$ | 5.50±1.17$^{a}$ | 2.80±2.20$^{a}$ | 3.20±2.48$^{a}$ | 4.70±2.26$^{a}$ |
| 27 | 4.50±1.84$^{abc}$ | 6.30±1.25$^{abc}$ | 6.30±1.63$^{a}$ | 4.50±2.27$^{ab}$ | 6.10±1.28$^{abc}$ | 6.80±1.31$^{a}$ | 3.60±2.41$^{ab}$ | 3.80±2.44 | 4.70±2.16$^{a}$ |
| Mean score | 4.44±1.16 | 5.91±0.83 | 5.75±0.56 | 4.89±0.96 | 5.71±1.02 | 6.06±0.59 | 3.97±1.25 | 4.82±1.28 | 5.04±0.62 |
| P-value | | 0.0008* | | | 0.0116* | | | 0.0702 | |

CMB: Control non-supplemented *mpedli* beer; BUB: Blended unpasteurized *mpedli* beer; BPB: Blended and pasteurized *mpedli* beer. For each sensory attribute, mean values in the column not followed by the same superscript lowercase letter (s) were different at $p < 0.05$ by storage time. (*) P-value lower than 0.05 indicating significant difference by processing method.

the highest in terms of bitterness (5.91±0.83), while BPB samples were better judged than CMB samples in terms of alcoholic (6.06±0.59 versus 4.89±0.96) and acidic taste (5.04±0.62 versus 3.97±1.25).

### 3.1.3 Viscosity, Texture and Overall Acceptability

The sensory ratings of the texture, viscosity and overall acceptability of *mpedli* beer during storage are presented in Table 3. Except for the overall acceptability of BUB samples which displayed significant variations ($p<0.05$), all the other attributes for both treated and untreated beer samples were not significant ($p>0.05$) throughout storage period. The viscosity and texture scores of the processed beer samples increased from the 0$^{th}$ to the 12$^{th}$ day of storage for BUB samples (6.30±1.82 to 7.00±0.93 and 6.50±1.43 to 7.20±1.03, respectively) and 27$^{th}$ day for BPB samples (5.40±2.45 to 6.70±1.76 and 4.10±2.46 to 6.80±2.27, respectively). In terms of viscosity (7.00±0.94 and 6.80±2.27) and texture (7.20±1.03 and 6.70±1.76), BUB and BPB samples were most graded on the 12$^{th}$ and 27$^{th}$ day of storage respectively, while CMB samples were most preferred on the 8$^{th}$ and 24$^{th}$ day of storage (6.40±1.34 and 6.90±1.37, respectively).

These results showed a significant difference in terms of viscosity ($p = 0.0340$) and texture ($p = 0.0025$), but BUB samples were most preferred for both attributes through the storage. These samples recorded 69% (mean score 6.23) compared to 65% (mean score 5.85) and 60% (mean score 5.42) viscosity rating for the control CMB and processed BPB samples respectively. In the same trends, 72% (mean score 6.49±0.54) of texture rating was attributed to BUB samples while 68% (mean score 6.15±0.45) and 62% (mean score 5.56±0.70) of the same attribute were shown for CMB and BPB samples, respectively.

The overall acceptability of BPB samples did not significantly change while relevant variations ($p<0.05$) were found for CMB and BUB samples during the storage. The unpreserved CMB samples were most appreciated when fresh, on the 0$^{th}$ day of storage (6.60±1.26) while the blended and unpasteurized BUB samples were most appreciated from the 2$^{nd}$ to 12$^{th}$ storage day (7.10±0.09 to 7.10±0.56, respectively), with the highest acceptance recorded on the 4$^{th}$ and 6$^{th}$ storage day (7.20) and the pasteurized and blended the BPB samples were most appealed from the 15$^{th}$ to the 27$^{th}$ storage day (6.40±1.17 to 7.00±1.33, respectively). Globally, the processed *mpedli* beer was significantly ($p = 0.0047$) more accepted than non-supplemented *mpedli* drink. The BUB and BPB samples recorded 72.3% (mean score 6.51) and 69% (mean

**Table 3.** Viscosity, texture and overall acceptability of *mpedli* beer samples during storage

| Storage time | Viscosity | | | Texture | | | Acceptability | | |
|---|---|---|---|---|---|---|---|---|---|
| (days) | CMB | BUB | BPB | CMB | BUB | BPB | CMB | BUB | BPB |
| 0 | 5.50±2.27$^{a}$ | 6.30±1.82$^{a}$ | 4.10±2.46$^{a}$ | 6.10±2.18$^{a}$ | 6.50±1.43$^{a}$ | 5.40±2.45$^{a}$ | 6.60±1.26$^{a}$ | 6.40±0.69$^{ab}$ | 5.50±1.90$^{a}$ |
| 2 | 5.90±1.37$^{a}$ | 5.60±1.77$^{a}$ | 4.70±2.49$^{a}$ | 6.40±1.77$^{a}$ | 6.40±1.50$^{a}$ | 4.40±2.36$^{a}$ | 6.80±1.13$^{a}$ | 7.10±0.99$^{a}$ | 5.20±1.93$^{a}$ |
| 4 | 5.80±1.68$^{a}$ | 6.90±0.87$^{a}$ | 4.40±2.06$^{a}$ | 5.70±1.82$^{a}$ | 6.90±1.52$^{a}$ | 4.80±1.81$^{a}$ | 5.60±1.95$^{ab}$ | 7.20±0.91$^{a}$ | 5.40±1.57$^{a}$ |
| 6 | 4.90±1.10$^{a}$ | 6.70±0.94$^{a}$ | 5.40±2.31$^{a}$ | 5.80±1.39$^{a}$ | 6.80±1.39$^{a}$ | 5.30±2.31$^{a}$ | 5.70±1.70$^{ab}$ | 7.20±0.78$^{a}$ | 6.40±1.07$^{a}$ |
| 8 | 6.40±1.34$^{a}$ | 6.30±1.76$^{a}$ | 4.70±1.56$^{a}$ | 6.40±1.77$^{a}$ | 6.20±1.61$^{a}$ | 5.10±1.72$^{a}$ | 5.90±1.19$^{b}$ | 6.70±0.48$^{ab}$ | 6.40±0.51$^{a}$ |
| 12 | 6.30±2.05$^{a}$ | 7.00±0.94$^{a}$ | 5.50±1.58$^{a}$ | 5.50±2.36$^{a}$ | 7.20±1.03$^{a}$ | 6.10±1.44$^{a}$ | 4.50±1.71$^{b}$ | 7.10±0.56$^{a}$ | 6.70±0.82$^{a}$ |
| 15 | 5.40±1.64$^{a}$ | 5.10±1.52$^{a}$ | 6.00±1.33$^{a}$ | 5.60±2.01$^{a}$ | 5.20±1.22$^{a}$ | 5.50±1.26$^{a}$ | 4.40±1.34$^{ab}$ | 5.30±1.25$^{b}$ | 6.40±1.17$^{a}$ |
| 18 | 6.30±1.49$^{a}$ | 5.90±1.59$^{a}$ | 5.10±1.37$^{a}$ | 6.20±1.47$^{a}$ | 6.70±1.33$^{a}$ | 5.40±1.71$^{a}$ | 5.10±1.19$^{ab}$ | 5.70±1.15$^{ab}$ | 6.20±1.13$^{a}$ |
| 21 | 6.20±1.98$^{a}$ | 5.70±0.94$^{a}$ | 6.50±1.26$^{a}$ | 6.50±2.06$^{a}$ | 6.00±1.69$^{a}$ | 6.10±1.37$^{a}$ | 5.70±0.94$^{ab}$ | 6.10±1.19$^{ab}$ | 6.50±1.17$^{a}$ |
| 24 | 6.20±1.75$^{a}$ | 6.80±1.87$^{a}$ | 6.40±1.68$^{a}$ | 6.90±1.37$^{a}$ | 6.80±2.09$^{a}$ | 6.40±2.17$^{a}$ | 5.20±1.03$^{ab}$ | 6.30±1.49$^{ab}$ | 6.90±1.19$^{a}$ |
| 27 | 5.50±1.77$^{a}$ | 6.20±1.31$^{a}$ | 6.80±2.2$^{7}$a | 6.60±1.77$^{a}$ | 6.70±1.76$^{a}$ | 6.70±1.76$^{a}$ | 5.20±1.13$^{ab}$ | 6.50±1.17$^{ab}$ | 7.00±1.33$^{a}$ |
| Mean score | 5.85±0.48 | 6.23±0.61 | 5.42±0.91 | 6.15±0.45 | 6.49±0.54 | 5.56±0.70 | 5.52±0.75 | 6.51±0.63 | 6.24±0.61 |
| p-value | | 0.0340* | | | 0.0025* | | | 0.0047* | |

CMB: Control non-supplemented *mpedli* beer; BUB: Blended unpasteurized *mpedli* beer; BPB: Blended and pasteurized *mpedli* beer. For each sensory attribute, mean values in the column not followed by the same superscript lowercase letter (s) were different at $p < 0.05$ by storage time. (*) P-value lower than 0.05 indicating significant difference by processing method.

score 6.24) overall acceptability rating respectively, while the CMB samples only had 61% (mean score 5.52) acceptance during the storage period at room temperature.

### 3.2 Radar Plots

Figure 2 represents the global view of the *mpedli* beer at the end of storage. It emerges that, the blended samples portrayed a similar radial representation against the control non-supplemented samples. It also has been noted that storage at room temperature upgrades general appreciation of the treated beer samples (BUB and BPB) compared to unpreserved ones (CMB). Therefore, the BUB samples were found most graded for its colour/appearance, bitterness, aroma, viscosity, texture and overall acceptability while the BPB ones were most liked for its acidic and alcoholic taste.

### 3.3 Correlation between the Sensory Attributes of the Treated Sorghum Beer Samples during Storage

The association between the organoleptic characteristics of BUB and BPB *mpedli* beer samples during storage is presented in both Tables 4 and 5, respectively. With the unpasteurized and blended BUB samples (Table 4), a positive and significant interrelation was found between colour and bitterness ($r = 0.797$; $p < 0.01$); odour and colour ($p = 0.906$; $p < 0.01$); odour and bitterness ($r = 0.868$; $p < 0.01$); odour and alcoholic taste ($r = 0.862$; $p < 0.01$). Aroma was highly correlated to odour ($r = 0.795$; $p < 0.01$), bitterness ($r = 0.870$; $p < 0.01$), and alcoholic taste ($r = 0.920$; $p < 0.01$). All the properties evoked above, positively influenced the overall acceptability of BUB samples; however, bitterness and odour attributes were found more loaded to the overall acceptability of the indigenous beer ($r = 0.898$; $p < 0.01$ and $r = 0.930$; $p < 0.01$, respectively). Colour and bitter taste were more correlated ($r = 0.819$; $p < 0.01$) in BPB samples than in BUB ones. Viscosity significantly affected bitterness ($r = 0.697$; $p < 0.05$) and colour ($r = 0.869$; $p < 0.01$) in the pasteurized and blended BPB samples. Aroma of BPB samples was mainly linked to the bitterness ($r = 0.779$; $p < 0.01$). A positive and relevant correlation was found between texture and viscosity ($r = 0.801$; $p < 0.01$) in BPB samples during storage. The acceptance of BPB samples was positively linked to viscosity ($r = 0.819$; $p < 0.01$), texture ($r = 0.862$; $p < 0.01$), bitterness ($r = 0.887$; $p < 0.01$) and colour ($r = 0.894$; $p < 0.01$).

### 3.4 Multivariate Analysis

To visualise the relationships of *mpedli* beer with their sensory attributes during the storage, principal component analysis (PCA) was carried out using 33 samples and 9 attributes that showed statistical significance (p-value < 0.0001) according to the sphericity test of Bartlett. The Kaiser-Meyer-Olkin (KMO) value of 0.832 indicated that the sampling was sufficient for PCA analysis. As shown by the PCA biplot, the main sample differences and similarities, as well as sensory attributes, were reduced to two main dimensions, F1 and F2, which accounted for 59.15% and 23.64%, respectively, and both explained 82.79% of

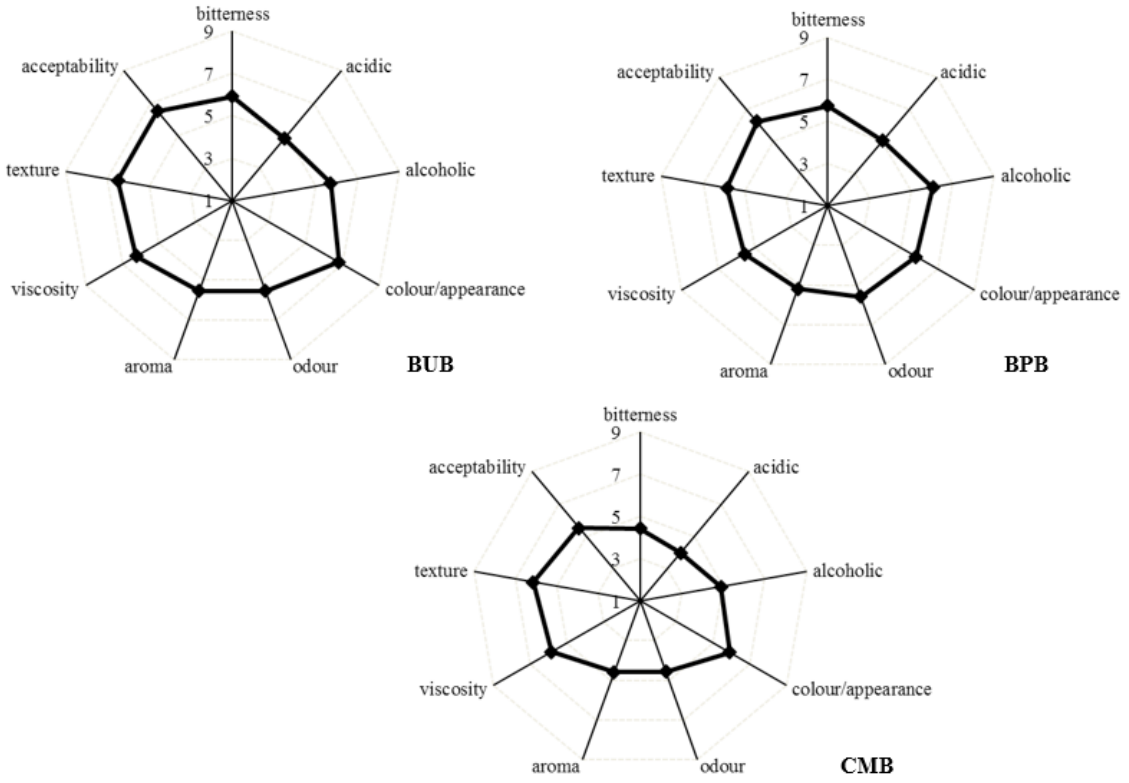


**Figure 2.** Radar plots for treated and control *mpedli* beer. CMB: Control non-supplemented *mpedli* beer, BUB: Blended unpasteurized *mpedli* beer and BPB: Blended and pasteurized *mpedli* beer

**Table 4.** Pearson coefficients between the sensory attributes of BUB samples during storage period

| Colour | Bitterness | Alcohol | Viscosity | Odour | Aroma | Texture | Acceptability |
|---|---|---|---|---|---|---|---|
| 1 | 0.797** | 0.696* | 0.497 | 0.90[6*]* | 0.611* | 0.406 | 0.794** |
| | 1 | 0.813** | 0.559 | 0.868** | 0.870** | 0.485 | 0.898** |
| | | 1 | 0.506 | 0.862** | 0.920** | 0.444 | 0.821** |
| | | | 1 | 0.556 | 0.439 | 0.859** | 0.679* |
| | | | | 1 | 0.795** | 0.557 | 0.930** |
| | | | | | 1 | 0.357 | 0.860** |
| | | | | | | 1 | 0.676* |
| | | | | | | | 1 |

(*): r values are statistically significant at $p < 0.05$; (**): r values are statistically significant at $p < 0.01$.

**Table 5.** Correlation between the sensory attributes of blended and pasteurized BPB *mpedli* beer samples

| Colour | Bitterness | Alcohol | Viscosity | Odour | Aroma | Texture | Acceptability |
|---|---|---|---|---|---|---|---|
| 1 | 0.819** | 0.265 | 0.869** | 0.604* | 0.684* | 0.763** | 0.894** |
| | 1 | 0.398 | 0.687* | 0.479 | 0.77[9*]* | 0.725* | 0.887** |
| | | 1 | 0.058 | 0.655 | 0.626* | 0.286 | 0.526 |
| | | | 1 | 0.504 | 0.483 | 0.801** | 0.819** |
| | | | | 1 | 0.610 | 0.537 | 0.696* |
| | | | | | 1 | 0.604* | 0.767** |
| | | | | | | 1 | 0.862** |
| | | | | | | | 1 |

(*): r values are statistically significant at $p < 0.05$; (**): r values are statistically significant at $p < 0.01$.

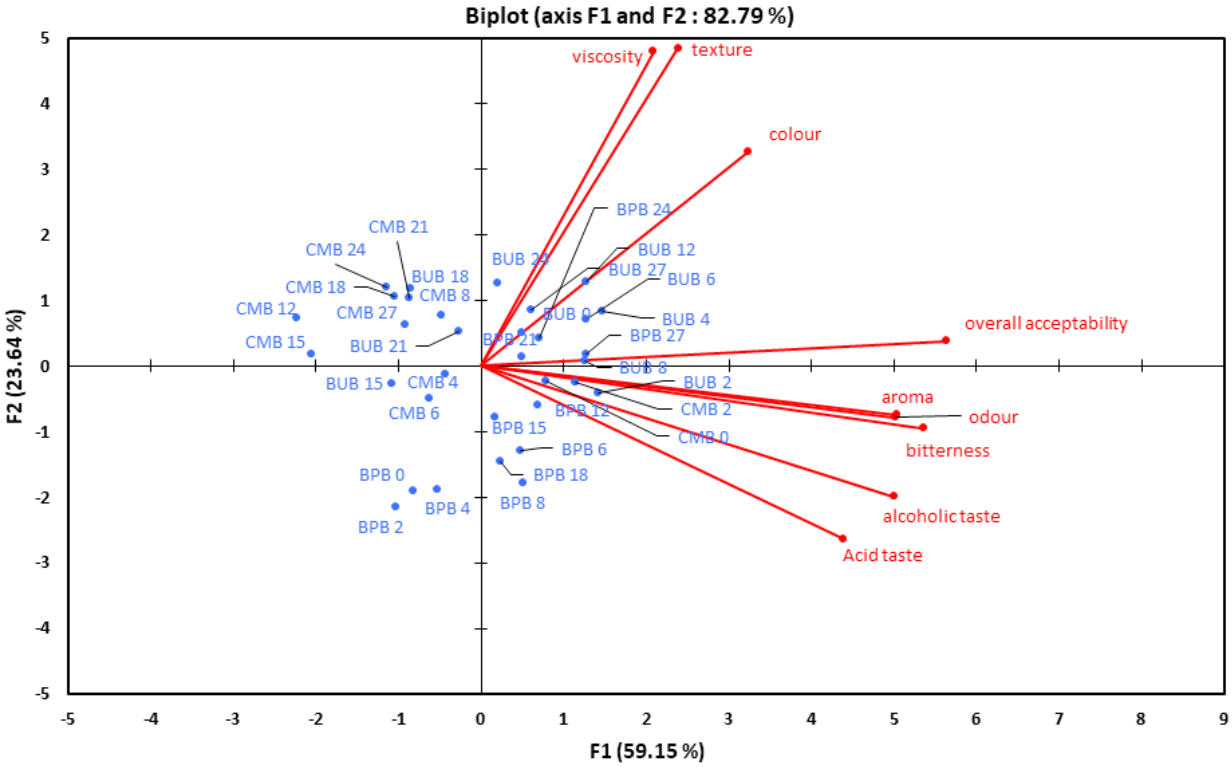


**Figure 3.** PCA loadings for sensory attributes and the scores of *mpedli* beer after varimax rotation ($p < 0.0001$; KMO = 0.832). Beer samples are coded as Figure 1. Digits 0 to 27 represent number of storage days

the total variation (Figure 3). The F1 dimension mainly accounted for the sensory attributes odour, aroma, bitterness, acidic taste, alcoholic taste, and overall acceptability which were loaded on the positive side of this factor. The unpreserved and control CMB 0, CMB 2, unpasteurized blended BUB 2, BUB 8, and pasteurized blended BPB 12, BPB 27 samples recorded high positive sensory scores for these attributes. While colour, viscosity and texture contributed positively to the F2 dimension and VA blended samples BUB 12, 24 and 27 registered high positive score for the three sensory attributes mentioned above.

## 4. Discussion

It was observed that the colour, odour, taste (bitterness and alcoholic), aroma, texture, and overall acceptability of beer samples varied significantly during the storage. This implies that pasteurization treatment and addition of aqueous extract of *Vernonia amygdalina* leaf had an effect on the organoleptic characteristics of the *mpedli* beer. As with some indigenous beverages, it has been shown that blending may increase both the shelf life and sensory acceptance of the product [26]. Though the blended samples witnessed relevant grade in the aforementioned attributes, the blended and unpasteurized *mpedli* beer samples registered the greatest scored in terms of colour/appearance, viscosity, bitter taste, texture and overall acceptability. According to Nielsen [27] and Salanţă et al. [28], colour is one of the important characteristics that reinforce quality and acceptability of food products. The difference in colour between treated and unpreserved beer samples resulted from the addition of *Vernonia amygdalina* leaf extract. Leaves of VA are well-known for their high concentration in phytochemicals and natural phytopigments like green chlorophyll. This last component was responsible for the greenly opaque appearance of the processed *mpedli* samples [29]. The increase in colour of treated beer samples with storage time matched with the observation done by Cao et al. [30], who showed that the colour of beer increased linearly during storage at room temperature. The relevant sensory scores registered by control *mpedli* samples only during the first 48 hours after the production should be the result of microbial alteration, which caused deterioration of the *mpedli* beer after this storage period. In line with Bayoï et al. [9], unpreserved local beverages have shelf life less than 48 hours at room temperature. According to Cao et al. [30] and Malfliet et al. [31], the quality of beers is greatly affected by temperature and storage time. The BPB blended and pasteurized *mpedli* beer recorded the highest scores

in the acidic and alcoholic taste, while the lowest grades were recorded for viscosity and texture. The heating use during the pasteurization process has greatly influenced the nature of processed beer. The heating accelerates evaporation as such BPB sample became more concentrated making it more viscous (porridge), high texture with an opaque colour. Two sensory characteristics very rejected by the consumers of sorghum-based beer like *mpedli* [32]. Moreover, the high scores for alcoholic and acidic taste attributes of pasteurized and blended *mpedli* beer were also associated to the heat treatment. During heating, there is hydrolysis of residual starch and disaccharides into glucose molecules which are easily converted in either alcohol or organic acids by fermenting microorganisms which escaped to the combined effect of heating treatment and antimicrobial action of the plant extract [33]. However, the panelist respondents reported that both alcoholic and acidic taste of pasteurized and blended BPB samples were just about right during storage period. According to *mpedli* consumers, this beverage is preferred when the alcohol content is moderately high. Therefore, most of indigenous beers are accepted at high level alcoholic [34]. Despite technological development, the production of manufactured beers with low levels of alcohol is more and more pertinent [12]. The bitter taste of *mpedli* samples positively affected the acceptability of the beers. So, blended samples were more accepted than non-blended ones. Bitterness is a characterisitic property of manufactured beers [28]. According to Pluháčková et al. [35], the bitter taste was the most appreciated and graded with beer samples fortified with some Czech medicinal herbs and plants. Plant secondary metabolites are rich in polyphenols, flavonoids, xanthones, phenolic acids, and resins with characteristics tastes [36]. Among the commercially used hops, polyphenols, flavouring agents and hops resins constitute the major bitter agents in the brewing process [37]. Unfortunately, bitterness is not among the sensory attributes found in traditional African beers [18]. Therefore, the use of bitter plant species as *Vernonia amygdalina* should be considered as a useful path to upgrade the processing of sorghum-based beer and improve the quality of the local indigenous beers [14]. The extracts from *Vernonia amygdalina* are known to contain secondary phytoconstituents like Vernoniosides which are a group of saponins likely responsible for bitter taste of the leaves. The bitter taste had been also associated with the presence of alkaloids, tannins, and glycosides [17]. These compounds made VA leaves act as a bittering agent and a hop substitute used for controlling microbial contamination in beer brewing without reducing the quality of malt [19]. Even with this supplementation action, the bitter taste of the processed beer varied greatly. Unpasteurized samples were more bitter than pasteurized ones. This variation resulted from the heating action (pasteurization) which led to the volatilisation of some metabolites responsible for the bitter taste of the blended *mpedli*. Odour was found as one of the major attributes contributing to the overall acceptability of the *mpedli* beer. The treated samples were more preferred than untreated control samples in terms of odour. This was consistent with increase in storage time. The reason may be attributed to off-odour produced from the 2$^{nd}$ day of storage altering quality and acceptance of untreated *mpedli* samples. While, both pasteurization and VA aqueous leaf extract, which are considered as two antimicrobial treatments, slow the formation of off-odour and reinforce the "leafy flavour" of the vegetable extract making processed *mpedli* more pleasant contrary to untreated *mpedli*, which was attractive freshly (0$^{th}$ day of storage). The unpasteurized blended BUB sample was highly preferred between 4$^{th}$ and 6$^{th}$ day of storage and the pasteurized blended BPB one recorded relevant acceptance from the 15$^{th}$ to 27$^{th}$ day of storage. This suggests that BPB sample registered the highest shelf life and confirmed the efficacy of combined preservation techniques compared to unifactorial one. This is in the same line with findings reported by Konfo et al. [14] with African traditional sorghum beers and Ayirezang et al. [39] with pito beer from Ghana and Nigeria.

## 5. Conclusions

The adjunction of the aqueous leaf extract of *Vernonia amygdalina* and pasteurization have greatly and positively influence most of the sensory characteristics and the shelf life of the *mpedli* beer. Irrespective of the treatment made, the colour, viscosity, texture, bitterness, acidic taste, aroma, odour and alcoholic taste affected the overall acceptability but the bitter taste and the odour were the key determining factor. With respect to these attributes, the BUB blended beer samples were the most appreciated but the BPB blended and unpasteurized *mpedli* beer had a milder appreciation. Apart from the trends in the sensory parameters, it was noticed that processed *mpedli* beer had extended shelf life over the untreated sample. Therefore, the pasteurized and blended *mpedli* beer registered the most valuable shelf-life extension. As with the sensory parameters, the aqueous leaf extract of VA and fabulous heating action during pasteurization contributed positively for it. Substantially, the aqueous leaf extract of VA contains

important elements necessary to enhance the sensory characteristics and shelf life of *mpedli* beer and more so, its bitterness makes it a potential source of natural hops which may be exploited in the Cameroonian brewing industry.

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This research received no external funding.

## Conflict of Interest

All authors declare that no competing interest exist. The beverage used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use this product as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the brewing company rather it was funded by private efforts of the authors.

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