

Effect of Moringa Stenopetala Leaf Extract on Antioxidant Capacity, Microbial Quality, and Consumer Acceptability of Different Lager Beer Brands in Addis Ababa, Ethiopia

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Abstract

Microbial spoilage from the brewing process or due to storage, oxidation, and flavor instability affects beer quality. Modern breweries apply synthetic antioxidants to overcome these problems. This study aimed to investigate the efficacy of moringa stenopetala leaf extract as an alternative to synthetic antioxidants. 200, 400, and 600 ppm of the extract were added to three beer brands (Walia, Harar, and St. George). A negative control (untreated beer) and a positive control (12 ppm potassium metabisulphite-treated beer) were also used. Total phenolic content was determined by the Folin-Ciocalteu method and total flavonoid by the aluminum chloride method. Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. Total plate count, yeast and mold, and potential beer spoilers were also analyzed. Consumer acceptability was assessed using a nine-point hedonic scale. The total phenolic content of untreated beer was 66.04 ± 1.67 , 68.83 ± 0.23 and 44.06 ± 0.27 mg GAE/L and total flavonoid was 102.37 ± 9.78 , 115.57 ± 3.49 and 86.87 ± 7.84 mg CE/L for Walia, Harar and St. George beer, respectively. Up to 600 ppm extract addition raised the total phenolic content to 75.24 ± 1.31 , 76.91 ± 1.46 , and 69.51 ± 2.52 mg GAE/L and the total flavonoid content to 167.09 ± 2.95 , 171.62 ± 0.89 , and 124.27 ± 1.16 mg CE/L for Walia, Harar, and St. George beer, respectively. The addition of 600 ppm extract resulted in up to 65.78 % DPPH inhibition. Total phenolic content, total flavonoid, and DPPH inhibitory capacity decreased with increasing storage time. All beer is treated with leaf extract up to 600 ppm and consumers have accepted KMS 12 ppm. Keywords: antioxidant activity, consumer acceptability, effect, lager beer, lea extract, microbial quality, moringa stenopetala

1. Introduction

Beer is one of the oldest and most often consumed alcoholic beverages in human history. Globally beer is the most consumed alcoholic beverage; it is the third preferred drink after water and tea (1). However, beer spoilage by bacteria of the genera *Lactobacillus* and *Pediococcus* and flavor instability are serious challenges for the brewing industry (2). In beer spoilage, bacteria have their effect starting from relatively minor changes to more serious physicochemical changes like off-flavor and aroma defects, turbidity problems, and ropiness. These undesirable changes bring significant economic losses annually (3).

One of the most important quality problems in the brewing industry is flavor instability resulting from beer storage due to oxidation (4). As a result, breweries are now applying synthetic antioxidants to prevent or minimize the effects of beer oxidation that can occur during storage. However, current research does not recommend the use of synthetic antioxidants in food and beverage industries because of the prolonged health effects and the evolution of food laws (5). Sodium metabisulphite, which is an artificial antioxidant, is effective in extending the shelf life of pito (sorghum beer in Ghana) but found to hurt tract irritation (6). Therefore, modern breweries should look into other natural and safe alternatives to artificial antioxidants. Polyphenolic compounds are found to be promising natural antioxidants, with mechanisms involving both atom scavenging and metal chelation and could replace the synthetic ones (7).

Several plant products and herb extracts including polyphenolic substances like flavonoids and tannins have proven to have antioxidant actions (8). The *Moringa* species like *Moringa stenopetala*, *Moringa peregrine*, *Moringa oleifera*, and *Moringa concanensis* are currently of wide interest due to their outstanding potential as nutritious vegetables, antioxidant properties, medicinal plants, and water clarification agents (9).

M. stenopetala is endemic to Southern Ethiopia and Northern Kenya and commonly known as the cabbage-tree (10). It is widely used in Ethiopian and Kenyan traditional medicine for treating a range of illnesses (11). *M. stenopetala* leaf powder and extracts exhibited anticoccidial activity against *Eimeriatenella* infection in broiler chickens (12). The hydroalcoholic extract of leaves was shown to inhibit intestinal α -glucosidase, pancreatic cholesterol esterase, and pancreatic lipase activities (13). The seed powder was also found to be effective in the removal of several heavy and toxic metals (14). In addition, *Moringa* is known to be a source of natural antioxidants. It contains a high concentration of phenolic acids, flavonoids, and alkaloid compounds (15, 16). The antioxidant and different medicinal properties of *M. stenopetala* extract could play a role in flavor stability, which is currently one of the determining factors for the shelf life of packed beer.

So far, an attempt has been made to investigate the effect of *M. stenopetala* leaf extract on the physicochemical properties of laagered beer during storage time. The limitation or gap was that the study was done only on a single beer brand. In addition, its effect on microbial load was not investigated (17, 18). Therefore, the present study aimed to investigate the effect of *M. stenopetala* leaf extract on the antioxidant capacity, microbial load, and sensory acceptability of different lager beers produced in Ethiopia

2. Materials and Methods

2.1. Study area

This experiment was conducted in St. George and Heineken breweries, in Addis Ababa, Ethiopia.

2.2. Beer sampling and sample collection

A total of 120 bottles of 3 different brands of lager beer (Walia, Harar, and St. George) were collected from two different brewery factories (St. George and Heineken breweries). 40 bottles of St. George beer from St. George Brewery and 80 bottles (40 Walia and 40 Harar) beer from Heineken breweries were collected to investigate the effect of *M. stenopetala* leaf extract (MSLE) on the microbial quality, antioxidant capacity, and sensory acceptability of beer during storage period. Bottles were selected by a simple random sampling technique.

2.3. Raw material preparation and extraction

2.3.1. *M. stenopetala* leaf extract (MSLE) preparation

Green leaves of *M. stenopetala* were collected from the Melkassa agricultural research center wrapped with aluminum foil and brought to Addis Ababa University, Center for Food Science and Nutrition Laboratory. Formal identification of the plant was carried out by a senior botanist at the Melkassa Agricultural Research Center, who confirmed the species based on morphological characteristics.

It was washed with water to get rid of impurities and air-dried in a dark room to avoid exposure to sunlight. The dried leaves were ground using an electrical grinder as described in Nadeem *et al* (16). One gram of the leaf powder was mixed with 10 mL of 80 % ethanol (1:10) (w/v) using a Pyrex beaker of 1000 mL. The beakers were closed with a cover bush and macerated using an electrical shaker for 18 hrs. The residue was separated by Whatman paper and the clear solution was dried at 50 °C using a rotary evaporator. A stock solution was prepared by using 80 % ethanol in a 5 mg/mL ratio and stored in the refrigerator at 4 °C for subsequent usage as described in Siddhuraju and Becke (19).

2.4. Experimental design and treatment

All the beer samples from both breweries were collected by using a simple random sampling technique. Then, different concentrations of MSLE and 12 ppm of the usual synthetic antioxidant called potassium metabisulphite (KMS) were added to each beer type using a safety hood.

MSLE concentration levels were determined based on the result of the pre-test sensory evaluation experiment. Selected and trained panelists participated to evaluate the maximum concentration of MSLE that could be added without significantly affecting the original flavor of the beer, and that concentration was determined as 600 ppm. Based on the pre-test experiment, three levels of treatments 200 ppm (C2), 400 ppm (C3), and 600 ppm (C4) of MSLE were selected. The factorial experimental design was used to investigate the effect of MSLE at different concentrations (C2, C3, and C4) with different storage times (1, 30, 60, and 90 days) on the microbial analysis, antioxidant potential and consumer acceptability of the beer (**Table 1**). In addition to the selected treatments, the experiment used a negative control for a beer with no antioxidant added (C1) and a positive control for a beer with conventional 12 ppm KMS (C5). The initial concentration of MSLE (200 ppm) was based on previous work (17, 18) and the concentration of KMS (12 ppm) was based on factory standards.

All samples were labeled and stored at room temperature. The microbial analysis and antioxidant potential of each sample were analyzed every 30 days for three consecutive months starting from the first day of sample preparation.

2.5. Determination of bioactive compounds

2.5.1. Total phenol

The total phenolic content of each beer sample was determined using the Folin–Ciocalteu reagent according to Pourmorad *et al* (20) with a slight modification. Beer samples were diluted to five-folds, from each diluted beer sample, 1 mL was taken and mixed with 9 mL of Folin-Ciocalteu reagent (diluted ten times) and the mixture was left for 5 min. Then 1 mL sodium carbonate (7.5 % w/w) was added and the mixture was incubated for 90 min at room temperature. A calibration curve was obtained by using gallic acid as standard. Different concentrations of gallic acid were prepared. After 90 min incubation, the absorbance of all standards and samples was measured at 765 nm using Cecil CE7410 UV-Vis spectrophotometer and results were expressed as milligrams of gallic acid equivalents (GAE) per liter of beer.

2.5.2. Total flavonoid

The total flavonoids of each beer sample were analyzed according to the method described by Pai *et al.* (21). Catechin was used to make the calibration curve. 10 mg of catechin was dissolved in 96 % ethanol and diluted to 2, 4, 6, 8, and 10 µg/mL. The beer sample was diluted five times and 1 mL of each concentration of standard solution as well as 1 mL of each diluted beer sample was mixed with 3 mL of 96 % ethanol, 0.2 mL of 10 % aluminum chloride, 0.2 mL of potassium acetate (1 M) and 5.6 mL of distilled water. The mixture was incubated at room temperature for 10 min with intermittent shaking. The absorbance was measured at 510 nm using a Cecil CE7410 UV-V is spectrophotometer. Results of total flavonoid were calculated as mean ± SD (n=3) and expressed as milligrams of catechin equivalents (CE) per liter of beer.

2.6. Determination of antioxidant potential

2.6.1. DPPH radical scavenging capacity

Free radical scavenging activity of MSLE was measured by 2, 2- diphenyl-1-picryl hydroxyl (DPPH). Briefly, a 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 mL) was added to 3 mL of different extracts in ethanol at different concentrations (200, 400, 600 ppm). The mixture was shaken vigorously and allowed to stand at room temp for 30 min then, absorbance was measured at 517 nm by using a spectrophotometer (UV-VIS Shimadzu) as described by Ahmad *et al.* (22). The Reference standard compound being used was ascorbic acid and the experiment was done in triplicate. The lower absorbance of the reaction mixture indicated higher free radical activity (23). The percent DPPH scavenging effect (I %) was calculated by using the following equation:

DPPH scavengi

ng effect or Percent inhibiti

on (I %) = $A_C - A_S / A_C \times 100$.

Where:

Ac = is the absorbance of the control reaction without the test sample

As = is the absorbance of the test sample

% I = Percent of inhibition

2.7. Microbial analysis

2.7.1. Total plate count

Modified Wallerstein Laboratory medium (m WLN); a medium for performing total counts within the brewery was used and samples were membrane filtered before being subjected to analysis. 37 g of m WLN agar was dissolved in 1 L of water by stirring and boiling. The pH was checked and adjusted to six with 1.0 N HCl and 1.0 N NaOH solution. The molten medium was dispensed into final screw-cap containers and sterilized in an autoclave for a quarter-hr at 121 °C. The medium was cooled to 45 °C poured into sterilized petri dishes and incubated for 3 days at 31 °C under aerobic conditions.

2.7.2. Yeast and mold count

Yeasts and molds were enumerated on YPD-Chloramphenicol (10 g yeast extract, 10 g peptone, 20 g glucose, 20 g agar, 0.5 g chloramphenicol, and 1000 mL distilled water) after 48 to 72 h of incubation at 30 °C.

2.7.3. Potential beer spoilers-Raka Ray

Raka Ray medium was used to provide a general count of the potential beer spoilage bacteria (lactic acid bacteria) that could be developed in brewing and fermentation processes, and in samples associated with the production process, over a wide pH range. It was formulated specifically for suitability within the brewing industry for the detection of lactic acid bacteria (*Lactobacillus* and *Pediococcus*).

Preparation of the medium

Agars of Difco (74.9 g) or Oxoid (77.1 g) of Raka Ray Agar medium were suspended in 1000 mL distilled water. The pH was checked and adjusted to 5.2-5.4 when necessary. The selectivity of the medium was increased by the addition of 3 g of 2-phenylethanol and 7 mg cycloheximide (Actidione) per liter prior to autoclaving. Then the medium was mixed by stirring and heated to dissolve properly. The molten medium was dispensed into final screw-cap containers and sterilized in an autoclave for 15 minutes at 121 °C. The medium was cooled to 45 °C and poured into sterile petri dishes. 100 mL of beer was

aseptically filtered through a sterile 0.45 µm pore size membrane filter. The membrane was transferred to a pre-poured Raka-Ray agar plate. Then, plates were incubated for 5 days at 31 °C under anaerobic conditions.

2.8. Sensory analysis

The sensory acceptability of the beer samples was estimated by 30 adult healthy volunteers, using a 9-point hedonic scale. They were selected at random from the potential beer consumers, and they were instructed to evaluate all three beer types based on the basis of appearance, beer color, bitterness, flavor, aroma, and overall acceptability using a nine-point hedonic scale where 1 = disliked extremely and 9 = liked extremely. Samples in each treatment were served in glass cups (50 mL) coded with three-digit numbers and they were instructed to clean their mouth with water before testing the next sample. Test evaluation was conducted at the Food Science and Applied Nutrition Sensory Laboratory, Addis Ababa Science and Technology University.

2.9. Statistical analysis

Statistical analyses of different tested parameters were computed using SPSS Version 24. The antioxidant activity as DPPH radical scavenging capacity, total phenolic content, and total flavonoid were carried out in triplicates and the average values and standard deviations were calculated. Differences between means of the parameters were evaluated using one-way analysis of variance (ANOVA) and Tukey's post hoc test was used to compare the mean values. Differences in means were considered statistically significant with a *P* value ≤ 0.05.

3. Result and discussion

3.1. Effect of MSLE on total flavonoid of different lager beer brands.

Initially, the total flavonoid content was compared for the three beer brands prior to adding any extracts (Untreated beer samples). The result showed a total flavonoid content of 102.37 ± 9.78 , 115.57 ± 3.49 , and 86.87 ± 7.84 mg CE/L for Walia, Harar, and St. George beer, respectively. Statistical analysis also revealed a significant difference in the total flavonoid content of the three brands **Table 2**.

The total flavonoid content of MSLE-enriched beer samples increased linearly with respect to MSLE concentrations, regardless of beer type. The addition of 200 ppm extract significantly increased the total flavonoid content of all three brands (from 102.37 ± 9.78 to 132.92 ± 1.55 , from 115.57 ± 3.49 to 139.74 ± 6.17 and from 86.87 ± 7.84 to 98.84 ± 4.27 mg EC/L) for Walia, Harar and St. George beer, respectively.

The addition of 400 ppm increased the total flavonoid content of Walia beer to 159.38 ± 5.1 mg EC/L and a further increase to 600 ppm increased the flavonoid content to 167.09 ± 2.95 mg CE/L. A comparative result has been also reported where treating the beer with 600 ppm of the extract raised the total flavonoid content from 147.51 up to 159.82 mg CE/L (18). The 12 ppm KMS-enriched Walia beer sample also had a significantly higher total flavonoid content (145.27 ± 3.49 mg EC/L) than the untreated sample (102.37 ± 9.78 mg EC/L).

Likewise, increasing MSLE concentrations resulted in a significant increase in flavonoid content for Harar and St. George. The addition of 600 ppm extract significantly raised the total flavonoid content to 171.62 ± 0.89 and 124.27 ± 1.16 mg EC/L for Harar and St. George beer, respectively. The addition of 12 ppm KMS also resulted in a significant increase in the total flavonoid content of both beers compared with untreated beer samples. The increase in flavonoid content with increasing MSLE concentration is undoubtedly due to the nature of the plant being rich in total flavonoids, as has been confirmed by several studies (9; 24; 25).

Harar beer was found to have the highest total flavonoid content, followed by Walia and then St. George; which maintained the trend for untreated samples. This clearly indicates that there is no interaction between beer brand and MSLE concentration.

Total flavonoid content decreased with increasing storage time for all of the three brands, regardless of MSLE and KMS concentrations (**Table 2**). For example, the total flavonoid content of untreated Walia beer significantly reduced to 73.24 ± 8.56 mg CE/L after 60 days of storage from 102.37 ± 9.78 mg CE/L (first-day storage). For Harar beer, it reduced from 115.57 ± 3.49 mg CE/L to 75.18 ± 4.83 mg CE/L and for St. George beer, it went from 86.87 mg CE/L to 61.88 mg CE/L. A significant reduction in flavonoid content was also observed for all beer samples treated with KMS and MSLE as shown in **Table 2**. Previously up to 30 % reduction of total flavonoid content was also reported on a single beer brand (18).

The total flavonoid content of beer samples treated with KMS (145.27 ± 4.47 , 147.71 ± 5 and 105.22 ± 4.2 mg CE/L) for Walia, Harar, and St. George beer, respectively were found to be lower when compared with flavonoid content of the same beer treated with 400 ppm of MSLE (159.38 ± 5.1 , 162.11 ± 1.02 and 109.24 ± 5.72 mg CE/L). A study conducted by Mazengia and his colleagues on the antioxidant potential of the extract of *M. stenopetala* leaf on a beer has also revealed that the addition of 400 ppm of the extract resulted in a higher flavonoid content than the 12 ppm KMS (18).

The results indicated the possibility of replacing synthetic antioxidants (KMS) with MSLE for beer treatment. This idea is also supported by recent researchers who discourage the use of synthetic antioxidants in the food and beverage industry due to their long-lasting health effects (5).

3.2. Effect of MSLE on the total phenolic content of different lager beer brands

Results of total phenolic content before adding any extract were 66.04 ± 1.67 , 68.83 ± 0.23 , and 44.06 ± 0.27 mg GAE/L for Walia, Harar, and St. George beer, respectively (**Table 3**). There was no significant difference in the total phenolic content between Walia and Harar beer. However, St. George beer had a significantly lower phenolic content than the two beer brands. This finding is supported by a previous study where an average of 70 mg GAE/L of total phenolic content was reported (26). Another study also reported a 46.79 mg GAE/L of total phenolic content, which is equivalent to one of our beer brands (18).

Like total flavonoids, the total phenolic content of MSLE-supplemented beer samples increased linearly with extraction concentration, regardless of beer type. The addition of 200 ppm extract increased the total phenolic content of all three beer brands (from 66.04 ± 1.67 to 69.17 ± 1.9 ; from 68.83 ± 0.23 to 71.88 ± 1.29 and from 44.06 ± 0.27 to 50.01 ± 0.21 mg GAE/L for Walia, Harar and St. George Beer, respectively).

Increasing the extract concentration from 200 to 400 ppm raised the total phenolic content of Walia beer from 69.17 ± 1.9 to 72.45 ± 1.84 mg GAE/L and a further increase to 600 ppm resulted in a 75.24 ± 1.31 mg GAE/L. The addition of 12 ppm KMS also resulted in a significant increase from 66.04 ± 1.67 mg GAE/L (untreated samples) to 71.64 ± 2.12 mg GAE/L. Similarly, increasing the MSLE concentrations up to 600 ppm significantly raised the total phenolic content of all the Beer. The Addition of 12 ppm KMS also significantly increased the total phenolic content of Harar beer from 68.83 ± 0.23 mg GAE/mL (untreated beer) to 70.76 ± 1.58 mg GAE/L and St. George beer from 44.06 ± 0.27 mg GAE/mL (untreated beer) to 50.88 ± 0.33 mg GAE/L.

All KMS-treated samples were found to have lower total phenolic content (71.64 ± 2.12 , 70.76 ± 1.58 and 50.88 ± 0.33 mg GAE/mL) than 400 ppm extract-treated samples (72.45 ± 1.84 , 74.52 ± 1.5 and 58.74 ± 0.3 mg GAE/mL) for Walia, Harar and St. George beer, respectively. This is also consistent with the study done by Mazengia and his colleagues where they reported that adding 400 ppm resulted in better phenolic content than adding the 12 ppm synthetic KMS, tested in a single beer brand (18). Our findings clearly showed that *M. stenopetala* extract has the potential to replace synthetic antioxidants (KMS) and can yield better total phenolic content for beer samples.

The increment of total phenolic content in beer samples enriched with MSLE might come from the potentially high accumulation of bioactive compounds in *M. stenopetala* leaf. A study conducted by Tebeka & Libsu on the antioxidant potential of the leaf extracts of *M. stenopetala* reported a maximum polyphenol content of 92.8 mg GAE/100 g of dry weight (27).

As was observed for flavonoid content, the total phenolic content also decreased with increasing storage time for all of the three beer brands, regardless of the concentrations of the extract and KMS used (**Table 3**). The total phenolic content of untreated Walia beer was reduced by 31 %, Harar by 30.5 % and St. George by 5 % during the 60-day storage period. Previously, up to 23 % of total phenolic content decrement was reported during 6 months of storage time (28). This decrease in total phenolic content will contribute to the reduction of the antioxidant activity of the beer samples (29). The decrease in total phenolic content is likely due to the reaction of phenolic substances with other beer constituents (29). Of

these, polyphenol–protein interactions have been researched most thoroughly due to their involvement in beer haze. This was also confirmed by Vanderhagen *et al.* (7). The total phenol content of beer samples treated with KMS and MSLE also decreased significantly within 60 days of storage as shown in **Table 3**.

3.3. Radicals scavenging activity of different lager beer brands with different concentrations of MSLE by DPPH

The DPPH scavenging activity of each beer sample during storage was expressed as a percentage inhibition and shown in **Figure 1**. Regardless of the type of beer used, the untreated beer samples were found to have significantly lower percent inhibition than that of KMS and MSLE-treated beer samples. Untreated Walia, Harar, and St. George beer inhibited the DPPH radicals by 51.65, 52.57 and 50.17 %, respectively with no significant difference between the beer samples. A comparative result was also reported by scholars, in which one of the Ethiopian beer brands inhibited DPPH radicals by 46.55 % (18). However, a significant decrease in inhibitory capacity was observed as the storage time increased. Reduction of 24.3, 14.3, and 16.2 % was observed for Walia, Harar, and St. George beer, respectively during the 60-day storage time. Treating the beer samples with 12 KMS has resulted in better percentage inhibition with 57.87, 57.74, and 56.89 % and there was a relatively slight reduction of DPPH scavenging activity during the two months storage time.

Looking at the DPPH scavenging activity of beer samples treated with MSLE, the result revealed a linear increase in percentage inhibition with increasing extract concentration (**Figure 1**). The addition of 200 ppm MSLE to Walia, Harar, and St. George beer resulted in 57.32, 58.98, and 56.34 % of inhibition, respectively. Statistically, there was no significant difference in inhibitory activity between samples treated with 12 ppm KMS and 200 ppm MSLE. It can be assumed that in terms of DPPH activity, 200 ppm extract obtained from *Moringa* leaves could act as KMS. Treating each beer brand with 400 ppm resulted in a significant reduction in free radicals compared with 12 ppm KMS and 200 ppm MSLE. The results showed inhibition levels of 61.88, 62.88.41 and 59.41 % for Walia, Harar, and St. George beer, respectively. Increasing the concentration of the extract further to 600 ppm resulted in 64.39, 65.78, and 63.11 % inhibition. The increased DPPH removal capacity of the extract treatment is attributed to the abundance of bioactive compounds and antioxidants present in the leaves of *M. stenopetala* (27). This also suggests that *M. stenopetala* leaf extract contains compounds that can readily donate electrons/hydrogens and stabilize free radicals (15). In all cases, the DPPH radical scavenging effects of the samples decreased as the storage period elongated. This may be due to the depletion of antioxidants brought about by reactions with hydroxyl radicals over time (30).

3.4. Effect of MSLE on the microbial quality of different lager beer brands

All the beer samples were subjected to be stored for 90 days. Results of microbial analysis showed no growth of any microorganisms during the three-month storage period. Therefore, the main purpose of adding either KMS or any concentration of MSLE is not to inhibit the growth of microorganisms such as bacteria and yeasts, since no microbial growth was also observed in any of the untreated beer brands. However, since MSLE has an advantage over KMS in terms of total flavonoid and total phenolic contents as well as free radical scavenging activity, it is still advisable to use the leaf extracts of *M. stenopetala* instead of KMS.

3.5. Effect of MSLE on the sensory quality of different lager beer brands

3.5.1. Pre-sensory analysis (overall acceptability) of different lager beers

The effect of MSLE concentrations on the overall acceptability of beer was assessed prior to determining the concentration to be added to the beer samples. Results showed that the addition of the extract up to 600 ppm was generally accepted by the panelists for all three beer brands (**Table 4**). Further treatment of the beer samples with the addition of 800 ppm made all of the beer brands unacceptable to the panelists. As a result, the effect of 800 ppm MSLE was not considered to evaluate its effect on the antioxidant capacity and anti-microbial quality of a beer.

3.5.2. Sensory analysis of different lager beer brands with different concentrations of MSLE

The sensory analysis results of the three beer brands supplemented with different concentrations of MSLE and 12 ppm KMS after 60 days of storage time are shown in **Table 5**. Since most of the parameters were significantly reduced after 60 days of storage, sensory analysis was not evaluated for the 90th day. All three beer brands treated with up to 600 ppm leaf extract and 12 ppm KMS were found to be within the acceptable range during the 60-day storage time. No significant difference in acceptability was also observed between untreated, KMS-treated, and MSLE-treated beer brands during storage. Considering the individual organoleptic parameters, bitterness increased linearly with increasing extraction concentration, and consumers rated the bitterness of all 600 ppm-treated beer samples as neither liked nor disliked, but moderately liked for KMS-treated and untreated samples. Consumer flavor and aroma preferences were also reduced in samples treated with high extract concentrations. However, the concentration of the extract did not affect the appearance and color during the 60-day storage period.

4. Conclusion

Beer is a relatively stable beverage, but storage and processing such as malting can cause oxidation and change its original flavour. The addition of synthetic antioxidants like KMS can reduce or prevent this effect, but due to its long-term negative impact on consumers` health, breweries are looking for a natural alternative to preserve beer. Alternatively, *M. stenopetala*, which is rich in antioxidants, can effectively replace the synthetic or chemical antioxidants as the present study highlighted that the addition of 400 and 600 ppm of its leaf extract resulted in a better bioactive compound (phenolic and flavonoid contents) and enhanced antioxidant activity of the beer as it is evidenced by high DPPH free radical scavenging activity. This moderate-level incorporation of the leaf extracts had effects similar to KMS on the microbial load over a three-month storage period. Our study has also clearly indicated that leaf extract of *M. stenopetala* could be used to improve the stability and shelf-life of commercial beers with better consumer preferences without incorporating artificial or chemical preservatives. Further studies on other quality characteristics of beer due to extract addition are recommended.

Limitation

A voucher specimen was not deposited in a public herbarium at the time of the study because the plant is not wild-harvested, endangered, or difficult to identify, and its cultivation at Melkassa is well-documented and accessible for verification. However, we fully recognize the importance of voucher deposition for transparency, reproducibility, and future reference. While a deposition number is not available, we are committed to depositing a representative specimen at the National Herbarium of Ethiopia, Addis Ababa University, for future research activities.

Declarations

Ethical considerations

This study was approved by the Institutional Review Board of the College of Natural and Computational Sciences of Addis Ababa University.

The *Moringa Stenopetala* leaves used in this study were collected from the Melkassa Agricultural Research Center. As the plant is cultivated on-site and routinely used for research purpose, we confirm that appropriate permission was obtained from the institutional management at the research center prior to plant material collection.

Informed consent: Written informed consent was obtained from all study participants for the sensory analysis

Author contributions

All authors equally contributed to the conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, writing, review & editing.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

The data used to support the findings of this study is available upon reasonable request from the corresponding author.

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Figures

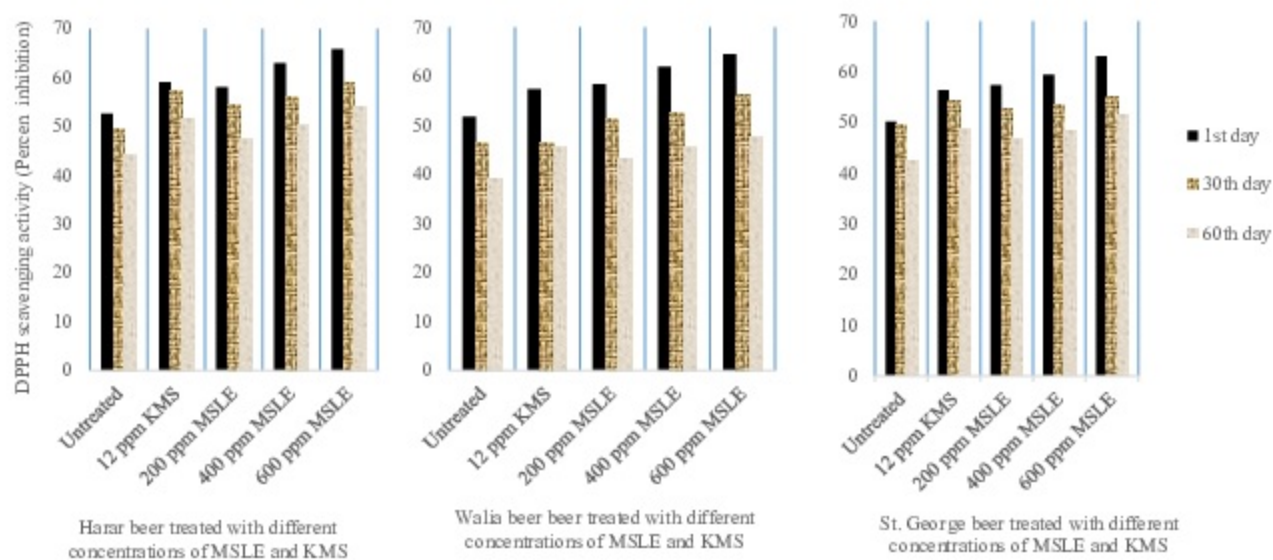


Figure 1. DPPH radicals scavenging activity of Harar, Walia and St. George beer treated with leaf extract of *M. stenopetala* and KMS.

Figure 1

Legend not included with this version.