

**Original Article**

Color Attributes and Total Phenolic and Flavonoids Contents of Canned Maz-type Common Bean Lines

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ABSTRACT

Background and Objectives: Canned common beans with good preservation of color and polyphenolic compounds are desirable for consumers, the canning industry and other end users. The objective of this study was to assess the color attributes and total phenolic and flavonoid contents of canned maz-type common bean lines.

Materials and Methods: Three maz type common bean lines were collected, cleaned and sorted manually to remove undersized and broken seeds. The prepared maz-type common bean lines were used in canning processes. The experimental setup was carried out using factorial design (9*3), where nine various canning treatments were used for three maz-type common bean lines and each treatment was repeated three times during the experiment. Color attributes and total phenolic and flavonoid contents of the raw and canned maz-type common bean lines were assessed using official standard methods.

Results: Results showed that the highest yellowness (b^*) (22.57) and lightness (L^*) (29.85) values were recorded for maz-153 common bean lines. Moreover, the present result revealed that yellowness (b^*) and lightness (L^*) color attributes increased by canning processes. Statistically, significant ($P < 0.05$) differences were reported for total phenolic and flavonoid contents due to variation of maz-type common bean lines. In addition, good preservation of total phenolic and flavonoid contents was demonstrated in samples soaked at ambient temperature for 30 min and blanched at 75 and 88 °C for 30 min each.

Conclusions: Common bean lines of the maz-type showed promising potentials in production of canned beans with improved color and preserved phenolic and flavonoid contents.

Keywords: Blanching, Color, Canning, Flavonoids, Phenolic, Soaking

Highlights

- MAZ-type Common beans are rich in essential nutrients and phytochemicals like flavonoids and phenolic acids.
- Color, flavonoids, and phenolic contents of the canned maz-type common beans were influenced by canning variables and beans' genetics.
- Canned beans showed better lightness and yellowness in color, enhanced flavor, and preserved their flavonoids and phenolic contents.
- The highest retention of phenolic and flavonoids content was observed in canned beans prepared by soaking beans for 30 minutes at room temperature followed by blanching at 75°C and 88°C, respectively, each for 30 minutes.

Introduction

Common beans include high levels of proteins, certain carbohydrates and fibers and are goodsources of minerals and vitamins (5). In addition to its nutrient composition, numerous phytochemicals, such

as polyphenolic compounds, alkaloids, proanthocyanidins, saponins, steroids, terpenoids, lectins and antioxidants are predominant bioactive compounds in common bean cultivars (14,26). Phenolic acids and flavonoids are two

examples of the phenolic compounds detected in common beans. These chemicals include a variety of antioxidant characteristics that are beneficial to human health associated with anticancer characteristics (2,22). The taste and color of beans are attributed to phenolic compounds and their concentrations vary by the environment and cultivars (10,22). The poly phenolic compounds, particularly flavonoids, provide the seeds with inherent defense abilities against pests, including insects and microbiological invaders.

These phytochemicals are bioactive components and include a potential role in preventing chronic diseases such as coronary heart disease, cancer, type II diabetes and obesity (8). Common bean types contain nonnutritive proteins that display insecticidal characteristics against bean bruchids. Bruchids are types of tiny beetles that are serious pests of stored pulse crops and cause large post-harvest losses (4). To decrease post-harvest losses caused by bruchids in warehouses, it is necessary to develop common bean seeds that are resistant to bruchids damage.

Despite their nutritional compositions, the exceptional resistance of maz-type common beans to bruchids makes them selected breeding stocks in development of better multifunctional common bean varieties. Like other varieties of beans, maz-type common beans can be used as boiled, protein-fortifying, canned and snack foods as well as other uses for human consumption. Similar to other bean categories, these lines of beans must be processed before consumption. Their nutritional composition, color and polyphenolic compounds can be altered during processing. Canning is a common process used for beans before consumption. The term of canning refers to a traditional food preservation technique where the food product is packed in hermetically sealed containers and then sterilized using heat (6).

The canning process consists of various operations such as soaking, blanching and autoclave cooking. These operations can contribute to improvements in nutritional profile, enhancements in flavors of beans, decreases in heat-labile anti-nutrients and elongations of the storage life of canned foods (11,17). According to White and Howard (25), soaking hydrates and softens cotyledon grain, which facilitates the grain absorption of 53–57% of water content. Blanching is another canning procedure that is a pretreatment method to eliminate gasses from the surface of and between the cells and deactivate enzymes to prevent oxidation and discoloration of the products (27). Canning needs cooking of beans in liquid water, steam water, or a combination of the two waters using an autoclave at 110–135°C to destroy harmful microorganisms. There are maz-type bean lines developed for canning purposes, for whom, color and polyphenolic compound preservation were not assessed after canning. Therefore, the objective of this study was to assess color attributes and total phenolic and flavonoid components of maz-type canned common beans.

Materials and Methods

Sample Collection and Preparation

Three maz type common bean lines (Maz-23, 153 and 200) were selected for the study. Nearly 9 kg (3 kg from each line) of maz-type common beans were received from Melkassa Agricultural Research Center, Ethiopia. Seed samples of maz-type common bean lines were cleaned and sorted manually to remove undersized and broken seeds. The cleaned and sorted seed samples of each bean line were stored in separate airtight containers until use. The experiment was designed as 9*3 factorial arrangement, with a total of 27 treatment groups and each treatment was repeated three times (Table 1).

Table 1. Treatment combination of an experiment

| Treatments | Soaking and blanching time in minutes | Blanching temperature in °C | Thermal processing (temperature °C *time in minutes) |
|------------|---------------------------------------|-----------------------------|--|
| 1 | 20 | 60 | 121*30 |
| 2 | 20 | 75 | 121*30 |
| 3 | 20 | 88 | 121*30 |
| 4 | 30 | 60 | 121*30 |
| 5 | 30 | 75 | 121*30 |
| 6 | 30 | 88 | 121*30 |
| 7 | 40 | 60 | 121*30 |
| 8 | 40 | 75 | 121*30 |
| 9 | 40 | 88 | 121*30 |

Canning Procedure

Canning of cleaned seed samples was carried out using modified laboratory canning method by Balasubramanian (1). Nearly 96 g from each maz-type common beans were weighed using clean plates and transferred to mesh bags. Weighted seeds were soaked in distilled water (DW) containing 10 mg Ca^{+2} kg^{-1} (10 ppm) as food-grade calcium chloride (1:3 g:ml) for 20, 30 and 40 min at room temperature (RT). This was then blanched for the highlighted time and temperature combination in water with 10 mg Ca^{+2} kg^{-1} (10 ppm) as food-grade calcium chloride. The seed samples within the mesh bag were weighed after cooled to RT. The weighed seed sample from each mesh bag was transferred to bottle cans. Then, DW with a calcium content of 10 mg Ca^{+2} kg^{-1} (10 ppm) from food-grade calcium chloride was used to prepare brine that comprised 1.3% (wt/vol) sodium chloride and 1.6% (wt/vol) sugar to fill the cans. Then, cans were sealed and processed for 30 min at 121 °C using retort autoclave and steam. The cans content was set to cool down in water at 20 °C for 20 min.

Color (C): Color attributes of the raw and canned maz-type common bean samples were estimated using HunterLab calorimeter (model ARS00073, Hunter Lab, USA). Color scale values were assessed using three international color units such as L^* represented lightness and extended from 0.0 (black) to 100.0 (white), a^* represented to redness (value) to greenness (-value) and b^* represented yellowness (value) to blueness (-value) (9).

Total Phenolic Content

The total phenolic compounds (TPC) of the extracts were assessed using Folin-Ciocalteu reagent (FCR) (23) and UV spectrophotometer. Briefly, 100 μl of a crude extract (10 mg/ml) were mixed with 0.2 ml of FCR (1: 9 ml: DW ratio), 2 ml of purified water and 2 ml of 7.5% Na_2CO_3 . The mixture measured at 765 nm after 2 h incubation at RT. Various concentrations of Gallic acid were used as standard. Various concentrations of Gallic acid solutions in methanol (0.005, 0.01, 0.02, 0.04, 0.08 and 0.1 mg/ml) were prepared from the standard solution. To each concentration, 0.2 ml of 10% FCR, 2 ml purified water and 2 ml of 7.5% Na_2CO_3 were added, making a final volume of 4.3 ml. The total polyphenolic compounds were calculated and expressed as mg Gallic acid equivalent per gram of sample (mg GAE/g). The assessment was carried out in triplicate and calculated using the following formula:

$$P \text{ (mg GAE/g)} = C1 * V / m$$

Where, P was the total polyphenolic content in mg/g expressed in GAE (Gallic acid equivalent) and C1 was the concentration of Gallic acid in mg/ml assessed from the standard curve, V was the volume of purified sample in milliliters (ml) and m was the sample extract mass (g).

Total Flavonoids

Flavonoid content of the flour samples was assessed using UV spectrophotometer method (18). Two milliliters (2 ml) of the extracted sample (extracted with 80% acidic methanol) with concentrations of 10 mg/ml were mixed with an equal volume of 2% AlCl_3 solution in methanol. Then, the mixed solution was incubated at RT for 1 h. Various concentrations of quercetin solution were used as standards and methanol was used as blank at maximum wavelength of 415 nm using spectrophotometer. Based on the measured absorbance, concentration of the flavonoid was read (mg/ml) on the calibration line and content of the flavonoid in each sample was calculated using the following formula:

$$Ft \text{ (mg QAE/g)} = C1 * V / m$$

Where, Ft was total flavonoid content in milligram of quercetin equivalent per gram of sample, C1 was the concentration of quercetin calculated from the standard calibration curve in mg/ml, V was the volume of purified sample in milliliters (ml) and m was the sample extract mass in gram (g).

Standard preparations

After dissolving 10 mg of quercetin in 10 ml of methanol to prepare a stock solution (1 mg/ml), the standard solution was serially diluted to prepare various solutions with concentrations of 0.5, 1, 2, 3, 4, 5 and 6 $\mu\text{g/ml}$. The total flavonoids content was expressed as quercetin equivalents using linear equation based on the calibration curve (quercetin equivalent (mg of QAE per gram of sample)).

Statistical Data Analysis

In this study, SAS statistical software was used to analyze the collected data and two-way analysis of variance (ANOVA) was carried out. The significant difference ($P < 0.05$) was assessed by estimating the critical difference. To separate the means, the least significant difference (LSD) test was used.

Results

Color attributes and total phenolic and flavonoids contents for the raw and canned maz-type common bean lines were assessed. Results were present in tables.

Table 2. Effects of the major factors (canning treatments and maz-lines) on color attributes of the maz-type common beans

| Maz- lines | | | L* | a* | b* |
|------------|----------------------|-----------------------------|---------------------------|--------------------------|---------------------------|
| Maz-23 | | | 20.84±3.02 ^b | 22.81±3.13 ^a | 20.37±3.54 ^b |
| Maz-153 | | | 29.85±6.90 ^a | 18.58±4.62 ^b | 22.57±3.51 ^a |
| Maz-200 | | | 19.59±6.23 ^b | 23.09±4.29 ^a | 20.44±4.26 ^b |
| CV | | | 10.76 | 9.22 | 11.43 |
| LSD | | | 1.30 | 1.02 | 1.25 |
| Treatments | ST and BT in minutes | Blanching temperature in °C | L* | a* | b* |
| 1 | 20 | 60 | 19.29±7.49 ^d | 20.92±3.45 ^{bc} | 21.21±3.33 ^{abc} |
| 2 | 20 | 75 | 23.53±5.30 ^c | 20.71±3.22 ^{bc} | 22.62±3.94 ^{ab} |
| 3 | 20 | 88 | 23.73±6.55 ^{bc} | 21.43±3.86 ^b | 22.97±2.56 ^{ab} |
| 4 | 30 | 60 | 24.01±6.04 ^{bc} | 19.36±1.98 ^c | 20.31±3.71 ^c |
| 5 | 30 | 75 | 25.94±7.56 ^{ab} | 20.29±4.07 ^{bc} | 20.85±3.36 ^{bc} |
| 6 | 30 | 88 | 27.21±7.72 ^a | 19.84±1.27 ^{bc} | 22.41±3.28 ^{abc} |
| 7 | 40 | 60 | 25.60±5.39 ^{abc} | 20.05±3.02 ^{bc} | 21.71±4.72 ^{abc} |
| 8 | 40 | 75 | 25.27±6.26 ^{abc} | 20.62±4.24 ^{bc} | 20.99±3.08 ^{bc} |
| 9 | 40 | 88 | 26.55±4.51 ^a | 20.56±3.83 ^{bc} | 23.31±2.34 ^a |
| 0 | Raw | | 13.16±5.52 ^e | 31.13±2.64 ^a | 14.94±1.85 ^d |
| CV | | | 10.76 | 9.22 | 11.43 |
| LSD | | | 2.37 | 1.87 | 2.28 |

Where, L* indicates lightness, a* refers redness, b* refers yellowness, CV=Coefficient of variation, LSD =Least significant difference, ST= Soaking time, BT= Blanching time, Means within same column followed by the same letters are not significantly different; ($P > 0.05$)

Color

Statistically, significant ($P < 0.05$) difference was observed for L*, which indicated lightness color attributes of maz-type common lines as shown in Table 2. The mean value of L* attributes for Maz-23, 153 and 200 included 20.84 ± 3.02 , 29.85 ± 6.90 and 19.59 ± 6.23 , respectively (Table 2). Accordingly, the highest value of L* was recorded for Maz-153, while the lowest value was observed in Maz-200 (Table 2). Moreover, the variation of canning processes ($P < 0.05$) included a significant range in the color attributes of L* (lightness) from 19.29 ± 7.49 to 27.21 ± 7.72 for canned maz-type common bean lines, which soaked at RT for 20 and 30 min and then blanched at 60 and 88 °C, respectively, irrespective to the raw maz-type common bean lines (Table 2). In the current result, a significant variation was observed for the color attributes of a* (redness) between the maz-type common bean lines from 18.58 ± 4.62 for Maz-153 to 23.09 ± 4.29 for Maz-200 (Table 2). A statistically significant ($P < 0.05$) variation was reported for the canned beans processed under various canning procedures. For Maz-153, 200 and 23, the average b* values, which corresponded to yellowness color attributes, were recorded as 22.57 ± 3.51 , 20.44 ± 4.26 and 20.37 ± 3.54 , respectively (Table 2). Regardless of raw maz-type common bean lines, the current findings showed that the color values of b* (yellowness) varied from 20.31 ± 3.71 to 23.31 ± 2.34 for canned maz-type common bean lines that were soaked for 30 and 40 min at RT before blanching at 60 and 88 °C, respectively (Table 2).

Total Phenolic Content

The results of TPCs of raw maz-type and canned one were present in Table 3.

The present study showed that the TPCs of maz-type common beans significantly ($P < 0.05$) changed due to the variation of maz-lines. Accordingly, the highest TPC as 0.73 ± 0.65 mgGAE/g was recorded in Maz-153, followed by Maz-23 and 200 (0.65 ± 0.43 and 0.55 ± 0.61 mgGAE/g, respectively). Statistically, significant ($P < 0.05$) decreases of phenolic contents for the canned maz-type common bean lines were recorded in the present study, compared to raw maz-type common bean flour (Table 3). Canned maz-type common bean lines that were blanched at 75 °C for 30 min after soaking at RT for 30 min included the second-highest TPC of 0.95 ± 0.52 mgGAE/g close to raw maz-type common bean lines including 1.88 ± 0.44 mgGAE/g TPC. The lowest (0.23 ± 0.06 mgGAE/g) total phenolic was reported for canned maz-type common bean lines that were soaked for 40 min at RT before blanching at 88 °C for 40 min (Table 3).

Total Flavonoid Content

The results of the present study demonstrated that the total flavonoid contents of Maz-type common beans were significantly ($P < 0.05$) affected by Maz-type common bean lines (Table 3). As a result, Maz-200 included the highest total flavonoid content (2.27 ± 0.73 mg QAE/g), whereas Maz-153 included the lowest total flavonoid content (1.05 ± 0.62 mg QAE/g). The present study detected a statistically significant ($P < 0.05$) variation in the total flavonoid contents for the canned maz-type common bean lines (Table 3). The canned maz-type common bean lines that were soaked for 30 min at RT before blanching at 88 °C for 30 min included the highest total flavonoid concentration (2.15 ± 0.96 mg QAE/g).

Table 3. Effects of the major factors (canning treatments and maz-lines) on the total phenolic and flavonoid contents of maz-type common bean lines

| Maz-lines | | | Total phenolic (mg GAE/g) | Flavonoids (mg QAE/g) |
|------------|-------------------------|--------------------------------|------------------------------|--------------------------|
| Maz-23 | | | 0.65±0.43 ^b | 1.71±1.03 ^b |
| Maz-153 | | | 0.73±0.65 ^a | 1.05±0.62 ^c |
| Maz-200 | | | 0.55±0.61 ^c | 2.27±0.73 ^a |
| CV | | | 8.83 | 5.35 |
| LSD | | | 0.03 | 0.05 |
| Treatments | ST and BT in minutes | Blanching temperature in °C | Total Phenolic | Flavonoids |
| 1 | 20 | 60 | 0.82±0.44 ^c | 1.12±0.19 ^g |
| 2 | 20 | 75 | 0.43±0.17 ^f | 1.72±0.58 ^d |
| 3 | 20 | 88 | 0.51±0.40 ^e | 1.08±0.43 ^g |
| 4 | 30 | 60 | 0.35±0.12 ^g | 1.44±1.04 ^e |
| 5 | 30 | 75 | 0.95±0.52 ^b | 1.42±0.81 ^{ef} |
| 6 | 30 | 88 | 0.64±0.54 ^d | 2.15±0.96 ^b |
| 7 | 40 | 60 | 0.34±0.03 ^g | 1.35±0.89 ^f |
| 8 | 40 | 75 | 0.28±0.09 ^h | 1.39±0.46 ^{ef} |
| 9 | 40 | 88 | 0.23±0.06 ^h | 1.85±1.09 ^c |
| 0 | Raw | | 1.88±0.44 ^a | 3.22±0.66 ^a |
| CV | | | 8.83 | 5.35 |
| LSD | | | 0.05 | 0.08 |

Note: Note: CV=Coefficient of variation, LSD =Least significant difference, ST= Soaking time, BT= Blanching time, Means within same column followed by the same letters are not significantly different; (P > 0.05)

Discussion

Color is a key attributes for consumer approval and breeding programs that use a distinct color scale to assess the degree of such respective color attributes (12). The quantity and abundance of polyphenols, including flavonols, glucosides, condensed tannins and anthocyanins, are responsible for the color of the bean seed coat. Additionally, the present finding revealed that L*, which reflected lightness, was significantly improved due to the use of various canning treatments for the selected maz-type common bean lines. The current result showed that L* value of the canned maz-type common beans were slightly higher than that of canned dry beans reported by Wang *et al.* (24). This might be attributed to less darker color of maz-type bean seed coat used in the present study. Color was reported as dark if the L* value was less than 50, according to Petracci *et al.* (16). In addition, the a*, which reflected redness color intensity, decreased due to various canning processes, compared to that of raw maz-type common bean sample. The highest (31.13 ± 2.64) a* value, which showed red color, was recorded for raw maz-type common bean lines while the lowest (19.36 ± 1.98) a* value was seen in canned maz-type common bean lines that soaked for 30 min at RT prior to blanching at 60 for 30 min (Table 2). The degree of color preservation in beans during canning process depends on genotype makeups and variation of canning treatments (19). The result of b* value, which referred to yellowness value in the present study, was closely linked to b* value reported by Bassett *et al.* (3) for fast cooking dry beans subjected to canning processes at 121 °C for 10–45 min. Additionally, the present finding revealed that b* (yellowness) value was significantly improved as a result of canning variables used

for the selected maz-type common bean lines, compared to that of the raw Maz-type common bean lines. Numerous health advantages such as prevent oxidative stress, allergy, cardiovascular diseases (CVDs) and antibacterial, antiviral, anti-carcinogenic and anti-inflammatory characteristics have been associated to phenolic compounds (20). The present study demonstrated the moderate decreases of the TPC of canned maz-type common bean lines. The losses of TPC were attributed to the thermal processing used for the bean sample during various canning steps. The decrease of phenolic compounds because of canning procedures in the present study was similar to that by Parmar *et al.* (15), who explained a significant decrease in phenolic compounds for canned kidney beans, chickpeas and field peas. Similar finding described degradation of the TPC of canned Mexican common beans were reported by Gallegos-Infante *et al.* (7); similar to the present study.

Statistically significant ($P < 0.05$) differences in the total flavonoid contents for the canned maz-type common bean lines were reported in the present study (Table 3). Compared to a previous study, the total flavonoid content detected in the present study was close to that of various legume seed varieties reported by Ren *et al.* (21). The flavonoid content of canned maz-type common bean lines was lower than that of raw maz-type common bean lines. Canning steps (blanching and autoclaved cooking temperature) might be contributed to the slight decrease of total flavonoids for the canned common bean lines in this study. In addition, heat-labile nature of phenolic compounds might possibly be the cause of decreases in flavonoid levels after cooking procedures (13).

Conclusions

The present results revealed that canning treatments and maz-type common bean lines included significant effects on color attributes and total phenolic and flavonoid contents of the bean samples. Results showed that the highest b^* and lightness L^* values belonged to Maz-153 common bean lines. For color attributes, a^* value decreased whereas L^* and b^* values increased for the canned Maz-type common bean lines. Good preservation of total phenolics and flavonoids in the canned maz-type common bean lines were reported for samples soaked at ambient temperatures for 30 min and blanched at 75 and 88 °C for 30 min each, respectively.

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