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WATER-SOLUBLE TOTAL SUGARS AND TOTAL FREE AMINO ACIDS OF THE TWO VARIETIES OF TEFF [*ERAGROSTIC TEF* (ZUCCAGNI) TROTTER] CULTIVATED IN DIFFERENT REGIONS OF ETHIOPIA

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ABSTRACT. Teff [*Eragrostis tef* (Zuccagni) Trotter] is cereal grain native to Ethiopia as staple food to millions of people. However, no research work has been reported for characterization with respect to water-soluble total sugars and total free amino acids in white and brown teff grains. The main objective of this study was to determine water-soluble total sugars and total free amino acids (TFAA) in the white and brown teff grain varieties. The determined water-soluble total sugar contents of white and brown teff grain sample extract was ranged between 2.69±0.12 – 4.56±0.08 g GE/100 g and 2.22±0.04 – 4.74±0.19 g GE/100 g, respectively. The mean TFAA contents for white and brown teff samples was found in the range between 181.4±30.0 – 638.9±42.6 mg AE/100 g and 471.7±37.0 – 927.1±32.4 mg AE/100 g, respectively. The mean value of the TFAA in brown teff samples was found higher than that of white teff samples. Pearson correlation was in the opposite direction, indicating that the biosynthetic pathways for the water-soluble total sugars and TFAA in both white and brown teff varieties might be opposite. Therefore, the present study indicated that teff is rich in the water-soluble total sugars and TFAA.

KEY WORDS: Teff, Sugars, Amino acids, Glucose, Ninhydrin, UV-Vis spectrophotometry

INTRODUCTION

Whole grains are highly encouraged as healthy and sustainable diet profile based on the need for higher intakes of plant-based dietary fiber-containing foods and resulting less consumption of higher fat meat and animal products [1]. Based on the observational and intervention studies made, higher consumption of whole grain has been linked to a lower incidence and mortality from cardiovascular diseases [2, 3]. It can help to maintain pancreatic function and increase glucose-stimulated insulin production in overweight/obese persons at risk of type 2 diabetes [4]. This has revealed that diabetic patients benefit from the slow release of carbohydrate constituents [5]. Many recent works of the literature also confirmed that teff contains complex carbohydrates with slowly digestible starch [6]. Indicating that those who consume whole grains are more physically active and healthy [7].

Cereals essentially consist of high proportions of carbohydrates, most come from starch and lower proportions come from total sugars. These carbohydrates make up the main source of energy in cereals [8]. They are one of the most important components in food and include a wide range of macromolecules that can be classified by their chemical structure as mono-saccharides, disaccharides, oligosaccharides and polysaccharides [9]. Sugars are the building blocks of carbohydrates and they are naturally found in many foods such as cereals, fruits, milk and vegetables [10]. The term 'sugar' in chemistry refers to mono-, di- and the lower oligosaccharides and as the number of monosaccharide units in a molecule increases beyond 2, the sweet taste is considerably less pronounced [11]. This is due to the fact that sugar content of a food item is defined as the sum of mono-saccharides and disaccharides [12]. The primary function of sugars in food products is to provide sweetness and energy. Besides, sugars play a very important role in preservation, fermentation, color and texture in food [10]. The amount of sugars (per 100 g edible

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portion) in cereals, therefore, was reported by some researchers in rice (1.0 g), wheat (2.1 g), maize (1.6 g), barley (1.8 g), sorghum (1.3 g), millet (4 g) and oats (1.2 g) [13]. Contrary to the advantages of the sugars in cereal grains, overconsumption of sugars can result disturbing the blood sugar level. Thus, reporting accurate information to consumers about the quantity of sugars present in various cereal food items is really essential [12].

Teff is a warm-season annual cereal and the only cultivated species in the genus *Eragrostis* which has originated in Ethiopia [14–17]. It is a whole grain and superior than other cereals due to being naturally gluten free and is the dominant source of nutrients like carbohydrates, amino acids, minerals, dietary fibers, proteins, dietary polyphenols, starch, and vitamins [5, 16, 18, 19], volatiles like aldehydes, ketones and alcohols [16], and is rich in unsaturated fatty acids like linoleic, oleic and linolenic acids [17, 20]. The ratio of ω-6/ω-3 found in the white and brown teff flour was almost consistent with the suggested 1-2:1 ratio and is recommendable for the normal human growth and development. Besides, the atherogenicity and thrombogenicity indices used to determine the lipid quality of the teff grain samples has indicated excellent status of the grains compared to other cereal grains which are useful for the human nutrition and health [17]. Teff flour is used extensively in Ethiopia to make injera, a soft flatbread prepared from slightly fermented batter, and the grains are also used in stews and porridges [15]. It is a nutritious food even better than the common staple cereals such as wheat, rice, oats and barley which is preferable by celiac disease patients and other types of gluten sensitivity as it is consumed as a whole grain [6, 15, 20].

Amino acids (AAs) are indispensable nutritional constituents for body growth and many biological activities. They function in regulating gene expression, preventing tumorigenesis, suppressing obesity, and reducing blood pressure [21–23]. In human organisms, amino acids take part in building the protein chain, but there is always a pool of free amino acids (FAA) [23] required for survival, growth and development, reproduction, lactation and maintaining health throughout life. The amino acids released during the digestion of food protein are essential for the synthesis of tissue protein which comprises approximately 16% of the human body [21]. The nutritional properties of gluten-free flours contain well balanced composition of the amino acids with high biological value [23]. Teff accounts for about two-third of the daily protein intake in the diet of Ethiopian population. The protein composition of teff offers an excellent balance among the essential amino acids [5, 6, 14, 15, 24]. Nowadays, there is a great interest in the free amino acids (FAA) because they have an important effect in food flavor, influence its palatability, and contribute to the formation of amines and volatile compounds. Changes in the free amino acid concentration are used for measuring proteolytic and hydrolytic activity in food processing [25]. The apparent relationship reported between the food levels of the free amino acids (FAAs) and organoleptic qualities has prompted scientific research in to FAA composition in food [26, 27]. On top of this, there is also growing demand for amino acids as value added substances since they have many potential applications in the food, clinical, pharmaceutical and cosmetic industries, among others [22].

To the best of the researchers' knowledge, the literature survey indicated that there was no study conducted and reported about the water-soluble total sugars and total free amino acids contents in the white and brown teff grain samples. Therefore, to address concrete information to consumers about the quantity of water-soluble total sugars and total free amino acids present in the two varieties of teff, determination of these parameters was found to be very crucial. Hence, the objectives of the study were: 1) to determine the level of water-soluble total sugars contents in the two varieties of teff cultivated in different parts of Ethiopia using sulfuric acid method, 2) to determine the total free amino acids contents in the two varieties of teff cultivated in different parts of Ethiopia using ninhydrin method, 3) to compare the levels of water-soluble total sugars and total free amino acids in the two varieties of teff with other reported cereal grains.

EXPERIMENTAL

Apparatus and instrument

The electronic balance (Model: ARA520, China), grinder (High speed multifunctional grinder, Shanghai, China), water deionizer system (Model: Molatom510d, Molewater System Co., Ltd), centrifuge (model: 80-2, China), thermostatic water bath (Model: HB10DS99, IKA), were used to conduct the experiment. UV–Vis–NIR spectrometer (Lambda 950, Perkin Elmer, UK) with a 1 cm path length quartz cuvette was used for measuring the absorbance of the prepared standards and samples.

Chemicals

The glucose standard (laboratory reagent; Merck Extra Pure, England), sulfuric acid (96%; Sigma-Aldrich, Germany), methanol (≥ 99.9%; Carlo Erba, Italy), alanine (99%; BDH Chemicals Ltd, Poole, England), pyridine (> 99%; Sigma-Aldrich, Germany), ninhydrin (Sigma-Aldrich, Germany) were used. Deionized water was used for the preparation of standards and dilution of samples.

Sample collection and pretreatment

Thirty six (36) teff samples were collected from three administrative zones of Amhara and Oromia regions each as well as one administrative zone of the Southern Nations Nationalities and Peoples Region (SNNPR) of Ethiopia. The selected areas are the major teff producing areas in the country. For the chemometric analysis, sampling regions are represented by group 1, group 2 and group 3 for Amhara, Oromia and SNNP Regions (Table 1), respectively. The two varieties of teff samples (white and brown teff) were collected from 18, December 2020 up to 18, January 2021 from the local markets. All samples, of each 500 g was collected and kept in airtight polythene bags at ambient temperature. After the samples were transported to the laboratory, contaminations like straw, soil, husk, immature seeds and dust particles, were removed by sieve and ground by an electronic grinder to mesh size 300 µm and then made ready for the extraction.

Extraction of total sugars and total free amino acids in the white and brown teff samples

To extract the water-soluble total sugars and total free amino acids contents in the two teff varieties, the adapted method reported by Behlil *et al.* [28] was employed. Briefly, 0.5 g of the teff flour sample was added to 20 mL of extraction solvent (80% methanol) and soaked for 30 min. The wetted sample has been mechanically shaken for 30 min and the mixture was centrifuged for 10 min at 3000 rpm. The supernatant was filtered using Whatman filter paper. Then, to determine the total sugars content, the modified procedure of Albalasmeh *et al.* [29] was used. Clearly, a 0.5 mL aliquot of the sample extract was diluted with 4.5 mL of 80% methanol followed by the addition of 3 mL of concentrated sulfuric acid (96%) in a vial and stirred using a glass rod for 1 min. The temperature of the mixture rapidly increased after 5 seconds stirring. The solution was cooled in ice for 2 min to bring it to room temperature. Finally, the solution was scanned by UV-Vis spectrophotometer.

To determine the total free amino acids, the modified procedure of Behlil *et al.* [28] was employed. Clearly, 0.5 mL of each of the samples solution was taken in separate test tubes and their volume was made 2 mL by using deionized water. Then, 1 mL of 2% ninhydrin and 1 mL of 10% pyridine solution were added to all test tubes. All the test tubes were heated in boiling water bath at 80 ºC for 40 min and the result was obtained in purple color. The solution of all the test tubes were made the volume up to 10 mL by deionized water and then the absorbance (λ_{max} = 570 nm) was recorded by UV-Vis spectrophotometer.

Table 1. Sampling areas and sample ID of the white and brown teff grains (*Eragrostis tef* (Zuccagni) Trotter)) varieties cultivated in Ethiopia.

No.	Geographical region	Administrative zone	District	Variety of teff	Sample ID
1	Group 1	North Shewa	Minjar Shenkora	White	AW1
				Brown	AB19
			Tar Mahber	White	AW2
				Brown	AB20
			Antsokiyana Gemza	White	AW3
				Brown	AB21
			Ankober	White	AW4
				Brown	AB22
		South Wollo	Dessie Zuria	White	AW5
				Brown	AB23
			Were Ilu	White	AW6
				Brown	AB24
			Legahida	White	AW7
				Brown	AB25
		East Gojam	Goncha Siso Enese	White	AW8
				Brown	AB26
2	Group 2	East Shewa	Boset	White	OW9
				Brown	OB27
			Adama Zuria	White	OW10
				Brown	OB28
		Arsi	Jeju	White	OW11
				Brown	OB29
		West Arsi	Negelle Arsi	White	OW12
				Brown	OB30
		East Shewa	Ada'a	White	OW13
				Brown	OB31
			Bishoftu	White	OW14
				Brown	OB32
			Gimbichu	White	OW15
				Brown	OB33
			Dugda	White	OW16
				Brown	OB34
3	Group 3	Haddiya	Soro	White	SW17
				Brown	SB35
			Gomibora	White	SW18
				Brown	SB36

Note: Amhara region = Group 1, Oromia region = Group 2, SNNP region = Group 3, ID = Identification.

Preparation of standard solution

To prepare glucose standard for the determination of water-soluble total sugar contents, a 0.1 g of glucose standard was measured using analytical balance and transferred in to 100 mL volumetric flask to prepare 1000 mg/L stock solution. The working standard solution was prepared by taking 100, 200, 400, 800 and 1000 µL in to 10 mL volumetric flask to get 10, 20, 40, 80, and 100 mg/L solution. Then, 1 mL of each standard solution was taken in to separate vials followed by the addition of 3 mL of concentrated sulfuric acid (96%) and stirred using a glass rod for 1 min. The temperature of the mixture rapidly increased after 5 seconds stirring. The solution was cooled in ice for 2 min to bring it to room temperature. Finally, the solution was scanned by UV-Vis spectrophotometer.

To prepare alanine standard for the determination of total free amino acids, a 0.1 g of alanine standard was measured and taken in to 100 mL volumetric flask to prepare 1000 mg/L stock solution. The working standard solution was prepared by taking 25, 75, 150, 225, and 300 µL in to 25 mL volumetric flask to get 1, 3, 6, 9, and 12 mg/L solution. Then, 1 mL of each standard solution was taken in to separate test tubes followed by the addition of 1.5 mL of 2% ninhydrin and 2 mL of 10% pyridine solution, respectively. All the test tubes were heated in boiling water bath at 80 ºC for 40 min and the result was obtained in violet color. The absorbance was recorded by the UV-Vis spectrophotometer.

Chemometric analysis

OriginPro 2015 64Bit was used to plot the calibration curves, overlay spectra of the standards and real samples. Data analysis was performed using Minitab 17 software. Results are presented as mean±SD from replicate measurements. One-way analysis of variance (ANOVA) was utilized to test the presence of significant differences ($\alpha = 0.05$), in the mean concentration of total sugars and total free amino acids among the sampling regions (groups). Tukey's test was used to check if there were significant differences among the three regions and varieties of teff samples.

RESULTS AND DISCUSSION

The overlay UV-Vis spectra and calibration curve ($y = 0.01083x + 0.06928$) of the concentrated glucose standard (λ_{max} = 323 nm) used and the comparison of the standard with that of the teff samples spectra and the diluted glucose spectra (λ_{max} = 280 nm) are revealed in Figure 1. Looking at the data points, an interesting linear response over a concentration range of 10–100 mg/L was noted, in which the regression coefficient ($R^2 = 0.99976$) indicated a strong relationship between the concentration ratio and response. As can be seen in Figure 2, the λ_{max} observed at 323 nm is due to the aggregation of the glucose standard concentration and the λ_{max} appeared at 280 nm is due to the monomeric species of the same compound. Figure 2 clearly revealed that the dimer peak appears at longer wavelength (λ_{max} = 323 nm) which is higher in concentration while the monomer peak occurred at lower wavelength (λ_{max} = 280 nm) as the concentration decreases for the same compound. Because of this reason, therefore, the dilution factor was taken in to account while determining the amount of the water-soluble total sugars in the teff samples.

The determined water-soluble total sugars contents of the white and brown teff grain samples were reported as gram of glucose equivalent per 100 g of teff samples (g GE/100 g) by further considering the dilution factors and are shown in Table 2. While the mean, minimum and maximum values of the sampling group 1, 2, and 3, respectively, are given in Table 3. The water-soluble total sugars contents of the extract in the white and brown teff grain samples ranged between 2.69 ± 0.12 – 4.56 ± 0.08 g GE/100 g and 2.22 ± 0.04 – 4.74 ± 0.19 g GE/100 g, respectively, which is in agreement with the content of total sugars in wheat (2.91 g GE/100 g), maize (3.66 g GE/100 g), sorghum (2.56 g GE/100 g), millet (3.31 g GE/100 g) [8], and rye (2.3 g/100 g) [30]. However, results of the total sugars in the present study were higher than barley (1.8 g/100 g), rice (1 g/100 g) and oat (1.2 g/100 g) [13], but lower than the wheat samples (7.8 g/100 g) [30]. Furthermore, the total sugar content determined for the rice samples at different hydrolysis times under the constant acid concentration and temperature was reported by Omar *et al.* [31] in the range of 7.93 - 23.34% which is higher than the present study. In this study, the lowest water-soluble total sugars content (2.22 g GE/100 g) and the highest water-soluble total sugars content (4.74 g GE/100 g) were found in the brown teff grain samples from group 1. This implied that the brown teff grain samples from group 1 contained higher total sugars than that of the white teff samples from group 1, 2 and 3 and brown teff samples of group 2 and 3.

The presence of significant differences in the mean concentration of water-soluble total sugars in the white and brown teff samples of the three locations (group 1, group 2, and group 3) in Table

2 was tested using chemometric analysis utilizing one-way ANOVA (α = 0.05). However, the ANOVA test revealed that there were no statistically significant differences (α = 0.05) among the three sampling locations and even between the mean value of the white and brown teff samples. The box plot distribution of total sugars level in the 36 teff grain samples from each of the three sampling groups is depicted in Figure 3(A). The content of the water-soluble total sugars in group 1 found to be the most dominant and revealing a wide distribution in range followed by group 2 and 3, respectively, while group 3 has shown a narrow distribution in total sugars as compared to group 1 and 2 of the sampling locations. Besides, the box plot in Figure 3(B), exhibited that the water-soluble total sugars contents in both teff samples were evenly spread. The outlier which is the minimum amount of total sugar and the maximum content shown in the Figure 3(B) was found in the brown teff samples.

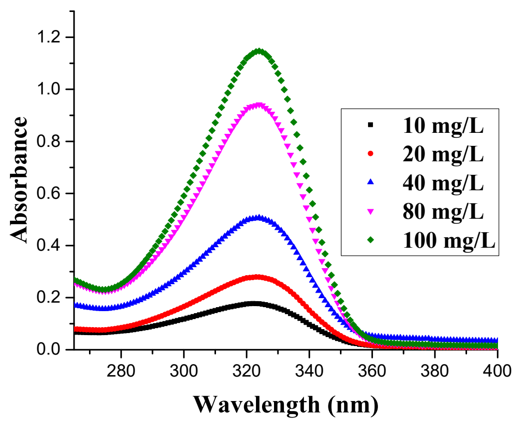


Figure 1. Overlay spectra of glucose standard.

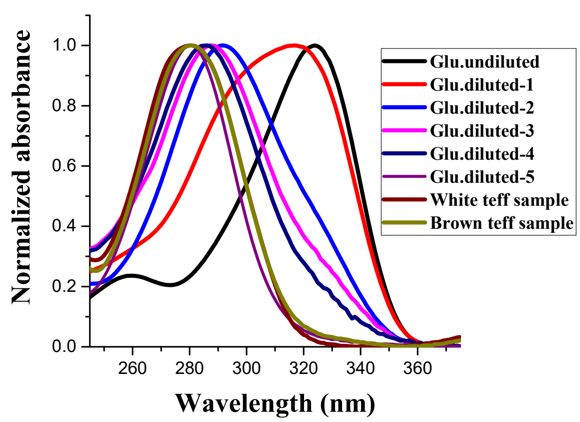


Figure 2. Overlay spectra of the total water-soluble sugars in the white teff sample, brown teff sample and glucose standard (Glu).

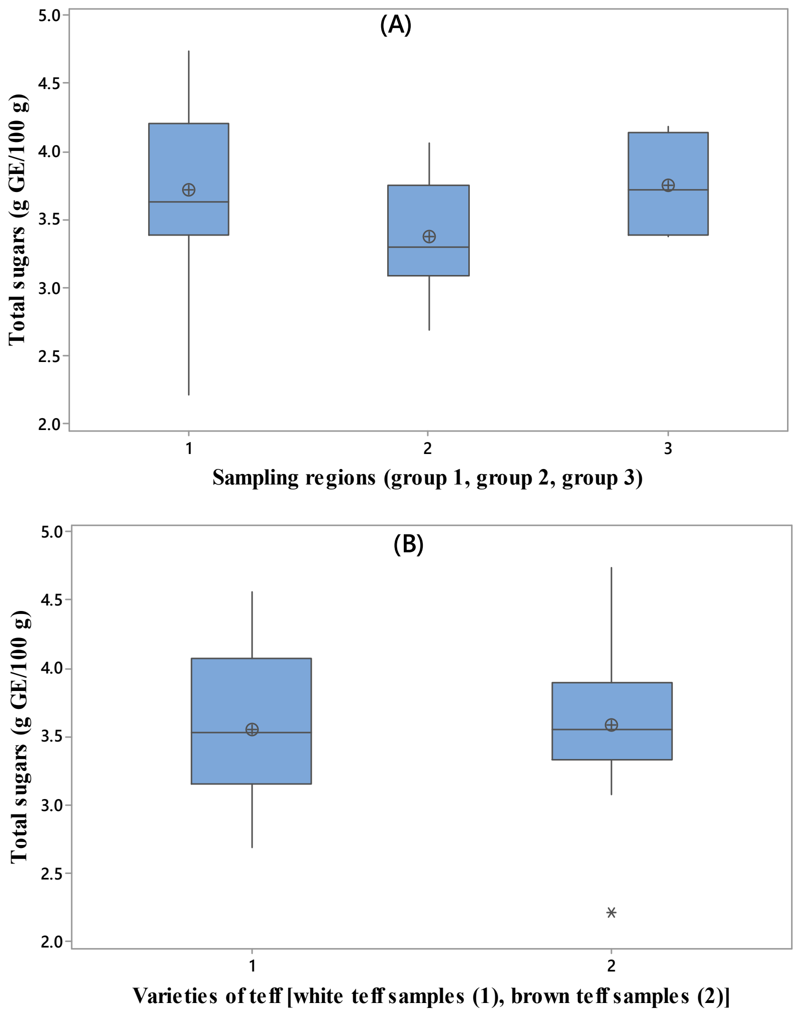


Figure 3. Boxplot revealing the distribution of the concentration (g GE/100 g) of the water-soluble total sugars in the two varieties of teff samples among the three sampling regions (A) and between the white and brown teff samples (B). Horizontal bars indicate the median for each group; vertical bars indicate the maximum and minimum value. The ball sign at the center indicate the average value of the teff samples.

The determination of total free amino acids (TFAA) content using spectrophotometric method requires ninhydrin [23]. Ninhydrin reacts with the α-amino group of the primary amino acids producing 'Ruhemann's purple', with the absorption maximum at 570 nm. The chromophore formed is the same for all primary amino acids. The intensity of the color formed depends on the number and chemical nature of the amino groups being analyzed. In the present study the overlapped UV-Vis spectra and calibration curve ($y = 0.04216x + 0.09257$) of the alanine standard used and the comparison of the standard with that of the teff samples spectra (λ_{max} = 570 nm) are revealed in Figure 4 and Figure 5, respectively. A concentration range of 1 – 12 mg/L having a regression coefficient (R^2 = 0.982) was established. The total free amino acids (TFAA) contents (Table 2) present in the white and brown teff samples were quantified through the calibration equation by considering the dilution factors and the min, max and mean values are presented in Table 3. The contents were reported as milligram of alanine equivalent per 100 g of teff samples (mg AE/100 g) to be suitable for comparison with literatures.

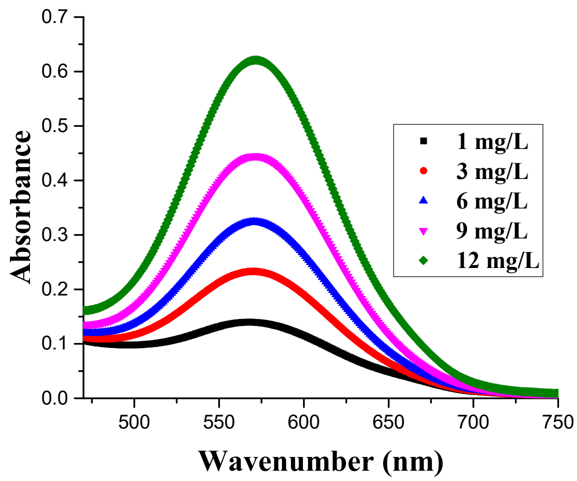


Figure 4. Overlay spectra of alanine standard.

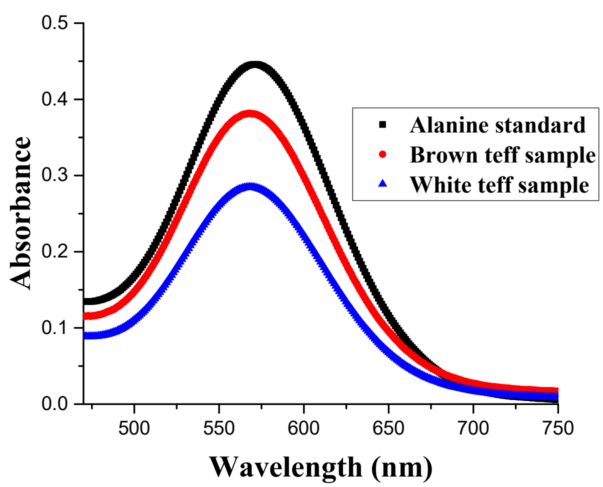


Figure 5. Overlay spectra of the total free amino acids in the white teff sample, brown teff sample and alanine standard.

The mean TFAA contents for the white and brown teff samples ranged between 181.4 ± 30.0 – 638.9 ± 42.6 mg AE/100 g and 471.7 ± 37.0 – 927.1 ± 32.4 mg AE/100 g, respectively. The observed TFAA levels of the present study are comparable to the findings reported by Culea *et al.* [25] for the corn seed (71.3 – 579.8 mg/100 g), but higher than the rice varieties (35.3 – 59.1 mg/100 g) stated by Kamara *et al.* [32], rice varieties (18.8 – 72.7 mg/100 g) [27] and lower than the different genotypes of wheat flour (6,300 – 10,900 mg/100 g) studied by Laze *et al.* [33]. In this study, the highest (927.1 mg AE/100 g) and the lowest (181.4 mg AE/100 g) total free amino acids contents were found in the brown and white teff samples of the group 1, respectively.

Table 2. Water-soluble total sugars and total free amino acids contents of the white and brown teff [*Eragrostis tef* (Zuccagni) Trotter] varieties cultivated in different parts of Ethiopia.

Region	Variety of teff	Sample ID	Total sugars (g GE/100 g)	Total free amino acids (mg AE/100 g)
Group 1	White teff (n = 8)	AW-1	3.63 ± 0.03	630.7 ± 41.9
		AW-2	3.52 ± 0.04	567.0 ± 28.7
		AW-3	3.37 ± 0.06	600.9 ± 26.6
		AW-4	4.56 ± 0.08	517.8 ± 20.3
		AW-5	4.52 ± 0.15	532.4 ± 33.6
		AW-6	3.64 ± 0.16	549.9 ± 34.0
		AW-7	3.24 ± 0.18	608.9 ± 21.6
		AW-8	4.09 ± 0.13	638.9 ± 42.6
	Brown teff (n = 8)	AB-19	3.48 ± 0.06	843.0 ± 49.3
		AB-20	2.22 ± 0.04	894.8 ± 42.8
		AB-21	3.40 ± 0.14	889.2 ± 28.0
		AB-22	4.74 ± 0.19	927.1 ± 32.4
		AB-23	3.82 ± 0.13	471.7 ± 37.0
		AB-24	3.38 ± 0.12	800.3 ± 34.9
		AB-25	3.77 ± 0.15	599.7 ± 54.8
		AB-26	4.25 ± 0.12	678.6 ± 30.2
Group 2	White teff (n = 8)	OW-9	3.54 ± 0.22	456.2 ± 31.6
		OW-10	2.69 ± 0.12	385.0 ± 17.9
		OW-11	3.14 ± 0.10	341.3 ± 40.6
		OW-12	2.80 ± 0.07	181.4 ± 30.0
		OW-13	3.56 ± 0.02	405.3 ± 38.0
		OW-14	2.96 ± 0.11	189.1 ± 62.0
		OW-15	3.16 ± 0.15	356.1 ± 39.4
		OW-16	4.07 ± 0.03	329.1 ± 62.1
	Brown teff (n = 8)	OB-27	3.40 ± 0.04	853.6 ± 95.9
		OB-28	3.17 ± 0.13	834.9 ± 57.0
		OB-29	3.20 ± 0.12	820.6 ± 55.4
		OB-30	3.08 ± 0.02	679.1 ± 74.2
		OB-31	3.86 ± 0.16	750.7 ± 63.6
		OB-32	3.80 ± 0.13	610.7 ± 49.4
		OB-33	4.03 ± 0.09	552.5 ± 39.8
		OB-34	3.63 ± 0.15	643.4 ± 121.9
Group 3	White teff (n = 2)	SW-17	4.19 ± 0.11	468.9 ± 35.9
		SW-18	3.38 ± 0.06	577.2 ± 67.5
	Brown teff (n = 2)	SB-35	3.43 ± 0.14	682.7 ± 66.2
		SB-36	4.01 ± 0.12	591.7 ± 53.5

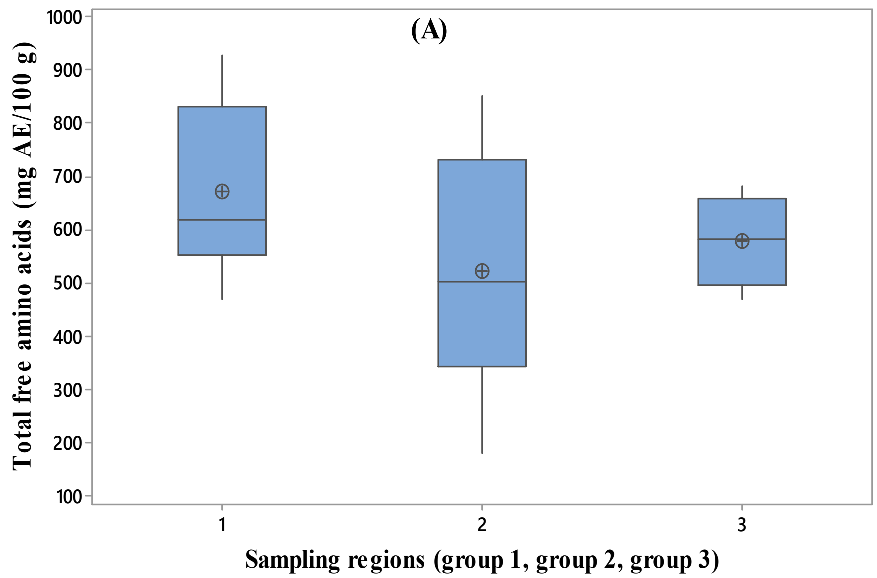
Note: Group 1 = Amhara, Group 2 = Oromia, Group 3 = SNNPR, GE = Glucose equivalent, AE = Alanine equivalent, SD = Standard deviation.

Table 3. Mean contents of the water-soluble total sugars and total free amino acids of the white and brown teff [*Eragrostis tef* (Zuccagni) Trotter] varieties cultivated in different parts of Ethiopia.

Region	Variety of teff		Total sugars (g GE/100 g)	Total free amino acids (mg AE/100 g)
Group 1	White teff (n = 8)	Mean	3.82	580.8
		SD	0.10	31.2
		Min	3.24	517.8
		Max	4.56	638.9
	Brown teff (n = 8)	Mean	3.63	763.1
		SD	0.12	38.7
		Min	2.22	471.7
		Max	4.74	927.1
Group 2	White teff (n = 8)	Mean	3.24	330.4
		SD	0.10	40.2
		Min	2.69	181.4
		Max	4.07	456.2
	Brown teff (n = 8)	Mean	3.52	718.2
		SD	0.11	69.7
		Min	3.08	552.5
		Max	4.03	853.6
Group 3	White teff (n = 2)	Mean	3.79	523.1
		SD	0.09	51.7
		Min	3.38	468.9
		Max	4.19	577.2
	Brown teff (n = 2)	Mean	3.72	637.2
		SD	0.13	59.9
		Min	3.43	591.7
		Max	4.01	682.7

Note: Group 1 = Amhara, Group 2 = Oromia, Group 3 = SNNPR, GE = Glucose equivalent, AE = Alanine equivalent, SD = Standard deviation, Min = Minimum, Max = Maximum.

Statistical analysis using one-way ANOVA ($\alpha = 0.05$) was employed to test the presence of significant differences among the mean concentration of TFAA in the two varieties of teff from the sampling regions (Table 2). Accordingly, the ANOVA test has shown that there was no statistically significant differences ($\alpha = 0.05$) among the mean concentration of the TFAA with respect to the sampling three groups. However, there was statistically significant difference ($\alpha = 0.05$) in the mean value of TFAA between the white and brown teff samples and the mean value of the TFFA in the brown teff samples was found higher than that of the white teff samples. This was further confirmed by Tukey's pair wise comparison test. The box plot distribution of TFAA mean value in the two varieties of teff among the three sampling groups is depicted in Figure 6(A). The content of the TFAA in group 2 was found to be the most dominant and revealing a wide in range followed by group 1 and 3, respectively, while group 3 has shown a narrow distribution in TFAA as compared to group 1 and 2 of the sampling locations. Besides, the box plot in Figure 6(B) indicated that the TFAA content between the white and brown teff samples was nearly similar in statistical distribution.



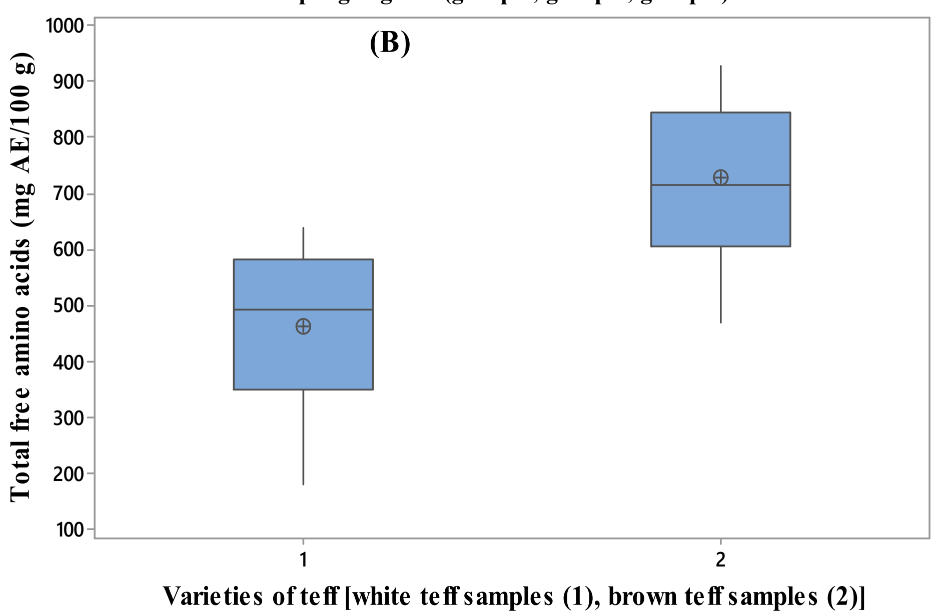


Figure 6. Boxplot revealing the distribution of the concentration (mg AE/100 g) of the total free amino acids in the two varieties of teff samples among the three sampling regions (A) and between the white and brown teff samples (B). Horizontal bars indicate the median for each group; vertical bars indicate the maximum and minimum value. The ball sign at the center indicate the average value of the teff samples.

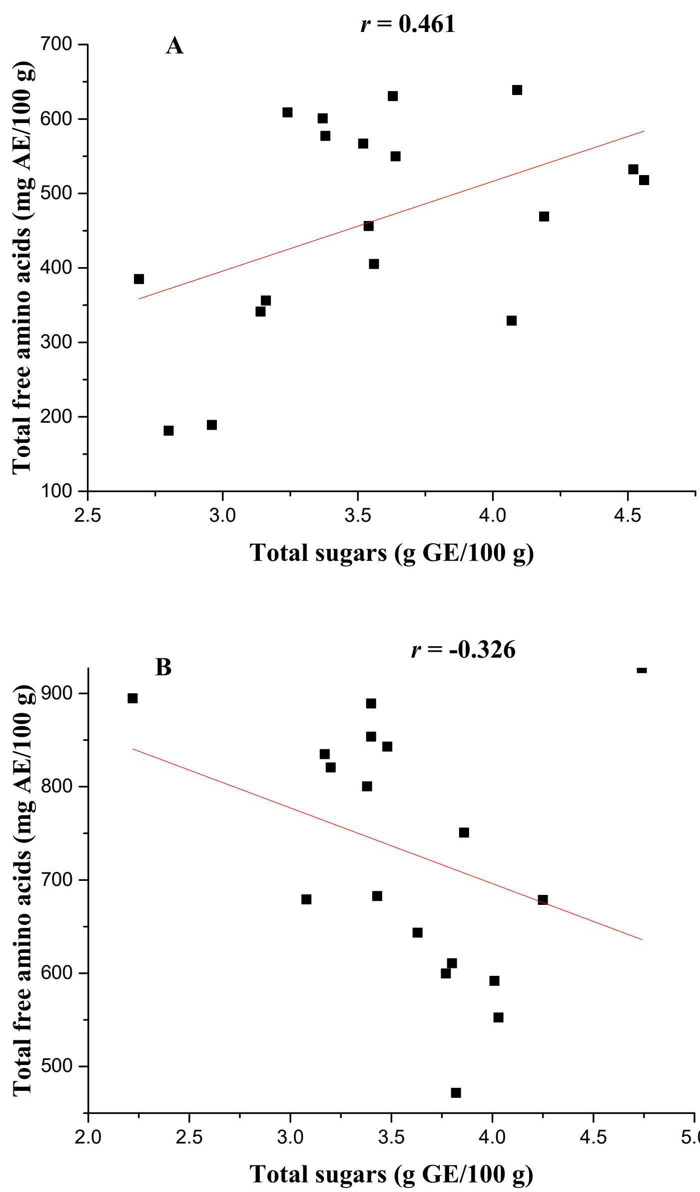


Figure 7. The correlation between the water-soluble total sugars (g GE/100 g) and total free amino acids (mg AE/100 g) in the 18 white teff (A) and 18 brown teff (B) varieties of the three sampling groups.

Correlation between the water soluble total sugars and total free amino acids contents

The Pearson correlation between the water-soluble total sugars and total free amino acids (TFAA) contents (Table 2) in the white and brown teff extracts were analyzed. The sample variety based correlation curves are depicted in Figure 7. As can be seen in Figure 7, the relationship test between the total sugars and TFAA contents in the 18 white teff variety is positive and moderately strong (r = 0.461); while in the 18 brown teff variety is negative and weak (r = -0.326). The Pearson correlation indicated that both the white and brown teff varieties might have opposite biosynthetic pathways for the synthesis of the water-soluble total sugars and TFAA, since the direction of the correlation was opposite for which a relationship was established. Besides, the composition of volatiles in the white and brown teff study reported by Yisak *et al.* [16] has confirmed clear differentiation between the two varieties of teff. Therefore, these experimental findings revealed that the two varieties of teff might have difference in terms of their quality.

CONCLUSION

The present study was intended for the determination of water-soluble total contents of sugars and total free amino acids (TFAA) in the white and brown teff varieties. The mean water-soluble total sugars contents of the two varieties of teff have revealed no statistically significantly different (α = 0.05) among the sampling locations and between the white as well as the brown teff samples. The lowest and highest water-soluble total sugars content were found in the brown teff grain samples from group 1. While, the highest and the lowest total free amino acids contents were found in the brown and white teff samples of the group 1, respectively. Besides, no statistically significant differences (α = 0.05) in the mean TFAA concentrations among the three sampling groups was found. However, there was significant differences (α = 0.05) between the white and brown teff samples and the amount of TFAA in the brown teff samples was found higher than that of the white teff samples. This implied that brown teff sample is rich in TFAA than the white teff samples. The Pearson correlation test was in the opposite direction showing that both the white and brown teff varieties might have opposite biosynthetic pathways for the synthesis of total sugars and TFAA. From the present study, therefore, it is possible to conclude that the two varieties of teff samples were found to be rich in the content of water soluble total sugars and TFAA. Hence, findings of this study can be useful for consumers to be aware about the nutritional value of the white and brown teff grains. Besides, it may pave a path for researchers to conduct further study on individual sugars and amino acids of the teff samples in more compressive manner.

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