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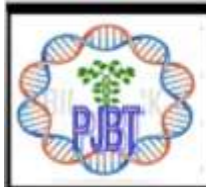# Pakistan Journal of Biotechnology
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PROXIMATE COMPOSITION, AMINO ACID PROFILING, AND SAFETY ASSESSMENT OF PROCESSED NATURAL VEGETATION OF THARPARKAR

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ABSTRACT

Some processing techniques tend to advance the overall nutritional quality of food commodities and thus may contribute to counter malnutrition in regions like Tharparkar. For scanning the efficacy of some selected processing treatments, the natural vegetation of Tharparkar (NVT) i.e., mung beans (MB), sesame seeds (SS), and pearl millet (PM), were sprouted, roasted, and blanched, respectively, and milled to flour after thermal drying. All fresh and processed NVT, i.e., sprouted mung bean flour (SMBF), roasted sesame seed powder (RSSP), and blanched pearl millet flour (BPMF), were analyzed for proximate composition, profiling of amino acids (AAs), and safety assessment. The results demonstrated statistically different (P<0.05) mean values of fresh and processed NVT for most of the analyzed parameters. It was found that processing significantly increased (P<0.05) ash, protein, fat, and fiber content whereas significantly decreased (P<0.05) phytic and oxalic acid in the NVT. In addition, some essential AAs significantly (P<0.05) enhanced in SMBF i.e., histidine (0.45g/100g), isoleucine (0.59g/100g), lysine (0.71g/100), methionine (0.23g/100g), phenylalanine (1.54g/100g), and valine (0.32g/100g); RSSP i.e., isoleucine (1.63g/100g), leucine (4.57g/100g), lysine (0.75g/100g), phenylalanine (3.14g/100g), tryptophan (0.81g/100g), and tyrosine (2.15g/100g); and in BPMF i.e., threonine (0.55g/100g), isoleucine (0.28g/100g), leucine (1.70g/100g), methionine (0.21g/100), tryptophan (0.11g/100g), and valine(0.17g/100g). Among non-essential AAs, the alanine and glycine remained significantly higher (P<0.05) in all processed NVT i.e., 1.56, 1.29, and1.43g/100g alanine in SMBF, RSSP, and BPMF, respectively. However, glycine remained at 0.56, 0.59, and 0.23g/100g in SMBF, RSSP, and BPMF, respectively. It is therefore concluded that processing techniques exerted a significant role in enhancing nutritional profile while mitigating the antinutrient load from the NVT. Consequently, it is recommended that SMBF, RSSP, and BPMF be utilized for developing various food products to counter malnutrition in regions like Tharparkar.

Keywords: Natural vegetation, Nutritional composition, Antinutrients, Food processing techniques

INTRODUCTION

In recent years, the consumption of plant-based foods has emerged phenomenally due to their immense health-related benefits (Dhull *et al*., 2023). The plant-based foods such as cereals, legumes, seeds, nuts, fruits, and vegetables are all enormously loaded with essential macro and micronutrients (Lim *et al*., 2020; Bhattacharya, 2024). However, selecting the right food processing technique can further enhance the overall nutritional composition, sensorial profile, and safety attributes by mitigating the antinutritional factors i.e., phytic and oxalic acid, etc. (Sá *et al*., 2020).

Application of different food processing techniques dates to the prehistoric eras and at that time commonly used conventional techniques were i.e., sun drying, salting, fermentation, pickling, etc. (Bonciu, 2018). Later with the advent of technology, new techniques rapidly continued to arrive such as sprouting, blanching, freezing, canning, irradiation,

etc. Among different food processing techniques, sprouting is an extremely inexpensive technique usually applied to seeds during which the seed absorbs water, develops an embryonic axis (Montemurro *et al*., 2019), and improves nutrient content, and food safety attributes. Consequently, sprout foods are generally referred to as functional foods (Reed *et al*., 2018). Blanching, on the other hand, is a hot water treatment that retards enzymatic activity, improves sensorial profile, and reduces microbial load in foods (Richter, 2017). Moreover, roasting is a dry-heat food processing technique that enhances the nutritional and sensorial attributes of food (El Hanafi *et al*., 2023). Although, risk-benefit concerns of food processing techniques are imperative to consider hence selecting the right food processing technique for the right food should be a prime focus of food technologists.

Malnutrition is a serious health-associated issue affecting millions of people globally (Webb *et al*.,

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2018). It is expected that by 2050, around 9 billion people worldwide are likely to face feeding challenges (Lanjwani *et al*., 2023). As per the National Nutrition Survey (2018), the situation of nutritional status among the population of Pakistan is not satisfactory. The Tharparkar district of Sindh province witnessed devastating outcomes of stunting, wasting, and micronutrient deficiencies however certain hospital-based findings have also exhibited a reportedly higher prevalence of malnutrition in the district (Ahsan *et al*., 2017). Among many of the factors associated with the prevalence of malnutrition, food insecurity, and inadequate dietary consumption are the main causes of malnutrition in Tharparkar (Lanjwani *et al*., 2023). The district is an arid region of Sindh province, merely 1.6% of its cultivatable land is irrigated either by ground or rainwater and produces natural vegetation like wheat, barley, pearl millet, maize, beans, pulses, sesame, etc. (Salam *et al*., 2023). The present study was carried out to counter malnutrition and its related consequences up to a certain extent and for this reason, the natural vegetation of Tharparkar i.e., mung beans (Vigna radiata), sesame seeds (Sesamum indicum), and pearl millet (Pennisetum glaucum) was selected owing to their abundance and subjected to various food processing techniques for evaluating their role in enhancing the nutritional quality and safety profile.

MATERIALS AND METHODS

Sample collection: The freshly harvested natural vegetation of Tharparkar i.e., MB, PM, and SS were collected from Tharparkar. The samples were packed properly in sterile Nasco easy-to-close sample bags, properly labelled, and brought to the IFST, Sindh Agriculture University, Tandoajm, Sindh, Pakistan.

Sample processing: Initially, the MB, SS, and PM were manually cleaned, washed thrice with distilled water, air-dried at ambient temperature (35±2 °C), and separated into two lots. The first lot of each NVT was served as fresh (or control) while the other was subjected to various processing treatments. The sprouting of MB, roasting of SS, and blanching of PM were performed as per the method described by Liu *et al*. (2020), Ahmed *et al*. (2021), and Pawase *et al*. (2021), respectively, with minor modifications (Figure 1). Later, sprouted MB, roasted SS, and blanched PM were thermally dehydrated at 55±1 °C for 12-18 hours, and milled to obtain sprouted MB flour (SMBF), roasted SS powder (RSSP), and blanched PM flour (BPMF) (Figures 1 and 2). Finally, the fresh and processed samples of NVT were subjected to analysis.
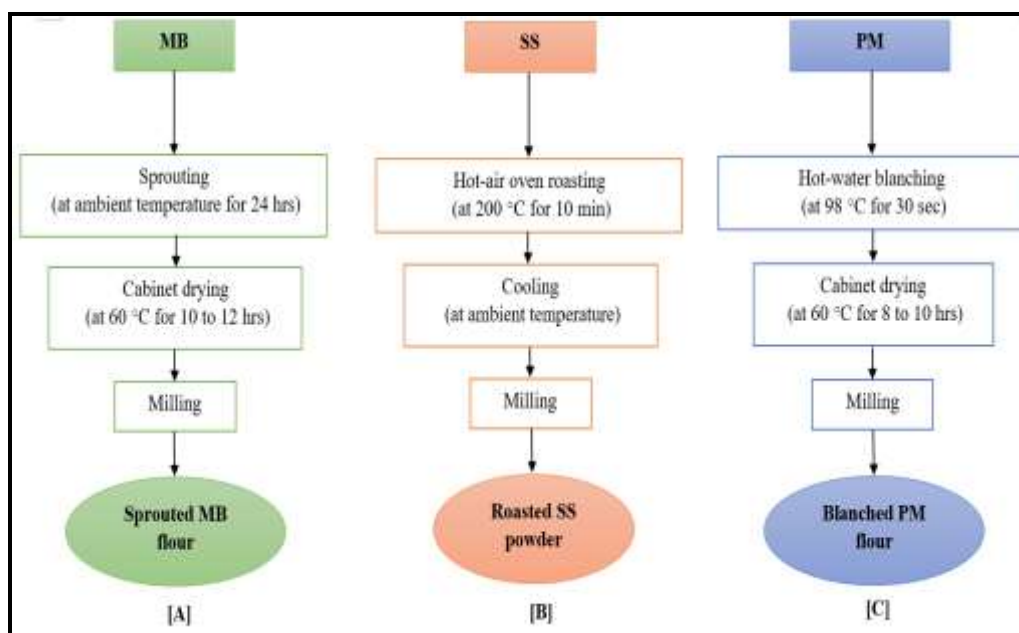

Figure 1   Flowchart showing processing techniques [A] sprouting of MB, [B] roasting of SS, and [C] blanching of PM

Sample analysis

Proximate composition: Fresh and processed MB, SS, and PM were determined for their proximate composition, amino acid profiling, and antinutrient load as per standard analytical procedures. The moisture, ash, and protein % of fresh and processed MB and SS were determined as per AOAC (2016), fat % was analyzed according to AOAC (2000), and carbohydrate was determined as per the difference method proposed by AOAC (1990). The method prescribed by Khalil and Durrani (1990) was followed to evaluate fiber % whereas the energy value as kcal/ 100 grams of sample was calculated by the method described by Paul and Southgate (1988). Fresh PM and BPMF were analyzed for proximate composition as per the methods proposed by AACC (2000).

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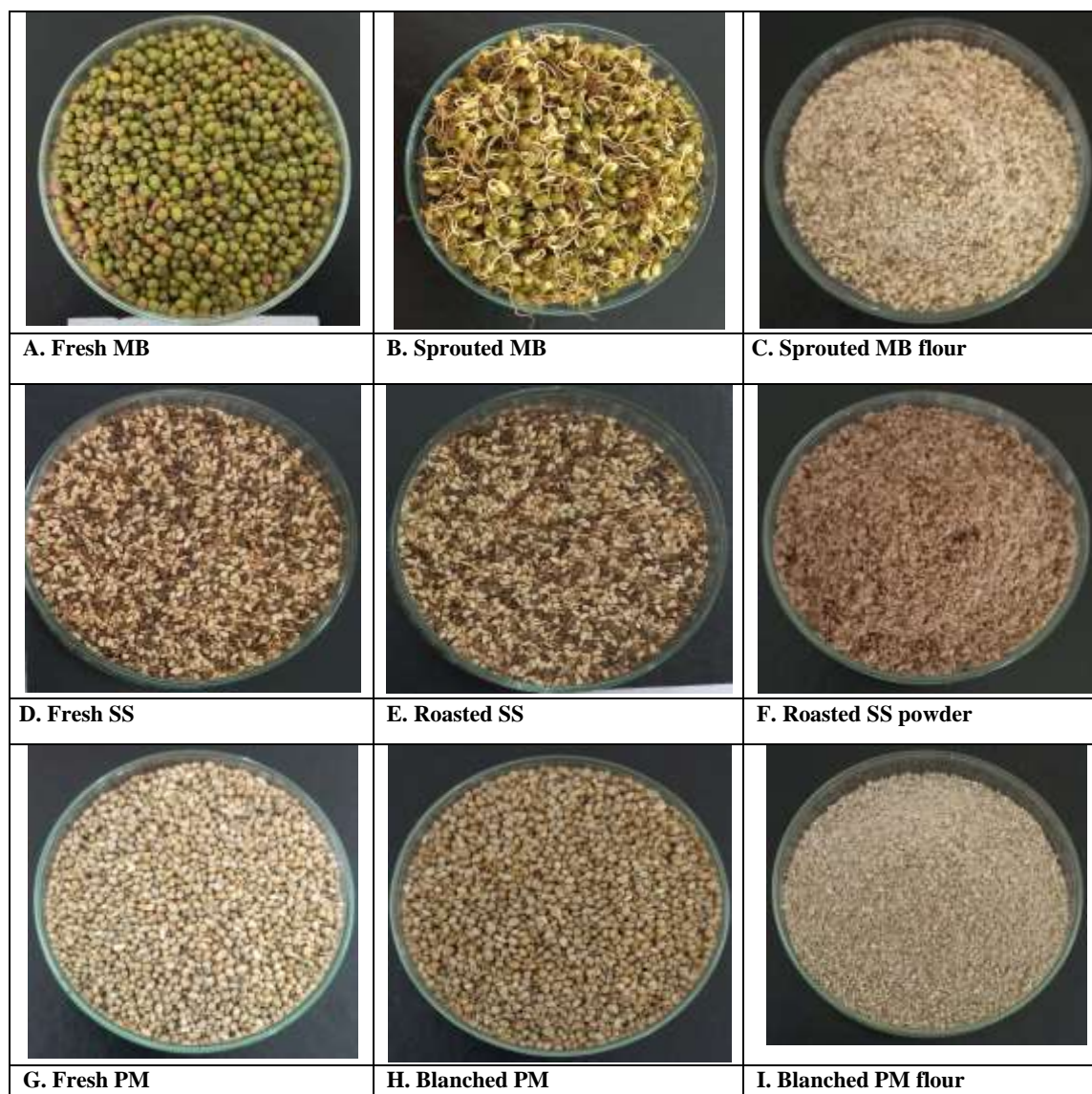
. **Figure 2   Fresh and processed natural vegetation of Tharparkar**

Antinutrients: For assessing safety attributes in fresh and processed NVT, the load of phytic and oxalic acid was examined. The phytic acid was determined as per the method described by Lucas & Markaka (1975) whereas oxalic acid was determined by calculating the titratable acidity of the samples according to the method of AOAC (2000). A 10 g sample was crushed with 30 ml of distilled water using a pestle and mortar. The mixture was stirred and filtered through Whatman filter paper No. 4. Ten ml of filtered sample was transferred to a prewashed conical flask and 3 to 4 drops of phenolphthalein were added. Next, the sample was titrated against 0.1 N NaOH. Oxalic acid (%) was determined as per the following formula:

Oxalic acid (%) = 1/10 × Eq. wt. of oxalic acid × Normality of NaOH × Titre /10
(The equivalent weight of oxalic acid = 45 g)

Amino acid profiling: The amino acid profiling was carried out at the laboratory of Food & Marine Resources Research Centre, PCSIR laboratories complex, Karachi. A total of 17 AAs were determined in fresh and processed NVT. The acid hydrolysis was carried out by the method described by AOAC (2000) official method 994.12 (ion exchange chromatography). About 100 mg sample was taken in a separate digestion tube with the addition of 5 ml 6N HCl carrying 0.1% phenol and kept for 18-24 hrs. at 110 °C. The hydrolyzed samples were evaporated to dryness on a rotary evaporator in a vacuum at 60 °C, washed with 20 ml water, and evaporated again. The washing and evaporating were repeated twice. The final volume was made up of 50 ml with sodium citrate buffer. The sample was filtered through a 0.22-micron filter before sample injection (20 μL) into the amino acid analyzer. Amino acid analysis was conducted on the Shimadzu HPLC amino acid analysis system through the application data book with Shim-Pack Amino-Na column (4.6mm, I.D x100mm). A post-column derivatization reaction of

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eluted AAs with o-phthaldehyde (OPA) was used to detect peaks through a fluorescence detector, adjusted at 350 nm (excitation wavelength), and 450 nm (emission wavelength).

Statistical analysis: A total of three replications were studied for all tests (i.e., proximate composition, antinutrients, and amino acid profiling) on different days in a similar manner. The data obtained from the analysis was statistically evaluated through one-way ANOVA using IBM-SPSS 22 to compare the average values (at P<0.05) between each fresh and processed natural vegetation of Thraparkar as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The proximate composition, anti-nutrient content, and amino acid profile of fresh and processed MB, SS, and PM are presented in Tables 1 to 3.

Proximate composition: Table 1 shows the proximate composition of fresh and processed NVT. It was seen that sprouting significantly increased (P<0.05) ash (3.78 %), protein (30.47 %), fat (1.97 %), fiber (8.12 %), and energy value (334.21 kcal/100g) while decreased moisture and carbohydrate in MB. The metabolic changes owing to sprouting affect the bioavailability of different nutrients, palatability, and digestibility in legumes. Masood *et al*. (2014) also reported an increasing trend of ash, protein, and fiber in sprouted MB. The moisture content in SMBF was lesser (7.00 %) owing to the thermal drying which lowered the moisture content in SMBF. Present results are analogous to the findings of Skylas *et al*. (2018), in their study the ash, protein, fat, and fiber % in differently processed MB flour ranged between 3.1 to 3.5%; 27.6 to 29.4%;1.8 to 2.0 %; and 3.4 to 13.1%, respectively. Although MB is a crucial source of protein however sprouting further enhances protein concentration up to 1.4% in MB (Ghavidel & Prakash, 2007). Liu *et al*. (2018) reported the effective role of MB sprouting in increasing the protein % in MB flour. The RSSP also showed a significant increase (P<0.05) in ash (5.07%), protein (29.22%), fat (42.79%), fiber (5.85%), and energy value (552.60 kcal/100g). Roasting SS exhibited a phenomenal increase in protein in comparison to fresh SS, however, it decreased moisture (5.45%) and carbohydrate (24.75%) in RSSP. Tenyang *et al*. (2017) also reported that the moisture and carbohydrate content reduced significantly while fat, protein, and fiber increased when SS were roasted at elevated temperatures. Significant variations (P<0.05) were noticed when PM was subjected to blanching treatment. It was seen that BPMF had lower moisture and carbohydrate content with higher ash (2.58 %), protein (12.27 %), fat (5.71 %), fiber (7.33 %), and energy value (357.35 kcal/100g). The trend of findings indicates that blanching treatment brings favorable modification in the overall proximate composition of PM. In contrast to present findings, Choudhary *et al*. (2023) reported significantly higher

ash, and carbohydrate while lower moisture, protein, and fat content in blanched PM.

Antinutrients: Phytic and oxalic acids are well known to function as antinutrients when consumed in higher proportions by impeding the absorption of important nutrients. Different food processing treatments behave differently in mitigating the load of anti-nutritional factors (Abbas & Ahmad, 2018). In the present findings, all processing treatments i.e., sprouting, roasting, and blanching exerted a significant impact on lowering the load of phytic and oxalic acids from NVT (Table 2). Sprouting significantly reduces phytic acid in MB (from 621.13 to 312.84mg/100g) while RSSF had lower oxalic acid content (59.63%) in comparison to fresh SS (102.14mg/100g). The findings from the current study align with the results reported by Abbas & Ahmad (2018).

Amino acid profiling: The results showed significant differences (P<0.05) among various AAs in fresh and processed NVT (Table 3). Out of 17 AAs, the sprouting enhanced the concentration of 11 AAs in SMBF while merely 03 AAs remained at par (i.e., threonine, glutamic acid, tryptophan), 02 reduced during sprouting (i.e., proline, tyrosine), and 02 remained non-detected (i.e., arginine, cysteine). Results confirmed sprouting as a promising technique in significantly increasing the essential AAs (i.e., histidine, isoleucine, leucine, lysine, methionine, phenylalanine, valine). It is for this reason that sprouting brings biochemical changes that are associated with enhancing the concentration of AAs in legumes. Wongsiri *et al*. (2015) also reported that sprouting increases the concentration of AAs in MB. Roasting SS before consumption is one common processing technique that anticipates enhancing the nutritional and sensorial profile of seeds (Makinde & Akinoso, 2014). In the present study, roasting persisted favorably in enhancing a total of 10 AAs out of 17 in RSSP with 05 essential AAs (i.e., isoleucine, leucine, lysine, phenylalanine, and tyrosine). Furthermore, 04 AAs (i.e., aspartic acid, threonine, serine, methionine) were decreased, 02 AAs remained at par (i.e., histidine, valine) whereas arginine and cysteine remained non-detected in RSSF. The SS roasting time and temperature are two crucial aspects since prolonged roasting at higher temperatures leads to protein decomposition, cross-reaction in AAs, and formation of compounds that react with protein in the Maillard reaction (Rizki *et al*., 2015). Among different cereals, the AAs content of PM is satisfactory (Pawase *et al*., 2021) however it may be further lifted if PM is subjected to blanching. The blanching employs higher temperature treatment which significantly induces the biochemical properties of cereals. A total of 10 AAs were increased in BPMF out of which 06 were essential AAs (i.e.,

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threonine, isoleucine, leucine, methionine, tryptophan, valine). Moreover, BPMF was reported to have a lower concentration of aspartic acid and serine. Aggregately, 04 AAs remained at par (i.e., histidine, lysine, phenylalanine, tyrosine) while cysteine was not detected in fresh and BPMF. It was further reported that the arginine was evacuated from BPMF might be due to blanching treatment. In a similar study, Mutiara, *et al.,* (2013) studied the impact of blanching on AAs content in drumstick plants and reported analogous findings.

Table 1   Proximate composition of fresh and processed natural vegetation of Tharparkar

Proximate composition	Mung beans		Sesame seeds		Pearl millet	
	Fresh	Sprouted	Fresh	Roasted	Fresh	Blanched
Moisture %	8.98 ± 0.10^a	7.00 ± 0.11^b	5.45 ± 0.14^a	4.40 ± 0.16^b	9.07 ± 0.08^a	7.89 ± 0.01^b
Carbohydrate %	54.00 ± 0.65^a	48.64 ± 0.32^b	24.75 ± 0.20^a	12.65 ± 0.10^b	67.48 ± 0.31^a	64.20 ± 0.09^b
Ash %	3.27 ± 0.09^b	3.78 ± 0.03^a	3.97 ± 0.11^b	5.07 ± 0.02^a	2.21 ± 0.07^b	2.58 ± 0.12^a
Protein %	25.54 ± 0.83^b	30.47 ± 0.19^a	20.85 ± 0.21^b	29.22 ± 0.05^a	10.14 ± 0.12^b	12.27 ± 0.27^a
Fat %	1.62 ± 0.08^b	1.97 ± 0.06^a	40.68 ± 0.41^b	42.79 ± 0.23^a	4.89 ± 0.32^b	5.71 ± 0.21^a
Fiber %	6.56 ± 0.25^b	8.12 ± 0.05^a	4.29 ± 0.14^b	5.85 ± 0.09^a	6.20 ± 0.11^b	7.33 ± 0.10^a
Energy value (kcal/100g)	332.83 ± 2.0^b	334.21 ± 0.38^a	548.59 ± 2.50^b	552.60 ± 1.95^a	354.49 ± 2.28^b	357.35 ± 1.04^a

Different superscript letters in the column show statistically significant differences at P<0.05 between each fresh and processed vegetation.
Mean± Standard Error

Table 2   Antinutrient content in fresh and processed natural vegetation of Tharparkar

Antinutrients (mg/100g)	Mung beans		Sesame seeds		Sesame seeds	
	Fresh	Sprouted	Fresh	Roasted	Fresh	Roasted
Phytic acid	$\mathbf{621.13\pm0.03^a}$	312.84 ± 0.04^b	$\mathbf{36.27\pm0.32^a}$	13.70 ± 0.35^b	$\mathbf{801.71\pm4.84^a}$	611.64 ± 6.90^b
Oxalic acid	$\mathbf{220.11\pm0.89^a}$	202.97 ± 2.51^b	$\mathbf{102.14\pm0.36^a}$	59.63 ± 1.56^b	$\mathbf{34.75\pm0.77^a}$	29.63 ± 0.49^b

Different superscript letters in the column show statistically significant differences at P<0.05 between each fresh and processed vegetation.
Mean± Standard Error

Table 3   Amino acid profile of fresh and processed natural vegetation of Tharparkar

AAs (g/100g)	Mung beans		Sesame seeds		Pearl millet	
	Fresh	Sprouted	Fresh	Roasted	Fresh	Blanched
Aspartic acid	3.11 ± 0.02^b	$\mathbf{3.93\pm0.03^a}$	$\mathbf{2.06\pm0.01^a}$	1.73 ± 0.01^b	$\mathbf{1.34\pm0.008^a}$	0.73 ± 0.23^b
Threonine	0.62 ± 0.08^a	0.65 ± 0.03^a	$\mathbf{2.66\pm0.36^a}$	0.38 ± 0.01^b	0.2 ± 0.01^b	$\mathbf{0.55\pm0.2^a}$
Alanine	1.43 ± 0.02^b	$\mathbf{1.56\pm0.01^a}$	1.18 ± 0.01^b	$\mathbf{1.29\pm0.01^a}$	0.82 ± 0.01^b	$\mathbf{1.43\pm0.47^a}$
Glutamic acid	6.41 ± 0.08^a	6.28 ± 0.03^a	4.73 ± 0.36^b	$\mathbf{5.11\pm0.01^a}$	2.10 ± 0.03^b	$\mathbf{3.97\pm1.32^a}$
Glycine	0.42 ± 0.02^a	$\mathbf{0.56\pm0.03^b}$	0.52 ± 0.01^b	$\mathbf{0.59\pm0.01^a}$	0.13 ± 0.01^b	$\mathbf{0.23\pm0.08^a}$
Proline	$\mathbf{0.76\pm0.005^a}$	0.30 ± 0.01^b	0.22 ± 0.01^b	$\mathbf{0.46\pm0.01^a}$	0.03 ± 0.03^b	$\mathbf{0.21\pm0.07^a}$
Serine	0.80 ± 0.08^b	$\mathbf{1.43\pm0.03^a}$	$\mathbf{1.99\pm0.36^a}$	1.07 ± 0.01^b	$\mathbf{0.48\pm0.02^a}$	0.23 ± 0.08^b
Histidine	0.36 ± 0.006^b	$\mathbf{0.45\pm0.008^a}$	0.24 ± 0.01^a	0.25 ± 0.006^a	0.12 ± 0.01^a	0.18 ± 0.05^a
Isoleucine	0.46 ± 0.01^b	$\mathbf{0.59\pm0.008^a}$	0.65 ± 0.005^b	$\mathbf{1.63\pm0.01^a}$	0.18 ± 0.02^b	$\mathbf{0.28\pm0.08^a}$
Leucine	2.19 ± 0.01^b	$\mathbf{2.53\pm0.008^a}$	1.71 ± 0.005^b	$\mathbf{4.57\pm0.01^a}$	1.32 ± 0.02^b	$\mathbf{1.70\pm0.55^a}$
Lysine	0.59 ± 0.003^b	$\mathbf{0.71\pm0.005^a}$	0.43 ± 0.01^b	$\mathbf{0.75\pm0.005^a}$	0.29 ± 0.01^a	0.30 ± 0.1^a
Methionine	0.18 ± 0.008^b	$\mathbf{0.23\pm0.01^a}$	$\mathbf{0.24\pm0.01^a}$	0.13 ± 0.01^b	0.08 ± 0.02^b	$\mathbf{0.21\pm0.06^a}$
Phenylalanine	1.37 ± 0.008^b	$\mathbf{1.54\pm0.02^a}$	1.15 ± 0.01^b	$\mathbf{3.14\pm0.02^a}$	0.51 ± 0.008^a	0.73 ± 0.25^a
Tryptophan	0.22 ± 0.02^a	0.23 ± 0.03^a	0.07 ± 0.005^b	$\mathbf{0.81\pm0.01^a}$	0.02 ± 0.003^b	$\mathbf{0.11\pm0.02^a}$
Tyrosine	$\mathbf{0.95\pm0.006^a}$	0.82 ± 0.008^b	1.12 ± 0.01^b	$\mathbf{2.15\pm0.008^a}$	0.55 ± 0.02^a	0.55 ± 0.18^a
Valine	0.23 ± 0.01^b	$\mathbf{0.32\pm0.01^a}$	0.18 ± 0.008^a	0.15 ± 0.006^a	0.08 ± 0.01^b	$\mathbf{0.17\pm0.05^a}$
Arginine	ND	ND	ND	ND	3.43 ± 0.01^a	ND
Cysteine	ND	ND	ND	ND	ND	ND

Different superscript letters in the column show statistically significant differences at P<0.05 between each fresh and processed vegetation.
Mean± Standard Error
ND= Not detected

CONCLUSIONS

This study demonstrated the promising impact of processing techniques applied on NVT (i.e., sprouting of MB, roasting of SS, and blanching of PM) therefore it is concluded that all processing techniques significantly enhanced ash, protein, fat, fiber contents, and energy value in MB, SS, and PM. More importantly, the antinutritional factors (i.e., phytic and oxalic acids) favorably lowered in all processed NVT. Among the processing techniques, sprouting significantly reduces phytic acid in MB while RSSF had lower oxalic acid content in comparison to fresh SS. Moreover, A total of 16 AAs were reported during AAs profiling or fresh and processed NVT.

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The processing techniques exhibited an effective role in enhancing the concentration of AAs particularly essential AAs. Among essential AAs, isoleucine and leucine remained the common AAs that were enhanced in response to processing techniques in all NVT. The sprouting enhanced the concentration of 11 AAs in SMBF, roasting of SS and blanching of PM increased the concentration of 10 AAs. It is therefore suggested that SMBF, RSSP, and BPMF are nutritionally rich and safer to consume, especially in regions like Tharparkar where malnutrition is an inordinate dietary challenge.

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