

## BIOCHEMICAL AND NUTRITIONAL CHARACTERIZATION OF DIFFERENT SOYBEAN VARIETIES CULTIVATED IN PAKISTAN

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Soybean is an oleaginous legume and endorsed source of complete plant protein that contributes highest gross output as an oil seed crop throughout the world. The aim of this study was to investigate effect of soybean varieties on physicochemical properties, compositional analysis, mineral contents, fatty acids, lipoxygenase, antioxidant and phytochemical screening with respect to functional product. The significant  $p < 0.05$  results recorded for composition and mineral contents varied in all varieties. Among fatty acids the most dramatic effect was seen in palmitic (8.23-12.47%), oleic (22.19-34.56%), linoleic (49.25-56.34%) and linolenic acids (5.23-8.23%). Three lipoxygenase isozymes (LOX-1, LOX-2 and LOX-3) in soybean showed inhibition potential; however, LOX-2 was the most inhibition prone enzyme. Phytochemical screening through phenolic contents showed variation in between 1.21 to 2.76 (mgGAE/g) and total flavonoid contents 0.42 to 1.78 (mgCE/g). Antioxidant activity by DPPH was 3.68 to 5.65, FRAP 20.78 to 29.56 and ABTS 9.63 to 13.48 (mgTE/g). Conclusively, results showed that soybeans have the potential as a functional food and nutritional component highest in Ajmeri followed by William-82 and NARC-II, Rawal-I and Faisal.

**Keywords:** Soybean, complete protein, phenolic compound, antioxidant potential, functional food

### INTRODUCTION

Soybean (*Glycine max* L.), is preeminent global agricultural crop due to its alluring application in the manufacturing of a vast variety of foods along with its economic significance on national and international markets (Mcclain *et al.*, 2018). Soy-based products have become novel and attractive source of high-quality proteins with uniqueness of free from lactose and cholesterol (Ahsan *et al.*, 2015). That's why it is a plenteous and economical cradle of nourishment for vegetarians, lactose-intolerant persons, and patients who are milk-allergic and hypercholesterolemics (Jeske *et al.*, 2017). Considering the compositional profile, soybean is a crucial source of edible protein and oil comprises about 36-40% protein that varies in different varieties, 20% oil contents, 15% soluble carbohydrates (raffinose, succrose and stachyose) and 15% insoluble carbohydrates (Leamy *et al.*, 2017). It contains phenolics including phenolic acids, flavonoids and isoflavonoids with putative health benefits making it a momentous functional food (Zaheer and Akhtar, 2017). Soybean also comprises many micronutrients and phytochemical *i.e.* isoflavones, phytate, saponins, phytosterol, vitamins and minerals. The six isoflavone in soybean are daidzin, genistin, glycitin, daidzein, genistein, and glycitein and three major groups of isoflavones found in soybeans were genistein, daidzein, and glycitein (Li *et al.*, 2018). Given the enormous agricultural importance of seed

composition in soybean, it is not surprising that nutritional profile of soybean is also significant to control the prevalence of malnutrition among the individuals belong to developing and developed countries. Pakistan is among those developing countries which has highest prevalence rate of child malnutrition. According to the national nutrition survey, 33% children were underweight, nearly 44% were stunted, 15% wasted and 33% were suffering from iron deficiency (Asim and Nawaz, 2018). Keeping in view this alarming situation, it is essential to meet the mandate of nutritional status in these suffered communities and provide alternative supplies of protein. In this regard, legume seeds are of great interest for researchers and scientific community. All these strategies are appealing to the consumers because people are more concerned about the nutritional attributes of product to reduce the prevalence of life style related ailments (Schmidt, 2016). Health claims of soybean seed and oil are based on physicochemical characteristics but they are not taken into deliberation in present era. Based on therapeutic significance of soybean, food industries are mainly focusing to attain products with exceptional functional, nutritional and sensory potential along with significant market value. These significant reasons have increased consumer demand for nutritious and wholesome food which can help to improve their health and protect them from various diseases (Singh *et al.*, 2018). Nature has blessed Pakistan with different seasons and has excellent growing conditions including soil, water,

fertilizer, etc. Soybean cultivation has been practicing in some regions of Pakistan especially Punjab province and acclimatized successfully to environmental conditions with 1.42-1.62 tons per acre yield. There is no research work done so far on functional and physiochemical characterization of Pakistani grown soybean for its nutritional as well compositional parameters. Current research has been carried out to augment and provide sufficient information about soybean acclimatized to Pakistani environment. Selection of the most appropriate variety for soybean processing industries through screening is one of the most important decisions that growers and processors must make in consideration.

## MATERIALS AND METHODS

Soybean varieties named NARC-II, Willium-82, Ajmeri and Rawal-I procured from National Agriculture Research Centre (NARC), Islamabad and Faisal from Ayub Agriculture Research Institute (AARI), Faisalabad. The chemicals and reagents used for analyses were acquired from Sigma Aldrich (USA), Oxoid (UK) and Merck (Germany).

**Compositional analyses:** The chemical composition of all soy flour samples was investigated for moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extract according to their particular method as described in (AOAC, 2016).

**Mineral profiling of soybean varieties:** Mineral profile of soybean for iron, zinc, magnesium and manganese was determined by using Atomic Absorption Spectrophotometer (AA240, Varian) whereas calcium, sodium and potassium was quantified using flame photo meter (Sherwood Scientific Ltd., Cambridge, Model 410) by following method of wet digestion as described in (AOAC, 2016).

**Fatty acids profile of soybean oil:** Oil was extracted from soybean flour by using hexane as a solvent in Soxhlet apparatus and afterwards oil was purified in rotary evaporator. Then Fatty Acid Methyl Esters (FAME) were prepared as; the samples of oil were merged in n-hexane (5 mL) by using vortex mixer. After that 250  $\mu$ L sodium methoxide was added and again vortex for 90 seconds. Then 5 mL of saturated NaCl was added and vortex for 15 seconds and let it to be stand for 10 min. Then hexane layer was used for analysis through gas chromatograph (GC) (Agilent 6890) by using flame ionizing detector (FID), fused silica capillary column at an oven temperature of 90°C for 7 min then raised to 240°C at a rate 5°C/min and then kept at this temperature for 15 min. However, the injector and detector temperature were 260°C. The flow rate of carrier nitrogen gas was 1.51 mL/min and the split ratio was 1/50  $\mu$ L/min (Ozcan and Juhaimi, 2014).

**Analysis of lipoxygenase (LOX) activity:** During this assay LOX activity was determined by UV-VIS spectrophotometer (AA240, Varian) of LOX-1 and LOX- 2 at 234nm while LOX-3 at 280 nm in soy flour by using substrate (linoleic acid). Soy flour samples were defatted so that fat in sample do not interfere during analysis. According to this method, 1g

defatted soy flour was extracted with 50 mL sodium phosphate buffer (0.2 M, pH 6.8) for 2 hours in orbital shaker at 25°C after that centrifuged at 15,000 rpm for 10 min. The supernatant was used to ascertain the lipoxygenase activities. Enzyme caused an increase in absorbance of 1.0/min. Samples were loaded on ELISA plates and immediately readings were noted at the interval of 0, 1, 2, 3, 4 and 5 minutes. Lipoxygenase in soybean samples starts the lipid oxidation of linoleic acid and produces H<sub>2</sub>O<sub>2</sub> that was recorded on time basis. and expressed as a change in absorbance per minute ( $\Delta A/\text{min}$ ) (Mandal *et al.*, 2014).

### Phytochemical screening test:

**Sample preparation:** Soy flour (5g) each sample was weighed in centrifuge tubes and extraction solvent acetone/water (50:50 v/v) was added in it and placed in orbital shaker: 300 rpm, 25°C for 3 hours. After that it was placed for 14 hours under dark environment. Later on, the extracts were centrifuged for 15 minutes at 3000 rpm. After filtration the retentate was re extracted by using the same assay and then both extracts were combined and preserved in the dark conditions at 4°C for further analysis of TPC, TFC, DPPH, FRAP and ABTS(Xu and Chang, 2007).

**Total phenolic content (TPC):** The phenolic compounds in the samples oxidized and reduce the Folin-Ciocalteu reagent in the solution and give the blue end color which was measured on UV-VIS spectrophotometer (AA240, Varian) at 765 nm by using gallic acid as a standard. TPC values of different samples were calculated based on a calibration curve of gallic acid and expressed as mg GAE/g (Xu *et al.*, 2015).

**Total flavonoid content (TFC):** A calorimetric method was conducted to assess total flavonoid contents on UV-VIS spectrophotometer (AA240, Varian) and catechin was used as an external standard (Xu *et al.*, 2015). The extracted sample and standard were mixed with 1250  $\mu$ L distilled water and 75  $\mu$ L solution of 5% NaNO<sub>3</sub>. After that it was left at room temperature for 6 min and 150  $\mu$ L of 10% AlCl<sub>3</sub>.6H<sub>2</sub>O was added. Later on, mixture was kept for another 5 min and 500  $\mu$ L 1 M NaOH and 275  $\mu$ L distilled water were added to the mixture. The mixture was measured immediately at 510 nm. The TFC values of different samples were calculated based on standard calibration curve, expressed as mg CAE/g (Xu *et al.*, 2015).

### Antioxidant analysis in soy flour samples:

**2,2-diphenyl-1-picrylhydrazyl DPPH free radical scavenging activity assay:** DPPH radical scavenging activity is estimation of non-enzymatic antioxidant activity. The stable and non-biological radicals were used in this assay for the estimation of total reducing capacity. Sample extracts/Trolox standard were mixed with DPPH solution. The absorbance was checked by UV-VIS spectrophotometer (AA240, Varian) at 517 nm and results were expressed as mgTE/g of samples (Prvulovic *et al.*, 2017).

**Ferric-reducing antioxidant power (FRAP) assay:** The antioxidant activity of sample was measured by measuring

change in absorbance at 595nm on UV-VIS spectrophotometer (AA240, Varian). The reagents 0.3 M acetate buffer at pH 3.6, 10mM 2,4,6-Tripyridyltriazine (TPTZ) solution and 20 mM ferric solution were used in this assay. The ferric reducing ability of the extracts was expressed as mgTE/g (Mandal *et al.*, 2014; Handa *et al.*, 2016).

**2,2-Azinobis (3-ethylene benzothiazoline 6-sulphonic acid (ABTS):** Sample (10 µL) and diluted ABTS (100 µL) solution were coated in 96 wells micro plates. The decrease in absorbance was recorded with interval of 10 sec on UV-VIS spectrophotometer (AA240, Varian) at 734 nm. The trolox solution was used to obtain calibration curve and results were expressed in mgTE/g (Handa *et al.*, 2016; Prvulovic, 2017).

**Statistical analysis:** Each parameter was repeated three times and subjected to statistical analysis. The Analysis of variance (ANOVA) under complete randomized design (CRD) was applied to ascertain level of significance  $p < 0.05$  in by using Statistix 8.1 software (Awan *et al.*, 2018).

## RESULTS

**Compositional analysis of soybean:** The physiochemical and compositional attributes of soybean depicted significant difference ( $p < 0.05$ ) among five soybean varieties as shown in Figure 1. The moisture contents for Faisal, NARC-II, Willium-82, Ajmeri and Rawal-I were  $9.15 \pm 0.43$ ,  $9.68 \pm 0.47$ ,  $9.95 \pm 0.48$ ,  $9.45 \pm 0.46$  and  $10.49 \pm 0.49\%$ , respectively. The results showed that maximum protein was recorded in Ajmeri ( $37.65 \pm 1.84\%$ ) followed by Willium-82 ( $35.76 \pm 1.68\%$ ), NARC-II ( $34.45 \pm 1.69\%$ ), Rawal-I ( $32.73 \pm 1.60\%$ ) and Faisal ( $30.61 \pm 1.44\%$ ). The results represented substantial variation

in crude fat contents as maximum crude fat was recorded in Ajmeri ( $22.20 \pm 1.02\%$ ) trailed by Willium-82 ( $21.3 \pm 0.94\%$ ), NARC-II ( $19.2 \pm 0.83\%$ ), Rawal-I ( $18.03 \pm 0.74\%$ ) and Faisal ( $16.27 \pm 0.67\%$ ). Crude fiber results showed that maximum fiber contents were in Rawal-I ( $17.67 \pm 0.87\%$ ) and minimum in Ajmeri ( $13.33 \pm 0.65\%$ ). In food industry, amount of ash or minerals is very important; the ash contents in Faisal were ( $4.00 \pm 0.19\%$ ), NARC-II ( $5.13 \pm 0.25\%$ ), Willium-82 ( $5.37 \pm 0.24\%$ ), Ajmeri ( $5.63 \pm 0.28\%$ ) and Rawal-I ( $4.56 \pm 0.22\%$ ). The nitrogen free extract was highest in Faisal as  $25.60 \pm 1.20\%$  and lowest in NARC-II as  $10.06 \pm 4.27\%$ .

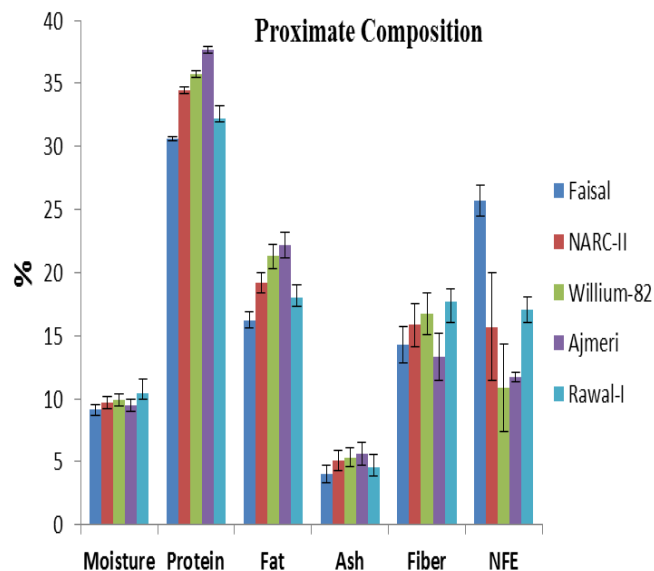


Figure 1. Proximate characteristics of soybean varieties.



Figure 2. A chromatogram of fatty acids peaks in Ajmeri through GC-MS.

**Table 1. Effect of soybean varieties on mineral contents (mg/100g).**

Varieties	K	Mg	Ca	Fe	Zn	Cu	Na	Mn
Faisal	1098.54±21.97 <sup>d</sup>	206.12±4.12 <sup>d</sup>	148.26±2.97 <sup>e</sup>	8.48±0.26 <sup>d</sup>	3.82±0.69 <sup>b</sup>	0.72±0.02 <sup>c</sup>	2.38±0.10 <sup>b</sup>	1.05±0.02 <sup>d</sup>
NARC- II	1894.37±37.89 <sup>a</sup>	328.27±6.57 <sup>b</sup>	202.43±4.05 <sup>c</sup>	16.94±0.52 <sup>b</sup>	4.64±0.81 <sup>b</sup>	1.44±0.04 <sup>c</sup>	3.69±0.15 <sup>b</sup>	1.66±0.23 <sup>b</sup>
William-82	1796.44±35.93 <sup>b</sup>	384.43±7.69 <sup>a</sup>	241.35±4.83 <sup>b</sup>	16.72±0.51 <sup>b</sup>	5.24±1.42 <sup>a</sup>	2.17±0.05 <sup>b</sup>	3.23±0.13 <sup>a</sup>	1.57±0.14 <sup>c</sup>
Ajmeri	1983.13±39.66 <sup>a</sup>	379.82±7.60 <sup>a</sup>	277.14±5.54 <sup>a</sup>	24.26±0.74 <sup>a</sup>	5.89±1.42 <sup>a</sup>	5.23±0.13 <sup>a</sup>	4.40±0.18 <sup>a</sup>	3.76±0.16 <sup>a</sup>
Rawal-I	1649.24±32.98 <sup>c</sup>	267.39±5.35 <sup>c</sup>	189.45±3.79 <sup>d</sup>	10.83±0.33 <sup>c</sup>	4.23±0.71 <sup>b</sup>	1.08±0.03 <sup>d</sup>	2.92 ±0.12 <sup>b</sup>	1.49±0.14 <sup>c</sup>

Results are expressed as mean ± standard deviation of means; n = 3 sets

**Table 2. Fatty acid (%) profiling of soybean varieties.**

Sr. No	Fatty Acids	Carbon Number	Faisal	NARC-II	William-82	Ajmeri	Rawal-I
1	Myristic	C14:0	ND <sup>D</sup>	ND <sup>D</sup>	0.08±0.06 <sup>C</sup>	0.098±0.01 <sup>B</sup>	0.12±0.03 <sup>A</sup>
2	Palmitic	C16:0	12.47±0.31 <sup>A</sup>	9.35±0.37 <sup>C</sup>	10.54±0.74 <sup>B</sup>	8.23±0.12 <sup>D</sup>	9.83±0.46 <sup>C</sup>
3	Stearic	C18:0	3.12±0.08 <sup>D</sup>	2.72±0.07 <sup>E</sup>	4.57±0.40 <sup>B</sup>	4.01±0.06 <sup>C</sup>	5.43±0.14 <sup>A</sup>
4	Arachdic	C20:0	0.36±0.01 <sup>A</sup>	0.12±0.03 <sup>E</sup>	0.16±0.02 <sup>D</sup>	0.264±0.04 <sup>C</sup>	0.34±0.09 <sup>B</sup>
5	Behenic	C22:0	ND <sup>D</sup>	0.20±0.05 <sup>B</sup>	0.23±0.16 <sup>A</sup>	0.18±0.03 <sup>BC</sup>	0.13±0.04 <sup>C</sup>
6	Oleic	C18:1	22.19±0.55 <sup>D</sup>	25.54±0.63 <sup>C</sup>	32.75±2.29 <sup>A</sup>	34.56±0.52 <sup>A</sup>	29.23±0.73 <sup>B</sup>
7	Linoleic	C18:2	53.67±1.34 <sup>AB</sup>	49.57±1.24 <sup>C</sup>	52.45±3.67 <sup>BC</sup>	56.34±0.84 <sup>A</sup>	49.25±1.23 <sup>C</sup>
8	Linolenic	C18:3	5.23±0.13 <sup>E</sup>	5.7±0.14 <sup>D</sup>	6.43±0.45 <sup>C</sup>	8.23±0.12 <sup>A</sup>	6.92±0.17 <sup>B</sup>
9	Erucic	C22:1	ND <sup>D</sup>	ND <sup>D</sup>	0.32±0.022 <sup>B</sup>	0.42±0.006 <sup>A</sup>	ND <sup>D</sup>

Results are expressed as mean ± standard deviation of means; n = 3 sets, <sup>abcd</sup> Different superscript in row vary significantly

**Minerals profiling:** The results showed that there was great variation ( $p < 0.05$ ) in varieties of soybean regarding the mineral contents expressed in Table 1 shows major and minor minerals in soybean. The highest value of potassium (K) was noted in Ajmeri as 1983.13±39.66 mg/100g, magnesium (Mg) was 379.82±7.60 mg/100g, calcium (Ca) was 277.14±5.54 mg/100g, iron (Fe) was 24.26±0.74 mg/100g, zinc (Zn) was 5.89±1.42 mg/100g, copper (Cu) was 5.23±0.13 mg/100g, sodium (Na) was 4.4±0.18 mg/100g and manganese was 3.76±0.16 mg/100g (Table 1) The results of all mineral elements that maximum mineral contents were found in Ajmeri trailed by William-82, NARC-II, Rawal-I and Faisal. The contents of minerals were as  $K > Mg > Ca > Fe > Zn > Cu > Na > Mn$ .

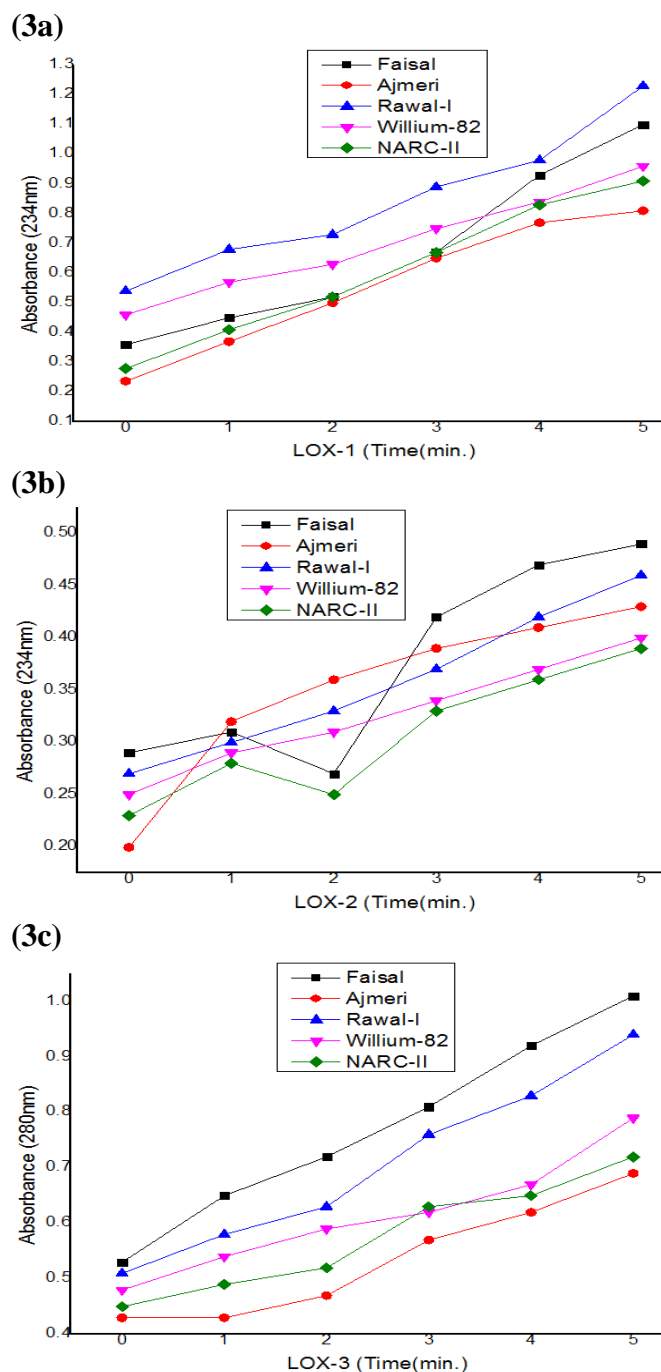
**Fatty acids profiling of soybean varieties:** The mean values of fatty acids detected in soybean oil are illustrated in Table 2 which showed that saturated fatty acids (SFAs) found were myristic, palmitic, stearic, behenic and arachdic. The mono unsaturated fatty acids were the oleic and erucic acids, while the poly unsaturated fatty acids determined were linoleic and linolenic fatty acids. The saturated fatty acids are considered as bad for health because they are related to synthesis of cholesterol in human body. The maximum concentration (0.12±0.03%) of myristic acid was noted in Rawal-I and minimum was in William-82 (0.08±0.06%) but not detected in NARC-II and Faisal. Palmitic acid was the 3<sup>rd</sup> dominant fatty acid in soybean oil. Its highest concentration (12.47±0.31%) was found in Faisal and lowest (8.23±0.12%) in Ajmeri. The stearic fatty acid was at the 5<sup>th</sup> number regarding its strength in in the soybean oil. The highest concentration of stearic acid was in Rawal-I as 5.43±0.14% and lowest was in NARC-II as

2.72±0.07%. Arachdic acid was higher in Faisal variety (0.36±0.01%) and minimum in NARC-II (0.12±0.03%). Behenic acid was higher in William-82 (0.23±0.16%) and minimum in Rawal-I (0.13±0.04%) and not detected in Faisal. The Oleic acid (monounsaturated fatty acid) is desirable for oil stability and is the 2<sup>nd</sup> major fatty acid of soybean oil. It was found highest in Ajmeri (34.56±0.52%) while the minimum level was noted in Faisal (22.19±0.55%) variety of soybean. The linoleic and linolenic are the two essential fatty acids, which are very important for the human health and must be obtain from diet. The quantity of linoleic was highest in soybean oil than other fatty acids, while linolenic was at the 4<sup>th</sup> position regarding the concentration of fatty acids in soybean oil. The maximum level of linoleic acid was in Ajmeri (56.34±0.84%) and minimum in Rawal-I as 49.25±1.23%. Likewise, linolenic acid was higher in Ajmeri as 8.23±0.12% and minimum in Faisal as 5.23±0.13%. Erucic acid is monounsaturated omega-9 fatty acid was higher in Ajmeri as 0.42±0.006% and was not detected in Rawal-I, Faisal and NARC-II.

#### **Lipoxygenase (LOX) activity:**

**LOX-I:** The statistical analysis of the results regarding the LOX activities shows the significant difference ( $p < 0.01$ ) in LOX activities among varieties like Faisal, NARC-II, William-82, Ajmeri and Rawal-I. The LOX-1 activity of five varieties is presented in Figure 3a. It is apparent from the graphical presentation that as the time increased the mean values of absorbance also increased in all the varieties which showed the lipoxygenase 1 activity of the varieties. It is also noted that minimum absorbance was recorded at 0 min in

Ajmeri as  $0.24 \pm 0.006$  and maximum at the same time was recorded in Faisal as  $0.36 \pm 0.01$ .



**Figure 3a–c. Comparison of inhibition potential of lipoxygenase. a) LOX-1 activity registered at 234 nm at pH 9.0; b) LOX-2 activity registered at 234 nm at pH 6.8; c) LOX-3 activity registered at 280 nm at pH 7.1.**

The highest value was recorded in Rawal-I as  $1.23 \pm 0.018$  and minimum value was noted in Ajmeri as  $0.87 \pm 0.013$  at 5 min. The Figure 3a elaborated the conventional shape of kinetic curves of all the varieties. The graph showed changes in curve from conventional ‘rectangular hyperbola’ to ‘plateau’ shaped with increasing time. Among the varieties Ajmeri showed minimum absorption as compared to other varieties which means it contains less LOX 1 and low rate of hydro peroxidation (Fig. 3a) followed by the NARC-II, Faisal, Willium-82 and Rawal-I.

**LOX-2:** The effect of varieties on LOX-2 is highly significant ( $p < 0.01$ ). It is mentioned in Figure 3b that at 0 min the minimum value was recorded in Ajmeri ( $0.20 \pm 0.0031$ ) and maximum in Faisal ( $0.29 \pm 0.0043$ ). However, as time goes and hydroperoxidation increased the values of absorbance also increased and maximum was ( $0.49 \pm 0.0073$ ) in Faisal and minimum in NARC-II ( $0.39 \pm 0.0058$ ) at 5<sup>th</sup> min.

The graphical presentation of LOX-2 showed a highest variation by showing nonconventional behavior that was slightly bursting at start and then shifted towards lag phase and at last it becomes plateau (Figure 3b). Overall, the maximum activity of LOX-2 was recorded in Faisal (0.38) followed by Rawal-I (0.36), Ajmeri (0.35), Willium-82 (0.33) and NARC-II (0.31).

**LOX-3:** The kinetic curve of conventional shape was attained for LOX-3 activity in all varieties of soybean and among all isozymes it was least inhibiting. It works best at pH 7.0 and causes less hydroperoxidation but produces ketodiene. The Figure 3c is depicting the results for LOX-3 and the results are in correspondence to other lipoxygenase activities because as time span increases the hydroperoxidation also increased from 0 to 5 min. The values were varied in the range of  $0.40 \pm 0.0062$  (Ajmeri) to  $0.53 \pm 0.0079$  (Faisal) at zero min and  $0.69 \pm 0.01$  (Ajmeri) to  $1.0 \pm 0.02$  (Faisal) at 5 min. Overall, the lowest activity of LOX-3 was noted in Ajmeri, followed by the NARC-II, Willium-82, Rawal-I and Faisal.

The overall comparison of inhibition potential of lipoxygenase is illustrated from Figure 4. The figure is depicting that LOX-1 showed minimum inhibition potential followed by LOX-3 and LOX-2.

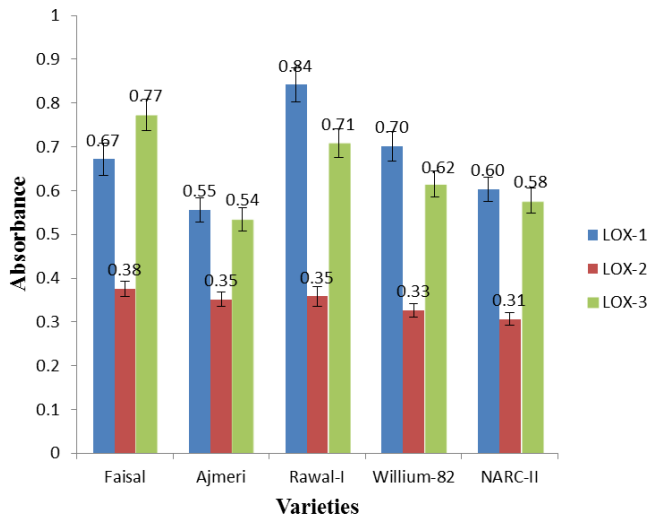
**Phytochemical screening:** It was demonstrated that maximum phenolic contents (TPC) and flavonoids contents (TFC) were recorded in Ajmeri as 2.76 mgGAE/g and 1.78 mgCE/g followed by the Willium-82, NARC-II, Rawal-I and Faisal (Table 3). Soybean varieties were also investigated for antioxidants activity including DPPH, FRAP and ABTS assay (Table 3). The results showed that DPPH values were generally found to be higher in Ajmeri ( $5.65 \pm 0.23$  mgTE/g) followed by Willium-82 ( $5.32 \pm 0.21$  mgTE/g), Rawal-I ( $4.56 \pm 0.18$  mg TE g), NARC-II ( $4.02 \pm 0.16$  mgTE/g) and Faisal ( $3.68 \pm 0.14$  mg TE/g). The mean values of FRAP activity given in Table 3 showed that the trend among the soybean was the same as for the antioxidant potential DPPH except for Rawal-I, which showed the higher potential of

**Table 3. Effect of soybean varieties on TPC, TFC, DPPH, FRAP and ABTS**

Varieties	TPC (mg GAE/g)	TFC (mg CE/g)	DPPH (mgTE/g)	FRAP (mg TE/g)	ABTS (mg TE/g)
Faisal	1.21±0.01 <sup>d</sup>	0.42±0.02 <sup>c</sup>	3.68±0.147 <sup>c</sup>	9.63±0.67 <sup>c</sup>	20.78±1.45 <sup>c</sup>
NARC- II	2.59 ±0.04 <sup>b</sup>	1.12±0.03 <sup>c</sup>	4.02±0.161 <sup>c</sup>	11.23±0.78 <sup>bc</sup>	26.54±1.86 <sup>ab</sup>
William-82	2.65±0.03 <sup>b</sup>	1.64±0.04 <sup>b</sup>	5.32± 0.213 <sup>a</sup>	12.41±0.86 <sup>ab</sup>	27.23±1.90 <sup>ab</sup>
Ajmeri	2.76±0.05 <sup>a</sup>	1.78±0.05 <sup>a</sup>	5.65±0.226 <sup>a</sup>	13.48±0.94 <sup>a</sup>	29.56±2.06 <sup>a</sup>
Rawal-I	1.52±0.02 <sup>c</sup>	0.58±0.02 <sup>d</sup>	4.56±0.182 <sup>b</sup>	11.34±0.79 <sup>abc</sup>	23.36±1.63 <sup>bc</sup>

Results are expressed as mean ± standard deviation of means; n = 3 sets; <sup>abcde</sup> Means in column with different superscripts differ ( $p < 0.01$ )

FRAP (11.34±0.79 mg TE/g) than NARC-II (11.23±0.78 mg TE/g). In the present exploration, the recorded values for ABTS were maximum in Ajmeri (29.56±2.06 mg TE/g) followed by William-82 (27.23±1.90 mg TE/g), NARC- II (26.54±1.86 mg TE/g), Rawal-I (23.36±1.63 mg TE/g) and Faisal (20.78±1.45 mg TE/g). However, there was non-substantial effect only between NARC-II and William-82, while Ajmeri contained the highest ABTS activity.



**Figure 4. Overall comparison of inhibition potential of lipoxygenase.**

Soybean has shown to possess high antioxidant properties and all other parameters that strengthen the hypothesis that different varieties behave differently in terms of nutritional aspects. The results obtained suggested that the widespread use of the soybean varieties mentioned could be beneficial for human health.

**DISCUSSION**

To accomplish recent research five samples of soybean varieties were taken from renowned research agriculture institutes of Pakistan. The goal of utilizing samples of soybean from Pakistan was to examine the hypothesis that is these varieties having strong nutritional potential and if they have then is there any significant difference among them. The

work herein is examination of quantitative measures of soybean seed and oil. The variation in moisture contents among each other may be due to the difference in the agronomic growing conditions of the lines, the level of moisture at the time of harvest and the processing conditions. However, in previous studies the variation in moisture contents were reported as 8.4-10.2% (Anwar *et al.*, 2016), 8.24-9.68% (de Barros *et al.*, 2014) and 9.19% (Lee *et al.*, 2013) moisture in soybean which was very close to Faisal variety as 9.15% in present finding. Nature has blessed soybean as a complete and impressive plant protein source. The crude protein contents of soybean were reported in between 41.67 to 45.64% (Anwar *et al.*, 2016). Likewise, Abubakar *et al.* (2014) studied the physicochemical properties of soybean seed and estimated maximum crude protein contents in the range of 44.81 to 47.46% which is higher as compared to present study. However, de Barros *et al.* (2014) identified protein in between 35.10 to 38.82% and Lee *et al.* (2013) has reported 34.19% of protein in soybean. The results of present finding for crude fat were supported by various scientists as 15.85-19.49%, 19.63-24.00% and 17.0-21.0% as declared by (Anwar *et al.*, 2016; de Barros *et al.*, 2014 and Sharma *et al.*, 2014), respectively. The fibrous food is considered as functional foods due to their therapeutically effect to cure certain ailments including cancer and heart diseases (Ferreira *et al.*, 2015). The Shurtleff and Aoyagi (2016) reported total dietary fiber content of soybean varied in between 10.64-19.50% and de Barros *et al.*, (2014) reported 16.01 to 17.68%. The findings of present research are in harmony with Anwar *et al.* (2016) who has reported ash contents in soybean varied from 5.50 ± 0.36 to 6.90±0.26%. Similarly, Lee *et al.* (2013) has reported ash in soybean was 5.24%. Nitrogen free extract consist of carbohydrates (starch and sugars). Researchers has reported nitrogen free extract in green soybean was 38.6% (Shurtleff and Aoyagi, 2016) and in another study (de Barros *et al.*, 2014) variation were in the range of 16.41 to 22.65%. The significant difference in minerals could be due to the difference in soil chemistry and agronomic practices as reported by the Porter and Jones (2003). This could be also due to rotation and continuous soybean cropping in the same field which could affect the seed composition (Chen *et al.*, 2015). Afterward, Jiao *et al.* (2012) studied the composition of different varieties of soybean grown in US and Argentina and reported that

potassium concentration varied from 1522.25±40.00 to 1663.54±24.45 mg/100g, Ca varied in between 126.26±7.1 to 228.05±8.40 mg/100g and Zn element varied from 2.21±0.08 to 3.88±0.24 mg/100g. Later on, Ozcan and Juhaimi (2014) worked on soybean composition and they also reported the highest level of potassium than other minerals that varied at the level between 1637.5 mg/100g to 2035.7 mg/100g. The results of present investigation are also in line with Costa *et al.* (2015) who reported 1.98 mg/100g manganese, 8.84 mg/100g iron, 1.91 mg/100g copper and zinc contents were 5.32 mg/100g. Overall the quantitative variation in composition can be due to geographical, environmental influence including biotic and abiotic factors and genetics of the soybean varieties (Mcclain *et al.*, 2018).

Soybean oil is a major source of the essential fatty acids (linoleic, and linolenic) for humans. A lack of the daily requirements of these fatty acids can lead to serious health problems like diminished growth, skin depigmentation, fatty livers, kidney degeneration and the loss of muscle tone. The comparative proportions of these fatty acids are of major concern that actually determine the overall quality of the oil, low proportion of saturated fatty acid and higher of unsaturated is considered as optimal for cardiovascular health (Leamy *et al.*, 2017). The findings of Jiao *et al.* (2012) are associated with present findings. They have studied on soybean oil from different varieties and reported oleic acid 27%, linoleic acid 53%, palmitic acid 11% and linolenic acid 6%, which were the major fatty acids of soybean oil relative to their contents. Afterwards, Ozcan and Juhaimi (2014) reported a wide range of fatty acids in soybean oil *i.e.* oleic acid contents varied between 19.07 and 35.31%, linoleic from 42.17 and 54.76% while linolenic acid varied from 5.09 to 6.48% and stearic acid from 0.16 to 4.48%. The results of current finding are also in harmony with the results of Chen *et al.* (2015) who studied the influence of soybean corn rotation on the fatty acid profile of soybean oil. They found that oleic acid varied from 28.50 to 20.0%, stearic acid 3.47 to 3.43%, palmitic acid 11.12 to 11.40%, linoleic acid 61.24 to 61.18% and linolenic acid 5.27 to 5.03% with cropping rotation. They stated that variation in fatty acids composition due to cropping rotation could be due to maintenance of optimum nutrient concentrations in soil.

Defatted flour is used in LOX analyses so that it might be favorable in reduction of rancidity. The rancidity in soybean oil is a major issue in oil industries and lipoxygenase acts as a good biocatalyst of cis poly unsaturated fatty acids and causes off flavor production. Normal mature soybean seed have isozymes of lipoxygenase named as LOX-1, LOX-2, and LOX-3 Chedea *et al.* (2008). The process involved is the reaction between LOX isozymes that require substrates containing polyunsaturated fatty acids, comprising linoleic and linolenic acid which undergoes hydro peroxidation, thus generating physiological reactive species, including LOO<sup>·</sup>, LO<sup>·</sup>, HO<sup>·</sup> and O<sub>2</sub>. However, the antioxidants in soybean help

to reduce the rate of oxidation. The polyunsaturated fatty acids contribute to beany or grassy flavor in soy foods. The major flavoring component in soybean is n-hexanal, produced from peroxidation of linoleic acid by lipoxygenase (LOX), which decompose and form hydroperoxide (de Barros *et al.*, 2014). LOX promotes the destruction of free and esterified linoleic and linolenic acids and inhibition of lipoxygenase particularly LOX-2 along with antioxidants can be helpful to solve the issue of off-flavor in oil.

Phenolic acids have been identified as embodying 28 to 72% of total phenolic contents in soybean (Chung *et al.*, 2008). Phenolic are predominantly acclaimed for their anti-inflammatory, anti-oxidant and anti-carcinogenic exertions (Duenas *et al.*, 2012). The recent findings are in corroboration with study of Josipovic *et al.* (2016) who documented TPC and TFC of different soybean genotypes and find out variation from 2.12 to 3.23 mg GAE / g and TFC were varied in between 0.43 to 0.66 mg CAE /g of dry weight. Likewise, Malencic *et al.* (2008) has studied polyphenol in the seeds of 20 soybean hybrids and the results were in the range of 189.1±12.9 to 384.0±43.7 mg catechin /100g and the results for TFC were in the range of 27.3±1.6 to 88.7±3.3 mg rutin/100g. Later, Malencic *et al.* (2012) worked on colored soybean seeds from central Europe and reported TPC in yellow soybean were 2.68±0.47 mg GAE/g of dry material and TFC were 0.48±0.02 mg of rutin/g of dry material. The findings of researchers proved that soybean is an excellent source of phenolic and flavonoids components that can protect from various diseases.

Intake of antioxidants is helpful in reducing different degenerative disorders. It is always recommended to use more than one method to find out the anti-oxidative potential which depends on which, when and where reactive oxygen species is released (Georgetti *et al.*, 2006). The findings are in agreement with Prvulovic *et al.* (2017) who computed DPPH in five different soybean cultivars and the results of their findings varied in between 3.76±0.03 to 6.37±0.03 (mg TE/g) and FRAP values were 10.79±0.36 to 12.85±0.65 mg TE/g while ABTS was 20.73±2.25 to 31.17±2.18 mg TE/g. FRAP test showed that soybean seeds have a significant reduction potential and all samples expressed different activity. In another research Chung *et al.* (2011) reported DPPH in the yellow soybean extracts (0.6-2.0 mmoles TE/g). Likewise, Handa *et al.* (2016) has investigated phenolics and antioxidant activities from fermented soy flour DPPH values varied in between 4.00-10.08 (µmol TE/g) and FRAP was 0.48 to 17.18 (µmol TE/g) and ABTS was 0-134.18 (µmol TE/g).

**Conclusion:** soybean an anonymous protein source can be exploited as potential tool for combating malnutrition in masses of Pakistan and prodigious food entity with reference to food security. It has also prospective worth as functional food to address various life style nutritional needs of Pakistan



community. The varieties tested exhibited wide variability in nutritional components, phenolic contents and a great free radical scavenging activity. Out of five tested varieties Ajmeri, Willium-82 and NARC-II were found to maintain maximum seed quality attributes while Rawal-I and Faisal were found to be deprived. The nutritional quality-oriented attributes in this study were competent as an index of their nutritional worth and can be recommended to particularly those communities of Pakistan who are deprived of economic power to fulfill their nutritional needs particularly for their malnourished children, farmers and consumers which may be graded as export quality soybean with good and exceptional nutritional values in international market. However, soybean is expected to expand gradually in new areas to increase its production and oil exports.

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