

## ENHANCEMENT OF FUNCTIONAL PROPERTIES AND BIOLOGICAL ACTIVITY IN BARLEY AND WHEAT GRAINS BY GERMINATION

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### Abstract

In recent years, sprouted grains have become very popular and widely accepted as a functional food because of their nutritious and health benefits. So, this study was performed to evaluate the phytochemical composition, and *in vitro* antioxidant capacity, reducing power, antihyperglycemic and anti-inflammatory activities, of seven-day old cereal sprouts (CS): Cultivars, barley NS565 (BSNS), barley Golozrni (BSG), wheat Spelta (WSSPE), wheat Simonida (WSSIM). Phenolic compounds were the most dominant bioactives in all CS. BSNS expressed significantly higher ( $p \leq 0.05$ ) content of total phenols, chlorophyll and carotenoids. The total flavonoids content (TFC) in CS showed that barley sprouts (BSNS, BSG) had the higher value ( $P \leq 0.05$ ) than wheat sprouts (WSSPE, WSSIM) respectively. The freeze-dried sprouts powders (FDSP) extracts were screened for possible antioxidant capacities using DPPH, ABTS, and reducing power (Rp) assays. The results indicated that the BSNS possessed higher antioxidant capacities in DPPH and ABTS assays, and reducing power ( $IC_{50}^{DPPH} = 0.54$  mg/ml;  $IC_{50}^{ABTS} = 0.79$  mg/ml;  $IC_{0.5}^{RP} = 9.35$  mg/ml) respectively. The inhibitory effect of FDSP extracts on  $\alpha$ -glucosidase activity was investigated. The BSNS extracts exhibited higher inhibitory activity ( $IC_{50}^{AHgA} = 1.43$  mg/ml) against  $\alpha$ -glucosidase ( $p \leq 0.05$ ). The anti-inflammatory activity (Denaturation of protein *in vitro*) showed significantly different between the FDSP, and Diclofenac sodium (DS). The  $IC_{50}^{AIA}$  of DS and BSNS was 0.79 and 1.86 (mg/ml) respectively. There was a strong positive correlation between TPC and antioxidant activities and reducing power, and also between TFC and anti-inflammatory activity.

**Keywords:** Cereal Sprouts, Phytochemicals, Free Radicals, Bioactivities.

### Introduction

A number of epidemiological studies have shown that regular consumption of whole grains reduces risks of various types of chronic diseases, such as cardiovascular disease (Anderson *et al.*, 2000), type 2 diabetes (Liu *et al.*, 2000), some cancers (Kasum *et al.*, 2002) and reduced mortality (Jacobs *et al.*, 2001). Seed germination is a primary step to generate a new plant. In this process triggered by the imbibitions of water, the plant embryo resumes growth after a period of quiescence (Barrôco *et al.*, 2005). During germination the amount of anti-nutritive compounds e.g., trypsin inhibitor, phytic acid and tannins decreased and after the germination also compounds with health-maintaining

effects and phytochemical properties (glucosinolates, natural antioxidants) could be detected that can have a considerable role among others also in the prevention of some diseases (Marton *et al.*, 2010). Thus, germination can lead to the development of such functional foods that have a positive effect on the human organism and that help in maintaining the health (Sangronis & Machado, 2007). As sprouts are consumed during the beginning of the growing phase, their nutrient concentration remains very high (Donkor *et al.*, 2012; Pajak *et al.*, 2014). Recently, the attention of experts dealing with the healthy nutrition turned more and more towards the determination of the biological value of the nutritional sprouts (Penas *et al.*, 2008). Currently, the consumption of the germinated seeds is increasing, and became common, which can be observed especially in Europe, as the sprouts meet the requirements of the modern nutrition where the consumers are not only interested in food of a high nutritive value but also in food with functional properties, i.e. with a high supply of antioxidant substances. It has been reported that barley leaves possess beneficial properties, such as the antioxidant, hypolipidemic, antidepressant, and antidiabetic effects (Kamiyama & Shibamoto, 2012). This diverse range of health benefits is probably due to the wide range of secondary metabolites contained within barley. Ilona *et al.* (2011) found that the content of total phenolic compounds of barley varieties increased after steeping, and germination compared with whole grains. Seo *et al.* (2013) reported that the concentration of Policosanols (PCs) increased with the growth stage of barley sprouts until ten days post-sprouting, and then decreased. PCs are well-known to lower blood cholesterol and prevent low-density lipoprotein (LDL) oxidation and platelet aggregation (Singh *et al.*, 2006; Viola *et al.*, 2008). Considering that there are few reports on phytochemical composition and *in vitro* bioactivity of cereal sprouts, especially barley and wheat. The purpose of this work was to investigate these properties of cereal sprouts (CS), hybrid and nonhybrid varieties.

## Materials and Methods

### Chemicals

Trolox, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazine (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), pepsin, pancreatin,  $\alpha$ -glucosidase, 4-nitrophenyl  $\alpha$ -D-glucopyranoside, diclofenac sodium, and standard phenolic compounds were purchased from Sigma-Aldrich Co. (St. Louis, USA). All other chemicals and solvents were of the highest commercial grade and obtained from Lach-Ner (Brno, Czech Republic).

### Plant Material and Sprouting Conditions

The two varieties of barley were: 'NS565' (*Hordeum vulgare* L.ssp. *distichum*) and non-hybrid 'Golozrni' (*Hordeum vulgare* var. *nudum*) and two varieties of wheat were: wheat Spelt (*Triticum aestivum* subsp. *Spelta*) and Simonida (*Triticum aestivum* L. ssp. *vulgare* var. *lutescens*) as shown in figure (1), were kindly donated by the Institute of Field and Vegetable Crops (NS seme), Novi Sad, Serbia. Sprouting was carried out according to the method described by Vale *et al.* (2014) with slight modifications. Clean seeds were

sanitized with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution (5%, v/v) for 5 minutes and then soaked in water for 6 h in the dark, drained and washed with distilled water. The sprouts were harvested after seven days of germination, and then frozen at -80 °C and freeze-dried (Alpha 2-4 LSC Martin Christ, Osterode, Germany). The varieties sprouts were ground by using the commercial mill (Moulinex, France), and then passed through a sieve of 0.5 mm and the obtained freeze-dried sprouts powders (FDSP), were packed in vacuumed plastic bags and stored at -20 °C until further analysis.



Wheat Simonida

Wheat Spelta

Barley NS565

Barley Golozrni

**Figure (1): The varieties of the investigated cereal**

### ***Extraction of FDSP***

A portion of 10g of each FDSP was extracted with 100ml of methanol (70%, v/v) in an ultrasonic bath for 20 min, followed by agitation using a laboratory shaker at 200 rpm (Unimax 1010, Heidolph Instruments GmbH, Kelheim, Germany) under light protection for 2 h at room temperature. Then, the extracts were filtered using Whatman paper No. 1, and the obtained extracts were stored at 4°C pending further analysis.

### ***Analytical Procedures***

All spectrophotometrical measurements were carried out using UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan), except  $\alpha$ -glucosidase assay which was executed using Multiskan GO microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA). For HPLC analysis a Shimadzu Prominence chromatographic system was used, which

consisted of LC-20AT binary pump, CTO-20A thermostat and SIL-20A autosampler connected to the SPD-20AV UV/Vis detector (Shimadzu, Kyoto, Japan).

### ***Determination of Phytochemicals Content in FDSP***

#### ***Total Phenolic Content (TPC)***

Total phenolic content (TPC) was estimated using the method adopted by Singleton *et al.* (1999). The absorbance of the resulting coloured solution was measured at 750 nm against distilled water as control. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/100 g FDSP dry weight.

#### ***Total Flavonoids Content (TFC)***

Total flavonoids content (TFC) was measured using a colourimetric assay developed by Zhishen *et al.* (1999). The absorbance was measured at 510 nm against distilled water as control. TFC content was expressed as rutin equivalents (RE) in mg/100 g FDSP dry weight.

#### ***Total Chlorophyll (TChl), Chlorophyll a (Chl a) and Chlorophyll b (Chl b) Contents***

The total chlorophyll (TChl), chlorophyll a (Chl *a*) and chlorophyll b (Chl *b*) contents were assayed spectrophotometrically according to Lichtenthaler (1987). Briefly, absorbances of FDSP acetone extracts were read at 645 and 663 nm and the chlorophyll contents were calculated according to the following formulas:

$$\text{TChl } (\mu\text{g/ml}) = 20.20 \times A_{645} + 8.02 \times A_{663}$$

$$\text{Chl } a \text{ } (\mu\text{g/ml}) = 11.24 \times A_{663} - 2.04 \times A_{645}$$

$$\text{Chl } b \text{ } (\mu\text{g/ml}) = 20.13 \times A_{645} - 4.19 \times A_{663}$$

where  $A_{645}$  and  $A_{663}$  are the absorbances of FDSP acetone extracts at 645 and 663 nm, respectively. Results were expressed as mg of chlorophyll per 100 g FDSP dry weight.

#### ***Total Carotenoids Content (TCX)***

The total carotenoids in FDSP acetone extracts, containing carotenes and xanthophylls (TCX) in addition to chlorophylls, read at 470 nm (the carotenoids region) were determined spectrophotometrically according to the method devised by Lichtenthaler (1987). The total carotenoids were calculated according to the following formula:

$$\text{TCX } (\mu\text{g/ml}) = [(1000 \times A_{470} - 1.90 \times \text{Chl } a - 63.14 \times \text{Chl } b) / 214]$$

where  $A_{470}$  is the absorbance of FDSP acetone extract at 470 nm. Results were expressed as mg of carotenoids per 100 g FDSP dry weight.

### **HPLC Analysis of Phenolic Compounds**

All analyte solutions and solvents were filtered prior to analysis through 0.45  $\mu\text{m}$  (pore size) membrane filters (Millipore, Bedford, MA). Chromatograms were recorded using different wavelengths for individual phenolic compounds: 280 nm for hydroxybenzoic acids (gallic, protocatechuic, vanillic and syringic acid), catechins (catechin, epicatechin and epicatechin gallate), and ellagic acid, 320 nm for hydroxycinnamic acids (caffeic, chlorogenic, coumaric, ferulic, isoferulic, synapic and rosmarinic acid), and 360 nm for flavonoids (quercetin, isorhamnetin, rutin, luteolin, myricetin and kaempferol). Separation was performed on a Luna C-18 RP column, 5  $\mu\text{m}$ , 250 x 4.6 mm (Phenomenex, Torrance, CA, USA) with a C18 guard column, 4 x 30 mm (Phenomenex, Torrance, CA, USA). The data acquisitions were carried out by the LC Solution Software (Shimadzu, Kyoto, Japan).

### **Determination of FDSP Bioactivity**

#### **Antioxidant Capacity by DPPH Assay ( $AC_{DPPH}$ )**

The antioxidant capacity of FDSP extracts was assessed by the evaluation of the free radical scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, according to the method proposed by Brand-Williams *et al.* (1995). The absorbance was read at 517 nm using methanol as the blank. The ability to scavenge DPPH radicals, i.e.  $AC_{DPPH}$ , was calculated following the formula:

$$AC_{DPPH} (\%) = [(A_C - A_S) / A_C] \times 100$$

where  $A_C$  is the absorbance of the control, and  $A_S$  is the absorbance of the sample. The  $IC_{50}^{DPPH}$  values were calculated as inhibitory concentration (mg/ml) of the extract necessary to decrease the initial DPPH $\bullet$  absorbance by 50%. The results were also expressed as mg or mmol Trolox equivalent (TE) per 100 g FDSP dry weight (DW).

#### **Antioxidant Capacity by ABTS Assay ( $AC_{ABTS}$ )**

This method is based on the antioxidant capacity of the sample to scavenge the ABTS $\bullet^+$  in a hydrophilic medium. The ability of FDSP extracts to scavenge ABTS $\bullet^+$ , i.e.  $AC_{ABTS}$ , was evaluated employing the modified method according to Šaponjac, *et al.* (2014) by measuring the variation in absorbance at 414 nm after 35 min. The antioxidant capacity ( $AC_{ABTS}$ ) of sample was calculated using the following equation:

$$AC_{ABTS} (\%) = [(A_C - A_S) / A_C] \times 100$$

where  $A_C$  and  $A_S$  are absorbance's of the control and the sample, respectively. The results were computed as the inhibitory concentration of the extract (mg/ml) necessary to decrease the initial ABTS $\bullet^+$  absorbance by 50% ( $IC_{50}^{ABTS}$ ). The results were also expressed as mg or mmol Trolox equivalent (TE) per 100 g FDSP DW.

### **Reducing Power Assay (RP)**

The RP was determined by the method adapted from Oyaizu (1986), by measuring the reduction of the  $\text{Fe}^{3+}$ /ferricyanide complexes to the ferrous ( $\text{Fe}^{2+}$ ) form. The capacity of the extracts to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by recording the absorbance at 700 nm. The sample concentration providing 0.5 of absorbance ( $\text{RP}_{0.5}$ ) was calculated from the graph of absorbance at 700 nm against extract concentration (mg/ml).  $\text{RP}_{0.5}$  value was also expressed as mg or mmol Trolox equivalent (TE) per 100 g FDSPDW.

### **Anti-hyperglycemic Activity (AHgA) by $\alpha$ -glucosidase Assay**

$\alpha$ -Glucosidase inhibitory potential was used to examine anti-hyperglycemic activity of FDSP extracts using method reported by Šaponjac, *et al.*, (2014). The absorbance of 4-nitrophenol released from 4-nitrophenyl  $\alpha$ -D-glucopyranoside was measured at 405 nm. The sample concentration providing 50% inhibition of  $\alpha$ -glucosidase enzyme activity ( $\text{IC}_{50}^{\text{AHgA}}$ ) was calculated from the graph of AHgA (%) against extract concentration (mg/ml).

### **Anti-inflammatory Activity (AIA) by Protein Denaturation Assay**

*In vitro* assessment of anti-inflammatory activity of FDSP extracts was determined by protein denaturation bioassay, using egg albumin (from fresh hen's egg), according to the method adopted by Ullah *et al.* (2014). The absorbance was measured at 660 nm by using a vehicle as blank, and diclofenac sodium as a drug reference. Anti-inflammatory property was calculated according to the following equation:

$$\% \text{ Inhibition} = [(A_c - A_s) / A_c] \times 100$$

Where  $A_c$  and  $A_s$  are absorbances of control and sample, respectively. The results were computed as the inhibitory concentration of the extract (mg/ml) necessary to inhibit 50% of protein denaturation ( $\text{IC}_{50}^{\text{AIA}}$ ).

### **Statistical Analysis**

Data were reported as mean  $\pm$  standard deviation of three independent experiments. Data were analyzed by one-way analysis of variance (ANOVA). The level of significance ( $\alpha$ -value) was 95 % in all cases ( $P \leq 0.05$ ) and more than two comparisons were separated by Tukey test.  $\text{IC}_{50}$  values were calculated by using the best-fit regression model. All the data were analyzed by using Microsoft Office Excel 2007 software.

## **Results and Discussion**

### **Total Phenolic Content (TPC)**

Phenolic compounds are produced in plants as secondary metabolites via the shikimic acid pathway. Phenylalanine ammonialyase is the key enzyme catalyzing the biosynthesis of

phenolics from the aromatic amino acid phenylalanine. The germination process implies a series of active and complex biochemical and physiological reactions, resulting in extensive changes in composition and/or morphology (Lin *et al.*, 2006; Chiou *et al.*, 1997). In the current study, the results for TPC of investigated sprouts were summarized in Table 1. It can be seen that the highest TPC is present in the BSNS ( $P \leq 0.05$ ) following by, WSSIM and BSG respectively. The lowest TPC was registered in WSSPE. It was estimated that the TPC in BSNS was 1.91-fold higher than that of WSSPE. The TPC of buckwheat sprouts (670 mg GAE/100 g DW) reported by Alvarez-Jubete *et al.* (2010), was found to be similar to that of BSNS while the values for wheat sprouts (110 mg GAE/ 100 g DW) were found to be lower than WSSIM, and WSSPE in the current study. Pajak *et al.* (2014) investigated TPC in five-day old sprouts of mung bean, sunflower, broccoli, and radish. It was noticed that the TPC of all sprouts in this study was higher than in mung bean (360 mg GAE/ 100g DW). BSNS was similar with broccoli sprouts (750 mg GAE/ 100 g DW), whereas that all the sprouts in this study were found to be lower than broccoli sprouts. Tian *et al.* (2010) reported that the total phenol content was increased in 6-day old germinated oats comparable with seeds, and raised up to (95 mg GAE/ 100 g. DW) it was found lower than all cereal sprouts in this study.

**Table (1): Total phenolic content (TPC) and total flavonoids content (TFC) for freeze-dried sprouts (FDSP).**

FDS	TPC	TFC
BSG	479.02 ± 11.70	285.05 ± 10.55
BSNS	713.25 ± 26.86	288.29 ± 23.27
WSSPE	373.37 ± 19.62	216.52 ± 3.25
WSSIM	607.21 ± 55.32	195.46 ± 5.21

Results are presented as mean ± SD (n = 3). where TPC in mg GAE/100 g DW. and TFC in mg RE/100 g DW.

The increment or decrement in TPC may depend on the genus, species, cultivar/genotype, harvesting time, and germination conditions.

#### **Total Flavonoids Content (TFC)**

Flavonoids are present in almost all plants and are known to possess anti-carcinogenic, anti-inflammatory and anti-allergic properties. Cereals have only small quantities of flavonoids, except that barley contains measurable amounts of catechin and some di- and tri-procyanidins (McMurrough & Baert, 1994). Alvarez-Jubete *et al.* (2010) observed that the flavone and flavonol glycoside contents of buckwheat were increased during sprouting. The TFC of FDSP in the current study is presented in Table 1. The results show that the BSNS and BSG had the higher value respectively, and there was no significant difference in TFC between those sprouts ( $P \leq 0.05$ ) following by WSSPE, and WSSIM respectively. There is no significant difference was found between WSSPE and WSSIM ( $P \leq$

0.05). Through these results it was observed that the TFC participated in irregular percentage of TPC it has the following order: BSG had the higher percentage and participated at about 59 % of TPC > WSSPE 58 % > BSNS 40 %, whereas in WSSIM participated at about 32 %. Generally, cereals have only small quantities of flavonoids, except barley, that contains measurable amounts of flavonoids such as catechin and some Di- and Tri-procyanidins. De- Nicola *et al.* (2013) determined TFC in seven-day old sprouts of (*Brassica oleracea L. spp*). These authors found that the TFC ranged from 560 to 970 mg catechin equivalents/100 g of sprouts. Similarly to TPC, it can be proposed that this increment or decrement depends on the genus, species, cultivar/genotype, harvesting time, and germination conditions.

### HPLC Analysis of Phenolic Compounds (PC)

Phenolic acids occur in plants in different forms, such as aglycones (free phenolic acids), esters, glycosides, and/or bound complexes (Ross *et al.*, 2009). In this study, FDSP samples were subjected to HPLC analysis to determine the profile of the most abundant bioactive compounds phenolics.

According to the results presented in Table 2. It can be seen that epicatechin is the dominant phenolic compound in both barley sprouts (BS). Besides epicatechin, both BS contain significant amounts of catechin, protocatechuic acid and gallic acid. Unlike BSNS, BSG contains *p*-hydroxybenzoic acid and notably higher amounts of ferulic acid. In agreement with spectrophotometrically results presented also in Table 1, HPLC analysis confirmed that BSNS contained significantly higher ( $P \leq 0.05$ ) total amount of phenolics than BSG.

**Table (2): Phenolic acids and flavonoids content of investigated cereal sprouts (mg/100g DW) determined by HPLC.**

PC	BSNS	BSG	WSSIM	WSSPE
Catechin	163.93	97.63	-	-
Gallic acid	145.65	71.99	25.91	26.04
Protocatechinic acid	152.86	52.53	29.63	29.77
P-hydroxybenzoic acid	Nd	15.83	7.60	8.04
Vanillic acid	19.94	11.04	70.20	54.20
Caffeic acid	-	-	2.07	2.34
P- coumeric acid	-	-	6.48	4.97
Ferulic acid	14.57	96.30	2.49	2.21
Syringic acid	-	-	26.49	44.50
Sinapic acid	19.50	2.95	20.02	22.75
Rutin	-	-	5.19	7.05
Myricetin	2.59	9.98	1.13	7.05
Quercetin	0.67	9.75	0.77	2.95



<b>Kempherol</b>	3.29	1.10	-	-
<b>Luteolin</b>	-	-	-	-
<b>Apigenin</b>	-	-	-	-
<b>Isoramnethin</b>	-	-	-	-
<b>Chlorogenic acid</b>	20.32	Nd	-	-
<b>Epicatechin</b>	555.59	227.32	-	-
<b>Total</b>	1098.91	596.42	197.98	267.99

Nd: not detected

Also, these results confirmed that flavonoids have a significant share in total phenolics available in BSNS and BSG. In both of wheat sprouts (WS) where vanillic, protocatechinic, syringic, gallic and sinapic acids were the predominant compounds respectively. Unlike BS, WS does not contain any amount of epicatechin. It is worth mentioning that the WS contained very small amounts of flavonoids, compared with barley sprouts. These results are relatively corresponded to the results of TFC, which were registered in Table 1. In general, BSNS had the highest value ( $P \leq 0.05$ ) in phenolic acid and flavonoids followed by BSG, WSSPE, and WSSIM respectively. Carvalho *et al.* (2015) reported significant amounts of catechin in different varieties of barley. Yu *et al.* (2001) investigated phenolic acids in hot water extract, acid, amylase and cellulase hydrolysates of different barley varieties and found chlorogenic acid, protocatechuic and *p*-hydroxybenzoic acids to be dominant, respectively. Pajak *et al.* (2014) reported that the free phenolic acids and flavonoids content of mung bean, radish, broccoli and sun flower sprouts (seven day old) determined by HPLC were; gallic, protocatechuic, caffeic, *p*-coumeric, ferulic, chlorogenic and sinapic acid, whereas flavonoids were; quercetin, kaempferol, luteolin and apigenin. The results obtained by the abovementioned authors indicate that the highest value of total free phenolic acids was registered by broccoli sprouts (39.94 mg/ 100g DW), while the lowest value was found in mung bean sprouts (9.95 mg/ 100g DW). Sunflower and radish sprouts were contained (27.46, 20.32 mg/ 100g DW) of free phenolic acids respectively. According to the above results, it can be seen that the sprouts in the current study were significantly higher than those reported by the abovementioned authors. The differences between phenolic contents reported in various studies could result from multiple factors, such as methodology (procedure of extraction, different susceptibilities to degradation, type of chromatography and quantification), plant species, growth and storage environments (Naczek & Shahidi, 2004; Perez-Balibrea, *et al.*, 2011; Ross, *et al.*, 2009).

#### **Total Chlorophyll (TChl), Chlorophyll A (Chl a) and Chlorophyll B (Chl b)**

The results of chlorophyll content in FDSP (Table 3) showed that the highest value of TChl content was registered in BSNS ( $P \leq 0.05$ ) following by WSSPE, WSSIM and BSG respectively. There were significant differences between all those sprouts. BSNS possesses 2.80 -fold higher values for TChl comparable with BSG, which had the lowest value. De- Nicola *et al.* (2013) determined the TChl in seven-day old sprouts of broccoli, daikon, sango, and Tuscan black kale, all *Brassica oleracea L.* spp. Their TChl values

were in the range from 20 to 70 mg/100 g DW. The BSNS in the current study possessed 2.80-fold to 9.81-fold higher values in TChl than those reported by De- Nicola *et al.* (2013), while BSG, was found within the range reported by the same authors. Generally, cereal sprouts are rich in chlorophyll, comparable with *Brassica* sprouts.

**Table (3): Total chlorophyll (TChl), chlorophyll a (Chl a), chlorophyll b (Chl b) and total carotenoids content (TCX) for FDSP.**

FDSP	TChl	Chl a	Chl b	TCX
BSG	70.16 ± 0.43	47.27 ± 0.23	14.96 ± 0.18	15.13 ± 0.11
BSNS	196.23 ± 2.13	130.55 ± 1.62	43.53 ± 0.33	37.58 ± 0.36
WSSPE	131.32 ± 1.82	88.17 ± 1.45	28.31 ± 0.17	22.84 ± 1.05
WSSIM	111.69 ± 1.42	75.52 ± 0.97	23.55 ± 0.30	22.01 ± 0.06

Results are presented as mean ± SD (n = 3). Where the units in mg /100 g. DW

Chlorophyll a (Chl a) is summarized in Table 3. The quantity of Chl a in BSNS is the highest ( $P \leq 0.05$ ) followed by WSSPE, WSSIM, and BSG respectively. There were significant differences between all those sprouts. The lowest Chl a content is present in BSG ( $P \leq 0.05$ ). It was observed that the Chl a in all FDSP investigated participated at about 66 and 67 % of TChl content. Chlorophyll b (Chl b) is also summarized in Table 3. The results of Chl b have shown significant difference, and it was indicated that BSNS possessed highest value in Chl b ( $P \leq 0.05$ ) comparable with other investigated sprouts flowing by WSSPE, WSSIM and BSG respectively. There is significant difference between all those sprouts. The quantity of Chl b represented about 20 - 21 % of the TChl in all investigated FDSP varieties. The quantity of Chl a in all investigated FDSP possesses about 3-fold higher than that of Chl b. As far as chlorophyll is concerned it is worth noting that this isoprenoid plant lipid may be both pro-oxidant, in the presence of light and antioxidant in the dark. Endo *et al.* (1985) reported that Chl a showed the highest antioxidant capacity with regard to Chl b, and their derived compounds. The weight ratio of Chl a, and Chl b (Chl a/b ratio) is an indicator of the functional pigment equipment and light adaptation of the photosynthetic apparatus (Lichtenthaler, 1987).

#### **Total Carotenoids Content (TCX)**

Total carotenoids included carotenes and xanthophylls (TCX) are yellow, orange and red lipid-soluble pigments that occur widely in plants, fruits and vegetables. Several of them are antioxidant nutrients that act mainly as secondary antioxidants in foods by quenching singlet oxygen. They may also prevent oxidation by trapping free radicals in the absence of singlet oxygen. The TCX of BSNS and BSG are presented in Table 3. BSNS had the highest value ( $P \leq 0.05$ ) in TCX flowing by WSSPE, WSSIM and BSG. There is no significant difference was observed between WSSPE and WSSIM. BSG had the lowest values in TCX. BSNS had 2.48, 1.71 and 1.65 -fold higher content of TCX than that of BSG, WSSIM and WSSPE respectively. In general, barley sprouts contain considerable quantities of carotenoids compared to *Brassica* vegetables. Podsedek (2007) reported that

the amount of carotenes in Brussels sprouts was in the range from 0.26 mg/100 g in white cabbage to 6.1 mg/100 g in broccoli sprouts, whereas the amount of xanthophylls was moderately high 0.78 - 3.50 mg/100 g in broccoli and Brussels sprouts. Our investigation indicated that barley, wheat sprouts in this study showed higher values of TCX than broccoli and cabbage sprouts. It is worth mentioning that the contribution of TCX content and TChl content in this study was compatible in all sprouts varieties.

### Antioxidant Capacity

#### DPPH Assay

In order to test the bioactivity potential of FDSP in this study antioxidant capacity using DPPH assay was investigated. *In vitro* assay based on DPPH<sup>•</sup> was used to determine the antioxidant capacity (AC) of FDSP extracts. This assay has been widely used to determine the free radical-scavenging capacity of various plants.

It was observed that the % of inhibition in DPPH assays of FDSP extracts were concentration related and increased through the increase in FDSP samples concentration, which depends primarily on the amount of polyphenols that plays as a key role of antioxidant. AC<sup>DPPH</sup> of FDSP was investigated. BSNS showed significantly higher ( $P \leq 0.05$ ) AC<sup>DPPH</sup> % 90.19 % when the concentration was 3.33 mg/ml compared to other varieties in all concentrations. Scavenging effect on the DPPH radical decreased to the order: BSNS > BSG > WSSIM, whereas WSSPE showed the lowest ( $P \leq 0.05$ ). The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Baumann *et al.*, 1979). IC<sub>50</sub>, defined as the necessary concentration at which the radicals generated by the reaction systems were scavenged by 50%, could be served as an indicator of radical-scavenging activity. The IC<sub>50</sub> value expresses the amount of FDSP extract necessary to decrease the absorbance of DPPH by 50% (Antolovich *et al.*, 2002). The value can be determined graphically by plotting the % DPPH inhibition against the concentration of FDSP extract than using the regression equation to calculate IC<sub>50</sub>. The higher IC<sub>50</sub> value corresponds to a lower scavenging activity on DPPH radicals. The IC<sub>50</sub> values of FDSP in all DPPH assays was calculated and listed in Table 4. The IC<sub>50</sub> values by the DPPH method were in the range of (0.54 ± 0.04 - 5.67 ± 0.75 mg/ml). The lowest IC<sub>50</sub><sup>DPPH</sup> was observed in BSNS and the highest IC<sub>50</sub><sup>DPPH</sup> in WSSPE ( $P \leq 0.05$ ) FDSP which had the lower IC<sub>50</sub><sup>DPPH</sup>, had the higher antioxidant capacity. As can be seen in Table 4. there is a high significant difference between FDSP sprouts ( $P \leq 0.05$ ), whereas no significant difference was observed between WSSEP and WSSIM. Shen *et al.* (2016) reported lower IC<sub>50</sub><sup>DPPH</sup> value of black highland barley (0.19 mg/ml) than in this study for FDSP.

**Table (4): The IC<sub>50</sub> values of FDSP in DPPH assay and other AC parameters.**

FDSP	IC <sub>50</sub> <sup>DPPH</sup> (mg/ml)	1/IC <sub>50</sub> <sup>DPPH</sup> ARP (ml/mg)	TEAC (IC <sub>50</sub> <sup>TEAC</sup> / IC <sub>50</sub> <sup>Sample</sup> )*10 <sup>5</sup>	mg TE/100 g DW.
BSNS	0.54 ± 0.04 <sup>a</sup>	1.88 ± 0.16 <sup>a</sup>	525.08 ± 44.16 <sup>a</sup>	600.77 ± 50.53 <sup>a</sup>

<b>BSG</b>	0.73 ± 0.02 <sup>b</sup>	1.36 ± 0.03 <sup>b</sup>	381.65 ± 8.22 <sup>b</sup>	426.14 ± 9.17 <sup>b</sup>
<b>WSSPE</b>	5.67 ± 0.75 <sup>d</sup>	0.18 ± 0.02 <sup>d</sup>	50.00 ± 6.84 <sup>d</sup>	59.67 ± 8.16 <sup>d</sup>
<b>WSSIM</b>	4.69 ± 0.53 <sup>d</sup>	0.22 ± 0.03 <sup>d</sup>	60.24 ± 7.12 <sup>d</sup>	66.62 ± 7.88 <sup>d</sup>

Results are presented as mean ± SD (n = 3). The same letters in columns represent statistically not significant difference ( $P \leq 0.05$ ).

The increment of  $AC^{DPPH}$  is probably due to the higher TPC in black highland barley (17177 mg GAE/100 g) than in FDSP. The possible reason for this, besides the origin and variety of the sample, could be the different extraction method used for black highland barley (alkaline hydrolysis). Anti-radical power (ARP) was the inverse of  $IC_{50}$  value, the larger the ARP the more efficient the antioxidant is. The highest ARP values were exhibited by BSNS and BSG (1.88, 1.36 ml/mg) respectively, following by WSSIM, and WSSPE respectively. WSSPE had the lowest ARP value (0.18 ml/mg). The antioxidant capacity of the sample was then expressed as Trolox equivalent antioxidant capacity values (TEAC) using the formula  $TEAC = (IC_{50}^{Trolox}/IC_{50}^{Sample}) \times 10^5$ , as previously outlined by Hagen *et al.* (2007).

BSNS showed significantly higher ( $P \leq 0.05$ )  $TEAC^{DPPH}$  compared with other FDSP varieties. WSSPE was the lowest ( $P \leq 0.05$ )  $TEAC^{DPPH}$ . In these assays TE values of BSNS were 1.40, 9.02 and 10.06-fold higher compared to BSG >WSSIM and WSSPE respectively. Alvarez-Jubete *et al.* (2010) investigated the antioxidant capacity of amaranth, quinoa, buckwheat and wheat sprouts. They reported that buckwheat and wheat sprouts possessed 666 and 73.7 mg TE/ 100g DW, respectively. Our results indicated that BSNS levels were similar to buckwheat sprouts, while BSG and other FDSP levels have been found to be lower than buckwheat sprouts. Both WSSIM and WSSPE in the current study found to be particularly similar to wheat investigated by Alvarez-Jubete *et al.* (2010). BSNS and BSG in the current study registered higher AC compared with wheat sprouts reported by Alvarez-Jubete *et al.* (2010). Pajak *et al.* (2014) studied the AC, DPPH assay of mung bean, radish, broccoli, and sunflower sprouts where data ranged from 141 in mung bean to 607 mg TE/100 g DW in radish sprouts. Pajak *et al.* (2014) also observed that the sunflower sprouts demonstrated the highest  $AC^{DPPH}$  (1147 mg TE/ 100 g DW), while broccoli sprouts registered the lowest  $AC^{DPPH}$  (365 mg TE/ 100 g DW). According to those results,  $AC^{DPPH}$  for BSNS and BSG were higher than mung bean and broccoli, and lower than sunflower, whereas BSNS was similar to radish sprouts.

### **ABTS Assay**

The antioxidant activity of the FDSP extracts was further monitored by the  $ABTS^{+\bullet}$  method, based upon the capacity of antioxidant compounds to scavenge radicals in  $ABTS^{+\bullet}$  solution in a hydrophilic medium. The ability of FDSP extracts to scavenge  $ABTS^{+\bullet}$ , i.e.  $AC^{ABTS}$ , was evaluated employing the modified method according to Šaponjac *et al.* (2014). Similar to DPPH, ABTS assays also has been widely used to determine the free radical-scavenging capacity of various plants.

The % of inhibition in ABTS assays of FDSP extracts were concentration related and increased through the increase in FDSP. The results of ABTS radical scavenging activities showed that BSNS exhibited the greatest free radical scavenging activity (77%) at concentration (1.25 mg/ml) in %  $AC^{ABTS}$  flowing by WSSIM, BSG and WSSPE, which registered (60, 58, and 50 %) respectively.

There is no significant difference was observed between BSG and WSSIM. WSSPE had the lowest values in %  $AC^{ABTS}$  at ( $P \leq 0.05$ ) Table 5. Regarding the  $IC_{50}$  values, all the FDSP extracts were depleted the initial ABTS concentration by 50%. The lower of  $IC_{50}$  value is the higher of free radical scavenging activity of a sample. The free radical scavenging activities of all extracts of FDSP started with lower value of  $IC_{50}$  were in this order: BSNS < WSSIM < BSG < WSSPE (Table 5).

Regarding to the results of ARP (Table 5), the larger the ARP the more efficient the antioxidant is. The highest ARP values were exhibited by BSNS and WSSIM (1.27, 1.00 ml/mg) respectively, following by BSG and WSSPE respectively. All the results are in agreement with %  $AC^{ABTS}$ . The antioxidant capacity of the sample was then expressed as Trolox equivalent antioxidant capacity values (TEAC) using the formula  $TEAC = (IC_{50}^{Trolox}/IC_{50}^{Sample}) \times 10^5$ , as previously outlined by Hagen *et al.* (2007).

**Table (5): The  $IC_{50}$  values of FDSP in ABTS assay and other AC parameters.**

FDSP	$IC_{50}^{ABTS}$ (mg/ml)	$1/IC_{50}^{ABTS} \text{ARP}$ (ml/mg)	TEAC ( $IC_{50}^{TEAC} / IC_{50}^{Sample}$ ) * $10^5$
BSNS	$0.79 \pm 0.02^a$	$1.27 \pm 0.03^a$	$241.83 \pm 5.24^a$
BSG	$1.04 \pm 0.03^b$	$0.96 \pm 0.03^b$	$183.28 \pm 5.24^b$
WSSPE	$1.21 \pm 0.03^d$	$0.82 \pm 0.02^d$	$156.60 \pm 3.29^d$
WSSIM	$1.00 \pm 0.01^b$	$1.00 \pm 0.01^b$	$189.81 \pm 1.33^b$

Results are presented as mean  $\pm$  SD (n = 3). The same letters in columns represent statistically not significant difference ( $P \leq 0.05$ ).

As shown in Table 5. BSNS showed significantly higher ( $P \leq 0.05$ )  $TEAC_{ABTS}$  compared to other FDSP varieties. WSSPE was the lowest ( $P \leq 0.05$ )  $TEAC_{ABTS}$ . In these assays  $TEAC_{ABTS}$  values of BSNS were 1.27, 1.32, and 1.54-fold higher compared to WSSIM > BSG and WSSPE respectively.

### **Reducing Power (Rp) Assay**

Reducing power is associated with antioxidant activity and may serve as a significant reflection upon the antioxidant activity (Oktay *et al.*, 2003). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Chanda *et al.*, 2009). The reducing power of all the FDSP extracts increased by an increase in concentration. BSNS exhibited the highest. Based on the results presented in Table 6. and regarding the  $IC_{0.5}$  values the lower of  $IC_{0.5}$  value is the higher of free radical scavenging activity of a sample. The free radical scavenging activities of all extracts of

FDSP started with lower value of  $IC_{0.5}$  were in this order: BSNS < WSSIM < BSG and WSSPE respectively (Table 6). A greater difference ( $P \leq 0.05$ ) was observed between BSNS, which had the lowest  $IC_{0.5}$  (9.35) and WSSPE, which had the highest  $IC_{0.5}$  25.83. Generally, there is significant difference between all FDSP.

**Table (6): The  $IC_{0.5}$  values of FDSP in reducing power (RP) assay and other RP parameters.**

FDSP	$IC_{0.5}^{Rp}$ (mg/ml)	RP 1/ $IC_{0.5}^{Rp}$ (ml/mg)	$TE_{Rp}$ ( $IC_{0.5}^{TE_{Rp}} / IC_{0.5}^{Sample}$ )* $10^5$
BSNS	9.35 ± 0.06 <sup>a</sup>	0.11 ± 0.00 <sup>a</sup>	764.86 ± 4.97 <sup>a</sup>
BSG	19.72 ± 1.08 <sup>b</sup>	0.05 ± 0.00 <sup>b</sup>	363.29 ± 19.34 <sup>b</sup>
WSSPE	25.83 ± 0.17 <sup>d</sup>	0.04 ± 0.00 <sup>d</sup>	276.78 ± 1.79 <sup>d</sup>
WSSIM	17.58 ± 0.49 <sup>c</sup>	0.06 ± 0.00 <sup>c</sup>	406.87 ± 11.25 <sup>c</sup>

Results are presented as mean ± SD (n = 3). The same letters in columns represent statistically not significant difference ( $P \leq 0.05$ ).

The result of the FDSP was then expressed as Trolox equivalent antioxidant capacity values ( $TE_{Rp}$ ) using the formula  $TE_{Rp} = (IC_{0.5}^{Trolox} / IC_{0.5}^{Sample}) \times 10^5$ , as previously outlined by Hagen *et al.* (2007). As shown in Table 6 BSNS showed significantly higher ( $P \leq 0.05$ )  $TE_{Rp}$  compared with other FDSP varieties. WSSPE was the lowest ( $P \leq 0.05$ )  $TE_{Rp}$ . In these assays TE values of BSNS were, 1.88, 2.11, and 2.76-fold higher compared to WSSIM > BSG > WSSPE respectively.

Alvarez-Jubete *et al.* (2010) investigated the antioxidant capacity of amaranth, quinoa, buckwheat and wheat sprouts. They reported that buckwheat and wheat sprouts possessed 739 and 210 mg TE/100 g DW when Rp assay was used. Our results indicated that BSNS levels were higher than buckwheat and wheat sprouts, whereas all the other FDSP in the current study had been found to be lower than buckwheat, but higher than wheat.

Pajak *et al.* (2014) studied the Rp of mung bean, radish, broccoli, and sunflower sprouts. This author observed that the sunflower sprouts demonstrated the highest value (11.05 mmol /100 g DW) in Rp assay and 1.20 in mung bean sprouts, while broccoli and radish sprouts consisted (8.64, 10.49) respectively. According to the results mentioned above comparable with our results, it was clear that all FDSP showed higher Rp than mung bean, lower than sunflower, radish, and broccoli sprouts. Based on the results of AC and Rp, we concluded that the effect increases with increasing scavenger concentration of polyphenols in the extract which leads to suggest that the antioxidant effect of FDSP extracted is related to the amount of polyphenols are present. This hypothesis is demonstrated by several researchers such as (Jayaprakash *et al.*, 2007; Hodzic *et al.*, 2009). The antioxidant effect of an extract may also differ depending upon the quality of polyphenols such as flavonoids are present that have shown an antioxidant activity (Wang & Mazza, 2002). The mechanism of the reaction between the antioxidant and ABTS, DPPH and Rp depend on the structural conformation of the antioxidant (Kouri *et al.*, 2007). Some compounds react rapidly with the ABTS, and DPPH reducing the number of ABTS, DPPH equal to that of the hydroxyl groups of the antioxidant.

### ***Anti-Inflammatory Activity (AIA) of FDSP***

Inflammation is a pathophysiological response to injury, infection or destruction characterised by heat, redness, pain, swelling and disturbed functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body's response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair.

It is triggered by the release of chemical mediators from injured tissue and migrating cells. The most commonly used drug for management of inflammatory conditions is non-steroidal anti-inflammatory drugs. There are certain problems associated with use of animals in experimental pharmacological research such as ethical issues and the lack of a rationale behind their use when other suitable methods are available, or could be investigated. Hence, in the present study, the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of hydroalcoholic (methanol) extract.

In this study, anti-denaturation of egg albumin method was chosen to evaluate anti-inflammatory activity (AIA) of FDSP. In anti-denaturation assay the denaturation of egg albumin is induced by heat treatment. Diclofenac sodium was used as positive control. It was already proved that conventional non-steroidal anti-inflammatory drugs (NSAID's) like phenylbutazone and indomethazine do not act only by the inhibition of endogenous prostaglandins production by blocking cyclooxygenase (COX) enzyme but also by prevention of denaturation of proteins (Ullahet *al.*, 2014). Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo* (Umopathy *et al.*, 2010). The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by FDSP throughout the concentration range of (2.5, 5, 10 and 20 mg/ml) there is no effect was observed against protein (albumin) denaturation at low concentration (0.25, 1.25), whereas Diclofenac sodium as the reference drug had a positive effect against protein denaturation (at the concentration range of (0.25, 1.25, 2.5, 5, 10 and 20 mg/ml); however, the effect of diclofenac sodium was found to be higher as compared with that of FDSP. At low concentration started with 2.5 up to 20 mg/ml BSNS exhibited a higher inhibition against protein denaturation ( $P \leq 0.05$ ), whereas WSSIM showed a similar behavior, followed by SWSPE which showed lower inhibition against protein denaturation. This was further confirmed by comparing their  $IC_{50}$  values. The AIA of FDSP, calculated in terms of  $IC_{50}^{AIA}$  values, was reported in Table 7. BSNS was found to be significantly more efficient ( $P \leq 0.05$ ) against protein denaturation than other FDSP varieties. The  $IC_{50}^{AIA}$  values ranged from  $1.43 \pm 0.07$  in BSNS to  $3.70 \pm 0.38$  in WSSPE, which had the highest  $IC_{50}$ . However, all FDSP showed lower AIA than diclofenac sodium ( $IC_{50}^{AIA} = 0.79$  mg/ml).

**Table (7): Anti-inflammatory activity calculated as  $IC_{50}^{AIA}$  value and Anti-hyperglycaemic activity (AHgA) calculated as  $IC_{50}^{AHgA}$  for FDSP.**

FDSP	$IC_{50}^{AIA}$ (mg/ml)	AHgA $IC_{50}$ (mg/ml)
BSG	1.86 ± 0.20	4.40 ± 0.55
BSNS	1.43 ± 0.07	1.97 ± 0.13
WSSPE	3.70 ± 0.38	14.61 ± 1.35
WSSIM	2.71 ± 0.03	17.28 ± 0.82
DS	0.79 ± 0.00	-

Data shown represent mean ± standard deviation (n = 3).

Ullah *et al.* (2014) concluded that ethanolic extract of the *Curcuma zedoaria* rhizome possessed marked AIA (300 µg/ml express 53.04% inhibition), using the same method, while for acetyl salicylic acid, the reported AIA was even higher (100 µg/ml shows 50.56% inhibition). All FDSP samples showed much lower AIA values than these literature findings. Reports for AIA of cereals or cereal sprouts, based on protein denaturation assay, were not found in literature. Chandra *et al.* (2012b) concluded that flower of *Mikania scandens* possessed marked anti-inflammatory effect against the denaturation of protein *in vitro*; it possessed  $IC_{50}$  0.007 mg/ml.

Chandra *et al.* (2012a) reported that dried ripe seeds of (*Coffea arabica* Linn. Family: Rubiaceae) has  $IC_{50}$  value 0.004 mg/ml and DS were found to be 0.63 mg/ml; it found to be similar with the  $IC_{50}$  of DS 0.79 mg/ml in the current study. According to the results computed as  $IC_{50}$  value, FDSP varieties showed lower AIA than seeds of *Coffea arabica*, and a flower of *Mikania scandens* which are used for some medicinal purposes at the Indian subcontinent.

#### **Anti-Hyperglycemic Activity (AHgA)**

Control of hyperglycaemia is essential treatment strategy for Enzyme inhibitors like  $\alpha$ -Glucosidase and  $\alpha$ -amylase inhibitors play a role in the management of post-prandial hyperglycaemia for diabetics (Tundis *et al.*, 2010). The inhibition of  $\alpha$ -Glucosidase is considered to be an effective strategy for the control of diabetes by diminishing the absorption of glucose (Hara & Honda, 1990).

The inhibitors of  $\alpha$  glucosidase are also of interest due to their promising therapeutic potential against diseases such as HIV infection, metastatic cancer and lysosomal storage diseases (Jabeen *et al.*, 2013). Polyphenolic rich extracts are known to have  $\alpha$ -Glucosidase inhibition potential ( $\alpha$ -GIP).  $\alpha$ -GIP is the useful assay to estimate a potential anti-hyperglycaemic effect of sprouts (Donkor *et al.*, 2012). Among of studies have already shown that intake of plant material rich in polyphenols can cause anti-hyperglycaemic effects in animals and in humans, possibly via  $\alpha$ -glucosidase and/or  $\alpha$ -amylase inhibition (Hogan *et al.*, 2010). The potential of FDSP cultivars tested for  $\alpha$ -Glucosidase inhibition.  $\alpha$ -GIP in the current study was tested in the concentration range 0.90 - 18.18 mg/ml. In all FDSP varieties, general trend showed that the inhibition of  $\alpha$ -glucosidase increased when



the concentration of FDSP increased. BSNS expressed significantly higher AHgA ( $P \leq 0.05$ ) than other FDSP followed by BSG. On the other hand, WSSIM and WSSPE showed low inhibitory effects. Based on  $IC_{50}^{AHgA}$  values (Table 7), it can be seen that the  $IC_{50}^{AHgA}$  values ranged from  $1.97 \pm 0.13$  in BSNS, which had the lowest  $IC_{50}$  to  $17.28 \pm 0.82$  in WSSIM, which had the highest  $IC_{50}$ . Regarding to the inverse of  $IC_{50}$  values ( $1/IC_{50}$ ), it can be concluded that BSNS had the highest activity of AHgA, while no significant difference was found between WSSIM, BSG and WSSPE ( $P \leq 0.05$ ). Donkor *et al.* (2012) reported that higher inhibitory  $\alpha$ -glucosidase activity was observed in germinated sorghum (20% AHgA), and germinated rye (12% AHgA). Germinated barley showed higher inhibitory  $\alpha$ -glucosidase activity, compared to oat and brown rice, while it was similar with wheat and buckwheat. Šaponjac *et al.* (2014) investigated the effect of phytochemicals in the blackberry and Raspberries press residues on  $\alpha$ -Glucosidase inhibition potential, those authors found that the  $IC_{50}^{AHgA}$  values were (0.078, 0.097) in blackberries ('Thornfree', 'Čačanska bestna') respectively, and (1.825, 0.198) in Raspberries ('Willamette', 'Meeker') respectively.

#### **Relationship (correlation) Among Phytochemicals and Biological Activities in FDSP**

For relationship (correlation),  $IC_{50}^{DPPH}$ ,  $IC_{50}^{ABTS}$  and  $IC_{0.5}^{RP}$  values were transformed into their reciprocal values,  $1/IC_{50}^{DPPH}$ ,  $1/IC_{50}^{ABTS}$  and  $1/IC_{0.5}^{RP}$ . Reciprocal values are more representative of the presented activities because they follow the increasing trend of the sample efficiencies in the tested assays. These values were investigated about the correlation with the amount of TPC, TFC, TChl, Chl *a*, Chl *b*, and TCX quantified in FDSP. In order to evaluate the relationship between those phytochemicals and antioxidant capacities and reducing power expressed by the different assays performed, a Pearson's correlation coefficient ( $r$ ) was analysed, and some strong significant correlations were achieved ( $0.8 \leq r < 1$ ). The results of correlation analysis are presented in Table 8. It has been proposed that TPC has strong correlation with antioxidant effect measured by ABTS radical scavenging activity (0.949), also strong correlation can be seen between TPC and reducing power ( $r = 0.876$ ). Whereas DPPH radicals scavenging activity showed moderate positive correlation ( $r = 0.561$ ) with TPC. These results suggest that phenolic compounds are good predictors of *in vitro* antioxidant activity. On the other hand, DPPH showed a strong correlation ( $r = 0.851$ ) with TFC. Moderate positive correlation was found among ABTS and TFC, while low Pearson's correlation was found between Rp and TFC ( $r = 0.461$ ). Vale *et al.*, (2014) reported that penca cabbage and red cabbage sprouts showed a maximum DPPH scavenging activity after 7 days of germination. These authors observed that DPPH scavenging activity was strong related in penca cabbage sprouts to the TPC ( $r = 0.987$ ), and with TFC ( $r = 0.889$ ). It should be noted that our results are in accordance with the reported by Aires *et al.*, (2011) who found moderate positive correlation between TPC and DPPH ( $r = 0.640$ ) in brassica vegetables (*Brassica oleracea L.* and *Brassica rapa L.*). As shown in Table 8. There is no correlation was seen between TChl, Chl *a*, Chl *b*, TCX and ABTS scavenging activity, also between those phytochemicals and reducing power, whereas moderate correlations were found between phytochemicals mentioned above, and DPPH ( $r$ ) ranged from 0.502 in TChl to 0.570 in TCX.

**Table (8): Pearson's correlation coefficient (r) between TPC, TFC, TChl, Chl a, Chl b, TCX and the inverse of IC<sub>50</sub> values for DPPH, ATBS, RP, AIA, and AHgA for FDSP.**

1/IC <sub>50</sub>	TPC	TFC	TChl	Chl a	Chl b	TCX
1/ IC <sub>50</sub> DPPH	0.561	0.815	0.502	0.520	0.522	0.570
1/ IC <sub>50</sub> ABTS	0.949	0.514	0.000	0.000	0.000	0.144
1/ IC <sub>50</sub> RP	0.876	0.461	0.000	0.000	0.000	0.011
1/IC <sub>50</sub> AIA	0.712	0.842	0.551	0.545	0.567	0.622
1/IC <sub>50</sub> AHgA	0.440	0.151	- 0.320	- 0.331	- 0.288	- 0.200

## Conclusion

The results of current study have shown those cereals sprouts can provide a high content of phytochemicals and considerable bioactivities. Moreover, data related to these cereals sprouts, here reported for the first time; show that they contain a unique pattern of bioactive molecules, which make these cereals, sprouts attractive functional foods for a health-promoting diet. To sum up, the potential beneficial effects of the consumption of cereals sprouts, especially BSNS may be particularly expressed by using these sprouts for the risk reduction of some diseases, in addition to their nutritional value that may use in food fortification.

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