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ARTICLE

Chemical, Antioxidant and Sensory Profiling of Vitamin K-rich Dietary Sources

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Abstract In present research, vitamin K-rich dietary food products were prepared from spinach & soybean and evaluated for nutritional factors, phylloquinone and menaquinone-7 (MK-7) contents and sensory attributes. For the purpose, two spinach-based products (fresh cooked spinach (FCS), reconstituted spinach (RS), and fermented soybean (natto A; NA and Natto B; NB) were formulated. Nutritional composition indicated that natto were rich in protein and fat compared to spinach-based products. They also contained appreciable amount of mineral i.e. potassium, magnesium, calcium zinc, sodium cupper, and iron. High performance liquid chromatography revealed that FCS contained 368.81±13.96 µg/100 g of phylloquinone followed by 270.07±9.45 µg/100 g in RS, whereas minimum value 26.90±0.94 µg/100 g was observed in NA. However, maximum MK-7 concentration was recorded in NA at 803.82±21.14 µg/100 g trailed by NB 681.35±16.85 µg/100 g. On the contrary, MK-7 was not detected in the spinach-based products. Antioxidant indices of products indicated that spinach-based products contained higher amount of the total phenolic content, DPPH free radical-scavenging activity, ferric reducing antioxidant power (FRAP), and antioxidant activity compared to natto. Sensory response of the products showed that spinach-based products attained higher scores than those of natto A and B. Conclusively, FCS and NA contained sufficient amount of phylloquinone and MK-7 along with antioxidant activity and has higher potential to modulate the coagulation and bone related abnormalities.

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Introduction

Vitamin K is fat-soluble, and abundantly present in green leafy vegetables, animals, and fermented products as phylloquinone (vitamin K_1) and menaquinones (vitamin K_2). Among these sources, spinach (*Spinacea oleraceae* L.) is recognized as a good reservoir of phylloquinone, the most common form of vitamin K occurring in green leaves (Yasin et al., 2013).

Fermented soybean (natto) is one of the richest sources of menaquinones among other plant- and animal-based food products (Schurgers et al., 2007). Menaquinone, rich natto, is prepared by the action of bacteria (*Bacillus subtilus*). They are responsible for the converting raw soybean into natto that is a sticky substance mainly due to glutamic residues (Kwaka et al., 2007). Menaquinones are also synthesized by the action of intestinal microflora *B, vulgates*, *B. fragilis*, and *E. coli* (Narumi et al., 1998). However, chemically synthesized vitamin K is referred as synthetic menadione (vitamin K_3) (Thijssen et al., 2006). Moreover, menaquinones synthesized through fermentation of soybean with *B. subtilis* are water-soluble, showing similar behavior such as protein apI, accordingly form intracellular complex with protein and release in the extracellular fraction during culture progression (Yanagisawa and Sumi, 2005).

Vitamin K is an essential cofactor in the synthesis of blood coagulation factors II, VII, IX, and X in the liver, osteocalcin in bone and matrix Gla protein in blood vessel walls and cartilage (Schurgers et al., 2007a). The hydroquinone form of vitamin K is indispensable for posttranslational γ-carboxylation of vitamin K-dependent proteins by the activation of γ-glutamyl carboxylase (Berkner and Runge, 2004).

The vitamin K deficiency is noticed in postmenopausal women.

Moreover, deficiency is also reported in coagulopathy, chronic renal failure, hemodialysis, advanced cancer, and cephalosporin or cephamycin antibiotics patients (Dougherty, et al., 2010). The cystic fibrosis and pancreatic paucity are also responsible for serum vitamin K deficiency in the humans (Dougherty, et al., 2010).

Vitamin K-rich dietary foods also provide the antioxidant along with vitamin K to the body (Kumar et al. 2010). However, fermented soybean showed higher antioxidant compared to non fermented soybeans (Moktan et al., 2008). In this context, Hu et al. (2010) reported that soybean fermentation with *Bacillus natto* attained higher 1-diphenyl-2-picrylhydrazyl (DPPH) activity and total phenolic contents than that of soaked and cooked soybeans. To overcome the vitamin K deficiency, vitamin K-rich dietary sources are helpful to manage the associated health problems. Considering the above discussion, present research work was planned to evaluate nutrition composition of vitamin K-rich dietary sources, antioxidant status, and their hedonic response with special reference to vitamin K content. No published literature that provides comparison of different form of cooked spinach and natto is available at present.

Materials and Methods

The present research work was conducted in National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan. Spinach variety Desi Palak and soybean cultivar namely Faisal Soybean were procured from Ayub Agriculture Research Institute Faisalabad. Analytical reagents and HPLC grade standards were obtained from Merck and Sigma-Aldrich (Germany).

Sample Preparation. Four different products were developed from the tested raw materials *i.e.* spinach and soybean. Cooked and reconstituted spinach were prepared from fresh and dehydrated spinach, respectively. However, the remaining two were formulated from soybean using different fermented conditions after inoculation with *Bacillus subtilis*.

Fresh cooked spinach (FCS). Spinach was washed and cut into small pieces. Cooking condition was estimated with preliminary trails and noted the minimum cooking time for an adequate palatability and taste. For cooking, 100 g cleaned spinach was cooked in covered stainless steel pan for 10 min on moderate flame. The prepared cooked spinach considered as FCS was stored at refrigeration temperature for further analysis (Sultana et al., 2008).

Reconstituted spinach (RS). For drying, 100 g of spinach was spread to a thickness of 1.4 cm on the tray (0.09 m^2) in the force air dry cabinet. The drying temperature was maintained at 50^{o}C with 1.2 m/sec air velocity. The drying process was continued until the moisture content of the sample reached 15±2% (Doymaz, 2009). The dried sample was cooled under room temperature and kept in airtight jar covered with aluminum foil. For reconstitution purpose, 50 g dry spinach was added to 100 mL of boiling water, heated for 10 min, and labeled as RS.

Preparation of Natto. For natto preparation, overnight soaking of soybean was done with 3 times volume of water. Afterwards, beans were steamed for 30–45 min until easily crushed and inoculated with prepared culture medium of *Bacillus subtilis* gifted from Nattomoto, Yuzo Takahashi Laboratory Co. Japan (0.1 g powder culture dissolved in 10 mL of cooled pasteurized water) at 45^{o}C in a sterilized bowl. The treated beans were packed in plastic box and covered with sterilized cotton cloth to prevent drying in the subsequent steps. Fermentation of the steamed soybean was undertaken at two different conditions *i.e.* 40^{o}C for 24 h (NA) and 35^{o}C for 30 h (NB) in the incubator.

Proximate composition. Cooked and reconstituted spinach and fermented soybean samples were analyzed for moisture, crude protein, crude fat, crude fiber, ash, and nitrogen-free extract by using triplicate samples (AACC 2000).

Minerals profile. The samples were subjected to mineral assay through wet digestion following the protocols of AOAC (2006). For the estimation of sodium and potassium, Flame Photometer-410 (Sherwood Scientific Ltd., UK) was used, whereas calcium, iron, magnesium, zinc, and copper were measured through Atomic Absorption Spectrophotometer (Varian AA240, Australia).

Vitamin K content and preparation of sample. For the extraction of vitamin K, sample was ground separately in a blender to uniform consistency. Five grams of anhydrous sodium sulfate was added in weighed amount of each raw material and further pulverized. The resultant powder and 20 μL of internal standard (200 ng of dihydrophylloquinone) were transferred to 50 mL centrifuge tube. Subsequently, 15 mL of 2-propanol/hexane (3:2 v/v) and 32 mL of H_2O were added into the mixture and centrifuged (4000 rpm) for 5 min. The supernatant layer containing phylloquinone and dihydrophylloquinone was transferred to a clean amber color tube and evaporated under nitrogen stream. The residue was redissolved in 10 mL hexane with further purification through solid-phase silica gel.

Quantification of vitamin K. Extracted samples were quantified by HPLC (Perkin Elmer, Series 200, USA) using C_{18} column (250 mm×4.6 mm, 5.0 μm particle size). For quantification, 10 μL aliquot of samples were injected via autosampler (WISP Model 710). The column temperature was maintained at 40^{o}C. The mobile phase comprised of dichloromethane (100 mL), methanol (900 mL), zinc chloride (1.37 g), sodium acetate (0.41 g), and acetic acid (0.30 g). The flow rate was adjusted at 1 mL/min. Quantification of vitamin K was carried out using UV/vis detector (model 481) at 249 nm (Majchrzak, and Elmadfa, 2001).

Antioxidant indices. For *in vitro* studies, methanol and ethanol extracts of spinach and soybean were tested for their antioxidative properties.

Total phenolic content (TPC) of each extract was determined by using Folin-Ciocalteu reagent (Singleton et al., 1999). Fifty microliters sample was mixed with 250 μL of 2 N Folin-Ciocalteu's reagent. Subsequently, 750 μL of 20% Na_2CO_3 solution and distilled water were added to make the volume 5 mL. After 2 h, absorbance was measured at 765 nm using UV/vis Spectrophotometer (CECIL CE7200). Total phenolic content was calculated and expressed as gallic acid equivalent (mg gallic acid/100g).

Antioxidant activity of vitamin K-rich dietary source extracts was assessed by coupled oxidation of β-carotene and linoleic acid as described by Taga et al. (1984). Briefly, β-carotene (1.0 mg) was dissolved in 10 mL of chloroform. One milliliter of the prepared solution was taken in flask containing linoleic acid (20 mg) and Tween 40 (200 mg). The chloroform was removed using Rotary Evaporator at 40°C. Gradually, 50 mL of distilled water was added to the flask with vigorous shaking to form an emulsion. Subsequently, 5 mL emulsion was mixed with 0.2 mL sample in a test tube. After shaking, absorbance was recorded at 470 nm. Test tube was placed in a water bath equipped with agitation at 50°C, and reading was measured at 10-min intervals up to 40 min.

$$\ln (a/b) \times 1/t = \text{degradation rate of sample}$$

ln=natural log
a=initial absorbance (470 nm) at time zero
b=absorbance (470 nm) after 40 min
t=time (min)

Antioxidant activity (AA) was expressed as % inhibition relative to control

$$AA = \frac{\text{Degradation rate of control} - \text{degradation rate of sample}}{\text{Degradation rate of control}} \times 100$$

Free radical scavenging activity of vitamin K-rich dietary extract sources was estimated using DPPH as described by Muller et al. (2011). Shortly, 125 µL sample was mixed with 4 mL DPPH (1.2 mM) in methanol solution. Absorbance was measured after 30 min at room temperature using spectrophotometer (CECIL CE7200) at 520 nm. The inhibition of free radicals by DPPH was calculated through following expression:

$$\text{Inhibition (\%)} = [100 \times (A_{blank} - A_{sample})/A_{blank}]$$

The ability of reduced ferric ions was measured by the method of Muller et al. (2011). An aliquot of spinach and soybean (50 µL each) was taken with 3 mL of ferric reducing antioxidant power (FRAP) reagent following incubation at 37°C for 30 min. The increase in absorbance was noted at 593 nm using a spectrophotometer. The results were compared with the calibration curve, prepared by using various concentrations of trolox as standard.

Sensory evaluation. The vitamin K-rich dietary sources (FCS, RS, NA, and NB) were tested for their sensory response using 9-point hedonic scale system *i.e.* 9=like extremely; 1=dislike extremely as mentioned in Appendix-I following the instructions of Meilgaard et al. (2007). Accordingly, hedonic behavior of vitamin K-rich dietary products was assessed for various quality traits such as color, taste, flavor, texture, and overall acceptability. For evaluation, prepared food products were presented to the sensory panelists in transparent plates labeled with random codes in separate booths equipped with fluorescent light. Mineral water and unsalted crackers were also provided to the panelist to neutralize their mouth receptors after testing the products. To avoid biasness, samples were offered to the judges randomly and requested to assign scores for the mentioned traits.

Statistical analysis. The obtained data were subjected to statistical analysis using completely randomized design (CRD) by Statistical Package (Costat-2003, Co-Hort, version 6.1). Level of significance was determined through one way analysis of variance (ANOVA) technique.

Results and Discussion

Proximate composition. Proximate composition of prepared vitamin K-rich dietary products depicted that FCS contained the maximum moisture content of 75.64±3.51% followed by reconstituted spinach 71.86±2.96% and natto B 62.72±3.11%, whereas the minimum moisture content was noticed in NA 56.47±2.81%. However, the protein content was highest 15.39±0.65% in NA trailed by 13.19±0.54% in NB and 6.11±0.21% for RS whereas, the lowest value of 5.29±0.18% was recorded in FCS. Crude fat content was observed in vitamin K-rich products as 0.81±0.03, 0.92±0.02, 7.31±0.31, and 7.61±0.32% for FCS, RS, NA, and NB, respectively (Table 1). Moreover, formulated vitamin K-rich dietary products showed non-significant differences for crude fiber content that ranged from 1.17±0.04 to 1.42±0.03% in tested products. The maximum ash content was observed in RS at 6.27±0.31% followed by FCS 5.41±0.23% and NA 1.59±0.07% however, the minimum value was recorded for NB 1.37±0.05%.

Cooking and fermentation are the important processing steps that inactivate the nutritional inhibitors present in spinach and

Table 1 Proximate composition (%) of vitamin K-rich products

Proximate composition	FCS	RS	NA	NB
Moisture	75.64±3.51^{a}	71.86±2.96^{b}	56.47±2.81^{c}	62.72±3.11^{bc}
Crude Protein	5.29±0.18^{b}	6.11±0.21^{b}	15.39±0.65^{a}	13.19±0.54^{a}
Crude Fat	0.81±0.03^{c}	0.92±0.02^{c}	7.31±0.31^{a}	7.61±0.32^{a}
Crude Fiber	1.23±0.04^{a}	1.42±0.03^{a}	1.41±0.06^{a}	1.17±0.04^{a}
Ash	5.41±0.23^{b}	6.27±0.31^{a}	1.59±0.07^{c}	1.37±0.05^{c}
NFE	11.47±0.47^{c}	13.35±0.52^{ab}	17.78±0.67^{a}	13.89±0.54^{b}

Means carrying same letter in a row differed non-significantly (p >0.05).
FCS: Fresh cooked Spinach; RS: Reconstituted Spinach; NA: Natto A; NB: Natto B.

Table 2 Mineral profile (mg/100 g) of vitamin K-rich products

Minerals	Fresh cooked spinach	Reconstituted spinach	Natto A	Natto B
K	514.23±23.78[c]	513±24.34[d]	671.47±29.74[a]	667.15±31.36[b]
Zn	76.72±3.84[c]	65.66±3.28[c]	284.93±13.55[a]	279.70±12.83[b]
Mg	646.28±32.31[a]	616.08±30.80[a]	225.44±10.52[b]	218.19±10.56[b]
Ca	86.14±3.56[b]	85.51±3.41[b]	181.69±8.83[a]	173.07±7.65[a]
Fe	2.37±0.19[c]	2.35±0.15[c]	7.25±0.31[a]	7.18±0.32[b]
Na	72.58±3.81[c]	71.74±3.73[c]	2.72±0.12[a]	2.64±0.12[b]
Cu	6.89±0.42[a]	6.84±0.45[b]	1.87±0.06[c]	1.76±0.05[c]

Means carrying same letter in a row differed non-significantly (p >0.05).
FCS: Fresh cooked Spinach; RS: Reconstituted Spinach; NA: Natto A; NB: Natto B.

soybean. The results regarding proximate composition of cooked spinach are in agreement with the finding of Kuti and Kuti (1999), who elucidated that spinach has 8.67±0.23% crude protein content. Similarly, the values documented by USDA (2010) regarding proximate composition of cooked spinach supports the present results. The published values for cooked spinach are 91.21, 2.97, 0.29, 2.4, and 3.75% for moisture, protein, fat, dietary fiber and carbohydrate, respectively. The conventionally cooked spinach has significantly less amount of moisture, whereas protein, lipid, ash, and total dietary fiber contents are higher as compared to the raw sample (Kala and Prakash, 2004).

These results are in harmony with the findings of Premarani et al. (2011); they illuminated the values for fermented soybean as 62.1 moisture, 1.42 ash, 8.2 crude fiber, 26.02 soluble protein, and 24.36% fat contents. Likewise, Jeff-Agboola and Oguntuase (2006) expounded that natto contains 40% protein and 24.68% fat contents. They were on the view that controlled fermentation process imparts significant differences on the nutritional composition of natto as compared to natural fermentation. However, hawaijar (fermented soybean) prepared from different soybean cultivars exhibited non-significant variations for lipid, fatty acid, and amino acid contents (Li et al., 2007). Earlier, Wei and Chang (2004) evaluated natto samples prepared from four different soybean cultivars using *B. natto "Itobiki"* for moisture, protein, lipid, ash, and carbohydrates and recorded the range 59.30–61.24, 36.63–42.72, 19.64–23.09, 4.38–4.97, and 30.80–34.23%, respectively. However, the natto prepared from *B. natto* NRRL B-3393 showed the values 61.07–61.75, 39.4–44.31, 25.00–27.28, 4.72–4.86, and 25.02–28.91%, respectively, for these attributes.

Mineral profile. The mineral profile of vitamin K-rich dietary products explicated significant differences due to treatments. In present exploration, spinach based vitamin K-rich products (cooked and reconstituted spinach) have values 514.23±23.78, 513±24.34, 76.72±3.84, 65.66±3.28, 646.28±32.31, and 616.08±30.80, 86.14±3.56, and 85.51±3.41, 2.37±0.19, and 2.35±0.15, 72.58±3.81, and 71.74±3.73, 6.89±0.42, and 6.84±0.45 mg/100 g for K, Zn, Mg, Ca, Fe, Na, and Cu in FCS and RS, respectively (Table 2). However, soybean-based vitamin K-rich dietary products *i.e.* NA and NB exhibited the highest concentrations 671.47±29.74 and 667.15±31.36 mg/100 g for K followed by 284.93±13.55 and 279.70±12.83 mg/100 g for Zn, whilst the lowest levels 1.87±0.06 and 1.76±0.05 mg/100 g were noticed for Cu in NA and NB, respectively.

Lisiewska et al. (2008) explored the mineral profile of cooked spinach and reported that potassium (258.0±51.0 mg/100 g), magnesium (19.8±2.2 mg/100 g), iron (0.94±0.18 mg/100 g), and zinc (0.74±0.06 mg/100 g) contents were not significantly different from those of raw spinach *i.e.* 335.0±24.4, 29.0±3.8, 1.11±0.14, and 0.80±0.11 mg/100 g, respectively. Recently, Sikora and Bodziarczyk (2012) expounded that green leafy vegetables showed variations in the mineral contents after cooking due to handling & processing losses and alteration in the moisture content of end product. Iron was non-substantially reduced to 2.97±0.75 mg/100 in conventionally cooked spinach compared to the raw sample 3.00±0.53 mg/100 g (Kala and Prakash, 2004).

The present results for potassium, magnesium, copper, iron, zinc, sodium, and calcium are in line with the earlier findings of Nikkuni et al. (1995). They delineated the values for tested minerals as 1697, 221, 1.46, 7.2, 4.55, 14.4, and 281 mg/100 g for natto. Moreover, kinema (fermented soybean) indicated the values for these minerals as 1768, 252, 1.71, 17.1, 4.52, 27.7, and 432 mg/100 g, respectively. Intake of natto containing poly-γ-glutamic acid resulted high percentage of Ca solubility in the intestine after inhibiting the formation of insoluble Ca complex (Tanimoto et al., 2001). The deviations in mineral conents are supposed to be linked with different greographical conditions, agronomic practices, cultivars, and harvesting time. During fermentation process bacteria are utilizing minerals for their growth, thereby minor variations in mineral profile of resultant product were found. Nevertheless, the fermented soybean (natto) showed higher amount of protein and fat than that of cooked and reconstituted spinach.

Vitamin K (Phylloquinone & menaquinone-7) contents. Means related to phylloquinone exhibited the highest value 368.81±13.96 for FCS followed by 270.07±9.45 in RS, whereas NA showed the minimum value 26.90±0.94 μg/100 g. However, maximum menaquinone-7 (MK-7) was recorded in NA 803.82±21.14 trailed by NB 681.35±16.85 μg/100 g. In contrary, menaquinone-7 was not detected in the cooked and reconstituted spinach (Table 3).

The results of instant investigation are comparable with the

Table 3 Phylloquinone and menaquinone-7 (µg/100 g) in vitamin K-rich products

Products	Phylloquinone	Menaquinone-7
FCS	368.81±13.96[a]	ND
RS	270.07±9.45[b]	ND
NA	26.90±0.94[c]	803.82±21.14[a]
NB	29.80±1.04[c]	681.35±16.85[b]

Means carrying same letter in a column differed non-significantly (p >0.05).
ND=Not detected.
FCS: Fresh cooked Spinach; RS: Reconstituted Spinach; NA: Natto A; NB: Natto B.

earlier work of Kamao et al. (2007) for phylloquinone and MK-7 contents of spinach and natto. The documented values for phylloquinone and MK-7 were 498 and 939 µg/100 g in spinach and natto, respectively. They concluded that vegetables and fermented foods are one of the prime sources of vitamin K in humans. Recently, Booth (2012) explicated the levels of phylloquinone and MK-7 as 380 and 998 µg/100 g for spinach and natto, respectively. Likewise, Damon et al. (2005) quantified phylloquinone concentrations in fresh, boiled, and microwaved spinach samples as 293-441, 533-547, and 348-544 µg/100 g, respectively. Earlier, Schurgers and Vermeer (2000) delineated that phylloquinone contents are 299–429 µg/100 g in spinach and 31.2–36.7 µg/100 g in soybean. Moreover, the concentration of MK-7 was reported in natto as 882–1034 µg/100 g. Tsukamoto et al. (2001) identified mutant strain of bacteria that has relatively higher productivity of menaquinone-7 *i.e.* 139-156% as compared to commercial strain that produces 864 µg/100 g of MK-7 in natto.

In addition, natto was shown to have almost 2.5 times higher MK-7 level than that of phylloquinone content of spinach (Schurgers and Vermeer, 2000; Booth, 2012). The phylloquinone and menaquinones are heat stable entities; however, phylloquinone is more susceptible to light and alkaline conditions (Fu and Booth, 2012). Nevertheless, phylloquinone and menaquinones are quite stable in cooked spinach and fermented soybean, respectively. Generally, green leafy vegetables contribute approximately 60% of the daily phylloquinone intake. However, allied health claims of long chain menaquinones enhance their importance in everyday diet (Schurgers et al., 2002).

Antioxidant potential. Means for TPC of vitamin K-rich dietary products (Table 4) indicated the highest value 714.94±32.10 mg GAE/100 g in fresh cooked spinach followed by 700.21±24.01 mg GAE/100 g in reconstituted spinach and 405.83±17.80 mg GAE/100 g in NA, whereas the lowest TPC value 395.59±14.51 mg GAE/100 g was observed in NB. Likewise, antioxidant activity and DPPH were maximum in cooked spinach (52.70±2.35 and 59.02±2.19%) trailed by reconstituted spinach (51.00±1.99 and 57.42±2.42%) and natto A (25.09±1.11 and 34.05±1.37%), whereas the minimum values in natto B were (23.75±0.92 and 32.35±1.08%). Similarly, the gathered data for FRAP of resultant products showed the values 2.04±0.10, 1.99±0.09, 1.27±0.04, and 1.23±0.06 µmol trolox Eq/100 g for FCS, RS, NA and NB, respectively.

The results of the present study are in harmony with the earlier finding of Bunea et al. (2008), they documented phenolic contents of spinach as 1067.4±7.3 to 2108.8±14.9 mg GAE/kg on fresh weight basis. Moreover, Ismail et al. (2004) also determined phenolics and antioxidant activities of raw and cooked spinach ethanolic extracts. They deduced that the raw spinach has higher phenolics and antioxidant activity: 7167±73 mg GAE/100 g and 66.4±1.1% than that of cooked treatment; 6168±41 mg GAE/100 g and 61.9±0.6%, respectively. Furthermore, they also observed that phenolic content has linear association with antioxidant activity.

Similarly, Jimenez-Monreal et al. (2009) observed 11.1 and 31.6% reduction for TPC and DPPH values, respectively, in thermally

Table 4 Antioxidant potential of vitamin K-rich products

Products	Total phenolic content (mg GAE/100 g)			DPPH free radical scavenging activity (%)		
	Methanol	Ethanol	Means	Methanol	Ethanol	Means
FCS	803.55±35.90	626.34±24.31	714.94±32.10[a]	62.40±2.39	55.65±2.01	59.02±2.19[a]
RS	785.85±37.41	614.58±20.62	700.21±24.01[b]	60.52±2.06	54.33±1.79	57.42±2.42[ab]
NA	466.37±21.88	345.30±15.73	405.83±17.80[c]	36.19±1.83	31.92±1.26	34.05±1.37[b]
NB	453.65±17.56	337.53±13.47	395.59±14.51[c]	34.65±1.05	30.05±1.11	32.35±1.08[b]
Means	627.35±26.60[a]	480.93±19.53[b]		48.44±2.14[a]	42.98±2.03[b]	
Products	**Antioxidant activity (%)**			**FRAP (µmol trolox Eq/100 g)**		
	Methanol	Ethanol	Means	Methanol	Ethanol	Means
FCS	55.74±2.45	49.67±2.24	52.70±2.35[a]	2.12±0.09	1.97±0.08	2.04±0.10[a]
RS	54.57±2.07	47.43±1.90	51.00±1.99[ab]	2.07±0.10	1.92±0.09	1.99±0.09[b]
NA	26.76±1.20	23.42±1.01	25.09±1.11[b]	1.36±0.06	1.19±0.05	1.27±0.04[c]
NB	24.97±10.98	22.54±0.85	23.75±0.92[b]	1.32±0.04	1.15±0.03	1.23±0.06[c]
Means	40.51±1.67[a]	35.76±1.52[b]		1.93±0.12[a]	1.82±0.10[b]	

Means carrying same letter in a column differed non-significantly (p >0.05).
FCS: Fresh cooked Spinach; RS: Reconstituted Spinach; NA: Natto A; NB: Natto B.

Table 5 Sensory response of vitamin K-rich products

Products	Color	Flavor	Taste	Texture	Overall acceptability
FCS	7.86±0.34^{a}	7.96±0.35^{a}	7.78±0.24^{a}	7.56±0.33^{a}	7.84±0.34^{a}
RS	6.94±0.26^{b}	7.34±0.32^{b}	7.18±0.27^{b}	7.14±0.31^{b}	7.74±0.34^{b}
NA	6.84±0.30^{b}	7.02±0.30^{c}	7.08±0.25^{c}	6.94±0.30^{c}	7.14±0.31^{c}
NB	6.04±0.25^{c}	6.14±0.26^{d}	6.98±0.29^{d}	6.16±0.26^{d}	6.74±0.29^{d}

Means carrying same letter in a column differed non-significantly (p >0.05).
FCS: Fresh cooked Spinach; RS: Reconstituted Spinach; NA: Natto A; NB: Natto B.

treated spinach. The current outcomes for antioxidant potential of cooked spinach are in coherence with Amin et al. (2006), who found that â-carotene inhibition, DPPH free radical activity, and total phenolic content are in the range of 21–43, 28–75%, and 32-68 g GAE/kg, respectively. Moreover, FRAP of uncooked spinach is 15.89 mmol Fe^{2+}/100 g that decreased to 13.89 mmol Fe^{2+}/100 g after 10 min cooking (Mazzeo et al., 2011). The heat treatment significantly breakdowns the lignocellulosic structure of the vegetables along with depolymerization and defibration of lignin components that reduce the antioxidant activity of resultant product. Consequently, phenolic molecules may leach down from the vegetable matrix to the boiling water (Xu and Chang 2008). The differences in the antioxidant potential of spinach-based products are probably due to variations in cooking methods and conditions *i.e.* time and temperature (Gazzani et al., 1998).

The findings of Yao et al. (2010) are synchronized with the current results that *Bacillus sp.* Fermented-Korean soybean has higher values for TPC, DPPH, and FRAP activity by 379.7 mg GAE/100 g, 58% and 250.04 µg trolox Eq/g as compared to non-fermented soybean 257.6 mg GAE/100 g, 42% and 307.03 µg trolox Eq/g FW, respectively. The higher antioxidant activity of fermented soybean is associated with the generation of isoflavones and their glycosides through microorganisms during fermentation. Similarly, DPPH radical scavenging capacity is uplifted by fermentation with various types of microorganisms. In this context, *Bacillus sp*. yields the highest FRAP activity due to the synthesis of iron chelating compounds during fermentation. Earlier, it has been observed that fermented soybean contains antioxidant peptides responsible for scavenging free radicals and exert inhibitory effect on β-carotene in linoleic acid model system (Wang et al., 2008). The results of present study regarding antioxidant indices are in corroboration with the work of Moktan et al. (2008), revealing that methanolic extract of soybean fermented with *B. subtilis* has higher metal chelating ability, lipid peroxidation inhibition, DPPH free radical scavenging activity, and reducing power as compared to raw soy and suggested that fermentation significantly enhances these attributes. Furthermore, Dajanta et al. (2011) explicated that fermented soybean (*Thai Thua nao*) has appreciable amount of phenolics and DPPH radical scavenging activity. Nevertheless, its antioxidant activity was estimated as 54.55 to 65.65% through β-carotene assay.

Sensory evaluation. The sensory response of resultant vitamin K-rich products showed significant variations in the tested traits including color, flavor, taste, texture, and overall acceptability. The highest color score was assigned to fresh cooked spinach (7.86±0.34) followed by reconstituted spinach (6.94±0.26), NA (6.84±0.30), and NB (6.04±0.25). The observed scores for flavor were 7.96±0.35 (FCS), 7.34±0.32 (RS), 7.02±0.30 (NA), and 6.14±0.26 (NB). Likewise, taste scores for FCS, RS, NA, and NB were 7.78±0.24, 7.18±0.27, 7.08±0.25, and 6.98±0.29, respectively. Means for texture in various treatments illuminated variations from 7.56±0.33 to 6.16±0.26 for FCS to NB, respectively. Lastly, the recorded scores for overall acceptability of the vitamin K-rich products *i.e.* FCS, RS, NA and NB were 7.84±0.34, 7.74±0.34, 7.14±0.31, and 6.74±0.29, respectively (Table 5).

Present results concerning sensory profile of the processed spinach are in harmony with the work of the Donadini et al. (2012), who elucidated that boiled spinach has better hedonic response as compared to raw sample in a vegetable-liking questionnaire assessment. Earlier, Bangash et al. (2011) inferred that amongst tested sensory parameters, color is exceptionally affected due to cooking. Likewise, the color of conventionally and microwave cooked spinach is fairer than pressure cooked spinach. Similarly, hedonic attributes of cooked green vegetables were evaluated using ranking test and quantitative descriptive analysis (QDA). The traits analyzed were color, appearance, aroma, taste, and texture. The comparison between conventional and microwave methods expounded that color of the microwave cooked spinach is comparatively better. Furthermore, aroma and texture of cooked spinach by pressure cooking is relatively inferior to conventionally cooked sample. Additionally, taste and overall acceptability are more appealing for conventionally cooked spinach than pressure cooked sample (Kala and Prakash, 2004).

The current results are in accordance with the findings of Youn et al. (2002) and Lee et al. (2005). They expounded that fermented soybean (chungkukjang) prepared with *B. licheniformis* attained higher hedonic scores for color, flavor, and taste as compared to traditional chungkukjang. Likewise, fermented soybean with *B. subtilis* TN51 showed better performance on aroma than conventionally fermented soy (Dajanta et al., 2011). Furthermore, significant differences for aroma and texture were observed in the prepared and commercially available natto. The sensory attributes of natto *i.e.* color, aroma, flavor, and texture were within acceptable limit estimated through continuous linear 10 cm intensity scale (Wei et al., 2004). It has been observed that fermentation conditions are the crucial factors affecting sensory response of the fermented products. In this context, controlled fermented soybean attained higher scores for hedonic response as compared to conventionally fermented sample. Additionally, controlled fermentation imparts non-significant variations for appearance, odor, taste, and stickiness of natto samples prepared from different soybean varieties (Luo et al., 2010). Earlier, Lee et al. (2007) assessed the sensory response of fermented soybean (chungkukjang) including odor, taste, and overall acceptability through 9-point hedonic scale and found scores within acceptable range. Previously, Lee et al. (2005) determined the hedonic response *i.e.* color, flavor, taste, and overall acceptability of the chungkukjangs prepared from different bacterial strains using 5-point hedonic scale. They noticed better sensory response for chungkukjang prepared from *B. subtilis* than when formulated with rest of the bacterial strains.

Conclusively, HPLC quantification showed higher phylloquinone content in fresh cooked spinach as compared to the reconstituted spinach. Similarly, menaquinone-7 was more pronounced in the natto A might be due to better fermentation conditions for *B. subtilis*. Furthermore, antioxidant activity of resultant spinach products is better than fermented soybean formulations owing to higher polyphenolic contents. The natto based products have attained comparatively lower scores for sensory attributes due to their sticky nature, bitter taste, and slimy structure as compared to spinach samples. The utilization of the vitamin K-rich dietary sources especially fresh cooked spinach and fermented soybean are considered to be beneficial to cope with its deficiency and related abnormalities.

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