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PHYTIC ACID, POLYPHENOLS AND POTENTIAL NUTRIENTS IN *BRASSICA* SEEDS

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Some exotic and indigenous *Brassica* seeds were analysed for proximate composition, trace elements, vitamins, phytic acid and phenolic compounds. While comparing results with that reported for soybean (taken as standard) *Brassica* seeds contained higher amounts of fat (32.06 - 37.13%), iron (67-136 ppm), manganese (26.1 - 44.1 ppm) nicotinic acid (129-161 ppm) riboflavin (3.20 - 5.88 ppm), phytic acid (2.30 - 3.58%) total phenols (0.74 - 1.20%) and sinapine (0.15 - 0.55%) but were lower in protein (25.57 - 30.69%), copper (5.00 - 8.55 ppm) and zinc (30.1 - 38.4 ppm) content. More variability in phytic acid and phenolics (16.79 - 33.33%) indicated a possibility of selection of low phytic acid and low polyphenol cultivars in a breeding programme.

Key words: *Brassica* cultivars, Trace elements, Total phenolics, Sinapine, Phytic acid.

Introduction

Rapeseed (*Brassica* sp.) is a good source of oil and protein. Quality of its protein is high as it has a well balanced amino acid profile [1]. However, its utility is limited due to the presence of toxic (erucic acid, glucosinolate) and antinutritive (phenols, phytate) factors [2-5]. Local as well as exotic *Brassica* seeds grown in Pakistan have already been extensively screened for erucic acid and glucosinolate content [6-10]. With the introduction of improved cultivars (Canola) containing low levels of erucic acid and glucosinolate [4,11], and successful elimination of glucosinolate [12] attention should be focussed on other constituents of the seed that might influence its nutritive value. Phytic acid and polyphenolic compounds can affect the bioavailability of minerals and proteins [5,13,14]. The object of the present investigation was to analyse exotic and indigenous cultivars of *Brassica* seeds for important nutrients and antinutrients.

Materials and Methods

Samples of Bangladesh sarson (*Brassica compestris*), SM 82001 (*B. juncea*), SM 83000 (*B. juncea*), Torch (*B. compestris*) Varuna (*B. juncea*), RD 80 (*B. juncea*), Porbi raya (*B. carinata*), Brown raya (*B. carinata*), Raya NS (*B. carinata*) and Peela raya (*B. carinata*) were procured from Ayub Agricultural Reserch Institute (AARI), Faisalabad, whereas that of Altex (*B. napus*), Tower (*B. napus*) and Wester (*B. napus*) were collected from Agricultural Research Institute, (North), Mingora, Swat, NWFP. The seed samples were cleaned and stored in polyethylene bags at room conditions.

Chemical analysis. Moisture, oil, protein and ash contents of the samples were determined by AOAC methods [15]. Total carbohydrate and dry matter were determined by calculation. Seed samples were digested in nitric acid and perchloric acid (5:1) and were read in an atomic absorption spectrophotometer (Hitachi, Model 170-10) for trace elements (Fe, Mn, Zn, Cu) determination. Riboflavin and niacin were determined in ground sample (passed through 20 mesh) by the use of methods as outlined by AACC [16].

Phytic acid content of the seed meal obtained by solvent extraction was determined by a rapid colorimeter method of Haug and Lantzsch [17]. The phytic acid was precipitated with an Iron- III solution of known iron content and the decrease of iron in the supernatant was measured by 2,2-bipyridine solution. Total phenols were determined by the method of Titto [18]. Ground samples (passed through 20 mesh) were extracted with methanol (absolute). Colour was developed in the extract with Folin-Ciocalteau phenol oxidizing reagent and absorbance was read at 760 nm. Tannic acid was used as standard. Sinapine content were determined by the method of Blair and Reichert [19]. The methanol extracts used for total phenol assay were diluted (1:50) and analysed for sinapine content using spectrophotometry. The concentration of sinapine in the methanol extract was calculated using the formula C=A/EL, where C=concentration in mol litre-1, A=absorbance at 330 nm and E=pathlength of spectroscopic cell.

Results of all the chemical constituents analysed in duplicate were expressed both on moisture free basis (reported as percent of seed) and moisture and fat free basis (reported as percent of seed meal).

Results and Discussion

Proximate composition: Results regarding proximate composition of different *Brassica* seeds are reported in Table 1. Average dry matter, fat, protein, ash and carbohydrate contents in seeds were found to be 93.29, 34.23, 27.77, 4.21 and 27.11% respectively. Fats and proteins are major components followed by total carbohydrate and ash contents. The increase

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in protein (42.24%), ash (6.40%) and carbohydrate (41.24%) content in seed meals was mainly due to the removal of fat. The values obtained for seed and seed meal generally agree with the results reported for Pakistani *Brassica* seed [20,21] and that of other countries [22,23]. Some variation in the amount of some constituents might have been due to genetic characters and agroclimatic conditions. While comparing present values with that of soybean (taken as standard) [23,24], *Brassica* seeds contained more fat but were lower in protein. Variation in proximate constituents of *Brassica* seeds was less than 7% (Table 1).

Trace elements. The important trace element contents of different *Brassica* seeds samples are given in Table 2. Out of the four trace elements determined iron was the highest followed by manganese, zinc and copper. Average values for these minerals were 107, 38.7, 34.19 and 6.97 ppm, respectively. Due to elimination of fat, the seed meal contained considerably higher amount of trace elements than seeds. The mean values for these elements were 164, 58.8, 52.1 and 10.58 ppm, respectively. Compared to soybean seed meal [23], *Brassica* seed meal is better source of Fe and Mn whereas the former is richer source of Cu and Zn. Coefficient of variability was higher for trace elements (6.64-21.38%) than constituents mentioned under proximate composition (Table 2).

Vitamins. Nicotinic acid and riboflavin content of some *Brassica* seeds and seed meals are reported in Table 3. Wester (3.2 ppm) and Altex (3.79 ppm) contained less riboflavin than other cultivars where it was more than 5 ppm. Nicotinic acid was lower in Altex (129 ppm) than Wester (161 ppm) and Tower (159 ppm). The seed meal samples contained significantly higher amount of these vitamins compared to corresponding seed samples. Present result reveal that *Brassica* seed meal contains higher levels of nicotinic acid and riboflavin than soybean seed meal [17].

Phytic acid. Phytic acid content ranged from 2.30-3.58% in seed and 3.53-5.44% in the seed meal samples (Table 4) which are higher than reported for soybean [25]. These values agree with those reported previously in seed [20] and seed meal [26]. It has been shown that phytic acid can reduce the availability of minerals like P, Ca, Mg, Zn, Cu and Mn [1,27]. Jones [2] recommends Zn supplementation to animal feed rations containing rapeseed meal, as phytate tends to make Zn unavailable to animals. Similarly, phytic acid form stable compounds with rapeseed proteins, hence decreases their solubility and affects their electric properties [26].

Phenolic compounds. Exotic as well as indigenous *Brassica* seeds and their meals were analysed for total phenolic and sinapine content (Table 4) Total methanol-extractable phenolics varied from 0.74 to 1.20% in seeds and 1.09-1.87% in seed meals of different samples. Values ranging from 9.8-1.5% in flour [28] and 0.1-3.0% in meal [29-30] have been reported. Genetic background, agroclimatic condition and analytical method used have been reported to affect the values [31].

TABLE 1. PROXIMATE COMPOSITION OF *BRASSICA* SEEDS.

Material	Dry(%) seed	Fat (%) Seed	Protein(%) Seed	Protein(%) Meal	Ash(%) Seed	Ash(%) Meal	Total CHO (%) Seed	Total CHO (%) Meal
Bangladesh sarson	93.69	32.06	27.55	40.55	4.31	6.34	29.77	43.82
SM 83001	92.77	32.42	25.57	37.84	4.32	6.39	30.46	45.07
SM 83000	92.71	35.42	26.79	41.48	4.37	6.77	26.43	40.93
Torch	92.98	34.25	29.72	45.20	3.91	5.95	25.10	38.17
Varuna	92.72	34.22	28.35	43.10	4.23	6.43	25.92	39.40
RD-80	93.76	33.87	27.85	42.09	4.29	6.49	27.05	40.90
Porbi raya	92.90	33.09	30.69	45.87	4.26	6.37	25.46	38.50
Peela raya	94.02	35.99	28.56	44.62	4.00	6.25	25.47	39.79
Brown raya	93.68	37.14	25.73	40.93	4.49	7.14	26.32	41.87
Raya NS	93.71	33.81	26.92	40.62	3.89	5.88	29.09	43.95
Mean	93.29	34.23	27.77	42.24	4.21	6.40	27.11	41.24
SD	0.49	1.50	1.53	2.35	0.19	0.34	1.82	2.27
CV (%)	0.53	4.38	5.51	5.56	4.51	5.31	6.71	5.50

TABLE 2. MINERALS OF *BRASSICA* SEEDS.

Material	Fe (ppm) Seed	Fe (ppm) Meal	Mn (ppm) Seed	Mn (ppm) Meal	Zn (ppm) Seed	Zn (ppm) Meal	Cu (ppm) Seed	Cu (ppm) Meal
Bangladesh sarson	67	99	38.0	55.9	32.7	48.1	5.00	7.36
SM 83001	95	141	42.5	62.9	36.8	54.5	8.41	12.44
SM 83000	98	152	43.0	66.6	38.4	59.5	7.72	11.95
Torch	122	186	44.1	67.0	35.3	53.7	5.59	8.50
Varuna	113	172	40.4	61.4	34.6	52.6	8.41	12.79
RD-80	120	181	42.8	64.7	33.9	51.3	8.10	12.25
Porbi raya	114	170	39.0	58.3	30.1	45.0	8.55	12.78
Peela raya	96	150	31.3	48.9	35.0	54.7	5.97	9.33
Brown raya	136	216	26.1	41.5	33.1	52.7	4.42	7.03
Raya NS	116	175	40.3	60.9	32.0	48.4	7.50	11.33
Mean	107	164	38.7	58.8	34.19	52.1	6.97	10.58
SD	18.29	29.65	5.45	7.04	2.27	3.88	1.49	2.17
CV (%)	17.09	18.10	14.08	11.79	6.64	7.45	21.38	20.51

TABLE 3. RIBOFLAVIN AND NICOTINIC ACID CONTENT OF *BRASSICA* SEEDS.

Material	Riboflavin (ppm) Seed	Riboflavin (ppm) Meal	Nicotenic acid (ppm) Seed	Nicotenic acid (ppm) Meal
Bangladesh sarson	5.88	8.67	–	–
SM-83001	5.44	9.51	–	–
SM-83000	5.81	9.02	–	–
Wester	3.20	7.76	161	220
Tower	5.64	9.85	159	213
Altax	3.79	8.19	129	168
Mean	4.96	8.83	149.67	200.33
SD	1.06	0.72	14.64	23.04
CV (%)	21.37	8.15	9.78	11.50

TABLE 4. PHYTIC ACID AND PHENOLICS OF *BRASSICA* SEEDS.

Material	Phytic acid (%)		Total phenolics(%)		Sinapine(%)	
	Seed	Meal	Seed	Meal	Seed	Meal
Bangladesh sarson	2.54	3.74	0.74	1.09	0.15	0.22
SM-83001	2.56	3.79	0.93	1.38	0.46	0.68
SM-83000	3.51	5.44	1.08	1.67	0.47	0.73
Torch	2.47	3.75	0.74	1.13	0.21	0.32
Varuna	3.58	5.44	0.91	1.38	0.37	0.56
RD-80	3.16	4.78	0.98	1.48	0.32	0.48
Porbi raya	2.36	3.53	0.72	1.08	0.32	0.48
Peela raya	2.38	3.72	1.20	1.87	0.33	0.52
Brown raya	2.30	3.66	1.13	1.80	0.55	0.87
Raya NS	2.51	3.80	0.81	1.22	0.34	0.51
Mean	2.74	4.17	0.92	1.41	0.35	0.54
SD	0.46	0.72	0.16	0.28	0.11	0.18
CV (%)	16.79	17.15	17.82	19.60	31.43	33.33

Sinapine content ranged from 0.15-0.55% in all *Brassica* seed samples while the values for corresponding seed meal samples varied from 0.22-0.87% (Table 4). Present values obtained for sinapine content of different *Brassica* seed meal samples were lower than those reported by other workers [31,32]. The main (80-98%) phenolic constituent of rapeseed is sinapine [31,33]. Sinapine is a bitter tasting compound and taste threshold of free sinapinic acid is reported to be 1 ppm [34]. Soybean cotyledons [35] and hulls [36] have been reported to show zero tannin value. Present results and those of other workers indicate small but significant amount of polyphenols in *Brassica* seed and its products. The presence of phenolic compounds can alter the sensory properties (off-colour), bitterness, astringency, reduce the digestibility and adversely change the functional properties of *Brassica* seed proteins in food and feed systems.

Present results on antinutrients suggest that *Brassica* seed and seed meal intended to be fed to animals should be improved by evolving cultivars with a reduced content of phytic acid and phenolics. As compared to other chemical compounds studied, phytic acid and phenolics exhibited more variability (16.79, - 33.33%), indicating a possibility of selection of low phytic and low polyphenol cultivars in a breeding programme. As an interim arrangement, the processing of meal to remove phytic acid [20] and sinapine [36] should be considered.

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