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VARIATION OF PHENOLIC COMPOUNDS IN RAPESEED VARIETIES GROWN AT PESHAWAR

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Total phenols, catechin, sinapine, flavan-4-ols, proanthocyanidine and procyanadine of 18 rapeseed varieties (BSA, Tobin, Salam, Marnoo, Tower, Altex, Tatyoon, PR-7, D.G.L, Toria, Raya N.S, Porbi Raya, Raya Raya, SM-83001, SM-83000, Torch, Varuna and RD-80) collected from Peshawar were determined from methanol and methanol-HCl (1%) extract. Variations in flavan-4-ols and proanthocyanidine content were much larger among the varieties as compared to other phenolic constituents. Maximum sinapine content (0.99%) which is the major constituent of rapeseed was found in the variety RD-80 while the minimum amount (0.58%) in variety Toria. The sinapine content extractable in absolute methanol is 52.2% and that extractable in acidic methanol is 20.4% of the total phenolics. This information will serve as a guide for processing of rapeseed for animal/human nutrition.

Key words: Polyphenols, Rapeseed varieties.

Introduction

There is considerable interest in the development of rapeseed meal as a source of edible proteins for animals and human nutrition. Rapeseed meal was found to be inferior to other oilseed meals as the sole source of protein when tested on mice, but the protein isolates from rapeseed meal were better sources of dietary protein than soybean, sunflower, flax and safflower protein isolates [1]. However, the usefulness of rapeseed as a source of food protein is limited by the presence of undesirable components such as glucosinolates, phytates and phenols [2]. The extraction of glucosinolates, which interfere with protein utilization [3] and air-classification of hulls from the meals [4], have been successfully achieved. However, rapeseed flour and protein isolates develop brown colour when their aqueous slurries were adjusted to an alkaline pH [5] indicating that phenolic compounds may be present in the hull free flours. The minimum amount of dietary tannin needed to elicit a negative growth response in animals has not been established. However, Chang and Fuller [6] found that 0.1% tannic acid in chick-diets exhibited no effect but that 0.5 and 2.0% caused 7-weeks growth depression of 3% and 32% respectively as compared to the control. Vohra *et al.* [7] found that 70% chick mortality occurred with a diet containing 5% tannic acid. A diet containing 3.9% of condensed tannin purified from *Vicia faba* seeds caused net weight losses in chicks [8].

The effects of dietary tannin on humans are unknown, although epidemiological considerations have led to the suggestion of correlation between condensed tannins and oesophageal cancer [9]. Sinapine and its hydrolytic product, sinapic acid, are common constituents of rapeseed species and their bitter flavour may have an adverse effect on the palatability of

rapeseed product [10]. They are also involved in the genesis of a fishy odour or taste in the eggs of certain brown egg laying hens [11, 12]. Tannins have also been linked to egg taint, through an adverse effect on hepatic oxidation of trimethylamine [13]. The occurrence of the phenolics in rapeseed grown in the Peshawar valley has not been studied. Hence it was felt necessary to monitor the available cultivars for their phenolic compounds.

Materials and Methods

Exotic (Tobin, Salam, Marnoo, Tower, Altex, Tatyoon, Torch and Varuna) and locally (BSA, PR-7, DGL, Toria, Raya N.S, Porbi Raya, Raya Raya, SM-8300, SM-83000 and RD-80) cultivated varieties of rapeseed were obtained from Mutation Breeding Division of this Institute. The seeds were dried to a moisture level of about 7%. They were cleaned to get rid off dust, dirt and other undesirable particulate matter. The seeds were grounded in a pestle and mortar and then passed through 40 mesh screen in a Wiley mill. The samples were extracted with methanol and 1% HCl in methanol and assayed for different polyphenols [14].

The samples were analysed for extractable total phenols using folin-ciocalteu phenol reagent [15] which contained sodium molybdate and sodium tungstate, 2.5 and 10% respectively. This reagent is nonspecific for any phenolics and the colour yield depends on phenolic hydroxyl groups and their place in the molecules. The sinapine content in the extracts were assayed spectrophotometrically according to the method of Blair *et al.* [16]. The concentration of sinapine in the methanol extracts was calculated using the formula $C = A/EL$; where C = concentration in mole litre⁻¹, A = absorbance at 330 nm, E = extinction coefficient (21390) at 330 nm and L = path-

length of the spectroscopic cell. The sinapine content determined by this procedure includes all sinapic acid esters plus free sinapic acid. Procyanidines were determined using HCL: formic acid (1:1) solvent as a complexing reagent [17]. Catechin contents were determined by the Vanillin method [18]] using (+) catechin as the standard.

With a solvent of 1-butanol and concentrated HCl, anthocyanidine formed from flavan-4-ols were measured at 550 nm [14] because flavan-4-ols are readily converted to anthocyanidine in acidic solvents at room temperature [19]. An estimate of relative variation in polyphenols in rapeseed varieties was made by determining the coefficient of variation (CV), which is a ratio of standard deviation to the mean [21].

Results and Discussion

Sinapine. The major phenolic content of the rapeseed is the sinapine [22]. The amount of sinapine recovered from rapeseed varieties in this study was 0.59% for absolute methanol and 0.23% for 1% concentrated HCl in methanol with a total of 0.82%. This constitutes about 78% of the total phenolics in rapeseed varieties, whereas, literature values for sinapine are more than 98% of the phenolics in Canola [2], 85% (as sinapine acid esters) in diffusion extracted rapeseed flour [23], and about 50% of the total extractable tannins [24] in rapeseed meals. Kozłowska *et al.* [25] have shown that trans-sinapic acid is the predominant phenolic acid in Candle and Tower flour (97.8-99.3%) of total phenolic acids. However, sinapine

which is a choline-ester of sinapic acid, is the major phenolic acid ester [26]. The highest value of 0.99% of total sinapine was for variety RD-80 and the lowest value of 0.58% for variety Toria. Blair *et al.* [16] reported values of 0.7-4.0% and 1.3-3.3% for the sinapine content in rapeseed and Canola cotyledons respectively while Mueller *et al.* [27] reported values for the sinapine content of rapeseed meal ranging between 1.0-2.5%. Similarly another study [28] observed 0.033% cis-sinapic acid in rapeseed flour.

Total phenols. The mean values for the total phenol extractable in pure methanol and methanol/HCl (1%) solution were 0.58 and 0.47% respectively with a total of 1.05% indicating that 55% of total phenols (0.38-0.80%) were extracted in pure methanol while 45% (0.21-0.69%) in acidic methanol solution with maximum value of 1.26% in variety SM-83000 and the minimum value of 0.76% in Toria. A study [23] conducted on span rapeseed varieties showed that total ether extractable compounds constituted 1.5 and 0.2% of dehulled and diffusion extracted flour. Similarly the values for tannin content of rapeseed meal on dry matter basis ranging between 0.1% to 3% have been reported [29, 30].

Catechin. Catechin content was determined by vanillin assay which is specific for the flavan-3-ols and flavan 3, 4-diol ring systems found in condensed tannin but does not distinguish between tannin and its monomeric components [31]. Variation in catechin contents extractable in acidic methanol were more with value of 0.02% (Toria, Porbi Raya, Torch and

TABLE 1. PERCENT PHENOLIC CONSTITUENTS OF RAPESEED VARIETIES.

Varieties	Sinapine			Total phenol			Catechin		
	1	2	3	1	2	3	1	2	3
1. BSA	0.50	0.20	0.70	0.41	0.57	0.98	0.04	0.03	0.07
2. Tobin	0.54	0.25	0.79	0.47	0.61	1.08	0.05	0.07	0.12
3. Salam	0.56	0.26	0.82	0.64	0.47	1.11	0.05	0.07	0.12
4. Marmoo	0.65	0.23	0.88	0.65	0.36	1.01	0.07	0.05	0.12
5. Tower	0.70	0.22	0.92	0.80	0.21	1.01	0.06	0.08	0.14
6. Altex	0.63	0.24	0.87	0.66	0.28	0.94	0.05	0.07	0.12
7. Tatyoon	0.62	0.24	0.86	0.75	0.42	1.17	0.05	0.10	0.13
8. PR-7	0.61	0.18	0.79	0.69	0.51	1.20	0.04	0.04	0.08
9. D.G.L.	0.60	0.17	0.77	0.70	0.42	1.12	0.04	0.03	0.07
10. Toria	0.43	0.15	0.58	0.38	0.38	0.76	0.03	0.02	0.05
11. Raya N.S.	0.58	0.18	0.76	0.50	0.51	1.01	0.05	0.03	0.08
12. Porbi Raya	0.56	0.22	0.78	0.51	0.45	0.96	0.09	0.02	0.11
13. Raya Raya	0.62	0.21	0.83	0.51	0.50	1.01	0.04	0.06	0.10
14. SM-83001	0.58	0.22	0.80	0.59	0.56	1.15	0.02	0.03	0.05
15. SM-83000	0.59	0.20	0.79	0.57	0.69	1.26	0.06	0.03	0.09
16. Torch	0.54	0.34	0.88	0.53	0.48	1.01	0.01	0.02	0.03
17. Varuna	0.60	0.36	0.96	0.52	0.43	0.95	0.02	0.06	0.08
18. RD-80	0.68	0.31	0.99	0.60	0.53	1.13	0.02	0.02	0.04
Mean	0.59	0.23	0.82	0.58	0.47	1.05	0.04	0.05	0.09
CV.	10.8	24.6	11.6	19.7	24.5	11.2	44.9	53.2	37.2

1. Methanol extract; 2. Methanol/HCl (%); 3. Total of methanol + methanol/HCl extracts. The results are on as such basis. CV= Coefficient of variation.

TABLE 2. PHENOLIC CONSTITUENTS OF RAPESEED VARIETIES (Δ A550/g).

Varieties	Flavan-4-ols			Proanthocyanidine			Procyanidine		
	1	2	3	1	2	3	1	2	3
1. BSA	0.12	0.75	0.87	0.88	1.68	2.56	3.45	3.61	7.06
2. Tobin	0.63	0.12	0.75	1.60	0.75	2.35	2.40	3.24	3.64
3. Salam	0.10	0.65	0.75	1.12	1.82	2.94	2.89	3.78	6.67
4. Marnoo	0.12	1.12	1.24	1.25	1.00	2.25	3.25	3.38	6.63
5. Altex	0.75	0.20	0.95	0.88	1.05	1.93	4.51	3.04	7.55
6. Tower	0.52	1.52	2.04	0.82	0.75	1.57	3.71	2.95	6.66
7. Tatyoon	0.30	0.08	0.38	0.28	0.42	0.70	3.75	4.11	7.86
8. PR-7	1.25	0.78	2.03	1.05	0.52	1.57	3.34	4.38	7.72
9. D.G.L.	0.40	0.65	1.05	0.40	0.68	1.08	2.91	3.24	6.15
10. Toria	0.35	0.08	0.43	0.45	0.40	0.85	4.54	3.32	7.86
11. Raya N.S.	0.05	0.05	0.10	0.25	0.45	0.70	3.64	2.72	6.36
12. Porbi Raya	0.18	0.18	0.36	0.45	0.40	0.85	3.78	2.94	6.72
13. Raya Raya	0.58	0.80	1.38	0.66	1.15	1.81	4.46	2.40	6.86
14. SM-83001	0.35	0.92	1.28	0.68	0.92	1.60	4.20	3.85	8.05
15. SM-83000	0.12	0.28	0.40	0.32	0.38	0.70	3.91	4.01	7.92
16. Torch	0.12	0.12	0.24	1.92	3.18	5.10	3.52	3.78	7.30
17. Varuna	0.40	0.48	0.88	1.20	2.55	3.75	3.75	4.05	7.80
18. RD-80	0.15	1.03	1.18	1.58	2.50	4.08	5.08	4.02	9.10
Mean	0.36	0.54	0.91	0.88	1.14	2.02	3.73	3.49	7.22
CV.	83.25	79.59	61.76	56.52	74.62	62.97	17.85	15.69	11.64

1. Methanol extract; 2. Methanol/HCl (1%); 3. Total of Methanol + Methanol/HCl extracts. The results are on as such basis. CV = Coefficient of variation.

RD-80 varieties) to 0.10% (Tatyoon) as compared to catechin extractable in pure methanol giving value of 0.01% (Torch) to 0.09% (Porbi Raya). The total catechin content was highest for variety Tower (0.14%) and lowest for variety Torch (0.03%) with a mean value of 0.09% for all rapeseed varieties studied.

The results of catechin content are in agreement to that of Blair *et al.* (16). His reported mean values with the vanillin and modified vanillin methods were 0.1 and 0.36% (defatted rapeseed) and 0.15 and 0.53% (defatted Canola), respectively. In another study [32] different solvents for the recovery and determination of condensed tannins as catechin equivalent in rapeseed meals were investigated. Solvents used were absolute methanol, 1% concentrated HCl in methanol and 70% (v/v) acetone in water. The amount of catechin found was 0.059% for absolute methanol and 0.124% for 1% concentrated HCl in methanol.

Flavan-4-ols, proanthocyanidine and procyanidine. The polymeric proanthocyanidine determined as anthocyanidine by heating in strong acid while the flavan-4-ols are readily converted to anthocyanidine in acidic solvents at room temperature. The condensed tannins have the common property of forming red coloured amorphous precipitates, the so called phlobaphenes [33].

The flavan-4-ols and proanthocyanidine were extracted more in acidic methanol as compared to absolute methanol with mean values of 0.54 and 1.14 Δ A550/g respectively for acidic methanol and 0.36 and 0.88 Δ A550/g respectively for

absolute methanol. According to literature [19] the flavan-4-ols and procyanidine content for sorghum varieties were more in HCl/methanol as compared to methanol alone. The highest values of total flavan-4-ols and proanthocyanidine were 2.04 Δ A550/g in Tower and 4.075 Δ A550/g RD-80 respectively. The mean value of total procyanidine content was 7.22 with maximum value of 9.1 Δ A550/g in RD-80 variety and minimum value of 5.64 Δ A550/g in Tobin.

The results of this and earlier study [34] on the contents suggest that rapeseed meal intended for animal feeding or as a source of protein for human nutrition should be improved by developing cultivars with a low level of phenolic contents. As an interim measure, the processing of meal to remove phenolic contents should be considered, since various processing techniques (e.g. ammoniation) have been shown to be beneficial in removing these constituents [29,30]. Similarly use of different solvents can be more useful and simple method [32].

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