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Biochemical assessment of nutritional status in Indian mustard

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Abstract: The present investigation was carried out to evaluate the nutritional potential of five different Indian mustard genotypes. Fatty acid composition was determined in the oil, whereas seed meal was analyzed for limiting amino acids (tryptophan and methionine), protein content, glucosinolate content and antioxidant potential (DPPH free radical scavenging activity, total antioxidant activity and iron chelating activity). The monounsaturated fatty acids (MUFA) were found to be maximum in RH 0749 (58.70 %) followed by RH (OE) 0801 (48.91 %), JM 6011 (47.03 %), EC 597328 and EC 597340 (45.77 %). Polyunsaturated fatty acids (PUFA) were observed maximum in EC 597340 (47.45 %).Glucosinolate content ranged from 42.80 (EC 597328) to 79.79 µmole/g defatted seed meal (EC 597340). The methanolic seed meal extract exhibited a concentration dependent elimination of DPPH free radicals. All the five genotypes showed about 50 % inhibition in 3.0 mg of dry seed meal. The highest total antioxidant activity (20.41mg/g) and metal ion chelating activity (32.58 %) was observed in RH 0749. Protein content varied from 33.57 [RH (OE) 0801] to 38.01 % (RH 0749). Maximum methionine and tryptophan content were recorded in RH 0749 (0.99 and 1.01 g/100g protein, respectively). Thus, RH 0749 was observed as a potent variety in terms of total antioxidant activity, metal ion chelating activity, protein content, methionine and tryptophan content.

Keywords: Antioxidant, Fatty acids, Glucosinolates, Methoinine, Protein

INTRODUCTION

Indian mustard [*Brassica juncea*(L.) Czern & Coss.] is one of the premier oilseed crop of India. It is the second largest oilseeds crop in India after soybean. It accounts for nearly 30 % of the total production and contributes about 27 % to edible oil of the country (Sutariya *et al.,* 2011). It is an important source of edible oil and meal as well. Nutritional properties of mustard oil are determined by the fatty acid profile which includes palmitic, stearic, oleic, linoleic, linolenic, eicosenoic and erucic acids. Both linoleic and linolenic acids are essential fatty acids; however, less than 3 % linolenic acid is preferred for oil stability. A balanced ratio of these essential fatty acids in mustard oil makes it desirable for edible purposes (Singh *et al*., 2014). Linolenic acid is being essential but it also reduces the shelf life of the oil. It causes auto-oxiadtion resulting in off-flavour (Priyamedha *et al.*, 2014). Edible oil with > 2 % erucic acid is undesirable for human consumption because it causes myocardial infarction and increased blood cholesterol.

Mustard seed meal is mostly used as animal feed but can also be utilized for production of value-added products (Bala and Singh, 2012). Seed meal contains high amounts of anti-nutritional compounds known as glucosinolates, the hydrolysis products of which have been reported to be dangerous to animal health, particularly in non-ruminants as they reduce the feed palatability by affecting the iodine uptake by the thyroid gland (Bell, 1984). The recommended concentration of glucosinolates is <30 µ mole/g defatted seed meal. Seed meal of mustard is a rich source of protein (32-40 %) also. Mustard protein has a high nutritive value compared to other vegetable products due to its high biological value. The seed meal of mustard also has flavonoids, tocopherols, ascorbic acid etc. which exhibit antioxidant properties. Antioxidant compounds may function as free radical scavengers, complex of pro-oxidant metals, reducing agent and quenchers of singlet oxygen formation (Andlauer and Furst, 1998). Food industries have used effective synthetic antioxidants, consumers of food, however, prefer natural antioxidants on the basis of the assumption that natural compounds are safe (Halliwell, 2010). Thus, Indian mustard seed meal is an important source of natural antioxidants in the food industry as well as in the livestock industry for animal feed. The present study was therefore, undertaken with an objective to determine the nutritional and fatty acid profile of five different

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Indian mustard genotypes.

MATERIALS AND METHODS

Freshly threshed seeds of five genotypes of Indian mustard [RH 0749, RH (OE) 0801, JM 6011, EC 597328 and EC 597340] were used for carrying out the present study. Finely powdered seeds were then defatted with n-hexane (1 g/40 ml) for 6 h in Soxhlet's apparatus.

The methanolic extract of mustard seed meal was used for the determination of antioxidant potential *i.e.* 2, 2-diphenyl-1-picryl hydrazine scavenging activity (DPPH), total antioxidant activity (TAA) and iron chelating activity. Free radical 2, 2-diphenyl- 1-picryl hydrazine (DPPH) scavenging activity was monitored as described by Yen and Duh (1993). The percent radical scavenging capacity was calculated by the formula; A_c-A_s/A_c*100 (where A_c= absorbance of control and A_s= absorbance of samples). The total antioxidant activity was estimated by the method of Prieto *et al.* (1999). The antioxidant activity was expressed relative to that of ascorbic acid. The chelating activity of Fe^{2+} was estimated by the method of Hsu *et al.* (2003). The method is based on the principle of the Fe^{2+} chelating ability of the antioxidant by measuring the ferrous iron -ferrozine complex formed at 562 nm. The Chelating activity was calculated by the equation; Scavenging effect (%) = [1-(A_t/A_0)]*100 (where A_t is the absorbance of the sample and A_0 is the absorbance of the control at 562 nm).

Tryptophan content was determined by the method of Spies and Chambers (1949) by addition of p-methylbenzaldehyde and 19N H_2SO_4 to defatted seed meal and then this mixture was kept in dark room for 12 hours. Finally, O.D. was taken at 454 nm after addition of 0.045 % $NaNO_2$. Colorimetric method by Horn *et al.* (1946) was used for the estimation of methionine content. Firstly defatted sample was acid hydrolyzed with 2.5N HCl for 18 hours. After that dried residue was used for decolourisation by activated charcoal and volume was made up with warm distilled water. Then 5N NaOH, 10 % sodium nitroprusside, 3 % glycine and orthophosphoric acid were added and shaken well and absorption was measured at 520 nm. Protein content was determined by the standard method of Kjeldahl using a VAP-50-Gerhardt apparatus. The fatty acids were analyzed by the gas chromatography. The methyl esters of fatty acids were prepared by method of Vasudev *et al*. (2008).

RESULTS AND DISCUSSION

The mustard seed meal is superior to soybean and groundnut seed meal in terms of having essential amino acids, minerals and less content of phytic acid which reduces the bioavailability of minerals. The mustard oil is mainly utilized for edible purposes; however, depending upon the fatty acid composition, it can also be utilized for a number of non-food, fuel/non -fuel industrial products. The fatty acid composition of different Indian mustard varieties is presented in Table 1. It is evident that the newly developed variety RH 0749 synthesized high content of erucic caid (44.70 %) and low content of oleic acid (11.20 %), whereas, it had lowest content of linoleic acid (15.10 %) and highest content of linolenic acid (19.70 %) among all the genotypes studied. Oil high in oleic acid has demand in commercial food-service applications due to a long self-life and cholesterol- reducing properities (Kaushik and Agnihotri, 2000). High erucic acid content, however, is beneficial for polymer industry, whereas, low erucic acid (<2 %) is recommended for food purposes. In the present study, the genotype RH (OE) 0801 and EC 597328 synthesized the erucic acid within the permissible limits (≤ 2.0 %) and with high oleic acid content of 42.51 and 39.00 %, respectively. The erucic acid content in remaining two genotypes (EC 597340 and JM 6011) was recorded slightly in higher side (2.50 and 5.03 %, respectively) of the recommended content. All the genotypes (except RH 0749) synthesized medium content of essential fatty acids, *i.e.* linoleic acid (28.19-30.98 %) and linolenic acid (15.84-17.91 %).

Table 1. Fatty acid composition of five different Indian mustard genotypes.

Genotypes	Fatty acid (%)			
	Oleic acid	Linoleic acid	Linolenic acid	Erucic acid
RH (OE) 0801	42.51	28.45	15.84	1.51
JM 6011	39.15	28.19	17.91	5.03
EC 597328	39.00	29.73	17.50	2.00
EC597340	40.53	30.98	16.47	2.50
RH 0749	11.20	15.10	19.70	44.70

Table 2. Protein content and other biochemical parameters of five different Indian mustard genotypes.

Genotypes	Protein content (%)	Glucosinolate content (µ mole/ g defatted seed meal)	Total antioxidant activity (mg/g AAE)	Metal ion chelating activity (%)
RH (OE) 0801	33.57	60.10	17.94	24.52
JM 6011	36.02	53.60	19.85	30.25
EC 597328	33.51	42.80	18.08	24.32
EC 597340	35.43	79.79	19.44	23.35
RH 0749	38.01	63.82	20.41	32.58

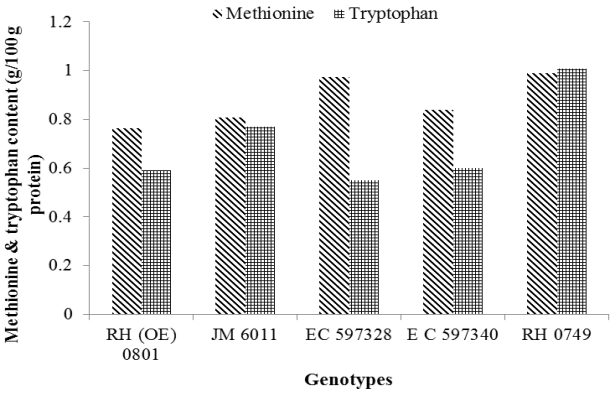

Fig. 1. *Methoinine and tryptophan content of five different Indian mustard genotypes.*

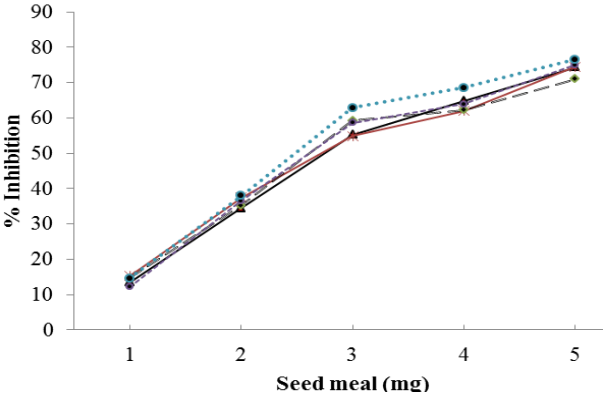

Fig. 2. *DPPH inhibition (%) in five different Indian mustard genotypes.*

The data on protein content and other biochemical parameters recorded in five different genotypes is presented in Table 2 which revealed that maximum protein content (38.01 %) was recorded in important variety RH 0749 followed byJM 6011 (36.02 %) and EC 597340 (35.43 %). Protein content in EC 597328 was minimum (33.51 %) which was at par with RH (OE) 0801 (33.57). Chauhan and his coworkers (2010) also found high amount of protein in Indian mustard. Besides having a good amount of protein, the seed meal is also rich in essential amino acids which increase its suitability for feed. The maximum tryptophan content was observed in RH 0749 (1.01 g/100g protein) followed by JM 6011 (0.77 g/100g protein), EC 597340 (0.60 g/100g protein), RH (OE) 0801 (0.59 g/100g protein) and EC 597328 (0.55 g/100g protein) (Fig. 1.) The methionine content ranged from 0.764 (RH (OE) 0801) to 0.990 g/100g protein (RH 0749) among all the tested genotypes (Fig. 1.) Verma and Baigh (2012) also observed methionine and tryptophan content, 1.129 and 0.850 g/16 g N_2, respectively in Indian mustard.

The antioxidant properties of seed meal also contribute in its increased uses in food industries and health management. To check the antioxidant potential all the five genotypes were analyzed for DPPH activity, total antioxidant activity and metal ion chelating activity. For determining the DPPH activity, IC_{50} value was determined in methanolic extract of seed meal. All the genotypes showed about 50 % inhibition in the concentration of 3.0 mg dry seed meal (Fig. 2). These results are in accordance with those of Kumari *et al*. (2016), while Dua *et al*. (2014) who reported IC_{50} value 2-25 mg of seed meal. Saxena and his coworkers (2011) also found that the methanolic extract of fenugreek was better for determining free radical scavenging activity than any other solvent. The highest antioxidant activity was recorded in RH 0749 (20.41 mg/g) followed by JM 6011 (19.85 mg/g), EC 597340 (19.44 mg/g), EC 597328 (18.08 mg/g) and RH (OE) 0801 (17.94 mg/g) (Table 2). Similarly, high antioxidant activity in Indian mustard was also reported by Bala *et al*., (2011) and Kumari *et al*., (2016). Natural plant based antioxidants are far better than the artificial one because of their less allergenic properties and ethical acceptance. Transition-metals like iron, nickel and copper are well known for their functions as biocatalyst and pro-oxidant functions, which promote the undesirable oxidation. The metal chelating activity in all the genotypes varied from 23.35 (EC 597340) to 32.58 (RH 0749) % (Table 2). Similar results were also reported earlier by Ishtiaque *et al*. (2013) in Indian mustard.

Apart being nutritional better, the major drawback of seed meal of Indian mustard is glucosinolates, which are well known for their antithyriod effect and reduction in feed palatability and iron absorption (Bille *et al*, 1983). The glucosinolate content ranged from 63.82 – 79.79 µ mole/g defatted seed meal. The highest glucosinolate content was recorded in the genotype EC 597340 (79.79 µ mole/g defatted seed meal) followed by RH 0749 (63.82 µ mole/g defatted seed meal**),** RH (OE) 0801 (60.10µ mole/g defatted seed meal), JM 6011 (53.60 µ mole/g defatted seed meal) while minimum glucosinolate content was observed in JM 6011 (42.80 µ mole/g defatted seed meal) (Table 2). Thus, the present study revealed that Indian mustard genotypes investigated had, in general, low level of saturated fatty acids (<7 %). The methanolic extract of seed meal had different levels of antioxidant activity, metal ion chelating activity and scavenging effect on free radicals. Among the tested genotypes, RH 0749 performed better in terms of protein content, antioxidant activity and limiting amino acids. Present study therefore, might be helpful in breeding of Indian mustard germplasm having desirable characterstics with enhanced health promoting and nutritive qualities.

Conclusion

The seed meal of Indian mustard genotypes was observed to be qualitatively better in terms of limiting amino acids (methionine (0.764 to 0.990 g/100g protein) and tryptophan (0.55 to 1.01 g/100g protein) and antioxidants (The total antioxidant activity ranged

from 17.94 mg/g to 20.41 mg/g, The metal chelating activity varied from 23.35 to 32.58 % *etc*.).This can be used as a source of natural antioxidant in food industries and animal feed as well. The variety RH 0749 was observed as a potent variety due to higher total antioxidant activity, metal ion chelating activity, protein content, methionine and tryptophan content.

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