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Quality characteristics in rapeseed-mustard and role of some anti-nutritional factors in plant defense: future strategies

Sandeep Kumar[1*], Dhiraj Singh[2] and M Dutta[1]

[1] *National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012;*
[2] *Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur, Rajasthan-321 303,*
Corresponding author: kumarsandeep_biochem@rediffmail.com

Abstract

Recommendations for use of rapeseed-mustard oil and seed meal for consumption purposes particularly presence of low saturated fat, high oleic acid, balanced ratio of SFA/MUFA/PUFA and antioxidants, and absence of trans-fat and minimum anti-nutritional factors, are generally different from those of industrial requirements. Further, some anti-nutritional factors present in rapeseed-mustard crops are known to be associated with some plant defense system and other important biological functions. These factors have brought the crop quality research on top with the objective of producing designer crops specific for consumption and industrial purposes. For producing such designer crops through conventional breeding, screening of the available germplasm for specific traits and their further utilization in crop improvement programme becomes necessary. An integrated approach including plant breeding, genetics, biochemistry and biotechnology including bioinformatics is essential in identifying new related biomarkers, or elucidating the chemistry of regulation of various biotic and abiotic stresses. In the present review, information regarding rapeseed-mustard quality characteristics and on correlation of some anti-nutritional factors with some biotic and abiotic stresses has been compiled.

Key words: *Anti-nutritional factors, plant defense, rapeseed-mustard, quality characteristics*

Introduction

Quality is a comparative term essential to identify a germplasm with better nutritive value. Now days, consumers are not only concerned about what they eat but also how the consumed ingredients affect their health. A large variability in nutritional quality parameters exists not only between different oilseed crops, but also between *Brassica* species/genotypes. Although, oil content is the major quality parameter, seed meal protein content is also very important as it can be used to eliminate protein malnutrition in animals. Advancement in the non-destructive techniques has greatly helped in rapid screening of germplasm for various traits, and also in the identification of isomeric forms (n-7 and n-9 etc.) of fatty acids. Rapeseed-mustard oil quality is determined by the constituent fatty acids including palmitic, stearic, oleic, linoleic, linolenic, eicosenoic and erucic acids. Linoleic and linolenic acids are essential fatty acids not synthesized by our body. Erucic acid, although, anti-nutritional and should be <2% in the edible oil, higher erucic acid is of considerable industrial importance. Information regarding concentration of saturated fat, trans fat, linoleic (ω6) to linolenic (ω3) acids ratio, saturated fatty acids (SFA)/monounsaturated fatty acids (MUFA)/polyunsaturated fatty acids (PUFA) ratio, concentration of anti-nutritional factors (erucic acid, glucosinolates, phytic acid, sinapic acid and tannins etc.), presence of various phenolic compounds acting as an antioxidants and taste determinants, reducing seed meal fibre content, association of glucosinolates with different biotic and abiotic stress factors and their role in plant defense systems and other biological activities including anti-cancerous activities, use of various *Brassica* plant parts as

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biofumigants due to presence of isothiocyanates, factors affecting glucosinolates synthesis and degradation have been reviewed. Further mechanism of myrosinase-sinigrin interaction along with active site mapping of myrosinase in *B. juncea*, importance of '00' i.e. canola oil, and usefulness of stage specific profiling of metabolites and their correlation with expression pattern using transcriptomics in identifying individual glucosinolates or other new biomarkers with respective functions have also been thoroughly reviewed to guide future research.

Quality characteristics of rapeseed-mustard

Quality comes from Latin word 'Qualitas' which means attribute, property or basic nature of an object. Quality also means degree of excellence or superiority. Therefore, quality is a relative term and should be defined in the context of the purpose e.g. a variety is rich in oleic acid, but the same variety may be deficient in linolenic acid. Nutritional quality of rapeseed-mustard seed is determined by oil content and its fatty acid constituents, protein content and respective amino acids concentration, crude fibre and various anti-nutritional factors including glucosinolates, phytic acid, sinapine etc.

One of the most remarkable characteristics of *Brassica* species is the high oil content in the seeds, ranging in wild types from about 17 - >40% (Kumar and Tsunoda, 1980) and 21.5-46.7% (Mandal *et al*., 2002). Rapeseed-Mustard seed protein content ranged from 17.8-22.0%. Fatty acids present in rapeseed-mustard oil are both saturated and unsaturated types. Saturated fatty acids contain only single bonds in their chemical structure, whereas unsaturated fatty acids also contain double or triple bonds. Higher concentration of saturated fatty acids in the edible oil is harmful for human health. Rapeseed-mustard oil contains <7% saturated fatty acids. The concentration of oleic acid (18:1), a beneficial monounsaturated fatty acid, ranges from 3.6-32.2% in rapeseed-mustard oil (Chauhan and Kumar, 2011). It also contains a considerable amount of essential fatty acids including linoleic (18:2) and linolenic (18:3) acids. Although, linolenic acid confers some nutritional benefits, its higher concentration makes the oil highly susceptible to oxidation Mead, 1977). Erucic acid (22:1) which constitutes <40 to 60% of the total fat (Mandal *et al*., 2002) is undesirable in mustard oil. Consumption of high erucic acid in the diet is known to cause impaired myocardial conductance, myocardial fibrosis, lipidosis, and increased blood cholesterol (Gopalan *et al*., 1974; Ackman *et al*., 1977). Its anti-nutritional property is mainly attributed for it's metabolically inertness, as it does not enter into the Beta-oxidation pathway to produce ATP (Rogers, 1978). So, oils with <2% erucic acid are recommended for consumption purposes. Quality characteristics of rapeseed-mustard oil have also been reported by earlier workers (Velasco *et al*., 1998; Mandal *et al*., 2002; Chauhan *et al*., 2007). Various quality characteristics of rapeseed-mustard oil in comparison to other oilseed crops are listed in Table 1. Latest technologies have led to the identification of isomeric forms of fatty acids. In our recent study regarding the distribution of (n-9) and (n-7) isomers of monounsaturated fatty acids in Indian mustard, we found that palmitoleic acid is an important intermediate component in the synthesis of long chain (n-7) fatty acids (Bhogal *et al*., 2014).

SFA/MUFA/PUFA and ω6/ω3 ratio

Rapeseed-mustard oil contains <7% saturated fat along with higher amounts of monounsaturated fatty acids. But for good quality edible oil, ratios of SFA, MUFA and PUFA, and linoleic /linolenic acids (ω6/ω3) play a very important role. SFA/MUFA/PUFA and ω6/ω3 ratios of rapeseed-mustard oil in comparison to some other major oilseeds are listed in Table 2. From the comparison, it appears that no single oil contains suitable ratios of SFA/MUFA/PUFA and ω6/ω3 ratios as per international recommendations i.e. 1:1-3:1 and 5-10:1, respectively.

Seed meal quality characteristics

After extraction of oil, the remaining portion of seed is known as seed meal. Seed meal of rabi oilseed crops is a rich source of protein (35-40%) and crude fibre (10-12%). Rapeseed-mustard seed meal protein is balanced with respect to all amino acids except methionine and can be used to eliminate protein malnutrition in animals. Rapeseed-mustard seed meal also contains high amount of glucosinolates (<30->120 µmole/g defatted seed meal), the

Table 1: Quality characteristics (%) of rapeseed-mustard seed oil in comparison to other crops

Oils	Oil content	Protein content	Palmitic + Stearic acids C16:0+C18:0	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3	Arachidic acid C20:0	Eicosenoic acid C20:1	Behenic acid 22:0	Erucic acid C22:1	References
Rapeseed-mustard	34.9-44.9	17.8-22.0	4.4	13.3	18.4	15.7		7.2		41.4	Chauhan *et al*., 2011
Soybean	8.1-27.9	34.1-56.8	14	18	55	13	-	-	-	-	Clemente and Cahoon, 2009
Groundnut	33.60-54.95	18.92-30.53	10.69	58.69	21.76	0.34	1.83	-	3.88	-	Asibuo *et al*., 2008, Aluyor *et al*., 2009
Sunflower	19.8-26.7	10-25	10.9	31.5	52.8	0.4	0.4	0.2	0.7	-	Rafalowski *et al*., 2008

Table 2: SFA:MUFA:PUFA* and Omega6/Omega3* ratio of rapeseed-mustard oil in comparision to other oils

Oils	SFA	MUFA	PUFA	SFA:MUFA:PUFA	ω6/ω3
Rapeseed-Mustard	4.4	61.9	34.1	1:14.07:7.75	1.17
Soybean	14	18	68	1:1.29:4.86	4.23
Groundnut	16.82	58.79	22.11	1:3.49:1.31	64
Sunflower	12	31.8	53.2	1:2.65:4.43	132

*Calculated on the basis of values listed in Table 1.

hydrolysis products of which have been reported to be detrimental to animal health, particularly in non-ruminants, as they reduce the feed palatability, adversely affect the iodine uptake by the thyroid glands and thus reduce feed efficiency and weight gains (Fenewick *et al.*, 1983; Bell, 1984). The recommended concentration of glucosinolates is <30 µmole/g defatted seed meal. In India, some *B. napus* varieties with double low '00' have been developed and efforts are also continuing in case of Indian mustard (AICRP-RM Annual Report, 2006-10). Rapeseed-mustard seed meal also contains ash (4–6%), minerals and vitamins (1.0–1.5%), tannins (1.6–3.1%), sinapin (1.0–1.5%) and phytic acid (3–6%) (Chauhan *et al.*, 2002). Glucosinolates, fibres, tannins, phytic acid, and sinapin, all reduce feed value of seed meal.

International quality standards

According to internationally accepted quality norms, saturated fat should be < 7% as the sum of all saturated fatty acids C16:0 + C18:0 + C20:0 + C22:0 etc., whereas oleic acid (C18:1) content should be high. SFA:MUFA:PUFA ratio should be in the range of 1:1-3:1, PUFA:SFA ratio should be between 0.8-1.0 and linoleic acid (/ω6): linolenic acid ω3) ratio should be between 5-10 (Kale, 2007; WHO/FAO, 2003). Trans fatty acids should be absent because of their ill effects on health, and antioxidants including tocopherols should be present to avoid the oxidation of oil. Both, oil and seed meal should be free or at non-toxic levels of anti-nutritional constituents including erucic acid <2% in edible oil and glucosinolates <30 µmole/g defatted seed meal (Thacker, 1990).

Glucosinolates, their degradation products, related complexity and biological functions

Glucosinolates are natural secondary metabolites belonging to S-glycosides class, which share a core structure containing a Beta-D-glucopyranose residue linked via a sulfur atom to a (Z)-N-hydroximino sulfate ester, and are distinguished from each other by a variable R group derived from one of several amino acids (Fahey *et al.*, 2001; Stoin *et al.*, 2009). Based on the chemical structure, they can be classified as aliphatic, aromatic, x-methylthioalkyl and heterocyclic (e.g., indole) glucosinolates (Fahey *et al.*, 2001). Myrosinase catalysed degradation involves cleavage of the thioglucoside linkage yielding D-glucose and an unstable thiohydroximate-O-sulphonate that spontaneously rearranged, resulting in the production of sulphate and one or more of a wide range of products (Kelly *et al.*, 1998). Their degradation products generally include a thiocyanate, isothiocyanate or nitrile depending on factors such as substrate, pH or the availability of ferrous ions. Till date, over 130 glucosinolates have been identified, of which more than 30 are present in *Brassica* species (Fahey *et al.*, 2001). Recently, we determined the mechanism of myrosinase-sinigrin interaction in *B. juncea* and also the amino acid residues constituting the myrosinase active site including those involved in the enzyme-substrate interactions (Kumar *et al.*, 2011). It was the first study of its kind in the Indian mustard which will help in deciding strategies for controlled expression of myrosinase-sinigrin system. The myrosinase catalyses sinigrin not via acid-base catalysis, but with the mechanism involving glycosylation and deglycosylation of the enzyme.

Both synthesis and degradation of glucosinolates are influenced by a number of factors. The quantitative as well as qualitative variations for glucosinolate content have been reported among various *Brassica* species, genotypes/cultivars of the same species (Kirkegaard and Sarwar, 1998; Rangkadilok *et al.*, 2002), and even in different tissues of the same plant with respect to plant age and environmental growth conditions (Clossais-Besnard and Larher, 1991; Sarwar and Kirkegaard, 1998). Further, developmental changes have also been reported in myrosinase expression patterns (Lenman *et al.*, 1993) which elucidate that the specific hydrolytic products of glucosinolates are required by the plant under certain situations or developmental stages.

In recent years, myrosinase catalysed degradative products of glucosinolates have gained considerable attention for their unique biological and chemical properties, including biodegradable biocides comprising antifungal, nematicidal and insecticidal activities (Brown and Morra, 1997; Buskov *et al.*, 2002; Kirkegaard and Sarwar, 1998; Yu *et al*., 2005), deterrents of generalist herbivores (Giamoustaris and Mithen, 1995), herbicidal (Vaughn *et al*., 2006), and allelopathic actions (Hill *et al*., 2003). Glucosinolates have been reported with anticarcinigenic activity (Keck and Finley, 2004; Gamet-Payrastre *et al*., 2000; Agerbirk *et al*., 1998; Bonnesen *et al*., 1999; Faulkner *et al*., 1998; Smiechowska *et al*., 2008); indole-3-carbinol (I3C) and its dimeric product, 3, 3´-diindolylmethane, have been widely investigated for their effectiveness against a number of human cancers *in vitro* as well as *in vivo* (Ahmad *et al*., 2011). Further, myrosinase is also involved in the production of some phyto-hormones from their inactive precursor glucosinolates (Bones and Rossiter, 1996), potentially in sulphur and nitrogen metabolism (Schnug, 1990) and growth regulation. In addition, some of the hydrolytic products could be used as an important intermediate in chemical synthesis (Gueyrard *et al*., 2000).

The complexity of the glucosinolate-myrosinase system also known as "mustard-oil bomb" suggests a diverse and multifunctional role in the life of cruciferous plants (Kelly *et al*., 1998). In foods developed from certain Brassicaceae vegetables (cabbage, cauliflower, and broccoli) and condiments (mustard, horseradish, and wasabi), the distinct taste and flavors are primarily due to isothiocyanates i.e. the hydrolysis products of glucosinolates. Indole glucosinolates and those with alkenyl R groups are especially known for causing bitterness (Engel *et al*., 2002). The defense mechanism in case of Brassicaceae is predominantly due to glucosinolates (Bjorkman *et al*., 2011) and their decomposition products (Broekgaarden *et al*., 2008; Pratt *et al*., 2008). Glucosinolates degradation products such as isothiocyanates and nitriles act as feeding repellents and toxic to herbivores (Ratzka *et al*., 2002). They affect individual group of pests, generalists or specialists, differently (Lankau, 2007; Muller *et al*., 2010). Research carried out at Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur, India also established a significant negative correlation between aphid infestation and total glucosinolates (AICRP-RM Annual Report, 2006-2010).

Challenges for Rapeseed-Mustard Workers

* Development of double low "00" Indian mustard varieties with erucic acid < 2% and glucosinolate content less than 30 µmole/g defatted seed meal and to achieve the balanced ratio of SFA/MUFA/PUFA and ω6/ω3.

* The recommendations for fat consumption particularly presence of low saturated fat, high oleic acid, balanced ratio of SFA/MUFA/PUFA etc. are different from that of industrial requirements. Research should be focussed towards development of varieties with different oil qualities for consumption purposes including <7% saturated fat, upto 70% oleic acid, 40-50% linoleic acid (High linoleic) and '0' erucic acid (<2%), and for industrial purposes including high stearic acid (20-40%) for margarines, very low linolenic acid (<3%) for prolonged shelf life and margarines, and high erucic acid (40-50%) for industrial polymers, lubricants and plastic industries and >50% erucic acid for cosmetics and pharmaceuticals.

* Glucosinolates are involved in a number of activities and since their synthesis and degradation is also influenced by a number of factors, there is a need to identify conditions leading to specific degradation products and also to determine specific compound responsible for a particular activity.

Future Strategies

Designer Crops

As, the quality characteristics required for consumption purposes are different from those of industrial or plant defense purposes, designer crops could provide a solution. Designer crops with varying chemical composition such as low (> 3.5%) and high (>20%) linolenic acid; mid (65-75%) and high (>75%) oleic acid; high erucic acid-high glucosinolate (HEAR); low erucic acid-high glucosinolate (LEAR); high erucic acid-low glucosinolate; low erucic acid-low glucosinolate (double low quality, canola); and balanced ratios of SFA/MUFA/PUFA and omega6/omega3 etc. can be produced depending upon the purpose. For production of these designer crops, large scale screening of rapeseed-mustard germplasm conserved in the national genebank is necessary for identification of trait specific germplasm and their further utilization in crop improvement programme.

Integrated use of different disciplines for identification of new biomarkers and to establish their functions

With the changing scenario, glucosinolates and their degradative products should be studied in more details to establish their roles. There is also a need to identify specific glucosinolates with respective functions. This is possible only with an integrated interdisciplinary approach with plant breeding, genetics, biochemistry, physiology, entomology, pathology and biotechnology using bioinformatic tools to identify new biomarkers for specific traits or to elucidate the chemistry of regulation. Stage specific untargeted profiling of metabolites and their correlation with expression pattern using transcriptomics can play a major role in identifying new biomarkers. This will allow production of designer *Brassica* crops with up- or down-regulation for synthesis of specific glucosinolates through breeding programs. Recently, translocators essential for transfer of glucosinolates to seeds have been identified (Eldin *et al*., 2012) which can be used as a mean to control allocation of defense compounds in a tissue specific manner.

In case of biotic stresses, other than untargeted approach, new methodologies can be applied on both, insect-pest or pathogen and the host plant, for studying various biochemical changes. Recently, we published a common method suitable for amino acid profiling of both, insect-pests and their host plant parts (Dhillon *et al*., 2014).

With the changing delopment scenario, demands for different rapeseed-mustard quality characteristics are changing. Designer crops with specific combinations of chemical quality characteristics will be required to fulfil the increasing future demands from various sectors. For anti-nutritional factors such as glucosinolates, there is a need to identify appropriate conditions conducive to synthesis and degradation of specific compounds and to establish their roles. An integrated interdisciplinary approach is required for identification of new biomarkers and chemistry of regulation etc.

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