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Carbohydrate composition of low dose radiation-processed legumes and reduction in flatulence factors

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Abstract

The effects of low dose γ-radiation processing, for insect disinfestation, on functionally important sugars, were investigated in commonly used legumes i.e. mung, Bengal gram, horse beans (val), horse gram, cowpeas and rajma. The separation profiles of legume carbohydrates were qualitatively comparable; distinct legume-specific quantitative changes were observed. The main flatulence-producing raffinose family oligosaccharides (RFO), stachyose and verbascose, which constituted 55–65% of soluble carbohydrates in these legumes, were degraded at different rates during 0–4 days of germination, with concomitant accumulation of easily metabolizable sucrose and reducing sugars. Radiation processing enhanced this in a legume-specific manner. Subtle differences in degradations of these oligosaccharides, between control and irradiated samples (0.25 and 0.75 kGy) were observed in the dry seeds of Bengal gram, horse beans (val), and cow peas; these were highly significant in mung and horse gram on the second day of germination and no change was noticed in rajma. Degradation of flatulence factors reflected an accumulation of sucrose in Bengal gram, cow peas and rajma rather than reducing sugars, which were more prominent in mung and horse gram. These results conclusively indicate that radiation processing of the six legumes, at disinfestation dose (0.25 kGy) and germination (0–2 days), results in rapid degradation of flatulence factors without affecting their sprout lengths; this improves their nutritional acceptability, though subtle varietal differences are noticed. At higher dose (0.75kGy), significant reductions in their sprout lengths compared to the control were noticed; however, their sensory attributes were not altered.

Keywords: Legumes; γ-irradiation; Germination; Flatulence-factors; Carbohydrates

1. Introduction

Legumes contain two- to three-fold more proteins than cereals, besides being good sources of dietary carbohydrates. Plant proteins are now identified as biologically active and functionally versatile dietary components and are cheaper substitutes than animal proteins (FAO, 1990). Legumes are widely grown in semi-arid regions while cereals require more water and intensive cultivation. Supplementing cereal-based diets with legumes improves overall nutritional status and is one of the best solutions to protein calorie malnutrition in the developing countries. Legumes also have the potential to lower cholesterol and serum glucose and quicken adjustment to high altitude induced stress (Mazur, Duke, Wahala, Rasku, & Adlerventz, 1998). However, the presence of certain antinutritional factors, such as flatulence causing raffinose family oligosaccharides (RFO), limits their biological value and acceptance as a regular food item (Reddy, Pierson, & Sathe, 1984).

Verbascose, stachyose and raffinose (RFO, α-galactosyl derivates of sucrose) are associated with desiccation tolerance and storability of seeds (Obendorf, 1997) and are also flatulence producing. Humans do not have the enzyme α-galactosidase to cleave the α-galactosyl linkage and the intact oligosaccharide is not absorbed by the digestive tract (Reddy et al., 1984). These oligosaccharides accumulate in the large intestine where the α-galactosidase containing intestinal bacteria degrade them and subsequent anaerobic fermentation (Fleming, 1981) results in production of H_2, CO_2 and traces of CH_4. These gases cause abdominal discomfort due to a flatus effect and sometimes result in diarrhoea. Essential removal of these compounds would improve the nutritional quality of legumes. This may be achieved by processing, plant breeding and molecular biology techniques. It is highly desirable to decrease the oligosaccharide content of legumes if they are to be more effectively exploited as relatively cheap substitutes for good quality proteins. Aqueous or alcoholic extraction of legumes, changes in pH, temperature or humidity leads to reduction in flatulence (Calloway, Hickey, &

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Murphy, 1971). Degradation of RFO could also be achieved by treatment with microbial or plant α-galactosidase. All these steps, however, affect their sensory attributes (Somiari & Balogh, 1995), thereby lowering their acceptability. Degradation of RFOs during germination by endogenously synthesized α-galactosidase, is also well documented (McCleary & Matheson, 1974).

Legumes are easily prone to insect infestation. It has been established that radiation processing of legumes with γ-rays, at low dose (0.25 kGy), effectively eradicates insect pests at all life stages and extends their shelf-life (Tilton & Burditt, 1983). γ-Radiation treatment of mung beans, at low dose for insect disinfestation (Machaiah, Pednekar, & Thomas, 1999), also enhances nutritional quality by rapid degradation of flatulence-causing RFO.

Commonly used legumes, such as mung, Bengal gram, horse beans (val), horse gram and cow peas, are consumed in our country, often in dry, as well as in sprouted conditions. They are also consumed in the early seedling stage along with salads and noodle preparations in many Southeast Asian countries. Legumes of the same species exhibit wide variations in their carbohydrate profiles. Many authors have reported studies on the carbohydrate composition of legumes (Eskin, Johnson, Vaisey Genser, & McDonald, 1980; Labaneiah & Luh, 1981). However, in some of these reports, which have characterized the degradation of flatulence factors, the quantitation of individual carbohydrates is not clear, due to inconclusive resolution of sugars, attributable to the techniques used (Aman, 1979). The oligosaccharide separation by GC, which requires derivatization, also gave problems due to their high molecular weight.

The current studies were undertaken to see the effect of radiation processing on the degradation patterns of flatulent factors in different legumes. Comparative profiles of degradation were obtained and individual sugars were quantitated using HPLC, all the sugars being separated into distinct peaks. The legume-specific variation in degradation of flatulent factors, in response to germination and radiation treatment, has been systematically evaluated.

2. Materials and methods

2.1. Materials

Legumes, mung (*Vigna radiata*), Bengal gram (*Cicer arietinum*), horse beans (Val, *Vicia faba*), horse gram (*Dolichos biflorus*), cow peas (*Vigna sinensis*) and Rajma (*Phaseolus vulgaris*), were purchased from a local market, packed in polythene pouches (100 g lots) and irradiated at 0.25 and 0.75 kGy dose levels in a Cobalt-60 Gamma Cell 220 (AECL, Canada) at 25 °C. The dose rate was 18 Gy/min and the overdose ratio was 40%.The overdose ratio is the amount of dose dissipated, as the dose is adjusted as the mean of minimum and maximum dose levels. The disinfestation dose being low and the package of the commodity big, the overdose ratio is kept at 40% to ensure that the minimum required dose is delivered. The unit 1 kGy = 1000 Gy; 1 Gy (Gray) is equivalent to 1 J of energy received by 1 kg of mass. The irradiated samples were stored for 2 weeks at 25 °C prior to the experiments.

All the authentic standards of sugars were obtained from Sigma Chemical Co., USA. All the reagents were prepared from analytical grade chemicals and the solvents used were all re distilled prior to the experiment.

2.2. Germination of legumes and extraction of carbohydrates

The germination of these legumes (in 2 g lots, 0–4 days) and extraction of total soluble carbohydrates was carried out essentially as described earlier (Machaiah et al. 1999). Rajma was not germinated for longer periods as it is generally not sprouted before consumption. Seedling height was measured on each day of germination, by marking the length of the seedling on a thread and reading it with a graduated scale. The total carbohydrates were estimated colorimetrically by the phenol sulfuric acid method (Dubois, Gilles, Hamilton, Robers, & Smith, 1956) using glucose as the standard. The total oligosaccharides were estimated by the thiobarbituric acid method, using sucrose as the standard (Tanaka, Thananunkul, Lee, & Chickester, 1975).

2.3. Separation and quantitation of carbohydrates

The concentrated crude extracts were passed through Dowex $50H^+$ and Amberlite IR-45 resins and evaporated to dryness under vacuum. The residue was dissolved in 300 μl of double-distilled water and aliquots of these samples [≅ 300 μg (10–15 μl)] were initially separated by paper chromatography (Trevelyan, Proctor, & Harrison, 1950) and the carbohydrates were identified and qualitatively analyzed. Further, they were separated by HPLC (LKB-Pharmacia LCC-225) on a micro bondapack carbohydrate column connected to a refractive index (RI) detector, using acetonitrile:water (75:25 v/v) as the eluting solvent, under the conditions described previously (Machaiah et al., 1999). Recording was done on a Varian 4290 integrator. Authentic standards of sugars identified by paper chromatography, were run separately at different concentrations and also in a mixture. Identification and quantitation were done by comparing the retention time with that of standards and multiplying by corresponding factors. The factor was derived by running standards at different concentrations and drawing a concentration curve for each sugar.

Standard deviation was calculated and probability was evaluated by the Student's *t*-test method.

3. Results

3.1. Seedling height

The seedling height increased rapidly in horse bean (Table 1). Mung and horse gram germinated very uniformly, followed by cow peas and Bengal gram; in the control samples, seedling heights were all comparable. On the 4th day of germination they were around 4 cm (Table 1). At the disinfestation dose (0.25 kGy), there was no difference in seedling height in mung, Bengal gram, horse gram or cow peas on the 4th day of germination, though some differences were observed earlier. In horse beans, which exhibited a higher rate of germination, the difference in seedling height between control and 0.25 kGy was not significant up to 3 days; it became highly significant on the 4th day ($P<0.0001$; Table 1). At the higher dose of radiation (0.75 kGy), the reduction in seedling height compared to control was highly significant (<0.0001) in all the legumes at different stages of germination (1–4 days).

3.2. Total carbohydrates

The total carbohydrates did not change significantly due to germination or radiation treatment up to 2 days of germination in these legumes. It was about 30–40 mg g^{-1} (dry weight). However, on the 4th day of germination it increased significantly in mung, horse beans and cow peas; the rate of increase was lower in the irradiated samples (0.25 and 0.75 kGy) compared to the control (Fig. 1). It remained unchanged in Bengal gram and horse gram up to 4 days of germination.

Total oligosaccharides in the extracts, which represent all the nonreducing sugars, were about 20 mg g^{-1} in dry seeds of mung, horse beans and horse gram; this content was higher (25 mg g^{-1}) in Bengal gram and cow peas (Fig. 2). As in the case of total carbohydrates, there was no change in the total oligosaccharides up to 2 days of germination. It increased on the 4th day in all the samples, except Bengal gram where a significant decrease was observed. Compared to other legumes, rajma had lower levels of soluble carbohydrates and oligosaccharides, (20–30 and 15 mg g^{-1}, respectively) and there was no change due to radiation treatment.

3.3. Quantitation of carbohydrates by HPLC

Legume carbohydrates were analyzed qualitatively by paper chromatography and quantitation was done by HPLC. A typical HPLC profile (Fig. 3) of Bengal gram and horse beans, clearly indicates the presence of 9–10 carbohydrates, i.e. xylose, fructose, glucose, galactose, sucrose, mellibiose, raffinose, stachyose and verbascose. The sugars were quantitated and their distribution was expressed as a percentage of total. One peak between raffinose and stachyose could not be identified; it constituted $<0.5\%$ in the dry seeds of horse beans and was higher in Bengal gram and cow peas (4–9%), but not detected in other legumes. Some authors (Bernabe et al.,

Table 1
Seedling height[a] (cm) of germinating legumes

Legumes	Radiation dose (kGy)	Germination time (days)			
		1	2	3	4
Mung	Control	0.73±0.12	1.86±0.22	2.84±0.28	4.24±0.75
	0.25	0.63±0.14 d	1.14±0.32 a	2.21±0.56 b	4.61 ±0.61 n.s.
	0.75	0.53±0.17 b	1.07±0.31a	1.24±0.2 a	3.21±0.33 a
Bengal gram	Control	0.61±0.1	2.95±0.2	3.51±0.62	3.61±0.61
	0.25	0.59 ±0.08 n.s.	2.48±0.36 a	3.39±0.6 n.s.	3.49 ±0.57 n.s.
	0.75	0.54±0.05 c	2.45±0.18a	2.86±0.35 c	3.01±0.43 b
Horse beans	Control	1.6±0.35	3.23±0.83	6.55±1.49	11.26±1.21
	0.25	1.35±0.56 n.s.	3.25±0.94 n.s.	6.06±0.99 n.s.	9.0±1.82 a
	0.75	1.15±0.36 b	3.01±0.61 n.s.	5.69±0.61 d	6.33±1.03 a
Horse gram	Control	1.28±0.13	2.13±0.31	4.17±0.53	4.13±0.06
	0.25	1.02±0.23 n.s.	1.8±0.17 b	3.79±0.32 a	4.14±0.42 n.s.
	0.75	0.77±0.13 b	1.35±0.34 a	2.94±0.38 a	3.55±0.5 b
Cow peas	Control	2.45±0.25	2.68±0.21	4.2±0.95	4.15±0.19
	0.25	2.26±0.23 d	2.28±0.16 a	2.65±0.32 a	3.9±0.48 n.s.
	0.75	1.62±0.13 b	2.2±0.16 a	2.32±0.17 a	3.22±0.17 a

[a] Seedling height of 12–15 sprouts from each group was measured (in cm) and the statistical evaluation was done as described in the text. The level of significance in each sample was expressed as a,b,c and d representing the P values <0.0001, <0.001, <0.01 and <0.05 respectively. n.s., not significant.

1993; Quemeuer & Brillonet,1983) have identified this peak to be ciceritol by enzymatic assays, GC/MS and NMR techniques. Recently Sanchez-Mata, Penuela-Ternel, Camara Hurtado, Diez-Marquis, & Torija-Isasa, (1998) reported ciceritol to be the main α-galactoside in chick peas (*Cicer arietinum* L.) but we found stachyose and verbascose to be the major sugars (55%). Though ribose has been reported to be a major pentose in peas and lentils (Sanchez-Mata et al., 1998), we found the presence only of xylose in some of the legumes. This was confirmed both by paper chromatography (RF values) and HPLC (retention time), using the authentic standard. Xylose was nearly absent in the dry and early germination stages. However, after 2 days of germination, it appeared in small amounts ($<2\%$) and increased to 5–8% on the 4th day of germination. In horse beans, which had a rapid rate of germination, the higher xylose content could be correlated with a greater seedling height (Table 1). Presence of xylose has been confirmed in horse beans (Cerning, Sapsonik, & Guilbot, 1975) and winged beans (Sajjan & Wankhede, 1981) and it is said to be a part of structural polysaccharides, such as like hemicellulose. The hexoses, fructose, glucose and galactose, constituting the main reducing sugars, were very low in dry seeds and increased with the onset of germination (Fig. 4A). This was a common observation in all the legumes; the increase is attributed to concomitant degradation of the RFO, which exhibited distinct legume specific and radiation related differences (Fig. 4B).

In mung, horse beans, Bengal gram and horse gram, the reducing sugars increased sharply after overnight soaking and accounted for 40–50% of the total sugars (Fig. 4A) after 2 days of germination. This corroborated with the reduction in raffinose family oligosaccharides from 50–60% in dry seeds (Fig. 4B) to less than 10% on the 4th day of germination in all the samples. Rapid degradation of RFO in irradiated samples, compared to the unirradiated controls, which was reflected in an increase of reducing sugars was observed in mung, horse beans, Bengal gram and horse gram, though the extent varied. In cow peas, however, there were no significant radiation-related changes in these components up to 4 days of germination (Fig. 4A). It was interesting to note that the increase in reducing sugars (40–50% of total), could be attributed mainly to fructose and glucose, while galactose, which was a main product of the action of α-galacatosidase on RFO, was present only in small

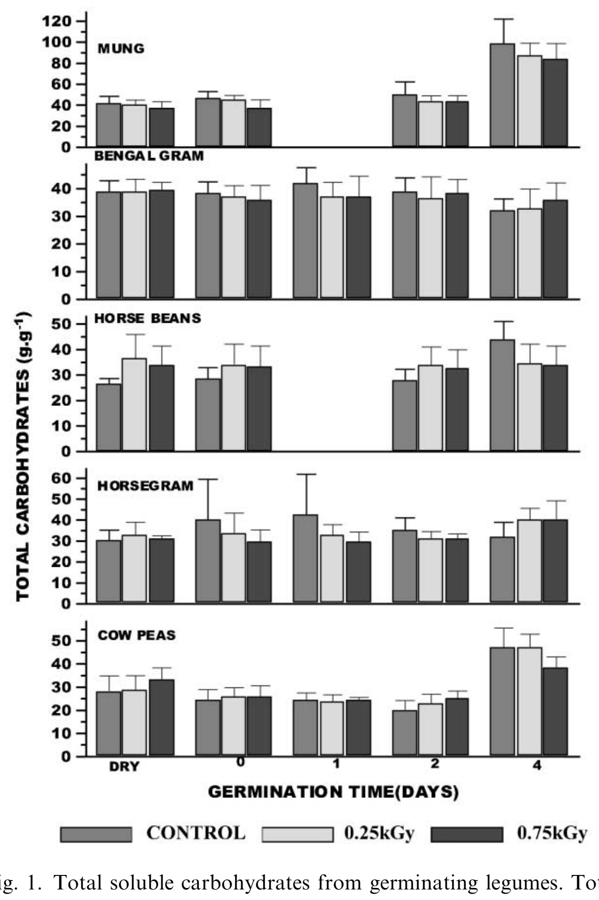

Fig. 1. Total soluble carbohydrates from germinating legumes. Total soluble carbohydrates were extracted using 80% ethanol as per standard procedure (Machaiah et al.,1999).They were estimated in the crude fraction before passing through the ion-exchange resins as described in the text. Each value represents mean ± SD of four independent experiments analysed in duplicate.

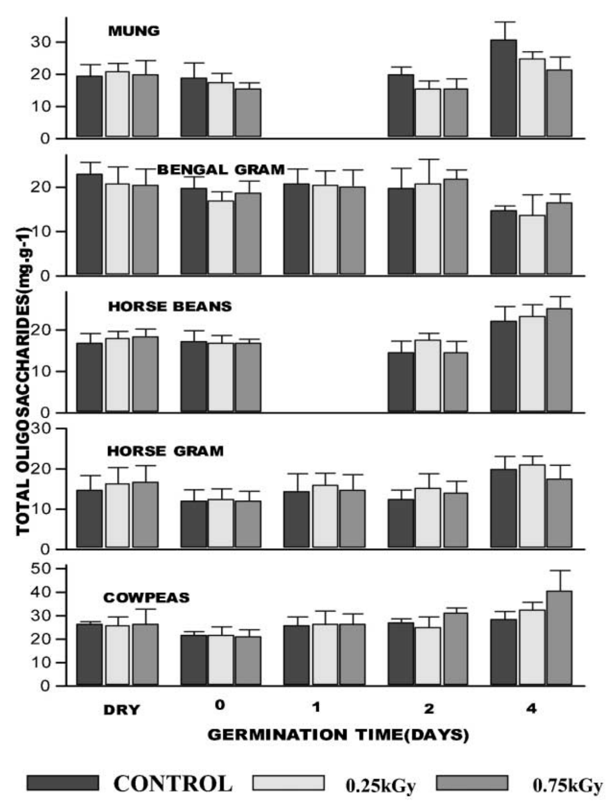

Fig. 2. Total oligosaccharides in the crude extracts of germinating legumes. Estimations were done by the thiobarbituric acid method. It estimates the total non reducing sugars, including oligosaccharides. Each value is mean ± SD of duplicate estimations of four individual extractions.

amounts (<2% in control and 0.25 kGy and about 5% in the 0.75 kGy samples). As some reports (Obendorf, 1997) indicate, it is probably metabolized very rapidly to prevent feedback inhibition of α-galactosidase, whose role in degradation of RFO, to ensure constant supply of germinative energy is well documented (McCleary & Matheson,1974).

The carbohydrate composition of rajma was found to differ from other legumes. The hexoses, xylose and raffinose, amounted to about 5% each, in the dry seeds and did not change significantly on overnight soaking. Sucrose was the major carbohydrate (65%) and there was no change due to radiation treatment.

The sucrose content was around 40% in mung, horse beans and horse gram but higher (50–60%) in Bengal gram and cow peas on the 2nd day of germination. High sucrose/reducing sugar ratio in seeds is correlated with seed desiccation and dormancy (Horbowicz & Obendorf, 1994). The ratio is low in the dry seeds and increases on germination. On the 2nd day of germination, in mung, Bengal gram, horse beans and horse gram (Fig. 5), the ratio was much greater in the irradiated samples, particularly at 0.75 kGy. However, this was not observed in cow peas, though it was the case at lower dose (0.25 kGy).

Mellibiose was present only in horse beans, Bengal gram and cow peas. It constituted about 2–3% of horse beans and cow peas; it was higher (5%) in Bengal gram and increased to about 16% on the 4th day of germination. There was no change due to radiation treatment. Raffinose content (5–6%), though an intermediary in the breakdown of stachyose and verbascose, did not change during germination. It is probably rapidly converted to galactose and sucrose to support further metabolic activity.

3.4. Reduction in flatulence factors

The reduction in the main flatulence factors, stachyose and verbascose, which formed 55–65% of the

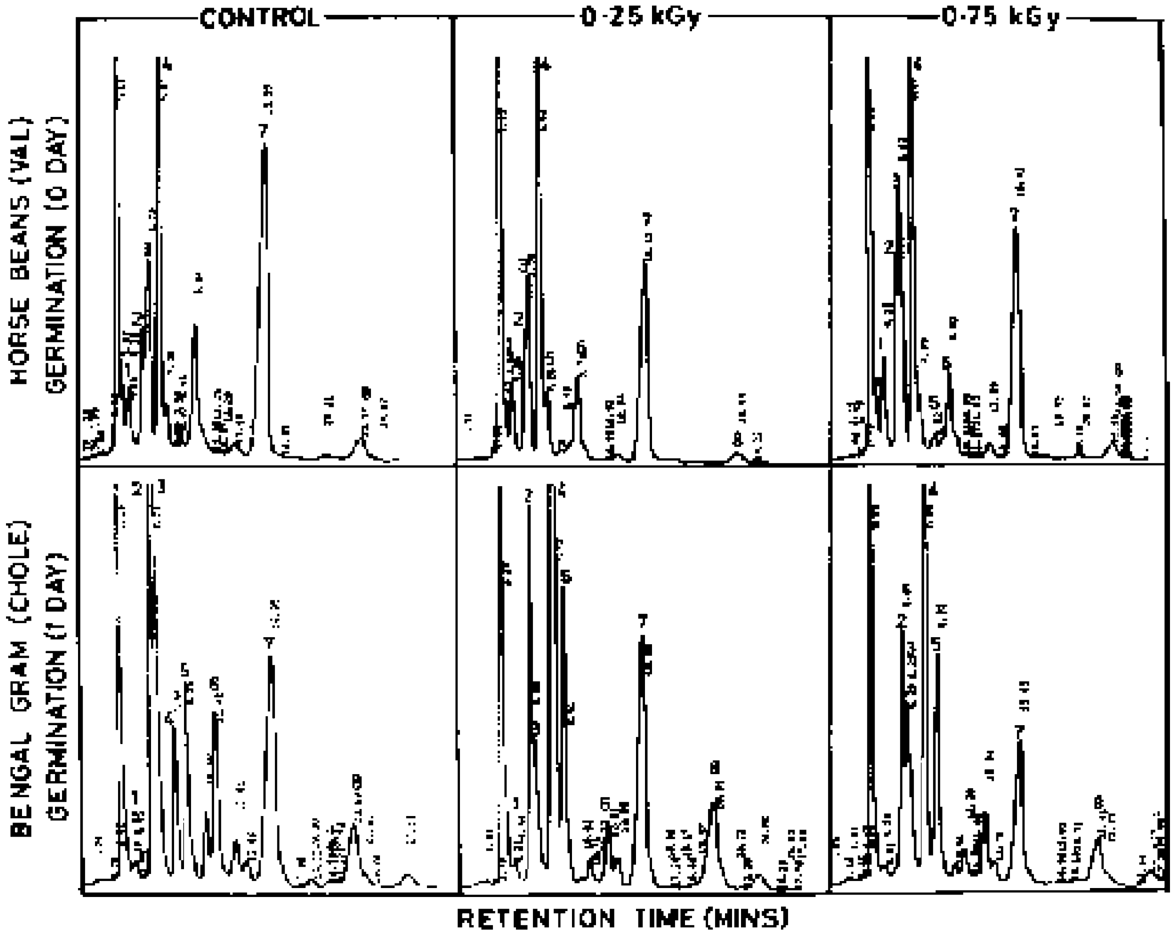

Fig. 3. The HPLC separation of total soluble carbohydrates. Separation was carried out on microBondapack carbohydrate column using acetonitrile:H_2O (75:25 v/v) as the eluting solvent. The fig. shows the typical profile for horse beans and Bengal gram germinated for 0 and 1 day, respectively. Glucose and galactose separated into different peaks in the standard mixture but were not resolved in many samples, particularly when galactose was present in small amounts as seen in the paper chromatograms. The peak numbers represent the sugars as follows: 1, xylose; 2, fructose; 3, glucose and galactose; 4, sucrose; 5, mellibiose; 6, raffinose; 7, stachyose; 8, verbascose.

total soluble carbohydrates, was accentuated by radiation treatment in all the legumes (Fig. 6). Stachyose was the major flatulence factor (>90%) in horse beans, horse gram and cowpeas; verbascose was greater (60%) in mung and Bengal gram. Rajma contained the least flatulence factors (15–17%). The difference in degradation between control and irradiated samples was observed in the dry state (Fig. 6A) in Bengal gram and horse beans (val); it continued at the same rate until the 2nd day of germination, when 80% of the flatulence factors were degraded in the irradiated samples compared to 65–70% in the control (Fig. 6A). In the irradiated mung samples, as reported earlier (Machaiah et al., 1999) the degradation occurred rapidly on the 2nd day; 70 and 85% of the flatulent factors were broken down in the 0.25 and 0.75 kGy samples, respectively, while only 5% was degraded in the control sample (Fig. 6B). In horse gram, the rate of degradation was higher. On the 2nd day of germination, 92 and 98% of the flatulent factors were degraded in 0.25 and 0.75 kGy samples, respectively, compared to 63.5% in the control (Fig. 6B). About 80% of the flatulence factors were degraded in the irradiated cow peas on the first day of germination compared 52% in the control; on the 2nd day, the difference was reduced and 90% RFO were degraded in the irradiated samples compared to 78% in the control. Degradation of the flatulence factors was noticeable in the irradiated Bengal gram (Cicer) in the dry state itself; nevertheless, as germination progressed, the hydrolysis of these oligosacharides plateaued around 80% in both control and irradiated samples (Fig. 6A, B). This observation was distinctly different from the other four legumes where, after 4 days of germination, about 95% of these factors were degraded in

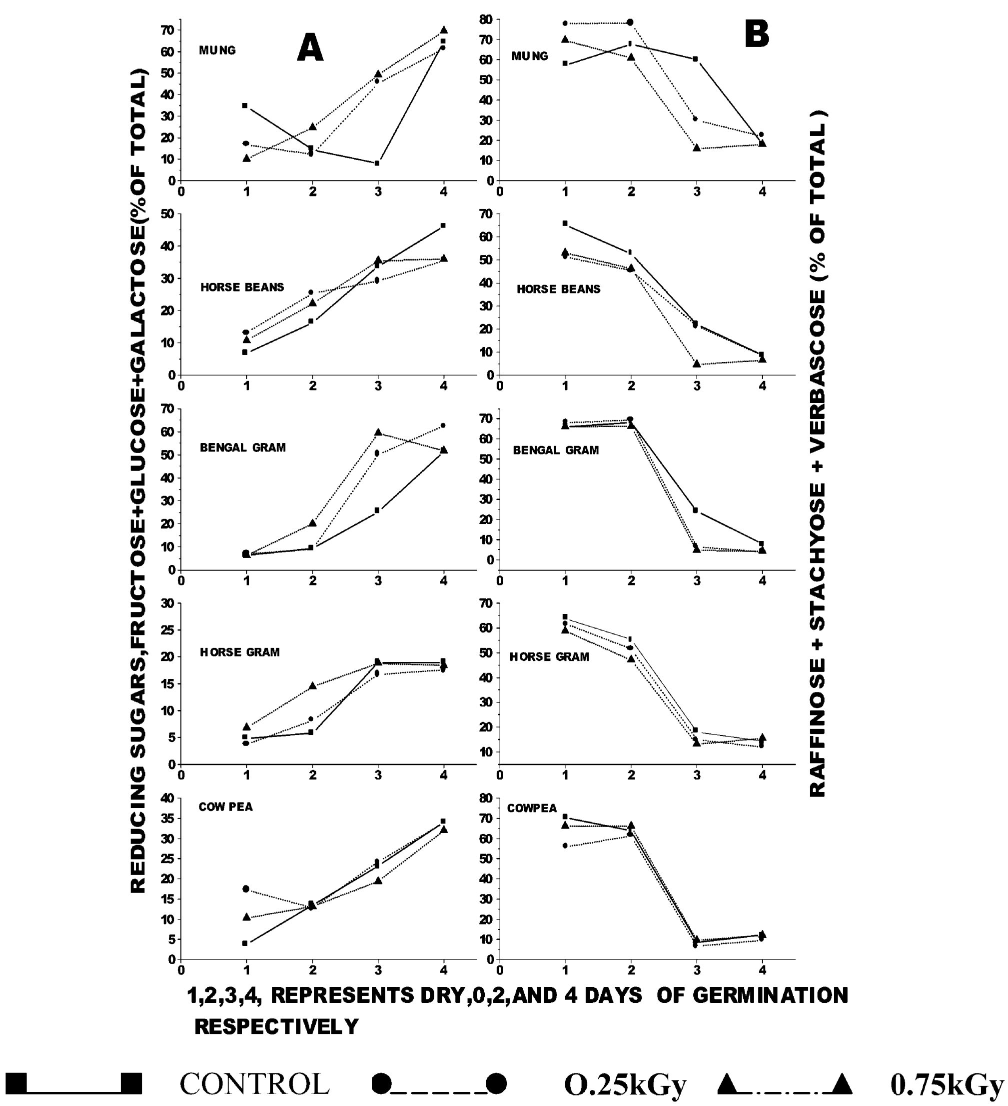

Fig. 4. The sugars were quantitated by HPLC and expressed as% of total carbohydrates. (A) represents the reducing sugars, fructose, glucose and galactose, which are increasing and (B) represents the raffinose family oligosaccharides which are degraded with the progress of germination. Each value represents the mean of four separate experiments.

all the samples, irrespective of radiation treatment. In these legumes, radiation-specific enhanced degradation of flatulence factors into easily metabolizable carbohydrates, such as hexose and sucrose (Fig. 4A) was highly significant ($P < 0.0001$) during early germination (1 and 3 days).

4. Discussion

Low dose (0.25 kGy) radiation processing is now widely recognized as an effective method for extending the shelf-life of cereals and legumes (Tilton & Burditt, 1983). Our studies on the commonly used legumes clearly indicate that near total degradation of flatulence factors occurs in radiation-processed legumes during early germination, compared to unirradiated controls (Fig. 6); besides, they are converted into easily metabolizable simpler carbohydrates (Fig. 4A). This would make them nutritionally more acceptable. Radiation treatment does not affect the acceptability of these legumes in terms of sensory attributes, such as taste, aroma, texture and cooking quality (Marathe, Rao, & Thomas, 1998), unlike other procedures used for reduction of flatulence factors (Calloway et al., 1971). Raffinose oligosaccharides are reported to be water-soluble, hence soaking and discarding the soak water will greatly (50–90%) reduce their content (Iyengar & Kulkarni, 1977; Iyer, Salunkhe, Sathe, & Rockland, 1980). Our results (Fig. 4B), however, show 10–20% decrease, only in horse gram and Bengal gram on overnight soaking, while no reduction was noticed in other legumes; on the other hand in mung, horse gram and rajma 10, 5 and 15% increases in flatulence factors were observed. Legumes accumulate RFO during maturation until desiccation occurs, when all the enzymes involved in their synthesis are active (Obendorf, 1997). Possibly the hydration of seeds during imbibition activated some of these residual enzymes and induced some synthesis during early germination (Wahab & Burris, 1975), until they were superseded by the activity of degradative enzymes, such as α-galactosidase (McCleary & Matheson, 1974), leading to their breakdown to supply the germinative energy.

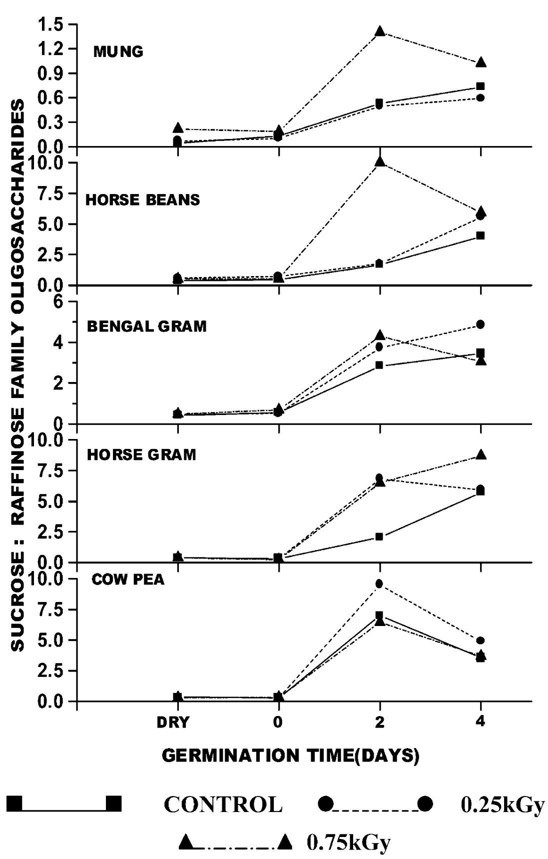

Fig. 5. Effect of radiation treatment on the ratio of sucrose to raffinose family oligosaccharides. The ratio was calculated after the quantitation by HPLC.The sucrose content was divided by the total content of Raffinose + Stachyose + Verbascose, as obtained for different days of germination.

Higher sucrose/red sugar ratio on the 2nd day of germination (Fig. 5) could be due to the increase in sucrose, a degradation product of RFO by α-galactosidase (McCleary & Matheson, 1974). At this stage increase due to sucrose synthesis, which is also linked to starch degradation (Kuo, Van Middlesworth, & Wolf, 1988), is unlikely, as in legumes it is established that first the RFO degradation products are utilized as sources of energy (McCleary & Matheson. 1974). As the germination proceeds, sucrose is translocated to the growing axis (Kuo et al., 1988). Our results clearly show that, at 0.75 kGy, the seedling height is significantly reduced (Table 1) which may also contribute to the observed increase in sucrose/reducing sugar ratio, as well as sucrose arising out of RFO degradation, which is greater in these samples than unirradiated controls.

The radiation-processed samples are well accepted in both dry and sprouted conditions. Legumes consumed as sprouts are least flatulent and nutritionally upgraded due to improved digestibility (Chitra & Rao, 1996) and increased vitamins and minerals (Prodanor, Sierra, & Vidal-Valuerde, 1997). It was interesting to note that, though the seedling height at 0.75 kGy was significantly reduced compared to unirraidated controls, its sensory properties were unaffected (Marathe et al., 1998). Sprouts from radiation-processed legumes, as such or in the form of powder, either alone or in combination with cereal flours, could be used to prepare easily digestible weaning and health foods with lower flatulence factors. Radiation-processed legumes, such as horse beans (val) and Bengal gram, exhibiting reduction in flatulence factors in the dry state, as well as providing good quality proteins at lower cost, would be more acceptable when incorporated into cereal-based snack items.

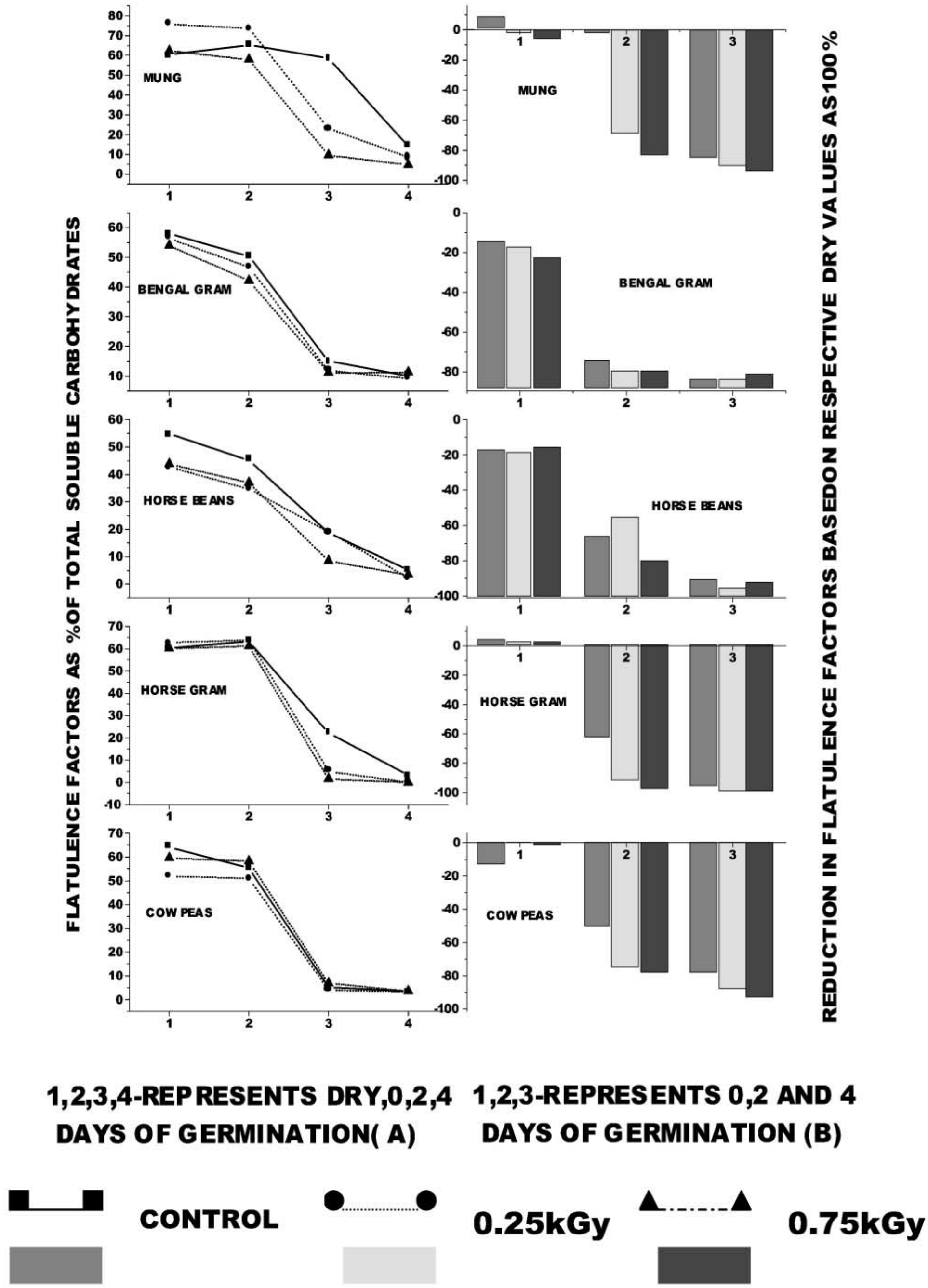

Fig. 6. Degradation of flatulenle factors with the progress of germination. Degradation of Stachyose + Verbascose is represented in (A). The% reduction of flatulence factors which is enhanced due to radiation treatment, is presented in (B).

Legumes contain oligosaccharides in their cotyledons, localized in the cytosol, while the hydrolytic enzymes, such as α-galactosidase are in the protein bodies (Calloway et al., 1971). Apart from the varietal differences in the degradation of oligosaccharides, our studies also show a radiation dose-related response, degradation being higher at 0.75 kGy. Previously, degradation of aqueous raffinose *in vitro* in a dose-related manner has been observed (Rao & Vakil, 1983). Our observation of greater degradation of flatulence factors in irradiated legumes could be a radiation-related phenomenon or due to enhanced activity of the associated degradative enzymes. This aspect is currently being investigated.

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