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Traditional methods of soaking or sprouting pulses reduce the flatulence causing raffinose family alpha-galactosides in regularly used nutritious edible legumes

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Pulses are an alternative sustainable and inexpensive source of protein. They are rich in carbohydrates, proteins, vitamins, and minerals. The total soluble sugars in pulses include monosaccharides, disaccharides, and oligosaccharides. Legume seeds contain raffinose family oligosaccharides (RFOs) as their primary source of sugar reserves, which drive seed germination and improve seed vigor and longevity. The RFOs are also critical during plant growth and development in unfavorable environments. However, because monogastric animals, including humans, lack the enzyme alpha-galactosidase, they cannot digest the RFOs present in legume grains, leading to the accumulation of flatus due to microbial fermentation of RFOs in the large intestine. Subsequently, large amounts of carbon dioxide, methane, and hydrogen build up in the intestine due to flatulence. Flatulence, being induced by water-soluble RFOs, it is possible to reduce the levels of RFOs in the dietary pulses by traditional soaking of legumes. Herein, we report the RFOs levels of 21 common dietary pulses consumed in various forms, such as dry grains, soaked grains, and seed sprouts. We show that seed sprouts accumulate lower levels of RFOs compared to fresh vegetables. We hypothesize that the RFOs levels increase when the filled grains desiccate to mature and decrease when the desiccated seed commits to germination. Our study suggests incorporating highly nutritious pulses, such as sprouts, into diets to combat flatulence.

Keywords: Diet, Flatulence, Legume, Nutrition, RFOs, Sprouts

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Human nutrition, malnutrition, and anti-nutritional factors are often addressed at multiple levels to improve nutritional quality, value-add nutritious constituents, and eliminate deterring anti-nutritional compounds. The Fabaceae or Leguminosae family consists of over 20,000 species[1] that provide dietary fibre, protein, carbohydrates, B vitamins, iron, copper, magnesium, manganese, zinc, and phosphorus, yet are low in fat, no saturated fat, and cholesterol-free. One serving of legume provides about 115 calories, 20 g of carbohydrates, 7-9 g of fibre, 8 g of protein, and 1 g of fat. The total soluble sugars in the pulses include monosaccharides, disaccharides, and oligosaccharides. The raffinose family oligosaccharides (RFOs) are the primary oligosaccharides in pulse grain, ranging from 2.7 to 5.9 g/100g in seeds[2]. Legume seeds contain raffinose family oligosaccharides (RFOs) as their primary source of sugar reserve, which drives seed germination and improves seed vigor and longevity. They belong to the category α-galactosides. The primary role of RFOs is to tolerate desiccation during seed maturity and supply energy during seed germination. The RFOs are also critical during plant growth and development in unfavourable environments[3]. However, monogastric animals, including humans lack the enzyme alpha-galactosidase that breaks down RFOs into simple sugars. Therefore, the gut microbiome ferments the RFOs to produce carbon dioxide, methane, and hydrogen causing flatulence. The RFOs are considered antinutritional factors for they cause flatulence in monogastric animals, reduce nutrient assimilation, and cause diarrhoea[4]. Although RFOs are anti-nutritional, they may have prebiotic-promoting effects when consumed in low concentrations[5,6].

The levels of RFOs vary depending on the genotype and cultivable environment. The pulses of faba bean and field pea contain a high amount of verbascose and stachyose[7,8], respectively. Kabuli-type chickpeas have a higher total RFOs content than desi-type chickpeas[9]. The RFOs accumulate to a higher concentration in the seeds of cowpeas and

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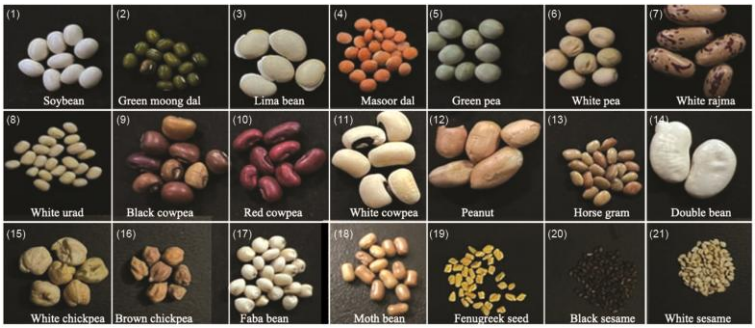

Fig. 1 — Pulses and other dietary grains that are commonly used for culinary purposes

soybeans[10,11], peas[12] and lentils[13]. There are many non-genetic ways to lower the RFOs content in pulses, such as microwaving, autoclaving, irradiation, dehulling, ethanolic and water extraction, enzymatic hydrolysis, and microbial fermentation[14]. However, enzymatic hydrolysis of RFOs, microbial fermentation, irradiation, and ethanolic extraction methods are impossible in cuisine. Interestingly, most Indians traditionally soak legumes before cooking or consume them as sprouts. Therefore, we hypothesised that soaking or sprouting pulses reduce flatus-causing RFOs. Therefore, we investigated the increase or decrease of RFOs in 21 common dietary agricultural raw products to advocate the correct pulse stage to reduce human flatus production.

Materials and Methods

Plant material and chemicals

Commonly used legume seeds obtained from the local market were studied (Fig. 1). Fine chemicals, enzyme substrate, enzymes α-galactosidase, and invertase were purchased from Sigma-Aldrich, USA.

Surface sterilization

Seeds were surface sterilized using 1% sodium hypochlorite followed by three washes in sterile water, later the seeds were imbibed for 16 h in 20 mL of water in the dark. Later the pulse sprouts were used for sugar analysis.

Preparation of different forms of pulses

The vegetable form of the pulse was dried to a constant weight at 40°C to prepare dry grains. To prepare pulse sprouts, these dry grains were soaked overnight and allowed to germinate at 25°C.

Extraction of soluble carbohydrates

100 mg of seed powder or the leachate was extracted with 10 mL of 50% ethanol (ethanol: water 1:1) overnight at 37°C in a shaker incubator. The mixture was centrifuged at 10,000 rpm for 20 min, the supernatant was transferred to a fresh tube. The pellet was re-extracted with 5 mL of 50% ethanol, the extracts were pooled. The extract was evaporated at 40°C to get a fine powder of soluble sugars. The powder was dissolved in 2 mL sterile water; the solution was filter sterilized using 0.44 µ filter before analysis[15].

Estimation of total alpha-galactosides

The total alpha-galactosides was estimated using the DNS acid method[15]. The concentration of RFOs was estimated by hydrolysing the extract using the enzymes invertase (0.003 Units) and α-galactosidase (0.05 Units). To estimate sucrose and α-galactosides, the extract was treated with invertase (0.003 Units) for one hour and sequential digestion of the extract with α-galactosidase (0.05 Units) and invertase (0.003 Units) each for one hour respectively. Subsequently, the enzyme-digested extract was used to estimate reducing sugars by the DNS method. The α-galactosides concentration was computed using the concentrations of reducing sugar, sucrose and galactosides.

Statistical analysis

The experiments were done in biological and technical replications (the number of seeds per replicate was 20). The Analysis of Variance was performed (One-way ANOVA) using Graphpad 8.0.

Results

We consume most of the dietary pulses in three different forms: vegetable, dry grain, and sprouts. Traditionally, the legumes are soaked for a few hours to overnight in water before using them for culinary purposes. Some also have a common practice of consuming legume sprouts instead of grains. We measured the amount of RFOs in dry grains, sprouts, raw vegetables, and the leachate of common dietary

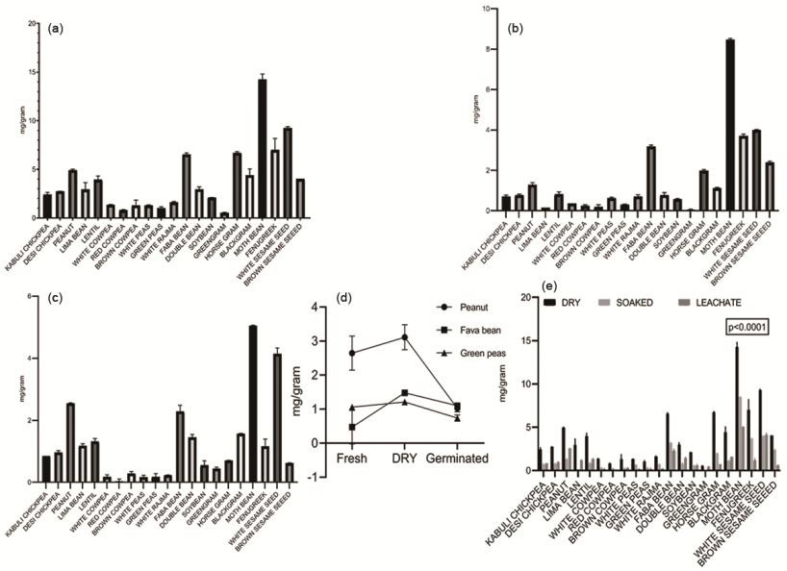

Fig. 2 — The alpha-galactosides were assayed in dry seeds (a), soaked seeds (b), leachate (c), respective vegetables, and sprouts (d) of edible legumes. The amount of galactosides leached by soaking is significant ($p<0.0001$) (e). All assays were performed in biological and technical duplicates. The alpha-galactosides are given in mg/gram of seed powder

pulses. RFOs (alpha-galactosides) are soluble oligosaccharides; soaking the seeds overnight in water reduces the total alpha-galactosides.

Among the dry grain pulses studied, moth bean showed high levels of alpha-galactosides (14.2 mg/gram of grain powder) followed by white sesame (9.2 mg), fenugreek (7.0 mg), and faba bean (6.5 mg) whereas; green gram contained the lowest amount of galactosides (0.5 mg) (Fig. 2a). However, soaking the pulses overnight drastically reduced the galactosides. After soaking, moth bean, white sesame, fenugreek, faba bean had 8.4, 4, 3.7, and 3.2 mg/gram of alpha-galactosides respectively. Post-soaking, green gram showed the lowest levels of galactosides (0.058 mg/gram) (Fig. 2b). The reduction in the alpha-galactosides between dry grain and soaked pulse was statistically significant ($p<0.0001$).

Furthermore, the analysis of alpha-galactosides in the soaked water (leachate) revealed that the sugars are leached out during soaking. The galactosides in the leachate of the moth bean, white sesame, peanut, fababean were 5.04 mg/gram, 4.15 mg/gram, 2.54 mg/gram, and 2.3 mg/gram of pulse powder respectively. The leachate of the red cowpea had the least amount of galactosides (0.034 mg/gram) (Fig. 2c). Since the pulses like peanut, fababean and green peas are used in three different forms such as green vegetable, dry grain and sprouts, the levels of alpha-galactosides in the three different forms revealed that seed sprouts of peanut and green peas contain low levels of alpha-galactosides compared to the vegetable and grain forms of the pulse (Fig. 2d). The analysis revealed that the alpha-galactosides in the dry grains are leached during overnight soaking in all the edible pulses studied ($p<0.0001$) (Fig. 2e).

Discussion

Pulses are the major source of vegetable protein acclaimed to be critical to a nutritious diet, Pulses are traditionally consumed in three different forms: fresh vegetables, dry grains or germinated sprouts. In this study, the accumulation levels of alpha-galactosides in the various dietary pulses were investigated in the three forms. Alpha-galactosides mainly consist of raffinose family oligosaccharides (RFOs), which build up in all legume seeds as they mature to help the seeds deal with the physiological stress that comes with maturation and to add to the energy reserves during germination. In addition, it is critical in abiotic stress tolerance and promotes plant growth and development. Despite its role in plant growth and development, RFOs (alpha-galactosides) are generally considered antinutritional causing flatulence, diarrhoea, and bowel discomfort in monogastric animals including humans.

Although various genetic and non-genetic approaches have been proposed to reduce the flatulence-causing galactosides in edible pulses, traditionally, in India, mere overnight soaking of the edible pulses and consumption of pulse sprouts is effective. Here, we provide scientific evidence for the

age-old traditional method of soaking to minimize galactosides in dietary pulses.

RFOs are water soluble, overnight soaking enables the leaching of soluble RFOs, drastically reducing them in grains. Besides being water soluble the RFOs are also hydrolysed by the enzyme alpha-galactosidase upon hydration[16,17]. Therefore it is evident that soaking leaches or hydrolyses the soluble RFOs in the grains reducing the total alpha-galactosides. Furthermore, it was interesting to note that some pulses like faba beans, the vegetable form accumulate low RFOs compared to grain and sprout forms. Other pulses like peanuts and green peas accumulate lower RFOs in the sprouts than the other forms. The accumulation of RFOs in various forms like fresh vegetables, dried grain, and sprouts is a characteristic of the individual dietary pulses. RFOs play a significant role in the physiology of seed maturity, and seed desiccation, and provide energy during germination[12]. Soaking of the dietary pulses can hydrolyse and leach considerable amounts of flatulence causing RFOs. The above knowledge on RFO composition in various forms of edible pulses will provide a choice of dietary form of pulses to overcome flatus production.

Conclusion

Legumes store RFOs in the seeds to tide over the physiological drought during seed maturity and to provide energy for germination. However, RFOs are antinutritional as humans lack the enzyme alpha-galactosidases to process them. Our study claims that soaking the legume seeds to sprout utilizes most of the accumulated RFOs compared to dry grain and raw vegetables. Therefore, we advocate soaking legume seeds overnight or raw vegetable forms of pulses to reduce flatus production in the human diet.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Author Contributions

The research was conceived, and designed by RA, performed by FT and SYN. The data was analysed by Yuvatha and RA. The manuscript was written by RA.

Data Availability

The authors approve that the data supporting the finding of this study may be obtained from the corresponding author upon reasonable request.

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