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Raffinose Family Oligosaccharides, Occurrence in Food Materials, Nutritional Implication and Methods of Analysis, a Review

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Abstract: Raffinose family oligosaccharides are important components in various plant products which 8have numbers of contributions to plant physiology and are non-digestible in monogastric 9animals and in human upper gut. Thus, they are passed into the lower gut without being digested where they are fermented and liberate a substantial amount of gas (CO_2, H_2 and CH_4) which result in flatulence and hence can be considered as anti-nutritional factors as they cause abdominal discomfort, diarrhea, flatulence and others. RFOs are widely distributed in various crops. Leguminous plants for example are one of the most important plant food materials, for they are the concentrated and low cost sources of protein for the vegan population and low income societies. However, they are under-utilized in various countries specially developed countries because of the anti-nutritional factors including flatulence factor, raffinose family oligosaccharides. Apart from these anti-nutritional properties, RFOs is also among the important food components when consumed moderately. It is among the health promoting components of foods as it facilitates the growth of important microflora in the gut, enhance the activities of antioxidant enzyme system, hepatoprotective and other multiple health benefits. Therefore, it is very important to reduce RFOs to certain optimal amount only to reduce their adverse anti-nutritional effect and utilize their advantages.

Keywords: Anti-nutritional, Flatulence, Inhibitors, Microflora, RFOs

1. Introduction

Raffinose family oligosaccharides (α-galactosides) are parts of the different compounds common in plant materials. Raffinose family oligosaccharides are important components in carbohydrates mainly found in seeds and other common storage organs of many plants. They are generally recognized as the most extensively distributed compound in the plant kingdom [1]. They have number of contributions to plant physiology. Raffinose family oligosaccharides fulfill multifarious functions in plant seeds. For instance, they protect cellular seed structures during desiccation and they also assist as a carbon reserve to be utilized at the time of germination of plant seed [2].

RFOs are non-digestible in monogastric and in human small intestine. Thus, they are passed into the lower gut without being digested where they are fermented with the liberation of a considerable amount of gas (containing CO_2, H_2 and CH_4) which cause flatulence and hence can be considered as anti-nutrient in this regard [3]. Leguminous seeds and some cereal like soybean and sorghum are known to contain these raffinose family oligosaccharides or α - D-galactosides (AG), responsible for gas producing fermentations and hence flatulence in the stomach. On the other hand, these oligosaccharides are not only negatively affecting human health; rather, they have number of beneficial health related effects as well.

The analysis of raffinose family oligosaccharides (RFOs) is very important for variety of reasons. It is sufficient to raise issues like development of food product and even plant breeding to indicate the importance of determining RFOs in plant food sources. Food carbohydrates including these RFOs on the other hand are known of possessing wide range of chemical reactivity and molecular size [4]. Farther more, carbohydrates do not possess chromophores or fluorophores, and hence, cannot be detected with uv- visible or

fluorescence techniques as elaborated in the above literature. Rather, refractive index detection technique can detect concentration up to ppm range and above and electrochemical detection is used in the analysis of sugars as low as ppb ranges. In these methods, more complex samples necessitate more detail sample preparation and processing, such as fat extraction and de-proteination. The HPLC method can be used to analyze mono-, di-, and trisaccharides as well as sugar alcohols. Taking the importance of RFOs analysis in food sources and complexity of the procedures, it is important to review different methods of analysis used in different journal articles worldwide.

There are varieties of factors responsible for occurrence of RFOs in food components. The types and varieties of plant foods for example are among the common factors. Furthermore, the growing environment is also among the factors determining the concentration of RFOs in plant foods. The varieties of stress are also reported to be responsible for determining the concentration of RFOs in foods [5]. Plant foods like legumes are known to cause flatulence specially when consumed raw or near raw. It is these RFOs that are causing the problem though they might have health related advantages as well [6]. These two concepts are opposing each other and therefore, it is important to review some possible literatures and indicate both the anti-nutritional and nutritional implication of RFOs to indicate some concluding remarks.

2. Occurrence of RFOs in Food Materials

2.1. Synthesis of RFOs

Raffinose family oligosaccharides (RFOs), abundantly occurring in plant seeds is known to accumulate during seed development and vanish swiftly at the time of seed germination [7, 8]. As further elaborated in these literatures, the synthesis of raffinose family oligosaccharides starts with the activity of galactinol synthase (GoIS) enzyme that galactosylates myo-inositol to produce galactinol. The Galactinol donates activated galactose moieties for addition to sucrose for synthesis of raffinose and the higher molecular weight families, Stachyose, Verbascose, and Ajugose subsequently. The whole process is clearly indicated in the following schematic representation of the process. Stachyose, verbascose and ajugose, the three higher raffinose families of the oligosaccharides next to raffinose are either synthesized by galactinol-dependent galactosyltransferases or by transfer of galactosyl units between two RFOs molecules.

Raffinose family oligosaccharides are synthesized from sucrose by the subsequent addition of activated galactose moieties donated by Galactinol [7, 8]. The enzyme of this process, galactinol synthase (GoIS) is functional only in the flowering plants and thus, it is assumed that the synthesis RFO is a specialized metabolic event in higher plants. However, it is not known at this level whether lower plant groups synthesize any galactinol or these RFOs [8].

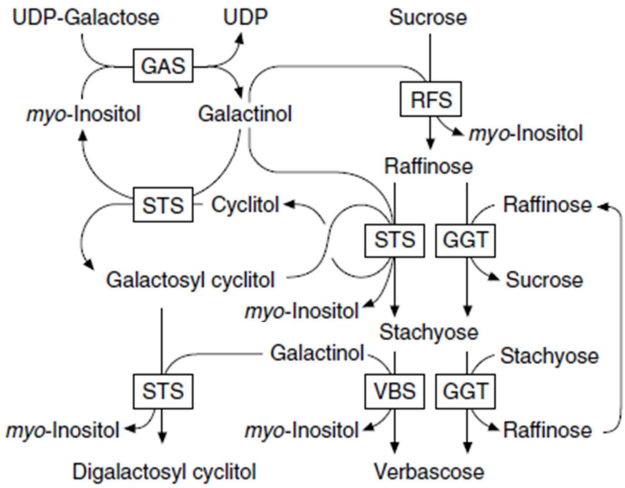

Figure 1. *Schematic representation of RFOs synthesis.*

GAS - galactinol synthase; RFS - raffinose synthase; STS - stachyose synthase; VBS - verbascose synthase; GGT - galactan: galactan galactosyltransferase. Cyclitol may stand for ononitol, pinitol or *chiro*-inositol, respectively. All reactions are reversible.
Sources: [7]

2.2. Occurrence of RFOs

Raffinose family oligosaccharides are widely distributed in plant based food sources and some other types of oligosaccharides are also found to certain extent in some animal products like honey and milk [9]. Legumes and sugar beet root among plant food sources are the main natural sources of raffinose family oligosaccharides. They are also found in other vegetables and cereal grains although in minor amounts [1]. The α-galactooligosaccharides from legume including pea and lupin have been considered as a main source of RFOs [10]. Raffinose family oligosaccharides as high as 4071 mg/100 g dry matter was reported in lentils in literature [11]. In legume lupin seed again, RFOs between 3.49 and 9.25 g kg−1 is reported in literature [2]. Raffinose family oligosaccharides are hence widely dispersed compound in different plant products including cereals and legumes as indicated in literatures [12, 7, 8, 13].

Table 1. *Occurrence of predominant RFOs in some legumes.*

Crops	RFOs			
	Raffinose	Verbastose	Stachyose	References
Soybean	0.67-2.56		2.09-7.10	a
Lupin	0.9 - 19.0	ND - 35.0	5.2 - 86.0	b, c
vine peas	2.95	1.29	1.44	d
Chickpea%	0.63-0.81		1.10-1.42	e
Dry bean Mg/g	2.29-4.43	ND	12.38-18.41	f
Field pea	6.0 - 14.0	23.0	17.1 - 27.0	b
Lentil	3.1 - 10.0	4.7 - 31.0	14.7 - 31.0	b
faba bean	0.27-1.3	0.67-5.03	0.90-2.50	g
Pink-mottled cream bean g/kg	1.6	ND	25.3	h
White bean g/kg	1.9	ND	24.8	h
Common bean	0.2-4.0	ND	10.7-24.2	I

a- [12], b- [5], c- [14], d- [15], e- [16], f- [17], g- [18], h- [19], i- [20]

Other type of oligosaccharides human milkoligosaccharide

(HMO) for example is naturally occurs in human breast milk [9]. Thus, it can be concluded that mammalian milk is the natural source of oligosaccharides more specifically GOS, Galactooligosaccharides. Oligosaccharides are also available in honey. Honey is reported to contain 0.75% fructooligosaccharides, one of the different kinds of oligosaccharides. However, RFOs are a distinct group of oligosaccharide widely available in plant and plant based products.

3. Nutritional Implications of RFOs

The RFOs is a group of prebiotic carbohydrates considerably available in varieties of legumes and cereals [11]. As the name prebiotic imply, it has multiple health advantages related to the growth of normal micro flora and even antioxidant capacity. When RFOs pass to the lower gut without being digested in the upper gut, they used as important substrates for fermentation caused by intestinal microflora [12]. However, there are also anti-nutritional implications with RFOs due to the fact that it is among the flatulence factors as well.

3.1. Health Promoting Property

Presently, the demand for foods having health benefits in addition to their nutrition advantages is increasing owing to the emerging and increasing food and nutrition related chronic problems. In line with this, RFOs are receiving extensive research attention due to their numerous healthy advantages. Studies indicated that, these functional oligosaccharides have positive effects in tackling nutrition related chronic problems [8]. Research finding has indicated that RFOs is one of the main active ingredients responsible for the hepatoprotective effect in plant products [21]. There is situation of oxidative stress which occurs when there is an imbalance between production of cellular oxidant species and antioxidant capability, one of metabolism related chronic problems. The RFOs were shown to markedly enhance the activities of antioxidant enzyme system of the host and exert a sparing effect on the antioxidant enzymes based on the study conducted on mice [21]. According to these authors, their results support existence of a beneficial relationship between antioxidant activity and hepatoprotective effect of RFOs. They are also reported to have significances on mineral absorption, immune response, lipid and glucose homeostasis, satiety regulation, body weight gain [1]. These authors finally concluded that there is high possibility of exploiting these functional oligosaccharides (RGOs) as the novel preventive and therapeutic ingredients for the mitigation of oxidative stress-induced liver injury and some chronic disease.

There is different research based convincing evidence that RFOs have beneficial effects on the survival of probiotic in fermented food products [22]. According to these authors, there is synergistic effect of both probiotics and prebiotics promoting health in such kinds of food based on evidence from their study on dairy products. As they cannot be digested in the upper gut, the pass into lower gut and undergo fermentation there at which its formation products will serve as very important substrate for the intestinal microflora [12].

3.2. Anti-nutritional Effect

RFOs have also anti-nutritional effect apart from their nutritional or health promoting advantages. It is listed as anti-nutritional factor as it causes flatulence when foods composed of it in higher amount like beans are eaten [20]. Literatures reported that, the presence of high concentration of raffinose family oligosaccharides (RFOs) in legumes limits their consumption and acceptance worldwide especially in developed countries despite the fact that they are excellent source of carbohydrates, proteins, dietary fibers, vitamins, minerals and other bioactive compounds [5, 23]. This is due to the fact that monogastric animals and human lack alpha-galactosidase enzyme which is needed to hydrolyze α (1→6) glycosidic linkages hence cannot digest RFOs. The undigested oligosaccharide in the upper gut will pass down to lower gut and contribute to production of gases carbon dioxide, hydrogen and methane through fermentation by anaerobic bacteria. The production of these fermentation gases in high amount bring about stomach discomfort, abdominal rumblings, cramps, pain, and diarrhea [3]. The reduction in net dietary energy, osmotic imbalance which reduced nutrient absorption and protein utilization is also other effect of consumption of RFOs in high amount. However, still low level of RFOs is very important nutritionally.

4. Methods of Extraction and Analysis of RFOs

The most commonly used methods for the analysis of oligosaccharides in common beans and other agricultural products can be classified into various categories. Some of these methods are discussed here under one after the other.

4.1. Extraction

The extraction procedure used for analyzing the RFOs is of great importance when determining RFOs in foods and food materials [15]. These authors optimized the extraction methods for determination of the RFOs in leguminous vine peas. They evaluated effect of different factors on raffinose family oligosaccharides by determining raffinose content. The authors also used different extraction conditions and evaluated the efficiencies of the conditions; two concentrations of ethanol (50% and 80% v/v), two temperatures (room temperature and boiling temperature) and three different times of extraction (15min, 30min and 60 min). So, according to these authors, temperature and solvent (both type and quantity) are among the different factors affecting raffinose extraction yield. Different authors followed the method while modifying in little as per their sample and experiment [14, 24]. The result of their experiment is quite

consistent for all the tested samples and valid even for the other oligosiccharides (verbarcose and stacchyose for example). The authors also come up with the conclusion; temperature had little or no effect on the extraction yield of raffinose family oligosaccharides in 50% ethanol. With respect to extraction time, their result indicates that the extraction of raffinose family oligosaccharides is complete after 30 min but sample extracted at 15 min is lower in amount than 30 and 60 min extraction though this difference is significant only in verbascose and not in raffinose.

4.2. Methods of Determinations

4.2.1. Enzymatic Assay

The enzymatic assay has been used for the determination of RFOs as indicated in literature across the world [3, 25]. Long ago, [26] for instance, analyzed the carbohydrate change in locust bean in his research on product from locust bean known as *iru* which is common in Nigeria. For the assay of α -galactosidase activity, the authors mixed 2ml enzyme extract with 1 mL of 1% (wt/vol) melibiose dehydrate and incubated it for 2h at 40°C. According to the author, the reaction was stopped by adding 3 mL dinitrosalicylic acid reagent (DNS), boiled in water bath for 5 min, cooled in cold water, and the diluted with 18 mL water. Then, optical density of the resultant solution was measured at 550nm using spectrophotometer. The blank was similarly treated except that the DNS was added before adding the sugar solution. Finally, the amount of reducing sugar formed was calculated from a standard curve with known concentration of maltose. The activities of α-galactosidase and sucrose were assayed using 1% (wt/vol) solutions of lactose and sucrose respectively. The unit of enzyme activity corresponded to 1 gram of reducing sugar liberated. Hence, enzymatic Assay is one of the different categories of raffinose family oligosaccharide analysis [27]. In the method in literature above, oligosaccharide is hydrolyzed to galactose, glucose, and fructose by α galactosidase and invertase. Then, glucose is determined by glucose oxidase enzyme or peroxidase reagent. The short coming with the method is that, it does not distinguish between raffinose and stachyose, but rather, measures these as a group. Because 1 mol of each of the raffinose-series oligosaccharides contains 1 mol of glucose, the concentrations are presented on a molar basis. Free sucrose and glucose in sample extracts are determined concurrently.

4.2.2. Ion Exchange Chromatography-Pulsed Amperometric Detection (IEC-PAD)

This is among the most sensitive methods existing for the determination of the sucrose, raffinose, and stachyose. The principle of separation in the technique is based on the separation of carbohydrates as anions using a highly alkaline mobile phase followed by detection using pulsed amperometric detection [27]. According to these authors, ground soy samples without defatting are extracted with 50% ethanol/water mixture and directly analyzed by anion exchange chromatography on a Dionex Carbopac 1 column using 150 m*M* NaOH as mobile phase in isocratic mode. All three sugars are baseline-resolved and separated in 16 min. Selectivity and sensitivity is major advantages of this approach.

There are different articles done and reported with the identification and characterization of raffinose family oligosaccharides in different accessions of soybeans following the method of Ion Exchange Chromatography-Pulsed Amperometric Detection (IEC-PAD) [28, 29]. These authors also used high performance liquid chromatography with pulsed amperometric detection (PAD) in order to quantify the oligosaccharides.

4.2.3. HPLC Separation on an Amino Column

The other important method of raffinose family oligosaccharide analysis is high performance liquid chromatography (HPLC) separation techniques. Oligosaccharides are separated on an amino column such as Spherisorb NH_2 column using an acetonitrile/water mobile phase using refractive index (RI) detection. However, the performance and ruggedness of amino columns in chromatography are always in question [27].

It is indicated in literature that extracted α -galactosidase or raffinose family oligosaccharide from 0.3g of lupine seed flour by the use of solvent (48% ethanol) by sonicated for 60 minute followed by centrifugation at 700g for 10minute [24]. According to the procedures outlined in this article, combined supernatants heated at 85°C under reflux for 30 min., cooled and centrifuged at 700μg for 5 min, then evaporated to dryness on rotary vacuum evaporator at 45°C, dissolving residue in water (4l), an aliquot of 0.5 mL was transferred into a glass-stoppered test tube followed by shaking and the mixture will steered over night at 4°C.

After filtering, the sample (100 ml) injected into the HPLC system equipped with RI detector and the RFOs is analyzed accordingly. For the separation of saccharose and α-galactosides, a column (Carbohydrate Analysis, Waters) with pre-column Adsorb sphere C18 (Altech) was used in the experiment of the authors above and the calculations for the amount of oligosaccharides in 0.1 ml of the analyzed sample is done. Finally, they isolate and purify α-galactosides from bitter lupin extract. Similar procedure was also used to determined raffinose oligosaccharides with little modifications in an experiment on effect of processing evaluation on the coated bean and fate of oligosaccharide evaluation [30]. According to them, raffinose and stachyose were monitored after 4, 8, and 12 h of soaking and in cooked bean. Then, the followed the procedures for the analysis of raffinose family oligosaccharides as per this method.

4.2.4. High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection

For the last 30 years, high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD) has been a widely used in carbohydrate analysis [31]. This method with CarboPac PA200 column is able to separate and determine soluble sugars within 6 min of

total run time (25 min) in both standard and chickpea sample [23]. Accordingly, sucrose, raffinose, stachyose and verbascose were detected at 4.1, 4.8, 5.1 and 6.0 min, respectively. However, there is shift in peak retention time observed by the authors above suggesting poor reproducibility of this method. Thus, imprecision in quantifying different sugars is the drawback with this method.

4.2.5. High Performance Liquid Chromatography Refractive Index Detector (HPLC–RI)

This method is based on separation of sugars by size exclusion chromatography (SEC). As the name imply the detection is using a RI detector (HPLC–RI) [23]. According to the authors, a good separation among raffinose, stachyose and verbascose was obtained using chromatogram resulted from SEC in standards' mixture having retention time of 86.7, 73.9 and 62.8 min respectively. Moreover, the authors elaborated that there are still several drawback with this method which are outlined as follows:

1) The method was not suitable for chickpea seed meal samples as peaks were not well separated in their specific experiment.
2) The long run time/sample (160 min run time + 30 min washing time) is also the other disadvantage of the method.
3) The blockage of guard column brings about frequent change of security guard cartridge after every 50 samples. This on the other hand cause high back pressure problem.

4.2.6. HPLC Separation on Polymeric Gel Column

Alltech, a well-known company in America recently introduced a novel Prevail Carbohydrate ES column (Alltech Associates, Inc., Deerfield, IL) that is packed with a rugged hydrophilic polymeric gel. This column with its high efficiency, excellent stability, good reproducibility, and long column lifetime reaches full potential when used with evaporative light scattering detection (ELSD). There are literatures reported with this method following the procedures explained as follows [32]. First of all, the authors finely ground the bean either extruded or the raw bean till it passes through 250 µm mesh. Citing [33], the authors added Ethanol of 70%v/v to each 10 g finely ground bean flour sample at a 10 mL /g dry mass ratio. Then samples were thoroughly mixed, sonicated for 1 h, and centrifuged. They collected the ethanol supernatant and filtered it through 50 µm nylone mesh filter. Then they analyzed the filtered supernatant for the oligosaccharide content using a light scattering detector (Polymer Labs, Amherst, MA, USA). A Prevail carbohydrate ES HPLC column (250 4.6 mm, Alltech Associates, Deerfield, IL, USA) was used to separate the oligosaccharides for their experiment; the injection volume was 20µL. The mobile phase used two solvents at a gradient flow of 1 mL /min that changed from 100% A to 50% A and 50% B over 20 min. Solvent A was 75% acetonitrile and 25% HPLC-grade water and solvent B was 25% Acetonitrile and 75% HPLC-grade water. The total run time was 30 min. and the standard curves for determined of these oligosaccharides is prepared using raffinose and stachyose standards according to the literatures.

4.2.7. High-Temperature Gas Chromatographic Detection

High-temperature GC (HTGC) was developed and first published in 1980s [34]. In this approach, the oligosaccharides are reduced with KBH_4, neutralized with acetic acid, and methylated with iodomethane in dimethyl sulfoxide and NaOH and analyzed by capillary gas chromatography using flame ionization detection. Although this analytical technique was initially not considered as sufficiently robust compared to HPLC or supercritical fluid chromatography, it has recently been successfully used for the analysis of hydrocarbons with the number of carbons in the molecule exceeding the value of 130 (simulated distillations), for the analysis of lipids, emulsifiers, detergents, polymeric additives, oligosaccharides, porphyrins and many other substances with a high boiling temperature [35, 36]. In HTGC, for the extension of the boiling point distribution range of substances with low volatility, a column with a thin-film silicone stationary phase of 0.1 µm or less and with 0.53 mm internal diameter of column is used. In addition, the temperature of analysis reaches up to 450°C. Under these conditions, the substances elute from the column by about 260–320°C before their boiling temperature calculated for atmospheric pressure [37]. These authors also used this high-temperature gas chromatography with mass spectrometry detection (HTGC-MS) for separation and identification of iridoids (aucubin, catalpol), flavonoid aglycone (quercetin, apigenin, luteolin) and flavonoid glycosides in standard mixtures and real natural matrices. There is developed two-step derivatization process in this method which allowed direct silanization of polar analytes in the plant material without the need of extraction. Apart from RFOs, the method was also used to analyze polyphenols, including glycosylated polyphenols, via a procedure based on injection-port derivatization coupled to gas chromatography–tandem mass spectrometry (GC–MS/MS) by extracting polyphenols in lyophilized fruit samples with an acidified MeOH mixture assisted by ultrasound [36].

5. Factors Determining Presence of RFOs in Food Crops

5.1. Crop Varieties

The raffinose family oligosaccharide composition is known to be dependent on the plant species and varieties under investigation. The leguminous plants are reported in literatures as highest raffinose sources among the different plant species [24, 10, 38]. Moreover, lupin, soybean, field pea, chickpea, lentils, common bean and mung bean and the like are considerable sources of raffinose family oligosaccharides [5]. The literature also imply that lupin among these leguminous are predominant source in these oligosaccharides composition. These authors also reported that the composition of these oligosaccharides both in

qualitative and quantitative term differ not only among family, rather also among species and varieties of these families. Different varieties of the same crops are also different in their raffinose family oligosaccharide compositions. For instance, there is high variability in RFOs contents among different soybean genotypes as indicated in a study conducted in India [12]. According to the study by these authors, RFOs content of different soybean varieties ranges between 0.60 and 2.62 mmol/100 g of samples. A study on RFOs in 13 lupin varieties in Spain also indicated that there are large variations in the levels of individual RFOs between lupin cultivars [22]. According to these authors, the total RFOs ranges between 5.30 and 12.30 in different varieties of lupin. In various black gram varieties, wide range of variability has been reported by scholars and RFOs is varied between 26.64 and 61.57 mg/g [39].

Table 2. *Variation of RFOs between cultivars of soybean and lupin.*

Crop	Cultivars	Raffinose	Stachyose	Verbascose	Total RFOs	References
Soybean	EC216379	1.6	7.1	ND	8.7	a
	Dada Cha-ma-me	2.53	4.80	ND	7.33	
	SL525	1.17	2.09	ND	3.26	
	PK1042	0.65	3.27	ND	3.92	
	Alankar	1.65	5.24	ND	6.89	
	G2129	1.78	5.31	ND	7.09	
Lupin	L.biteus LO-4500	0.54	6.13	2.79	9.46	b
	L.abgustifolus LO-4820	0.89	3.62	0.79	5.30	
	L.albus cv multolupa	0.62	5.74	0.19	7.56	
	L.albus cv marta	0.33	7.24	0.94	8.51	
	L.albus LO-3844	0.44	7.26	ND	7.71	
	L.albus LO3855	0.48	4.98	ND	5.46	

a- [12], b- [22]

Raffinose family oligosaccharides are soluble low-molecular weight oligosaccharides which occur in plant and plant products in various forms such as raffinose (trisaccharide), stachyose, verbascose. It has been discussed that they occur to different extent in different types of crops.

5.2. Processing Methods

The different types of processing can reduce anti nutritional factors in general and raffinose in particular. For example, raffnose family oligosaccharide was reported to be lowered during blanching leguminous vine peas [15]. Hence, this is why different agricultural products known for their raffinose content in particular and antinutrient content in general should pass through different processing stages before consumption. As indicated in a research reported from India, cooking of soybean reduced raffinose and stachyose content by 45.0% and 25.0% respectively [3]. Authors farther reported a 26.6% decrease in raffinose and 20.1% decrease in stachyose content. Treatments like gamma radiation are also reported in literatures as affecting RFOs contents of soybean [40]. Moreover, enzyme treatment can also reduce raffinose and stachyose content by 80.0% and 85.0%, respectively according to the authors. The sprouting of crops like soybeans has also been reported to reduce RFOs and improve its nutritional values [41]. From these literatures, it is possible to understand that different processing methods have various level of influence on RFOs content and compositions of food sources. The extent of reduction in RFOs content of soybean sample was indicated in the following table as an example of effect of processing methods on RFOs compositions.

Table 3. *Influence of some processing methods on RFOs in soybean.*

Treatment	Raffinose g/kg	Stachyose g/kg
Raw	9.84	16.46
Soaked	7.22	13.15
Cooked	5.41	12.34
Enzyme treated	1.96	2.46

Source: [3]

5.3. Growing Environment

The growing environment is also among important factors affecting levels of availability of raffinose family oligosaccharides. The growing environment can impose effects on raffinose and stachyose contents of seed of dry beans by interacting with the variety as literatures imply [42]. The synthesis of RFOs is considerably high in environment where there is abiotic stress and even induced by this abiotic stress [8]. However, RFOs content in soybean seeds is not statistically significantly affected by environmental factor alone as implying in a literature [12]. Nevertheless, growing environment is reported to affect RFOs by interacting with other factors including crop varieties.

6. Conclusion

Sucrose content in various leguminous crop seeds is anticipated to be high due to its sweetening properties; and its benefits in acceptability food products. On the other hand, galactosyl derivatives of sucrose which in called raffinose family oligosaccarides including raffinose, stachyose and verbascose, which are flatulence factors need to be in low concentration in the seeds of crops both for enhancing utilization of these crops for food and providing improved utilizable energy for human being and monogastric animals.

The antinutritional properties of RFOs is due to the fact that they cause flatulence, stomach ache, diarrhea and other similar discomfort owing to the absence of α-galactosidase enzyme in human and monogastric animals. It will pass down to lower gut and undergo fermentation where they contribute to gas production and hence different kinds of stomach discomfort. Consequently, RFOs is anti-nutritional factor in this regard.

The natural sources RFOs may include but not only limited to legumes, cereal and vegetables. RFOs can be considered prebiotics as they support growth of normal micro flora. Apart from their antinutritional effect, RFOs still have nutritional advantages. There are also reports indicating the role of RFOs in minimizing or preventing the risk of chronic diet related problems. Therefore, RFOs can potentially benefit human health. The literatures in this field across the world have reported relationship between the consumption of RFOs and the positive effect in gut microflora and metabolic activity. It is reported in literatures that there are farther positive effects related to consumption of foods containing RFOs among which significances on satiety regulation, body weight gain, mineral absorption, immune response, lipid and glucose homeostasis, and oxidative stress are indicated. There are various methods used for extraction and quantification of RFOs some of which are reviewed in this paper each having their respective strength and drawback. There are different factors determining the level of RFOs in food reported in literatures. Crop species, variety, processing methods and growing environment are reviewed among these factors. Farther research however, is very important in the field to characterize the varieties of different crops for these oligosaccharides, evaluated processing methods that better minimize their level and the related health consequences especially in developing countries including Ethiopia.

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