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# Role of Probiotic α-galactosidases in the Reduction of Flatulence Causing Raffinose Oligosaccharides (RFOs)

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## Abstract

Raffinose family oligosaccharides (RFOs) are a class of carbohydrate oligosaccharides in which the galactose residues are attached to sucrose moieties *via* α-glycosidic linkage. These compounds are present in high quantities in many plant-based foods like pulses. As the human digestive system is devoid of α-galactosidase activity, it cannot use these oligosaccharides as an energy source. Therefore, these oligosaccharides pass through the human digestive system and are metabolised in the lower intestinal tract by gut microbes, which produce $CO_2$ and methane as by-products causing flatulence. Therefore, these oligosaccharides are considered undesirable in the human diet. Various strategies have been used to decrease the content of these RFOs, like genetic engineering to inhibit RFOs biosynthesis and the use of post-harvesting and cooking methods. Here we summarise an attractive alternative strategy using which the content of RFOs can be decreased while providing added nutritional advantages by using probiotic microbes.

## Introduction

Human consumption of legumes like soybean has been hampered by the presence of non-digestible oligosaccharides (NDOs), which are not eliminated by processing. In humans, these NDOs are metabolised by microorganisms in the large intestine, liberating large amounts of gas, which can cause gastrointestinal disorders in sensitive individuals. These oligosaccharides include α-galactosyl derivatives of sucrose, such as raffinose and stachyose. Raffinose is an oligosaccharide composed of galactose, glucose, and fructose residues that can be hydrolysed to D-galactose and sucrose by the enzyme α-galactosidase. This trisaccharide is non-digestible to humans but can be metabolised by colon bacteria. Similarly, other RFOs too escape the digestion process and are utilised by gut microbes (bacteria) to synthesise by-products such as hydrogen ($H_2$), carbon dioxide ($CO_2$), and methane ($CH_4$). Therefore, they are flatulence-inducing components and need to be in low concentration in soybean seeds not only for augmenting utilisation of the crop in food uses but also for delivering soy meal with improved metabolisable energy for monogastric animals. RFOs, unlike sucrose, are not digested in the human gastrointestinal tract due to the absence of the α-galactosidase enzyme in the small intestine required to break down the α-glycosidic linkage. As a result, RFOs pass to the lower gut, where they serve as ideal substrates for fermentation caused by intestinal microflora.

## RFOs as Prebiotics

These RFOs are considered excellent prebiotic candidates, as they can be used as prebiotic molecules using microbial enzymes *via* catalytic transformations. Recent

studies have reported the prebiotic potential of RFOs in the human gut, as they promote the growth of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli* and reduce the harmful bacteria present in the colon. The fermentation of lactulose and raffinose significantly increases the total amount of lactate and post-biotic compounds such as short-chain fatty acids (SCFAs). RFOs positively affect the gut microbiota, large intestines, and colon health and can be used as therapeutic agents to reduce inflammation, diabetes and allergies.

## RFOs and Probiotics

Microbial α-galactosidase is a promising solution for degrading these NDOs. α-Galactosidase (galactohydrolase, EC 3.2.1.22) is widely distributed in microorganisms, plants and animals. Most α-galactosidases are acidic proteases; however, an alkaline α-galactosidase from melon (*Cucumis melo*) fruit tissue with a substrate preference for raffinose have also been reported in the literature. This enzyme is generally produced in culture conditions supplemented with one or more α-D-galactopyranosyl groups as carbon sources and is inducible, intracellular, and partially membrane-bound in most microorganisms. Lactic acid bacteria (LAB) have been consumed in fermented foods by humans for centuries without any apparent adverse effects. LAB such as *Lactobacillus plantarum*, *L. fermentum*, *L. brevis*, *L. buchneri* and *L. reuteri* can hydrolyse α-galactosides into digestible carbohydrates during vegetable fermentations (Sasi *et al.*, 2022). The molecular characterisation of an α-galactosidase from *L. plantarum* has also recently been published. Furthermore, *Weissella* spp. strains with a potential to use raffinose have also been characterised. The role of endogenous α-Gal in RFOs degradation in fava bean flour have also been reported previously. Therefore, it has been hypothesised that the combined action of endogenous and microbial enzymatic activity leads to the breakdown of RFOs. An *in vivo* study on mice showed that an intragastric administration of raffinose oligosaccharides improved both beneficial microbes and immunological functions.

## RFOs Metabolism by Microbes

α-Galactosidase acts upon gal-gal bonds in the tetrasaccharide stachyose, releasing galactose and raffinose, and acts upon gal-glu bonds with the release of sucrose (Figure 1). Sucrose is, in turn, split by invertase, producing fructose and glucose. α-Gal production was observed to be higher at pH 5.5, 5.0, and without pH control than at pH 4.5 and 6.0. This result was again proportional to final bacterial concentrations after fermentation since more bacteria are found at pH 5.5 and 5.0 than at the other pH values. An advantage of using only α-galactosidase to hydrolyse RFOs is that there is no loss in soluble solids, and the RFOs are converted into digestible sugars. This means that the vitamins and minerals that confer nutritional quality to the navy beans remain in the final product. Enzymatic degradation by immobilised α-galactosidase can also be used to reduce RFOs.

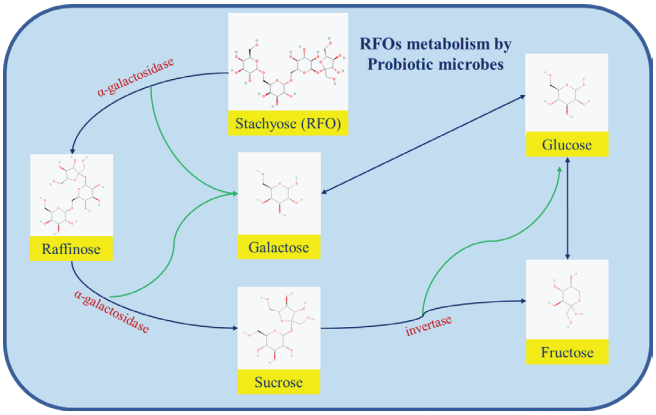


Figure 1: Fermentative metabolism of RFOs by the probiotic microorganisms

## Regulation of RFOs Metabolism

However, it has been reported that in *Pediococcus pentosaceus,* the fermentation of some sugars, including raffinose, is plasmid-encoded, and the presence of α-Gal is inducible (Gonzalez and Kunka, 1986). Raffinose was the most effective inducer, followed by melibiose and galactose; the enzymes were partially inhibited by fructose and sucrose. In Gram-negative bacteria, α-galactosidase can be induced by melibiose and raffinose and is subject to catabolite repression by other simple sugars. Significant repression of enzyme activity can also be observed in the presence of sucrose, glucose, and fructose. The minimal growth rate and α-galactosidase activity obtained in the presence of simple sugars were due to a carbohydrate repression effect (Mabinya *et al.*, 2004). This is consistent with a reportswhich showed that adding 1% glucose or lactose to soymilk decreased α-galactosidase activity. Invertase hydrolyses sucrose and produces one molecule each of glucose and fructose. Hence, the invertase activity produced by microorganisms can also cause repression of α-galactosidase activity. Based on these results, it is probable that the enzyme was inhibited by the intermediates released during soymilk fermentation. In addition, when glucose was added at the logarithmic growth phase of the strains in MRS media, a marked decrease in enzyme activity was observed. This suggests that glucose has a suppressive effect not only at the start time but also throughout fermentation. Some forms of α-galactosidase can also exhibit galactose inhibition andthis phenomenon occurs when galactose is produced as a product of the hydrolysis of stachyose, and raffinose binds to the active site of the

enzyme, preventing the access of substrates and thereby inhibiting the rate at which the substrates are hydrolysed. Therefore, the effects of monosaccharides and disaccharides on the α-galactosidase activity should be highly considered for estimating the reduction of NDO in soy products.

## Conclusion

To summarise, RFOs could be exploited as functional foods due to their prebiotic nature. Its multifunctional benefits are still yet to be realised in human and animal well-being. Several food scientists have suggested improving soy foods' nutritional value by reducing the RFO content. We need to strike the right balance of RFO content in crops to promote them as functional foods. Therefore, it is essential to determine the proper dose of raffinose intake through *in vivo* models, consisting of the recommended amount that maximises the prebiotic effect while presenting minimal secondary effects. α-Galactosidase enzyme treatment with RFOs could be efficient, inexpensive, and reliable.

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