Degradation of Raffinose and Stachyose in Solid State Fermented Lathyrus sativus Seeds

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Introduction

The Galactose containing oligosaccharides such as raffinose and stachyose are abundant in nature. They are found most frequently in seeds, roots, and under grounds stems. The quantity of raffinose and stachyose are greater in the seeds of legumes like soybeans, chickpeas and other seeds[1]. Though the legumes have been considered as leading groups in protein supply of the developing countries of the world where malnutrition prevails at a high rate and the seeds contain 2 to 3 times more protein than cereal grain; these legumes have not become popular due to the presence of flatulence causing oligosaccharides and other antinutritional factors[2].

The processing methods such as washing, cooking, fermentation and germination are reported to cause considerable reduction of these oligosaccharides in the legumes[3] like chickpeas; but there was no study of the effect of solid state fermentation by Rhizopus oligosporus on the flatulence causing oligosacchardies like raffinose and stachyose of Lathyrus sativus seeds. The purpose of this study was to see the effect of solid state fermentation of Lathyrus sativus on Raffinose and Stachyose in relation to improvement of the nutritional quality of these seeds which are cheap in Bangladesh, are easily grown by the people of Bangladesh and are eaten by the poor class of people of the country during famine[4].

Materials and Methods

Lathyrus sativus seeds were purchased from the local market of Bangladesh and stored at room temperature until all tests could be made. The seeds 50 gm portions were soaked in 150 ml of water for overnight, washed twice and steamed for 10 minutes and inoculated with 0.5% tempeh inoculum (Rhizopus oligosporus). The inculum was obtained from the Nutrition Research Development Centre, Bogor, Indonesia. Inoculated seeds were packed in glass petridishes (100 x15 mm dia) and fermented for 10, 20, 30, 40 and 50 hours at 30°C[5]. The control seeds were processed and inoculated by the same procedure and kept at 4°C in the refrigerator. The fermented 10, 20, 30, 40 and 50 hours samples (Petridishes) were kept out of incubator and steamed for 10 minutes to kill the mold and kept in the refrigerator at 4°C.

Carbohydrate was done according to the Method of Hallab[6] (1970). In this method, carbohydrate was extracted from the samples with 95% hot alcohol in presence of $CaCo_3$, and boiled on steam bath for 30 minutes. After cooling, extract was decanted, then extraction was repeated, combined together and filtered. Dry residue was grinded, extracted again and total extracts were collected and made to a certain volume with 80% ethanol.

Separation of sugars and nonsugars: Extracts were placed in a beaker and

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evaporated all alcohol in steam bath and transferred to a volumetic flask. Precipitations were made after adding saturated lead acetate (0.5-1.5 ml), filtered and made to a certain volume. The extract was deleaded by adding potassiusm oxalate, filtered and were ready for sugar analysis. Simple sugars and oligosaccharides were determined according to the method of Michel Dubois et al [7]. In this method simple sugars were analysed from the sample by the reaction of phenol sulfuric acid and read at 490 mμ in Perkin Elmer (Perkin Elmer, 35 spectophotometer, Coleman Instruments Division, Oak Brook, Illinois, USA).

Table 1. *Raffinose equivalent sugar content of during soild state Fermentation.*

Sample : Hours of fermentation		Raffinose equivalent sugar (mg/g)	Times increase in sugar content
1	O hours (control)	8.72	
2	10 hours	16.82	1.93
3	20 hours	19.08	2.2
4	30 hours	18.25	2.1
5	40 hours	15.63	1.8
6	50 hours	14.10	1.6

Table 2 . *Stachyose equivalent sugar content of Lathyrus sativus seeds during soild state formentation.*

Sample	Fermentation hours	Stachyose equivalent sugar (mg/g)	Times increase in sugar content
1	0 hours	8.65	
2	10 hours	10.61	1.23
3	20 hours	19.31	2.23
4	30 hours	19.90	2.30
5	40 hours	15.26	1.8
6	50 hours	14.10	1.6

Results

The results are given in Table 1 and Table 2.

Discussion

In the Table No. 1, it can be seen that after solid state fermentation of Lathyrus sativus seeds, the amount of raffinose is degraded to simple sugars. The stachyose equivalent sugar is also increased with the increasing of fermentation hours. In 0 hour, stachyose equivalent sugar content was 8.65, where as 10 hours fermentation, it increased to 1.23

time, 20 hours to 2.23 times, 30 hours 2.30 times. Then gradually raffinose equivalent sugar content was decreasing slightly in 40 and 50 hours. The maximum raffinose equivalent sugars increased in 30 hours in the solid state fermented Lathyrus sativus seed at 30 hours at 30°C-36°C. In 40 and 50 hours, the products become ovefermented and are not suitable for eating purpose, If the sugar content of raffinose increases after fermentation, the flatulence will be decreased. In the similar way, the stachyose equivalent sugar content also increases during the increasing of fermentation hour and it reaches maximum in 30 hours and decreases slightly downwards in 40 and 50 hours. But, the solid state fermented products become edible in 30 to 36 hours[8] fermentation and flatulence decrease after fermentation. Raffinose and stachyose metabolise to equivalent sugars after fermentation to improve the nutritional status of Lathyrus sativus seed.

Summary

Flatulence causing oligosacchardies of the solid state fermented and unfermented Lathyrus sativus were studied in this paper and was found that the flatulence causing oligosaccharides raffinose and stachyose are degraded to simple sugars after fermentation. The maximum degradation to sugars of raffinose and stachyose occurs at 20 and 30 hours fermentation. The fermented products of the legumes are acceptable for human consumption upto 30 hours fermentation and there is no off flavor of the product at this fermenting period. Moreover, by this solid state fermentation, the other nutritional cosntents are also increased and at the same time flatulence causing oligosaccharides are reduced. Therefore, there are more benefits in fermenting the legume seeds to improve the seeds in terms of Nutrition.

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