# An improved method to obtain pure α-galactosides from lupin seeds

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**ABSTRACT**

Improvement of a previous described method of purification of α-galactosides, raffinose family oligosaccharides (RFOs) has been developed for lupins. The considerable amount of sucrose present in the RFO preparations obtained by previous method has been removed by modifying the purification stage on diatomaceous earth and charcoal. The present method allows for the preparation of high purity RFOs containing about 99.4% of RFOs in form of white fine powder which provides new perspectives for the production of pure α–galactoside preparations for their use as prebiotics in functional foods.

**Keywords:** α–galactosides, raffinose family oligosaccharides (RFOs), lupins

## INTRODUCTION

α-Galactosides, called also raffinose family oligosaccharides (RFOs) are widely distributed in the plant kingdom. Large amounts of RFOs occur in the generative parts of higher plants where perform protective physiological functions *(1-4)*. In legumes, RFOs accumulate during seed development and raffinose and stachyose are formed *de novo* during seed maturation *(5, 6)* and are carbon reserves utilised during germination *(7, 8)*. Legumes seeds contain a large α-galactoside content and their consumption has been associated with the production of flatulence, since these oligosaccharides are not hydrolysed in the small intestine, due to the absence of α-galactosidases in monogastrical animals, and they are fermented in the lower intestine *(9, 10)*. This problem has been considered to be the single most important factor that deters people from eating this nutritious food *(10)*.

It has been considered recently that pure RFOs are prebiotics. RFOs promote the growth of bifidobacteria population *(11,12)* and, consequently, there is a great deal of interest to use prebiotic oligosaccharides as ingredients in functional foods in order to manipulate the composition of colonic microflora, contributing to human health in many ways.

There are many prebiotic oligosaccharides known, principally in the Japanese market and they are added as ingredients in a large number of products such as soft drinks, cookies, cereals and candies *(12)*. α–Galactosides from soybean are the only legume oligosaccharides on the market and the main producer is Japan *(13)*. In the USA and Europe RFOs are also available on the market, however the market leaders are fructo-oligosaccharides in these regions.

These low-molecular weight oligosaccharides are present in different seeds, but lupins are one of the legumes with the most of α-galactoside content (7-15%) *(8; 14-17)*. The sweet lupins, containing very low levels of alkaloids, can be a good source of RFOs. In 2000 a paper was published describing a simple method for the isolation and purification of α-galactosides from lentils and peas *(18)*, but the obtained RFO preparations contained sucrose in considerable amounts (11 and 12%, respectively). In the present work, a modification of the previous published method is presented that allows obtaining lupin RFOs free of sucrose. It creates new perspectives for production pure α–galactoside preparations for their use as prebiotics in functional foods.

## MATERIALS AND METHODS

**Samples and Chemicals**. Sweet lupin seeds of *Lupinus albus* L. cv. Multolupa, were obtained from the Agricultural Research and Technology Development Service of the Agriculture and Commerce Council of the Junta de Extremadura (Spain). Seeds were cleaned and stored in polyethylene container at 4ºC until used. Diatomaceous earth, charcoal, and naphthoresorcinol were purchased from Sigma, Darmstadt (Germany). Dowex 50WX8, 100-200 mesh was purchased from BDH Laboratories (England). Ethanol, 2-propanol and acetic acid were supplied by Scharlau (Germany). Acetonitrile (HPLC grade) was purchased from ACROS-ORGANIC (Belgium). Silica gel 60 $F_{254}$ TLC plates, sucrose, raffinose, and stachyose were obtained from Merck, Darmstadt (Germany). Milipore FH (0.45 µm) membranes were obtained from Millipore (Bedford, MA).

**Isolation of RFOs.** Lupins seeds were submitted to different selective extractions according to a previous procedure described by Gulewicz et al. *(18)*, (Figure 1).

**Purification of RFOs**. The purification scheme of RFOs is shown in Figure 1. RFOs precipitate was dissolved in 25 mL of distilled water and suspended in a glass funnel (pore size G4, 7cm x 5 cm i.d.) with diatomaceous earth and charcoal (1:1 w/w) and connected to vacuum. The funnel was washed with 6% ethanol to remove sucrose. The absence of sucrose in the eluat was confirmed by thin-layer chromatography (TLC) according to Dey, *(7)*. TLC of carbohydrates was performed on silica gel 60 F254 plates with 2-propanol:acetic acid:water (5:2:3 v/v). Carbohydrates were visualised with naphthoresorcinol. After confirming the absence of sucrose, RFOs were eluted with 70% ethanol until negative naphthoresorcinol reaction. The ethanol RFOs fraction was concentrated to dryness on a vacuum evaporator at 50ºC. The RFOs were dissolved in 10 mL of distilled water, transferred into a Dowex 50WX8 column (12 x 1.5 cm i.d.), and washed with distilled water (50 mL) until negative naphthoresorcinol reaction. The acid solution of RFOs (pH 2) was adjusted to pH 7 with 4% freshly prepared $Ca(OH)_2$. The solution was then boiled for 2 minutes and centrifuged for 10 minutes at 10.000 rpm. Supernatant containing a high purity RFOs was then evaporated to dryness on a vacuum evaporator at 50ºC.

**Determination of total soluble carbohydrate content.** Sucrose and α-galactosides content were determined by HPLC following the procedure described by Granito *et al*. *(19)*.

## RESULTS AND DISCUSSION

Figure 2 shows chromatograms of RFO preparations from lupins obtained as previously described by Gulewicz et al., *(18)* (method A) and improved method described in this work (method B). It can be observed that chromatographic peak of sucrose was sharply reduced using method B, whilst raffinose, stachyose and verbascose increased. These peaks were quantified and results are shown in Table 1. The improved method described in this paper increased to 99.4% the purity of RFO preparations, compared with a 75.1 % obtained using method A. The RFO powder obtained presented a 12.6% of raffinose, a 62.3% of stachyose and a 24.5% of verbascose, and only a trace amount of sucrose (0.8%) was detectable (Table 1).

There are five stages in the improved method of isolation and purification α-galactosides from legume: (i) imbibition of seeds, (ii) extraction of RFOs, (iii) RFO precipitation, (iv) purification of RFOs on diatomaceous earth and charcoal, and (v) cation-exchange chromatography. In the purification stage with diatomaceous earth and charcoal (iv) percolation of RFOs with water used in method described by Gulewicz *et al*., *(18)* was not efficient for total removal of monosaccharides, sucrose and other impurities. Using the 6% ethanol solution makes possible entire elution of sucrose, that has been confirmed by HPLC analysis. Finally, using a higher concentration of ethanol (70%) conducted to the total elution of RFOs and the obtained preparation shows very high purity (> 99%).

The removal of RFOs from legume seeds has been widely studied from the nutritional point of view, in order to obtain legume flour with low flatulent-causing compounds *(20, 21, 22),* but little attention has been paid to the extraction and purification of these α-galactosides as prebiotic substances. Lupin seeds have been previously used as source of RFOs but in those studies purity of the isolated have

not been reported *(15)*. Kim et al. *(23)* optimizated the extraction of oligosaccharides from soybean meal with either water at 50ºC or 10% ethanol and purification was carried out by ultrafiltration. These authors found fructose, sucrose, raffinose and stachyose in their concentrates. Gulewicz *et al*., *(18)* obtained soluble sugars preparations containing 11.5% sucrose, 7.2% raffinose, 29.3% stachyose and 40.3% verbascose from peas and 12.2% sucrose, 5.6% raffinose, 9.3% ciceritol, 39.5% stachyose and 11.5% verbascose from lentils. The improved method described in this paper offer a simple and rapid procedure for obtaining high purity RFO preparations free on monosaccharides and disaccharides from lupin seeds and it could be apply to other legumes. The pure RFOs can be offered as functional food ingredient like prebiotic, which can contribute to human health.

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**Table 1.** Percentage of α-galactosides and sucrose in the RFO preparations from lupin obtained by method A and method B

| **Sugars** | **Method A*** | **Method B**** |
|---|---|---|
| Sucrose | 20.22 ± 0.11 | 0.82 ± 0.01 |
| Raffinose | 9.65 ± 0.33 | 12.56 ± 0.91 |
| Stachyose | 52.99 ± 2.13 | 62.33 ± 0.48 |
| Verbascose | 12.50 ± 1.01 | 24.47 ± 0.05 |
| **Total of α-galactosides** | **75.14 ± 2.86** | **99.36 ± 1.43** |

*) Method A: According to Gulewicz et al. *(18)*

**) Method B: Present improved method