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# Optimization of soaking condition of blackgram to minimize flatogenic sugar content in blackgram-based products

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Verbascose (PubChem CID: 441434)
Xylose (PubChem CID: 644160)

ABSTRACT

Raffinose family oligosaccharides (RFOs), comprising 41.7 g/kg blackgram dal (dry wt basis), are important antinutritional factors. In this study, response surface methodology was used to optimize the soaking conditions of blackgram dal in order to reduce these flatogenic sugars. A central composite rotatable design was used to study the effect of four separate soaking parameters. The optimum soaking condition obtained was: bean-water ratio of 1:10, and soaking temperature, time and pH being 16 °C, 21 h and 6.0, respectively, in which the total RFO content predicted in soaked dal was 1.97 g/kg (dry wt basis). The experimentally obtained value was 2.02 g/kg (95.16% reduction over raw dal). Lack of significant difference between the experimental and predicted values validates accuracy of the model. While fermentation of mixed batter prepared by using traditionally soaked dal led to 57.35% decrease in the total RFO content, that prepared by using optimally soaked dal reduced the contents of each of the RFOs below their respective limits of detection (decrease of total RFOs by >93.85%). Steaming of the fermented batter for 15 min had no significant effect on the RFO content.



## 1. Introduction

Grain legumes are a nutritionally rich and inexpensive source of dietary protein in many parts of the world, especially in countries like India where the majority of the population is vegetarian. Besides protein, legumes are a good source of dietary fibre, vitamins and micronutrients. The functional oligosaccharides, like raffinose family oligosaccharides (RFOs) present in legumes, also have some beneficial health effects like maintaining the population of gastrointestinal microbiota and reducing the risks of gut infections (Ngo, Kim, & Kim, 2008). For this reason, these act as good prebiotics. In spite of this, they are under-utilized due to the presence of several antinutritional factors (Dahiya et al., 2015). The oligosaccharides present in blackgram (*Vigna mungo* (L.) Hepper; synonym *Phaseolus mungo* L.), like in other legumes, are the RFOs which include raffinose, stachyose, verbascose and ajugose. In mature legume seeds, RFOs are present at a level of 31–76% of the total soluble sugar content (Reddy, Pierson, Sathe, & Salunkhe, 1984). Blackgram contains 7.6, 6.5, 33.2 and 16.6 g/kg (dry wt basis) raffinose, stachyose, verbascose and ajugose, respectively (Girigowda, Prashanth, & Mulimani, 2005), while raw soybean (*Glycine max* (L.) Merr.) contains 19.2 and 43.2 g/kg (dry wt basis) raffinose and stachyose, respectively (Sarkar, Jones, Craven, & Somerset, 1997) and kidney bean (*Phaseolus vulgaris* L.) contains 2.9 and 18.4 g/kg (dry wt basis) raffinose and stachyose, respectively (Shimelis & Rakshit, 2007). These RFOs consist of one or more galactose units attached to a sucrose unit via α-D-1,6 linkages.

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Ajugose, being a higher homologue, is present in traces in the seeds (Girigowda et al., 2005). The RFOs are the principal cause of flatulence in humans and monogastric animals (Jood, Mehta, Singh, & Bhat, 1985). The gastro-intestinal tract in them lacks the enzyme α-galactosidase which hydrolyzes the α-1,6-galactosidic linkage of these RFOs (Tachibe, Ohga, Nishibata, & Ebihara, 2011). Consumption of inadequately processed legumes containing higher contents of RFOs in them leads to gastronomical discomfort. These RFOs escape digestion and absorption in the stomach and the small intestine and reach the colon where they undergo anaerobic fermentation by bacteria to produce several gases such as $CO_2$, $H_2$ and a small amount of $CH_4$ (Rackis, 1975). Besides social implications, flatulence is characterized by abdominal cramps, diarrhoea and nausea (Albersheim & Darvill, 1985). Hence, it is necessary to determine the conditions for reducing the effect of these RFOs and to improve the nutritional quality and digestibility of legumes. There are several practices like malting, dehulling, cooking and enzyme treatment which help to reduce the RFO content in legumes (Price, Lewis, Wyatt, & Fenwick, 1988). There are reports of the effects of soaking, germination and cooking on the RFO content of blackgram (Iyengar & Kulkarni, 1977; Rao & Belavady, 1978).

The most popular mode of consumption of blackgram dal (dehulled and split seeds) is in the form of a soup and the most common household practice of processing blackgram prior to the preparation of soup is soaking them in water, preferably overnight. The soak water is drained and soaked beans are cooked in fresh water. However, the cook water, which contains further leached out RFOs, is not discarded before consumption. Hence, reduction of RFO content at the time of soaking is imperative. Besides soup, a mixture of soaked blackgram dal and rice is used in the preparation of popular fermented foods such as idli and dosa.

Traditionally, an optimization process is achieved by an empirical method in which one factor is varied at a time while others are kept constant. This one-factor-at-a-time approach is cumbersome, expensive and less effective as interactions between the variables cannot be studied. When many factors and interactions are studied to obtain a desired response, response surface methodology (RSM) is the preferred and effective optimizing tool that helps to understand and quantify the interactions between variables (Kristo, Biliaderis, & Tzanetakis, 2003).

To the best of our knowledge, there is no report on the optimization of blackgram processing with respect to reduced RFO content during the production of blackgram-based products. This study aimed at a comparative evaluation of the effects of different process parameters, viz. soaking, fermentation and cooking on the RFO content, and response surface optimization of soaking condition of blackgram in order to minimize the amount of RFOs in this representative blackgram-based product.

## 2. Materials and methods

### 2.1. Preparation of idli

Blackgram dal and white polished rice grains, purchased from a local market in Siliguri, were washed with tap water and then with distilled water followed by soaking separately in distilled water (Fig. 1). While soaked rice was coarsely ground, dal was ground to a smooth, mucilaginous paste. The rice slurry and dal paste (2:1) were stirred well with added common salt (8 g/kg) to form a thick batter which was put in a closed container and left to ferment spontaneously at 30 °C for 18 h. For the preparation of idli, the leavened batter was dispensed in cups (7 cm in diameter having holding capacity of 40 ml) of stainless steel-made idli pan and steamed under cover for 15 min to prepare soft and spongy idli cakes with a honey-comb appearance.

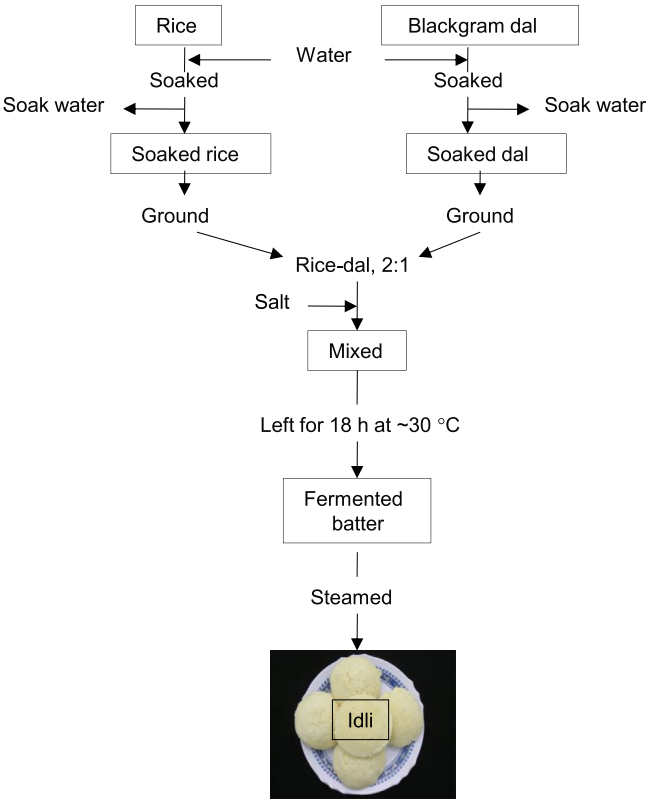


**Fig. 1.** Flow sheet for the production of idli. Boxed texts are the sites of sampling for oligosaccharide assay.

### 2.2. Preparation of sample

Raw beans and rice grains were ground to powder, while the soaked beans and grains were made into paste using a waring blender (Bajaj, India). All these samples as well as mixed batters and macerated products were frozen overnight at −20 °C, lyophilized (Eyela freeze dryer, model FDU-506, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) and pestled to pass through a 60-mesh sieve. The fine powders were defatted with distilled petroleum ether (Merck 61782225001730) in Soxhlet extractors. The extracts were evaporated at ≤45 °C in a rotary vacuum evaporator and quantified gravimetrically to use for fat correction during calculation of RFO content of the defatted samples on dry weight basis. Defatting was followed by deproteination and RFO extraction by a procedure based on that of Knudsen (1986). Approximately 1 g of the defatted sample was mixed with 10 ml water (HPLC grade, Merck 61765010001730) and brought just to boiling. The mixture was shaken in a 60 °C water bath for 5 min, made up to 10 ml with distilled water and centrifuged at 1100 g for 10 min. The supernatant (3.5 ml) was mixed thoroughly with 6.5 ml acetonitrile (SDFCL 25251 L25) and left overnight at 4 °C. After filtering (G3 sintered glass), an aliquot of the filtrate was placed in a 5-ml glass vial for HPLC analysis.

### 2.3. Chromatographic analysis

The estimation of RFOs was done following the method described by Sarkar et al. (1997). The chromatographic system (Waters Associates, Milford, MA, USA) consisted of an isocratic 515 pump, a Rheodyne manual injector equipped with a 5-μl sample loop, a carbohydrate column (3.9 mm i.d. × 30 cm) having gel particle size of 10 μm, a column heating attachment, a guard-pak