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Quantification of rice and black gram *dal* proportions in *idli* batters by estimation of starch, daidzein and trypsin inhibitor activity

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ABSTRACT

The traditionally used proportion of 3:1 (rice: black gram *dal*, g/g) of ready-made wet *idli* batters available in the Indian market, might be altered due to a large price difference between rice and *dal*. In this work, differences between starch, daidzein and trypsin inhibitor activity (TIA) of rice and *dal* have been utilized to quantify their proportions in *idli* batters. Starch ($R^2 = 0.980$) and daidzein ($R^2 = 0.999$) served as reliable indicators for quantification of rice and *dal* ratio in unfermented batters while TIA could be used for both unfermented ($R^2 = 0.996$) and fermented batters ($R^2 = 0.995$). Different varieties of rice and *dal* were used for making batters, to study varietal influence on these constituents. For unfermented batter, starch content increased from 62 to 89 g/100 g batter as rice proportion increased. Daidzein content reduced from 2.6 to 2.0 mg/100 g batter in unfermented batter while TIA of batter decreased from ~3.0 to ~1.4 U/g sample with decreased *dal* proportion. Robustness of the methods was successfully validated on coded samples and analysis of locally sold *idli* batters using these parameters indicated the proportion of rice and *dal* to be in the range of 2:1 to 5:1.

1. Introduction

Idli is a south Indian breakfast food item, traditionally prepared from rice (*Oryza sativa* L.), especially parboiled and decorticated black gram (*Phaseolus mungo* L.) *dal* (Rekha & Vijayalakshmi, 2011). It is nutritious as it is steamed and involves a blend of a cereal rich in carbohydrates and a legume rich in proteins. Legumes, when consumed in combination with cereals, serve as a source of nutritional and functional proteins having a well-balanced essential amino acid profile (Sharma, Kumari, Nout, & Sarkar, 2017).

Preparation of *idli* is a time-consuming process (Mulmule et al., 2017). Rice and black gram *dal* are soaked in water for 4 h and ground separately. Ground rice and black gram *dal* are then mixed to form a fine batter, salt is added and batter is optimally fermented for about 12 h at 30 ± 2 °C. The fermented batter is then steamed in moulds in the form of small cakes to prepare *idlis*. Due to the elaborate process involved in the preparation of *idli*, popularity of readymade wet *idli* batters as well as ready-to-cook instant mixes is increasing in the Indian market (Ohariya, Singh, & Rajput, 2017). Readymade batters of rice and black gram-based products such as *idli, dosa* and *medu vada* of different brands as well as local supplies are available in the market.

Traditionally, *idli* batter is prepared using three parts of rice and one part of decorticated black gram *dal* (Rekha & Vijayalakshmi, 2011). There is a large price difference between rice and black gram *dal* because of which, manufacturers may be tempted to reduce the proportion of black gram *dal* in batters, without/slightly affecting the texture of the final product, for monetary benefit. There exists no method for quantifying the proportions of rice and black gram *dal* in wet *idli* batters (Paradkar, Singhal, & Kulkarni, 2002), further encouraging this practice. Altering the ratio might not cause any harmful effects to the body, but addition of more rice will dilute the black gram *dal* proportion, thereby reducing the protein content of the product and may also affect the taste and texture of *idli*. Hence, there is a need to develop a robust method which can help in quantification of rice and black gram *dal* proportions in wet *idli* batters.

Rice has a high amount of starch as compared to black gram *dal*. Legumes are a rich source of isoflavones, especially daidzein (Esch, Kleider, Scheffler, & Lehmann, 2016). Trypsin inhibitor is present in both rice and black gram *dal*; however, its proportion is higher in black gram *dal*. Hence, estimation of these components can be correlated to the proportions of the two raw materials i.e. rice and black gram *dal* used in the preparation of *idli* batter.

In the present study, we aimed to estimate the concentrations of starch, daidzein and trypsin inhibitor activity in *idli* batter variants prepared by varying proportions of rice and black gram *dal*. Starch and daidzein served as good indicators for quantifying the proportions of

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rice and black gram *dal* in fresh *idli* batters while trypsin inhibitor activity could be used as an indicator for both unfermented (fresh) and 12 h fermented *idli* batters. Market samples of locally sold wet *idli* batters were analyzed for the above three constituents. The values of starch, daidzein and trypsin inhibitor activity obtained for market samples were compared with values obtained for self–made batters, which helped in accurately estimating the ratio of rice and black gram *dal* in readymade batters.

2. Materials and methods

2.1. Raw materials

Local varieties of dehulled black gram *dal* (*Vigna mungo* L.) and parboiled rice (*Oryza sativa* L.), were purchased from the local market of Mumbai city. Experimental varieties of rice and black gram were procured from Konkan Krishi Vidyapeeth, Dapoli and Mahatma Jyotiba Phule Krishi Vidyapeeth (Pulses Improvement Project), Rahuri, Ahmednagar respectively. Locally sold wet *idli* batter samples were collected from different localities of Mumbai city.

2.1.1. Reagents

Ethanol, perchloric acid, anthrone, sulphuric acid, glucose, hydrochloric acid, toluene, ethyl acetate, formic acid, Tris, calcium chloride were purchased from SD Fine Chemicals Limited (Mumbai, India). Daidzein and *N*-Benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA) were purchased from Sigma Aldrich (St. Louis, MO, USA). Thin Layer Chromatography (TLC) aluminum sheets pre-coated with silica gel 60 F254 (MERCK, Mumbai, India) were cut to 20 × 10 cm and used as a stationary phase. 3× crystallized trypsin was purchased from SRL (Mumbai, India). All chemicals used were of AR grade and all reagents were freshly prepared during each estimation. Methanol and acetone (HPLC grade) were procured from SD Fine Chemicals Limited (Mumbai, India).

2.2. Methods

2.2.1. Preparation of idli batter

Experimental varieties of black gram were crushed slightly in mortar and pestle to remove the hull and split the seeds into *dals*. *Idli* batters were prepared as mentioned by Shrivastava and Ananthanarayan (2014). Wet *idli* batter variants were prepared using various ratios of rice: black gram *dal* (g/g) such as 2:1, 3:1, 4:1 and 5:1, using different varieties of rice and *dal*. Rice and black gram *dal* for each of these proportions were soaked separately for 4 h, followed by draining of water, grinding, using 10, 15, 20 and 25 mL of distilled water for 20, 30, 40 and 50 g rice respectively and 15 mL for 10 g black gram *dal* and mixing the resulting batters to yield different proportions of rice: black gram *dal* (g/g) such as 2:1, 3:1, 4:1 and 5:1 with addition of 0.9 g of salt per 100 g of batter. Fresh batters (0^{th} h of fermentation) and optimally fermented batters (12 h, 30 ± 2 °C) were used for the study.

2.2.2. Determination of moisture content of batters

Moisture content of all the batter samples was determined using the method described by AOAC (2005). Batter samples were kept in hot air oven (Labline, model BST/HAO-1124, Mumbai, India) at 105 °C, till constant weight was obtained.

2.2.3. Extraction and estimation of starch

Extraction and estimation of starch from batter samples was done using the method described by Hedge and Hofreiter (1962) in which 10 μL of test solutions (starch containing extracts from batter samples) were made to 1 mL with distilled water and assayed. Standard curve was obtained using glucose (0–0.1 mg/mL). Starch content of *idli* batter samples was expressed on moisture free basis.

2.2.4. Extraction and estimation of daidzein

Daidzein was extracted from *idli* batter samples with a modification in the protocol (mentioned below) as described by Sukanya and Gayathri (2014). Daidzein standard (0.1 mg/mL in methanol) was used for estimation by HPTLC (Camag® Linomat 5, serial no. 141124, Muttenz, Switzerland). Mobile phase used for analysis was toluene: ethyl acetate: acetone: formic acid (20 mL: 4 mL: 2 mL: 1 mL) (Gour, Nathawat, Arya, & Patni, 2012).

2.2.4.1. Extraction of daidzein. *Idli* batters were tray dried at 50 °C till constant weight (5 h). Dried *idli* batters (4 g each) were extracted with acidified methanol (27 mL of methanol and 3 mL of 6 mol/L HCl) by sonicating the mixture in bath sonicator (Citizon Ultrasonic Cleaner, CUB 2.5) for 1 h. Following sonication, mixture was kept on shaking water bath at 60 °C, 120 rpm for 2 h. The mixture was then centrifuged (J2-MC; Beckman Coulter, Brea, CA, USA.) at 5018 × g at 4 °C for 15 min. Supernatant was collected and concentrated 2.5-fold on vacuum evaporator (IKA® RV 10 rotary evaporator, Bangalore, India) at 26664.4 Pa. Concentrated extract was filtered through PVDF filter of 0.22 μm.

2.2.4.2. Estimation of daidzein content. Filtered extract was subjected to HPTLC analysis. Standard and samples were applied with the aid of Linomat Camag syringe (100 μL). Six microliters each of standard and samples (in triplicates) was applied on pre-coated TLC plates. Band length was maintained as 6.0 mm, application position 8.0 mm with solvent position 85.0 mm. Measurement mode was UV absorbance/reflectance with scanning wavelength 254 nm, tank saturation 20 min with filter paper, scan start position was at 5.0 mm, scan end position was at 85.0 mm and slit dimensions were 6.00 × 0.45 mm with scanning speed of 20 mm/s.

Mobile phase used for analysis was toluene: ethyl acetate: acetone: formic acid in the ratio of 20 mL: 4 mL: 2 mL: 1 mL (Gour et al., 2012). Plates were observed at 254 nm to check for the presence of daidzein. The area under peak was calculated, using standard graph of daidzein, with daidzein concentration ranging from 0 to 0.5 μg/mL. Daidzein content of *idli* batters was expressed on moisture free basis.

2.2.5. Extraction of trypsin inhibitor and estimation of trypsin inhibitor activity (TIA)

2.2.5.1. Extraction of trypsin inhibitor. The method described by Senanayake, Ranaweera, Bamunuarachchi, and Gunaratne (2013) was followed. Three grams of wet *idli* batter variants containing different proportions of rice: black gram *dal* were extracted with 30 mL of 0.01 mol/L NaOH by shaking the mixture on an orbital shaker (Global Corporation, model GMP-GC-80, Mumbai, India) at 120 rpm for 3 h at room temperature. The slurry was centrifuged (J2-MC; Beckman Coulter, Brea, CA, USA.) at 7840 × g at 4 °C for 30 min. Supernatant was collected and used for estimation of TIA.

2.2.5.2. Determination of trypsin inhibitor activity (TIA). The extracts (supernatant) obtained were diluted 20 times in distilled water. Tubes were prepared in triplicates as per the compositions mentioned by Senanayake et al. (2013). The absorbance due to release of *p*-nitro aniline in each tube was recorded at 410 nm. All results of TIA in *idli* batter samples were expressed on moisture free basis.

2.2.6. Preparation of idli batter variants containing different proportions and varieties of raw materials

Keeping the black gram *dal* variety same (commercial variety 2), commercial rice varieties such as parboiled rice, super *Patni*, old *Parimal* and experimental varieties such as Ratnagiri 1 and Karjat 1 were used separately, for preparation of *idli* batters in the ratios of 2:1, 3:1, 4:1 and 5:1 (rice: black gram *dal*, g/g). Starch was extracted and estimated from these batters as described in section 2.2.3.

Next, keeping the rice variety same (parboiled rice), three