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Development of black rice (*Oryza sativa L. Indica*) based crackers for irritable bowel syndrome (IBS) patients

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Abstract
Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder marked by abdominal discomfort and changes in bowel patterns, occurring without a distinct and specific organic pathology. There is no definitive cure for irritable bowel syndrome (IBS), but managing the condition can be achieved by identifying and eliminating factors that like stressful situations, specific medications and most importantly making dietary adjustments. The objective of the current research work was to develop gluten-free crackers for IBS conditions and to carry out proximate, calcium, zinc, and polyphenol content of the most acceptable product. Three variants of crackers *viz*., CV1, CV2, CV3 with black rice, ragi and quinoa flour respectively in different proportions. Descriptive sensory analysis was used to score the developed crackers and CV2 products with black rice and finger millet flour scored highest on the sensory descriptors. AOAC methods were used to analyse proximate and mineral composition and total polyphenol was analysed using the Folin-Ciocalteau method. The analysed CV2 had carbohydrate 71.3±1.0 g/100g, protein 7.95±0.1 g/100g, calcium 41.76±0.1 mg/100g and zinc 13.93±0.1 mg/100g. There lies a great potential and market for developing savory products for IBS conditions as IBS patients tend to have limited food choices.

Keywords: Irritable Bowel Syndrome (IBS), crackers, gluten, black rice, finger millet, quinoa, proximate analysis, calcium, zinc, polyphenols

1. Introduction
The Functional Gastrointestinal Disorders (FGIDs) include a diverse range of chronic conditions that have significant implications for public health as it affects quality of life. Irritable bowel syndrome (IBS) stands out as the most observed FGID in the global population [1]. IBS can be categorized into different types: IBS-D (diarrhoea-predominant), IBS-C (constipation-predominant), IBS-M (mixed), and IBS-U (unclassified) [2]. It presents itself with symptoms like chronic abdominal pain or discomfort, altered bowel habits like bloating, diarrhoea, and constipation [3, 4] and seen mostly in populations above 50 years and common in females than men. The understanding of the causes of IBS remains limited and generally considered to be multifactorial. Several factors have been proposed to contribute to the development of irritable bowel syndrome (IBS), like gastrointestinal (GI) dysmotility, visceral hypersensitivity, increased intestinal permeability, interactions between foods and intestinal bacteria, and biopsychosocial factors [5-7]. Evidence also indicates that serotonin regulation is disrupted in individuals with irritable bowel syndrome (IBS) [8], thereby affecting the overall quality of life.
The Rome Criteria III for irritable bowel syndrome (IBS) was established through consensus based on clinical studies. According to the criteria a patient should have two or more of the following features: Improvement of symptoms with defecation, onset of symptoms related to a change in stool frequency, or onset of symptoms related to a change in stool consistency and also that the patient must experience recurring abdominal pain or discomfort for at least 3 days per month over the past 3 months [9]. However, a 2009 position statement issued by the American Council of Gastroenterology states that no symptom-based criteria have ideal accuracy for diagnosing IBS.

The main objective of interventions for irritable bowel syndrome (IBS) is to alleviate the patient's symptoms and enhance their overall quality of life. Low FODMAP (fermentable oligo-di-mono-saccharides and polyols), gluten-free diets, exclusion diets continue to be the foremost non-pharmacological clinical interventions used by many clinicians for patients with irritable bowel syndrome (IBS) [9-11]. The diet provides substrates for microbial fermentation, and, as the composition of the intestinal microbiota is disturbed in IBS patients, the link between diet, microbiota composition, and microbial fermentation products might have an essential role in IBS etiology. Hence, there is great potential to explore the area of product development that caters to IBS patients. The current research work was designed to develop gluten-free and low-FODMAP savoury crackers.

2. Materials and Methods

2.1 Raw materials and conduction of experiment

Ingredients needed for making crackers *viz*., quinoa flour, finger millet flour, Italian seasoning, mango powder, salt, sodium bicarbonate, butter were bought from local market in Mysuru while black rice flour was procured from a local market at Imphal, Manipur. The experiments were conducted in Nutrition lab, Department of Nutrition and Dietetics, JSS University of Higher Education and Research, Mysuru.

2.2 Preparation of crackers

The cracker dough was made with black rice, ragi and quinoa flour in different proportions as shown in the table 1. Butter, salt, sodium bicarbonate, amchur powder and Italian seasonings were added for flavour and texture development and the quantity remained same for all the variations prepared. The prepared dough was then sheeted and moulded into a cracker shape. The crackers were baked at 150 °C for 25 minutes. Before storing the crackers were allowed to cool down.

Table 1: Cracker dough composition

Cracker variation	Black rice flour	Finger millet flour	Quinoa flour
CV1	100 g	-	-
CV2	60 g	40 g	-
CV3	60 g	-	40 g

2.3 Sensory Analysis

The formulated variations were subjected to sensory analysis by semi-trained panel members with the help of individual scorecards. The parameters considered were appearance, texture, colour, aroma, taste, and overall acceptability for the three different variations of the crackers i.e., black rice flour cracker (CV1), black rice-ragi flour cracker (CV2) and black rice-quinoa flour crackers (CV3) respectively. The most acceptable product was only subjected to proximate, mineral and polyphenol estimation.

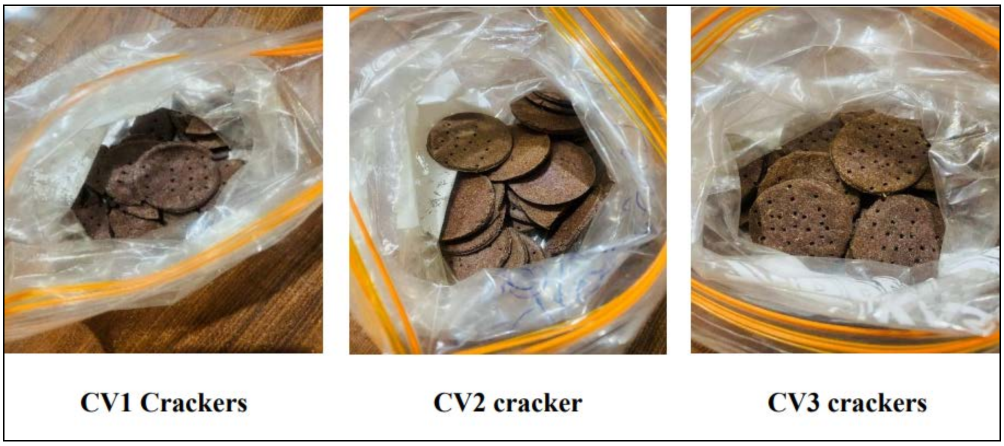

Fig 1: Show CV1 Crackers, CV2 Crackers and CV3 Crackers

2.4 Proximate Analysis

2.4.1 Moisture and Ash Content

Moisture was determined using the oven drying method and expressed as a percentage difference between fresh sample to dried sample. The moisture-free sample was subjected to ashing in a muffle furnace at 550◦C for 6 hours (AOAC 2016) to determine ash [12].

2.4.2 Protein and Fat

Protein content was determined using Kjeldahl method (AOAC 2016) [13]. It is estimated by quantifying the conversion of nitrogen present in the sample to ammonia by titrimetric method. SOCS PLUS method based on the solvent extraction principle was used for fat analysis [12].

2.4.3 Carbohydrate

Anthrone method was used to estimate total carbohydrate content. In the method first, the carbohydrates are hydrolyzed by diluting hydrochloric acid into simple sugars. Then these simple sugars (glucose) are dehydrated to hydroxymethyl furfural in a hot acidic medium. The compound thus formed in presence of Anthrone reagent gives a blue-green color complex which is measured spectrophotometrically at 630nm [14].

2.4.4 Total Dietary Fiber

Defatted dried sample was treated with glucosidases and proteases, which hydrolyzed the starch and proteins. This was followed by treatment with solvent to remove hydrolyzed and depolymerized protein and glucose thereby precipitating fiber. The residue was filtered, washed with solvents, dried and weighed. One duplicate is used to analyze the protein and the other ash content. Total Dietary Fiber is the weight of the filtered and dried residue minus the weight of protein and ash [12].

2.5 Calcium and Zinc

Zinc and iron content was determined by atomic absorption spectrometry. Calibration of the mineral measurements was performed using iron and zinc standards and appropriate acid blanks. All measurements were carried out with standard flame-operating conditions as recommended by the manufacturer [15].

2.6 Estimation of total polyphenols
The FC reagent contains complexes of phosphomolybdic and phosphotungstic acid. In an alkaline environment, electrons are transferred from phenolic compounds, leading to the formation of a blue chromophore comprising a complex of phosphotungstic/phosphomolybdenum. The absorption of this complex is maximized depending on the concentration of phenolic compounds. The reduced Folin-Ciocalteu reagent can be detected using a spectrophotometer within the wavelength range of 690 to 710 nm. To expedite the colour development, a reaction temperature of 37 °C is commonly employed. Gallic acid is used as the reference standard compound, and the results were expressed in as gallic acid equivalents (mg/mL) [16].

2.7 Statistical Analysis
The sensory data was subjected to statistical analysis. Organoleptic characteristics of the formulated different variations of the crackers were statistically analysed by calculation of mean and standard deviation. The different variations of the formulated crackers were compared using one-way ANOVA.

3. Results and Discussion
Food is typically not regarded as a direct cause of irritable bowel syndrome (IBS), but rather as a factor that can trigger symptoms. However, it plays a crucial role in the management of IBS for many individuals. Exclusion of gluten and low FODMAP products has been shown to be beneficial in some IBS patients. Consequently, there is significant potential for exploring, reformulating, modifying, incorporating new ingredients, or eliminating certain ingredients from existing products to make them suitable for individuals with irritable bowel syndrome (IBS). The category of baked savoury products is one area that can be actively explored for potential modifications and adaptations.

The current research work primarily focused on the development of cracker products. Crackers are crispy, thin baked goods made from dough that is unsweetened and unleavened. Depending on the ingredients and the way they are made, crackers can be classified into three primary categories: Soda crackers (such as saltines or cream crackers), snack crackers (also known as sprayed crackers), and flavoured or savoury crackers [17]. Three variations of crackers were created for the study. The first variation, CV1, served as the control and was made using 100g of black rice flour. The second variation, CV2, incorporated 60g of finger millet flour, while the third variation, CV3, used 60g of quinoa flour.

3.1 Sensory evaluation of the cracker variations
Sensory attributes are a major consideration in acceptance and commercial success of a product, therefore subjecting new conceptualized food products must undergo sensory evaluation. The sensory quality of the three different variations of the crackers were evaluated and the results are depicted in Table 1. The sensory analysis was conducted by 30 semi-trained panel members.

Table 1: Sensory analysis of the three developed products

Variations	Appearance	Texture	Colour	Aroma	Taste	Overall Acceptability
CV1	7.86±0.5	7.6±0.92	8±0.87	7.6±0.4	7.86±0.09	8.2±0.84
CV2	8.56±0.5	8.4±0.89	8.3±0.8	8.1±0.58	8.4±0.42	8.56±0.4
CV3	7.5±0.77	7.2±1.12	7.4±1.22	7.2±0.14	7.3±0.14	7.6±0.44

The sensory attributes included appearance, texture, colour, aroma, taste and overall acceptability. The panel members liked the cracker variation CV2 made with 40% finger millet flour over CV1 made with 100% black rice flour and CV3 with 40% quinoa. CV1 and CV2 scored 8 and 8.3 on colour indicating no difference in the colour score and this could be due to black rice and finger millet having similar colour. Colour is a major parameter that is considered by consumers when choosing a product. The colour of the cracker biscuits top surface was generated in the baking process possibly due to nonenzymatic browning (Maillard reactions) between reducing sugars and amino acids, but also possibly to starch dextrinization. On taste attribute CV2 scored the highest sensory score followed by CV1 and CV2 respectively. Overall, CV2 scored better followed by CV2 and CV3 was the least accepted product by the semi-trained panel members.

A study conducted on Rheological and qualitative characteristics of pea flour-incorporated wheat-based cracker biscuits showed that higher levels of pea flour incorporation into the products adversely affected all the sensory attributes studied odour, taste, firmness, colour and overall acceptance of final products [18].

A study aimed to create gluten-free crackers using a combination of chickpea, green and red lentil, yellow pea, pinto and navy bean flours, as well as isolates of pea protein, starch, and fiber from pulses concluded that gluten-free crackers made from pulse-based ingredients have significant potential to attract consumers and offer health advantages. The study revealed that the iron content per serving in chickpea crackers was 3-6 times greater than that of current products [19].

3.2 Proximate analysis
Fig 2. Demonstrates the nutritional composition of variation CV2. The energy content was determined to be approximately 458±10 kilocalories (kcal), while the moisture content was found to be approximately 1.72±0.1 grams (g). The variation also contained approximately 71.3±1.0 grams of carbohydrates, 7.95±0.1 grams of protein, 15±0.1 grams of fat, and 5.9±1.0 grams of dietary fiber. A comparable research study was carried out with the aim of creating and assessing rice cookies using various rice varieties, such as Brown Rice, Njavara Rice, and Burma Black rice. The objective of the study was to improve dietary diversity and provide an alternative snack option for individuals with gluten sensitivity. As a comparison, cookies made with white rice flour were used as the control. Both descriptive and inferential statistical analysis revealed that cookies made with Brown rice flour were the closest to the control and were perceived as the most acceptable. The proximate analysis conducted on the brown rice cookies indicated that they contained around 397.18 kilocalories (kcal) of energy, 60.30 grams of carbohydrates, 10.97 grams of protein, 17.38 grams of fat, and 0.07 grams of total fiber per 100 grams of the sample [20].

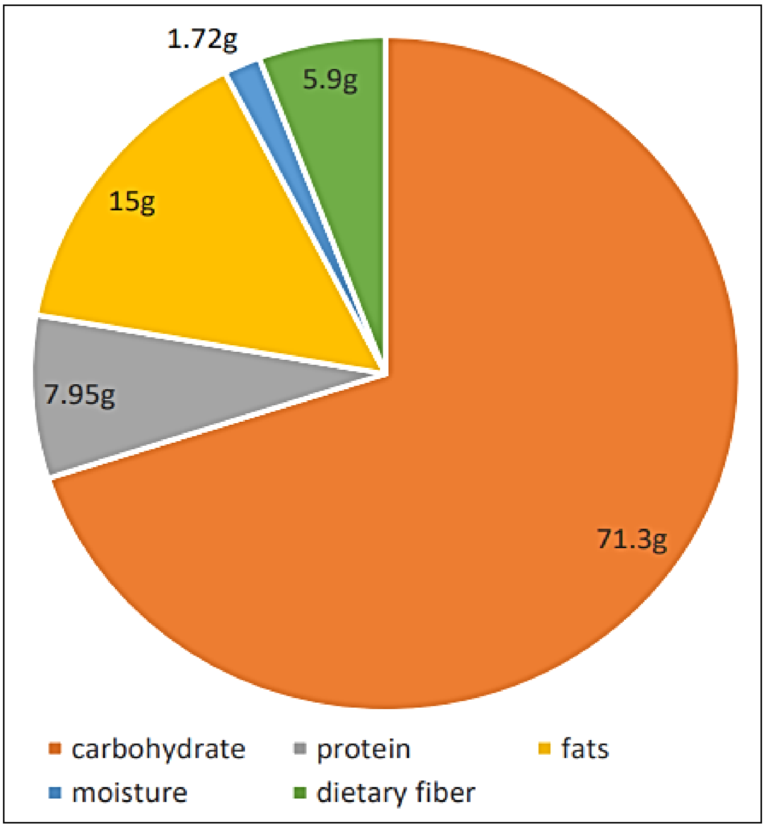

Fig 2: Proximate composition of CV2 sample per 100g

3.3 Mineral and polyphenol content

Crackers are convenient and universally enjoyed food across different populations. When crafted using whole, natural ingredients and minimal processing, biscuits can serve as both a nutritious snack and a source of essential nutrients. Table 2 presents two dietary minerals, the analysis of the cracker CV2 variable, indicating a calcium content of 41.76±0.1 mg/100g and a zinc content of 13.93±0.1 mg/100g.

Table 2: Mineral and polyphenol content of CV2 sample (mg/100g)

Sample	Calcium	Zinc	Total Polyphenols
CV2	41.76±0.1	13.93±0.1	2.06±2.0

Calcium is essential for maintaining optimal bone and teeth health. Moreover, it plays a critical role in a range of physiological functions, including muscle contraction and relaxation, transmission of nerve impulses, blood clotting, and activation of enzymes. Finger millet is considered one of the highest plant-based sources of calcium and study showed that biscuits made from a composite flour containing finger millet seed coat to contain a significant amount of calcium, specifically ranging from 700 to 860 mg/100 g [21]. A study conducted on sixteen varieties of finger millet found that the zinc content varied between 0.92 and 2.55 mg% (milligrams per 100 grams), with an average value of 1.34 mg% [22].

In addition to being a rich source of dietary minerals, finger millet is also known for its abundance of polyphenols, which offer various health benefits. These polyphenols demonstrate significant antioxidant activity and possess anti-inflammatory, antiviral, anticancer, and platelet aggregation inhibitory properties. Finger millet contains approximately 0.3 to 3% of phenolic compounds [23] and the CV2 cracker variant, as indicated in Table 2, contained a polyphenol content of 2.06±2.0 mg/100g. Black rice used in the study to make crackers have been found to possess higher levels of phenolic compounds, such as total anthocyanins (327.60 mg per 100 g). Anthocyanins extracted from black rice, particularly cyanidin and peonidin-3-glucoside, demonstrated a significant inhibitory effect on cancer cell proliferation in laboratory tests [24].

3.4 Shelf life and cost analysis

The shelf life of the developed product was analysed by keeping them at room temperature in an airtight zip lock cover which showed the shelf to be 60 days. This is attributed to the cooking technique employed that is baking which is based on dry heat cooking principle and the moisture content was 1.72g/100g (fig 2). The greater the moisture content of a product, the more susceptible it becomes to microbial spoilage. Cost plays an important role in determining the market success of a food product and the cost analysis of CV2 for 100g of product was rupees 23.85 which is comparable to similar products in the market.

4. Conclusion

The aim of the study was to develop a low FODMAP and gluten-free product for IBS patients. There are limited snack product lines in the market for IBS patients as the elimination of certain food plays a major role in managing IBS. Therefore, there lies a great market potential to develop IBS-friendly snacks while keeping in mind the broader needs of managing IBS. The developed product was made with black rice and finger millet flour which are gluten-free. Finger millet does contain some oligosaccharides, but the amount used is 40% and thus can be tolerated by few as tolerance for FODMAP in IBS condition is dependent on the severity of symptoms and individual tolerance levels.

5. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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