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Antidiabetic Potential of Peanut-Based Snack (Kulikuli) Fortified with Encapsulated Essential Oil from Basil (*Ocimum gratissimum* L.)

Falodun, A.I.*, Ayo-Omogie, H.N., Awolu, O.O. and Badejo, A.A.
Department of Food Science and Technology, Federal University of Technology, P. M. B 704, Akure, Nigeria
*Corresponding author: adedolapofalodun@gmail.com

ABSTRACT

The bulk of the diet in developing countries is starchy which results in overweight, poor metabolic health and an increased risk of cardiovascular diseases and diabetes mellitus. Plant-based foods that supply high protein and are affordable have been sought after to complement the starchy intake. This current study developed kulikuli (peanut-based snack) coated with biofilm made from Cardaba banana starch and fortified with microcapsules containing Ocimum gratissimum essential oil. The incorporation of O. gratissimum essential oil had a significant ($P \leq 0.05$) effect on the proximate composition, glycemic index, α-amylase and α-glucosidase inhibitory activities of the kulikuli. The glycemic index of the fortified kulikuli ranged from 24.4 to 32.9%. The microencapsulation and coating resulted in 10-58% increase in the alpha-glucosidase inhibition and 20-100% increase.in the alpha-amylase inhibition potential of the snacks. The kulikuli coated with the biofilm containing O. gratissimum essential oil may serve as a potential functional food for individuals suffering from diabetes mellitus.

Keywords: diabetes, essential oil, kulikuli, *Ocimum gratissimum*.

INTRODUCTION

The bulk of the diets in developing countries are majorly carbohydrate-laden and lack adequate amount of protein and minerals. In order to augment for this deficiency in diets, since animal protein are expensive and beyond the reach of the group suffering from Protein Energy Utilization, most people resort to plant-based affordable alternatives such as groundnut (Karigidi et al., 2022). The consumption of high-caloric-dense food has been implicated in the sudden rise in people suffering from diabetes mellitus (Ademosun et al., 2021).

Groundnut, commonly known as peanut (Arachis hypogaea), is a tropical legume rich in oil (35–56%), protein (25– 30%), minerals (especially P, Ca, Mg and K) and vitamins (E, K and B) (Kolapo et al., 2012). Nigeria contributes 10% of the global production and 35% in Africa. Groundnut is consumed after various processing operations. Industrially, it is processed for oil extraction. The residual cake obtained after oil extraction is laden with protein and commonly processed into an indigenous edible snack called peanut cake and locally called kulikuli in Nigeria (Ayo-Omogie et al., 2022).

Kulikuli (peanut-cake) is the byproduct of oil extraction from groundnut and is indigenous to the West African coasts. Kulikuli is rich in protein and crude fat similar to its parent material, groundnut (Kolapo et al., 2012). Peanuts and their derivatives are often classified as street food which satisfies the essential protein need of the urban population due to its affordability (Boli et al., 2017). Thus, it may be utilized as a means to incorporate significant bioactive phytochemicals into the diet of the general public (Karigidi et al., 2022).

Arising from excessive consumption of carbohydrate-dense food, over 180 million people in developing nations have become diabetic and the number has been projected to quadruple by 2030 (Ullah et al., 2022). Several strategies such as regular use of different antidiabetic medications, dietary modifications and lifestyle change have been adopted to manage diabetes (Hawley & Gibala, 2012; Yoon et al., 2006). However, the use of synthetic medications is expensive and laden with side effects (Pooja, 2014). As such, several plant-based foods and dough meals have been developed for the management of diabetes (Oladebeye et al., 2023; Ullah et al., 2022).

Functional foods, are usually high in bioactive compounds that help in health promotion. These health-promoting compounds are often obtained from plant and their by-products. One of such plants currently receiving attention due to its high contents of bioactive compounds is the Basil leaf (*Ocimum gratissimum* (OG)).

Essential oils are extracts from plants that are widely used for their ability to fight illnesses, preserve food, and heal wounds (Sousa et al., 2022). However, essential oils are volatile liquids and hydrophobic, thus leading to loss of their functionality and making it difficult to incorporate them into food products. As such, encapsulation is one of the more effective strategies used to address this challenge by trapping the essential oil within a biodegradable polymer to enable controlled release and enhanced bioavailability. (Sundar & Parikh, 2023).

Biodegradable edible coatings made from starch are applied to the outer part of food to preserve the food for consumption. Apart from the flavours and nutrients, they are also laden with antibacterial and antibrowning agents that can lessen pathogen growth on food surfaces, thereby prolonging shelf life. (Restrepo et al., 2018).

Cardaba banana is rich in resistant starch and indigestible carbohydrates leading to improved insulin sensitivity, lower blood sugar levels and also reduced appetite in diet (Falodun et al., 2019). It has been used for the encapsulation of active substances such as carotenoids and essential oils (Liao & Chen, 2021). Furthermore, its indigestible fraction with higher resistant starch, are slowly digestible food components that assist in the prevention and management of type 2-diabetes. (Ayo-Omogie et al., 2022; Badejo et al., 2023).

The present study has investigated the proximate composition, glycemic index, invitro α-glucosidase, and α-amylase contents of kulikuli as influenced by edible biofilm coating fortified with OG essential oil.

MATERIALS AND METHODS

Materials

Groundnuts (*Arachis hypogaea* L.) and Cardaba banana were sourced from Oja–oba market, in Akure, Ondo State, Nigeria, and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria.

Basil leaves (*Ocimum gratissimum* L.) were obtained from the teaching and research farm in the university and authenticated by botanists at the Department of Crop, Soil and Pest Management of the Federal University of Technology Akure, with the herbarium number 0362. It was washed to remove dirt, sand and debris. All reagents and chemicals used were of analytical grade.

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Extraction of Essential Oil

The essential oil was extracted using the method described by Sousa et al. (2022). The washed basil leaves were subjected to hydro-distillation in a modified Clevenger apparatus for 2 h. Extracts were stored in amber flasks, and refrigerated.

Isolation of Cardaba Banana Starch

Cardaba banana starch was produced using the method described by Ayo-Omogie et al. (2022).

Microencapsulation of Essential Oil

The method described by Awolu et al. (2022) was adopted for the production of the micro-capsulation of the OG essential oil. Cell wall material (49.25 g of Cardaba banana starch), gum Arabic (0.75 g), Tween 80 (0.5 mL) and water (200 mL) were weighed respectively into a 25 mL plastic beaker and mixed evenly until a liquid mix was obtained. The mixture was homogenized at 12,000 rpm for 25 min using a Lab homogenizer (GEN 700 Cole Parmer) and thereafter frozen at -20 ºC for 24 h and lyophilized in a freeze dryer. The freeze-dried microencapsulates were stored in an airtight container in a refrigerator at 4oC.

Production of Kulikuli

The method of Achimugu & Okolo, (2020) was used in the production of kulikuli with slight modifications. Fig. 1 shows the flow chart for the production of kulikuli.

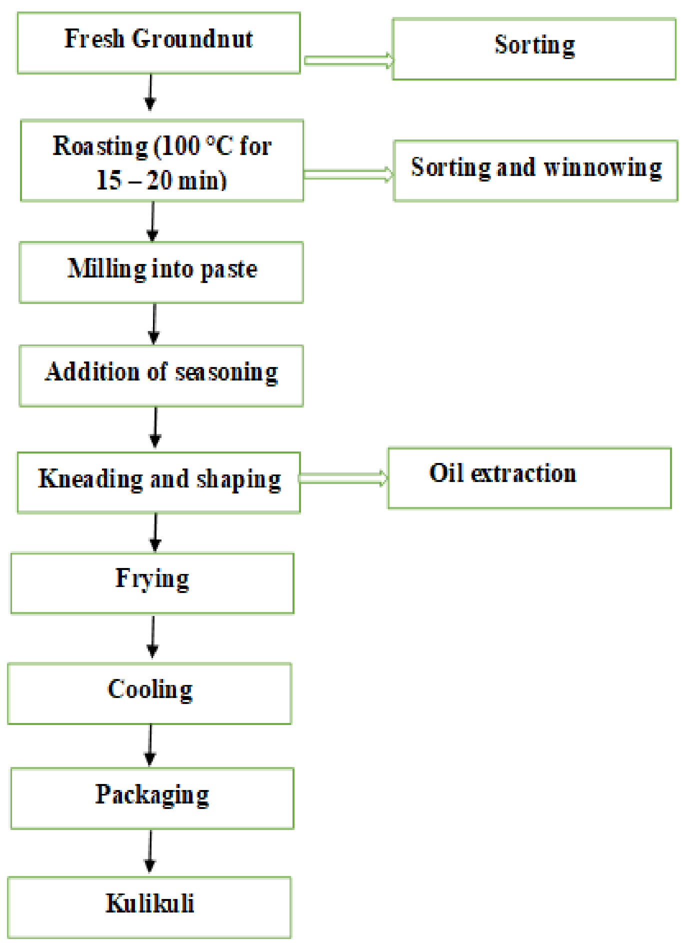

Fig. 1: Flowchart for the production of kulikuli

Coating of the Kulikuli samples

The kulikuli samples were coated with the biofilm by using the dipping method as described by Senturk et al., (2018) and thereafter dried in hot air oven at 60 ˚C for 2 h.

Proximate Analysis

The proximate composition was carried out according AOAC, (2006)

Determination of in-vitro Glycemic index

The method described by Camelo-Méndez et al. (2017) was used for measuring the estimated glycemic index (eGI) using in vitro hydrolysis by pancreatic alpha-amylase. The reducing sugars released were measured at 530 nm in parallel with a standard curve of maltose. Glycemic index was estimated from the hydrolysis curves, using the empirical formula of eGI = 39.21 + 0.803(H90) (Gondi & Prasada Rao, 2015) .

Invitro Alpha-Glucosidase Inhibitory Assay

The effect of the samples on α-glucosidase activity was determined according to the method described by Etsassala et al. (2020) using α -glucosidase from Saccharomyces cerevisiae. The substrate solution p-nitrophenylglucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 100 µL of α-glucosidase (0.3 U/mL) was pre-incubated with 50 µL of the sample for 10 min. Then 50 µL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na2CO3. The α -glucosidase activity was determined by measuring spectrophotometrically the yellow-coloured paranitrophenol released from pNPG at 405 nm.

Invitro Alpha –amylase Inhibitory Assay

The method used involved estimating the amount of reducing sugar produced by the activity of each enzyme on buffered starch. Alpha amylase was assayed as reported by Etsassala et al. (2020). The substrate for assay was 0.5 mL of 0.5% soluble starch, buffered with 0.2 mL of 0.1 M sodium acetate (pH 5.6). Crude enzyme extract (0.3ml) was added to the mixture, mixed and incubated at 40oC for 30min in a water-bath. The DNSA (colorimetric) method as described by Miller (1959) was thereafter employed for estimation of reducing sugars produced. One milliliter (1mL) of DNSA solution was added to the mixture, boiled for 5 min, cooled and 4 ml of distilled water was added. Absorbance was read at 540 nm in spectrophotometer. A Blank consisting of 0.3 ml distilled water, 0.5 ml of 0.5% soluble starch, and 0.2 ml of buffer was subjected to similar treatments.

Statistical Analysis

The experimental data were tabulated and statistically analyzed using GraphPad Prism 8.2.1. The values were expressed as mean ± standard deviation. Means were separated using SPSS and significance was accepted at ($P \leq 0.05$).

RESULTS AND DISCUSSION

Proximate Composition of the Fortified Kulikuli Samples

The proximate composition of the kulikuli samples is presented in Table 1. The protein content ranged from 34.56 – 50.75%. The highest protein content was observed in sample KE1.25. The protein content of the kulikuli samples increased upon the introduction of microcapsules containing essential oil from OG, although the coating did not show significant increase in the protein content. The protein contents of the kulikuli samples were within the range of 31.8% to 40.8% as reported by Baah-Tuahene, (2015), and a value of 30.98% for commercial kulikuli reported by Karigidi et al. (2022). The kulikuli samples exhibited high protein contents, which is consistent with studies conducted by Arya et al. (2016) that found that the cake's protein content might increase by up to 50% following oil extraction. Additionally, *Ocimum gratissimum* has been reported to be a fairly rich source of protein (8.60%) and may be used as a protein supplement for patients with protein energy malnutrition (Mgbeje et al., 2019). The moisture content of the kulikuli samples were generally very low and ranged between 0.79 to 4.76%. The moisture contents of the samples are below the recommended moisture content level of 10% thus showing that limited amounts of moisture will be available for microbial activities, hence, good storability and product quality will be guaranteed.

The fat contents (25.49 – 29.26%) of the kulikuli samples were within the reported average range of Baah-Tuahene (2015) for kulikuli samples from different locations. The fat content increased with the introduction of encapsulation with oil from *Ocimum gratissimum* and this correlates with the reports of Mgbeje et al. (2019) that *Ocimum gratissimum* leaves contained (15.06 – 29.43%) fat on dry weight basis and its presence could contribute to the increase in fat content of the samples. The fat play a critical role in giving many peanut products their unique texture, flavor, and aroma.

The results also showed that the fiber content for kulikuli ranged from 1.18 to 8.51%, with the highest value recorded in sample KE1.25. Fiber content has also been shown to lower blood cholesterol, promote bowel movement and bulk, avoid constipation and piles, and prevent colon cancer. (Oluwafemi et al., 2021).

Table 1: Proximate composition (%) of fortified kulikuli samples

Sample	Moisture	Crude Fat	Ash	Crude Fibre	Protein	CHO	Energy (Kcal/g)
KC	0.79±0.31[e]	28.89±0.89[a]	1.94±0.23[ab]	3.73±0.00[c]	36.53±0.22[c]	28.12±0.83[b]	518.59±5.62[a]
$KE_{0.63}$	3.92±0.02[c]	27.55±0.11[b]	1.96±0.04[ab]	3.55±0.00[d]	47.91±0.22[b]	15.13±0.27[c]	500.07±0.63[b]
$KE_{1.25}$	1.79±0.28[d]	28.89±0.71[a]	1.94±0.00[ab]	8.51±0.00[a]	50.75±0.44[a]	8.13±0.16[d]	495.49±4.67[b]
$KE_{0.63}F_{0.6}$	4.08±0.31[b]	25.49±0.49[c]	1.55±0.51[b]	4.32±0.00[b]	34.56±0.44[d]	29.99±0.69[a]	487.62±4.79[c]
$KE_{1.25}F_{0.6}$	4.76±0.52[a]	29.26±0.15[a]	2.27±0.60[a]	1.18±0.00[e]	34.56±0.44[d]	27.97±0.21[b]	513.45±3.00[a]

Values are Mean±SD (n=3). Values with same alphabets within a column are not significantly different (p>0.05). KC= 100% Kulikuli; $KE_{1.25}$= Kulikuli with 1.25g microencapsulated EO, $KE_{0.63}$= Kulikuli with 0.63% microencapsulated EO, $KE_{1.25}F_{0.6}$= Kulikuli with 1.25% microencapsulated EO and Biofilm containing 0.6ml EO, $KE_{0.63}F_{0.6}$= Kulikuli with 0.63% microencapsulated EO and Biofilm containing 0.6ml EO

Glycemic Index of the Fortified Kulikuli Samples

Glycaemic index (GI) is used for rating foods rich in carbohydrate and compares with glucose as a reference on the rate at which blood sugar level rises after consumption. Foods that are regarded as having a low GI include legumes, which have been demonstrated to improve postprandial glucose levels; (Okoduwa & Abdulwaliyu, 2023). GI of less than 55 is considered low, 56-69 is considered medium and greater than 70 is high (Kaviani et al., 2020). As shown in Fig. 2 all the encapsulated and biofilm-coated kulikuli samples had GI values ranging from 24.4 to 32.9%. These are lower than values reported for kulikuli supplemented with ginger (Karigidi et al., 2022).

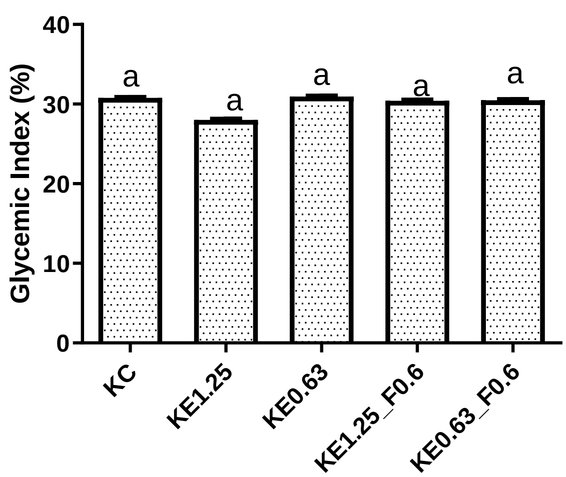

Fig. 2: Glycemic index of fortified kulikuli samples.
Values are Mean±SD (n=3). Bars with same alphabets are not significantly different (p>0.05). KC= 100% Kulikuli; $KE_{1.25}$= Kulikuli with 1.25g microencapsulated EO, $KE_{0.63}$= Kulikuli with 0.63% microencapsulated EO, $KE_{1.25}F_{0.6}$= Kulikuli with 1.25% microencapsulated EO and Biofilm containing 0.6ml EO, $KE_{0.63}F_{0.6}$= Kulikuli with 0.63% microencapsulated EO and Biofilm containing 0.6ml EO

Foods with low GI are considered to be advantageous to maintain balanced blood glucose levels and metabolic control while consumption of a diet with high GI is associated with an increased risk to Type 2 diabetes mellitus and other related diseases (Bohl et al., 2024).

Alpha-amylase and Alpha-glucosidase Inhibition Properties of the Fortified Kuliluli Samples

Alpha-amylase and alpha-glucosidase are digestive enzymes in the human system. Alpha-amylase is responsible for the initial hydrolysis of starch into smaller components. The inhibition of the activity of α- amylase delays the rate at which carbohydrate is hydrolyzed and consequently slows down the fast uptake of glucose in the blood stream. On the other hand, the enzyme alpha-glucosidase ensures the conversion of oligo- and/or disaccharides into monosaccharides, because monosaccharides are the type of carbohydrates that are absorbed via the mucosal border in the small intestine (Kazeem et al., 2015), the inhibitory activity of this enzym results in a drop in blood glucose levels. Several studies have reported that the inhibition of α-amylase and α-glucosidase is an essential approach in the management of blood glucose levels (Ajayi et al., 2020; Ben Lamine et al., 2019). When these enzymes' functions are compromised, complex sugars like starch may take longer to break down, thereby lengthening the time it takes for all carbohydrates to be absorbed and avoiding an excessive rise in blood glucose levels after meals. (Ajayi et al., 2020).

Fig 3 and 4 showed the inhibitory activities against α-amylase and α-glucosidase enzymes. It ranged from 14.11 - 40.3% and 13.72 - 96.07 for α- amylase and α- glucosidase respectively. A high α- amylase and α- glucosidase inhibition (40.3 & 90.13%) was exhibited by kulikuli samples with 0.63% microencapsulated EO and biofilm containing 0.6mL EO, followed by samples that had 1.25g microencapsulated EO (31.55 & 76.96%) as compared with the control sample (20.87 & 59.3%). This may be an indication of the in vitro antidiabetic potential of the encapsulated samples and may suggest that the inhibitory ability of α-glucosidase was much higher than α-amylase. There was a higher increase in the ability of the samples to inhibit these enzymes upon encapsulation and coating with the biofilm.

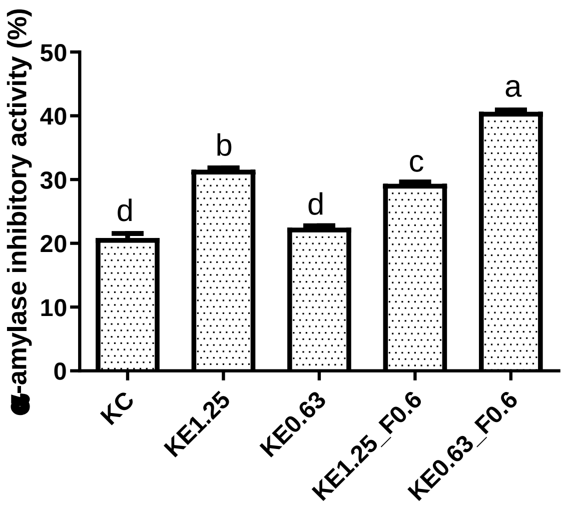

Fig. 3: α-Amylase inhibitory activity (%) of fortified kulikuli samples

Values are Mean±SD (n=3). Bars with same alphabets are not significantly different (p>0.05). KC= 100% Kulikuli; $KE_{1.25}$= Kulikuli with 1.25g microencapsulated EO, $KE_{0.63}$= Kulikuli with 0.63% microencapsulated EO, $KE_{1.25}F_{0.6}$= Kulikuli with 1.25% microencapsulated EO and Biofilm containing 0.6ml EO, $KE_{0.63}F_{0.6}$= Kulikuli with 0.63% microencapsulated EO and Biofilm containing 0.6ml EO

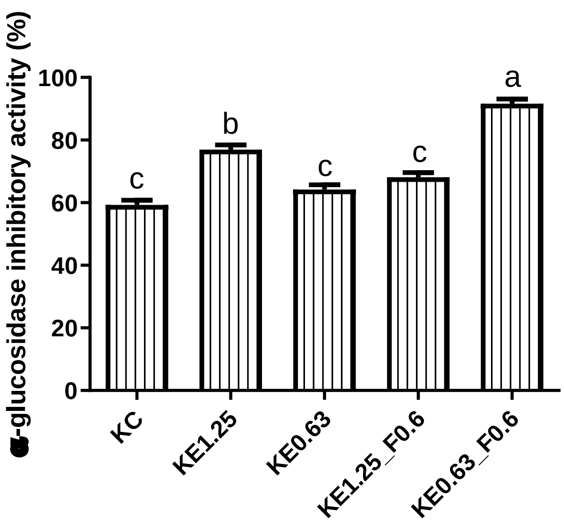

Fig. 4: α-Glucosidase inhibitory activity (%) of fortified kulikuli samples

Values are Mean±SD (n=3). Bars with same alphabets are not significantly different (p>0.05). KC= 100% Kulikuli; $KE_{1.25}$= Kulikuli with 1.25g microencapsulated EO, $KE_{0.63}$= Kulikuli with 0.63% microencapsulated EO, $KE_{1.25}F_{0.6}$= Kulikuli with 1.25% microencapsulated EO and Biofilm containing 0.6ml EO, $KE_{0.63}F_{0.6}$= Kulikuli with 0.63% microencapsulated EO and Biofilm containing 0.6ml EO

Kanmaz et al. (2023) reported α-amylase inhibitory effect of purple basil extract and purple basil essential oil of 64.71 ± 3.88% and 79.61 ± 4.99%, respectively. The α-glucosidase inhibitory activity of extract (68.47 ± 1.03%) was greater than that of essential oil (62.64 ± 1.34%). Karigidi et al. (2022) reported that IC50 for α-amylase enzyme ranged from 422.10 to 680.10 µg/ml while it ranged from 306.50 to 410.16 µg/ml for α-glucosidase enzymes for kulikuli supplemented with ginger.

Previous studies have reported that extracts from Ocimum species are rich in bioactive components such as eugenol, ursolic acid, oleanolic acid, limonene, rosmarinic acid, linalool, carvacrol, and β-caryophyllene which possess anti-hyperglycemic effect and were effective in the management of diabetes mellitus (Antora & Salleh, 2017; Kanmaz et al., 2023; Pant & Pandey, 2018). These bioactive compounds might be responsible for the observed α-glucosidase and α-amylase inhibitory properties.

Kulikuli with microencapsulated EO and biofilm containing EO contributed to the hypoglycemic and antidiabetic effects, based on the findings in this study. These effects were conceivable through the inhibition of α-amylase and α-glucosidase enzymes. Moderate inhibition of α- amylase is preferable to low or excessive inhibition. Excessive inhibition is linked to side effects such as diarrhoea and flatulence due to undigested starch being fermented by abnormal bacteria in the colon (Oluwafemi et al., 2021). Low inhibition on the other hand is associated with too rapid breakdown of starch increasing postprandial blood glucose level (Adeloye et al., 2021).

CONCLUSION

This study showed that the complementary qualities of edible films made from *Ocimum gratissimum* essential oil and Cardaba banana starch with its inclusion in the production of the groundnut-based snacks (kulikuli) possessed health-promoting properties. The inhibitory ability of the encapsulated kulikuli against α-amylase and α-glucosidase enzymes were enhanced while glycemic index was lowered. Consequently, *Ocimum gratissimum* essential oil may be used as a functional component in groundnut-based snack recipes for the management of diabetes mellitus.

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