Abbas A., Akhtar S., Ismail T., Raza H., Okoduwa S.I.R. 2024. "Evaluation of chapattis fortified-diet with raw and fermented carrot powder in persons with type 2 diabetes: A randomized control pilot study." *World Nutrition*, June, 134–145. https://doi.org/10.26596/wn.2024152134-145

Research

Evaluation of chapattis fortified-diet with raw and fermented carrot powder in persons with type 2 diabetes: A randomized control pilot study

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Keywords: Type 2 diabetes, Carrot flour, fermented carrot flour, whole wheat flour, fasting blood sugar, fortified-diet

https://doi.org/10.26596/wn.2024152134-145

World Nutrition 2024;15(2):134-145

Background

Diabetes is a metabolic disease that is common and growing rapidly. It is characterised by high blood sugar levels and related complications to vital organs such as the kidneys, heart, blood vessels, and eyes. The most common kind of diabetes affecting people globally is type 2 diabetes (T2D). An increasing number of people are looking at natural foods and dietary supplements to manage their T2D.

Objective

The aim of this pilot study was to assess the glycemic index, glycemic response, sensory qualities, nutritional composition, and possible antidiabetic benefits of chapattis fortified with raw and fermented carrot powder.

Methods

Seven preparations of chapattis were prepared into three variants from whole wheat flour chapattis (WWFC) as control group, raw carrot-supplemented chapattis (RCSC) with subtypes T1, T2, and T3, and fermented carrot-supplemented chapattis (FCSC) with subtypes T4, T5, and T6. The variants were provided to 11 professionally trained sensory panelists for organoleptic evaluation. The chapattis subtype with the best organoleptic properties together with the WWFC were then given randomly to a group of 30 persons with T2D respectively to see how their blood sugar levels were affected over a period of 4 weeks.

Results

The panelist adjudged RCSC (T2) and FCSC (T5) as the best organoleptically. The randomised control investigation demonstrated that the fasting blood glucose levels for WWFC, RCSC, and FCSC decreased (P<0.05) significantly by 6.42%, 7.24%, and 12.75%, respectively. However, there was no significant different in the fasting blood sugar between the WWFC and RCSC. As for the glycemic load values of WWC, RCSC, and FCSC, which were 21, 14, and 13, respectively, there was no statistically significant difference between RCSC and FCSC, suggesting that both types of chapattis could help T2D patients regulate their blood sugar levels. The most significant changes were consistently shown by the FCSC: 17.50% reduction in random blood sugar (p = 0.03), 21.85% reduction in serum low-density lipoprotein (p = 0.051), 16.67% increase in serum HDL (p = 0.01), 11.09% decrease in serum cholesterol (p = 0.02), and 12.575% reduction in serum triglycerides (p = 0.048).

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Conclusions

A beneficial dietary intervention for people with T2D may involve mixing fermented carrot powder into regular chapattis. These findings contribute to the growing body of evidence in favour of using natural food-based treatments for the management of diabetes.

INTRODUCTION

T2D is a serious public health concern that has an impact on life expectancy and healthcare expenses (Zimmet et al. 2014, Williams et al. 2020). According to data from the most recent 10th edition of diabetes atlas, diabetes is one of the 21st-century global health concerns with the greatest rate of prevalence (Magliano et al. 2021). About 537 million people worldwide were estimated to have diabetes in 2021; by 2030, that number is expected to rise to 643 million, and by 2045, it is predicted to reach 783 million. Furthermore, it is estimated that 541 million individuals had impaired glucose tolerance in 2021. Additionally, nearly 6.7 million adults between the ages of 20 and 79 died in 2021 from diabetes-related causes. Yearly, more children and adolescents (those under the age of 19) are diagnosed with diabetes. More than 1.2 million young people worldwide had type 1 diabetes in 2021. This disease was responsible for over 1 million deaths in 2017, making it the ninth leading cause of death (Khan et al., 2020). With 8.7 million cases of diabetes (20–79 years) in the Middle East and North Africa region, Pakistan is ranked third out of 21 countries in the International Diabetes Federation diabetes atlas 2021 (Magliano et al. 2021).

Diabetes significantly increases morbidity and mortality because it negatively impacts a person's physical capacities and general quality of life (Okoduwa et al. 2015a, 2015b; Khan et al. 2015). These trends are often attributed to the rising consumption of unhealthy diets and the adoption of sedentary lifestyles, which contribute to elevated body mass index (BMI), increased postprandial glucose levels, and higher fasting plasma glucose levels (Lone et al. 2017; King et al. 2010). In this context, wheat and rice are widely utilized sources of carbohydrates within the traditional food systems of South Asia and are equally popular among Asian immigrant communities worldwide (Spohrer 2013; Shekhawat 2022). In contrast, carrots are low glycemic index (GI) foods that are high in soluble fibers and resistant starch (Varshney and Mishra, 2022, Okoduwa and Abdulwaliyu, 2023) and are involved in delaying gastric emptying which improves metabolic control in diabetic patients (Lin et al. 2022, Huang et al. 2023). Therefore, many patients are shifting towards natural food-based approaches for managing T2D (Okoduwa and Abdulwaliyu, 2023). In this respect, previous studies have shown that meals with a low glycemic load are effective in improving T2D-related complications (O’keefe et al. 2016; Walker et al. 2010; Lal et al. 2021; Okoduwa and Okoduwa, 2023).

Previous research indicates that vegetable flour-supplemented chapattis can significantly reduce postprandial glucose and hyperglycemia (Akhtar et al. 2019; Priyadarshini et al. 2021). Pakistanis are used to whole wheat, a very common staple food consumed as chapattis on daily basis at each meal. However, in recent years, flours are increasingly being produced at higher levels of refinement by roller milling (Khurshid et al. 2019). Refined wheat is known to contain high concentration of starch which results in hyperglycemia, hence highly unsuitable for persons with diabetes as it instigates insulin resistance on the long-run causing T2D (Isharwal et al. 2009). The need to modify this staple food through supplementation and/or fortification becomes crucial for the people.

Studies have showned that the consumption of vegetables evokes positive effects by improving postprandial glucose (PPG) excursions, hyperglycemia, and insulin insensitivity (Akhtar et al. 2019; Komati et al. 2022; Survay et al. 2010). Carrots are nutrient rich and low GI, aligning with the goal of creating a healthier, blood sugar-friendly chapatti option, potentially appealing to health-consciousness for diabetic patients.

In this regard, carrot roots are rich in minerals, soluble fibers, and polyphenols which act as cofactors for improving insulin sensitivity and glucose metabolism (Varshney and Mishra 2022). In addition, carrots have been studied for their hypoglycemic and potential with insulin-sensitization and proposed as an adjuvant to oral hypoglycemic drugs (Survay et al. 2010; da Silva Dias and Imai 2017). Fermented carrot powder in chapattis could enhance the nutritional value and functional properties of the chapattis. It may increase the bioavailability of its nutrients and antioxidants and contribute to gut health. Fermented carrot powder added to chapattis not only adds flavor and color but also aligns with cultural culinary traditions, potentially appealing to consumers seeking both nutritious and flavorful food options. The remarkable attributes of carrots have sparked significant interest among researchers and healthcare experts in the creation of low-glycemic response cereal-based food items (Wan et al. 2019). The current pilot study aims to assess the GI, glycemic response, sensory qualities, nutritional composition, and possible antidiabetic benefits of chapattis fortified with either raw or fermented carrot powder.

MATERIALS AND METHODS

RAW MATERIALS

Raw carrots and whole wheat flour (WWF) were obtained as a single batch from a local market in District Multan, Pakistan, and subsequently taken to the laboratory located at the Institute of Food Science and Nutrition, BZU, Multan for further processing.

PREPARATION OF RAW CARROT POWDER (RCP)

The raw carrots were washed with tap water to remove dust, sliced into 2.5 cm length and 4 mm thick, and dipped into a solution of sodium hypochlorite with distilled water (100 ml/L) (Onwurafor et al. 2022; Sun et al. 2012). The final washing of carrots was achieved with tap water for 5 minutes to eliminate chlorine odor and thereafter spread for drying at room temperature. The dried carrot cubes were placed directly in a cabinet dryer at 50^{o}C and the dehydrated product was pulverized using a hammer grinding mill in a food processing laboratory to form the RCP.

PREPARATION OF FERMENTED CARROT POWDER (FCP)

The starter culture of *Lactobacillus plantarum* was prepared in the Food Microbiology Laboratory at Faculty of Food Science and Nutrition BZU, Multan. The dried carrot slices were added to a fermentation jar and mixed with a starter culture of *Lactobacillus plantarum* (1g (2.4 × 109 CFU) per 1000 g) in 0.85% normal saline (Chung 2011). Higher cell numbers (108 or 109 CFU/mL) of starter cultures modify the nutritional and sensory properties of starter cultures (Scheers et al. 2016; Swain et al. 2014). The *Lactobacillus plantarum* was allowed to ferment for 7 days at 20 °C. The fermented carrot slices were spread in cabinet dryer trays and allowed to dehydrate at 50 °C. The dehydrated fermented product was pulverized using a hammer grinding mill to form the FCP. The prepared FCP was packaged in plastic bags and preserved in a deep freezer at −8 to −10 °C temperature until required for use.

PREPARATION OF THE DIFFERENT VARIANTS OF CHAPATTIS

The WWF, RCP and FCP were reconstituted, ready for the production of the chapattis variants. These blends involved the substitution of portions of the WWF, being the base material, with varying levels of both RCP and FCP (Table 1, Figure 1). The reconstituted blends were kneaded with water until soft dough was made. The soft dough was put into a pan and covered with a lid for 30 minutes for proofing. After proofing, the dough balls were made from newly developed blends. The dough balls of all seven treatments were rolled out into chapattis (18 cm in length). The newly developed flat chapattis were baked on a hot plate at 210° C, having 3 mm thickness for approximately 150 ± 5 seconds. The Figure 2 represents the schematic diagram for the intervention study in human T2D subjects.

Table 1. Intervention treatment models based on the composition of ingredients

Groups	WWFC	RCSC			FCSC		
Ingredients (g)	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Whole Wheat Flour	80	76	72	68	76	72	68
Raw Carrot Powder	0	04	08	12	0	0	0
Fermented Carrot Powder	0	0	0	0	04	08	12

T_0 = WWF (100%)
T_1= WWF (95%) + RCP (5%)
T_2 = WWF (90%) + RCP (10%)
T_3 = WWF (85%) + RCP (15%)
T_4 = WWF (95%) + FCP (5%)
T_5 = WWF (90%) + FCP (10%)
T_6 = WWF (85%) + FCP (15%)

WWFC: Whole wheat flour chapattis, RCSC: Raw carrot supplemented chapattis, FCSC: Fermented carrot supplemented chapattis

*Assessment of glycemic response was performed only on treatments groups with the best organoleptic responses.

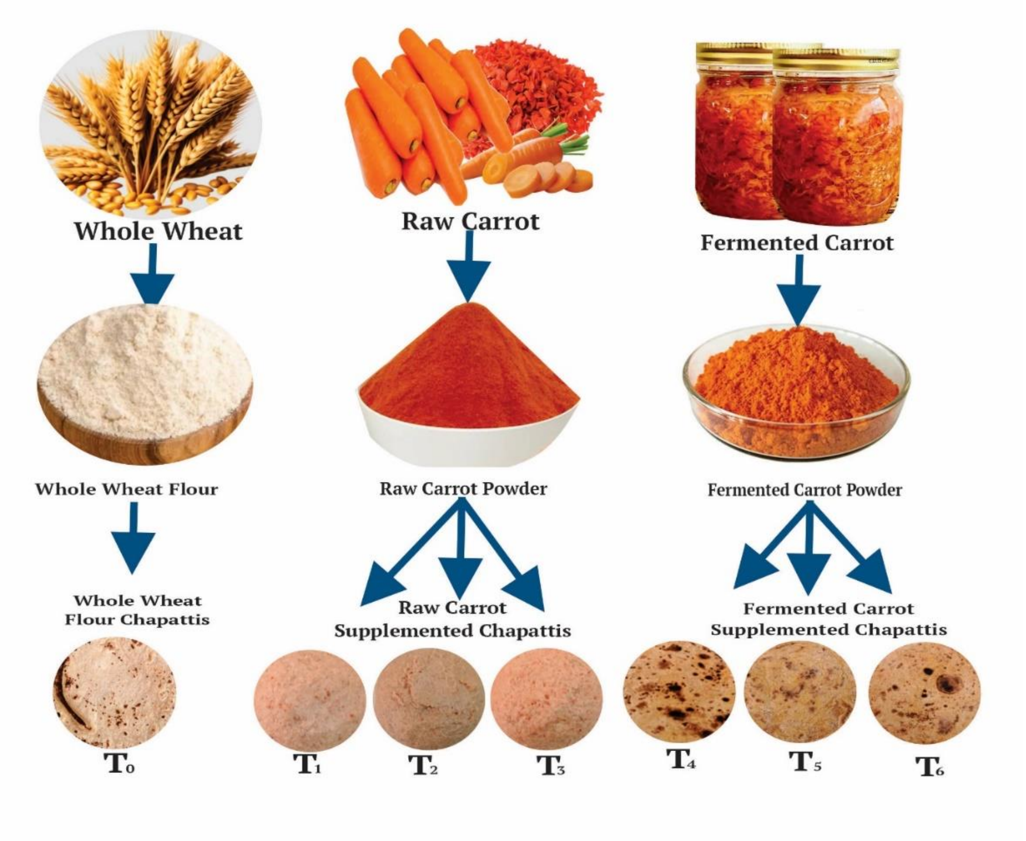

Figure 1. Experimental design for the preparation of the chapattis variants

T_0 = WWF (100%); T_1= WWF (95%) + RCP (5%); T_2 = WWF (90%) + RCP (10%); T_3 = WWF (85%) + RCP (15%); T_4 = WWF (95%) + FCP (5%); T_5 = WWF (90%) + FCP (10%); T_6 = WWF (85%) + FCP (15%)

WWF: Whole wheat flour, RCP: Raw carrot powder, FCP: Fermented carrot powder

EVALUATION OF CHAPATTIS

ORGANOLEPTIC EVALUATION

Eleven trained sensory panelists experts from the Faculty of Food Science and Nutrition at (BZU), Multan, evaluated the seven different variants of chapattis prepared from WWF, RCP and FCP for appearance, taste, texture, color, foldability, and overall acceptability, to select the best combination. The chapattis were evaluated on a rating scale of hedonic scale 1–9 (Akhtar et al. 2019). The sensory panelists were asked to judge the samples for sensory attributes. The chapattis with seven treatments were served on a plate, in the absence of any odor (food or chemical) and white light, which had been numbered according to the sample codes as mentioned in Table 1. The mouth was cleaned by all expert panelists with crackers and distilled water between every treatment chapatti analysis, and a printed questionnaire was provided to every analyst to mention their observations based on the hedonic scale. Based on the results obtained from the organoleptic assessment, the best chapattis with the highest score from each group were used for further assessment (glycemic response assessment).

NUTRITIONAL PROFILING OF THE PREFERRED CHAPATTIS

Analysis of chapattis exhibiting the most favorable organoleptic characteristics involved the determination of moisture, ash, protein, fat, and kilocalories content by using standard methods of proximate composition. These analyses were conducted following the methods outlined in the AOAC International manual of food analysis by Latimer (2012).

DETERMINATION OF AVAILABLE CARBOHYDRATES

The available carbohydrates were determined using the phenol sulfuric acid method (Akhtar et al. 2019; Dubois et al. 1956). Briefly, a total of 100 mg of homogenized sample was taken in a test tube and put into boiling water in a water bath for three hours to hydrolyze with 5 mL of 2.5 N HCl. Neutralization of the hydrolyzed sample was carried out by crystals of sodium carbonate. These crystals were added until effervescence ceased and then samples were cooled at 25 °C. Samples were then centrifuged for 30 minutes, and the supernatant was separated. Afterward, 2 mL of supernatant from each sample was taken in a test tube and 1 mL of 5% aqueous solution of phenol was added followed by 5 mL of sulfuric acid (97%). The contents of the test tubes were allowed to settle for 10 minutes and vortexed for 30 seconds and, the test tubes were kept in a water bath at 25 to 30 °C for 10 to 20 minutes. A spectrophotometer (UV-Vis 3000 model, made in Germany) was used to measure the color absorbance against a blank at 490 nm. All samples of chapatti were tested thrice, and the carbohydrate proportion was determined using known concentrations of glucose standard solutions. The following equation was used to calculate the amount of food needed to achieve the correct available carbohydrate content:

Amount of food = 100y / x, where x represents the total available carbohydrates in g/100 g of a sample, and y is the amount of required available carbohydrates.

For example, the whole wheat powder needed for 50g carbohydrate content was the amount of the available carbohydrate that food is required to provide was approximately 65g calculated by the amount of food: 100/77* 50 = 64.94 g wheat flour.

RANDOMIZED CONTROL TRIAL INVESTIGATION OF THE PREFERRED CHAPATTIS

QUESTIONNAIRE

A research questionnaire was designed under the supervision of trained staff at the Faculty of Food Science and Nutrition, BZU, Multan. It encompassed personal information (marital status, occupation), sociodemographic information (socioeconomic status, educational status, number of children), and anthropometric profile (height in cm, weight in kg, BMI).

PARTICIPANTS

The rule of thumb that provide the general guidelines of 30 participants per group for a pilot study was adopted for this randomized control trial investigation (Van Voorhis et al. 2007; Schulz et al. 2010; Eldridge et al., 2015). The 30 participants were confirmed persons with T2D. They were randomly assigned using lottery method into three groups, subject to meeting the selection criteria for the study (Lichtenstein et al. 2021). The fortified chapattis intervention diet being tested in at this stage were selected the best based on the organoleptic properties as adjudged by the panelists describe above. These are;

1. WWFC group (T0): control chapattis made from 100% WWF.
2. RCSC group (T2): chapattis formulated from 90% WWF supplemented with 10% RCP, and
3. FCSC group (T5): chapattis formulated from 90% WWF supplemented with 10% FCP.

The Participants who did not fulfill the inclusion criteria were excluded from this study. The participants included in this study were between 20 to 60 years of age. They were recruited from the South Punjab Hospital, Multan. All the participants provided written informed consent after being informed about the beneficial aspects and methodology of the current study. Moreso, the study got ethical approval from the Bioethics Committee of Bahauddin Zakariya University, Multan, Pakistan (Reg. No. 06-21/2019) and the study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki (General Assembly of the World Medical Association, 2014).

SELECTION CRITERIA

The inclusion criteria included T2D patients (both male and female) between 20 to 60 years of age, with a BMI greater than 23 kg/m^2 (overweight and obese category), and glycated hemoglobin (HbA1c) levels of greater than 7 or 7.5% (Sato et al. 2017; Tay et al. 2015). Participants should be non-insulin-dependent or on oral medications (Tay et al. 2015).

The exclusion criteria encompassed patients with known ischemic heart disease, nephropathy, neuropathy, hepatic issues, insulin-dependent patients, and pregnant mothers (Sato et al. 2017; Santos et al. 2022).

TREATMENT INTERVENTION APPROACH

Participants in both the intervention treatment and control groups were advised to consume the designated chapattis twice daily, at lunch and dinner, for 28 days to allow for a meaningful assessment of its impact on appropriate biochemical markers (Game et al. 2012). A standard menu plan was given to all participants. Participants were closely monitored all through the 28 days of dietary intervention and their biochemical measurement at the end (Maryniuk 2017).

A 50 g bioavailable carbohydrate content was obtained by standardising the quantities of each meal. The benchmark for GI calculation was a glucose beverage that could provide 50 g of readily available carbohydrates. The FAO/WHO-recommended procedure (FAO/WHO, 1998) was adopted as the experimental protocol to assess GI. Individual instructions to abstain from smoking and strenuous exercise were given to the research participants. To guarantee that the individuals consumed a balanced evening meal, a brief behavioural questionnaire and a 24-hour recall were employed. Subjects were instructed to fast for around 12 hours before visiting the study centre in the morning; however, they were free to consume water throughout the night. Standard glucose beverages or freshly made chapattis were given to the fasted participants. A 15-minute time limit was given to all participants to complete their test meals. For the purposes of PPG and PPI analysis, blood was drawn 2 hours after the test meal.

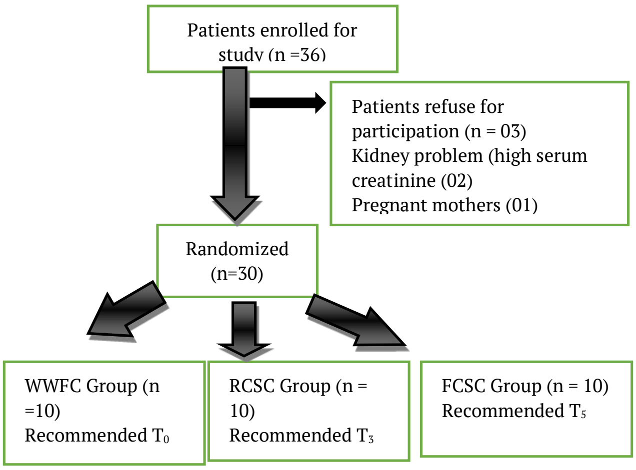

Figure 2. Schematic diagram for study design involving persons with type 2 diabetes

WWFC: Whole wheat flour chapattis, RCSC: Raw carrot supplemented chapattis, FCSC: Fermented carrot supplemented chapattis

MONITORING OF COMPLIANCE

Participants were provided with clear instructions and support to help them adhere to the dietary regimen. They were asked to maintained daily food diaries to record their intake and ensure compliance with the study protocol. Regular follow-ups and check-ins were conducted to monitor adherence, compliance rate and to address any challenges faced by the participants.

EVALUATION OF BIOCHEMICAL PARAMETERS

BLOOD PROFILE ANALYSIS

Fasting and random plasma glucose concentration, complete blood count, serum lipid profile, liver functioning test, kidney functioning test, and serum electrolyte count were monitored at baseline and after 28 days of intervention (Sato et al. 2017; Tay et al. 2015). A total of 5 mL of arterial blood was obtained and sent to the Pathology Department of Nishtar Hospital, Multan for blood tests.

PROTOCOLS FOR GLYCEMIC INDEXING

The glycemic values of eligible participants based on the inclusion criteria were measured. Before ingestion of food, a baseline blood sample was analyzed at 0 minutes (FAO/WHO 1998). Thereafter, 50 g of WWFC, RCSC and FCSC were given to the participants after overnight fasting. The post-meal blood glucose (BG) levels were monitored over two hours at 15, 30, 45, and up to 120 minutes.

STATISTICAL ANALYSIS

All measurements were performed in triplicate, and the collected data were analyzed using STATISTIX 8.1 23.0. A 3 X 2 factorial design was employed, with sensory attributes (color, aroma, flavor, off-flavor, and overall acceptability) as the primary factors of interest. For the nutritional composition and GI analysis of the product, data were subjected to a one-way analysis of variance (ANOVA). Post-meal responses for each treatment were recorded in an Excel spreadsheet, and the area under the curve was calculated using Microsoft Excel. The results are presented as the mean ± standard deviation (SD). To assess the comparison of blood profiles between baseline and the 28-day mark, a paired t-test was applied, with a p-value of less than 0.05 considered statistically significant.

RESULTS

The inclusion of vegetable flour affected the appearance and color ($P< 0.05$) of the product, resulting in a distinct visual aspect as perceived during sensory evaluation. Likewise, the incorporation of both raw and fermented carrot flour at a 15% level led to a minor decline in the texture characteristics of raw carrot supplemented chapattis (RCSC) and fermented carrot supplemented chapattis (FCSC), but the chapattis were still considered acceptable by the evaluators. The Table 2 presents the results of the sensory analysis of chapattis fortified with raw and fermented carrot powder.

A significant ($P > 0.05$) variation was detected in WWF, RCSC, and FCSC carbohydrate contents. As shown in Table 3, the addition of raw and fermented carrot flour, both being good sources of dietary fiber, significantly ($P< 0.05$) improved the fiber contents of RCSC and FCSC (10.21 g/100 g and 11.02 g/100 g, respectively). Calories decrease as the whole wheat flour concentration decreases.

The sociodemographic characteristics of participants are shown in Table 4. The mean age of study participants was 34.5 years and non-significant differences were found in the age of the participants between the three groups ($P>0.05$). The participants in the control group had a BMI of 28.23 while the average BMI of groups RCSC and FCSC was 28.02 and 28.15, respectively. Most of the participants were in the overweight category with BMI ranges between 23–33.

The waist circumference did not vary significantly between the three groups (p = 0.53).

The mean values of GI and glycemic load along with the incremental area under the curve (iAUC) are shown in Table 5. The mean iAUC values of 10% RCSC and 10% FCSC were reduced significantly (p-value < 0.01) as compared to WWFC (control).

Table 2. Sensory characteristics of raw and fermented carrot flour-supplemented chapattis

Groups	WWFC	RCSC			FCSC			
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	F-Ratio
Color	7.18 ± 0.36^{a}	6.96 ± 0.35^{ab}	7.21 ± 0.36^{a}	6.45 ± 0.32^{bc}	6.37 ± 0.32^{bc}	7.35 ± 0.37^{a}	6.26 ± 0.31^{c}	5.28^{**}
Appearance	7.83 ± 0.39^{a}	6.40 ± 0.32^{cd}	7.31 ± 0.37^{ab}	6.35 ± 0.32^{cd}	6.90 ± 0.35^{bc}	7.38 ± 0.37^{ab}	6.04 ± 0.30^{d}	10.7^{**}
Taste	7.23 ± 0.36^{a}	6.26 ± 0.31^{b}	7.26 ± 0.36^{a}	6.15 ± 0.31^{b}	6.46 ± 0.32^{b}	7.36 ± 0.37^{a}	6.10 ± 0.31^{b}	2.25^{NS}
Texture	7.63 ± 0.38^{a}	6.12 ± 0.31^{b}	7.50 ± 0.38^{a}	5.94 ± 0.30^{b}	6.29 ± 0.31^{b}	7.54 ± 0.38^{a}	5.98 ± 0.30^{b}	16.7^{**}
Folding ability	7.66 ± 0.38^{a}	6.13 ± 0.30^{b}	7.37 ± 0.37^{a}	5.84 ± 0.29^{b}	6.29 ± 0.31^{b}	6.39 ± 0.31^{b}	5.92 ± 0.30^{b}	4.8^{*}
Overall acceptability	7.50 ± 0.37^{a}	6.37 ± 0.31^{b}	7.33 ± 0.37^{a}	6.14 ± 0.31^{b}	6.46 ± 0.32^{b}	7.17 ± 0.36^{a}	6.06 ± 0.31^{b}	9.44^{**}

Value sharing the same superscript in a row do not differ significantly, (P < 0.05); NS = non-significant; * = Significant (P < 0.05); ** = Significant (P< 0.01).

T0 = WWF (100%), T1= WWF (95%) + RCP (5%), T2 = WWF (90%) + RCP (10%), T3 = WWF (85%) + RCP (15%), T4 = WWF (95%) + FCP (5%)
T5 = WWF (90%) + FCP (10%), T6 = WWF (85%) + FCP (15%), WWF: Whole wheat flour; RCP: Raw carrot powder; FCP: Fermented carrot powder., WWFC: Whole wheat flour chapattis, RCSC: Raw carrot supplemented chapattis, FCSC: Fermented carrot supplemented chapattis.

Table 3. Proximate composition (g/100g) of raw and fermented carrot flour supplemented chapattis

Treatments	T_0	T_2	T_5	F Ratio
Moisture	9.64 ± 0.48^{a}	9.76 ± 0.49^{a}	9.72 ± 0.48^{a}	0.05^{NS}
Ash	1.71 ± 0.09^{b}	2.04 ± 0.10^{a}	1.88 ± 0.09^{ab}	8.97^{*}
Dietary fiber	8.57 ± 0.43^{b}	10.21 ± 0.52^{a}	11.02 ± 0.51^{a}	12.8^{**}
Protein	10.56 ± 0.53^{a}	10.12 ± 0.51^{a}	10.24 ± 0.51^{a}	0.57^{NS}
Fat	1.94 ± 0.10^{a}	1.83 ± 0.09^{a}	1.84 ± 0.09^{a}	1.27^{NS}
Calories	259.70 ± 12.99^{a}	256.96 ± 12.85^{a}	257.54 ± 12.88^{a}	0.74^{NS}

Value sharing the same lettering in a row does not differ significantly (P < 0.05); NS = non-significant; * = Significant (P < 0.05); ** = Significant (P< 0.01)

Table 4. Sociodemographic characteristics of study participants

Participants Characteristics	WWFC (n=10)	RCSC (n=10)	FCSC (n=10)
Age (years)	33.80 ± 6.51	34.90 ± 6.64	34.80 ± 6.96
Height (cm)	170.02 ± 9.55	170.63 ± 6.60	168.30 ± 7.07
Weight (kg)	80.93 ± 14.70	81.10 ± 12.57	79.30 ± 11.27
Body Mass Index	28.23 ± 5.9	28.02 ± 5.17	28.15 ± 4.99
Ideal body weight (kg)	60.02 ± 9.52	60.63 ± 6.60	58.30 ± 7.04
Waist circumference (cm)	35.36 ± 5.38	35.40 ± 4.53	36.10 ± 5.17

Table 5. The incremental area under the curve, glycemic index and glycemic load of experimental chapattis

Groups	iAUC	Glycemic Index	Glycemic Load	Glycemic ranking
	Mean ± SD	Mean ± SD	Mean± SD	
WWFC	147.10 ± 16.60^{a}	60 ± 9.62^{b}	21 ± 3.92^{a}	High
RCSC	105.03 ± 13.10^{b}	48 ± 7.84^{a}	14 ± 2.13^{b}	Low
FCSC	99.75 ± 15.96^{b}	46 ± 7.55^{b}	13 ± 2.73^{b}	Low

Value sharing the same superscript letters in a row do not differ significantly (P<0.05)

[1]WW = Whole Wheat; [2]RCS = Raw Carrot Supplemented; [3]FCS = Fermented Carrot Supplemented. GL= Glycemic load; GI= Glycemic Index; iAUC= Incremental Area Under the Curve

The GI of all chapattis (WWC, RCSC, and FCSC) is calculated via incremental area under the curve over 120 minutes of PPG response compared to standard glucose beverages. Our findings indicated that the GI of the products is low in RCSC and FCSC and medium in WWC. The GI values were reduced significantly in RCSC and FCSC. Similarly, glycemic load values of WWC, RCSC, and FCSC were 21, 14, and 13, respectively. These observations suggested that RCSC and FCSC are non-significantly different and that both chapattis are equally beneficial for T2D patients in controlling blood sugar.

Comparisons of post-meal blood glucose concentrations of tested chapattis (RCSC and FCSC) versus control chapatti (WWFC) are shown in Figure 3. the PPG iAUC0-120 minutes of tested chapattis (RCSC and FCSC) were significantly lower than the control chapatti. Both tested chapattis (RCSC and FCSC) showed lower post-meal blood glucose concentration compared to the control chapatti, and the post-meal concentrations of RCSC and blood glucose at 15, 30, 45, 90, and 105 min were also significantly reduced (p = 0.001, p = 0.008, p=0.0023, p=0.0035, and p=0.002, respectively). In addition, the standard glucose beverage (GB) did not attain lower values of post-meal concentration as compared to the RCSC. The results also indicate a significant association between the post-meal blood glucose concentration and FCSC and GB at 30, 45, and 105 minutes (P<0.05).

Shown in Table 6 are our results of the impact of WWC, RCSC, and FCSC on the biochemical profile of T2D patients. The mean fasting and random glucose levels, serum low-density lipoprotein (HDL) and serum cholesterol concentrations, and triglyceride levels have improved, but these reductions were not statistically significant among all blood markers (p-value > 0.05). By the 4th week, all groups showed improvements in these markers, with The FCSC Group consistently exhibiting the most significant changes: a 12.8% reduction in fasting blood sugar (p=0.04), a 17.5% decrease in random blood sugar (p=0.03), a 21.9% reduction in serum low-density lipoprotein (LDL) (p=0.051), a 16.7% increase in serum HDL (p=0.01), an 11.0% decrease in serum cholesterol (p=0.02), and a 12.6% reduction in serum triglycerides (p=0.048). These results highlight that the dietary intervention, particularly for The FCSC Group, significantly improved the biochemical profiles of the participants. The implication of the change in values from baseline to 4th week for the RCSC and FCSC compared to control is indicated under the inference in Table 6.

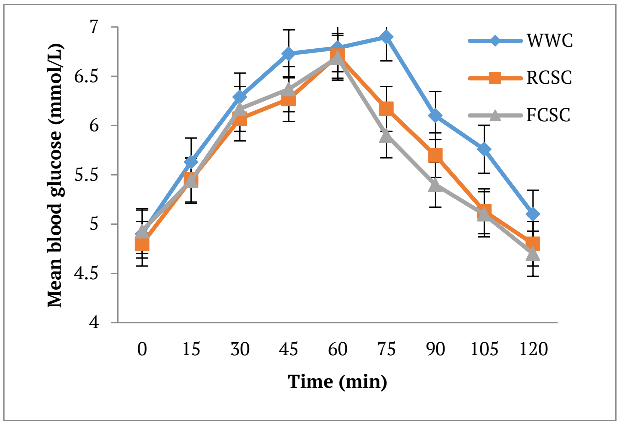

Figure 3. Comparison of the postprandial glucose response post consumption of the different chapattis variants

WWC Whole wheat chapattis, RCSC Raw carrot supplemented chapattis, FCSC Fermented carrot supplemented chapattis

Table 6. Impact of on the biochemical profile of the control group compared to the raw and fermented carrot powder groups

Biochemical Profile	Baseline	4th week	Change %	Within group *P*-value (baseline)	Within group *P*-value (4th week)	Inference
Fasting Sugar				0.06	0.04	
WWFC group	140 ± 9.5	131 ± 9.15	6.43			
RCSC Group	152 ± 8.35	141 ± 6.4	7.24			ND
FCSC Group	149 ± 7.75	130 ± 6.5	12.8			HS
Random blood sugar				0.054	0.03	
WWFC group	262 ± 13.1	246 ± 12.3	6.10			
RCSC Group	276 ± 18.8	244 ± 12.2	11.6			HS
FCSC Group	240 ± 17	198 ± 9.9	17.5			HS
Serum LDL				0.062	0.051	
WWFC group	142 ± 7.1	136 ± 6.8	4.23			
RCSC Group	148 ± 8.8	121 ± 6.45	18.2			HS
FCSC Group	151 ± 7.75	118 ± 5.8	21.9			HS
Serum HDL				0.059	0.01	
WWFC group	44.0 ± 2.45	47.0 ± 2.75	−6.81			-
RCSC Group	46.0 ± 2.95	52.0 ± 2.15	−13.0			HS
FCSC Group	48.0 ± 3.23	56.8 ± 2.84	−16.7			HS
Serum Cholesterol				0.053	0.02	
WWFC group	209 ± 9.75	198 ± 10.3	5.26			-
RCSC Group	212 ± 10.6	192 ± 9.15	9.43			HS
FCSC Group	218 ± 12.9	194 ± 10.1	11.0			HS
Serum Triglycerides				0.061	0.048	
WWFC group	161 ± 8.05	148 ± 8.35	8.08			-
RCSC Group	158 ± 7.5	142 ± 6.9	10.13			HS
FCSC Group	167 ± 8.65	146 ± 6.3	12.6			HS

ND represented as non-significant difference, HS represent highly significant difference

DISCUSSION

Dietary modifications have been recommended to prevent the onset and progression of chronic conditions such as obesity, diabetes, cancer, and cardiovascular diseases. The current randomized control trial was conducted to investigated the impacts of newly formulated chapattis as a source of dietary approach to manage T2D. In this study, a 28-day time frame was chosen partly based on evidence from existing literature indicating that significant changes in diabetic health markers can be observed within this period. This duration allows for capturing short-term effects of the dietary intervention, while being manageable for participant compliance and minimizing dropout rates.

Additionally, it takes into account practical considerations such as resource availability, budget constraints, and logistical feasibility, ensuring the study can be conducted effectively and ethically. The findings from this study are comparable with previous investigations that reported a range of GI values for wheat flour chapattis, spanning from low (55) to high (70) (Carneiro et al. 2020; Odunlade et al. 2017; Okoduwa and Abdulwaliyu 2023).

These variations in GI values can be attributed to several factors, including differences in starch and fiber content, variations in processing or cooking methods, the presence of anti-nutritional components, and the presence of resistant starch, all of which can influence the GI values (El Khatib et al. 2020). The information presented above showed that adding carbohydrates to vegetable flour can lower levels of blood sugar. Findings from the current study demonstrated that RCSC and FCSC have low GI values while WWC has a medium GI value. In raw and fermented carrot powder, the GI values were significantly lower.

Whole wheat flour chapatti has been reported to have medium GI values, or 56 to 69, and fiber-supplemented whole wheat flour has been reported to have low GI values, or 46% (Dodd et al. 2011; Akhtar et al. 2019). The purpose of using two different formulations was to compare and evaluate the potential differences in nutritional benefits, bioavailability of nutrients, and functional properties, such as enhanced digestion and probiotic content, provided by fermentation.

The findings of the current study agree with those of Odunlade et al. (2017), Khan et al. (2015), and Radhika et al. (2010), who reported that wheat flour formulations mixed with vegetable flour (spinach powder) and leafy vegetable powder demonstrate GI values lowering potential of chapattis. Therefore, given their lower glycemic response, both RCSC and FCSC could potentially serve as valuable tools in the management of diabetes, particularly in regions like Pakistan where the consumption of high glycemic load staples has been observed (Akhtar et al. 2019).

Our findings revealed that vegetable flour supplementation undeniably reduces hyperglycemia and enhances PPG concentrations in both healthy and T2D subjects (Onwurafor et al. 2022; Santos et al. 2022). Because carrot flour contains soluble fibers, particularly starch granules rich in amylose, it may have maintained some of its post-prandial glucose-lowering properties during simulated digestion (Gao et al. 2019). Likewise, the slowest-release starch among vegetables is found in carrot flour, which lowers the post-meal gastric emptying rate and absorption of glucose in modulating postprandial insulin (PPI) and post prandial glucose (PPG) responses (Lal et al. 2022). The fact that there was no statistically significant difference between RCSC and FCSC demonstrated that both chapattis are equally helpful for type 2 diabetic patients in controlling blood sugar. RCSC and FCSC can improve post-prandial glucose, fasting glucose, and random glucose. Therefore, these chapattis were selected for type II diabetic participants. This was due to the low GI of tested chapattis while the GI of control chapatti was high which presented the enhanced PPG response curve. Another study depicted similar findings where vegetable powder chapatti presented low post-meal blood glucose concentration as compared to all-purpose flour chapatti (APFC) (Akhtar et al. 2019). Similarly, Imai et al. (2014) found that eating vegetables before a meal that contains carbohydrates significantly lowers glucose excursions and PPG responses (39% reduction in incremental area under the curve (iAUC0)-3 hours) in T2Dpatients (Imai et al. 2014). The current finding builds on the findings of earlier exploratory studies, which showed that vegetable flour mix formulations significantly ($p>0.01$) reduced post-meal glucose response and iAUC0-120 minutes compared to wheat flour (Boers et al. 2017). In this respect, it is possible to assume that the fibers and bioactive compounds in carrot flour alter gastric conditions for hydrolytic enzyme activity, causing a slower release of nutrients and a decrease in PPG and PPI responses.

Furthermore, the present study revealed that the mean fasting blood glucose concentration improved significantly in the RCSC and FCSC groups (7.23 % and 12.75%, respectively) (p-values 0.042 and 0.033, respectively) but the non-significant change was observed among the control group. Similarly, FBG was improved as all participants were following a controlled diet. In this regard, Rothberg et al. (2017) and Ruggenenti et al. (2017) employed consistent research methods and reported a significant decline in blood glucose of 9 mg/dL and 18.1 mg/dL, respectively. Ingestion of a low glycemic diet in T2D patients is linked with improving fasting blood glucose, serum cholesterol, serum triglycerides, and low-density lipoproteins (Yao et al. 2017; Hodaei et al. 2019). The GI of both chapattis was low, and fasting glycemic control was improved on the 28th day among groups RCSC and FCSCwhile random blood sugar levels declined non-significantly. Animal studies were also in favor of the results of the current study as Ekpe et al. (2021) reported that carrots help mitigate high glucose levels in diabetic rats. Raw and fermented carrots also improved hyperglycemia and lipid concentration in diabetic rats (Louis et al. 2018). In another study, a significant decline of 15% was observed in random blood sugar among albino rats that were fed a carrot powder-supplemented diet as compared to the control diet (McClinton et al. 2020).

The current study indicated a significant decline in high-density lipoproteins in groups RCSC and FCSC ($P < 0.05$). Similar findings were also reported that a significant decline in cholesterol and HDL was observed in patients who were taking vegetable-supplemented meals (Wright et al. 2013). Tiwari et al. (2018) reported that raw carrot supplementation elevates high-density lipoprotein levels significantly ($P < 0.05$) (Tiwari et al. 2018). In contrast, findings reported by Soleti et al. (2021) in animal models showed a non-significant change in high-density lipoprotein, cholesterol, low-density lipoprotein, and triglycerides through supplementation. In contrast to the clear low-GI diet benefits over a high-GI diet concerning its effects on PPG metabolism, our findings indicated a non-significant improvement in random blood sugar, LDL, and serum triglycerides. On the other hand, low-GI diets are clinically useful for people with T2D (Carneiro et al. 2020).

In this respect, our findings showed that replacing high-GI foods with low-GI foods may also help improve glycemic control indices in people with metabolic syndrome traits. Our findings are consistent with those of a previous study in which individuals with diabetes showed improved random blood glucose control in response to a low-GI diet (Bergia et al. 2022). According to our findings, dehydrated vegetables' higher fiber content may reduce PPG and PPI responses. In this context, future research should explore the effect of resistant vegetable powder starch on these responses in foods like chapattis. Exploratory research is recommended to understand the physiological effects of these flour formulations in individuals with T2D.

LIMITATIONS

The present study did not comprehensively assess confounding variables, such as participants' physical activity levels, which could influence health outcomes independently of the supplementation intervention. Thus, caution is warranted in interpreting the findings, and future research should prioritize comprehensive assessment of confounding variables to enhance the validity and generalizability of results in the context of T2D management. Furthermore, the current study proposes an exploratory investigation aimed at assessing the metabolic effects of consuming a vegetable powder mix among individuals with T2D. We suggest that future research examine the responses of key hormones such as ghrelin, incretin, and glucagon to these specific flour formulations.

CONCLUSIONS

The partial substitution of wheat flour with dehydrated fermented carrot flour could be a promising strategy for reducing postprandial glucose responses and improving biochemical markers like hyperglycemia and serum lipid profile. This is likely due to the lower glycemic nature and higher fiber content of carrot flour. Future research should explore the metabolic effects of carrot flour mix consumption in individuals with T2D, such as responses of hormones like ghrelin and glucagon.

AUTHOR CONTRIBUTIONS

Conceptualization, A.A., S.A. and H.R.; **Design:** A.A., S.I.R.O.; **Data curation**, A.A., and H.R.; **Formal analysis**, A.A., and H.R.; **Investigation**, S.A. and T.I.; **Methodology**, S.A., T.I., and S.I.R.O.; **Supervision**, T.I., S.I.R.O.; **Validation**, A.A., and H.R.; **Visualization**, A.A.; **Writing—original draft**, A.A. **Writing—review and editing**, S.I.R.O., T.I., and S.A., All authors have read and agreed to the published version of the manuscript.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

CONFLICT OF INTEREST

The authors declare that they have no other potential conflicts of interest.

ACKNOWLEDGEMENTS

S.A. and H.R. worked together as co-principal, making them co-senior authors. All study participants who volunteered their time and attention are gratefully acknowledged by the study authors.

FUNDING

The present study was not funded by any project or agency or university.

Submitted: March 07, 2024; Accepted: June 25, 2024; June 30, 2024

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