

Inhibitory Potential Against Advanced Glycation End Products and Metabolic Syndrome-Related Enzymes of Bread Incorporated with Caralluma Extract Powder

Aimal Khan¹, Imran Khan², Shah Fahad², Sabina Khan, Muhammad Onais Inam, Shehar Yar Shafi, Malik Abdul Haseeb

Department of Human Nutrition. The University of Agriculture, Peshawar-Pakistan

Abstract- Wheat bread is a commonly consumed bakery item known for its high energy content. Nevertheless, bread produced from refined flour may lack in nutritional quality. To boost the nutritional profile of bread, various cereals, legumes, and medicinal herbs have been blended with wheat flour. This research aims to evaluate the overall polyphenol content (TPs), antioxidant potential, antiglycation characteristics, inhibition of enzymes linked to metabolic syndrome (such as α -amylase, α -glucosidase, and pancreatic lipase), and the sensory appeal of bread incorporated with caralluma extract powder (CEP). Different proportions of CEP (2%, 4%, and 6%) were incorporated in wheat flour to create composite bread. The bread was prepared following a standard recipe, dried, and stored for subsequent analysis. TPs were quantified using the Folin-Ciocalteu method, and antioxidant potential was checked by measuring its ability to scavenge ABTS and DPPH radicals. The Bovine Serum Albumin-Glucose (BSA-Glu) assay was used to ascertain the impact on antiglycation. Additionally, in vitro examinations were conducted to measure the inhibitory effect of CEP-enriched bread on alpha-amylase, alpha-glucosidase, and pancreatic lipase. To determine the palatability of the bread samples among consumers, a 9-point hedonic scale was used for the evaluation. Statistical analysis involved ANOVA (One-way analysis of variance) followed by the LSD post-test to compare and analyze the outcomes of bread with different CEP levels against the control bread. Bread containing 2%, 4%, and 6% CEP showed significantly higher levels of TPs (147.15 mg GAE/100g) and antioxidant capacity (ABTS 132.81 μ mol TE/100g, DPPH 172.36 μ mol TE/100g) compared to the control bread. CEP-containing bread also exhibited significantly stronger inhibitory effects ($p < 0.05$) against the formation of advanced glycation end products (20.46%-32.24%) compared to the control (10.18%). Inhibitory effects on metabolic enzymes— α -amylase (6.26%-17.24%), α -glucosidase (10.26%-22.24%), and pancreatic lipase (8.26%-19.24%)—increased significantly with higher levels of CEP in the bread. Sensory analysis indicated that all CEP-incorporated bread samples received an acceptable score (≥ 6), suggesting their palatability. In conclusion, bread enriched with CEP displayed significantly improved phytochemical properties at all incorporation levels, as well as substantial inhibition of advanced glycation end products and metabolic syndrome-related enzymes. Further research should investigate the effects of maximum CEP incorporation in bread.

Index Terms- wheat bread, caralluma extract powder (CEP), metabolic syndrome, polyphenol content (TPs), ABTS and DPPH radicals

I. INTRODUCTION

Metabolic syndrome, often known as syndrome X, is a prevalent metabolic disease that is linked to the worldwide epidemic of obesity and diabetes. The metabolic syndrome (MS), which comprises insulin resistance, abdominal obesity, dyslipidemia and hypertension, is a collection of conditions rather than a single disease. The comorbidities linked to metabolic syndrome include a prothrombotic state, proinflammatory condition, reproductive issues and non-alcoholic fatty liver disease. Clustering components like over-nutrition, excess adiposity and sedentary lifestyle reflects the metabolic syndrome (Cornier *et al.*, 2008). The National Cholesterol Education Program's Adult Treatment Panel III categorized metabolic syndrome as a significant public health concern in the United States with an impact on approximately 34% of the population. (Sae-Tan *et al.*, 2011). Patients with metabolic syndrome are typically counselled to abstain from cigarette use and consume nutritious foods, but changing their overall lifestyle choices will be far more crucial to reducing their chance of developing metabolic syndrome (VanWormer, *et al* 2017).

Since ancient times, people have used plants to heal a variety of illnesses. Being natural, plants have less adverse effects and are more palatable to human bodies. Even before the invention of synthetic modern medicine, many diseases including typhoid, measles, cholera and other microbiological diseases were treated using medicinal herbs. These herbs were utilized by people as a pain reliever, a cure for poison, a therapy for many sorts of inflammation, a remedy for snake bites and for many other issues. Because of the negative side effects of modern medications, society is turning back to herbal therapy. (Oladeji, 2016). Medicinal plants contain a variety of compounds, including polyphenols, flavonoids, isoflavonoids, terpenoids, carotenoids, phytosterols, and glucosinolates. Because of the existence of these bioactive substances, medicinal plants can be used therapeutically for purposes such as anti-cancer, anti-aging, anti-diabetic, analgesic, anti-allergic, anti-inflammatory and hepatoprotective. (Chopra and Doiphode, 2002; Maurya *et al.*, 2008). Medicinal plants find applications in the production of modern medications, both through direct and indirect means (Leitzmann, 2016). Caralluma (*Caralluma fimbriata*) stands out as a valuable medicinal plant. This edible succulent cactus belongs to the

Asclepiadaceae family, as documented by Qayyum *et al.* in 2018. Its habitat predominantly spans across semi-arid regions in southern Europe, India, Africa, Afghanistan, Arabia, and Pakistan. In Pakistan, it goes by the name "choogan" in Urdu, while in India, particularly in Tamil, it is referred to as "karallamu.". It is eaten in Pakistan and India in a variety of ways, including as a pickle or chutney and occasionally as a regular vegetable. Western Indian hunters have been utilizing caralluma to satiate their hunger and thirst for centuries. In India, it is commonly known as "famine food," as indicated by Asmi *et al.* in 2017. Up to this point, there have been no reported adverse effects associated with the consumption of caralluma. Caralluma is said to have therapeutic effects due of its phytonutrient concentration. It contains tannins, saponins, flavonoids, phenolic compounds, pregnane glycosides and ascorbic acid as well as other antioxidants (Priya, *et al* 2011).

As the exceptional nutritional profile of Caralluma, it is utilized to treat a variety of disorders. Research on Caralluma extract powder has shown that in addition to other health advantages, it also possesses anti-obesogenic, anti-diabetic and renal-protecting qualities. Besides its role in alleviating oxidative stress, the presence of antioxidants, such as phenolic compounds and flavonoids in caralluma, contributes to its effectiveness in treating inflammation and certain types of cancer, as highlighted by Gupta and Prakash in 2014. Furthermore, a study involving streptozocin-induced diabetic rats aimed to evaluate the antidiabetic properties of methanolic extract from Caralluma. The results indicated a significant reduction in the diabetes levels of the tested animals, as reported by Latha *et al.* in 2014.

Wistar rats were used in a further investigation. These rats were fed a high-fat diet in order to modify their lipid profiles. These rats were then administered a hydro-alcoholic extract of caralluma for the following 90 days. The outcomes shown that powdered caralluma extract may be useful in lowering lipid alterations. (Gujjala *et al.*, 2016). It indicates that Caralluma has been known for having anti-inflammatory, antioxidant, anti-obesogenic and anticancer properties and has never been associated with any negative side effects (Chester *et al.*, 2017). The purpose of this study is to assess how adding Caralluma extract powder to bread affects the production of AGEs and enzymes activity linked to the metabolic syndrome, such as α -amylase, α -glycosidase, and pancreatic lipase.

II. MATERIALS AND METHODOLOGY

1.1 Study Location

Research was conducted at the University of Agriculture Peshawar, in the Departments of Human Nutrition and Animal Health.

1.2 Procurement of ingredients

The standard bread ingredients, including wheat flour, salt, yeast, oil and sugar, were procured from market in Peshawar. Commercially available Caralluma fimbriata extract was also utilized in the study.

1.3 Bread making

To prepare the dough for bread making, the following steps were followed. Wheat flour, 100g, was placed in a clean bowl and other baking ingredients including 5g of oil and 1g of salt were added and mixed thoroughly. Yeast activation was performed by taking 60ml of lukewarm water in a separate clean glass. To this, 1g of yeast and 6g of sugar were added and stirred using a spoon. The glass containing the yeast mixture was subsequently placed in a dry area for 5 minutes to initiate yeast activation. Following this duration, the activated yeast mixture was blended with the flour to create the dough. The mixture underwent a 10-minute kneading process and was then left in a dry location for 40 minutes to facilitate fermentation. After this initial 40-minute period, the dough was kneaded again for an additional 10 minutes and allowed to ferment for another 40 minutes. Meanwhile, the baking oven was preheated at 220°C for 15 minutes. After the second fermentation period, the dough was placed in a baking pan and the pan was put in the preheated oven for baking. The baking process was carried out for 15 minutes at 220°C. For the formulation of the bread, wheat flour was substituted at different levels in combination with caralluma extract powder (CEP) (2% CEP, 4% CEP, 6% CEP). The remaining baking ingredients including water, yeast, salt, oil and sugar were added in the proportions as specified in the Table number 1.

Table 1. Formulation of wheat & caralluma extract powder composite bread (/100g flour).

Ingredients	Control Bread	Treatment Bread		
		2% CEP	4% CEP	6% CEP
Wheat Flours (g)	100g	98g	96g	94g
Caralluma extract powder (g)	0g	2g	4g	6g
Oil (g)	5g	5g	5g	5g
Yeast (g)	1g	1g	1g	1g
Salt (g)	1g	1g	1g	1g
Sugar (g)	6g	6g	6g	6g
Water (ml)	60ml	60ml	60ml	60ml

CEP: caralluma extract powder

1.4 Drying of Bread

To find the total polyphenols, enzymes inhibition and antioxidants activity all the four breads were dried in a 50°C oven for 24 hours.

1.5 Milling of bread

The dried bread was ground into a fine powder using a professional grinder.

1.6 Sample preservation

The powder was carefully transferred into pre-labeled airtight plastic bottles made of polyethylene. These plastic bottles were

subsequently wrapped with aluminum foil and stored in a refrigerator at a temperature of 4°C in preparation for analysis.

1.7 Chemical Analysis

1.7 (a) Extraction of samples to assess total phenolics and antioxidant activity

Sample extracts for the determination of antioxidant activity, total phenolics and inhibition of enzyme were prepared following the procedure proposed by Awika *et al.* (2004). A total of 1g of samples (including wheat flour, Caralluma extract powder in its raw form, control bread, 2% CEP bread, 4% CEP bread and 6% CEP bread) were accurately weighed and placed into pre-labeled Falcon tubes. Next, 10ml of a 99% Methanolic HCl solution (comprising 99ml of methanol and 1ml of HCl) was added to each Falcon tube containing the samples. The contents were thoroughly mixed by gently agitating the Falcon tubes, ensuring proper blending. To facilitate thorough mixing, the Falcon tubes were placed horizontally in a test tube rack. The Falcon tubes, containing the sample-extraction solution, were then immersed in a water shaker bath for a duration of 1 hour at a temperature of 25°C, with continuous shaking at 100 oscillations per minute. Following the one-hour extraction period, the samples in Falcon tubes were transferred to a centrifuge machine for the purpose of separation. Centrifugation was carried out for 20 minutes at a speed of 3000 rpm. Following centrifugation, the upper part, known as the supernatant, was carefully separated from the extraction tubes using a micropipette with a clean tip. The extracted supernatant was then placed into pre-labeled sterilized glass vials, covered with aluminum foil to protect it from light exposure and finally stored in a refrigerator at a temperature of -20°C. These vials were kept for further analysis.

1.7 (b) Total Phenol determination

Total phenolic content was examined using the colorimetric method involving the Folin-Ciocalteu reagent, as developed by Lin *et al.* (2007). The study included six samples for analysis: extracts of wheat flour, Caralluma extract powder, control bread, 2% CEP bread, 4% CEP bread and 6% CEP bread. The procedure was consistent for all samples. To prepare a 10-fold diluted FC reagent solution, 1ml of FC reagent was mixed with 10ml of distilled water. For each experiment, 0.2ml of the sample extract was placed into sterilized glass test tubes. Subsequently, 0.8ml of the diluted FC reagent solution was added to each test tube using a micropipette. The test tubes were then placed in the dark for a period of 3 minutes. Following a 3-minute incubation period, 2ml of a 15% sodium carbonate solution was introduced into each test tube. The mixture's final volume was adjusted to 5ml by incorporating 2ml of distilled water. Subsequently, the resulting solution was kept in darkness at room temperature for 1 hour. Following the 1-hour incubation, the absorbance was measured in duplicate at a wavelength of 760nm using a spectrophotometer (Genesys 10 UV). Distilled water was used as a blank reference. Gallic acid in concentrations ranging from 0 to 0.5mg/ml was used as a standard. The results were expressed as milligrams of Gallic acid equivalent per sample.

1.7 (c) Assessing antioxidant activity using ABTS assay

The ABTS (2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-sulfonic Acid) radical scavenging activity of both composite bread and raw sample extracts was determined using a method adapted from Ren *et al.* (2016) with slight modifications. Here's the procedure. To create the ABTS radical stock solution, potassium persulfate (K₂S₂O₈) powder and ABTS powder were meticulously weighed using a top-loading balance (CY 510C, Poland) in separate clean glass beakers. A total of 33mg of potassium persulfate and 9.6mg of ABTS were used for this purpose. These substances were then combined in distilled water to form the ABTS stock solution. In one glass beaker, 33mg of potassium persulfate was dissolved in 50ml of distilled water, while in another glass beaker, 9.6mg of ABTS powder was dissolved in 2.5ml of distilled water. Both solutions were stirred using a magnetic stirrer. After thorough stirring, 5ml of the potassium persulfate solution was mixed with 5ml of the ABTS solution in a conical flask at a 1:1 ratio. The flask was subsequently covered with aluminum foil and placed in a dark environment for a duration of 2 hours to induce the generation of free radicals. Following this 2-hour incubation period, the ABTS solution was diluted with methanol until it reached a final absorbance falling within the range of 0.7nm to 0.8nm. This absorbance was determined using a spectrophotometer (Genesys 10 UV) at a wavelength of 734nm. Subsequently, 2.85ml of the prepared ABTS stock solution with an optical density of 0.7nm was introduced into each pre-labeled small test tube. To these test tubes, 150ul of the sample extract was added. Absorbance measurements of the test samples were taken at time intervals of 0, 10, 20, and 30 minutes. The absorbance was measured at a wavelength of 734nm using a spectrophotometer. All measurements were recorded in triplicate.

1.7 (d) Assessing antioxidant activity via the DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay, as described by Yang *et al.* (2003), was utilized to assess the antioxidant activity of both the composite bread and raw sample extracts. To prepare the DPPH solution, 3.943mg of DPPH powder was accurately weighed using a top load balance (CY 510C, Poland). The DPPH powder was placed in a clean volumetric conical flask, which was then wrapped with aluminum foil. To this flask, 100ml of methanol was added and the contents were thoroughly mixed. This mixture resulted in a dark purple-colored solution. From the prepared DPPH solution, 2.85ml was dispensed into sterilized small glass test tubes. To each test tube, 150ul of the sample extract was added. The initial absorbance of these samples was recorded in triplicate using a spectrophotometer at a wavelength of 517nm. Following the initial absorbance measurement, the test samples, containing the DPPH solution, were placed in a dark location. After a 30-minute incubation period, the final absorbance of the samples was recorded. Control samples were created by combining 2.85ml of the DPPH solution with 150ul of methanol, in place of the sample extract. A standard substance, Trolox (ranging from 0 to 1mM), was employed for reference purposes, which was dissolved in methanol. The antioxidant capacity results obtained from the

DPPH assay were calculated as percent inhibition (%I) using the following equation:

$$\% I = [(Ac - As) / As] \times 100$$

1.7 (e) Extraction of samples for Advanced Glycation End Products analysis

Sample extracts for the determination of advanced glycation end products (AGEs) were prepared from both breads and flour samples following the procedure proposed by Awika *et al.* (2004). Weighed 1g of the respective samples, which included wheat flour, Caralluma extract powder in its raw form, control bread, 2% CEP bread, 4% CEP bread and 6% CEP bread. Added the weighed samples into separate Falcon tubes. Prepared an 80% Methanol solution by mixing 80ml of methanol with 20ml of distilled water. This solution was used for the extraction process. Added 5ml of the 80% Methanol solution to each Falcon tube containing the samples. Ensured that the Falcon tubes were properly adjusted in a test tube rack horizontally to facilitate thorough mixing of the samples with the extraction solution. Placed the Falcon tubes with the sample-extraction solution into a water shaker bath. The extraction was carried out for 40 minutes at a temperature of 25°C with continuous shaking at 100 oscillations per minute. After the 40-minute extraction period, the Falcon tubes with the samples were transferred to a centrifuge machine. Centrifugation was performed for 10 minutes at a speed of 5000 rpm. Following centrifugation, the upper part of the supernatant was separated from the extraction tube using a micropipette with a clean tip. The separated supernatant was then placed into pre-labeled sterilized glass vials. The glass vials containing the supernatant were placed in an oven for 36 hours at a temperature of 40°C. During this time, the methanol was removed from the samples. Following the methanol removal, 5ml of a 0.1M sodium phosphate buffer with a pH of 7.4 was introduced into the glass vials. Subsequently, the vials were placed in a refrigerator at -20°C for subsequent analysis.

1.7 (f) Assessment of the inhibitory impact on advanced glycation end products

The procedure for assessing the inhibitory effect of composite bread at different percentages and raw flour samples on the formation of advanced glycation end products (AGEs) followed the method outlined by Szawara-Nowak *et al.* (2014). To create a 22% Bovine Serum Albumin (BSA) solution, 10ml of 22% BSA was mixed with 10ml of 0.1M sodium phosphate buffer (pH 7.4) and 0.6mg of sodium azide (NaN₃) in a glass vial to prevent microbial activity. The solution was thoroughly mixed. A blank sample was prepared by combining 1ml each of BSA, D-glucose, and 0.1M sodium phosphate buffer (pH 7.4) in a cleaned and labeled glass vial. Test samples were prepared in cleaned, labeled glass vials by combining 1ml each of BSA, D-glucose, and the sample extract. A standard sample was prepared by combining 1ml each of BSA, D-glucose, and rutin solution in a labeled glass vial. All samples were incubated in an incubator at 55°C for 40 hours. After the incubation period, the samples were transferred to a clean microplate with 12 wells in the horizontal position and 8 wells in the vertical position, and their arrangement

was recorded. The fluorescence intensity of each sample was measured using a microplate reader (ELISA reader ALLshang Model-100) with an excitation wavelength of 330nm and an emission wavelength of 410nm. Quadruplicate samples were analyzed for each set, and the percentage inhibition of AGEs by each bread extract and rutin solution was calculated using the appropriate equation.

$$\% \text{ Inhibition} = (\text{fluorescence of the solution with inhibitors} / \text{fluorescence of the solution without inhibitors}) * 100$$

1.7 (g) Assessment of α -Amylase inhibitory activity

The alpha-amylase inhibition assay was conducted following the method outlined by Apostolidis *et al.* (2006). To assess the inhibitory effect of wheat bread enriched with caralluma extract powder on alpha-amylase activity, a control solution was prepared by combining 500ul of 0.02M sodium phosphate buffer (pH 6.9) and 50ul of alpha-amylase enzyme in a labeled glass vial, which was then incubated at 25°C for 10 minutes in an oven. After the initial incubation, 500ul of 1% starch mixture was added to the reaction mixture, followed by an additional 10-minute incubation at the same temperature. A control blank, lacking the enzyme, was similarly prepared by mixing 500ul of 0.02M sodium phosphate buffer and 500ul of 1% starch solution in a pre-labeled glass vial and incubating it as well. For the sample solution, 500ul of the sample extract and 50ul of alpha-amylase enzyme were combined in a labeled glass vial and incubated under the same conditions. Following the initial incubation in the sample vial, 500ul of 1% starch solution was added and the mixture was subjected to an additional 10-minute incubation. To halt the reactions, 1.0 ml of dinitrosalicylic acid (DNSA) color reagent was added to all vials and they were placed in a boiling water bath at 100°C for 5-10 minutes before being cooled to room temperature. The spectrophotometer was used to measure the absorbance at 540nm for each solution. A buffer was used as a control we also recorded sample blanks, which contained buffer instead of enzyme. The total blank sample was subtracted from the final extract absorbance (A₅₄₀ extract) to get the final value. In the end the following equation was used to find out the alpha-amylase inhibitory activity.

$$\% \text{ Inhibition} = (A_{540} \text{ control} - A_{540} \text{ extract} / A_{540} \text{ control}) * 100$$

1.7 (h) Assessment of α -Glucosidase inhibitory activity

The Alpha-glucosidase inhibition test as reported by Godavari *et al.* (2018) was used for the investigation. We made solutions, including 1% sucrose solution, α -glucosidase enzyme, 0.02 M sodium phosphate buffer (pH 6.9), and 96 mM 3,5-Dinitrosalicylic Acid solution (DNS). The following method was used to find out that whether the Caralluma containing wheat bread can affect the alpha-glucosidase activity. We made many a number of solutions that is 20ul of α -glucosidase solution, 20ul of 1% sucrose solution, and 20ul of 0.02M sodium phosphate buffer (pH 6.9) were mixed in a labelled vial to make the first solution, named as control solution. A 60ul of 0.02M sodium phosphate buffer (pH 6.9) to a vial, I made a control blank. This blank was free from both enzyme and substrate. In marked

jars, we blend 20ul of sample extract with 20ul of the enzyme α -glucosidase and 20ul of 1% sucrose solution to make sample solution. We also added 20ul of sample extract and 40ul of 0.02M sodium phosphate buffer (pH 6.9), without the enzyme or substrate, to a labelled vial to make the sample blank. Then, each sample mixture was kept in oven and heated to 37 °C for 60 minutes continuously. Following the incubation period, the reactions were terminated by adding 1.0 ml of dinitrosalicylic acid (DNSA) color reagent. The vials were then subjected to a boiling water bath at 100°C for 5-10 minutes. After cooling, the solutions were diluted with 10ml of distilled water, and their absorbance was measured at 540nm using a spectrophotometer. Recordings were made for sample blanks, which contained buffer instead of enzyme, and for a control, which contained buffer instead of sample extract. The α -glucosidase inhibitory activity was subsequently calculated using the relevant equation.

$$\% \text{Inhibition} = (A_{540} \text{ control} - A_{540} \text{ extract} / A_{540} \text{ control}) * 100$$

1.7 (i) Determination of inhibitory activity of Pancreatic lipase

The determination of lipase inhibitory activity was conducted using a spectroscopic method with adjustments inspired by the procedure outlined by Worsztynowicz *et al.* (2014). For the lipase inhibition assay, the following solutions were employed: hog pancreatic lipase enzyme, 4-Nitrophenyl palmitate (pNPP), and a buffer. To prepare the hog pancreatic lipase enzyme solution, 2mg of lipase enzyme was carefully weighed and added to 2ml of 0.1M sodium phosphate buffer (pH 6.9), which was subsequently mixed using a vortex. For the preparation of 4-Nitrophenyl palmitate (pNPP), 6mg of 4-Nitrophenyl palmitate was weighed and combined with 2ml of 0.01M isopropanol, followed by mixing using a vortex. Regarding the buffer solution, 0.55mg of Arabic gum and 1.15mg of sodium deoxycholate were weighed and blended in 1.8ml of 0.1M sodium phosphate buffer (pH 6.9), with stirring facilitated by a magnetic stirrer. To evaluate the inhibitory effect of wheat bread enriched with caralluma extract powder on the enzyme, a sample was created by adding 20ul of sample extract and 20ul of hog pancreatic lipase enzyme to a pre-labeled test tube, while a blank was prepared without the sample. In both the sample and blank, 20ul of buffer and 20ul of hog pancreatic lipase enzyme were added to separate pre-labeled test tubes. Subsequently, both sets of reaction mixtures were incubated at 37°C for 10 minutes in an oven. After incubation, 20ul of 4-nitrophenyl palmitate and 1800ul of buffer were introduced to the solutions of both the sample and the blank. The reaction mixtures were once again incubated in the oven at 37°C for 10 minutes. The absorbance of the solutions was measured at 410nm using a spectrophotometer, and the lipase inhibitory activity was calculated using the appropriate equation.

1.8 Sensory Evaluation

Sensory evaluation process involved 50 untrained subjects who participated at the University of Agriculture Peshawar, human nutrition department. Both males and females participated in the assessment. Hedonic scale (as illustrated in Appendix-I), utilized to conduct sensory evaluation. Before the assessment, participants

were given instructions to abstain from consuming food or beverages for at least 3 hours. All four bread types, including the control, CEPB 2%, CEPB 4%, and CEPB 6% were subjected to evaluation concerning attributes like texture, color. Participants were presented with plates that were pre-labeled and coded, each containing slices of the various bread samples. Before tasting each piece of bread, participants were instructed to sip water. The evaluation was conducted using a nine-point hedonic scale questionnaire. The codes used for labeling the bread samples were 013 for control, 012 for CEP B 2%, 011 for CEP B 4% and 010 for CEP B 6%. All participants were informed about the study's protocols and objectives before engaging in the sensory evaluation.

1.9 Statistical Analysis

For the statistical study, we used version 21 of the Statistical Package for the Social Sciences (SPSS). Various characteristics, including total phenolics, antioxidant capacity, anti-glycation impact, enzyme inhibition (alpha-amylase, alpha-glucosidase, and lipase), as well as sensory acceptability were examined. We performed a statistical examination utilizing a one-way analysis of variance (ANOVA) and a least significant difference (LSD) post-hoc test to assess the effect of bread infused with Caralluma. All data analysis methods were subjected to a significance cutoff of $P < 0.05$.

III. RESULTS AND DISCUSSION

The purpose of this study was to evaluate the effects of incorporating powdered caralluma extract into wheat flour bread on various parameters. The study examined several criteria, including the measurement of total phenolics, antioxidant activity via DPPH and ABTS tests, inhibition of pancreatic lipase, alpha-amylase, and alpha-glucosidase, prevention of advanced glycation end product (AGE) formation, and sensory acceptability. The bread samples were categorized into four groups based on the amounts of caralluma extract powder used as raw materials: 100% wheat flour bread with CEP levels of 2%, 4%, and 6%.

2.1 Phenolic content analysis in raw ingredients and bread samples

Both raw ingredients and bread samples were analyzed for total polyphenols and expressed in milligrams of gallic acid equivalent per 100 grams (mg GAE/100g). Gallic acid served as the standard for this measurement and the standard curve for Gallic acid concentration (mg) is illustrated in Appendix-I. In the analysis, it was found that caralluma extract powder contained a significantly higher ($p < 0.05$) total phenolic content (284.75 mg GAE/100g) compared to wheat flour (50.10 mg GAE/100g). Among the bread samples, the one containing 6% caralluma extract powder (147.15 mg GAE/100g) exhibited significantly higher total phenolic levels compared to the control bread (100% WF) (36.12 mg GAE/100g). This observation suggests that the total phenolic content of composite bread increased with the addition of caralluma extract powder. Such results are consistent with expectations due to the presence of phytochemicals in caralluma.

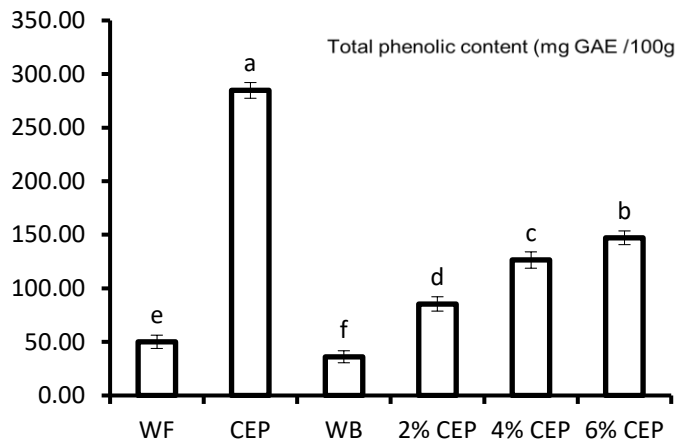


Figure 1. Total phenolic contents of raw materials and bread samples (dry basis). Values are means \pm SD of quadruplicate analyses. WF: wheat flour, CEP: caralluma extract powder, WB: white bread.

2.2 Evaluation of the Antioxidant Potential in Raw Ingredients and Bread Sample

The antioxidant properties of both the raw constituents and the composite breads were assessed using the ABTS and DPPH methods and the results are expressed in micromoles of Trolox equivalent per 100 grams ($\mu\text{mol TE}/100\text{g}$) as shown in table 2. Trolox served as the standard for these measurements and the standard curve for Trolox concentration (μmol) for the ABTS and DPPH assays is provided in Appendix-II and Appendix-III, respectively. In the ABTS scavenging activity analysis, caralluma extract powder exhibited significantly higher antioxidant activity ($291.31 \pm 6.41 \mu\text{mol TE}/100\text{g}$) after four different time intervals. Similarly, in the DPPH scavenging activity analysis, caralluma extract powder displayed significantly higher antioxidant activity ($356.33 \pm 10.19 \mu\text{mol TE}/100\text{g}$) in comparison to wheat flour ($86.17 \pm 7.72 \mu\text{mol TE}/100\text{g}$). Furthermore, the bread samples containing caralluma extract powder exhibited significantly higher antioxidant activity compared to the control bread. These findings suggest that the incorporation of caralluma extract powder into bread significantly enhances its antioxidant properties, potentially contributing to its health-promoting effects.

Table 2. Antioxidant activity of raw materials and bread samples (dry basis) *

Sample	ABTS ($\mu\text{mol TE}/100\text{g}$)	DPPH ($\mu\text{mol TE}/100\text{g}$)
WF	44.42 ± 4.63^e	86.17 ± 7.72^e
CEP	291.31 ± 6.41^a	356.33 ± 10.19^a
WB	23.21 ± 4.16^f	67.51 ± 9.31^f
2% CEP	79.53 ± 5.39^d	101.41 ± 10.35^d
4% CEP	95.04 ± 4.76^c	137.44 ± 9.33^c
6% CEP	132.81 ± 5.14^b	172.36 ± 9.41^b

*Values are means \pm SD of triplicate analyses. Means in the same columns with different letters are significantly different ($P < 0.05$, LSD test). WF: wheat flour, CEP: caralluma extract powder, WB: white bread.

2.3 The Suppression of Sample on Advanced Glycation End Product Formation

The impact of both raw materials and wheat bread enriched with caralluma extract powder on the generation of advanced glycation end products (AGEs) as illustrated Figure 2. The inhibitory effect of caralluma extract powder (49.43%) was notably greater ($p < 0.05$) in comparison to wheat flour (14.23%), underscoring the significant AGEs inhibitory potential of caralluma extract powder. Moreover, bread containing caralluma extract powder demonstrated a significantly superior ($p < 0.05$) ability to inhibit AGEs production when compared to the control bread. In all samples, most pronounced inhibition effect observed in the bread which contained the highest level caralluma extract, exhibiting inhibition rates ranging from 20.46% to 32.24% for incorporation levels of 2% CEP, 4% CEP, and 6% CEP, respectively.

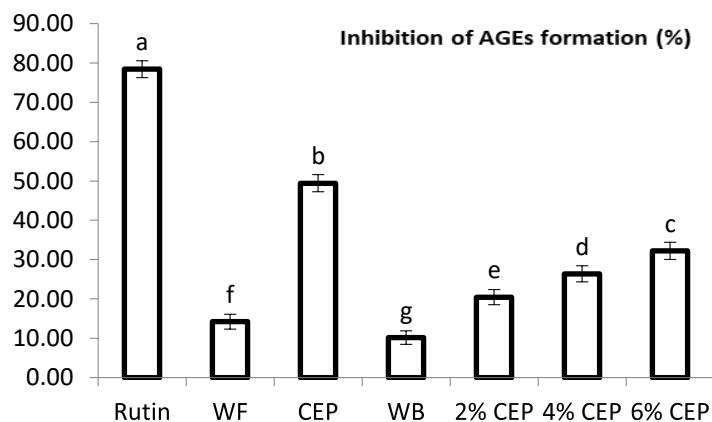


Figure 2. Inhibitory effects of raw materials and bread samples on the formation of AGEs in BSA-glucose model. Values are means \pm SD of triplicate analyses. WF: wheat flour, CEP: caralluma extract powder, WB: white bread.

2.4 Inhibition of α -Amylase Activity in Raw Ingredients and Bread Samples

The inhibitory activity against the α -amylase enzyme expressed as a percentage for both the raw materials and various bread samples as displayed in Figure 3. Notably, bread containing caralluma extract powder exhibited a significantly higher inhibitory effect on α -amylase compared to the control group ($p < 0.05$). Among all the bread samples tested, the greatest inhibitory effect was observed in the bread with higher levels of caralluma extract powder, showing inhibitions ranging from 6.26% to 17.24% for incorporation levels of 2% CEP, 4% CEP, and 6% CEP, respectively.

These findings align with existing literature, such as a study conducted by Chester *et al.* in 2017. In their research, it was reported that *C. fimbriata* extract displayed a noteworthy dose-dependent inhibition of glucose metabolism enzymes. They observed an alpha-glucosidase inhibitory effect of 83.05% at 1000

g/mL and an alpha-amylase inhibitory effect of 77.37% at 1000 g/mL for *C. fimbriata* extract. This study provides further support for the idea that caralluma extract, similar to *C. fimbriata*, possesses beneficial antihyperglycemic properties, which can be attributed to its ability to inhibit enzymes involved in glucose metabolism.

Some in-vitro evidences were also revealed in the study conducted by (Bellamakondi *et al.* 2014) the study was designed to assess Caralluma, a traditional medicinal herb, for its anti-hyperglycemic properties using in vitro tests. Caralluma extracts in methanolic, aqueous and hydro methanolic forms were created and their anti-hyperglycemic action was investigated. In vitro tests on the extracts' ability to absorb glucose in L6 myotubes were conducted. Also tested was the inhibitory efficacy against pancreatic lipase and alpha amylase. It was discovered that the methanolic extract (MCU) significantly absorbed glucose. MCU demonstrated a notable improvement in glucose absorption among the extracts studied, with 662.58% uptake over baseline control.

Other extracts, including water (ACU) and methanol: water (60:40) (HCU), demonstrated modest increases in glucose absorption (8.21.49 and 16.31.3, respectively); as a result, MCU was further separated to yield four fractions. Chloroform (CHCU) showed a large uptake with 562.40% uptake over baseline control while the other fractions showed modest activity. Fractions of MCU also showed an increase in glucose absorption. The powerful extract MCU was assessed in the following series of studies to determine its mode of action and the tests were carried out both with and without wortmannin. The absorption of glucose by MCU was shown to be lower in the presence of wortmannin, at $39 \pm 2.09\%$ over basal control, than it was in the absence of wortmannin, at $58 \pm 7.97\%$. Additionally, insulin uptake was decreased when wortmannin was present. Additionally, it was discovered that MCU had a promising effect on alpha-amylase inhibition.

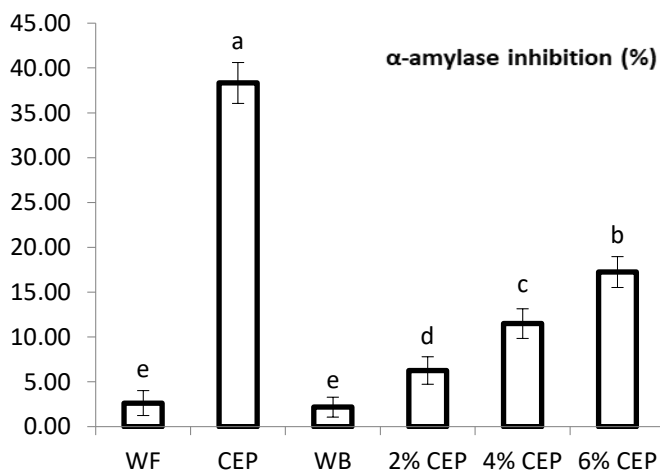


Figure 3. α -amylase inhibitory activity of raw materials and bread samples. Values are means \pm SD of triplicate analyses. WF: wheat flour, CEP: caralluma extract powder, WB: white bread.

2.5 Inhibition of α -glucosidase activity in both the raw materials and bread samples

The percentage of inhibitory activity against the α -glucosidase enzyme for both raw materials and bread samples as illustrates in Figure 4. Bread enriched with caralluma extract powder displayed a significantly elevated inhibitory effect on α -glucosidase inhibition ($p < 0.05$). Among all the bread samples examined, the highest level of inhibition was observed in those containing greater quantities of caralluma extract powder, exhibiting inhibitions ranging from 10.26% to 22.24% for inclusion levels of 2% CEP, 4% CEP, and 6% CEP, respectively. This increased inhibitory effect in these composite breads can be attributed to their elevated polyphenolic content.

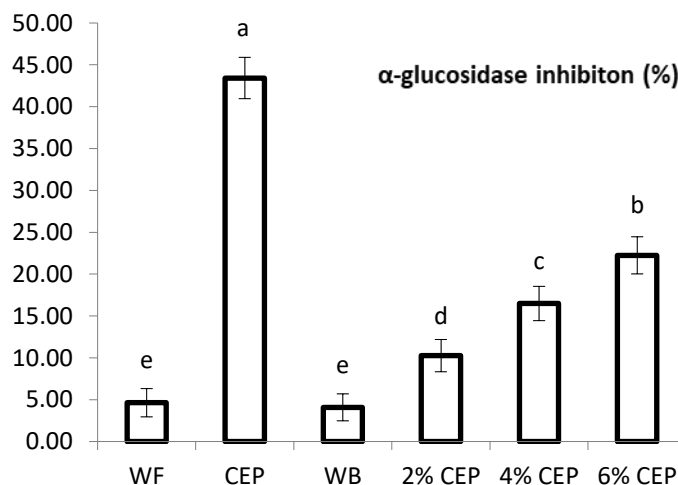


Figure 4. α -glucosidase inhibitory activity of raw materials and bread samples. Values are means \pm SD of triplicate analyses. WF: wheat flour, CEP: caralluma extract powder, WB: white bread

The study conducted by Ouassou *et al.* in 2018 produced similar findings. They aimed to determine the nature of enzyme inhibition by assessing enzyme kinetics with increasing concentrations of sucrose after investigating the inhibitory effect of caralluma on α -glucosidase activity in vitro. Additionally, they validated this effect in vivo using rats as test subjects. Among the five fractions of caralluma tested, only the ethyl acetate fraction significantly inhibited α -glucosidase and it was determined to be a competitive inhibitor. To establish the oral glucose tolerance test (OGTT), they conducted in vivo experiments using glucose and sucrose as substrates in mice and rats. The ethanolic and aqueous extracts of caralluma exhibited strong α -glucosidase inhibition at two different dosages compared to the control group. Notably, the ethyl acetate caralluma (EAC) at a dosage of 328 μ g/mL exhibited the most potent inhibitory action, with a 66% inhibition compared to acarbose's 69%. A kinetic analysis of caralluma's inhibition of α -glucosidase revealed that the rate of glucose release is highest in the absence of an inhibitor.

Presence of the ethyl acetate caralluma extract (EEC) at concentrations of 500 and 1000 g/mL led to a reduction in the rate of glucose release. In both normal and diabetic mice, the impact of the aqueous caralluma extract (AEC) on the oral glucose

tolerance test (OGTT) showed that in the control group, postprandial hyperglycemia resulting from a 1 g/kg glucose load reached 1.28 g/L 30 minutes after glucose administration, increased to 1.8 g/L after 90 minutes and then decreased to an average of 1.16 g/L at the 150-minute mark. In contrast to the normal control group, the administration of AEC at a dosage of 200 mg/kg significantly reduced postprandial hyperglycemia levels. Furthermore, glibenclamide had a significant ($p < 0.01$) effect on lowering blood glucose levels at 90 minutes. These findings demonstrate that both the caralluma ethanolic extract (EEC) and the caralluma aqueous extract (AEC) effectively reduced postprandial hyperglycemia following sucrose and glucose loading in both normal and diabetic rats. Additionally, AEC substantially decreased in situ intestinal glucose absorption. These results highlight caralluma's potent antihyperglycemic effects, which may be attributed to its ability to inhibit α -glucosidase activity and impede glucose absorption through the intestinal pathway.

A study conducted by Shalabi *et al.* (2020), researchers isolated three newly discovered pregnane glycosides and identified four previously known compounds from the methylene chloride fraction of Caralluma. The newly found compounds included 12,20-di-O-benzoyl-3 β ,8 β ,12 β ,14 β ,20-pentahydroxy-(20R)-pregn-5-ene-3-O- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-digitaloside, 3 β ,8 β ,14 β ,20-tetrahydroxy-(20R)-pregn-5-ene-3-O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-digitaloside-20-O- β -D-glucopyranoside, and 3 β ,8 β ,14 β ,20-tetrahydroxy-(20R)-pregn-5-ene-3-O- β -D-glucopyranosyl-(1 \rightarrow 6)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-digitaloside-20-O- β -D-glucopyranoside.

Furthermore, they identified known compounds, including luteolin 4'-O-neohesperidoside, apigenin-8-C-neohesperoside, β -sitosterol, and β -sitosterol glucoside. The study employed a bio-guided fractionation approach, assessing various Caralluma extracts (water, methanol, and methylene chloride) using in vitro inhibitory assays targeting α -glucosidase and lipase enzymes.

Most potent component was the methylene chloride fraction, displaying significant inhibitory effects on α -glucosidase and lipase enzymes, with inhibition rates of 94.66% and 48.82%, respectively. To isolate seven distinct chemical compounds, a bioassay-guided isolation process was applied to the methylene chloride fraction. These compounds encompassed two well-established flavone glycosides, three novel pregnane glycosides (labeled as 1-3) and two familiar sterols. The IC₅₀ values for the overall extracts, various fractions and the isolated compounds derived from Caralluma against α -glucosidase and lipase enzymes were 2.66 ± 0.22 mg/mL and 27.13 ± 2.23 at a concentration of 100 g/mL, respectively. Conversely, the aqueous extract exhibited comparatively lower inhibitory effects on both enzymes, with inhibition rates of $28.44 \pm 3.25\%$ for α -glucosidase and $4.67 \pm 1.98\%$ for lipase at the same concentrations.

The methylene chloride fraction obtained from the methanolic extract displayed the most potent inhibitory activity against α -glucosidase and lipase enzymes. Interestingly, the fractions eluted from the diaion column showed minimal to negligible inhibitory activity against either enzyme. The isolated compounds exhibited varying degrees of inhibition against alpha-glucosidase and lipase enzymes, with 50% inhibition percentages ranging from $3.62 \pm 3.19\%$ to $55.18 \pm 0.13\%$ at a concentration of

1 mM and from $3.62 \pm 3.47\%$ to $63.70 \pm 2.60\%$ at a concentration of 100 μ M. Compound 1 demonstrated the highest inhibitory activity among the isolated compounds, while compounds 2 and 3 exhibited the weakest inhibition, possibly due to the presence of acyl groups in compound 1. Initial evaluations of the crude methanolic extract and the methylene chloride fraction revealed inhibitory effects against both α -glucosidase and pancreatic lipase enzymes. Notably, compound 5 demonstrated the most potent inhibition of α -glucosidase, boasting an IC₅₀ value of 0.82 ± 2.50 μ M, whereas acarbose had an IC₅₀ value of 0.81 ± 0.86 μ M, for comparison. Conversely, compound 1 exhibited the highest inhibitory activity against pancreatic lipase, with an IC₅₀ value of 23.59 ± 2.49 μ M, while orlistat had an IC₅₀ value of 7.41 ± 2.26 μ M, for reference.

These findings from the current study underscore the superior inhibitory efficacy of the methylene chloride fraction compared to diaion eluted fractions against both α -glucosidase and pancreatic lipase enzymes. It is suggested that Caralluma, by hindering the digestion and absorption of dietary fats and carbohydrates, could potentially offer a safe complementary approach for the treatment and management of obesity.

2.6 Inhibition of pancreatic lipase activity in both the raw materials and bread samples

The inhibitory activity against the pancreatic lipase enzyme, expressed as a percentage, for both raw materials and different bread samples as illustrated in Figure 5. Significantly, bread containing Caralluma extract powder exhibited a notably stronger inhibitory effect on pancreatic lipase inhibition ($p < 0.05$). All the samples of bread which were tested, most prominent inhibitory effect was observed in that bread which contained high levels of Caralluma extract, the inhibition percentages were ranging from 8.26% to 19.24% for incorporation levels of 2% CEP, 4% CEP, and 6% CEP, respectively.

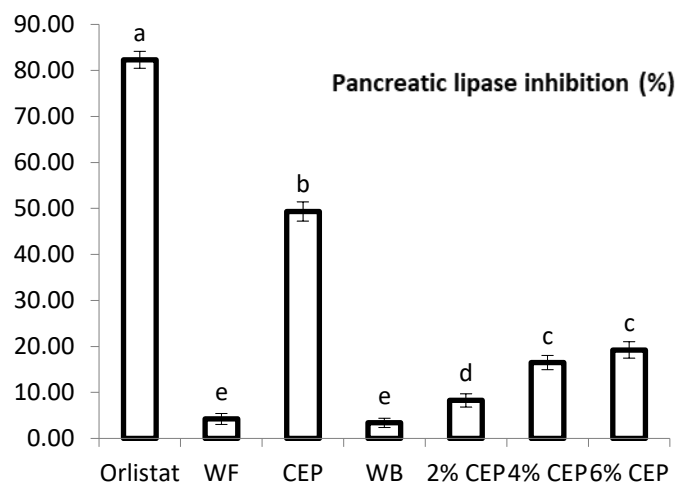


Figure 5. Pancreatic lipase inhibitory activity of raw materials and bread samples. Values are means \pm SD of triplicate analyses. WF: wheat flour, CEP: caralluma extract powder, WB: white bread.

Bellamakondi *et al.* (2014) reviewed that the Caralluma methanolic extract (MEC) was discovered to have a promising effect in alpha amylase and pancreatic lipase inhibition. The activity of the porcine pancreatic lipase was assessed using the substrate p-nitrophenyl butyrate (p-NPB). Aqueous, Methanol: Water (60:40) and Methanol: Caralluma (MEC, AEC and HEC) extracts were each incubated with PPL and then p-NPB to ascertain their inhibitory efficacy. By comparing test samples to the control reaction without the test sample, the percentage of PPL residual activity was calculated for the test samples. As a positive control, orlistat was utilized. The logarithmic regression analysis was used to determine IC50 values. Methanol: water (60:40) (HEC) shown greater efficacy against pancreatic lipase, with an IC50 of >1000 g/ml, compared to conventional orlistat's IC50 of 0.15 ± 0.03 g/ml.

The findings demonstrated that suppressing pancreatic lipase leads in less monoglyceride and fatty acid absorption. Therefore, it is discovered that pancreatic lipase inhibition is a useful target for the treatment of diet-induced hyperglycemia in humans, highlighting its useful role in treating obesity-related problems associated with diabetes. It's interesting to note that in vitro, MEC also inhibited pancreatic lipase and alpha amylase, both in a dose-dependent way. Thus, the polyphenols contained in the extracts may be the cause of the inhibitory action that was reported. These findings suggest that MEC has the capacity to function at many locations, including increasing glucose absorption and suppressing pancreatic lipase and alpha amylase.

2.7 Evaluation of the sensory acceptability of bread samples, including both regular white bread and bread containing caralluma extract powder

The sensory analysis results of bread samples containing varying levels (2% CEP, 4% CEP, 6% CEP) of Caralluma extract powder, along with a control bread sample made entirely from wheat flour as presented in Table 3. In this sensory evaluation, 50 subjects were invited through flyers and posters to assess the white bread and CEP-containing bread samples. All participants provided their consent (Appendix-IV) before participating in the bread sample testing. The samples were evaluated based on sensory attributes such as color, texture, flavor and overall acceptability. It's worth noting that the overall acceptability scores for all composite bread types were significantly lower compared to the control bread. However, Appendix-V illustrates the palatability questionnaire used in the study. Despite the lower scores, the bread samples containing Caralluma extract powder still achieved a minimal mean overall acceptance score of 6, which denotes their sensory attribute acceptability.

The results showed that the CF-treated samples received the highest scores throughout the holding period and were favored by the panelists. In contrast, the control and raw potato French fry samples were rated the least favorable. Those samples which were treated with Caralluma maintained their crispiness and a significant difference was observed with longer holding times. This suggests that natural antioxidants present in CF may have an impact on the taste and texture of the samples after treatment, contributing to the prevention of lipid oxidation and enhancing sensory attributes.

Table 3. Assessment of the sensory attributes of bread samples, comparing white bread with bread samples containing Caralluma extract powder (CEP) (n = 50) *

Sample	Colour	Texture	Flavor	Overall acceptability
WB	8.12 ± 2.06^a	8.31 ± 2.12^a	7.94 ± 2.08^a	8.33 ± 2.22^a
2% CEP	7.05 ± 1.97^b	7.14 ± 2.23^b	7.41 ± 2.02^b	7.31 ± 2.11^b
4% CEP	6.51 ± 2.29^c	6.35 ± 1.94^c	6.28 ± 2.26^c	6.44 ± 2.33^c
6% CEP	6.11 ± 2.21^d	5.91 ± 2.03^d	6.15 ± 2.01^c	6.27 ± 1.98^c

Values are means \pm SD. Means in the same columns with different letters are significantly different ($P < 0.05$, LSD test). CEP: caralluma extract powder, WB: white bread. *Data collected on a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely).

IV. CONCLUSION AND RECOMMENDATIONS

Conclusion

Bread containing caralluma extract powder demonstrated significantly elevated levels of polyphenolics and antioxidant capacity across all incorporation levels, surpassing those of the control bread.

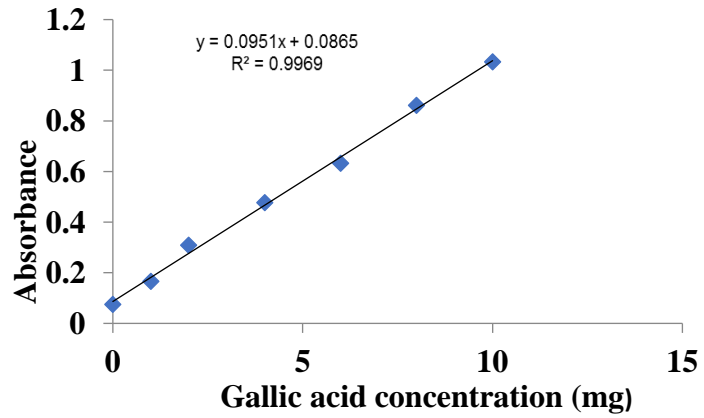
- 1). Composite bread with varying levels of caralluma extract powder displayed enhanced inhibitory effects against AGEs, α -amylase, α -glucosidase and pancreatic lipase. This inhibitory potential increased with higher levels of caralluma extract powder in the bread.
- 2). The sensory assessment of composite bread containing caralluma extract powder at all degrees of inclusion resulted in average 6 out of 9 on the hedonic acceptability scale. It means that overall, the research participants enjoyed and found the bread to be satisfactory.

Recommendation

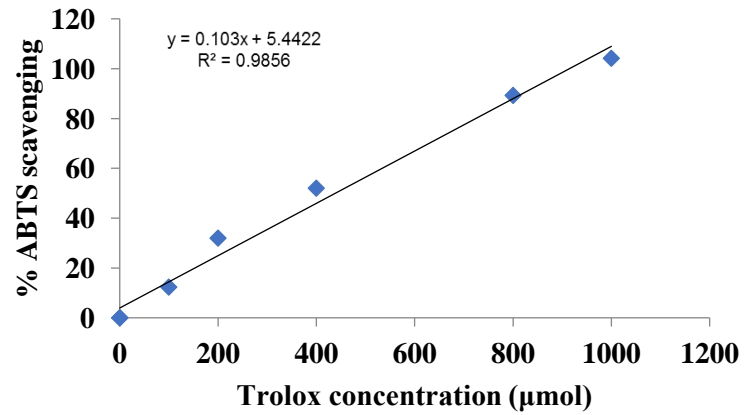
- 1). Conduct additional research to assess starch digestibility, protein digestibility and fiber content in bread containing caralluma extract powder. These factors can provide valuable insights into the bread's nutritional profile and potential health benefits.
- 2). Investigate individual phenolic compounds present in caralluma extract powder, such as flavonoids, glycosides and triterpenoids, to gain a deeper understanding of the specific bioactive constituents contributing to its health-promoting properties.
- 3). Evaluate the mineral content of composite bread. Analyzing the mineral composition can provide insights into the bread's nutritional value and its potential as a mineral source.
- 4). Conduct further studies to assess the clinical efficacy of caralluma extract powder-containing bread on biomarkers associated with metabolic syndrome. This research can help establish the potential therapeutic benefits of this bread in managing metabolic disorders.

APPENDICES

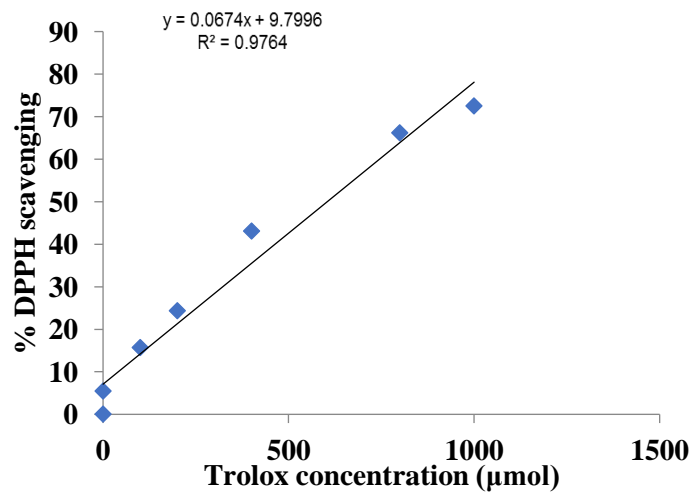
Appendix – I Standard curve for Gallic acid concentration (mg)



Appendix – II Standard curve for (ABTS) Trolox concentration (µmol)



Appendix – III Standard curve for (DPPH) Trolox concentration (µmol)



LITERATURE CITED

Apostolidis, E., Y.-I. Kwon, K. Shetty, E. Apostolidis and Y.-I. Kwon. 2006. Potential of cranberry-based herbal synergies for diabetes and hypertension management. *Asia Pacific Journal of Clinical Nutrition* 15.

Asmi, S., T. Lakshmi and R. Parameswari. 2017. *Caralluma fimbriata*-Pharmacological review.

Bellamakondi, P.K., A. Godavathi & M. Ibrahim, 2014. Anti-hyperglycemic activity of *Caralluma umbellata* haw, *BioImpacts* 4(3), 113–16.

Chester, K., S. Zahiruddin, A. Ahmad, W. Khan, S. Paliwal & S. Ahmad, 2017. Bioautography-based Identification of Antioxidant Metabolites of *Solanum nigrum* L. and Exploration Its Hepatoprotective Potential ag Chester, K. (2017) 'Bioautography-based Identification of Antioxidant Metabolites of *Solanum nigrum* L. and Explorati, *Pharmacognosy Magazine* 13 (Suppl (62), 179–88.

Chopra, A. and V.V. Doiphode. 2002. Ayurvedic medicine: core concept, therapeutic principles and current relevance. *Medical Clinics* 86: 75-89.

Godavari, A., K. Amutha and N.M. Moorthi. 2018. In-vitro hypoglycemic effect of *Foeniculum vulgare* Mill. Seeds on the carbohydrate hydrolysing enzymes, alpha-amylase and alpha-glucosidase. *International Journal of Pharmaceutical Sciences and Research* 9: 4441-4445.

Gujjala, S., M. Putakala, R. Ramaswamy and S. Desireddy. 2016. Preventive effect of *Caralluma fimbriata* vs. Metformin against high-fat diet-induced alterations in lipid metabolism in Wistar rats. *Biomedicine & Pharmacotherapy* 84: 215-223.

Gupta, C. and D. Prakash. 2014. Phytonutrients as therapeutic agents. *Journal of Complementary and Integrative Medicine* 11: 151-169.

Leitzmann, C. 2016. Characteristics and health benefits of phytochemicals. *Complementary Medicine Research* 23: 69-74.

Maurya, R., G. Singh and P.P. Yadav. 2008. Antiosteoporotic agents from natural sources. (eds.), *Studies in Natural Products Chemistry: Elsevier*, pp. 517-548.

Oladeji, O. 2016. The Characteristics and Roles of Medicinal Plants: Some Important Medicinal Plants in Nigeria. *Natural Products: An Indian Journal* 12: 102.

Ouassou, H., T. Zahidi, S. Bouknana, M. Bouhrim, H. Mekhfi, A. Ziyat, A. Legssyer, M. Aziz & M. Bnouham, 2018. Inhibition of α -Glucosidase, Intestinal Glucose Absorption and Antidiabetic Properties by *Caralluma europaea*, *Evidence-based Complementary and Alternative Medicine* 2018.

Priya, D., K. Rajaram and P. Suresh Kumar. 2011. Phytochemical studies and GC-MS analysis of *Caralluma fimbriata* wall. *International journal of Pharmaceutical Research and development* 3: 105-110.

Qayyum, N., H. Rani, K.B. Mir and A.Q. Khan. 2018. *Caralluma* Pharmacological Attributes. *Journal of Food* 2: 13.

Shalabi, A.A., A.M. El Halawany, M.A. Choucry, F.S. El-Sakhawy, H. Morita, D.W. Ki & E. Abdel-Sattar, 2020. New pregnane glycosides from *Caralluma hexagona* Lavranos and their in vitro α -glucosidase and pancreatic lipase inhibitory effects, *Phytochemistry Letters* 36(January), 49–57.

Szawara-Nowak, D., G. Koutsidis, W. Wiczowski and H. Zieliński. 2014. Evaluation of the in vitro inhibitory effects of buckwheat enhanced wheat bread extracts on the formation of advanced glycation end-products (AGEs). *LWT-Food Science and Technology* 58: 327-334.

Worsztynowicz, P., M. Napierała, W. Białas, W. Grajek and M. Olkowicz. 2014. Pancreatic α -amylase and lipase inhibitory activity of polyphenolic compounds present in the extract of black chokeberry (*Aronia melanocarpa* L.). *Process Biochemistry* 49: 1457-1463.

AUTHORS

First Author – Aimal Khan, M.Sc. (Hons.) Human Nutrition, The University of Agriculture, Peshawar-Pakistan.

Second Author – Imran Khan, Ph.D. Human Nutrition, The University of Agriculture, Peshawar-Pakistan.

Third Author – Shah Fahad, B.Sc. (Hons.) Human Nutrition, The University of Agriculture, Peshawar-Pakistan.

Fourth Author – Sabina Khan, M.Sc. (Hons.) Human Nutrition, The University of Agriculture, Peshawar-Pakistan.

Fifth Author – Muhammad Onais Inam, M.Sc. (Hons.) Human Nutrition, The University of Agriculture, Peshawar-Pakistan.

Sixth Author – Shehar Yar Shafi, B.Sc. (Hons.) Human Nutrition, The University of Agriculture, Peshawar-Pakistan.

Seventh Author – Malik Abdul Haseeb, B.Sc. (Hons.) Human Nutrition, The University of Agriculture, Peshawar-Pakistan.