World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Caralluma-enhanced bread: A novel approach to combat advanced glycation end products and metabolic syndrome-related enzymes

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World Journal of Biology Pharmacy and Health Sciences, 2025, 21(01), 001-012

Publication history: Received on 24 November 2024; revised on 30 November 2024; accepted on 01 January 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.21.1.1111

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 enhanced with caralluma extract powder (CEP). Different proportions of CEP (2%, 4%, and 6%) were substituted for 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 gauged 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 µmol TE/100g, DPPH 172.36 µmol TE/100g) compared to the control bread. CEP-enriched 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.

Keywords: Caralluma Extract Powder (CEP); Functional Bread; Antiglycation; Metabolic Syndrome Enzymes; Polyphenols and Antioxidants; Sensory Evaluation

1. 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

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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 [1]. 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 [2]. 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 [3].

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 [4]. 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 [5,6]. Medicinal plants find applications in the production of modern medications, both through direct and indirect means [7].

Caralluma (Caralluma fimbriata) stands out as a valuable medicinal plant. This edible succulent cactus belongs to the Asclepiadaceae family, as documented by [8]. 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 [9]. 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 [10].

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 [11]. 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 [12]. 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 [13]. 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 [14]. 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.

Bread is a widely consumed staple food in various forms. Its primary ingredient, wheat, is recognized for its rich energy content and nutritional value. However, when compared to other grains and pseudocereals, wheat falls short in terms of its overall nutritional and functional qualities. Regularly including white bread in one's daily diet may increase the risk of developing metabolic conditions that is type II diabetes, heart conditions, and high blood pressure. It is possible to add various grains and medicinal herbs to white bread and other wheat-based foods to increase their nutritional benefits. This study is focused on incorporating caralluma extract powder into bread to augment its nutritional content. Consequently, this research aims to evaluate the impact of bread enriched with caralluma extract powder on total polyphenolic content, antioxidant properties, antiglycation effects, inhibition of α-amylase, α-glucosidase and lipase enzymes and its acceptable sensory quality.

2. Material and methods

2.1. Study Location

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

2.2. 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.

2.3. 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 5 g of oil and 1 g of salt were added and mixed thoroughly. Yeast activation was performed by taking 60ml of lukewarm water in a separate clean glass. To this, 1 g of yeast and 6 g 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 (/100 g flour).

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

CEP: caralluma extract powder

2.4. 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.

2.5. Milling of bread

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

2.6. 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.

2.7. 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 [15]. 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.

2.8. Total Phenol determination

Total phenolic content was examined using the colorimetric method involving the Folin-Ciocalteu reagent, as developed by [16]. 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.

2.9. 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 [17] 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 persulphate 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 persulphate 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.

2.10. Assessing antioxidant activity via the DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay, as described by [18], 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.

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

2.11. 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 [19]. 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.

2.12. 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 [20]. 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 (NaN_3) 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.

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

2.13. Assessment of α-Amylase inhibitory activity

The alpha-amylase inhibition assay was conducted following the method outlined by [21]. 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.

$$\%\text{Inhibition} = (\text{A540 control} - \text{A540 extract} / \text{A540 control}) * 100$$

2.14. Assessment of α-Glucosidase inhibitory activity

The Alpha-glucosidase inhibition test as reported by [22] 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.

$$\%\text{Inhibition} = (\text{A540 control} - \text{A540 extract} / \text{A540 control}) * 100$$

2.15. Assessment of inhibition of Pancreatic lipase activity

The determination of lipase inhibitory activity was conducted using a spectroscopic method with adjustments inspired by the procedure outlined by [23]. 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.

2.16. 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 was 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.

2.17. Statistical Analysis

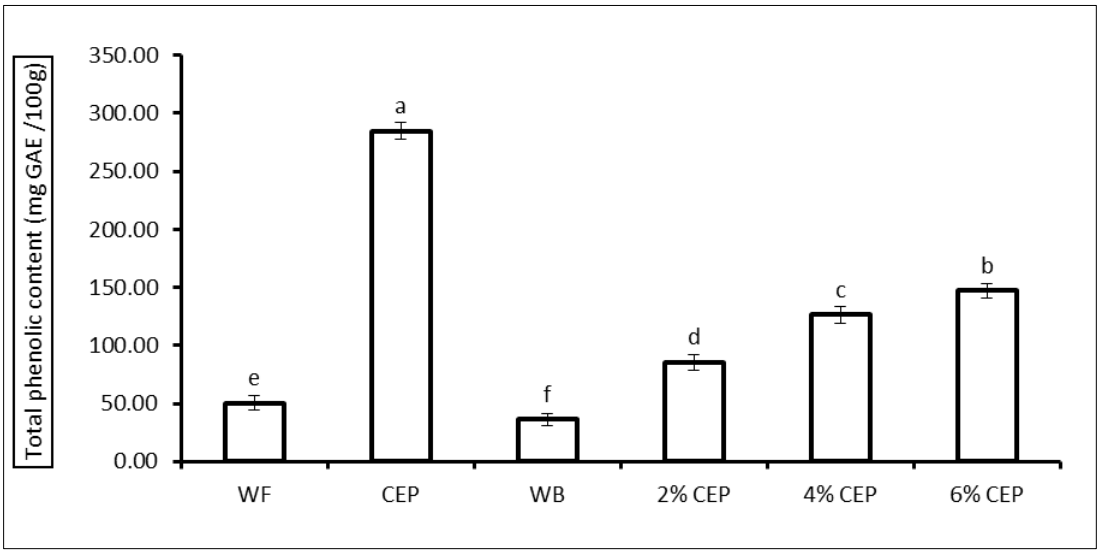
For the statistical study, we used version 21 of the Statistical Package for the Social Sciences (SPSS). Various characteristics, including total phenolics, antioxidant capacity, anti-glycation impact, enzyme inhibition (alpha-amylase, alpha-glucosidase, and lipase), as well as sensory acceptability 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 $P < 0.05$.

3. 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%.

3.1. 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 Figure-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.



Values are means ± SD of quadruplicate analyses. WF: wheat flour, CEP: caralluma extract powder, WB: white bread.

Figure 1 Total phenolic contents of raw materials and bread samples (dry basis)

3.2. 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 (µmol TE/100g) 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 Figure-II and Figure-Ⅲ, respectively. In the ABTS scavenging activity analysis, caralluma extract powder exhibited significantly higher antioxidant activity (291.31 ± 6.41 µmol TE/100g) after four different time intervals. Similarly, in the DPPH scavenging activity analysis, caralluma extract powder displayed significantly higher antioxidant activity (356.33 ± 10.19 µmol TE/100g) in comparison to wheat flour (86.17 ± 7.72 µmol TE/100g). 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.

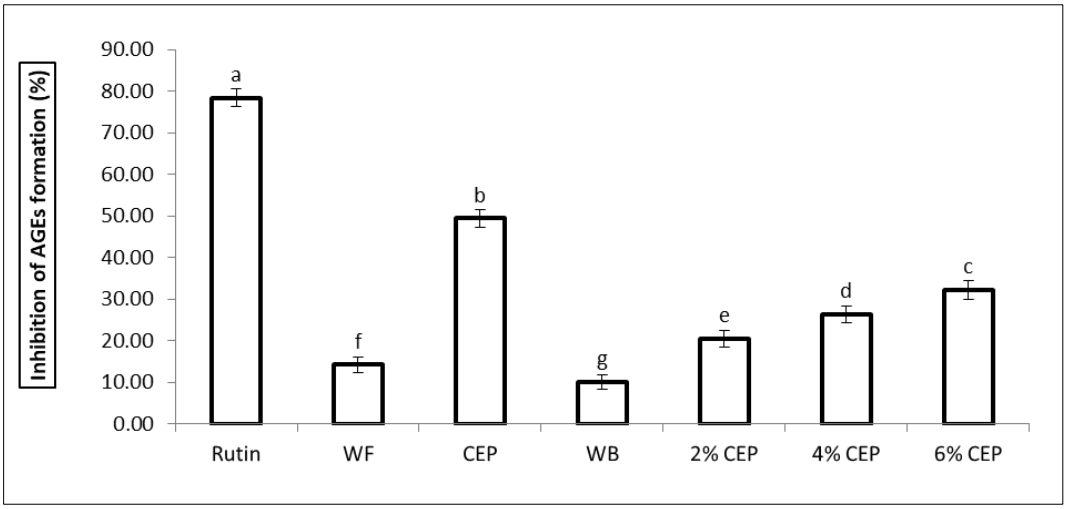
Table 2 Antioxidant activity of raw materials and bread samples (dry basis) *

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

*Values are means ± 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.

3.3. 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.

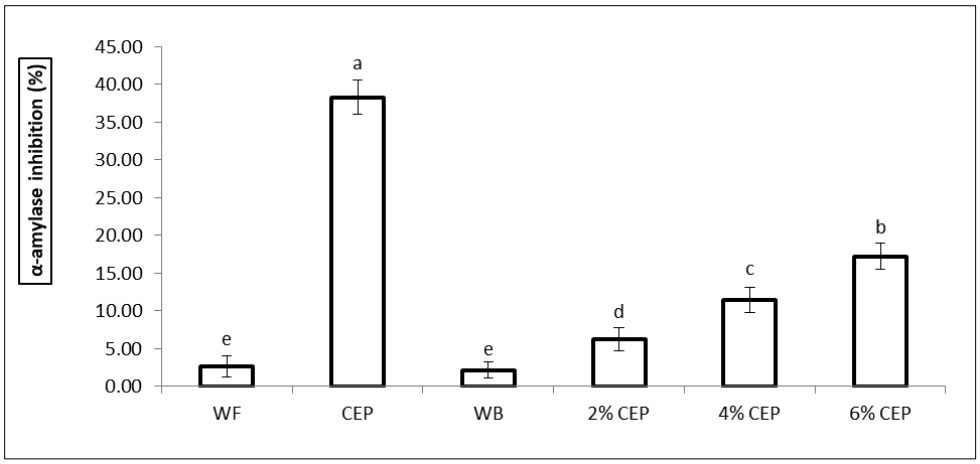


Values are means ± SD of quadruplicate analyses. WF: wheat flour, CEP: caralluma extract powder, WB: white bread.

Figure 2 Inhibitory effects of raw materials and bread samples on the formation of AGEs in BSA-glucose model

3.4. 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.

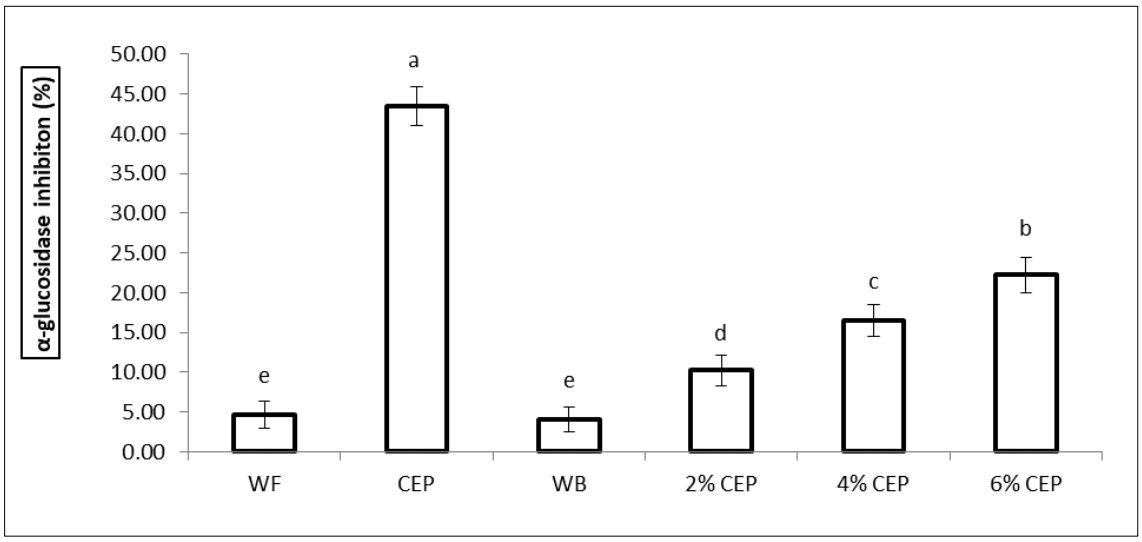


WF: wheat flour, CEP: caralluma extract powder, WB: white bread.

Figure 3 α- amylase inhibitory activity of raw materials and bread samples.Values are means ± SD of quadruplicate analyses

3.5. 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.

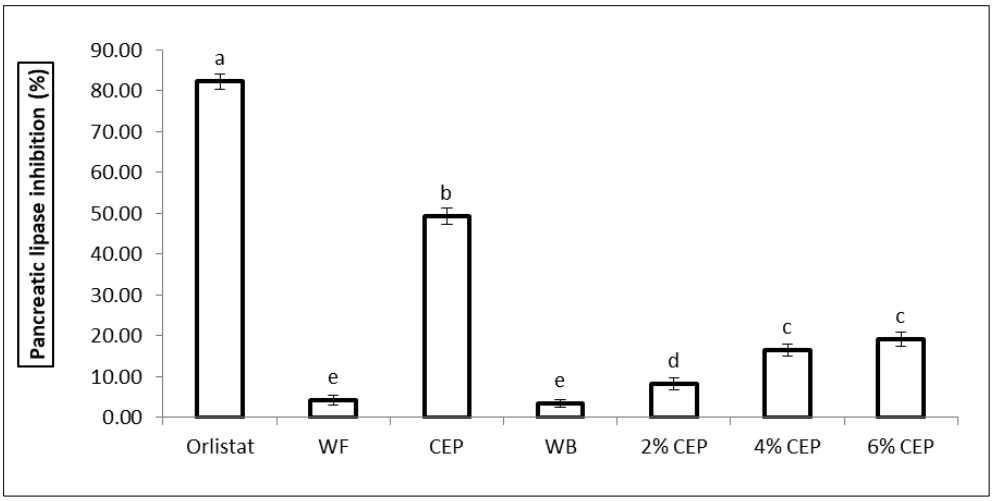


Values are means ± SD of triplicate analyses. WF: wheat flour, CEP: caralluma extract powder, WB: white bread.

Figure 4 α- glucosidase inhibitory activity of raw materials and bread samples

3.6. 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.



Values are means ± SD of triplicate analyses. WF: wheat flour, CEP: caralluma extract powder, WB: white bread.

Figure 5 Pancreatic lipase inhibitory activity of raw materials and bread samples

3.7. Evaluation of the sensory acceptability of bread samples

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 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. 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.

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

Values are means ± 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).

4. Conclusion

This study demonstrated that bread enriched with Caralluma extract powder (CEP) significantly improved its phytochemical properties, antioxidant capacity, and inhibitory potential against advanced glycation end products (AGEs) and enzymes linked to metabolic syndrome, including α-amylase, α-glucosidase, and pancreatic lipase. Sensory evaluation confirmed the palatability of CEP-incorporated bread, making it a viable functional food option. These findings highlight the potential of CEP-enriched bread to contribute to the prevention and management of metabolic disorders, benefiting public health. Future research should focus on optimizing CEP incorporation levels for maximum efficacy and broader consumer acceptance.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared no conflict of interests.

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Appendices

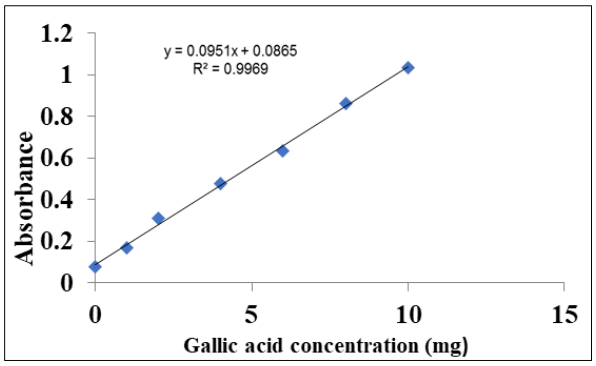

Figure - I Standard curve for Gallic acid concentration (mg)

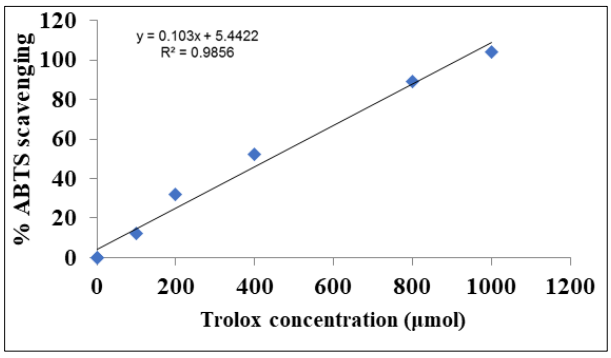

Figure – II Standard curve for (ABTS) Trolox concentration (µmol)

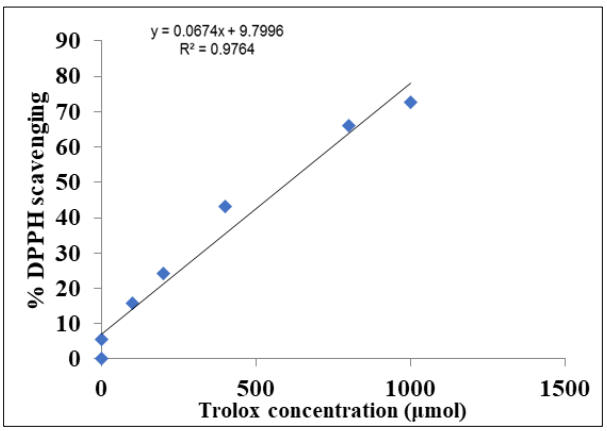

Figure – III Standard curve for (DPPH) Trolox concentration (µmol)