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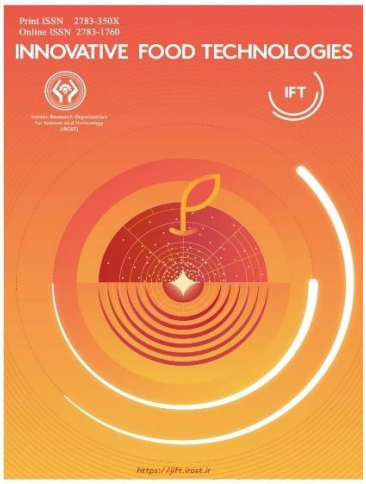

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Development of cookies using the under-utilized fruit *Carissa carandas*: Evaluation of sensory profile, nutritional value, and product safety

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Running title: Nutritional potential of karonda fruit

ABSTRACT
Carissa carandas, commonly known as karonda, is an underutilized fruit that is rich in nutrients but has not yet been fully explored as a ready-to-eat food product. This study aimed to develop cookies fortified with karonda fruit and also to evaluate their nutritional, sensory, phytochemical, and microbial quality. Cookies were prepared with varying levels of karonda incorporation: 0%, 10%, 20%, and 30%. Among these formulations, the 20% karonda cookies demonstrated the best overall acceptability. Cookies made with karonda fruit displayed a lower total bacterial count (0.02 vs. 0.13 CFU/g), reduced lipid peroxidation levels (2.23 vs. 3.58 nM TBARS), and decreased protein carbonyl content (1.09 vs. 1.57 nM) compared to control cookies because of antimicrobial and antioxidant compounds present in *Carissa carandas* ingredient. Overall, cookies containing 20% karonda fruit not only offered enhanced nutritional benefits but also maintained acceptable sensory quality, highlighting the fruit's potential for use in functional bakery products.

Keywords: *Carissa carandas*, Karonda fruit, Cookies, Sensory value, Nutritional value, Microbial load, Oxidative end-products.

1. Introduction

Nature has generously provided us with a wide variety of nutritious fruits that are essential for a healthy life. Among the widely known fruits, there are many wild and underutilized species that still hold untapped nutritional potential. One such fruit is *Carissa carandas* L., commonly known as karonda in English (Arif et al., 2016) and also referred to as *kilakkai* in Tamil Nadu, India (Arora et al., 2023). Karonda is a thorny shrub that belongs to the Apocynaceae family (Mahajan et al., 2022; Singh et al., 2020). This fruit is native to several countries, including India, South Africa, Nepal, Afghanistan, Bangladesh, Malaysia, and Sri Lanka. In India, karonda can be found growing naturally in the plains of the Western Ghats and in the Himalayas, typically at elevations ranging from 300 to 1,800 meters (Mahajan et al., 2022; Arora et al., 2023).

Karonda fruit is seasonal and is harvested from July to November (Arora et al., 2023). It has a shelf life of 7 days due to its soft flesh and high moisture content, which is 77.99% (Shaheel et al., 2015; Nisha et al., 2025). The fruit can be eaten raw and is commonly used in households across India for making pickles and chutneys due to its sour taste. Additionally, Karonda fruit is widely utilized in the production of jelly, jam, and syrup (Arif et al., 2016). It has also been reported that karonda can be bioprocessed to produce alcoholic beverages (Arora et al., 2023). Karonda is considered a valuable fruit because of its impressive nutritional profile, which

includes sugars (7.35-11.58 g), vitamin C (51.27 mg), total acids (11 mg), total flavonoids (0.44 mg of quercetin equivalents), manganese (0.2 mg), iron (150 mg), phosphorus (66 mg), potassium (81.26 mg), zinc (3.26 mg), calcium (115 mg), and copper (1.92 mg) per 100 g of fruit (Arif et al., 2016; Arora et al., 2023). Furthermore, the fruit contains various phytochemicals, including β-sitosterol, carisol, linalool, carissic acid, carindone, α-amyrin, β-caryophyllene, carissone, lupeol, carinol, and ursolic acid (Azeez et al., 2016).

Fruit-based ingredients have been extensively studied for their potential to enhance the nutritional and sensory qualities of bakery products. A functional fruit snack made with figs and coated in sugar-free chocolate showed improved nutritional quality and sensory attributes (Yeganehzad et al., 2025). Similarly, cookies enriched with jackfruit rind powder and ascorbic acid demonstrated higher levels of dietary fiber and increased antioxidant activity (Ngo et al., 2024). The incorporation of red banana peel powder into cookies added nutritional value and functional properties, highlighting its potential as a sustainable ingredient (Aravind et al., 2025). Additionally, the use of freeze-dried Japanese quince further improved the antioxidant activity, sensory qualities, and volatile aroma profiles of cookies (Antoniewska et al., 2019). Likewise, adding banana by-products to gluten-free corn flour cookies enhanced their nutritional and functional characteristics, supporting the use of underutilized fruit resources in value-added food products (Pomasa et al., 2025). Amazonian palm fruit flour cookies have been assessed for their phenolic content and antioxidant activity (Santos et al., 2023). The addition of clarified grape juice, processed through microfiltration, along with freeze-dried grape pomace flour, has been found to enhance the functional and nutritional properties of gluten-free cookies (Jofre et al., 2024). Fortifying sorghum cookies with *Garcinia mangostana* peel extract improved their antioxidant and antidiabetic potential while maintaining desirable product quality (Indrianingsih et al., 2025). Additionally, incorporating pineapple pomace into cookies enhanced their physicochemical and textural properties, promoting sustainable by-product utilization (Jose et al., 2022). Furthermore, cookies made from optimized pumpkin flour blends showed improved nutritional quality and significant antidiabetic potential (Adelerin et al., 2024). However, no report available on the development of karonda fruit-based cookies till date.

Although karonda is highly nutritious, it is not widely known and remains under-utilized. This project aims to highlight the nutritional benefits of karonda fruit and improve its accessibility to the public by incorporating it into cookies. Cookies are popular among various groups, particularly toddlers and preschoolers, making them an ideal vehicle for introducing the nutritious karonda fruit. Therefore, the main objective of this project is to develop cookies made with *Carissa carandas* fruit and to analyze their sensory qualities, nutritional content, phytochemical properties, and shelf life.

2. Materials and methods

2.1. Collection of karonda fruit

Fruits of Karonda (*Carissa carandas* L.) were collected (300 g) from Nattarmangalam, Perambalur, Tamil Nadu, India during the month of July. The plant was identified and authenticated based on its morphological characteristics and taxonomical keys by Dr. Vadivel Vellingiri from SASTRA Deemed University, Thanjavur, India. The collected fruits were cut in half, and the seeds were removed. The weight of the pulp approximately 200 g was taken and

used to prepare a paste. The pulp was placed in a mixer jar and blended for 10 minutes to achieve a fine paste, which was then incorporated into the cookie formulation.

2.2. Cookies preparation

The cookies were prepared using three different concentrations of karonda paste: 10%, 20%, and 30% (Supplementary Figure 1). Control cookies were made without any addition of karonda paste. The dough for 1 kg of cookies consisted of 250 g of butter, 250 g of sugar, 500 g of maida, and approximately 10 g of baking powder (Hu et al., 2022). The prepared dough was divided into four equal portions of 250 g each and kneaded in a blender for about 5 minutes. For the control cookies, the 250 g of dough was used without any karonda paste. For the 10% sample, 25 g of dough was removed from a 250 g portion and replaced with 25 g of karonda paste. For the 20% and 30% samples, 50 g and 75 g of dough were removed from their respective portions and replaced with equivalent amounts of karonda paste. All four dough samples were placed in molds and baked in an oven at 180 °C for 12 minutes. The control and karonda-based cookies were then used for the sensory test and further nutritional and product safety studies.

2.3. Sensory analysis

Sensory analysis was conducted to evaluate the appearance, color, aroma, and texture of the cookie samples. A total of 15 sensory panelists, aged 20 to 22 years (both male and female), participated in the study (Nikpour et al., 2025). During the evaluation, the panelists received different cookie samples (control, 10%, 20%, and 30% karonda fruit cookies) and rated them on a 9-point scale, where a score of 9 indicated "very good," 5 indicated "moderate," and scores below 5 indicated "poor." We calculated the mean score for each sensory attribute across all four cookie formulations (0%, 10%, 20%, and 30% karonda cookies). By ranking the overall acceptability mean scores, we determined which concentration was most preferred by the panel. Based on the scores, cookies with 20% karonda received the highest mean score for acceptability. Therefore, only the control (0% karonda) and the 20% karonda cookies were used in further experiments.

2.4. Nutritional analysis

2.4.1. Moisture content

The moisture content was measured for both the control cookies and the cookies containing 20% karonda using the methodology outlined by Ahn et al. (2014). One gram of each cookie type was placed into a pre-weighed petri dish and dried in a hot air oven at 105°C for 6 hours. After drying, the samples were reweighed, and the moisture content was calculated based on the loss in weight.

2.4.2. Total carbohydrates

Total carbohydrate extraction was conducted following the methodology outlined by Devi et al. (2024) for both the control cookies and the 20% karonda cookies. A sample of 0.5 grams was placed in a conical flask, and 25 mL of 2.5 N HCl was added. The mixture was thoroughly mixed and boiled for one hour at 80 °C, then allowed to cool to room temperature. The contents were filtered, and approximately 15 mL of the filtrate was collected. The volume was measured and adjusted to 100 mL using distilled water. For estimation, 0.25 mL of the prepared solution was taken in a test tube and diluted to 2 mL with water. Then, 4 mL of 0.2% anthrone reagent was added and mixed gently. The blank sample showed no color change, while the test sample

developed a distinct green color. The contents were mixed thoroughly using a pipette, and the test tubes were placed in a water bath at 80 °C. After heating, the absorbance was measured at 630 nm using a spectrophotometer. Dextrose was used as the standard for calculations.

2.4.3. Estimation of starch

For the control cookies and those with 20% karonda, starch estimation was conducted following the methodology outlined by Karmakar et al. (2025). A sample of 0.5 g from both the control and 20% karonda cookies was measured and mixed with 50 mL of 80% ethanol. This mixture was heated in a water bath at 80°C, covered with aluminum foil, to extract soluble sugars. After heating, the mixture was cooled to room temperature and then filtered to separate the filtrate. The filtrate was evaporated, and the residue transferred to a petri dish, which was placed in an oven for 20-30 minutes. Once dried and powdered, the residue was re-dissolved in distilled water and treated with 52% perchloric acid. This solution was heated at 80°C for 30 minutes and then filtered through muslin cloth. The volume of the resulting filtrate was adjusted to 100 mL. From this solution, 0.1 mL of the sample was diluted to 2 mL with distilled water. Subsequently, this was mixed with 4 mL of 0.2% anthrone reagent, heated at 80°C for 5 minutes, and the absorbance was recorded at 600 nm using a spectrophotometer. Dextrose was used as the standard for calculation.

2.4.4. Total lipids

For the control and 20% Karonda cookies, lipid estimation was conducted following the methodology outlined by Hewavitharana et al. (2020). Each cookie sample, weighing 2 grams, was combined with 20 mL of chloroform-ethanol solution in a 2:1 ratio. The mixtures were then covered with aluminum foil and incubated overnight. After incubation, the solutions were filtered using filter paper, and the resulting filtrates were dried on a hot plate. Finally, the gross weight of the petri plates, along with the lipids, was measured.

2.4.5. Total soluble sugars

The total soluble sugars present in the control cookies and those containing 20% karonda were estimated using the methodology described by Feng et al. (2023). For this analysis, 0.5 g samples of both the control and the 20% karonda cookies were measured and mixed with 50 mL of 80% ethanol. The mixture was heated in a water bath at 80 °C, covered with aluminum foil, to facilitate the extraction of soluble sugars. After heating, the mixture was allowed to cool to room temperature and then filtered to separate the filtrate containing the soluble sugars. The filtrate was transferred to a petri dish and evaporated on a hot plate at 60 °C. Once dried, 10 mL of distilled water was added to dissolve the sugars, and the solution was then transferred to a beaker, adjusting the final volume to 25 mL. From this prepared solution, 0.1 mL was diluted with distilled water to a total volume of 2 mL. Next, 4 mL of 0.2% Anthrone reagent was added, and the mixture was heated for 5 minutes to develop color. Finally, the absorbance was measured at 600 nm using a spectrophotometer to determine the concentration of soluble sugars. Dextrose was used as the standard for this calculation.

2.4.6. Total soluble proteins

Protein extraction and estimation were conducted following the methodology outlined by Ghais et al. (2018) for both the control cookies and the 20% karonda cookies. A sample weight of 0.5 g was taken from each batch, and 10 mL of 1X Phosphate Buffered Saline (PBS) buffer was

added. The samples were homogenized for five minutes and then centrifuged at 2000 rpm for ten minutes. The supernatant was collected, and 10 mL of 10% trichloroacetic acid (TCA) was added to each sample. The test tubes were then refrigerated for one hour to allow the pellets to form. Once the hour was up, the pellets were collected, and 10 mL of 0.1 N NaOH was added and mixed thoroughly. From each test tube, 0.5 mL of the sample was aliquoted, and 4.5 mL of Lowry's reagent was added. After incubating for ten minutes, 0.5 mL of Folin's reagent was introduced. Following another ten-minute incubation period, the absorbance was measured at 600 nm. Bovine serum albumin (BSA) was used as the standard for calculations.

2.4.7. Estimation of crude fiber

Crude fiber estimation was performed on both control and 20% karonda cookies using the methodology outlined by Cadavid et al. (2017). A sample of five grams from each type of cookie was weighed, and 25 mL of hexane was added for defatting. The mixture was thoroughly mixed and then filtered using filter paper. The resulting residue was dried in an oven, and its weight was recorded. Next, the dried residue was transferred to a conical flask containing 100 mL of 2.5% sulfuric acid (H_2SO_4) solution, which was then boiled at 80°C for 45 minutes. After boiling, the mixture was allowed to cool to room temperature, and the pH was adjusted to neutral by gradually washing with hot water. The solution was filtered to collect the residue, which was then treated with 100 mL of 2.5% sodium hydroxide (NaOH) solution and boiled again at 80°C for 45 minutes. The pH was adjusted to neutral once more by washing with hot water. Finally, the residue was thoroughly washed with ethanol, dried in an oven, and the final weight was measured to calculate the crude fiber content.

2.4.8. Total ash content

The total ash content was estimated for both the control cookies and those with 20% karonda using the methodology described by Quirino et al. (2023). A 5 g sample from each type of cookie was placed in a crucible and then incinerated in a muffle furnace at 650 °C for 5 hours. After cooling, the resulting white ash was collected and weighed. The ash content was expressed as a percentage of the original sample weight.

2.4.9. Estimation of free amino acids

Free amino acids in the control and 20% karonda cookies were estimated using the methodology described by Kowalska et al. (2021). For this analysis, 0.5 grams of each type of cookie were measured and mixed with 10 mL of 70% ethanol. The mixture was homogenized for five minutes and then centrifuged at 20,000 rpm for another five minutes. The supernatant was collected and transferred to a petri dish, which was placed on a hot plate at 60 °C until it dried completely. Next, 10 mL of citrate buffer was added to each petri dish to dissolve the residue. From this solution, 1 mL was transferred to a test tube and diluted to a total volume of 2 mL by adding more citrate buffer. Then, 1 mL of ninhydrin reagent was added to the test tube, and the mixture was heated in a boiling water bath at 70 °C. After heating, 5 mL of water was added, and the optical density was measured at 570 nm. Calculations were performed using glycine as the standard.

2.4.10. Estimation of Vitamin A

Vitamin A was analyzed in both the control cookies and the cookies with 20% karonda, following the methodology outlined by Slowik-Borowiec et al. (2023). For the analysis, 2 grams

of each sample (control and 20% karonda cookies) were weighed and placed into separate centrifuge tubes. Ethanol (3 mL) was added to each sample, which was mixed thoroughly before adding 6 mL of petroleum ether. The mixtures were then homogenized for five minutes and centrifuged at 2000 rpm for ten minutes. The resulting supernatant was collected and dried in petri dishes at 50 °C for ten minutes. After drying, 2 mL of chloroform was added to each petri dish, and the contents were thoroughly dissolved. From each mixture, 1 mL was transferred, and 2 mL of 50% trichloroacetic acid (TCA) was added. A color change was observed, and the absorbance was measured at 600 nm. Retinyl acetate was used as the standard for the calculation.

2.4.11. Estimation of Vitamin B2

Vitamin B2 (riboflavin) was quantified using the methodology described by Anaduaka et al. (2021) for both the control cookies and those made with 20% karonda. First, 3 grams of each cookie type were accurately weighed and mixed with 20 mL of 0.2 M hydrochloric acid (HCl). This mixture was then placed in a water bath at 80 °C for 1 hour to facilitate proper extraction. After incubation, the mixture was allowed to cool to room temperature, and the pH was adjusted to 4.0. The solution was filtered, with the filtrate collected and adjusted to a final volume of 25 mL using distilled water. For the estimation of vitamin B2, 1 mL of the prepared filtrate was taken, to which 0.5 mL of 3% potassium permanganate solution and 1 mL of glacial acetic acid were added. The mixture was incubated at room temperature for 3 minutes, after which 0.5 mL of 3% hydrogen peroxide was added. The absorbance of the solution was measured at 440 nm using a spectrophotometer, with riboflavin used as the standard for calculations.

2.4.12. Estimation of Vitamin C

The estimation of vitamin C was conducted for both the control cookies and the 20% karonda cookies, following the methodology outlined by Khadka et al. (2023). To prepare the samples, 0.5 g of each cookie - both the control and the 20% karonda - was taken, and 5 mL of 5% trichloroacetic acid (TCA) was added to each sample. The mixtures were then homogenized for 10 minutes and centrifuged at 2,000 rpm for another 10 minutes. The supernatants were collected, and 0.5 mL from each extract was mixed with 0.5 mL of 1% thiourea and dinitrophenyl hydrazine (DNPH). These mixtures were incubated at 60 °C for 20 minutes, after which the tubes were cooled using tap water. Next, 1 mL of ice-cold sulfuric acid was added to each tube, and the contents were mixed until crystal formation was observed. An equal volume of water was then added, and the solutions were centrifuged at 2,000 rpm for 10 minutes. Finally, the absorbance of each supernatant was measured at a wavelength of 520 nm, using ascorbic acid as the standard for calculations.

2.4.13. Estimation of Vitamin E

The estimation of Vitamin E was carried out following the methodology outlined by Ghasemzadeh et al. (2015) for both control cookies and 20% karonda cookies. One gram of each sample was accurately weighed and placed into clean test tubes. To each tube, 20 mL of absolute ethanol containing 5% ascorbic acid was added. The samples were then thoroughly homogenized for 5 minutes to ensure uniform mixing and heated at 78 °C for 30 minutes to facilitate the extraction of Vitamin E. After heating, the tubes were manually mixed for an additional 5 minutes and allowed to cool to room temperature. Next, 2.5 mL of distilled water was added to each tube, and the samples were cooled in an ice bath for 15 minutes. The tubes were then centrifuged at 2000 rpm for 10 minutes, and the supernatant was carefully collected. To this

supernatant, 10 mL of a 5.35% potassium chloride (0.77 M) solution and 2.5 mL of hexane containing 0.02% BHT were added. The mixture was vortexed for 10 minutes to ensure complete phase separation and underwent a second centrifugation at 2000 rpm for 10 minutes. The supernatant layer was transferred to petri dishes and allowed to evaporate at room temperature. The resulting dried residue was then dissolved in 3 mL of ethanol for further quantification of Vitamin E content. For the estimation, 1 mL of the extracted solution was mixed with 0.5 mL of ferric chloride (0.002 M), 0.5 mL of phosphoric acid (0.037 M), and 1 mL of a 2,4-dipyridyl solution (0.002 M). This mixture was incubated at room temperature for 15 minutes to allow for color development. The absorbance of the resulting solution was measured spectrophotometrically at 520 nm.

2.5. Microbial analysis

A total bacterial count was conducted to evaluate the microbial load in both the control cookies and those containing 20% karonda (Suhasini et al., 2015). For this analysis, nutrient agar was prepared, sterilized, and poured into sterile Petri plates. To prepare the samples, 1 g of each cookie type—control and 20% karonda—was measured and placed in a centrifuge tube. The samples were then mixed with 9 mL of phosphate-buffered saline (PBS) and thoroughly homogenized. A series of dilutions was created up to a dilution factor of 10^{-5}. From each dilution, 100 µL of the suspension was spread evenly onto the nutrient agar plates using a sterile L-rod. The plates were incubated at 37 °C for 24 hours. After incubation, the number of colonies on each plate was counted.

The total fungal load of the control cookies and the 20% karonda cookies was determined using the standard plate count method on Potato Dextrose Agar (PDA), as described by Ribeiro et al. (2019). One gram of each cookie sample was homogenized in 10 mL of sterile PBS to create the initial suspension. From this suspension, serial dilutions (10^{-1} to 10^{-5}) were prepared by transferring 1 mL of the initial suspension into 9 mL of sterile PBS. From each dilution, 100 µL was aseptically spread onto sterile PDA plates and incubated at 37 °C for 48 hours. After incubation, plates that exhibited between 30 and 300 colonies were selected, and the fungal load was calculated and expressed as CFU/g using the formula: CFU/g = (Number of colonies × Dilution factor) / Volume plated.

2.6. TBARS test

The TBARS (Thiobarbituric acid reactive substances) test was conducted for both the control and 20% karonda cookies, following the methodology outlined by Abeyrathne et al. (2021). For the test, one gram of each sample was weighed, mixed with 10 mL of water, and then homogenized. The mixture was filtered, and the filtrate was adjusted to a final volume of 10 mL with water. From this solution, 2 mL was treated with 2 mL of 10% TCA (trichloroacetic acid) and incubated at 4°C for one hour. After incubation, the sample was centrifuged at 2000 rpm for five minutes. Next, 1 mL of the supernatant was combined with 1 mL of 10% TCA and 1 mL of 0.8% TBA (thiobarbituric acid), and the mixture was heated in a water bath at 90°C for 20 minutes. Finally, the absorbance was measured at 520 nm using a spectrophotometer.

2.7. Carbonyl test

The carbonyl test was conducted to estimate the protein oxidation levels in both control cookies and those made with 20% karonda, following the methodology outlined by Soglia et al. (2016).

From each type of cookie, 1 gram was measured and mixed with 10 milliliters of water. This mixture was then homogenized, filtered, and made up to 10 milliliters with water. Next, 2 milliliters of the filtrate were treated with 2 milliliters of 10% trichloroacetic acid (TCA) and incubated at 4°C for 1 hour. Following this incubation, the mixture was centrifuged at 2000 rpm for 5 minutes. The pellet obtained after centrifugation was dissolved in 2 milliliters of 1 N sodium hydroxide (NaOH). From this solution, 1 milliliter was mixed with 0.5 milliliters of 5 mM 2,4-Dinitrophenyl hydrazine (DNPH) reagent and incubated in the dark for 1 hour to allow for color development. The mixture was then centrifuged again at 2000 rpm for 5 minutes, and the absorbance of the supernatant was measured at 490 nm to determine the protein carbonyl content.

2.8. GC-MS analysis

GC-MS (Gas Chromatography-Mass Spectrometry) analysis of Carissa carandas extract was conducted following the methodology outlined by Sudha et al. (2024). For extract preparation, 50 g of Carissa carandas fruits were weighed and blended with 200 mL of distilled water. The mixture was then subjected to extraction using a water distillation unit to obtain the aqueous extract. To this extract, 10 mL of ethyl acetate was added, and the upper organic layer was collected for GC-MS analysis. The analysis was performed using a GC-MS-QP Series (GC-2010) instrument equipped with an Rtx-5MS column (30 m x 0.25 mm ID, 0.25 µm film thickness). A 1 µL aliquot of the sample was injected in split mode (10:1) at an injector temperature of 280 °C. The carrier gas flow rate was maintained at 1.20 mL/min, with a total flow of 16.2 mL/min and a purge flow of 3.0 mL/min. The oven temperature was programmed to start at 50 °C (held for 1 minute), then increased at 5 °C/min to 220 °C (held for 5 minutes), raised at 6 °C/min to 280 °C (held for 10 minutes), followed by an equilibration period of 3 minutes. The ion source and interface temperatures were set at 230 °C and 280 °C, respectively. The mass spectrometer operated in scan mode over an m/z range of 50-650, with an event time of 0.40 seconds and a solvent cut-off time of 5 minutes. The resulting peaks were identified by comparing their mass spectra with those available in the NIST library, and compounds were characterized based on their spectral similarity and retention time.

2.9. Statistical analysis

All parameters were measured in triplicates, and the results are presented as mean ± standard deviation (SD). Statistical analysis of the data was performed using one-way analysis of variance (ANOVA). The symbol * indicates a significant difference ($p < 0.05$) in the mean values between the control group and the group that received 20% karonda fruit cookies.

3. Results and Discussions

3.1. Sensory value

Sensory analysis was conducted to evaluate the appearance, color, aroma, and texture of control and karonda fruit-based cookie samples, with the results presented in Figure 1. The evaluation indicated that the cookies with 20% karonda provided the most balanced and desirable qualities, achieving hedonic scale scores of 7.40 ± 1.00 for color, 7.87 ± 1.59 for appearance, 7.90 ± 0.60 for texture, and 7.33 ± 1.22 for aroma. This was attributed to the optimal presence of volatile esters and phenolic antioxidants that help maintain fruity notes. The hedonic values for appearance, texture, and color of the 20% karonda-based cookies were significantly higher ($p < 0.05$) than those of the control cookies. Color evaluation showed that the 20% sample exhibited

the most appealing and stable color. The natural pigments present at this level enhanced the visual quality without causing excessive darkening. In contrast, the cookies with 30% karonda exhibited accelerated browning, likely due to their higher phenolic content, as reported by Pedziwiatr et al. (2024). Interestingly, the highest appearance scores were recorded for the 30% karonda cookies (8.37 ± 1.06), which was primarily due to the intensified anthocyanin content and enhanced Maillard browning, resulting in a rich, glossy, golden-red surface. Conversely, the 10% (6.17 ± 1.16) and 20% cookies (7.87 ± 1.59) appeared lighter and less uniform due to lower pigment concentrations and milder browning reactions, leading to comparatively duller surfaces, as mentioned previously by Krajewska and Dziki (2023).

Texture analysis indicated that the 20% formulation of cookies was the most acceptable. The moderate level of fruit powder did not significantly disrupt the dough structure. In contrast, the cookies with 30% fruit powder became firmer and drier. This change was attributed to the increased fiber and solids, which affected the water-binding capacity, as reported by Najjar et al. (2022). Additionally, the 30% cookies experienced a decline in aroma due to the excessive fruit powder and higher phenolic levels, which altered the volatile profile and introduced mild off-odors, as noted in a previous study (Kausar et al., 2024). Similar findings have been reported for other fruit pulps, such as shea (Chikpah et al., 2023), dates (Tahir et al., 2023), and various other fruits (Krajewska and Dziki, 2023), which were found to enhance the sensory profile of cookies. Overall, while the 30% fortification improved only the visual appeal of the cookies, the 20% formulation demonstrated the most favorable overall sensory profile. It offered superior aroma stability, better texture, and a more desirable color. Therefore, only the 20% karonda-included cookies were selected for further experiments on nutritional value and shelf life studies.

3.2. Nutritional value

The control cookies had a moisture level of 5.50 ± 0.71 g/100 g, while cookies containing 20% karonda fruit exhibited a significantly lower moisture content of 1.80 ± 0.28 g/100 g ($p < 0.05$) (Figure 2). The *Carissa carandas* (Karonda) fruits contain sugars, fibers, and pectin, which can retain water tightly, making this bound water unmeasurable in standard proximate analysis. Additionally, the water activity decreases during baking, resulting in cookies made with *C. carandas* (Karonda) pulp having lower moisture content, as noted in a previous study (Canalis et al., 2020). Our findings regarding moisture content align with an earlier study on cookies incorporating sapota (Asadi et al., 2022). Regarding carbohydrate content, the control cookies contained 24.40 ± 1.13 g/100 g, while those with 20% karonda pulp showed an increased carbohydrate level of 29.60 ± 2.26 g/100 g (Figure 2). This increase can be attributed to the natural sugars and soluble polysaccharides found in *C. carandas* fruit. The karonda pulp is rich in dietary fiber, which undergoes partial hydrolysis during the baking process, contributing to the elevated carbohydrate level in the cookies, as reported in another study (Rafique et al., 2023). The addition of fruit pulp enhances the carbohydrate content in cookies primarily due to the natural sugars, such as glucose, fructose, and sucrose, as well as the fiber present in the fruit. When baked, fruit pulps may contribute a higher concentration of these carbohydrates compared to wheat flour (Krajewska and Dziki, 2023).

The cookies made with karonda fruit exhibited a significantly higher starch content (8.5 ± 0.21 g/100 g) compared to the control cookies (6.05 ± 0.35 g/100 g), with a *p*-value less than 0.05 (Figure 2). The pulp of *C. carandas* contains sugars and polysaccharides, which interact with

wheat starch during baking and influence the gelatinization of starch. These interactions may promote the formation of resistant starch. Previous studies have indicated that adding fruit pulp can increase the total starch content in cookies, as these pulps are concentrated sources of resistant starches and enhance the nutritional profile by increasing the amounts of slowly digestible starch and resistant starch (Agama-Acevedo et al., 2012). Additionally, the heat and acidity from the fruit, along with the interaction between fiber and starch, alter the structure of starch granules. As a result of these modifications, the proximate analysis shows a slightly higher starch value in cookies made with *C. carandas*, consistent with findings from previous research (Rafique et al., 2023). The cookies containing karonda also displayed a slightly higher lipid concentration (25.50 ± 5.66 g/100 g) compared to the control cookies (21.75 ± 1.06 g/100 g) (Figure 2). The increase in fat content in the karonda cookies is primarily due to the natural presence of a small amount of crude fat in *C. carandas* pulp. When this pulp is incorporated into the cookie formulation, its inherent lipids contribute to the total fat content measured. Although some fruits are low in fat, the dehydration process used to create pulp powder concentrates the lipids, and many fruit pulps may contain essential fatty acids. This results in a higher overall lipid percentage compared to standard wheat flour (Mouafo et al., 2024). Consequently, the cookies made with karonda showed a slight increase in total lipid content, as previously reported by Arif et al. (2016).

The control cookies contained a total soluble sugar content of 2.58 ± 0.00 g/100 g, while the cookies enriched with 20% karonda showed a slightly higher value of approximately 2.63 ± 0.01 g/100 g (see Figure 3). The fruit *C. carandas* is naturally rich in soluble sugars, including glucose, fructose, and sucrose, which contribute to its sweet taste and nutritional profile (Rafique et al., 2023). When karonda pulp is incorporated into the cookie formulation, these additional soluble sugars are introduced into the dough, as noted in an earlier study (Makroo et al., 2019). As a result, the final baked product exhibits a higher soluble sugar content compared to the control. In terms of protein, the control cookies contained 0.14 ± 0.01 g/100 g, whereas the cookies enriched with 20% *C. carandas* pulp showed a significantly ($p < 0.05$) higher value of 1.15 ± 0.10 g/100 g (Figure 3). This increase is likely due to the moderate protein content naturally found in karonda fruit, which contributes additional amino acids and soluble proteins to the cookie dough. The incorporation of fruit pulp enhances the overall protein content of the product without causing denaturation during baking (Mahajan et al., 2024). This aligns with the nutritional profile of *Carissa* species, which contain significant levels of proteins along with other macronutrients (Rafique et al., 2023).

The control cookies had a crude fiber content of approximately 1.30 ± 0.14 g/100 g, while the cookies enriched with 20% karonda displayed a slightly higher value of about 1.40 ± 0.28 g/100 g (Figure 3). This increase is expected, as *C. carandas* naturally contains a high amount of dietary fibers, such as cellulose, hemicellulose, lignin, and pectin. Incorporating fruit pulp into cookies boosts the fiber content because these ingredients are rich in both insoluble and soluble dietary fibers, which are either absent or present in very low amounts in refined wheat flour (Dhingra et al., 2012). When fruit pulp is added to the cookie formulation, these plant fibers become part of the final product and remain stable even after baking. Consequently, the overall fiber level in the sample cookies increases. This observation aligns with findings reported by Goli et al. (2023), which indicate that underutilized fruits like karonda are inherently rich in fiber and can enhance the nutritional profile of bakery products. The cookies containing 20% karonda

pulp had a higher ash content (0.61 ± 0.03 g/100 g) compared to the control cookies (0.44 ± 0.01 g/100 g), with a statistical significance of $p < 0.05$ (see Figure 3). *C. carandas*, is naturally rich in minerals such as calcium and iron, which contribute to the total ash residue measured after combustion. Incorporating fruit pulp into cookies increases the total ash content primarily because fruit pulp has higher mineral concentrations, such as potassium, magnesium, calcium, and iron, compared to refined wheat flour (maida) (Mounjouenpou et al., 2018). When karonda pulp is added to the cookie formulation, these mineral components remain stable during the baking process, enhancing the overall inorganic matter in the final product. As a result, cookies enriched with karonda pulp exhibit a significantly higher ash content than the control cookies. This finding is consistent with previous research conducted by Mishra et al. (2024).

The karonda-based cookies contained 13.22 ± 1.44 mg/100 g of total free amino acids, whereas the control cookies only had 12.20 ± 0.00 mg/100 g (Figure 4). The pulp of *C. carandas* has a higher concentration of free amino acids compared to wheat flour. This concentration effect enhances the nutritional profile of the cookies when the pulp is added. Fruit pulps, in general, can provide a more diverse and greater amount of amino acids - such as arginine and phenylalanine - compared to wheat flour alone, helping to create a more balanced protein profile in the final product (Egydio et al., 2013). Although heat during baking can cause partial degradation of certain amino acids, the cookies made with *C. carandas* pulp actually showed a slight increase in amino acid levels, as observed in other foods (Krajewska and Dziki, 2023). Additionally, the cookies containing karonda fruit had a higher vitamin A content, measuring 9.00 ± 1.16 mg/100 g, compared to the control cookies, which contained 7.36 ± 1.16 mg/100 g (Figure 4). The pulp of *C. carandas* is rich in carotenoids and provitamin A compounds, especially beta-carotene, which increases the carotenoid content in the final product, resulting in elevated vitamin A equivalents (Tanska et al., 2016). Furthermore, the fat in the cookie matrix enhances the measurement of fat-soluble carotenoids, contributing to the increased vitamin A content in the *C. carandas* cookies, as noted earlier by Rafique et al. (2023).

The control cookies contained 10.17 ± 1.60 mg of Vitamin B2 per 100 g, while the cookies enriched with *C. carandas* showed a significantly ($p < 0.05$) higher concentration of 17.01 ± 1.52 mg per 100 g (Figure 4). The pulp of *C. carandas* naturally contains B-group vitamins, including riboflavin, which increases the vitamin B2 content in the cookies. Generally, fruits serve as nutrient-dense fortifiers, providing vitamins such as vitamin B2 that are often absent in refined wheat flour (Kausar et al., 2024). When part of the flour is replaced with fruit pulp, the riboflavin content increases. Additionally, baking can concentrate heat-stable vitamins like B2 as moisture is reduced. Consequently, cookies made with *C. carandas* pulp exhibit a significant increase in riboflavin levels, a finding supported by previous research on other foods (Singh et al., 2020). The *Carissa*-enriched cookies contained 4.70 ± 0.10 mg of vitamin C per 100 g, while the control cookies had a significantly ($p < 0.05$) higher level of 5.78 ± 0.17 mg per 100 g (Figure 4). Vitamin C, also known as ascorbic acid, is highly sensitive to heat and can degrade during the baking process. According to Lee et al. (2017), while Vitamin C is heat-sensitive, the addition of fruit pulp in the cookies contributes a significant amount of Vitamin C to the final product. Although *C. carandas* is rich in Vitamin C, much of it breaks down during baking, resulting in lower Vitamin C levels in the finished cookies. This aligns with findings reported by Rafique et al. (2023). In terms of Vitamin E, the control cookies contained approximately 0.56 ± 0.02 mg/100 g, while cookies with 20% karonda pulp showed a slightly higher value of 0.57 ± 0.01

mg/100 g (see Figure 4). Typically, adding fruit pulp to cookies does not significantly increase Vitamin E content, largely because the heat, light, and oxygen involved in baking can degrade this sensitive nutrient. Furthermore, most fruit pulps are not high in Vitamin E (Asadi et al., 2021). However, the slight increase in this case can be attributed to the natural Vitamin E content in *C. carandas*, which enhances its nutritional profile. When karonda pulp is included in the cookie formulation, the Vitamin E remains stable during baking, resulting in comparable levels of Vitamin E in the enriched cookies.

3.3. Microbial safety

The control cookies recorded a bacterial load of 0.13 ± 0.01 CFU/g, while the *Carissa* cookies showed a significantly lower value of 0.02 ± 0.01 CFU/g (Table 1). This reduction is attributed to the natural presence of antimicrobial phenolic compounds, flavonoids, and organic acids in *C. carandas*, which suppress bacterial growth by disrupting cell membranes and reducing microbial survival, as indicated in previous studies (Kumar et al., 2023). Additionally, Rafique et al. (2023) reported that karonda fruit exhibits strong antibacterial activity, which explains the decreased bacterial load in the cookies enriched with *Carissa* pulp compared to the control cookies. Both the control and *Carissa* cookies recorded a fungal load of 0.00 ± 0.00 CFU/g (Table 1). This outcome is expected because cookies are low-moisture baked products with reduced water activity, effectively preventing fungal contamination. The baking process also eliminates fungal spores. Although karonda fruit contains antifungal phenolics, the effect is not measurable in this context since both samples showed no fungal growth. Rafique et al. (2023) noted the antifungal potential of *Carissa* fruit. Furthermore, the bioactive compounds in karonda fruit can act as natural preservatives, reducing the necessity for chemical additives while improving the shelf life of the cookies.

3.4. Oxidative products

The 20% *Carissa* cookies exhibited a significantly ($p < 0.05$) lower value of 2.23 ± 0.14 nM for TBARS compared to the control cookies, which measured 3.58 ± 0.14 nM (Table 1). This reduction indicates a decrease in lipid oxidation in the cookies made with *Carissa*. *C. carandas* contains natural antioxidants, such as phenolic and flavonoid compounds, which help inhibit free-radical formation during baking. As a result, the lipid content in the cookies enriched with karonda fruit is better protected. This aligns with previous findings by Rafique et al. (2023) that reported the antioxidant properties of *Carissa* fruit. In terms of carbonyl content, the control cookies had a measurement of 1.57 ± 0.16 nM, while the cookies made with 20% *Carissa* showed a lower value of 1.09 ± 0.13 nM (Table 1). This decrease may be attributed to the prevention of protein oxidation during baking, which ultimately reduces carbonyl formation in the cookies enriched with karonda fruit. The presence of natural antioxidants and organic acids from *Carissa* pulp helps shield proteins from oxidation, thereby contributing to the lower carbonyl levels. Adding karonda fruit pulp to cookies has been shown to reduce oxidative end products of both lipids and proteins. This is primarily due to the introduction of high levels of natural antioxidants that neutralize free radicals and delay the oxidation of fats and proteins (Zuniga-Martinez et al., 2022). Therefore, incorporating antioxidant-rich fruits can stabilize the cookie matrix, reduce rancidity, and mitigate the oxidative spoilage of the final product during storage.

3.5. GC-MS bioactives profile

C. carandas fruit extract exhibited several bioactive compounds, as revealed by the GC-MS profile analysis (see Figure 5 and Supplementary Table 1). This fruit is rich in terpenoids, long-chain fatty acids, phenolic derivatives, and fruity esters, all of which are prominently featured in the chromatogram of the *Carissa* cookies (Figure 5). The cookies displayed a richer and more diverse volatile profile, with terpenoids and esters contributing to the fruity aroma noted during sensory evaluation. Phenolic compounds, on the other hand, are responsible for the antioxidant properties. Fatty acids such as palmitic acid, which is commonly found in *Carissa* pulp, also appeared as prominent peaks in the analysis. Furthermore, bioactive compounds like azulene (retention time: 13.10 min, peak area: 0.43%), tetradecane (retention time: 18.97 min, peak area: 4.96%), and caryophyllene (retention time: 19.50 min, peak area: 0.24%) were detected in the GC-MS profile (Figure 5). Azulene is recognized for its powerful anti-inflammatory effects, antioxidant protection, and antimicrobial properties. It is used to treat conditions like irritated skin, sunburns, acne, rosacea, stomatitis, tonsillitis, and gingivitis (Slon et al., 2020). Tetradecane, a bioactive hydrocarbon compound found in plants, is noted for its antioxidant, antimicrobial, and antifungal properties, and it is associated with supporting immune responses in the body (Taher et al., 2023). Caryophyllene is widely acknowledged for its therapeutic benefits, including potential anti-inflammatory, analgesic, and anti-anxiety effects (Scandiffio et al., 2020). In summary, the GC-MS analysis confirms that incorporating *Carissa* pulp introduces bioactive volatile phytochemicals such as azulene, tetradecane, and caryophyllene. These compounds could enhance both the aroma and functional quality of the cookies, as previously reported by Saeed et al. (2024).

4. Conclusions

This project successfully demonstrated the development of nutrient-enriched cookies through the incorporation of *Carissa carandas* fruit pulp, offering a practical and innovative way to include indigenous fruits in bakery products. Among the tested formulations, the 20% incorporation level provided the best balance of taste, texture, color, and nutritional enhancement, making it the most suitable for consumer acceptance. The enriched cookies exhibited significantly higher levels of starch, total soluble proteins, total ash, vitamin B2, and vitamin C compared to the control cookies, confirming their superior nutritional value. The addition of *C. carandas* fruit to the cookies effectively inhibits microbial growth and prevents lipid and protein oxidation, thereby improving the product's shelf life. Furthermore, the presence of bioactive compounds detected through GC-MS analysis suggests additional health benefits for consumers. These cookies have strong commercial potential, as *C. carandas* can be integrated into the functional food and bakery sectors as nutritious snack options suitable for health food markets, institutional catering, and commercial manufacturing. Future research may include broader sensory evaluations, detailed shelf-life studies, packaging assessments, and stability analyses to support commercial scalability. A key innovation of this project is the unique application of *C. carandas,* a traditionally underutilized fruit, in a widely consumed bakery product. This work showcase the potential of karonda fruit as a functional ingredient that enhances nutritional, sensory, and antioxidant profiles while addressing the demand for healthier snack alternatives.

Supplementary file

Supplementary file includes Supplementary Figure 1 and Supplementary Table 1.

Author contribution statement

All authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Declaration

The authors declare that they have no conflict of interests.

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Ethical statement

There are no animal / human studies involved in the reported work.

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Figure 1. Sensory analysis of control and karonda incorporated (10, 20 & 30%) cookies. In each bar, values with asterisk symbol (*) indicates they are statistically significant ($p < 0.05$) while values with "ns" superscript are not significant when compared to control.

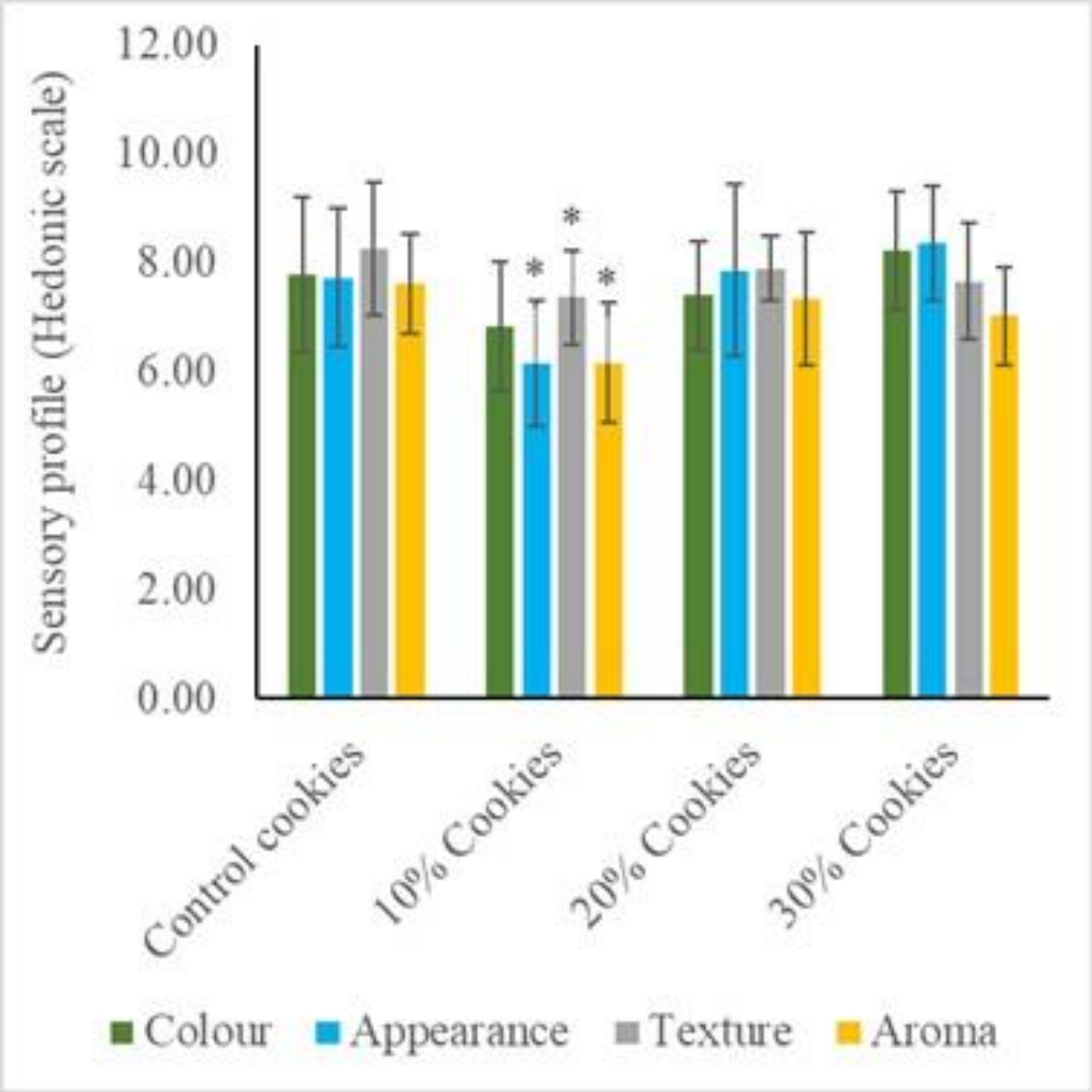


Figure 2. Moisture, total carbohydrate, starch and total lipid contents of control and karonda incorporated (20%) cookies. In each bar, values with asterisk symbol (*) indicates they are statistically significant ($p < 0.05$) while values with "ns" superscript are not significant when compared to control.

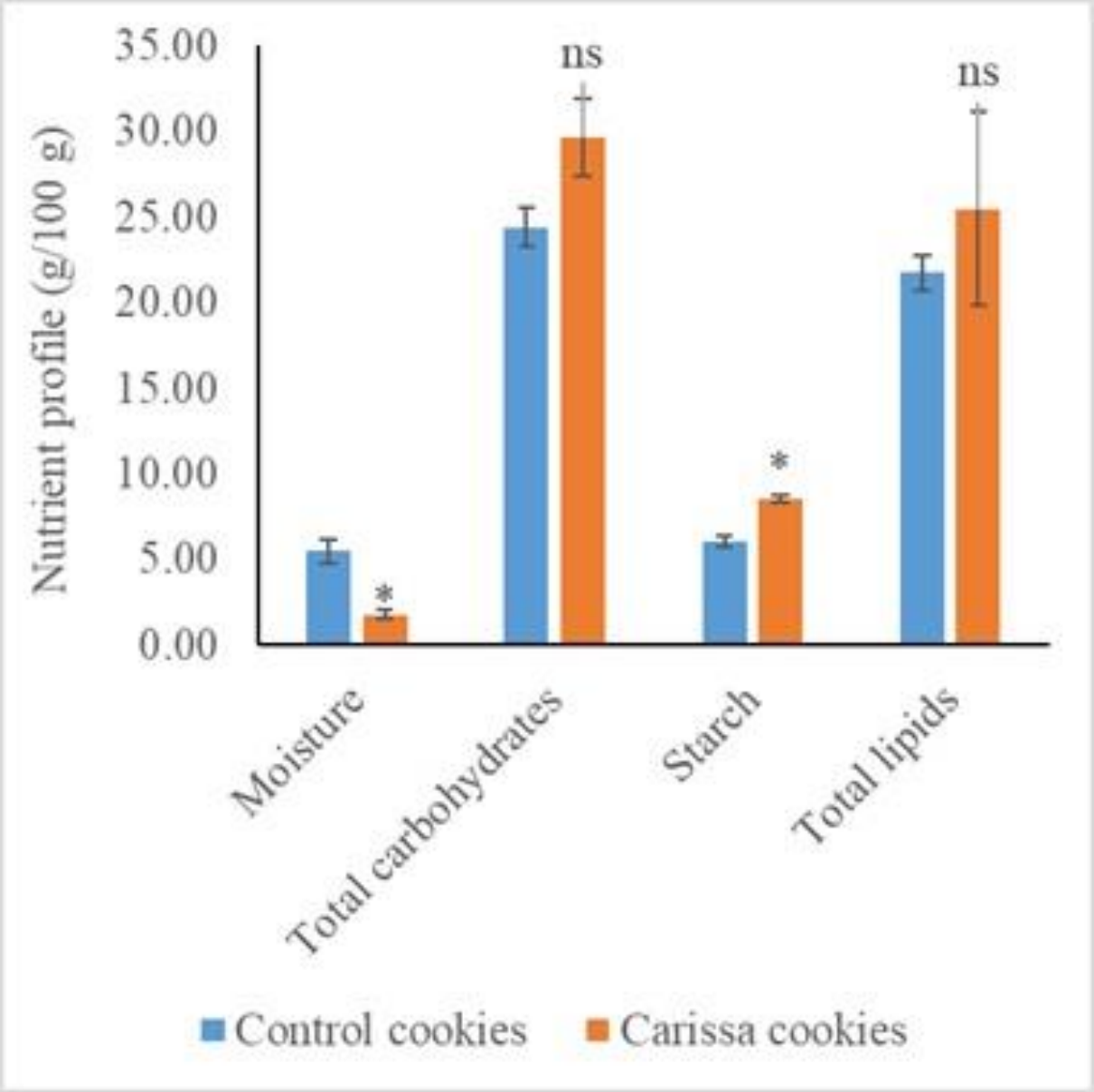


Figure 3. Soluble sugars, total soluble proteins, crude fibre and total ash contents of control and karonda incorporated (20%) cookies. In each bar, values with asterisk symbol (*) indicates they are statistically significant ($p < 0.05$) while values with "ns" superscript are not significant when compared to control.

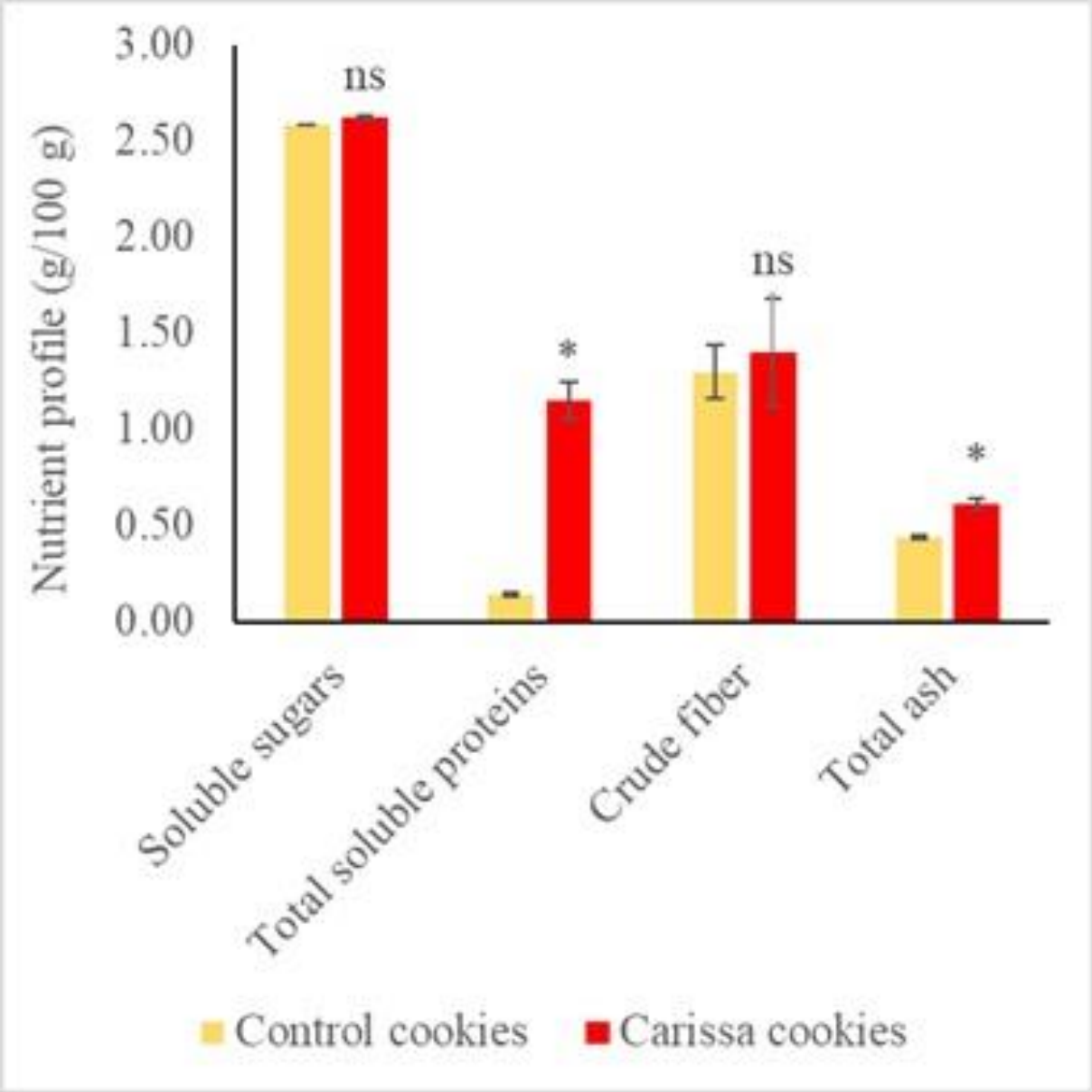


Figure 4. Free amino acids, vitamin A, vitamin B2 and vitamin E contents of control and karonda incorporated (20%) cookies. In each bar, values with asterisk symbol (*) indicates they are statistically significant ($p < 0.05$) while values with "ns" superscript are not significant when compared to control.

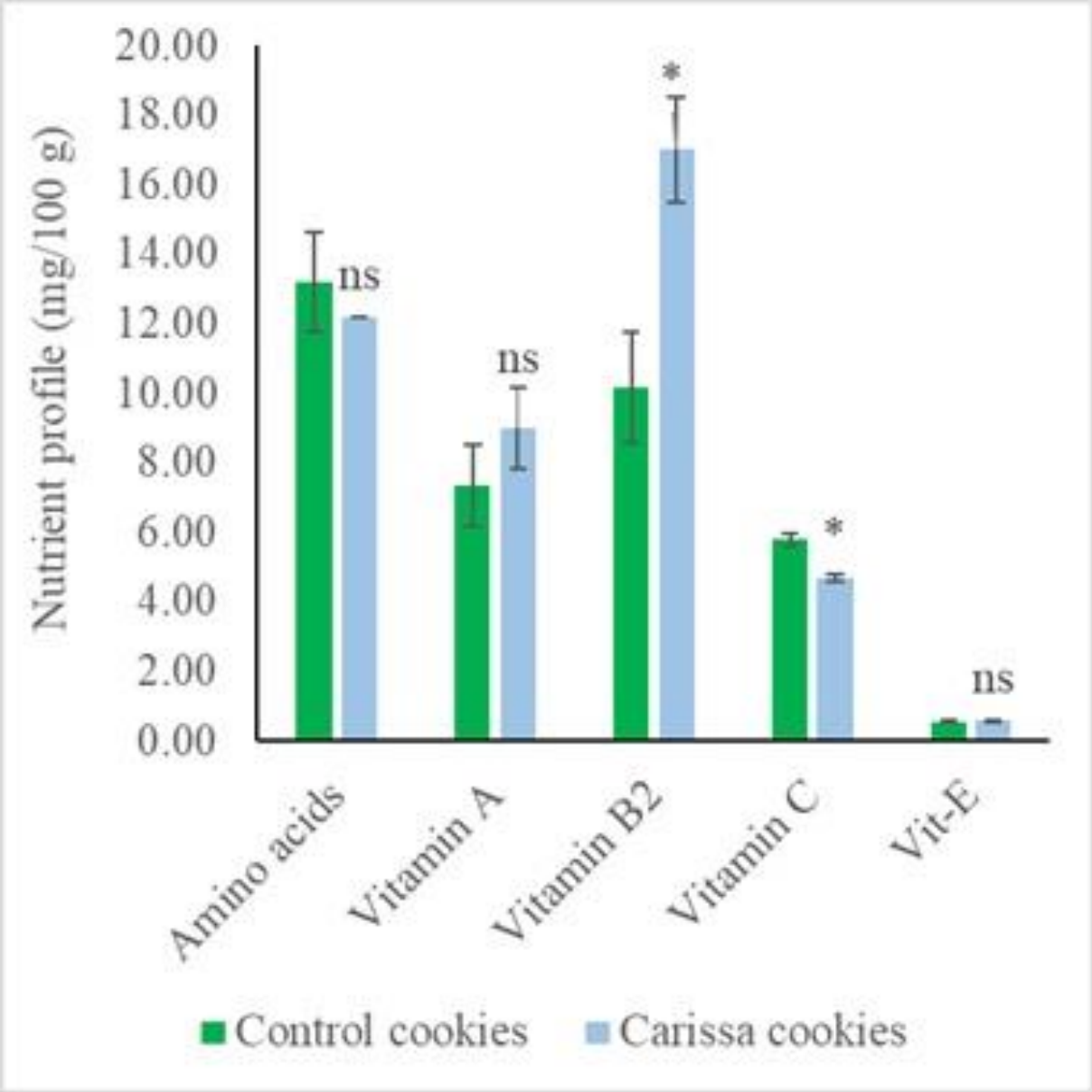
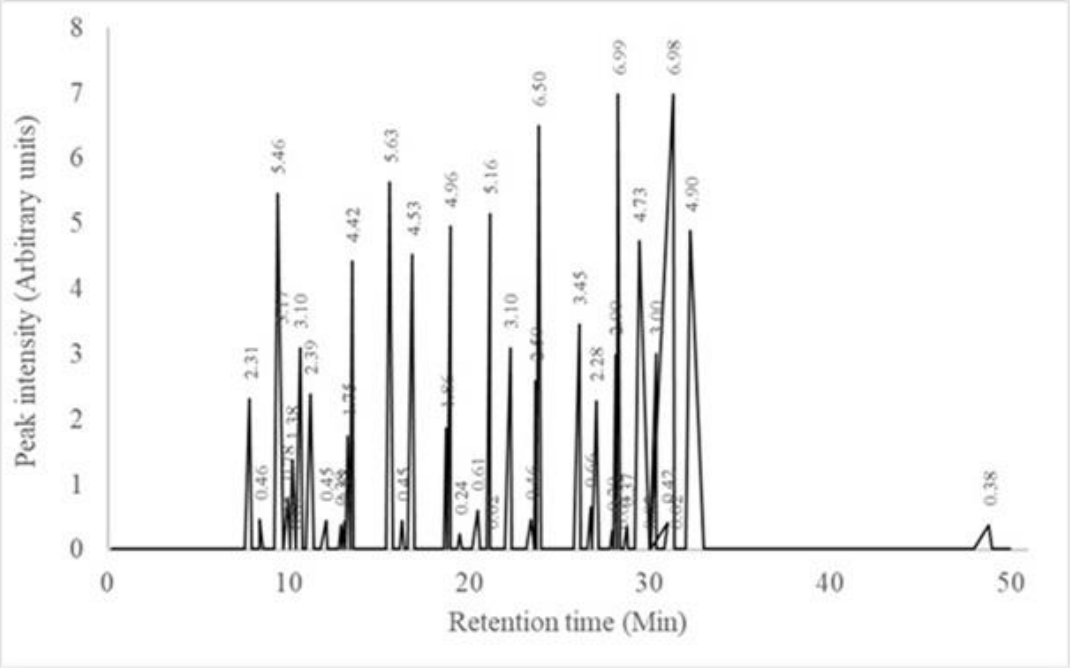
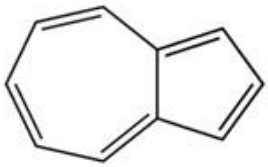


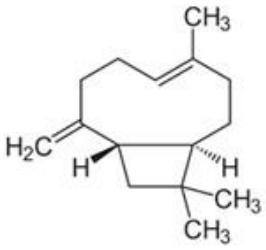
Figure 5. GC-MS profile of phytochemicals in *Carissa carandas* fruit extract and the the details of identified bioactive phytochemicals.





Azulene
Retention time: 13.10 min
Area: 0.43%

Tetradecane
Retention time: 18.97 min
Area: 4.96%



Caryophyllene
Retention time: 19.50 min
Area: 0.24%

Table 1. Microbial load and chemical oxidation levels of lipids (TBARS) and proteins (Carbonyls) in control cookies and 20% *Carissa* included cookies. In each parameter, values with asterisk symbol (*) indicates they are statistically significant ($p < 0.05$) while values with "ns" superscript are not significant when compared to control.

S. No.	Parameter	Control cookies	*Carissa* cookies
1	Total bacterial load (CFU/g)	0.13 ± 0.01	0.02 ± 0.00*
2	Total fungal load (CFU/g)	0.00 ± 0.00	0.00 ± 0.00
3	Lipid oxidation (TBARS, nM)	3.58 ± 0.14	2.23 ± 0.14*
4	Protein oxidation (Carbonyls, nM)	1.57 ± 0.16	1.09 ± 0.13^{ns}