



Nutritional, antinutritional, and phytochemical profiling of raw and fermented *Carissa carandas* puree and its utilization in ice cream development

Wisha Saeed^a, Muhammad Qamar^{a,*}, Tariq Ismail^a, Muhammad Zulqarnain Khan^a, Tuba Esatbeyoglu^{b,**}

^a Department of Food Science & Technology, Faculty of Food Science & Nutrition, Bahauddin Zakariya University, 60800, Multan, Pakistan

^b Department of Molecular Food Chemistry and Food Development, Institute of Food and One Health, Gottfried Wilhelm Leibniz University Hannover, Am Kleinen Felde 30, 30167, Hannover, Germany

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ABSTRACT

Carissa carandas, an underappreciated fruit, is abundant in vital nutrients and secondary metabolites but still overlooked and not fully utilized. The study aimed to assess nutritional composition, antinutritional contents, and biological potential of raw *C. carandas* puree and the effects of fermentation on said parameters. Moreover, raw and fermented puree were utilized in the development of functional ice cream as natural alternatives to artificial color and flavor. Results declared *C. carandas* raw puree a good carrier of primary and secondary metabolites but also with intrinsic toxicants. Fermentation caused a slight increase in protein (13 %), crude fiber (5 %), and ash (25 %) but a decline in moisture (1.2 %), crude fat (12 %) and carbohydrate (9 %) as compared to raw sample. Likewise, increase in Ca (6 %), Fe (5.5 %), P (6.3 %), Na (21 %), Mg (14 %), Mn (1.3 %), Zn (30 %), Cu (25 %) but decline in K (25 %) and Cr (11 %) among fermented samples was observed as compared to raw samples. The study found a significant decrease in anti-nutrient concentrations among fermented samples wherein phytates were the most affected (– 6.8 %) followed by oxalates (– 5 %) saponins (– 4.6 %) and tannins (– 1.9 %) with non-significant impact on antioxidant potential. Physical analysis of the *C. carandas* raw (12 %) and fermented (6 %) puree-based ice-cream showed non-significant difference among freezing point, overrun, melting point, viscosity, acidity, and pH as compared to control. The ice cream made with *C. carandas* puree not only provided a natural color and flavor but may also demonstrate potential as a functional product due to its minerals content and the presence of bioactive compounds but in vivo studies and clinical trials are warranted. In future, optimization of fermentation may help reduce toxicants and retain more nutrients. Whereas for ice cream commercialization, it is important to have shelf life assessment and trials about consumer acceptance to support sustainable agriculture and functional food development.

1. Introduction

In recent years, increased awareness among consumers regarding the importance of diet in maintaining a healthy life style has boosted the demand for functional foods (FF). FF provide health benefits beyond basic nutrition by delivering bioactive metabolites known to manage chronic ailments [1]. Dairy products have always been a valuable component of the human diet because of their high nutritional and energy value, with a good amount of macro- and micronutrients. Ice

cream, i.e., a famous dairy product known around the globe for its splendid taste, texture, aroma, nutrition, and unique sensory appeal, is mostly produced and consumed during the summer months [2]. Interestingly, ice cream provides a suitable medium to add fruits/functional ingredients owing to its low-temperature storage and stabilizing aspects [3]. However, the available ice cream in the market contains a significant amount of added color and flavors, which impart negative health implications [4]. The constant demand of replacing the synthetic additives in ice cream with natural ones has led to the production of

* Corresponding author.

** Corresponding author.

E-mail addresses: muhammadqamar@bzu.edu.pk (M. Qamar), esatbeyoglu@foh.uni-hannover.de (T. Esatbeyoglu).

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functional ice cream [5]. Previously, attempts have been made to develop new ice cream recipes by adding several functional ingredients to address specific health ailments [6–8].

C. carandas is a berry fruit known to have a good amount of iron, vitamin C, minerals, sugars, and protein [9,10]. Phenolic acids, anthocyanins, and flavonols are the key secondary metabolites present in the mentioned berry fruit. *C. carandas* extracts (ethanol, methanol) are reported to exert antioxidant, anti-inflammatory, anticancer, anti-diabetic, and enzyme inhibition activities in a range of studies [11–15]. *C. carandas* is already being processed for jams, jellies, beverages, juices, candies, and colored wines [16]. However, various regional varieties of this wild fruit have shown the presence of anti-nutrients in them [16,17]. A study showed the comparison between the nutrient value of both cultivated and wild fruits and concluded that wild fruits have a higher amount of nutrients and anti-nutrients, and the latter contributes adversely to the bioavailability of the nutrients in the body [18]. Anti-nutrients result in multiple nutrient deficiencies along with health issues such as headaches, bloating, rashes, and nausea [19–21]. Research is under process to figure out effective techniques that can alleviate anti-nutrients in fruits along with retention of quality attributes to curb the health challenges induced by these compounds. Fermentation is one of the ancient and effective methods to preserve foods. This process not only imparts unique flavor and texture but also improves nutritional profile of the product by activating endogenous and microbial enzymes (phytases, tannases, oxalate decarboxylase) that holds the potential to hydrolyze or degrade antinutritional factors, thus enhancing bioavailability [22]. Consequently, present study was planned to check the nutritional, antinutritional, and phytochemical profile of raw and fermented *C. carandas* puree and to investigate its potential use in ice cream production as a natural functional ingredient.

2. Materials and methods

2.1. Procurement of material

C. carandas fruit berries were collected from the gardens of Bahauddin Zakariya University, Multan, Pakistan in the month of July. The taxonomic identification was performed by botany department and reference sample as kept under ID CC-01. Berries were washed, cut into pieces using knife to remove seeds, and finally mashed to obtain puree. The fermentation was carried out by using *Lactiplantibacillus plantarum* strain (v.299; DSM 9843). *Lp. plantarum* culture was prepared in MRS (De Man–Rogosa–Sharpe) broth and incubated at 30 °C for 48 h. The biomass was centrifuged (Z326-K, Hermle, Wehingen, Germany) (1200 g, 20 min, 4 °C) and suspended in 0.9 % saline water solution to preserve a bacterial cell count of 10^7 CFU/mL. In order to initiate the fermentation process by intermittent shaking, 0.2 mL of the previously prepared *Lp. Plantarum* inoculum suspension was added to 100 g puree. The fermentation process was carried out in an incubator (model 100–800, Memmert, Schwabach, Germany) for 24 h at 32 °C. The fermentation process was monitored by controlling the physical conditions, i.e., temperature, pH and titratable acidity of the sample after every 6 h period.

2.2. Determination of pH and acidity

During the process of fermentation after the period of 6 h about 5 mL of the fermentation solution was taken out aseptically and its pH was measured using a pH meter (S-PH200 model:ST3100, Ohaus corporation, Parsippany, USA). To calculate acidity, fermentation solution (10 mL) was diluted with 50 mL distilled water and titrated to pH 8 using 0.1 N NaOH solution. The amount of titrate used for the neutralization was recorded. Titratable acidity was measured as lactic acid content [23, 24].

2.3. Proximate analysis

AOAC (Association of Official Analytical Collaboration) approved methods were used to determine proximate nutrient composition, i.e., crude ash (protocol no. 923.03), fiber (protocol no. 32–10), moisture (protocol no. 925.10), protein (protocol no. 920.87), and fat (protocol no. 920.85) [25]. Each parameter was analyzed in triplicate for both raw and fermented samples (n = 3).

2.4. Mineral analysis

Mineral analysis of raw and fermented puree of *C. carandas* was performed by the already described method by Hossain, 2015 [26]. Three replicates were performed per sample for each mineral (n = 3).

2.5. Phytochemical analysis and antioxidant activity

2.5.1. Total phenolic content

The water and ethanol extracts of *C. carandas* puree were evaluated for total phenolic contents by Folin-Ciocalteu (FC) colorimetric method [27]. In this experiment, ethanol was used as a blank and gallic acid as standard and absorbance was measured at 756 nm using a spectrophotometer (SPECORD 200 PLUS; VWR, Darmstadt, Germany).

2.5.2. DPPH radical scavenging activity

Determination of DPPH free radical scavenging activity of *C. carandas* fruit was done according to Qamar et al. [28]. Briefly, methanol was used for the preparation of 1 mM DPPH stock solution and was stored at –20 °C for further analysis. 0.1 mM DPPH working solution was obtained by mixing 10 mL stock and 90 mL methanol. In the test tubes, solution of 2 mL DPPH, 2 mL of graded methanol and 0.2 mL of extract were added. Test tubes were placed in the dark for incubation for a period of 30 min. After incubation, the absorbance was recorded at 517 nm via spectrophotometer (SPECORD 200 PLUS; VWR, Darmstadt, Germany). Moreover, for blank and standard recordings methanol and ascorbic acid were used, respectively. The DPPH inhibition activity (IC₅₀) of food grade extracts i.e., in ethanol and water, was determined using the following equation.

$$\% \text{ inhibition} = (\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100$$

2.5.3. Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power of *C. carandas* ethanol and water extracts was determined adopting the methodology of Benzei and Strain (1996) [29]. Firstly, 100 µL (1g/20 mL) ethanol and water extracts of *C. carandas* each were mixed with FRAP working solution (containing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/L HCl, and 20 mmol/L FeCl₃ in a ratio of 10:1:1, v/v/v) followed by incubation for 35 min at room temperature under dark to read the absorbance at 596 nm using spectrophotometer. The results values were given as Fe mmol/g of dried weight.

2.6. Anti-nutrients analysis

2.6.1. Determination of phytates contents

In order to prepare reference solution, initially stock solution was prepared by diluting sodium phytate (0.15 g) in deionized water (100 mL) and then 0.2 N HCl was diluted with aforementioned stock solution in series of 3–30 µg/mL (phytate phosphorus). Secondly, to prepare ferric solution about 0.2 g ammonium iron (III) sulfate dodecahydrate was mixed with 100 mL (2N HCl) and the total volume of the solution was raised up to 1000 mL with de-ionized (DI) water. Thirdly, potassium thiocyanate (KSCN) solution (15 % w/v) was prepared by adding 15 g (KSCN) in DI water and making total volume up to 100 mL. After that,

extraction of 0.06 g sample with 10 mL (0.2 N HCl) was performed. Out of this extracted solution about 0.5 mL was taken into test tube and then 1 mL of prepared ferric solution was added into the same test tube followed by adding 2 mL of DI water. After covering test tubes tightly, they were heated for 30 min in water bath and then cooled in ice water until it achieved room temperature. In the last step, 0.25 mL was taken from the already prepared KSCN solution and was added to the cooled test tube followed by proper mixing. After 30 min, the absorbance was recorded at 480 nm via spectrophotometer. The results were expressed as mg/100g dried weight [30,31]. Values were taken thrice and presented as mean.

2.6.2. Determination of tannins contents

C. carandas (0.5 g) puree powder was added with distilled water (75 mL), heated for 35 min, and centrifuged (25 min; 2000 rpm) to obtain the supernatant. After that, supernatant (1 mL), distilled water (75 mL), Folin-Denis reagent (5 mL), 35 % sodium carbonate (10 mL) were mixed together to have final volume of 100 mL by adding distilled water and then allowed to stand for 30–35 min at room temperature. A standard curve was obtained by making a stock solution of 1000 ppm (mg/dm³) tannic acid prepared by dissolving 100 mg of tannic acid in 100 mL of distilled water. The standard curve was developed using working solutions of 0, 10, 20, 40, 60, 80 and 100 ppm (mg/dm³) distilled water. The blue colored complex formed was measured at an absorbance of 760 nm via spectrophotometer [32,33].

2.6.3. Determination of oxalates contents

In the first step standard solution was prepared by adding 100 mg oxalic acid with diluting it up to 100 mL distilled water, using this standard solution, 0.1, 0.2 and so on up to 1 mL/mg solutions were obtained with proper dilution. A solution of 0.003 M KMnO₄ in 100 mL was prepared followed by the preparation of 2N H₂SO₄ in 500 mL distilled water. To formulate standard curve about 5 mL and 2 mL (0.003 M KMnO₄, 2 N H₂SO₄) were mixed with standard solutions (0.1, 0.2, up to 1 mg/mL) in two separate test tubes and were kept at 37 °C in incubation for about 10 min. For the preparation of 0.25 N HCl in 100 mL about 2.1 mL of HCl was diluted in 100 mL distilled water.

About 0.5 g of *C. carandas* powder was mixed with 30 mL (0.25 N HCl) and was subjected to boiling for 15 min in a water bath. Afterwards, its total volume was increased to 50 mL with already prepared 0.25 N HCl. About 1 mL was taken from this prepared *C. carandas* extract and 5 mL (2 N H₂SO₄), 2 mL (0.003 M KMnO₄) were added and mixed in volumetric flasks giving an incubation of 10 min at 37 °C. The absorbance was recorded at 519 nm via spectrophotometer [34].

2.6.4. Determination of saponins contents

In order to determine the saponins contents of *C. carandas* puree powder, vanillin solution was prepared by dissolving about 8 g of vanillin in 100 mL of ethanol. *C. carandas* sample (0.25 mL) was mixed with that prepared vanillin solution and 0.25 mL (72 % H₂SO₄). The mixture was heated in the water bath at 60 °C. After 20 min it was cooled in iced water. The sample absorbance was recorded at 560 nm via spectrophotometer and the results were calculated as mg/100 g dried weight. A standard curve was made from 100 mg of aescin dissolved in 100 mL of distilled water. Standard curve was prepared by using working solutions of 0, 10, 20, 40, 60, 80 and 100 ppm (mg/dm³) in distilled water [35].

2.7. Manufacturing of ice-cream

The ice cream was comprised of milk (56 %), cream (22.4 %), milk powder (10.5 %), sugar (10.5 %), and 0.3 % each stabilizer and emulsifier. All the aforementioned ingredients were homogenized using a homogenizer (T25 Ultra-Turrax, IKA, Staufen im Breisgau, Germany) at 5000 rpm for 5 min and then pasteurized between 75 and 80 °C for 10–12 min. After that, in order to have complete hydration of all

ingredients the pasteurized mix was aged at 4 °C for 24 h. Fruit puree concentrations in the ice creams optimized and set to 12 % (raw) and 6 % (fermented) based on pre-trials evaluating aroma, mouthfeel, and color. Higher levels of fermented puree led to excess acidity and off-flavors, while 6 % retained acceptability and improved functionality. Then, fruit purees (pre-pasteurized at 80 °C for 10 min) were added to the aged ice-cream mixes and ice-creams were produced using machine (Promag, ice cream batch machines, Italy) and stored in a freezer (−18 °C). The samples were subjected to physical, chemical, and sensory analyses [36].

The percentages for other ingredients were adjusted proportionally to maintain a total of 100 %.

2.8. Physical analysis of ice cream

Each physical attribute (viscosity, overrun, melting rate, pH, and acidity) was measured in triplicate for each formulation (T0, T1, T2). Mean ± standard deviation values are reported (n = 3).

2.8.1. Determination of overrun

Overrun of the ice cream was evaluated following the guidelines [37] by taking 100 mL cup as standard. The results were expressed in percentage using the following equation:

$$\text{Overrun \%} = (\text{Volume of ice-cream}) - (\text{volume of mix}) / \text{volume of mix} \times 100$$

2.8.2. Determination of melting rate

First dripping and complete melting time was measured by taking 25 g of tempered samples and then left to melt at 20 °C on a 0.2 cm wire mesh screen above a beaker. First dripping and complete melting times of the samples were accepted to be seconds [38].

2.8.3. Determination of pH and acidity

The pH and acidity were measured with same protocols adopted for fermentation solution [23,24].

2.8.4. Determination of viscosity

The viscosity of the ice cream mixes was determined at 4 °C using a digital viscometer (Brookfield LVDVE, MA, USA) with spindle number 4 [39]. Before determination, air was removed from the sample by stirring it gently.

2.8.5. Sensory evaluation

Ice cream made with 12 % raw and 6 % fermented puree were examined for sensory aspects wherein color, texture, aroma, mouthfeel, and overall acceptability were considered. The evaluation was done in respective laboratory at ambient temperature (25 ± 1 °C). by a panel of 10 judges (3 women and 7 men aged between 35–60 and 32–45, respectively) on a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Ice cream samples were served in coded cups in randomized order.

2.9. Statistical analysis

All the experiments were conducted in triplicates (n = 3) and expressed as mean ± standard error (S.D). Analysis of variance followed by Student's *t*-test was used to determine significant differences, and *p* < 0.05 was considered significantly different using GraphPad Prism (Graph Pad Software, V8, San Diego, CA, USA).

3. Results

3.1. Nutritional composition

Present investigation outlines *C. carandas* proximate composition on a fresh weight basis, revealing high moisture, protein, and fat, with a moderate amount of fiber and ash. In the case of fermented samples, results showed no change in moisture and carbohydrates contents, although declining trend was observed, decrease in fat but a rise in fiber, protein, and ash contents (Table 2).

Raw *C. carandas* puree noticed to be a great source of minerals including iron, magnesium, potassium, calcium, phosphorus, sodium, zinc, manganese, copper, and chromium in the range from 0.44 to 61 mg/100 g (Table 3). Importantly, fermentation observed to cause a significant increase in the levels of Zn (40.8 %), Na (25.6 %), and Mg (15.9 %) and a moderate increase in the levels of Ca (5.4 %) and P (6.2 %), compared to the raw *C. carandas* puree. Moreover, in the present analysis, iron levels experienced a slight increase (1.6 %) subsequent to fermentation, while potassium and chromium levels were decreased and manganese remained unchanged.

3.2. Antinutrients

Importantly, analysis of the raw (T_0) and fermented (T_1) samples depicted a significant decrease in the concentrations of several anti-nutrients among fermented samples. Starting with oxalates, the raw sample contained 638 (mg/100g), while the fermented sample showed reduced levels of 605 (mg/100g), reflecting a 5.06 % decrease. Among all anti-nutrients studied, phytates showed the most significant reduction wherein its level decreased from 352 (mg/100g) in the raw sample to 328 (mg/100g) in the fermented sample. Similarly, saponins concentration was reduced from 407 (mg/100g) in the raw sample to 388 (mg/100g) in the fermented sample. However, tannins exhibited the smallest reduction among the studied anti-nutrients as their levels dropped from 1405 mg/100g in the raw sample to 1378 mg/100g in the fermented sample (Table 4).

3.3. Phytochemical contents and antioxidant activity

Results presented in Table 5, showing phytochemical and antioxidant potential of two green solvent extracts i.e., water and ethanol for both raw and fermented *C. carandas* puree. Declining impact of fermentation was observed on aforementioned parameters. However, a direct relationship noticed between total phenolic content and antioxidant activity (see Table 6).

3.4. Physical and organoleptic properties

The incorporation of raw and fermented *C. carandas* puree exhibited

Table 1
Adjustments in ice cream mix composition with raw and fermented *Carissa carandas* puree.

Ingredient (%)	T_0	T_1	T_2
Milk	56.0	49.3	52.6
Cream	22.4	19.7	21.1
Milk Powder	10.5	9.2	9.9
Sugar	10.5	9.2	9.9
Stabilizer	0.3	0.3	0.3
Emulsifier	0.3	0.3	0.3
Raw <i>C. carandas</i> Puree	–	12.0	–
Fermented <i>C. carandas</i> Puree	–	–	6.0
Total	100.0	100.0	100.0

T_0 , Standard Recipe (%).

T_1 , Ice cream with 12 % Raw Puree (%).

T_2 , Ice cream with 6 % Fermented Puree (%).

distinct effects on ice cream's physical properties. Compared to the control (T_0), both T_1 and T_2 resulted in a decrease in overrun and pH, and an increase in viscosity and acidity. Notably, the fermented puree (T_2) led to a more pronounced drop in pH and overrun compared to the raw puree (T_1), while the raw puree showed slightly lower melting rate. These differences indicate the unique functional impact of raw versus fermented puree.

The results of sensory evaluation related to ice cream samples i.e., T_0 (control), T_1 (with 12 % raw puree), and T_2 (with 6 % fermented puree) are available in Table 7. It has been observed that variation in puree type had a variable impact on organoleptic aspects. Though, all the tested recipes were rated as acceptable by our panel of sensory experts. In detail, highest color and aroma scores were given to the T_1 , followed by T_2 , and T_0 (due to the absence of puree) outlining the impact of *C. carandas* puree on olfactory perception. Moreover, regarding the texture and mouthfeel, T_0 received the highest score, followed by the T_1 , while the T_2 had a slightly lower texture rating due to minor structural differences introduced by fermentation. The aroma score was also highest for the T_0 , followed by the T_1 , which was well-balanced between sweetness and tanginess. T_2 had a slightly lower aroma score due to its mild acidic undertone. Regarding overall acceptability, T_1 was the most preferred among the supplemented formulations, though the control remained the highest-rated due to its classic ice cream profile. It is important to note that sensory evaluation was conducted using a small, untrained panel ($n = 10$), which may limit the generalizability of results.

4. Discussion

The analysis revealed *C. carandas* is rich in moisture (88.0 %), protein (3.8 %), and fat (3.2 %), with a moderate amount of fiber (1.36 %) and ash (1.1 %). Results are in accordance with the previous findings [40–43] recorded higher amounts of moisture, protein, and fat among *C. carandas* on a fresh weight basis, comparing to fiber and ash contents. Prominently, fermentation influenced the proximate composition wherein it caused a slight decline in moisture, fat, and carbohydrates, but a rise in fiber, protein, and ash contents (Table 1). The reduction in fat contents might be due to prolonged fermentation, which causes the breaking down of fats and the utilization of lipids as a source of energy by microbes [44]. Fermentation in *C. carandas* caused an increase in protein contents due to hydrolysis, which may result in the release of proteins [45]. Children need protein for growth and development, and fermentation enhances the body's ability to break down and absorb it [46]. However, carbohydrates serve as the primary fuel source for the human body. Present analysis found that unfermented samples of *C. carandas* contained more carbohydrates than fermented samples. The reduction in carbohydrates observed during fermentation is a result of the microbes metabolizing starch, leading to a drop in the carbohydrate content [45]. These results align with the observations made by Nimra et al. [47]. Adeela et al. [48] and Younis et al. [49] reported elevated levels of protein, fiber, and ash and a decline in fat and carbohydrate contents as a result of fermentation.

Moreover, fermentation also resulted in a significant increase in the levels of Mg (15.9 %), Zn (40.8 %), and Na (25.6 %) and a moderate increase in the levels of Ca (5.4 %) and P (6.2 %), compared to the raw *C. carandas* puree, in accordance with Olagunju et al. [50] corroborated this observation by reporting that fermented tamarind seeds contain higher mineral levels, such as calcium (11.6 %), magnesium (10.3 %), zinc (8 %), phosphorus (9.3 %), iron (33.3 %), copper (20 %), and manganese (13.8 %). Onwurafor et al. [51] similarly observed that fermentation influenced mineral concentrations, resulting in fluctuations in calcium (8.8 %), iron (22.8 %), and zinc (–19.7 %). The disintegration of anti-nutrient–mineral chelation complexes, which is facilitated by the metabolic activity of fermenting microorganisms, is likely the cause of the increase in mineral content from fermentation [52]. Furthermore, the increase in mineral content can be attributed to the reduction in dry-matter content that occurs as a consequence of the

Table 2

Proximate composition of fermented and raw puree (fresh weight %).

Treatment	Moisture	Protein	Crude Fat	Crude Fiber	Ash	Carbohydrate
T ₀	88.0 ± 0.1 ^a	3.8 ± 0.1 ^b	3.2 ± 0.2 ^b	1.4 ± 0.1 ^b	1.1 ± 0.1 ^b	2.6 ± 0.1 ^a
T ₁	87.7 ± 0.2 ^a	4.0 ± 0.1 ^a	2.4 ± 0.1 ^a	1.9 ± 0.2 ^a	1.5 ± 0.2 ^a	2.5 ± 0.3 ^a

T₀, Raw. T₁, Fermented. Values are presented as mean ± standard deviation. Different letters between columns showed a significant difference between treatments.**Table 3**Mineral composition of raw and fermented *C. carandas* puree.

Minerals (mg/100g)	Treatment	
	T ₀	T ₁
Calcium	14.2 ± 0.2 ^b	15.01 ± 0.05 ^a
Iron	61.24 ± 0.25 ^b	62.3 ± 0.5 ^a
Phosphorous	10.44 ± 0.09 ^b	11.1 ± 0.2 ^a
Sodium	10.16 ± 0.02 ^b	12.8 ± 0.2 ^a
Magnesium	19.5 ± 0.1 ^b	22.58 ± 0.04 ^a
Potassium	19 ± 2 ^a	15.80 ± 0.02 ^b
Manganese	1.63 ± 0.01 ^a	1.65 ± 0.01 ^a
Zinc	6.66 ± 0.01 ^b	9.36 ± 0.06 ^a
Copper	0.88 ± 0.01 ^a	1.04 ± 0.03 ^a
Chromium	0.44 ± 0.02 ^a	0.25 ± 0.01 ^a

T₀, Raw. T₁, Fermented. Values are presented as mean ± standard deviation. Different letters between rows showed a significant difference between treatments.**Table 4**

Anti-nutrient composition of raw and fermented puree.

Treatment	Anti-nutrients (mg/100g)			
	Oxalates	Phytates	Saponins	Tannins
T ₀	637.6 ± 2.5 ^a	352.3 ± 2.5 ^a	407 ± 4 ^a	1405 ± 5 ^a
T ₁	605 ± 2 ^b	328 ± 2 ^b	388 ± 3 ^b	1378 ± 3 ^b

T₀, Raw. T₁, Fermented. Values are presented as mean ± standard deviation. Different letters between columns showed a significant difference between treatments.**Table 5**Phytochemical and antioxidant activity of raw and fermented *C. carandas* puree.

Type of puree	Extract	TPC mg GAE/g	DPPH % inhibition
T ₀	Ethanol	198.0 ± 1 ^a	55.1 ± 0.1 ^a
	Water	152.1 ± 0.2 ^c	47.3 ± 0.5 ^b
T ₁	Ethanol	175.1 ± 0.1 ^b	49.2 ± 0.3 ^b
	Water	143.0 ± 1 ^c	38.1 ± 0.2 ^c

T₀, Raw. T₁, Fermented. Values are presented as mean ± standard deviation. Different letters between columns showed a significant difference between treatments.

degradation of proteins and carbohydrates during fermentation [53]. Moreover, in the present analysis, iron levels experienced a slight increase (1.6 %) subsequent to fermentation, while potassium, chromium, and manganese levels decreased. This decline may have been a result of their conversion to less soluble forms or their loss during the fermentation process.

Likewise, fermentation caused a notable decline in several anti-

nutrients i.e., oxalates, phytates, saponins, tannins in *C. carandas* puree that may adversely impact the bioavailability of nutrients. Phytates mainly found in cereal based foods are known as nutrient inhibitors due to their potential to hinder the absorption of important minerals i.e., iron, calcium, and zinc by digestive system [54]. Next, tannins are basically present in all plants and are regarded to disturb the normal functioning of enzymes named lipase, amylase, trypsin, and chymotrypsin [55]. Saponins are bitter, at high doses are not safe to consume, and exhibit the abilities to block nutrient absorption mainly iron, zinc, and vitamin E along with other essential chemicals [56]. Oxalate (oxalic acid) forms insoluble salts when it combines with minerals including sodium, potassium, calcium, iron, and magnesium. Plants appear to produce oxalates for three main reasons including managing calcium levels, defense, and to detoxify heavy metals [57]. In our study, 5.02 %, 6.82 %, 4.68 %, 1.92 % reduction was noticed among oxalates, phytates, saponins, and tannins in fermented puree separately. Fermentation lowers the pH to 4–5 that results in the activation of endogenous phytase in addition to bacterial phytates activity resulting in the reduction of phytates. Moreover, saponins reduction may be linked to β -glucosidase activity causing its structural degradation [58,59]. Aforementioned reductions specify a directional trend toward reduced antinutritional content, but the absolute reductions were modest and may not translate to substantial biological impact when consumed in typical serving sizes. Previous studies which showed that fermentation treatment causes a significant decrease in phytates (78 %), oxalates (72 %) and alkaloids (66 %). In addition, 86 % and 89 % reduction in phytates and tannin contents were also reported by fermentation treatment [47–49]. Therefore, although fermentation shows potential for improving nutritional quality, further optimization may be required to achieve greater detoxification. Importantly, increase in nutritional and reduction in toxicants after fermentation did not impact the total phenolic contents and antioxidant activity. Ethanol and water extracts of *C. carandas* raw puree showed slightly higher potential as compared to ethanol and water extracts of fermented puree. The antioxidant activity was found in accordance with previous findings of [9] wherein *C. carandas* ethanol extract outlined notable potential in DPPH, FRAP, CUPRAC, and ABTS assay. In another investigation, *C. carandas* water extract outlined antioxidant activity comparable with standards i.e., curcumin and ascorbic acid in nitric oxide and DPPH assay, supports the findings of present research [10].

In the present study, physical parameters of ice cream samples made with raw (T₁) and fermented (T₂) *C. carandas* puree were not significantly different from raw samples (T₀). The addition of raw (12 %) and fermented (6 %) puree led to decrease in pH from 6.4 to 5.5 (raw) and 6.4 to 5.1 (fermented). Moreover, reduction was also recorded for melting rate (g/min) and overrun (39.9 %–35.2 %), but increase in acidity (0.11–0.20 %) and viscosity (76.2–81.1 cP). Overrun and viscosity are important attributes that may influence the texture,

Table 6Physical analysis of the *C. carandas* puree-based ice-cream formulations with raw and fermented puree.

Treatment	Viscosity (cP)	Overrun (%)	Melting rate gram/minute	pH	Acidity (% lactic acid)
T ₀	76 ± 1 ^b	40 ± 1 ^a	4.4 ± 0.1 ^a	6.4 ± 0.2 ^a	0.10 ± 0.02 ^a
T ₁	80 ± 1 ^a	37.8 ± 0.3 ^a	3.7 ± 0.2 ^b	5.5 ± 0.9 ^b	0.18 ± 0.01 ^b
T ₂	81.2 ± 0.3 ^a	35.2 ± 0.3 ^b	4 ± 1 ^a	5.1 ± 0.4 ^b	0.20 ± 0.01 ^b

T₀, Control. T₁, Raw. T₂, Fermented. Different letters between columns showed a significant difference between treatments.

Table 7Sensory evaluation of ice cream with raw and fermented *C. carandas* puree on a 9-point hedonic scale.

Treatment	Color	Texture	Mouthfeel	Aroma	Overall acceptability
T ₀	4.9 ± 0.1 ^c	8.4 ± 0.2 ^a	8 ± 1 ^a	5.8 ± 0.1 ^b	8 ± 1 ^a
T ₁	8.1 ± 0.2 ^a	8.1 ± 0.1 ^b	8.1 ± 0.1 ^a	8.20 ± 0.01 ^a	8 ± 1 ^a
T ₂	6.8 ± 0.1 ^b	7.2 ± 0.5 ^c	6.9 ± 0.3 ^b	6.9 ± 0.2 ^b	7 ± 2 ^b

T₀, Control. T₁, Raw. T₂, Fermented. Values are presented as mean ± standard deviation. Different letters between columns showed a significant difference between treatments.

mouthfeel, and overall quality of ice cream. In current investigation, decrease in overrun is due to the decline in fat contents in recipe after the addition of *C. carandas* puree. In previous reports by Refs. [3,36,60] recorded decreasing trend in overrun among ice cream samples made with amla, kiwifruit, blackberry, and raspberry fruit purees. Aziz et al. [61] believed that overrun is an integral aspect that influence physical parameters of product and reduction in fat content led to decrease in overrun. Moreover, increase in solid contents led to rise in viscosity after addition of minerals rich *C. carandas* puree. The results are in alliance with the previous findings wherein addition of fruit purees including gooseberry, minced blackthorn, besnigrape, sloe berry, blackberry, and raspberry caused to increase the viscosity of ice cream [36,62–65]. In summary, *C. carandas* addition came out as potential option to be a functional ingredient in addition to improving the sensory attributes by maintaining the physical features.

5. Conclusion

The development of ice cream using *C. carandas* puree identifies the prospective of this unappreciated and wildly grown berry for the functional food industry. *C. carandas* is rich in antioxidant compounds and minerals as well as source of natural color. On the other hand, presence of certain antinutrients mainly tannins, phytates, oxalates, and saponins hinders the bioavailability of certain nutrients and may limit its broader application in food products. Conversely, fermentation came out as wonderful technique to downregulate aforementioned toxicants and increase the body absorption of nutrients and making *C. carandas* potential ingredient for functional food formulations, although these claims remain preliminary and require further validation through in vivo or clinical studies. Importantly, *C. carandas* wild growth and resilience pave way for its commercial cultivations that may offer farmers a high-value low-input crop with great economic benefits. Controlled cultivation of *C. carandas* can provide farmers an improved fruit quality with better yields and ultimately access to emerging market of functional foods. Moreover, investing in the commercial production of *C. carandas* can contribute to both agricultural sustainability and the food industry, positioning it as an important crop for future food innovations.

CRediT authorship contribution statement

Wisha Saeed: Writing – original draft, Visualization, Investigation, Formal analysis. **Muhammad Qamar:** Writing – review & editing, Visualization, Software, Investigation, Formal analysis. **Tariq Ismail:** Writing – review & editing, Visualization, Validation, Resources. **Muhammad Zulqarnain Khan:** Investigation, Formal analysis. **Tuba Esatbeyoglu:** Writing – review & editing, Funding acquisition.

Consent to publish

All authors consented to publish this work with Journal of Agriculture and Food Research.

Ethics approval and consent to participate

This study does not involve human subject and consent to participate

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All data supporting the findings of this study are available within the paper.

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