Effect of incorporation of *Cyperus rotundus* L. rhizome powder on quality attributes of minced beef meat

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Abstract The primary objective of this study was to investigate the biological effectiveness of *C. rotundus* rhizome powder (CRRP) in improving the quality, storability, and safety of minced beef meat. CRRP was analyzed for its flavonoid and polyphenol content, antioxidants and antimicrobial activity, and its effect on the chemical properties, microbiological and sensory quality of minced beef meat. The results revealed that CRRP had a flavonoid and polyphenol contents of 24.30 mg CE/g and 353.10 mg GAE/g, respectively. The aqueous extract of CRRP showed inhibition zones of 16.3 and 11.7 mm against *E. coli* and *S. aureus*, respectively. The CRRP exhibited greater increase in TRPA, chelation of Fe^{2+} ions, and scavenging of H_2O_2 with the increase in CRRP extract concentration. Incorporation of various concentration of CRRP in minced beef meat significantly affected the physicochemical properties, microbial load, and sensory quality of the product. The effect was concentration dependent, and the highest level of CRRP (5 %) was more effective in retarding microbial growth and reducing peroxidation, but it negatively affected the sensory quality of the product. Therefore, CRRP at 2.5 % concentration was recommended as a functional additive to preserve minced meat.

Keywords Antimicrobial · Antioxidant · *Cyperus rotundus* · Minced beef meat · Peroxide value

Introduction

In the last decades, the production and consumption of minced meat products received great demands worldwide as it used in various fast foods. The rapid growth in the fast food market, the substantial nutritional quality of meat products as it considered as a primary source of protein, vitamins, and minerals in the diets, and the palatable flavors, aromas, and appearance of these products are the main reasons behind the increased demands for minced meat products. Subsequently, the international trade of minced meat products has focused attention on objective measures of the safety and quality attributes of these commodities. In an intact form of a meat piece, microorganisms are usually on the surface only, and oxygen penetrates only a few millimeters thus the interior is prevented from microbial growth and lipid peroxidation (Honikel 2014). However, mincing meat disrupts the integrity of muscle membrane, increases the surface area, and promotes oxygen and microorganisms distribution throughout the mince and thus decreases the shelf life of stored minced meat (Honikel 2014). Microbial spoilage and lipid peroxidation are the chief causes of food deterioration, poisoning, and wastages, and thus, they strongly affected the safety, nutritious, sensory, and economic potentials of stored minced meats (Jayawardana et al. 2015). Hence, limiting lipid peroxidation and microbial growth in minced meat is a vital approach to maintaining the safety, nutritional and sensory qualities, and economic potentials of these products. Accordingly, numerous researchers have investigated the utilization of natural or synthetic

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antioxidant and antimicrobial agents for controlling lipid peroxidation and microbial activity in minced meat products (Magrinyà et al. 2015; Tang et al. 2001). Synthetic antioxidants and antimicrobials are widely used in the food industry, but their safety is questioned by both human health professionals and consumers (Tang et al. 2001), which have prompted strict regulations for their use in food formulations. Consequently, the development and use of naturally occurring antimicrobial and antioxidant compounds or extracts derived from plant sources to meat products have increased in recent years due to their potential health benefits and safety (Das et al. 2012). In addition, natural antioxidants and antimicrobial have greater application potential for consumer's acceptability, palatability, stability, and shelf life of minced meat products (Jung et al. 2010). For the above reasons and alongside with increased consumer worries on the use of synthetic additives, researches on the application of natural antioxidants and antimicrobials in minced meat products are critical for both consumers and meat processors.

Cyperus rotundus L. (family: Cyperaceae) is one of the most invasive, problematic, economically damaging, and widely distributed agronomic weeds in tropical, subtropical, and temperate regions of the globe (Pirzada et al. 2015). However, this weed is considered as a favorable medicinal herb that has traditionally been used for treating numerous clinical diseases such as diarrhea, diabetes, pyresis, inflammation, malaria, and stomach and bowel disorders (Ahmad et al. 2012; Pirzada et al. 2015). Phytochemical and pharmacological studies revealed that *C. rotundus* contains a high concentration of essential oils, phenolic acids, ascorbic acids, and flavonoids demonstrating its antibacterial, anticancerous, antidiabetic, antidiarrheal, antiinflammatory, antilipidemic, antimalarial, antimutagenic, antiobesity, antioxidant, cardioprotective, and neuroprotective capabilities (Pirzada et al. 2015). This weed is also considered as an appetizer, thirst reliever and food flavor enhancer (Das et al. 2015; Pirzada et al. 2015). Toxicological studies demonstrated that rhizomes of *C. rotundus* are not toxic and reliably safe for use in various applications (Badgujar and Bandivdekar 2015; Pirzada et al. 2015; Sheik et al. 2013).

In Sudan, *C. rotundus* (locally known as Siada) is the worst weed that widely distributed throughout the country and adversely affecting most of the cultivated crops. However, its tubers are traditionally used in treating stomach disorders, bowels irritation, dyspepsia, diarrhea, dysentery, ascites, vomiting, cholera, wounds, ulcers, sores, and scorpion stings (El-Ghazali et al. 1994; Kabbashi et al. 2015). Recent investigations demonstrated that the whole plant extracts and rhizome oils of *C. rotundus* collected from different areas of the Sudan exhibited several phytochemicals having substantial antimicrobial and

antioxidant capabilities (Eltayeib and Um Ismaeel 2014; Kabbashi et al. 2015). Although much research has been done on the pharmacological, phytochemicals, antimicrobial, and antioxidant properties of *C. rotundus*, its application as a preservative in meat and meat products has not been studied. Therefore, this study was conducted to investigate the utilization of *C. rotundus* rhizome powder as a natural source of antioxidant and antimicrobials to extend the shelf life of minced beef meat and to determine its influence on the physicochemical, microbial, and sensory attributes of the formulated products.

Materials and methods

Materials

The fresh beef meat was purchased from Bahri meat market, Khartoum North, Sudan. *C. rotundus* rhizome was obtained from the Agricultural Demonstration Farm, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan. Spices were purchased from the local market, Khartoum North, Sudan. All chemical were of analytical grade.

Preparation of CRRP methanolic and water extract

The rhizomes of *C. rotundus* were cleaned from dust and foreign materials by hand and separate other parts of the plant, washed with water and then distilled water, dried at room temperature and then crushed by electrical machine into a fine powder and kept in polyethylene bags. To prepare methanolic and water extracts, 30 g of CRRP was extracted with either 300 ml methanol–water (4:1, v/v) or 150 ml of ddH₂O, at room temperature (25 °C) for 5–8 h using an orbital shaker. The extract was then filtered through filter paper and centrifuged (Hettich Zentrifugen, Tuttlingen, Germany) at 4000 × g for 10 min. The supernatant was concentrated under reduced pressure at 40 °C for 3 h using a rotary evaporator (IKA-WERKE-RV06ML) and kept at 4 °C until used for analysis.

Minced beef sample preparation

Fresh beef meat was sliced then minced by electric blender and stored at 4 °C. The minced beef samples were then mixed with different concentrations of CRRP (0, 2.5, and 5 %). Immediately after preparation (day 0), samples from the different treatment were taken for microbiological and physicochemical analysis. The rest of the samples were stored at 4 °C for 5 and 10 days. At the end of each storage period, samples were taken from each treatment and subjected physicochemical, microbiological and sensory analysis.

pH measurement

Samples were periodically removed from storage and homogenized in distilled water (1:10 dilution). The mixture was filtered through Whatman number 1 filter paper to obtain a clear filtrate for pH measurement. The pH was measured using a digital pH meter (model 210, HANNA instruments microprocessor pH meter).

Approximate analysis

Moisture, crude protein, fat, ash and crude fiber contents of the samples were determined according to the official methods (AOAC 2003). Carbohydrates were calculated by subtracting the sum of moisture, protein, ash, fat, and fiber from 100 as described elsewhere (Grosso et al. 2000).

Peroxide value determination

Peroxide value was determined according to the standard official method (AOAC 2003). Briefly, 50 g of minced beef meat samples were macerated with 200 ml chloroform for 8 h and then filtered through Whatman No. 4 filter paper. After that, a mixture of filtrate and chloroform/acetic acid (2:1.5, v/v) is reacted with a saturated potassium iodide solution in the dark. The released iodine was titrated with a sodium thiosulfate solution, and the peroxide value was then expressed as milliequivalents of active oxygen per kilogram of the sample (meq/kg).

Determination of total flavonoids

The determination of flavonoids was performed according to the colorimetric assay as described by Kim et al. (2003). Briefly, distilled water (4 ml) was added to 1 ml of CRRP methanolic extract. Then, 0.3 ml of 5 % sodium nitrite solution was added, followed by 0.3 ml of 10 % aluminum chloride solution. After that, the mixture was incubated at ambient temperature for 5 min, and then 2 ml of 1 M sodium hydroxide was added and immediately the volume of reaction mixture was made up to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink color developed was determined at 510 nm. A calibration curve was prepared with catechin, and the results were expressed as mg catechin equivalents per g sample (mg CE/g).

Determination of polyphenols

Total polyphenols of CRRP extract were determined according to Purssion Blue Spectrophotometric method (Price and Butler 1977). The results were expressed as

milligram gallic acid equivalents per g sample (mg GAE/g).

Determination of antioxidant activities

The antioxidant activities of CRRP were measured in vitro using three complementary methods, namely: the total reducing power method, the chelation of Fe^{2+} ions, and the hydrogen peroxide scavenging activity as described elsewhere (Mohamed et al. 2014). All readings were made spectrophotometrically using UV–VIS spectrophotometer (1061-Shimadzu, Japan).

Microbiological analysis

Determination of microbial load, coliform bacteria, *Escherichia coli* and *Staphylococcus aureus* counts were carried out using the method described by Harrigan (1998). Briefly, 10 g of each sample was homogenized with 90 ml of sterile peptone water (0.1 %). Appropriate serial dilutions were prepared, and 1 ml of each dilution were inoculated onto total plate count (Petrifilm^R 3 M), MacConkey broth and Brilliant green bile lactose broth, *E. coli* count plates (3 MTM Petrifilm), Baird Parker medium (Oxoid, UK) to obtain the total plate count, coliform count, *E. coli* and *S. aureus* count, respectively. The plates were incubated at 37 °C for 24–48 h.

The antibacterial activity of CRRP water extract against pure cultures of *Escherichia coli* and *Staphylococcus aureus* was measured following the disc diffusion method (Kilani et al. 2005). Briefly, the impregnated filter paper discs with the CRRP extract were suitably placed on the surface of the plate containing indicator organisms. Subsequently, the plates were incubated at 37 °C for 18–24 h, and they were then examined for the presence of inhibition zones of the bacterial growth around discs.

Sensory evaluation

Sensory analysis was done using a 7-point hedonic scale as described by Trindade et al. (2009). In this assay, the samples were randomly selected, cooked and kept warm for evaluation. Then, 13 semi-trained panelists (6 males and 7 females, age between 25 and 35) were used to evaluate the samples in terms of color, texture, flavor, juiciness and overall acceptability. A cut-off score of 4.0 was set for the rejection of the sensorial parameter measured, i.e., any sensory parameter that scores less than 4.0 is rendered unacceptable. Every treatment was given a code number and placed under natural light. Tap water was available for panelist used to flush mouth after each sample testing.

Statistical analysis

A completely randomized block design with three treatments (control, 2.5 % CRRP, and 5 % CRRP) was used, and the measurements were taken on three storage days (0, 5, and 10 days). Three independent replicates for different treatments and storage times were used to determine the effect of treatments, storage periods, and the interaction between them on the physicochemical, microbial, and sensory characteristics of minced beef meats. The data collected from different treatments was subjected to analysis of variance and whenever appropriate the mean separation procedure of LSD was employed (Steel and Torrie 1980). The SAS program (SAS, v. 8.1; SAS Institute Inc., Cary, NC) was used to perform the General linear method (GLM) analysis, and significances were accepted at $P \leq 0.05$.

Results and discussion

Chemical composition and antimicrobial activities of CRRP

The chemical composition and antimicrobial activities of CRRP are presented in Table 1. The results showed that CRRP contained high amounts of carbohydrates (63.70 %) and considerable amounts of moisture (12.30 %), fiber (8.80 %), protein (6.17 %), oil (5.40 %), and ash (3.67 %). These results were in disagreement with those reported previously for the tubers of *C. rotundus* grown in Nigeria (Emelugo et al. 2011). These variations could be attributed to the differences in the environmental conditions mainly

temperature and soil fertility as well as to agronomic practices such as irrigation and fertilization.

The phytochemical analysis of the aqueous extract of CRRP revealed the presence of considerable quantities of total polyphenols (353.10 mg GAE/g) and flavonoids (24.30 mg CE/g) (Table 1). The polyphenols contents in this study were higher than those reported previously for various CRRP extracts (Bashir et al. 2012; Jain and Aggarwal 2012), while the flavonoids contents were comparable to those reported by Bashir et al. (2012). However, the polyphenols in the current study were within the range 260–670 mg GAE/g, whereas the flavonoids are lower than the range 200–340 mg QE/g of CRRP extracted with various solvents (Kilani-Jaziri et al. 2011). Bashir et al. (2012) observed that different parts of *C. rotundus* had different amounts of polyphenols and flavonoids with the rhizome having the highest contents. The discrepancies in the phytochemical content of *C. rotundus* between these studies could be attributed to varietal differences, agronomical practices, environmental condition, and stage of maturity alongside with the extraction method and solvents. The tuber and rhizomes of *C. rotundus* contain high concentration of active ingredients such as essential oils, cyperol, furochromones, phenolic acids, ascorbic acids, alkaloids, saponins, sesquiterpenes, sitosterols, myristic acid, nootkatone, tannins, coumarins, and flavonoids with several therapeutic, pesticidal, insecticidal, and fungicidal capabilities (Pirzada et al. 2015).

The in vitro antibacterial activity of aqueous extract of CRRP against a Gram-positive (*Staphylococcus aureus*) and a Gram-negative (*Escherichia coli*) bacteria revealed potential activity against the two strains (Table 1). Water extract showed inhibition zones of 16.3 and 11.7 mm against *E. coli* and *S. aureus*, respectively, indicating high and mild antimicrobial activities against the two organisms. The antimicrobial activity of CRRP extract can be attributed to its polyphenols and flavonoids. Aqueous extract of CRRP of the current study was more potent against *E. coli* compared to the ethanolic extract (7.0 mm inhibition zone) of *C. rotundus* as reported previously (Ahmad et al. 2012). Kilani-Jaziri et al. (2011) indicated that *C. rotundus* extracts possess an inhibitory effect on the growth of *Enterococcus faecalis*, *S. aureus*, *Salmonella typhimurium*, *Salmonella enteritidis*, and *E. coli*. By contrast, Sharma and Singh (2011) stated that *E. coli* was the most resistant gram-negative bacteria against all *C. rotundus* extracts. The antibacterial activities of *C. rotundus* was attributed to the presence of polyphenols, tannins, coumarins and flavonoids in the crude extracts of this weed (Kilani-Jaziri et al. 2011). The antibacterial effects of CRRP may pave the way for its application as a natural preservative in food products.

Table 1 Chemical composition and antimicrobial activity of CRRP

Parameters	Values
Chemical composition	
Moisture content (%)	12.30 ± 0.80
Ash content (%)	3.67 ± 0.06
Crude protein (%)	6.17 ± 1.25
Oil content (%)	5.40 ± 0.80
Crude fiber (%)	8.80 ± 0.20
Carbohydrates (%)	63.70 ± 1.55
Flavonoids (mg CE/g)	24.30 ± 0.16
Polyphenols (mg GAE/g)	353.10 ± 0.80
Antimicrobial activity (mm inhibition zone)	
<i>Staphylococcus aureus</i>	11.7 ± 0.25
<i>Escherichia coli</i>	16.3 ± 0.15

Values are mean ± SD of triplicate samples

Antioxidant capacity

The results of total reducing power ability (TRPA), chelation of Fe^{2+} ions and scavenging of H_2O_2 of *C. rotundus* rhizome extract (CRRE) are shown in Table 2. The greater ($P \leq 0.05$) increase in TRPA, chelation of Fe^{2+} ions, and scavenging of H_2O_2 with the increased in CRRP extract concentration indicated that these assays were concentration dependent. The magnitude of the increased of TRPA, chelation of Fe^{2+} ions, and scavenging of H_2O_2 were 15-, 4-, and 2-folds, respectively, following the increased in CRRP extract concentration from 125 to 1000 $\mu\text{g/ml}$. Similar observation on the increase of TRPA, chelation of Fe^{2+} ions, and scavenging of H_2O_2 with the increased in the concentration of *C. rotundus* extracts were previously reported (Bashir et al. 2012; Jain and Aggarwal 2012). The activity of antioxidants could be assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock 1997). Jahan et al. (2013) assumed that CRR has a good combination of phenolic acid (Gallic, P- Coumaric, Ferulic Chlorogenic acid) and flavonol (Quercetin, Kaempferol Myricetin) which would contribute collectively to its strong antioxidant capacity. The results of the current study demonstrated an antioxidant potential of CRRP that could pave the way for its uses in food formulation as therapeutic agents in preventing or slowing the progress of aging and age-associated oxidative stress-related degenerative diseases. The overall antioxidant activity of CRRP might be attributed to its polyphenolic content and other phytochemicals constituents.

Approximate composition of minced beef incorporated with CRRP

Incorporation of CRRP in minced meat significantly ($P \leq 0.05$) affected the chemical composition of the product (Table 3). Crude protein, fat and ash content of minced beef concomitantly ($P \leq 0.05$) increased with the increase of CRRP concentration, whereas, moisture content showed a simultaneous ($P \leq 0.05$) reduction. The increase

in the protein, fat, and ash contents of minced beef meat extended with CRRP could be attributed to the reduction in moisture content that might lead to the concentration of these constituents in treated samples. In addition, CRRP contained appreciable amounts of protein, fat, and ash, which could also contribute to the increment of these elements in the CRRP-containing minced meat. The inverse relationship between moisture content and CRRP concentration may be related to the increase in the total soluble solids, or probably other mechanisms like increased moisture binding ability or modification of the pH of minced beef—CRRP system. Recent reports showed a similar trend of the decrease of the moisture content of beef patties following the increase of moringa seed flour (Al-Juhaimi et al. 2016) and Bambara groundnut seed flour (Alakali et al. 2010). The same reports also indicated that the incorporation of moringa seed powder or Bambara groundnut seed flour showed increases in the protein, fat and ash contents of the patties (Alakali et al. 2010; Al-Juhaimi et al. 2016).

Peroxide and pH values of minced beef extended with CRRP

The peroxide value of minced meat increased ($P \leq 0.05$) with the increase of storage period, however it decreased ($P \leq 0.05$) with the increase of CRRP level (Table 4). At 0 day of storage, a minced beef sample with or without CRRP had similar ($P \geq 0.05$) peroxide values, most probably this was due to insufficient contact time i.e. the time elapsed from the incorporation of CRRP to the time of PV measurement. The inclusion of CRRP in the minced beef formulation significantly ($P \leq 0.05$) delayed lipid oxidation. The changes in peroxide values was followed with the change in storage period, from 0 to 5 and from 5 to 10 days within each treatment i.e. control 0, 2.5, or 5 % CRRP. Within samples treated with CRRP, it is worth noting that the rate of change in peroxide value settled in the test storage period i.e. irrespective of CRRP level. Similarly, several reports revealed that incorporation of moringa leaves and seeds extracts in meat products significantly reduced lipid oxidation during cold storage (Al-Juhaimi et al. 2016; Das et al. 2012; Shah et al. 2014).

Table 2 Total reducing power ability (TRPA), chelation of Fe^{2+} ions, and scavenging of hydrogen peroxide at different concentration of water extract of CRRP

Parameter	Concentration of CR extract ($\mu\text{g/ml}$)			
	125	250	500	1000
Total reducing power ability (m/ml)	2.87 ± 0.06^d	6.10 ± 0.30^c	18.47 ± 0.15^b	45.90 ± 0.10^a
Chelating of Fe^{2+} ions (%)	20.33 ± 0.06^d	44.73 ± 0.38^c	64.00 ± 0.60^b	83.00 ± 0.40^a
Hydrogen peroxide scavenging (%)	26.37 ± 0.47^d	44.63 ± 0.35^c	50.93 ± 0.31^b	56.07 ± 0.40^a

Values are mean $\pm$ SD of triplicate samples. Mean in a row having different superscript letter(s) are significantly different ($P \leq 0.05$)

Table 3 Approximate composition of minced beef extended with CRRP

Parameter	Level of CRRP		
	0 %	2.5 %	5.0 %
Moisture content (%)	76.57 ± 0.15 ^a	75.07 ± 0.35 ^b	73.67 ± 0.15 ^c
Crude protein (%)	19.70 ± 0.06 ^c	20.30 ± 0.10 ^b	21.87 ± 0.09 ^a
Fat content (%)	1.47 ± 0.02 ^c	1.57 ± 0.04 ^b	1.87 ± 0.06 ^a
Ash content (%)	1.10 ± 0.00 ^c	1.30 ± 0.00 ^b	1.37 ± 0.06 ^a

Values are mean ± SD of triplicate samples. Mean in a row having different superscript letter(s) are significantly different ($P \leq 0.05$)

Table 4 Changes in peroxide value (meq/kg) and pH of minced beef as affected by different levels of CRRP and storage period

Storage period (days)	Level of CRRP			Average of storage period
	0 %	2.5 %	5.0 %	
Peroxide value				
0	1.07 ± 0.12 ^c	1.00 ± 0.89 ^c	1.00 ± 0.20 ^c	1.02 ^C
5	1.80 ± 0.00 ^b	1.53 ± 0.12 ^{cd}	1.33 ± 0.12 ^d	1.56 ^B
10	2.60 ± 0.20 ^a	1.93 ± 0.12 ^b	1.73 ± 0.12 ^{bc}	2.09 ^A
Treatments average	1.82 ^A	1.49 ^B	1.36 ^C	
pH				
0	5.53 ± 0.03 ^c	5.65 ± 0.00 ^b	5.70 ± 0.00 ^a	5.63 ^A
5	5.05 ± 0.00 ^g	5.23 ± 0.03 ^e	5.30 ± 0.00 ^d	5.19 ^B
10	4.80 ± 0.01 ⁱ	4.98 ± 0.03 ^h	5.09 ± 0.06 ^f	4.96 ^C
Treatments average	5.12 ^C	5.29 ^B	5.36 ^A	

Values are mean ± SD of triplicate samples. Any two mean value(s) having different superscript letter(s) in columns and rows are significantly different ($P \leq 0.05$)

Reduction of peroxide values in the present study may be due to inhibition of lipid peroxidation by polyphenols and flavonoids of CRRP that have antioxidant effects. The antioxidant activity of polyphenols and flavonoids was mainly because of their high redox potentials that allowed them to act as reducing agents, singlet oxygen quenchers and hydrogen donors (Diplock 1997). It could thus be assumed that inclusion of CRRP in minced meat may have contributed to the extension of the shelf life of the products.

The pH of minced meat samples decreased ($P \leq 0.05$) with the progress of storage period whereas it increased ($P \leq 0.05$) with CRRP concentration (Table 4). Initially at 0 day of storage, the pH of the control minced meat sample (0 % CRRP) was lower ($P \leq 0.05$) than that extended with 2.5 and 5 % CRRP. The same trend was noticed for all storage periods, and consequently, the overall mean of the treatments revealed that increasing the level of CRRP greatly ($P \leq 0.05$) increased the pH value of minced meat. The overall mean of storage period indicated that the progressing in storage time significantly reduced ($P \leq 0.05$) the pH value of minced meat. The changes in the pH of the samples might result from the endogenous and exogenous activities that led to the formation and

accumulation of low molecular weight metabolites. Formation of nitrogenous compounds such as ammonia may caused an increase in the pH while the accumulation of organic acids caused a reduction in the pH of stored minced meat. Similar observations on the reduction in pH during storage of moringa leave containing herbal sausages, Turkish dry fermented sausages, and tea polyphenol treated sausages have been reported (Jayawardana et al. 2015; Karabacak and Bozkurt 2012; Wenjiao et al. 2014). In addition, other reports showed increased in the pH of meat products containing moringa leaves and seed extracts (Al-Juhaimi et al. 2016; Muthukumar et al. 2014; Shah et al. 2014) compared to untreated controls. By contrast, Jayawardana et al. (2015) reported that addition of moringa leaves reduced the pH of formulated sausages. Meat pH has a profound influence on meat quality since it determines traits responsible for the processing suitability and eating attributes of meat products.

Microbial load of minced beef extended with CRRP

The microbial load of minced beef meat varied significantly ($P \leq 0.05$) among the treatments and between the days of storage (Table 5). The total viable count, and

coliform count, *E. coli*, and *S. aureus* of control samples had significantly ($P \leq 0.05$) increased with the increase of storage period, but that of CRRP-treated samples showed a significant ($P \leq 0.05$) decrease when the storage period progressed. All the samples incorporated with CRRP showed significantly ($P \leq 0.05$) lower microbial load compared to untreated control indicating that CRRP used in the present study exhibited significant ($P \leq 0.05$) antimicrobial property. This supports the above findings that the extracts of CRRP showed antimicrobial activity against a representative of Gram-negative and Gram-positive bacteria (Table 1). Apparently, the growth of microorganisms in minced meat decreased concomitantly with an increase of CRRP concentration. This could be due to the inhibition of microbial growth or prolongation of the lag phase by polyphenols and flavonoids of CRRP that exhibited antimicrobial activities. Previously, various investigators have reported antimicrobial properties of *C. rotundus* extract against strains of Gram-positive and Gram-negative bacteria (Eltayeib and Um Ismael 2014; Kilani-Jaziri et al. 2011). Similar to our findings, previous reports demonstrated that the total plate counts of meat products were substantially reduced by the incorporation of

leaves of various plants (Al-Juhaimi et al. 2016; Hazra et al. 2012; Jayawardana et al. 2015; Najeeb et al. 2015). In contrast, other reports have shown that incorporation of moringa leaves extracts in meat products did not reduce the microbial load in treated samples, which assumed to be due to the small doses of moringa leaf extracts applied (Muthukumar et al. 2014; Shah et al. 2014). In the current study, the lower counts of total viable bacteria, total coliform, *E. coli*, and *S. aureus* in minced beef extended with CRRP throughout the storage period might be due to the antimicrobial properties of CRRP. Although there has been no large-scale systematic investigation of the relationship between bacterial inhibition and total phenolic content of CRRP, the antimicrobial properties of CRRP could result from its phytochemicals compounds such as flavonoids and polyphenol as well as low weight peptides and proteins. Despite the protective role of CRRP on minced meat during storage, the detection of *E. coli*, and *S. aureus* in control and CRRP containing meat indicated the low hygiene during the preparation of these samples. However, the CRRP containing samples showed a greater low load of these pathogenic bacteria compared to control samples indicating the safety role of CRRP in the meat product.

Table 5 Changes in microbiological load of minced beef as affected by different levels of CRRP and storage period

Storage period (days)	Level of CRRP			Average storage period
	0 %	2.5 %	5.0 %	
Total viable count of bacteria (log CFU/g)				
0	6.80 ± 0.10 ^c	5.10 ± 0.60 ^d	4.97 ± 0.00 ^d	5.62 ^A
5	7.70 ± 0.10 ^b	4.80 ± 0.00 ^d	3.87 ± 1.00 ^{ef}	5.46 ^C
10	8.87 ± 0.06 ^a	4.10 ± 0.60 ^e	3.60 ± 0.10 ^f	5.52 ^B
Average treatments	7.79 ^A	4.67 ^B	4.14 ^C	
Total coliforms (MPN/g)				
0	43.00 ± 1.00 ^c	35.17 ± 0.50 ^d	24.50 ± 1.50 ^e	34.22 ^B
5	58.50 ± 5.50 ^b	27.50 ± 0.50 ^e	8.00 ± 1.00 ^{fg}	31.33 ^C
10	85.00 ± 10.00 ^a	14.00 ± 1.00 ^f	4.50 ± 0.1.50 ^g	34.50 ^A
Average treatments	62.17 ^A	25.56 ^B	12.33 ^C	
<i>E. coli</i> (MPN/g)				
0	17.50 ± 1.50 ^c	12.50 ± 0.50 ^d	11.50 ± 0.50 ^d	13.83 ^A
5	20.50 ± 0.50 ^b	6.50 ± 0.50 ^e	1.50 ± 1.50 ^{fg}	9.50 ^{BC}
10	26.00 ± 3.00 ^a	3.00 ± 0.00 ^f	0.00 ± 0.00 ^g	9.67 ^B
Average treatments	21.33 ^A	7.33 ^B	4.33 ^C	
<i>Staphylococcus aureus</i> (log cfu/g)				
0	3.77 ± 0.06 ^c	2.87 ± 0.06 ^d	2.80 ± 0.00 ^d	3.14 ^B
5	3.97 ± 0.06 ^b	2.70 ± 0.10 ^e	2.67 ± 0.60 ^e	3.11 ^B
10	4.97 ± 0.06 ^a	2.67 ± 0.06 ^e	2.50 ± 0.00 ^f	3.38 ^A
Average treatments	4.23 ^A	2.74 ^B	2.66 ^{BC}	

Values are mean ± SD of triplicate samples. Any two mean value(s) having different superscript letter(s) in columns and rows are significantly different ($P \leq 0.05$)

Sensory evaluation of minced beef (Kebab Shame) extended with CRRP

Sensory panelists detected significant differences ($P \leq 0.05$) on the sensory attributes of minced meat with or without CRRP (Table 6). Apparently, a reduction in scores of color, tenderness, juiciness, and overall acceptability was noticed concurrently with increasing concentration of CRRP, whereas that of flavor showed a significant increase with the CRRP level. None of the measured parameters of minced meat treated with 2.5 % CRRP had a score less than 4.0 (the set cut-off score) which mean that they were all acceptable to the consumer. However, 5 % CRRP insignificantly affected most of the sensory attributes of minced meat. The reduced consumer acceptance for color was likely due to the brownish color of CRRP that resulted in of dark brown color of treated minced meat, whereas the reduction in tenderness and juiciness could be attributed to moisture losses of CRRP-containing minced meat compared to untreated control. However, better flavor scores obtained for CRRP-containing minced meat could be associated with the flavor contributing compounds present in the rhizome of *C. rotundus* as reported to contain essential oils, flavones, phenols and steroids and was used as a food flavoring agents in many countries (Pirzada et al. 2015). Our findings are comparable to those reported by Jayawardana et al. (2015) who observed the highest consumer preference for appearance, color, odor and taste in the control (0.04 % BHT) and 0.25 and 0.50 % moringa leaves incorporated chicken sausages and increasing the moringa leaves percentage above 0.50 % negatively affected the sensory attributes. In addition, Al-Juhaimi et al. (2016) formulated beef patties with various concentrations of moringa seed powders, and they found that the sensory characteristics of cooked patties decreased concomitantly with increasing the levels of moringa seed powder. However, Das et al. (2012) stated that treating goat patties with 0.1 % moringa leaves did not

have any adverse effect on sensory attributes. Furthermore, Muthukumar et al. (2014) did not observe any changes in sensory attributes of cooked pork patties following the incorporation of moringa leave extract. The variations in these studies might be due to the differences in the percentage of the added amounts of plant materials, the used parts of the plant (seeds or leaves), the age of the trees, and the procedures of incorporation of plant materials in the formulated meat products. In the current study, the sensory characteristics of cooked minced meat revealed that incorporation of up to 2.5 % CRRP has resulted in acceptable sensory scores.

Conclusion

To the best of our knowledge, this study is the first report on the utilization of CRRP as a natural preservative in meat products. This study concludes that CRRP is a reliable source of polyphenols and flavonoids and have considerable antioxidant and antibacterial activities that preserve minced beef meat during cold storage. The addition of CRRP at 2.5 % concentration significantly improved the physicochemical properties, shelf life, and sensory quality of minced meat. Therefore, CRRP could be exploited as a potential source of antioxidant and antimicrobial that considered as natural and healthier preservative to extend the shelf life of meat products without adverse effect on the sensory quality. Additionally, the beneficial utilization of CRRP in preserving meat products could both satisfy the modern consumer's demands for natural, safe and healthy food ingredients and add commercial value to this problematic weed.

Compliance with ethical standards

Conflict of interest None.

References

- Ahmad M, Mahayrookh M, Asif BR, Jahan N (2012) Analgesic, antimicrobial and cytotoxic effect of *Cyperus rotundus* ethanol extract. Pak J Pharmacol 29(2):7–13
- Alakali JS, Irtwange SV, Mzer MT (2010) Quality evaluation of beef patties formulated with bambara groundnut (*Vigna subterranean* L.) seed flour. Meat Sci 85:215–223
- Al-Juhaimi F, Ghafoor K, Hawashin MD, Alsawmahi ON, Babiker EE (2016) Effects of different levels of Moringa (*Moringa oleifera*) seed flour on quality attributes of beef burgers. CyTA J Food 14(1):1–9
- AOAC (2003) Official methods of analysis of AOAC international, 17th edn. Association of the Official Analytical Chemists (AOAC), Gaithersburg
- Badgujar SB, Bandivdekar AH (2015) Evaluation of a lactogenic activity of an aqueous extract of *Cyperus rotundus* Linn. J Ethnopharmacol 163:39–42

Table 6 Sensory evaluation of minced beef extended with CRRP

Quality attribute	Level of CRRP		
	0 %	2.5 %	5.0 %
Colour	6.39 ± 0.65 ^a	4.85 ± 0.99 ^b	4.39 ± 0.50 ^b
Flavour	2.69 ± 0.65 ^c	4.62 ± 0.39 ^b	6.23 ± 0.17 ^a
Tenderness	6.08 ± 0.50 ^a	4.69 ± 0.25 ^b	4.00 ± 0.63 ^b
Juiciness	5.77 ± 0.30 ^a	4.00 ± 0.35 ^b	3.85 ± 0.28 ^b
Overall acceptability	6.23 ± 0.73 ^a	4.46 ± 0.13 ^b	4.31 ± 0.32 ^b

Values are mean ± SD of triplicate samples. Any two mean value(s) having different superscript letter(s) in a row are significantly different ($P \leq 0.05$)

- Bashir A, Sultana B, Akhtar FH (2012) Investigation on the antioxidant activity of Dheela Grass (*Cyperus rotundus*). *Afr J Basic Appl Sci* 4(1):1–6
- Das AK, Rajkumar V, Verma AK, Swarup D (2012) *Moringa oleifera* leaves extract: a natural antioxidant for retarding lipid peroxidation in cooked goat meat patties. *Int J Food Sci Technol* 47:585–591
- Das B, Pal D, Haldar A (2015) A review on *Cyperus rotundus* as a tremendous source of pharmacologically active herbal medicine. *Int J Green Pharmacy* 9(4):198–203
- Diplock AT (1997) Will the “good fairies” please prove to us that vitamin E lessens human degenerative disease. *Free Radic Res* 27(5):511–532
- El-Ghazali GEB, El-Tohami MS, El-Egami AAB (1994) Medicinal plants of the White Nile provinces. Medicinal plants of the Sudan
- Eltayeib AA, Um Ismael H (2014) Extraction of *Cyperus rotundus* rhizomes oil, identification of chemical constituents and evaluation of antimicrobial activity of the oil in North Kordofan State. *Int J Adv Res Chem Sci* 1(9):18–29
- Emelugo BN, Umerie SC, Okonkwo IF, Achufusi JN (2011) Evaluation of the tubers and oil of *Cyperus rotundus* Linn (Cyperaceae). *Pak J Nutr* 10(2):147–150
- Grosso NR, Nepote V, Guzman CA (2000) Chemical composition of some wild peanut species (*Arachis* L.). *J Agric Food Chem* 48:806–809
- Harrigan WF (1998) Laboratory method in microbiology. Academic Press, London
- Hazra S, Biswas S, Bhattacharyya D, Das SK, Khan A (2012) Quality of cooked ground buffalo meat treated with the crude extracts of *Moringa oleifera* (Lam.) leaves. *J Food Sci Technol* 49:224–240
- Honikel KO (2014) Minced meat. In: Dikeman M, Devine C (eds) *Encyclopedia of meat sciences*, vol 2, 2 edn. Elsevier Academic Press, London, pp 422–424
- Jahan N, UR-Rahman K, Ali S, Asi RM (2013) Phenolic acid and flavonol contents of gemmo-modified and native extracts of some indigenous medicinal plants. *Pak J Bot* 45(5):1515–1519
- Jain P, Aggarwal V (2012) Evaluation of antioxidant activity of an important medicinal plant *Cyperus rotundus*. *Int J Mod Biol Med* 2(2):84–90
- Jayawardana BC, Liyanage R, Lalantha N, Iddamalgoda S, Wethasinghe P (2015) Antioxidant and antimicrobial activity of drumstick (*Moringa oleifera*) leaves in herbal chicken sausages. *LWT Food Sci Technol* 64:1204–1208
- Jung S, Choe J, Kim B, Yun H, Kruk ZA, Jo C (2010) Effect of dietary mixture of gallic acid and linoleic acid on antioxidative potential and quality of breast meat from broilers. *Meat Sci* 86:520–526
- Kabbashi AS, Mohammed SEA, Almagboul AZ, Ahmed IF (2015) Antimicrobial activity and cytotoxicity of ethanolic extract of *Cyperus rotundus* L. *Am J Pharmacy Pharmaceut Sci* 2(1):1–13
- Karabacak S, Bozkurt H (2012) Effects of *Urtica dioica* and *Hibiscus sabdariffa* on the quality and safety of sucuk (Turkish dry fermented sausage). *Meat Sci* 78(3):288–296
- Kilani S, Abdelwahed A, Ammar R, Hyder N (2005) Chemical composition, antimicrobial and antimutagenic activities of essential oil from Tunisian *C. rotundus*. *J Essent Oil Res* 19:368–379
- Kilani-Jaziri S, Bhouri W, Skandrani I, Limem I, Chekir-Ghedira L, Ghedira K (2011) Phytochemical, antimicrobial, antioxidant and antigenotoxic potentials of *Cyperus rotundus* extracts. *S Afr J Bot* 77:767–776
- Kim DO, Jeong SW, Lee CY (2003) Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem* 81:321–326
- Magrinyà N, Terjung N, Loeffler M, Gibis M, Boua R, Weiss J (2015) Influence of fat addition on the antimicrobial activity of sodium lactate, lauric arginate and methylparaben in minced meat. *Int J Food Microbiol* 215:86–94
- Mohamed RMA, Fageer ASM, Eltayeb MM, Mohamed Ahmed IA (2014) Chemical composition, antioxidant capacity, and mineral extractability of Sudanese date palm (*Phoenix dactylifera* L.) fruits. *Food Sci Nutr* 2(5):478–489
- Muthukumar M, Naveena BM, Vaithiyanathan S, Sen AR, Sureshkumar K (2014) Effect of incorporation of *Moringa oleifera* leaves extract on quality of ground pork patties. *J Food Sci Technol* 51(11):3172–3180
- Najeeb AP, Mandal PK, Pal UK (2015) Efficacy of leaves (drumstick, mint and curry leaves) powder as natural preservatives in restructured chicken block. *J Food Sci Technol* 52(5):3129–3133
- Pirzada AM, Ali HH, Naeem M, Latif M, Bukhari AH, Tanveer A (2015) *Cyperus rotundus* L.: traditional uses, phytochemistry, and pharmacological activities. *J Ethnopharmacol* 174:540–560
- Price ML, Butler LG (1977) Rapid visual estimation and spectrophotometric determination of tannin in sorghum grain. *J Agric Food Chem* 25:1268–1273
- Shah MA, Bosco SJD, Mir SA (2014) Effect of *Moringa oleifera* leaf extract on the physicochemical properties of modified atmosphere packaged raw beef. *Food Pack Shelf Life* 3:31–38
- Sharma SK, Singh AP (2011) Antimicrobial investigations on rhizomes of *Cyperus rotundus* Linn. *Der Pharmacia Lettre* 3(3):427–431
- Sheik HS, Vedhaiyan N, Singaravel S (2013) Acute and sub acute toxicity studies of some indigenous medicinal plants. *Int J Pharm Phytopharmacol Res* 3:166–169
- Steel RG, Torrie JH (1980) Principle and procedures of statistics. MC Grow Hill, New York
- Tang SZ, Kerry JP, Sheehan D, Buckley DJ, Morrissey PA (2001) Antioxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle. *Int J Food Sci Technol* 36:685–692
- Trindade RA, Lima A, Andrade-Wartha ER, Oliveirade Silva AM, Mancini-Filho J, Villavicencio ALCH (2009) Consumer’s evaluation of the effects of gamma irradiation and natural antioxidants on general acceptance of frozen beef burger. *Radiat Phys Chem* 78(4):293–300
- Wenjiao F, Yunchuan C, Junxiu S, Yongkui Z (2014) Effects of tea polyphenol on quality and shelf life of pork sausages. *J Food Sci Technol* 51(1):191–195