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Evaluation of Chitosan/Fructose Model as an Antioxidant and Antimicrobial Agent for Shelf Life Extension of Beef Meat During Freezing

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In the present study the effect of chitosan/fructose Maillard reaction products (CF-MRPs) as antioxidant and antimicrobial agents was evaluated and applied on minced beef meat during frozen storage. Antioxidant and antimicrobial properties of chitosan-fructose complexes were tested. Antioxidant properties were measured by the DPPH, β-carotene and ABTS methods. These three methods showed the same profile of antioxidant activity. Chitosan with 4% fructose autoclaved for 45 min (CF9) showed to have the most effective antioxidant activity. It was demonstrated that the browning product exhibited antioxidant activity. For antimicrobial activity, most chitosan-fructose complexes were less effective than chitosan. Thus, MRPs derived from chitosan-sugar model system can be promoted as a novel antioxidant to prevent lipid oxidation in minced beef. Chitosan-sugar complex could be a potential alternative natural product for synthetic food additive replacement that would additionally meet consumer safety requirement.

INTRODUCTION

The Maillard reaction occurring between amino group and carbonyl group produces neo-formed compounds having certain levels of antioxidant activity depending on the reaction conditions and the type of reactants. The meat products have low oxidative stability and are very susceptible to rancidity during production and storage as any proteinaceous foods. Microbial activity is generally responsible for the deteriorations in meats and meat products accompanied with biochemical and enzymatic alterations [Devlieghere *et al.*, 2004]. Applying antimicrobial agents either by dipping or spraying on the surface of the product is one of many traditional ways to control microbial spoilage and improve food products [Kerry *et al.*, 2006].

Quintavalla & Vicini [2002] reported that activity of the antimicrobial stuffs is limited due to uncontrolled passage into the food matrix and partial inactivation of the active compounds due to interaction with food components. Many reports have pointed to lipid oxidation in meat and meat products which may be regulated by using different antioxidants [Nissen *et al.*, 2004]. The quality of meat and meat products were improved by adding some natural antioxidant compounds as chitosan and its derivatives [Ouattara *et al.*, 2000; Darmadji & Izumimoto, 1994; Jo *et al.*, 2001]. Numerous studies reported the antimicrobial efficacy of chitosan against a variety of spoilage and pathogenic organisms in meat and meat products [Roller *et al.*, 2002; Soultos *et al.*, 2008]. Prashanth & Tharanathan [2007] reported that chitosan has some characters, such as being non-toxic, biodegradable and biocompatible which make chitosan have a broad range of application in many areas. Also, it has exhibited some antimicrobial and antioxidative properties, so it is used as a food preservative and has been shown to have a broad-spectrum of antimicrobial activities against gram-positive and gram-negative bacteria and fungi [Prashanth & Tharanathan, 2007]. The most widely used synthetic antioxidants as butylated hydroxyanisole (BHA), butylated hydroxytoulene (BHT), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ), and metal chelating agents can be added to food products to avoid or delay autoxidation process [Kamil *et al.*, 2002]. However, the growing consumer demand for food devoid of synthetic antioxidants has focused research on the development of new natural preservatives [Matsugo *et al.*, 1998]. Several sources of natural antioxidants are known [Shahidi, 1997], and some of them, such as those of rosemary and sage, are currently used in a variety of food products.

Different antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. Some analytical methods measure the radical scavenging activity of antioxidants against free radicals like 1,1-diphenyl-2-pic-

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rylhydrazyl (DPPH) [Phisut & Jiraporn, 2013], others determining the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested as the thiobarbituric acid-reactive substances (TBRS) and also, methods using free radical traps as the ABTS [2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation [Thaipong *et al*., 2006].

The Maillard reaction products (MRPs) produced in an amino acid–sugar model system have been known to be accompanied with the formation of neo-formed and desirable compounds with prominent antioxidant activity [Jayathilakan & Sharma, 2006]. Chitosan has amino groups which can react easily with the carbonyl group of reducing sugar (glucose, fructose, maltose and lactose) leading to MRPs formation [Phisut & Jiraporn, 2013].

Chang *et al*. [2011] indicated that MRPs which were produced by autoclaving (121°C) chitosan (1%) and glucose (1.0%, 1.5%, or 2.0%) for 15 min, had significantly higher antioxidative activity when compared with chitosan or glucose alone. Glucosamine derivative derived from chitosan/glucose model system has been reported to have a relatively higher antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, when compared with chitosan [Chang *et al.*, 2008].

The main objective of our research was to determine the potential of MRPs of chitosan/ fructose model system as an antioxidant and antibacterial agent to prevent the deterioration of meat and meat products *via* lipid oxidation and food borne microorganisms. To reach the target, our work aimed to: (1) evaluate the antioxidative and antimicrobial properties of the chitosan–fructose Maillard reaction products, with various levels of fructose (1%, 2%, or 4%), and (2) evaluate its preservative effect on fresh minced beef meat during chill storage.

MATERIALS AND METHODS

Materials

Chitosan was obtained in a powder form (Fluka, Germany). It had a deacetylation degree of 96% and the moisture content below 10%. Tert-butyl hydroquinone (TBHQ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-Carotene, linolenic acid, Tween-20 (polyoxyethylene sorbitan monopalmitate), chloroform, acetic acid and 2,2-azino-*bis*(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS•) were purchased from Aldrich-Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). Fresh beef meat was obtained from local market.

Preparation of chitosan–fructose Maillard reaction products (CF-MRPs)

One gram of chitosan (96% degree of deacetylation); was dissolved in 100 mL of acetic acid (1%, Merck) in which 1.0%, 2%, or 4.0% fructose (Merck) were added. The pH value of each solution was adjusted to 6.0 by adding 1 N NaOH. After being autoclaved at 140±1°C, for 15, 30 and 45 min and cooled, the CF-MRP solutions with fructose levels of 1%, 2%, or 4% were assigned codes of CF1 to CF9, and chitosan only was assigned code of C, respectively. The samples were assigned the following keys, CF1: chitosan+fructose 1% at 15 min; CF2: chitosan+fructose 1% at 30 min; CF3: chitosan+fructose 1% at 45 min; CF4: chitosan+fructose 2% at 15 min; CF5: chitosan+fructose 2% at 30 mins; CF6: chitosan+fructose 2% at 45 min; CF7: chitosan +fructose 4% at 15 min, CF8: chitosan+fructose 4% at 30 min; and CF9: chitosan+fructose 4% at 45 min.

Fourier-Transform-Infra Red spectroscopy analysis

Chitosan-fructose complex formation was lyophilized then evaluated using Fourier Transform Infrared Spectroscopy (Shimadzu, Japan). The obtained spectral peaks were recorded between wave numbers of 400–4000 cm^{-1}.

Determination of antioxidant activity

DPPH radical-scavenging method

The DPPH radical-scavenging activity of the test samples was estimated by the method of Yamaguchi *et al*. [1998]. The diluted sample (200 μL) was mixed with 800 μL of *tris*--HCl buffer (100 mmol/L, pH 7.4), then 1 mL of 500 mmol/L $DPPH^{\cdot}$ in methanol was added and the mixture was vortexed. The absorbance was measured at 517 nm using a spectrophotometer (model UV-1601PC, Shimadzu Co., Tokyo, Japan), after 30 min of incubation in the dark. Inhibition % was calculated as:

$$\text{Inhibition } \% = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/ \text{Abs}_{\text{control}}] \times 100$$

β-Carotene radical-scavenging activity method

The antioxidant activity of CF-MRPs was evaluated acc. to Jayaprakasha *et al*. [2001] with some modifications. 0.1 mg of β-carotene in 0.2 mL of chloroform, 10 mg of linoleic acid and 100 mg of Tween-20 (polyoxyethylene sorbitan monopalmitate) were mixed. The solvent was evaporated under vacuum at 40°C and the resulting mixture was diluted with 10 mL of water and was mixed well. To this mixture, 20 mL of oxygenated water were added. Four milliliter aliquots mixtures were pipetted into different test tubes containing 1 mL of CF-MRPs. Control (0.2 mL of ethanol was added to 4 mL of the above emulsion) and blank (mixture without β-carotene) tubes were also prepared. All tubes were placed in a water bath (50°C). The absorbance was measured at 470 nm (zero time (t= 0), t=60 min and 15 min intervals) until the color of β-carotene vanished in the control tubes. All determinations were carried out in triplicates and the antioxidant activity (AA) of CF-MRPs were evaluated in terms of bleaching of the β-carotene using the following formula,

$$\text{Inhibition\%} = [(A_B\text{-}A_A)/A_B] \times 100$$

where: A_B: absorption of blank sample (t=0 min) and $A_{A:}$ absorption of sample solution (t=60 min).

ABTS radical cation decolorization assay

The spectrophotometric analysis of ABTS• radical scavenging activity of tested chitosan solutions were determined according to the method described by Chien *et al*. [2007].

The ABTS$^{\bullet}$ radical cation was produced by the reaction between 7 mmol/L ABTS$^{\bullet}$ in H_2O and 2.45 mmol/L potassium persulfate, stored in the dark at room temperature for 12 h. Before usage, the ABTS$^{\bullet}$ solution was diluted to get an absorbance of 0.7±.025 at 734 nm with phosphate buffer (0.1 mol/L, pH 7.4). Then, 2 mL of ABTS$^{\bullet}$ solution was added to 1 mol/L of CF-MRPs. The reaction mixture was allowed to stand at room temperature for 6 min, and the absorbance was immediately recorded at 734 nm using UV--spectrophotometer. A standard curve was obtained by using Trolox standard (0.25–1 mmol/L) in ethanol. The absorbance of the resulting oxidized solution was compared as mmol/L Trolox equivalents (TE).

Thiobarbituric acid-reactive substances (TBARS)

Fresh local beef meat, which was divided to small particles (1 cm^3) and dipped in the CF1 to CF9, and C solutions for 10 min. Samples without any dipping treatment and samples dipped in deionized water for 10 min were assigned codes of CON and DW, respectively. After dipping, the samples were placed in plastic bags, and stored at 4°C for 7 days. The TBARS values of the samples were determined according to the methods described by Liu *et al.* [2009]. Malondialdehyde (MDA) and other aldehydes, formed during lipid oxidation in the beef meat were measured, and reported as TBARS values in units of MDA equivalent/kg beef meat samples. The amount of the pink-colored TBA complex was measured with a spectrophotometer at 510 nm. Triplicate samples were analyzed.

Antimicrobial activity assay

Pure cultures of the bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella paratyph* and *Escherichia coli*) and fungi (*Aspergillus niger, Aspergillus parasiticus,* and *Candida albicans*) were provided by the Department of Microbiology, National Research Centre (NRC) (Dokki, Giza, Egypt). The antimicrobial activity was determined by measuring the diameter of inhibition zone [Bauer *et al*., 1996].

Determination of Minimum Inhibitory Concentration (MIC)

The MIC is defined as the minimum level of a sample that inhibits the growth (populations) of microbial colonies by 90%. The MIC was determined by the serial dilutions method. For the studies, chitosan solution was diluted with a sterile physiological saline (pH 7.2) in relation to the active substance. In order to establish MIC values, chitosan solutions in the ranges from 0.1 to 2.5 mg/mL (the concentration increasing by 0.1 mg/mL) were prepared. One mL of the breeding-ground was poured into the test tubes, and then 0.5 mL of the investigated preparation was added. Subsequently, 0.5 mL of given microorganisms culture was added to the test tubes. In the case of *C. albicans* and all the bacterial strains, cultures were diluted 1:1000. Each time, a sample controlling the growth of investigated microorganisms was made. Cultures of all the bacteria and *C. albicans* were incubated at 37°C for 24 h, while other mycotic strains were incubated at 25°C for 7 days.

RESULTS AND DISCUSSION

FT-IR spectra analyses of chitosan-fructose Maillard reaction products (CF- MRPs)

FT-IR spectroscopy has been used to determine the structure of chitin and chitosan [Kurita, 1986]. Figure 1 showed the FT-IR spectra of chitosan, fructose and CF-MRPs. Chitosan-fructose complexes were evaluated by FT-IR analysis, spectra of the reacted chitosan-fructose systems were compared with the corresponding spectrum of both purified fructose and chitosan. Figure 1 showed the data obtained for fructose, chitosan and chitosan with 1, 2, and 4% of fructose autoclaved for 15, 30 and 45 min at 140±1°C. The characteristic absorption bands at 3460–3411, 3366–3336 and 2942–2929 cm^{-1} are corresponding to OH, NH and CH stretching regions of chitosan were observed in all samples. CF-MRPs showed a decrease of the band at 1573 cm^{-1} corresponding to primary amine groups [Chang *et al*., 2008] and indicating the successful interaction between the carbonyl group of the fructose (reducing sugar) and the amine group of chitosan.

On the other hand, the 1620–1640 cm^{-1} band showed a sharp increase due to the appearance of a new band corresponding to C=N linkage indicating the formation of Schiff base during the interaction between fructose and chitosan [Umemura & Kawai, 2007]. Our results showed also intense browning indices. Data obtained from chitosan-fructose systems indicated the successful cleavage of fructose with chitosan chains during storage.

It is well-known that the Maillard reaction is mainly divided into three stages. The initial reaction between sugar-amino compound forming Amadori product through the Schiff base, then the latter is broken into numerous compounds. Finally, these products condense with amino compounds forming brown polymers (melanoidins) [Martins *et al.,* 2001]. From FT-IR spectral data, it is suggested that the amino group of chitosan covalently attached to fructose forming a glycated product. This glycated product or Schiff base, rearranges to a more stable ketoamine or Amadori product. The Amadori products can then form cross-links with other amino groups resulting in polymeric aggregates named advanced glycation end-products [Friedman, 1966].

Antioxidant activity assays

DPPH radical-scavenging activity

The DPPH stable radical is used for the determination of primary antioxidant activity of fruits, vegetables, aromatic and medicinal plants and also MRPs *in vitro*. The DPPH radical is scavenged by antioxidants through the hydrogen donation to form a stable DPPH-H molecule, measured as a decrease of DPPH absorbance at 517 nm (purple to yellow) [Wong *et al*., 2006].

Results in Table 1 showed the DPPH radical-scavenging activity of the different CF-MRPs, compared with that of TBHQ. The CF9 showed the strongest ability to scavenge DPPH radicals followed by the CF8, CF5 and CF3. On the other hand, both chitosan and fructose exhibited weak ability to scavenge the DPPH radicals (5 and 3%, respectively), depending on their concentration.

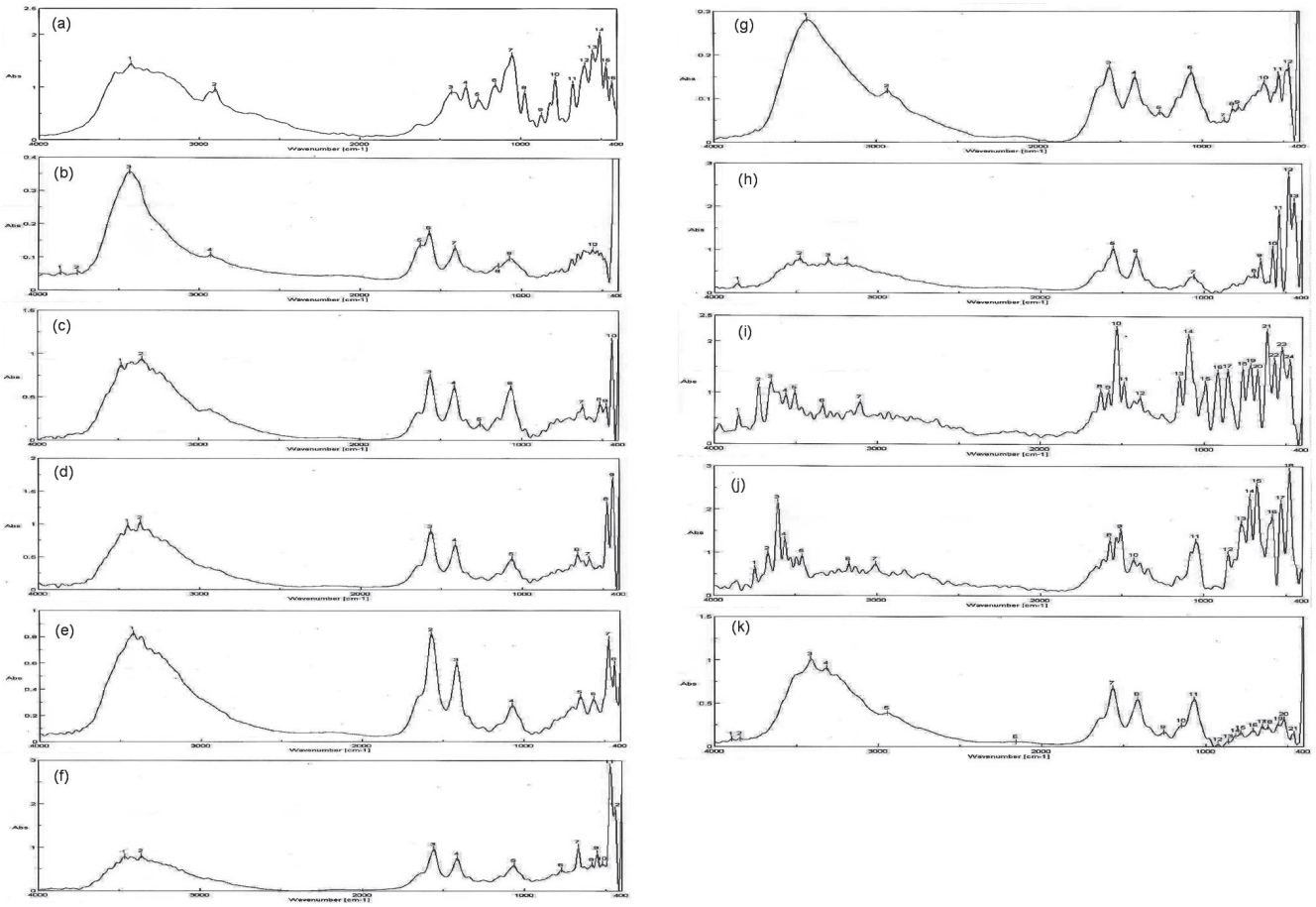

FIGURE 1. FT-IR spectral bands of: (a) fructose, (b) chitosan, and (c) to (k) chitosan-ftuctose complexes (from CF1 to CF9, respectively).

This result indicated that MRPs derived from the chitosan-fructose model system were free radical inhibitors, which can work as the primary antioxidant. The present results were in agreement with previous observations reported by Kanatt *et al*. [2007] who stated that the glucose/chitosan complexes had high antioxidant activity in comparison with chitosan or glucose alone.

Literature revealed that the chitosan–sugar MRPs exhibited significantly higher DPPH scavenging activities when compared to chitosan and sugar alone [Kanatt *et al*., 2008; Chang *et al.*, 2011] and the scavenging activity increased by increasing the concentration of sugar used (reaching the plateau at 30 mg/mL ribose) [Sumaya-Martinez *et al*., 2005]. Caramelization reaction that may occur during heating of chitosan--sugar model system contributes to its antioxidant activity [Phisut & Jiraporn, 2013].

β-Carotene radical-scavenging activity (BC)

Peroxyl free radicals produced from linoleic acid oxidation oxidize the highly unsaturated β-carotene and the presence of antioxidants will minimize that in β-carotene/linoleic acid assay. β-Carotene bleaching inhibition effects of different CF-MRPs are shown in Table 1. The results showed that there were significant differences in the radical scavenging activities of the different CF-MRPs prepared with various levels of fructose autoclaved at different times (15, 30 and 45 min).

CF9 (high fructose content at 140°C for 45 min, model system) exhibited the highest antioxidant activity among other model systems (38.9%). This result could be attributed to some brown complexes of the Maillard reaction formed during thermal interaction between fructose and chitosan. According to our knowledge, there are no studies carried out on β-carotene free radical scavenging activity of chitosan/ sugar Maillard reaction products.

ABTS radical-scavenging activity

ABTS assay is more practical than that of DPPH and β-carotene-linoleate assay [Miller, 1996]. When an antioxidant is added to the radicals, there is a degree of decolorization and reversing the formation of the DPPH radical and $ABTS^{\bullet+}$ cation as:

$$ABTS^{\bullet+} + AH \rightarrow ABTS^{+} + A^{\cdot}$$

The results gained using $K_2S_2O_8$ as an oxidant show that the presence of peroxodisulfate increases the rate of $ABTS^{\bullet+}$ autobleaching in a concentration-dependent manner. $ABTS^{\bullet+}$ radicals were generated in the ABTS/ $K_2S_2O_8$ system [Kaviarasan *et al.,* 2007]. The results in Table 1 showed that there were significant differences in the ABTS radical-scavenging activities of the CF-MRPs as obtained in the DPPH and β-carotene assay. The activities of the CF-MRPs scavenging the ABTS radicals were in the descending order: CF9 > CF8 > CF6 >

CF3 > CF7 > CF5 > CF4 > CF2 > CF1 and their activity values were 0.294, 0.192, 0.185, 0.172, 0.128, 0.125, 0.095, 0.08 and 0.0038 mmol/L Trolox Equiv., respectively.

Lipid oxidation (TBARS values)

Concentrations of MDA in the lipid fraction of the minced beef meat during the four-week storage period are presented in Table 2. MDA values were significantly ($P \leq 0.05$) different in all treatments during the whole storage period. CF9 treatments (1%, 2% and 4% v/w) exhibited the lowest MDA values for all measured samples. CF9 (4%) has exhibited the best antioxidative effect ($P \leq 0.05$) (710 μg/kg MDA) at the end of storage period and this result could be ascribed to the occurrence of CF-MRPs which induce a synergistic effect preventing the lipid oxidation. Our results are in agreement with findings of Darmadji & Izumimoto [1994] who reported that the TBA value (expressed as mg MDA/kg) of beef containing 1% chitosan was at the same level after 10 days of storage at 4°C. Furthermore, Chang *et al*. [2011] revealed a significant decrease of TBARS values ($P < 0.05$) during storage for five or more days under refrigeration of samples dipped in the chitosan-glucose-MRPs solutions.

In addition, CF-MRPS antioxidant activity could be ascribed to the results of Shahidi *et al*. [1999] who explained that chitosan derivatives chelate the free iron, which are released from hemoproteins during heat processing or storage and thus inhibit the lipid oxidation of products.

Antimicrobial activity assay

The results of antibacterial and antifungal activities of chitosan solutions in terms of Minimal Inhibitory Concentration (MIC) are presented in Table 3. All bacteria and fungi, which have been used in these studies, were susceptible to chitosan. The results of antibacterial and antifungal activities of chitosan after treated by CF-MRPs are presented in the Table 4. All treatments showed very low effect against all tested microorganisms. Chemically-modified chitins including partially deacetylated and carboxymethlyated chitins were found to have potent immunological and antibacterial activities [Nishimura *et al*., 1984; Ryan *et al*., 2001].

The protection of the host against bacterial infection is stimulated by chitosan [Iida *et al*., 1987]. The effectiveness of chitosan bacteriostatic properties was tested against bacterial strains and a common skin fungus. Powered chitin, chitosan or whole crab shells were not effective in any of the tests, but the solution of chitosan in acetic acid inhibited the bacterial and fungal strains [Cheng & Li, 2000].

The antimicrobial activity of chitosan-sugar complexes was proposed by several mechanisms. The inhibition of microbial growth is referred to the availability of an amino group (cation charged groups) on chitosan that interacts with N-acetylmuramic acid, sialic acid and neuraminic acid (anionic components), on the microbial cell membrane, results in changes in its permeability for essential nutrients and in inhibiting some enzymes [Mahae *et al.*, 2011].

Chitosan possesses high chelating capacity for various metal ions (including Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Mg^{2+}, and Cu^{2+}) in acid conditions, and it has been widely applied for the removal or recovery of metals in different industries. Metal ions that combine with the cell wall molecules of microorganisms are crucial for stability of the cell wall. Chitosan-mediated chelation of such metal ions has often been implicated in antimicrobial action [Kurita, 1998].

The inhibition of *Candida tropicalis* growth by chitosan--fructose CF9 MRPs, is in agreement with findings obtained by Allan & Hardwiger [1984] who stated that the solution of chitosan (1%) with acetic acid had completely inhibited the growth of *Candida tropicalis*.

TABLE 1. Antioxidant activity of chitosan-fructose model systems by DPPH, BC and ABTS scavenging assays.

C-F MRPs	DPPH (I%)	BC (I %)	ABTS (Trolox Eq. mmol/L)
CF1	12.4±0.28	11.4±0.99	0.0038±0.00
CF2	15.8±0.15	15.2±1.7	0.08±0.001
CF3	22.8±2.1	26.3±2.3	0.172±0.008
CF4	16.7±1.8	15.7±1.2	0.095±0.001
CF5	23.7±2.3	20.4±1.8	0.125±0.008
CF6	4.2±0.1	27.3±3.4	0.185±0.003
CF7	23±2.6	23.9±2.0	0.128±0.007
CF8	29.5±3.1	29.7±2.4	0.192±0.01
CF9	37.8±3.2	38.9±2.8	0.294±0.06

CF-MRPs: chitosan/fructose Maillard reaction products, CF1: chitosan +fructose 1% at 15min; CF2: chitosan +fructose 1% at 30min; CF3: chitosan +fructose 1% at 45min; CF4: chitosan +fructose 2% at 15min; CF5: chitosan +fructose 2% at 30min; CF6: chitosan +fructose 2% at 45min; CF7: chitosan +fructose 4% at 15min, CF8: chitosan +fructose 4% at 30min; CF9: chitosan+fructose 4% at 45min, I% :Inhibition percentage.

TABLE 2. Thiobarbituric acid-reactive substances (TBARS) of CF9 (1%, 2% and 4%) during frozen storage of the fresh minced beef meat.

Treatments	TBARS (MDA μg/kg)			
	1 week	2 week	3 week	4 week
C	607.3	1350	2843	3420
MBM+1% CF9	462	852	1215	1589
MBM +2% CF9	342	523	729	1123
MBM +4% CF9	235	345	523	710

C: control minced beef meat; MBM: minced beef meat and CF9: chitosan/fructose MRPs at 1, 2 and 4% fructose.

TABLE 3. Antibacterial and antifungal activity of chitosan.

Microorganism	Inhibition zone (mm)	MIC (mg/mL)
Escherichia coli	17	1.5
Pseudomonas aeruginosa	14	1.9
Staphylococcus aureus	18	0.8
Salmonella paratyphi	12	2.0
Candida albicans	19	0.7
Aspergillus niger	10	2.3
Aspergillus parasiticus	15	1.8

MIC: minimum inhibitory concentration.

TABLE 4. Antibacterial and antifungal activity of CF- MRPs.

Sample	Microorganism / Inhibition zone (mm)						
	E. coli	*Salmonella paratyphi*	*Candida albicans*	*Pseudomonas aeruginosa*	*Staphylococcus aurus*	*Aspergillus niger*	*Aspergillus parasiticus*
CF1	+	+	+++	+	ND	+	++
CF2	+	++	+	ND	ND	ND	ND
CF3	ND	++	++	+	++	+	+
CF4	ND	++	+	ND	+	ND	+
CF5	ND	+	+	ND	ND	ND	ND
CF6	+	++	+	++	+	+	+
CF7	ND	++	++	+	+	+	+
CF8	ND	++	+	+	+	ND	+
CF9	+	+	+	+	ND	ND	+

(ND): Negative, (+): 1 mm, (++): 2 mm, (+++): 3mm.

TABLE 5. Statistical analysis of sensory properties of minced beef meat treated with C-F MRPs.

Samples	Taste (10)	Odor (10)	Color (10)	Mouth feel (10)	Appearance (10)
MBM with CF1	6.2^{b}±0.74	7.7^{a}±0.25	8.2±0.79	8.4±1.17	8.7±0.82
MBM with CF2	5.8^{b}±0.49	7.4^{a}±0.33	8.0±0.94	7.9±0.99	8.0±0.94
MBM with CF3	7.6^{a}±0.37	8.1^{a}±0.66	8.7±0.82	8.5±0.53	8.8±0.79
MBM with CF4	6.0^{b}±0.42	6.5^{b}±0.21	8.0±1.05	7.6±0.52	8.0±1.05
MBM with CF5	5.5^{c}±0.95	6.3^{b}±0.13	7.5±0.84	7.9±0.56	7.5±0.84
MBM with CF6	7.2^{a}±0.92	8.3^{a}±0.16	8.5±0.71	8.4±1.26	8.5±0.71
MBM with CF7	7.8^{a}±0.48	5.8^{c}±0.35	8.1±0.99	7.9±0.99	8.6±0.84
MBM with CF8	8.2^{a}±0.40	7.2^{a}±0.96	8.6±1.26	8.0±1.05	8.6±1.26
MBM with CF9	7.8^{a}±0.44	8.0^{a}±0.76	8.6±0.84	8.2±1.23	8.6±0.99
LSD at.05%	0.941	1.02	NS	NS	NS

CF1: chitosan +fructose 1% at 15min; CF2: chitosan +fructose 1% at 30min; CF3: chitosan +fructose 1% at 45min; CF4: chitosan +fructose 2% at 15min; CF5: chitosan +fructose 2% at 30min; CF6: chitosan +fructose 2% at 45min; CF7: chitosan +fructose 4% at 15min, CF8: chitosan +fructose 4% at 30min; CF9: chitosan+fructose 4% at 45min.

TABLE 6. Statistical analysis of sensory properties of minced beef meat treated with C-F MRPs after two-weak storage.

Samples	Taste (10)	Odor (10)	Color (10)	Mouth feel (10)	Appearance (10)
C	7.60^{b}±0.09	8.20^{b}±0.15	6.80^{b}±0.13	6.20^{b}±0.2	6.30^{b}± 0.09
MBM+1% CF	9.30^{a}±0.11	9.80^{a}±0.12	8.00^{a}±0.19	7.80^{a}±.16	8.90^{a}±0.13
MBM +2% CF	9.10^{a}±0.13	9.50^{a}±0.17	8.20^{a}±0.07	7.20^{a}±0.08	8.50^{a}±0.21
MBM +4% CF	9.25^{a}±0.12	9.40^{a}±0.14	8.30^{a}±0.08	7.32^{a}±0.11	8.70^{a}±0.15
LSD at.05%	1.11	1.17	1.01	1.06	1.03

C: control minced beef meat; MBM: minced beef meat and CF: chitosan-fructose MRPs at 1, 2 and 4% fructose at 45min.

Organoleptic characteristics of the samples

The effects of chitosan/fructose Maillard reaction products (CF-MRPs) at different levels (1, 2 and 4%) autoclaved for 15, 30 and 45 min at 145°C on the organoleptic properties of the minced beef meat are presented in Table 5. With increasing CF-MRPs levels, the sensory scores for the color, taste, odor, mouth feel, and appearance of the samples sharply increased. There were no significant differences between

TABLE 7. Statistical analysis of sensory properties of minced beef meat treated with C-F MRPs after four-weak storage.

Samples	Taste (10)	Odor (10)	Color (10)	Mouth feel (10)	Appearance (10)
C	$5.00^{c}\pm0.13$	$6.10^{b}\pm0.22$	$4.80^{b}\pm0.11$	$5.20^{b}\pm0.12$	$5.10^{b}\pm 0.19$
MBM+1% CF	$7.50^{b}\pm0.22$	$7.20^{a}\pm0.18$	$7.20^{a}\pm0.13$	$6.30^{a}\pm.11$	$6.90^{a}\pm0.29$
MBM +2% CF	$7.10^{b}\pm0.13$	$7.10^{a}\pm0. 09$	$7.40^{a}\pm0.09$	$6.90^{a}\pm0.14$	$7.20^{a}\pm0.25$
MBM +4% CF	$8.05^{a}\pm0.12$	$7.40^{a}\pm0.29$	$7.60^{a}\pm0.15$	$7.02^{a}\pm0.16$	$7.50^{a}\pm0.22$
LSD at.05%	0.68	1.00	2.15	1.10	1.80

C: control minced beef meat; MBM: minced beef meat and CF: chitosan-fructose MRPs at 1, 2 and 4% fructose at 45min

the samples containing CF-MRPs at different levels with respect to color, mouth feel, and appearance tested. Also, the results showed significant differences in taste and odor between the samples treated with CF-MRPs.

Organoleptic characteristics of the samples during storage for month

Ten trained panelists were asked to detect differences between the control samples and those containing CF-MRPs at levels 1, 2 and 4% for 45 min. The mean scores from the sensory evaluation test showed that there were no significant differences ($p\geq0.05$) in color, taste, odor, mouth feel, and appearance for samples treated with CF-MRPs at deferent levels (Table 6 and 7). On the other hand, significant differences were reported during storage when, color, taste, odor, mouth feel, and appearance of samples treated with CF-MRPs scored higher than the control. Moreover, samples treated with CF-MRPs at levels 4% showed higher score at all concentrations. Decreased sensorial values in all characteristics were observed after 15 days or 30 days of storage ($p\leq0.05$). Results showed that very comparable scores were recorded among the different samples for each tested sensorial attribute, suggesting that the investigated active agents could be advantageously used to control the microbial quality without affecting sensorial properties.

Physical and sensorial characteristics of samples were comparable with control samples. The use of such CF-MRPs on processed meat products, however, can act as a "hurdle" or "barriers" that act synergistically to inhibit or retard microbial growth. Being effective against the spoilage proliferation, without affecting the sensorial properties of the prepared minced beef meat, it is possible to assess that the technique could be advantageously used to prolong the shelf life.

CONCLUSIONS

MRPs derived from chitosan-fructose model were good in antioxidant and antimicrobial properties for shelf life extension of beef meat during freezing. These properties may vary with different ratios of sugar used. Among them, CF9 seemed the most effective for participating in the formation of Maillard reaction products as evidenced by the antioxidant and antimicrobial activities. On the basis of the results obtained, MRPs from chitosan-fructose with presumed antioxidant and antimicrobial properties could be a potential alternative natural product for synthetic food additive replacement and also could meet consumer safety requirement.

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