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Antioxidative properties of a chitosan–glucose Maillard reaction product and its effect on pork qualities during refrigerated storage

H.L. Chang [a], Y.C. Chen [a], F.J. Tan [b,*]

[a] Department of Animal Science, National Pingtung University of Science and Technology, Pingtung 902, Taiwan, ROC
[b] Department of Animal Science, National Chung Hsing University, Taichung 402, Taiwan, ROC

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ABSTRACT

The objective of this study was to evaluate the antioxidative properties of a chitosan–glucose Maillard reaction product (CG-MRP), and its effect on pork qualities during refrigerated storage. Chitosan (1%), which was dissolved in acetic acid (1%) with 1.0%, 1.5%, or 2.0% glucose, pH adjusted to 6.0, autoclaved (121 °C, 15 min) and cooled, was prepared. The results showed that the 2,2-dipheny1-1-picrylhydrazy1 (DPPH) radical scavenging activities, ferrous ion chelating abilities, and reducing powers of various CG-MRP solutions were not significantly different. Pork loins soaked in the CG-MRP solutions or deionized water for 10 min and without dipping were stored at 4 °C for 7 days. Little influence was observed on the L^*, a^*, and b^* colour values of the samples. Dipping in CG-MRP tended to retard the increases in volatile basic nitrogen (VBN) and thiobarbituric acid-reactive substances (TBARS) values, and resulted in lower microbial counts during storage. No detrimental influence on the sensory characteristics was found.

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1. Introduction

Chitosan, which is a cationic polysaccharide made from alkaline N-deacetylation of chitin, has been commercially prepared from shellfish-processing waste. A purified chitosan product (Chito-Clear®), which is a shrimp-derived product (Primex Ingredients ASA, Norway), has achieved a GRAS (Generally Recognised As Safe) self-affirmed status in USA in 2001, and thus removes some of the earlier regulatory restrictions on its use in foods (Sagoo, Board, & Roller, 2002). Some characteristics, such as being non-toxic, biodegradable and biocompatible make chitosan have a broad range of application in many areas (Harish Prashanth & Tharanathan, 2007). In addition, chitosan has exhibited some antimicrobial and antioxidative properties and therefore, has received a great deal of attention for its use as a potential food preservative of natural origin. Chitosan has been shown to have a broad-spectrum of antimicrobial activity against gram-positive and gram-negative bacteria and fungi (Harish Prashanth & Tharanathan, 2007). Several possible explanations have been proposed for the antimicrobial activity of chitosan (Harish Prashanth & Tharanathan, 2007). For example, the polycationic chitosan molecule might interact with the predominantly anionic cell wall components of the microorganism, and result in the leakage of intracellular components. Some nutrients might not be able to enter the microbial cell due to changes in the permeability of barriers. Moreover, upon entering into the microbial cell, some chitosan molecules might bind to DNA, inhibit its RNA and protein synthesis, and thus influence the survival of the microorganisms. In addition, the chelation of some free metal ions enables chitosan to inhibit lipid oxidation of many foods (Shahidi, Arachchi, & Jeon, 1999).

Meat and meat products are comparatively highly susceptible to rancidity development caused by oxidation due to their contents of unsaturated lipids. In addition, meat and meat products are highly perishable due to higher moisture and protein contents. As a food component of natural origin, chitosan and its derivatives have been added to some meat and meat products to improve their qualities (Harish Prashanth & Tharanathan, 2007; Jo, Lee, Lee, & Byun, 2001; Ouattara, Simard, Piette, Bégin, & Holley, 2000). Previous studies have indicated that chitosan could be used effectively to inhibit the oxidation of meat and meat products both alone (Darmadji & Izumimoto, 1994; Jo et al., 2001) and in combination with some natural or artificial antioxidants (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007; Soultos, Tzikas, Abrahim, Georgantelis, & Ambrosiadis, 2008). The antimicrobial effects of chitosan against a variety of spoilage and pathogenic organisms in meat and meat products have also been reported in many studies (Darmadji & Izumimoto, 1994; Roller et al., 2002; Sagoo et al., 2002; Soultos et al., 2008). In addition, little detrimental effects on the textural and sensory properties of meat and meat products due to the addition of chitosan have been reported (Jo et al., 2001; Lin & Chao, 2001).

* Corresponding author. Tel.: +886 4 22870613x246; fax: +886 422860265.
E-mail address: tanfj@dragon.nchu.edu.tw (F.J. Tan).

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The Maillard reaction, which is a chemical reaction resulting from the condensation between a carbonyl group of reducing sugars, aldehydes or ketones, and an amino group of amino acids, proteins or any nitrogenous compound, has been utilised in many food processing and manufacturing procedures (Kanatt, Chander, & Sharma, 2008). A typical brown colour development due to the Maillard reaction when heating a model system containing chitosan and glucose has been observed (Tanaka, Huang, Chiu, Ishizaki, & Taguchi, 1993). In addition to contributing to the formation of specific colour and flavour and to changing some functional properties of the food products, many studies have also been reported on the contribution of the Maillard reaction compounds to the antioxidative and antimicrobial effects (Maillard, Billaud, Chow, Ordonaud, & Nicolasb, 2007; Usui et al., 2004). Having an amino group makes chitosan a candidate to react with the carbonyl group of a reducing sugar (e.g. glucose), and allows it to be a participant in the Maillard reaction.

Kanatt et al. (2008) reported that a Maillard reaction product, which was produced by autoclaving (121 °C) chitosan (1%) and glucose (1%) for 15 min, had significantly higher antioxidative activity than chitosan or glucose alone. The reduction to the oxidation and the numbers of spoilage microorganisms could be used to extend the shelf life of lamb meat and cocktail pork salami. A chitosan–glucosamine derivative produced by using the Maillard reaction has been reported to have a relatively higher antibacterial activity against *Escherichia coli* and *Staphylococcus aureus,* when compared with the native chitosan (Chung, Kuo, & Chen, 2005). Enhanced antimicrobial activity of the Maillard-type soy protein–chitosan conjugates has also been reported (Usui et al., 2004).

Therefore, the objectives of this study were (1) to evaluate the antioxidative properties of the chitosan–glucose Maillard reaction products, which were prepared by autoclaving (121 °C, 15 min) chitosan (1%) with various levels of glucose (1%, 1.5%, or 2%), and adjusting the pH to 6, and (2) to evaluate its preservative effect on fresh pork during refrigerated storage.

2. Materials and methods

2.1. Preparation of chitosan–glucose Maillard reaction product (CG-MRP) solutions and pork samples

The preparation of chitosan–glucose Maillard reaction product (CG-MRP) solutions was carried out according to the methods of Kanatt et al. (2008) with some modifications. One gram of chitosan (MW 250 kDa and 96% degree of deacetylation; China League Biotechnology Associates Ltd., Taipei, Taiwan) was dissolved in 100 ml of acetic acid (1%, Merck) in which 1.0%, 1.5%, or 2.0% glucose (Merck) were added. The pH value of each solution was adjusted to 6.0 by adding 1 N NaOH. After being autoclaved (121 °C, 15 min) and cooled, the CG-MRP solutions with glucose levels of 1.0%, 1.5%, or 2.0% were assigned codes of CG10, CG15, and CG20, respectively. Fresh porcine *longissimus* muscle, which was obtained from a local meat processing company was cut into cubes of 1 cm^3, and dipped in the CG10, CG15, or CG20 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 gently drained on a tissue paper, placed in plastic bags, and stored at 4 °C for 7 days.

2.2. 2,2-dipheny1-1-picrylhydrazy1 (DPPH) radical scavenging activity, ferrous ion chelating ability and reducing power

The DPPH radical scavenging activity and ferrous ion chelating ability were determined according to the methods described by Chien, Sheu, Huang, and Su (2007). The DPPH scavenging was determined according to: DPPH scavenging (%) = [1 − (absorbance$_{sample}$/absorbance$_{blank}$)] × 100, at a wave-length of 517 nm, using a spectrophotometre (U-2000, Hitachi, Japan). The ferrous ion chelating ability was determined according to: ferrous ion chelating (%) = [1 − (absorbance$_{sample}$/absorbance$_{blank}$)] × 100, at a wave-length of 562 nm, using the same spectrophotometer. The reducing power of samples was determined according to the method of Oyaizu (1986) at a wave-length of 700 nm, using the same spectrophotometer.

2.3. Instrumental colour measurement

Ground samples were placed in a measuring container, and then the L^* (lightness), a^* (redness), and b^* (yellowness) values of the samples were measured using a colorimeter (Nippon Denshoku Ze 2000, Japan). A standard plate with '*Y*' = 94.81, '*X*' = 92.83, and '*Z*' = 111.27 was used as a reference.

2.4. pH values and drip loss

Ten-gram ground samples were blended with 90 ml distilled water in a polyethylene bag for 1 min using a stomacher (Stomacher 400, Seward Ltd., England), and then the pH value of the mixture was measured using a pH meter (Micro-computer pH meter, Model 6210, Taiwan), which had been calibrated previously. Dipped samples were gently drained onto tissue papers, placed in plastic bags, weighted, stored at 4 °C for 48 h, and re-weighted. The drip loss was determined according to: drip loss (%) = (Weight$_{\text{before refrigeration}}$ − Weight$_{\text{after refrigeration}}$)/Weight$_{\text{before refrigeration}}$ × 100%.

2.5. Thiobarbituric acid-reactive substances (TBARS) and volatile basic nitrogen (VBN)

The TBARS and VBN values of the samples were determined according to the methods described by Liu, Tsau, Lin, Jan, and Tan (2009), and expressed as mg malonaldehyde/kg and mg/100 g, respectively.

2.6. Microbial evaluation

At a specified sampling time, samples were aseptically removed from the bags. Ten-gram samples were placed in a sterile bag, which contained 90 ml of sterile water, and homogenised with a stomacher (Stomacher blender, Model 400, Seward) for 2 min. Serial dilutions were then made. Plate count agar (PCA, Merck) was used for the enumeration of total plate counts and psychrotrophic bacteria counts, using the pour plate method. The plates for the total bacteria and psychrotrophic bacteria were incubated at 37 °C for 48 h and 7 °C for 5 days, respectively. The microbial counts in this study were expressed as $\log_{10}$ colony forming units (CFU) per gram of sample.

2.7. Sensory evaluation

At days 0, 3, 5, and 7 during storage, the samples were served to a sensory panel, which consisted of 10 meat science faculty and students. Sensory attributes, including colour, off-odour, and overall acceptance, were evaluated using a 1 to 7-point scale test, with 1 representing light colour, less off-odour and less overall acceptance.

2.8. Statistical analysis

The data were analysed using the general linear model (GLM) of Statistical Analysis System's Procedures (SAS Institute Inc., Cary,

NC) with a 5% level of significance. Means were separated using the least square means.

3. Results and discussion

3.1. DPPH scavenging activity

The results showed that there were no significant differences in the DPPH free radical scavenging activities of the chitosan–glucose Maillard reaction products (CG-MRP), which were prepared with various levels of glucose (Fig. 1a). Kanatt et al. (2008) indicated that the chitosan–glucose complex showed significantly higher DPPH scavenging activities when compared to chitosan and glucose alone. When evaluating the effect of the Maillard reaction conditions on the DPPH scavenging activity, Sumaya-Martinez, Thomas, Linard, Binet, and Guerard (2005) reported that the scavenging activity increased with increasing the concentration of sugar, and reached the plateau at a 30 mg/ml ribose concentration. Similarly, significantly higher DPPH scavenging activities were observed when this chitosan–glucose complex concentration increased from 20 to 100 μg/ml; however, this increase in DPPH scavenging activities was not observed when the chitosan–glucose complex increased to 150 or 200 μg/ml (Kanatt et al., 2008). In addition, Kanatt et al. (2008) reported that the addition of the chitosan–glucose complex at the level of 150 μg/ml had equivalent DPPH scavenging activities when compared to the artificial antioxidant (i.e. butylated hydroxyl toluene, BHT). They explained that this chitosan–glucose complex possesses hydrogen donating ability, and has the potential to react with free radicals. The influences of thermal treatments and amino acids of the Maillard reaction products on the radical scavenging activities have also been reported in some studies (Maillard et al., 2007). Disaccharide chitosan derivatives, which were prepared with lactose, maltose, cellobiose, chitosan and various substitution degrees of disaccharide, exhibited different free radical scavenging activities (Lin & Chou, 2004). Maillard et al. (2007) indicated that the glucose–cysteine Maillard reaction products had higher DPPH scavenging activities amongst the various glucose–amino acid mixtures, and suggested that this was probably due to the sulfhydryl group of cysteine. Rao, Chander, and Sharma (2005) reported an increase in the free radical scavenging activity of chitosan, which was irradiated at a dose up to 30 kGy of gamma radiation, and explained that this was probably due to the increased exposure of free amino groups during the chitosan depolymerisation after irradiation.

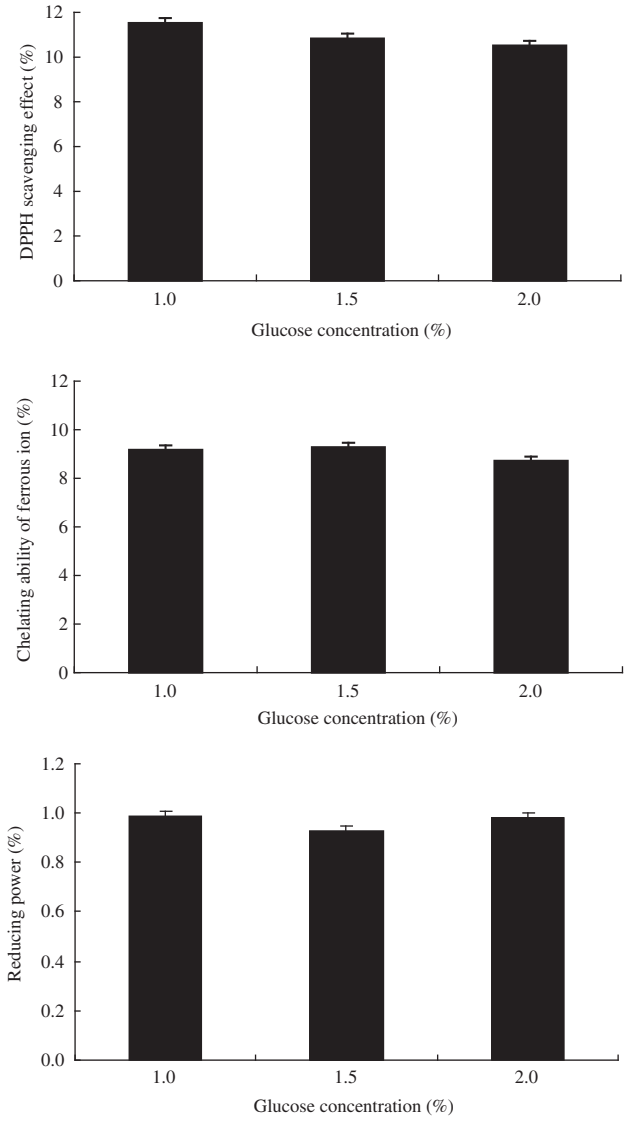

Fig. 1. (a) DPPH scavenging activities, (b) ferrous ion chelating abilities and (c) reducing powers of chitosan–glucose Maillard reaction products with different glucose levels added.

3.2. Ferrous ion chelating abilities

The results showed that ferrous ion chelating abilities were not significantly different between the samples with different levels of glucose added (Fig. 1b). Chitosan, which possesses high chelating capacity for various metal ions in an acidic environment, has been suggested as being responsible for the removal of metal ions in many industries (Kurita, 1998). Similarly, Winterowd and Sandford (1995) explained that by chelating the ferrous ions presented in the system, chitosan eliminates the prooxidant activity of ferrous ions or its conversion to ferric ion, and eventually retards lipid oxidation. The metal ion-chelating effect of chitosan and its Maillard reaction products, which was concentration related, was also reported by Chung et al. (2005). They illustrated that the Cu^{2+} chelating capacity of chitosan and its derivatives, which were made of chitosan (dissolved in acetic acid) and glucosamine, heated at 65 °C for 2 days, increased with higher concentration, and leveled off to a saturated chelating capacity at a sample concentration of 0.3%. The authors stated that some extra functional groups, such as amino groups from saccharides, might have contributed to the chelating capacities of samples. In addition, higher chelating capacities were observed when chitosan–glucosamine reacted with Cu^{2+} and Fe^{2+} than when reacted with Zn^{2+} (Chung et al. 2005).

3.3. Reducing power

There was no significant difference in the reducing powers between the chitosan–glucose Maillard reaction products, which were prepared with various glucose levels in this study (Fig. 1c). Kanatt et al. (2008) reported that the chitosan–glucose complex showed a significantly higher reducing power when compared to chitosan and glucose alone. In the same study, significantly higher reducing power was observed when this chitosan–glucose complex concentration increased from 1.25 to 2.5 mg/ml; however, this increase in reducing power was not observed when the chitosan–glucose complex increased to 2.5 or 3.75 mg/ml (Kanatt et al., 2008). Some brown complexes of the Maillard reaction, which were formed upon γ-irradiation of glucose and amino acid solution, might contribute to the increase in the reducing power of samples (Chawla, Chander, & Sharma, 2007). Hwang, Shue, and Chang (2001) explained that some of the Amadori products

produced in the primary phase of the Maillard reactions were responsible for the reducing activity.

3.4. Instrumental colour evaluation

Table 1 illustrates that fresh pork loin treated with CG-MRP or DW had significantly higher L^* values when compared to CON samples. This increase was probably because more solutions were absorbed during dipping, thus increasing the reflection of light on the surfaces of samples, which increased the L^* values (i.e. lightness) of samples. No significant difference was observed between the CG-MRP (i.e. CG10, CG15, and CG20) samples. In addition, the a^* values (redness) decreased after stored for 7 days ($P < 0.05$). During storage, the a^* and b^* values of the CG-MRP samples did not differ significantly ($P > 0.05$), except for some minor exceptions.

The direct addition of chitosan into the formula had little influence on the colours of meat and meat product, and this has been previously reported (Jo et al., 2001; Lin & Chao, 2001). In the current study, the chitosan–glucose Maillard reaction product (CG-MRP) solutions were prepared as 1% chitosan (dissolved in 1% glacial acetic acid) with glucose (1, 1.5, or 2%), adjusted to pH 6, and autoclaved at 121 °C for 15 min, according to the method of Kanatt et al. (2008). A visual colour change, from uncoloured to yellow brown, was observed during the preparation of CG-MRP during heating, and this agreed with many other studies. Browning, which was determined at 420 nm indicated that the formation of intermediate compounds of the Maillard reaction had occurred, and increased with the concentration of the chitosan–glucose complex (Kanatt et al., 2008). In addition, these authors reported that the fluorescent products, which are the precursors of the brown pigments, were formed during the Maillard reaction of this chitosan–glucose complex (Kanatt et al., 2008). In the current study, even though the CG-MRP solutions themselves showed typical browning colours of the Maillard reaction products, as described in many studies (Manzocco & Maltini, 1999; Zeng et al., 2007), however, limited dipping time (i.e. only 10 min in this study) of the samples in the CG-MRP solution might be the reason for a comparatively less typical browning colour changes in the CG-MRP-treated pork samples. Many factors, including the heating temperature, heating time, pH, storage environment (i.e. moisture and oxygen), reducing end groups and others, have been reported to influence the browning of chitooligomers, which were manufactured using chitosan (dissolved in acetic acid and pH adjusted to 5.5), and hemicellusase, and heated at 50 °C for 8 h (Zeng et al., 2007). Similarly, Chawla et al. (2007) indicated that the extent of browning of the Maillard reaction products was related to heating time, types and concentrations of amino acids and sugars, pH values, and other factors. Manzocco and Maltini (1999) reported that the colour change of a glucose/glycine solution due to the Maillard reaction was associated with the formation of heat-induced compounds.

3.5. pH values and drip loss

Table 2 illustrates that the pH values of fresh pork, treated with the CG-MRP solutions with different glucose levels added, did not differ significantly during refrigerated storage ($P > 0.05$). The drip losses of samples increased after storage for 3 or more days when compared to day 1. The CG-MRP treated samples (i.e. CG10, CG15, and CG20) tended to have higher drip losses numerically. In this study, the CG-MRP solutions, which were comparatively more sticky than deionized water, were likely absorbed to a larger extent into the pork samples during dipping, and were subsequently released during further refrigerated storage, thus increased the drip losses of samples. On the other hand, the solubility of chitosan by the Maillard reaction has been shown to be influenced by many factors, such as heating temperature, pH, reaction time, and others (Chung et al., 2005). Specifically, Umemura and Kawai (2007) indicated that the free amino groups of the glucose-added chitosan film were consumed during the Maillard reaction, so the amounts of free amino groups decreased rapidly when an increased amount of glucose was added. The authors explained that the decrease of the free amino groups could be contributed to the increase of the insoluble matter when adding too much glucose. In this study, when the adding level of glucose in the solution increased to

Table 1
Changes of L^*, a^* and b^* values of fresh pork treated with chitosan–glucose Maillard reaction product solutions during refrigerated storage at 4 °C.

Treatment[e]	Storage time (day)			
	1	3	5	7
	L^* value			
CON	59.76 ± 1.31^{cy}	59.57 ± 1.01^{dy}	61.76 ± 0.17^{bw}	60.91 ± 0.85^{bx}
DW	63.47 ± 0.62^{aw}	63.18 ± 0.53^{aw}	63.04 ± 0.94^{aw}	63.63 ± 1.39^{aw}
CG10	61.67 ± 0.63^{bx}	61.57 ± 0.66^{cy}	62.46 ± 0.66^{abx}	63.45 ± 0.53^{aw}
CG15	61.95 ± 0.61^{bx}	62.32 ± 0.52^{bx}	61.91 ± 0.50^{abx}	63.77 ± 0.45^{aw}
CG20	61.41 ± 0.53^{by}	62.16 ± 0.61^{bcxy}	62.56 ± 0.46^{awx}	63.28 ± 0.56^{aw}
	a^* value			
CON	14.59 ± 0.68^{aw}	12.85 ± 0.52^{ax}	11.33 ± 1.18^{bz}	12.14 ± 0.67^{ay}
DW	12.39 ± 0.60^{cw}	11.46 ± 0.32^{bx}	11.53 ± 1.18^{abx}	11.00 ± 0.94^{bx}
CG10	13.09 ± 0.50^{bw}	11.89 ± 0.63^{bx}	11.35 ± 0.43^{bx}	11.33 ± 0.61^{bx}
CG15	12.58 ± 0.59^{bcw}	12.04 ± 0.69^{bw}	12.14 ± 0.46^{aw}	11.23 ± 0.57^{bx}
CG20	13.45 ± 0.57^{bw}	11.76 ± 0.62^{bx}	10.92 ± 0.47^{by}	11.27 ± 0.67^{bcy}
	b^* value			
CON	16.74 ± 0.29^{aw}	16.78 ± 0.22^{aw}	16.34 ± 0.37^{aw}	16.61 ± 0.36^{aw}
DW	16.32 ± 0.47^{abw}	16.68 ± 0.35^{aw}	16.49 ± 0.45^{aw}	16.41 ± 0.80^{aw}
CG10	16.79 ± 0.39^{abw}	16.78 ± 0.41^{aw}	16.51 ± 0.32^{aw}	16.55 ± 0.40^{aw}
CG15	16.67 ± 0.27^{abw}	16.80 ± 0.58^{aw}	16.75 ± 0.44^{aw}	16.37 ± 0.38^{aw}
CG20	16.43 ± 0.28^{bw}	16.70 ± 0.41^{aw}	16.52 ± 0.66^{aw}	16.59 ± 0.50^{aw}

[a-d] Means within a column for the same test with different superscripts are significantly different ($P < 0.05$).
[w-z] Means within a row for the same test with different superscripts are significantly different ($P < 0.05$).
[e] Treatments: CON – no dipping; DW – dipped in deionized water for 10 min; CG10 – dipped in an autoclaved solution consisted of chitosan (1%) and 1.0% glucose for 10 min; CG15 – dipped in an autoclaved solution consisted of chitosan (1%) and 1.5% glucose for 10 min; CG20 – dipped in an autoclaved solution consisted of chitosan (1%) and 2.0% glucose for 10 min.

Table 2
Changes in the pH values and drip loss of fresh pork treated with chitosan–glucose Maillard reaction product solutions during refrigerated storage at 4 °C.

Treatment[d]	Storage time (day)			
	1	3	5	7
	pH value			
CON	5.67 ± 0.03^{aw}	5.63 ± 0.02^{aw}	5.61 ± 0.03^{bw}	5.63 ± 0.02^{aw}
DW	5.68 ± 0.02^{aw}	5.66 ± 0.01^{aw}	5.64 ± 0.02^{aw}	5.64 ± 0.01^{aw}
CG10	5.65 ± 0.05^{aw}	5.64 ± 0.02^{aw}	5.63 ± 0.01^{aw}	5.64 ± 0.03^{aw}
CG15	5.67 ± 0.06^{aw}	5.63 ± 0.02^{aw}	5.64 ± 0.02^{aw}	5.65 ± 0.04^{aw}
CG20	5.67 ± 0.03^{aw}	5.64 ± 0.01^{aw}	5.63 ± 0.01^{aw}	5.63 ± 0.04^{aw}
	Drip loss			
CON	3.57 ± 1.11^{ax}	4.83 ± 1.09^{cwx}	5.18 ± 0.48^{bw}	6.44 ± 1.12^{abw}
DW	4.14 ± 0.66^{ax}	5.31 ± 1.60^{bcw}	5.39 ± 0.73^{bw}	5.27 ± 1.11^{bw}
CG10	4.17 ± 1.03^{ax}	7.89 ± 0.77^{aw}	8.35 ± 1.18^{aw}	7.49 ± 0.73^{aw}
CG15	4.16 ± 1.35^{ax}	7.00 ± 0.52^{abw}	7.09 ± 0.28^{abw}	7.74 ± 1.25^{aw}
CG20	4.48 ± 0.94^{ax}	5.48 ± 0.59^{abwx}	5.58 ± 1.01^{bwx}	6.63 ± 1.27^{abw}

[a-c] Means within a column for the same test with different superscripts are significantly different ($P < 0.05$).
[w-x] Means within a row for the same test with different superscripts are significantly different ($P < 0.05$).
[d] Treatments: CON – no dipping; DW – dipped in deionized water for 10 min; CG10 – dipped in an autoclaved solution consisted of chitosan (1%) and 1.0% glucose for 10 min; CG15 – dipped in an autoclaved solution consisted of chitosan (1%) and 1.5% glucose for 10 min; CG20 – dipped in an autoclaved solution consisted of chitosan (1%) and 2.0% glucose for 10 min.

Table 3
Changes of TBARS and VBN values of fresh pork treated with chitosan–glucose Maillard reaction product solutions during refrigerated storage at 4 °C.

Treatment[c]	Storage time (day)			
	1	3	5	7
	TBARS (mg MDA/kg)			
CON	0.55 ± 0.24^{az}	0.78 ± 0.15^{ay}	1.20 ± 0.01^{ax}	1.28 ± 0.02^{aw}
DW	0.32 ± 0.06^{bz}	0.62 ± 0.19^{by}	1.07 ± 0.17^{ax}	1.23 ± 0.06^{aw}
CG10	0.26 ± 0.12^{bz}	0.53 ± 0.15^{by}	0.79 ± 0.15^{bx}	0.99 ± 0.04^{bw}
CG15	0.24 ± 0.11^{bz}	0.51 ± 0.13^{by}	0.75 ± 0.11^{bx}	0.89 ± 0.04^{bw}
CG20	0.24 ± 0.13^{bz}	0.50 ± 0.11^{by}	0.70 ± 0.14^{bx}	0.86 ± 0.06^{bw}
	VBN (mg/100 g)			
CON	8.09 ± 1.04^{ax}	8.55 ± 0.56^{abx}	9.01 ± 0.76^{ax}	10.17 ± 1.13^{aw}
DW	7.86 ± 1.43^{ax}	9.01 ± 0.76^{aw}	9.24 ± 0.71^{aw}	9.24 ± 0.71^{aw}
CG10	6.70 ± 1.05^{bx}	7.86 ± 0.72^{bw}	7.86 ± 1.13^{bw}	8.09 ± 1.04^{bw}
CG15	7.16 ± 1.05^{abw}	8.09 ± 0.57^{abw}	7.86 ± 0.71^{bw}	7.62 ± 1.45^{bw}
CG20	7.16 ± 1.05^{abx}	8.09 ± 1.04^{abwx}	7.63 ± 0.76^{bwx}	8.55 ± 1.04^{abw}

[a–b] Means within a column for the same test with different superscripts are significantly different ($P < 0.05$).
[w–z] Means within a row for the same test with different superscripts are significantly different ($P < 0.05$).
[c] Treatments: CON – no dipping; DW – dipped in deionized water for 10 min; CG10 – dipped in an autoclaved solution consisted of chitosan (1%) and 1.0% glucose for 10 min; CG15 – dipped in an autoclaved solution consisted of chitosan (1%) and 1.5% glucose for 10 min; CG20 – dipped in an autoclaved solution consisted of chitosan (1%) and 2.0% glucose for 10 min.

2.0%, this would cause the solution to have less solubility, thus comparatively less quantity of CG20 solution was absorbed when compared to the CG10 and CG15 samples. Finally, less drip loss of samples dipped in CG20 solution was observed when compared to samples dipped in the CG10 and CG15 solutions after storage for more than 3 days.

3.6. TBARS and VBN values

The TBARS values, which are indicators of lipid oxidation, are shown in Table 3. The TBARS values increased significantly ($P < 0.05$) during storage, as expected. When compared to CON and DW, the samples dipped in the CG-MRP solutions had significantly lower TBARS values when stored for five or more days under refrigeration. No significant differences in the TBARS values were observed for the CG-MRP samples (i.e. CG10, CG15, and CG20) during storage ($P > 0.05$). The effectiveness of chitosan on the oxidative stability of meat and meat products has been demonstrated in many studies (Darmadji & Izumimoto, 1994; Shahidi et al., 1999). Many factors, including the heating temperature and time, pH values, reducing sugars, and amino acids, might affect the effectiveness of antioxidative activities on the Maillard reaction (Manzocco & Maltini, 1999). A combination of chitosan (0.5% or 1%) and nitrites (150 ppm) showed a synergistically antioxidative effect on Greek-style fresh pork sausages during refrigerated storage (Soultos et al., 2008). The TBARS values of pork sausages with the addition of a chitosan oligomer (0.2%) were significantly lower after aerobically refrigerated storage for 3 weeks, when compared to the control (Jo et al., 2001). With a combination of a rosemary extract, chitosan exerted significantly better antioxidative effects, and extended the shelf-life of fresh pork sausages stored at 4 °C (Georgantelis et al., 2007). Shahidi et al. (1999) explained that chitosan would chelate the free irons, which are released from hemoproteins during heat processing or storage, and thus inhibit the lipid oxidation of products. Darmadji and Izumimoto (1994) reported that the addition of 1% chitosan resulted in a 70% reduction of the TBARS values in beef after storage at 4 °C for 3 days.

Increased amount of volatile basic nitrogen (VBN), which is the result of the decomposition of proteins during storage by microorganisms, can be an index of meat product freshness. Table 3 illustrates that the VBN values increased during storage. In addition, fresh pork dipped in the chitosan–glucose Maillard reaction products tended to have lower VBN values than the CON and DW treatments, especially after storage for five days. No significant difference in the VBN values between the treatments with various glucose levels was observed ($P > 0.05$). Fresh degutted silver carp, which was dipped in a 2% chitosan solution and maintained at −3 °C for 30 days, had significantly lower VBN values when compared to the control samples (Fan et al., 2009). The authors indicated that the reduction of the VBN values of samples might be due to a faster reduction of the bacterial population and a decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds when adding chitosan to the fish samples.

3.7. Microbial qualities

The results showed that the total microflora counts of the samples increased during storage, as expected (Table 4). When compared to CON and DW, the samples dipped in the CG-MRP (i.e. CG10, CG15, and CG20) had significantly ($P < 0.05$) lower total microflora counts. Especially at day 1, there was less than 30–300 CFU/g detected at a dilution of 10^{-1} for the CG-MRP samples. No significant differences in the total microflora counts were observed between the CG-MRP samples during storage ($P > 0.05$). Similarly, the psychrotrophic bacteria counts of samples increased during storage, as expected. The CG-MRP samples had significantly ($P < 0.05$) lower psychrotrophic bacteria counts than the CON and DW samples after storage for 7 days. No significant differences of the psychrotrophic bacteria counts were observed for the CG-MRP samples during storage ($P > 0.05$). The results in this study demonstrate that the chitosan–glucose Maillard reaction products could retard the growth of microorganisms of fresh pork samples during refrigerated storage, and this result agrees with other studies. A chitosan–glucose complex has been shown to have antimicrobial activities against *E. coli*, *Pseudomonas* and *S. aureus* (Kanatt et al., 2008). The authors reported that the addition of a chitosan–glucose complex increased the lamb meat shelf-life by more than 2 weeks during chilled storage and enhanced the

Table 4
Changes in the microbial counts of fresh pork treated with chitosan–glucose Maillard reaction product solutions during refrigerated storage at 4 °C.

Treatment[d]	Storage time (day)			
	1	3	5	7
	Total microflora count (log CFU/g)			
CON	3.32 ± 0.05^{ay}	3.39 ± 0.03^{ay}	3.53 ± 0.06^{ax}	4.24 ± 0.18^{aw}
DW	3.17 ± 0.05^{by}	3.26 ± 0.04^{bxy}	3.32 ± 0.02^{bwx}	3.56 ± 0.08^{bw}
CG10	ND[e]	2.64 ± 0.11^{cy}	2.93 ± 0.02^{cx}	3.02 ± 0.03^{cw}
CG15	ND	2.62 ± 0.06^{cy}	2.87 ± 0.02^{cx}	3.00 ± 0.05^{cw}
CG20	ND	2.66 ± 0.10^{cx}	2.89 ± 0.03^{cx}	3.00 ± 0.01^{cw}
	Psychrotrophic bacteria count (log CFU/g)			
CON	2.97 ± 0.48^{ay}	3.63 ± 0.07^{axy}	4.27 ± 0.64^{ax}	5.84 ± 0.25^{aw}
DW	ND	3.55 ± 0.50^{aw}	4.52 ± 1.23^{aw}	4.64 ± 0.95^{bw}
CG10	ND	ND	2.87 ± 0.18^{bw}	3.28 ± 0.03^{cw}
CG15	ND	ND	2.73 ± 0.03^{bw}	3.27 ± 0.03^{cw}
CG20	ND	ND	2.76 ± 0.10^{bw}	3.37 ± 0.11^{cw}

[a–c] Means within a column for the same test with different superscripts are significantly different ($P < 0.05$).
[w–y] Means within a row for the same test with different superscripts are significantly different ($P < 0.05$).
[d] Treatments: CON – no dipping; DW – dipped in deionized water for 10 min; CG10 – dipped in an autoclaved solution consisted of chitosan (1%) and 1.0% glucose for 10 min; CG15 – dipped in an autoclaved solution consisted of chitosan (1%) and 1.5% glucose for 10 min; CG20 – dipped in an autoclaved solution consisted of chitosan (1%) and 2.0% glucose for 10 min.
[e] ND: not determined, less than 30 CFU/g at a dilution of 10^{-1}.

shelf-life of pork cocktail salami to 28 days (Kanatt et al., 2008). A Maillard reaction product, prepared from chitosan and xylose, after heating to 95 °C for up to 60 h, showed antibacterial activities against *Bacillus subtilis* and other Gram-positive bacteria, and extended the shelf-life of fresh noodles (Huang, Huang, Huang, & Chen, 2007). A soy protein-chitosan conjugate, which was formed by the Maillard reaction at 60 °C for 2 weeks, was found to enhance bactericidal action (Usui et al., 2004).

Previous studies have indicated that chitosan alone or in combination with other components could be used to function effectively on the microbiological qualities of meat and meat products. Dipping in chitosan solutions (1%) reduced the native microflora (total viable counts, yeasts and moulds, and lactic acid bacteria) of pork sausages by approximately 1–3 log CFU/g during storage at 7 °C, for 18 days (Sagoo et al., 2002). In the same study, they reported that the addition of 0.3 and 0.6% chitosan reduced the microbial counts of the unseasoned minced pork mixture up to 3 log CFU/g during refrigerated storage for 18 days when compared to the untreated control (Sagoo et al., 2002). When containing both chitosan and rosemary extract, Georgantelis et al. (2007) reported there was a synergistic inhibition of microbial growth of *Enterobacteriaceae*, *Pseudomonas* spp., total viable bacteria, yeasts and moulds, and lactic acid bacteria on fresh pork sausages stored at 4 °C for 20 days. With or without the incorporation of lauric acid or cinnamaldehyde, a chitosan-based antimicrobial film was developed to inhibit surface spoilage bacteria in bologna, cooked ham, and pastrami (Ouattara et al., 2000). When combined with the low levels of sulphite (170 ppm), the addition of 0.6% chitosan resulted in a more effective retardation of the growth of spoilage microorganisms of chilled pork sausages than high levels (340 ppm) of sulphite alone (Roller et al., 2002). Microorganisms, including *Staphylococci*, coliform, gram-negative bacteria, *Micrococci*, and *Pseudomonas* in meat were inhibited by the addition of chitosan during storage at 30 °C for 48 h and 4 °C for 10 days (Darmadji & Izumimoto, 1994). The authors suggested that the interaction of chitosan with the membranes or the cell wall components of microorganisms, resulted in an increased permeability of the membranes and leakage of cell material. Alternatively, the water-binding capacity and the inhibition of various enzymes by chitosan, might contribute to the antimicrobial effects of chitosan. Furthermore, Harish Prashanth and Tharanathan (2007) explained that the polycationic chitosan molecule would interact with the predominantly anionic cell wall components (lipopolysaccharides and proteins) of microorganisms, and result in the leakage of intracellular components, due to changes in the permeability barrier of the microorganisms. In addition, entering of chitosan (especially low molecule weight) into the cell, binding to DNA, and inhibiting RNA and protein synthesis, might also contribute to the antimicrobial properties of chitosan.

Many factors, such as molecule weight, concentration, viscosity, deacetylation degree, pH values, temperature, and others, have been reported to influence the effectiveness of antimicrobial ability of chitosan (Kanatt et al., 2008; No, Park, Lee, & Meyers, 2002). For example, depending on the bacteria and molecule weight of chitosan, No et al. (2002) reported that the minimum inhibitory concentration (MIC) of chitosans ranged from 0.05% to more than 0.1%. In general, chitosan showed stronger bactericidal effects on Gram-positive bacteria than Gram-negative bacteria.

3.8. Sensory evaluation

Table 5 illustrates that the sensory colours of the samples treated with CG-MRP were not significantly different after storage for 5 days. The CON samples, which were not dipped, tended to have darker sensory colours than the dipping treatments (CG10–20 and DW) during storage. Some water-soluble pigments of meat might have dissolved in the solutions during dipping in the CG-MRP or deionized water, and might have led to the lighter colours of the DW and CG-MRP samples. In the current study, the off-odour refers to any unpleasant odour detected in fresh pork, where 1 represents the lowest intensity of an off-odour. The off-odour of the CG10, CG15, and CG20 samples increased numerically, but not significantly during storage. The off-odour of CON and DW increased significantly after refrigerated storage at 4 °C for 7 days. No significant difference in the off-odour between the CG-MRP treatments was observed during storage ($P > 0.05$). The decrease in the overall acceptance after storage for 7 days was probably due to the increase in off-odour. No significant difference in the overall acceptance was observed between the CG-MRP samples (i.e. CG10, CG15, and CG20) during storage ($P > 0.05$).

Table 5
Changes of the sensory characteristics[d] of fresh pork treated with chitosan–glucose Maillard reaction product solutions during refrigerated storage at 4 °C.

Treatment[e]	Storage time (day)			
	1	3	5	7
	Colour			
CON	4.13 ± 1.30^{aw}	3.69 ± 1.49^{awx}	3.25 ± 1.00^{axy}	3.50 ± 1.15^{ax}
DW	3.06 ± 1.12^{bcw}	2.69 ± 0.70^{cw}	2.69 ± 1.08^{aw}	2.56 ± 0.89^{bw}
CG10	2.88 ± 0.89^{cw}	2.56 ± 0.63^{cw}	2.69 ± 1.35^{aw}	2.69 ± 0.79^{bw}
CG15	3.63 ± 1.15^{abw}	3.56 ± 0.89^{bw}	2.63 ± 0.96^{ax}	2.94 ± 0.85^{abw}
CG20	3.69 ± 1.25^{abw}	2.94 ± 1.12^{bcx}	2.63 ± 1.02^{ax}	2.88 ± 1.02^{abx}
	Off-odour			
CON	2.56 ± 1.03^{ax}	2.81 ± 1.17^{ax}	2.75 ± 1.13^{ax}	3.31 ± 0.95^{aw}
DW	2.44 ± 0.96^{ax}	2.75 ± 1.06^{awx}	2.56 ± 0.89^{ax}	3.31 ± 0.95^{aw}
CG10	2.81 ± 1.33^{aw}	2.56 ± 1.03^{aw}	3.00 ± 0.89^{aw}	3.12 ± 0.95^{aw}
CG15	2.56 ± 1.03^{aw}	2.88 ± 1.20^{aw}	2.63 ± 1.02^{aw}	3.25 ± 0.77^{aw}
CG20	2.88 ± 1.24^{aw}	3.13 ± 0.96^{aw}	2.69 ± 0.70^{aw}	3.25 ± 0.86^{aw}
	Overall acceptance			
CON	4.25 ± 1.44^{aw}	4.00 ± 1.26^{awx}	3.81 ± 1.11^{awx}	3.19 ± 1.22^{ax}
DW	4.06 ± 1.24^{aw}	3.63 ± 1.15^{awx}	3.44 ± 1.09^{awx}	3.00 ± 1.10^{ax}
CG10	4.06 ± 1.18^{aw}	3.44 ± 1.36^{aw}	3.50 ± 0.97^{aw}	3.25 ± 1.18^{aw}
CG15	4.00 ± 1.37^{aw}	4.00 ± 1.37^{aw}	3.63 ± 1.02^{awx}	3.06 ± 1.12^{ax}
CG20	4.31 ± 1.30^{aw}	3.63 ± 1.26^{awx}	3.88 ± 1.41^{awx}	3.06 ± 0.85^{ax}

[a-c] Means within a column for the same test with different superscripts are significantly different ($P < 0.05$).
[w-y] Means within a row for the same test with different superscripts are significantly different ($P < 0.05$).
[d] A 7-point scale test, with 1 representing light colour, less off-odour and less overall acceptance.
[e] Treatments: CON – no dipping; DW – dipped in deionized water for 10 min; CG10 – dipped in an autoclaved solution consisted of chitosan (1%) and 1.0% glucose for 10 min; CG15 – dipped in an autoclaved solution consisted of chitosan (1%) and 1.5% glucose for 10 min; CG20 – dipped in an autoclaved solution consisted of chitosan (1%) and 2.0% glucose for 10 min.

The addition of chitosan in a Greek-style fresh pork sausage resulted in lower malondialdehyde (MDA) values and microbial growth during refrigerated storage, thus to decreased rancidity and spoilage flavours, and presented a more acceptable odour and taste than the control samples (Soultos et al., 2008). Jo et al. (2001) reported that the addition of a chitosan oligomer did not influence the sensory colour, flavour, texture and overall acceptance of the emulsion-type pork sausages. Similarly, Lin and Chao (2001) indicated that the incorporation of 0.1% chitosan with molecular weights ranging from 150 to 1250 kDa did not significantly affect the sensory parameters, including the off-odour, hardness, juiciness, oiliness and overall acceptability of the reduced-fat Chinese-style sausages.

4. Conclusions

In conclusion, no significant differences in the antioxidative properties were observed between the chitosan–glucose Maillard reaction products (CG-MRP) solutions, which were prepared in this

study. However, the fresh pork samples dipped in the CG-MRP for 10 min and stored at 4 °C tended to have lower TBARS values, VBN values, and microbial counts during refrigerated storage, without negative influences on the sensory qualities of the samples. Further studies of application of this CG-MRP on pre-cooked meat and poultry products would be useful.

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