Analytical Methods

Effects of chitosan, aqueous extract of ginger, onion and garlic on quality and shelf life of stewed-pork during refrigerated storage

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A B S T R A C T

Effects of 1% or 0.5% chitosan (CHI), 10% or 5% aqueous extract of ginger, onion and garlic (GOG) and their composite solutions (1% CHI + 10% GOG, 0.5% CHI + 5% GOG) on quality and shelf life of stewed-pork were evaluated. Microbiological (total bacterial count), chemical (pH, total volatile basic nitrogen (TVB-N), peroxide value (POV), 2-thiobarbituric acid (TBA)) and sensory characteristics were analysed periodically during refrigerated storage at 4 °C for 12 days. CHI and/or GOG treatments retarded the increases in pH, TVB-N, POV, TBA and total bacterial count. CHI showed better antibacteria but weaker antioxidation than GOG. Composite treatment had possible synergistic effect while the high concentration of composite solution (Mix1) had adverse effect on odour and overall acceptance. Mix2, the diluted solution of Mix1, could be a natural promising preservative for the stewed-pork considering the comprehensive effects of antioxidation, antibacteria and sensory quality, which could extend the shelf life for about 5–6 days.

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1. Introduction

Pork and its products are rich in protein, lipids and have suitable moisture content, which make them ‘natural media’ of microorganisms. Pork also contains high level of unsaturated fatty acids which are prone to oxidation. Microbial growth and lipid oxidation are the two leading factors for quality deterioration of meat.

To prevent or reduce lipid oxidation, discoloration and flavour deterioration, synthetic antioxidants, such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT), have been applied in meat products (Khalil & Mansour, 1998). Synergistic effect of BHA and BHT was also confirmed on antioxidation (Shelef & Liang, 1982). However, their application on foods had been restricted because of their potential health risks and toxicity (Sun & Fukuhara, 1997). Concerns about synthetic antioxidants’ safety have recently attracted special interest in a high demand for natural additives alternative to synthetic antioxidants (Zhou, Xu, & Liu, 2010).

Ginger, onion and garlic are traditional edible and medicinal plants. They are regularly used as seasonings to enhance the sensory quality of foods in China. Besides their health benefits have been studied widely (Chandrashekar, Prashanth, & Venkatesh, 2011; Shariatpanahi, Taleban, Mokhtari, & Shahbazi, 2010), the extracts of ginger, onion, and garlic have also been individually studied on their antioxidant properties in food preservation (Naznin, Maeda, & Morita, 2010; Shirin Adel & Prakash, 2010; Suh, Lee, Cho, Kim, & Chung, 1999). For example, the brined onion extracts could enhance the quality of turkey breast rolls during refrigerated storage (Tang & Cronin, 2007). Phytochemicals presenting in the three spices and their antimicrobial activity against some pathogenic bacteria were also investigated (Tagoe, Nyarko, & Akpaka, 2011). However, combined addition of these three extracts in meat products preservation has not been reported to date.

Chitosan [β-(1,4)-2-amino-2-deoxy-D-glucopyranose], which is mainly made from crustacean shells, is the second most abundant natural polymer in nature after cellulose (Shahidi, Arachchi, & Jeon, 1999). It has been widely used as a natural food additive in the food industry due to its non-toxic nature, biocompatibility, antibacterial and film-forming properties (Majeti & Ravi, 2000). Function of chitosan differs from its molecular weight and degree of deacetylation. The antimicrobial activity of chitosan with high molecular weight and high degree of deacetylation was well documented against a number of food spoilage and pathogenic microorganisms with concentration varying from 0.5% to 1.5% (No, Park, Lee, & Meyers, 2002). Chitosan has been more and more generally used as antimicrobial agents to prolong the shelf life of meat products (Dutta, Shupa, Mehratra, & Joydeep, 2009; Fan et al., 2009). But there are different views on the antioxidant capacity of chitosan. Kanatt, Chander, and Sharma (2008) reported that chitosan had no significant antioxidant activity, whereas effectiveness of chitosan treatment on oxidative stability of minced meat...
beef was reported by Darmadji and Izumimoto (1994), who observed that the addition of chitosan at 1% concentration could result in a 70% decrease in the 2-thiobarbituric acid (TBA) value of meat after 3 days of storage at 4°C.

The objective of this study was to investigate the quality changes of stewed-pork under refrigerated storage, and evaluate the effect of chitosan (CHI), and aqueous extract of ginger, onion and garlic (GOG), applied individually and/or in combination, on delaying lipid oxidation and inhibiting microbial growth.

2. Materials and methods

2.1. Preparation of preserved solutions: aqueous extract of ginger, onion and garlic (GOG), chitosan solution (CHI) and their composite solution

2.1.1. Preparation of aqueous extract of ginger, onion and garlic

Ginger (Zingiber officinale Rosc.), onion (Allium cepa L.) and garlic (Allium sativum L.) were purchased from a local superstore (Hangzhou, Zhejiang Province, China). After cleaning and draining, approximately 2000 g of minced samples was homogenised with 50 mL of 7.5% (w/v) trichloroacetic acid (TCA) before use. 5% (w/v) aqueous extract of ginger, onion and garlic (GOG5) was made by mixing GOG10 with equal volume of distilled water.

2.1.2. Chitosan solution preparation

Chitosan, with molecular weight of 1.6 × 10⁵ Da and 95% degree of deacetylation, was purchased from Jinke Company (Taizhou, Zhejiang Province, China). 1% (w/v) chitosan solution in glacial acetic acid (CHI1) was prepared as follows: aliquots (100 g each) of three chopped spices were mixed into 600 mL of distilled water and extracted for 30 min at 40°C in enclosed flasks with ultrasonic extract (200 W, 40 kHz). After filtration with Whatman No. 1 filter paper, the residue was re-extracted with an additional 400 mL of distilled water for additional 30 min and then filtered. The supernatant was combined and stored at 4°C before use. 5% (w/v) aqueous extract of ginger, onion and garlic (GOG5) was made by mixing GOG10 with equal volume of distilled water.

2.1.3. Preparation of composite solution of ginger, onion, garlic and chitosan

Chitosan was dissolved by adding 1% (w/v) glacial acetic acid and mixed with the proposed 10% (w/v) aqueous extract of ginger, onion and garlic (GOG10) mentioned above, which made chitosan a final concentration of 1% (w/v), and this was assigned as Mix1 (10% aqueous extract of ginger, onion and garlic–1% chitosan composite solution). Mix2 (5% aqueous extract of ginger, onion and garlic–0.5% chitosan diluted solution) was prepared by mixing CHI1 with equal volume of distilled water.

2.2. Preparation, treatment and storage of stewed-pork samples

M. longissimus dorsi muscle from local cross swine (5–6 months, 90–100 kg), was purchased from a local supermarket (Hangzhou, Zhejiang Province, China) at 48 h postmortem and iced transportation to the lab. After removing of subcutaneous, intramuscular fat and visible connective tissues, the lean pork was firstly cut into small sections with uniform size (3 × 2 × 1 cm). Then all the sections were cleaned with distilled water. After that Chinese traditional cuisine was used to cook pork meat as follows: sections of pork meat (selected approximately 2000 g) soaked in 3000 mL distilled water (mixed with 30 g salt), Clay pot (JYZS-M201, Joyoung Ltd., China) was employed to heat the pork patties until the internal temperature reached 75°C which were measured with infrared radiation thermometer (826-T4, Testo, Germany) and stewed for 2 h. The stewed time was decided by fuzzy mathematic evaluation (Amerine, Pangborn, & Roessler, 1965) which was investigated in our previous study.

After cooling to room temperature, the stewed-pork were placed in styrene foam trays, and divided into seven portions (each about 250 g) and gently drained on a tissue paper. Six portions were dipped in the GOG5 (5% aqueous extract of ginger, onion and garlic), GOG10 (10% aqueous extract of ginger, onion and garlic), CHI0.5 (0.5% chitosan), CHI1 (1% chitosan), Mix1 (10% aqueous extract of ginger, onion and garlic–1% chitosan), and Mix2 (5% aqueous extract of ginger, onion and garlic–0.5% chitosan) solutions for 10 min (Chang, Chen, & Tan, 2011), respectively. Samples dipped in deionized water for 10 min were as a control and assigned code of CON. All samples were drained on tissue paper after soaking treatments, placed in polyethylene bags (50 g each) and stored under aerobic conditions at 4°C for a period of 12 days. The experiment was performed twice, and every 3 days a sample was taken from each treatment in which the pH, TVB-N, POV, TBA values, total bacterial count and sensory characteristics of stewed-pork were analysed.

2.3. Chemical analysis

2.3.1. Determination of pH

pH determination was according to AOAC (1995), 10.0 g sample of the meat muscle was homogenised in 100 mL distilled water and the mixture was filtered. The pH of filtrate was measured by a pH meter (Mettler Toledo 320-S, Shanghai Mettler Ltd., China).

2.3.2. Determination of total volatile basic nitrogen (TVB-N)

TVB-N content was determined by distillation after the addition of MgO to the minced sample. The distillate was collected in a flask containing 3% (w/v) aqueous solution of boric acid and a mixed indicator produced by dissolving 0.1 g of methyl red and 0.05 g methylene blue to 100 mL ethanol. The boric acid solution turned green when the distilled TVB-N made it alkaline. Finally, the boric acid solution was titrated with a 0.01 mol/L HCl solution until it turned pink (Goulas & Kontominas, 2005). The quantity of TVB-N in mg/100 g sample was calculated from the volume (V) of HCl added and its concentration (C) as followed:

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TVB-N (\text{mg/100 g}) = \left( V \times C \times 10 \times 100 \right) / 10
\]

2.3.3. Determination of peroxide value (POV)

Lipid was extracted from minced samples with chloroform/methanol mixture (2:1, v/v) according to the procedure of Folch, Lee, and Stanley (1957). The lipid extracts were evaporated with a rotary evaporator (RE-5220, Yarong Biochemical Analysis Co., Ltd., China). The peroxide values (POV) of the lipids were determined by AOAC (1995). The results were expressed as milliequivalent (meq)/kg lipid.

2.3.4. Determination of 2-thiobarbituric acid (TBA)

2-Thiobarbituric acid (TBA) was determined by the method of Mielnik, Olsen, Vogt, Adeline, and Skrede (2006), with slight modification. Approximately 10.0 g of minced samples was homogenised with 50 mL 7.5% (w/v) trichloroacetic acid (containing 0.1% EDTA), with a homogenisation at 15,000 rpm. The dispersion was filtered through Whatman No. 1 filter paper. The supernatant (5 mL) was mixed with 5 mL 0.02 mol/L TBA reagent first; then it was heated in boiling water for 30 min to develop the rose-pink colour and cooled for 10 min in cold flowing water immediately. The absorbance was measured at 532 nm against
of meat sample (mg MDA/kg sample).

Thiobarbituric acid reactive substances (TBARS) were calculated from a standard curve of malondialdehyde (MDA), which was freshly prepared by acidification of 1,1,3,3-tetraethoxypropane (TEP). TBA value was expressed as milligrams of malondialdehyde per kilogram of meat sample (mg MDA/kg sample).

2.4. Microbial analysis

Approximately 10.0 g of minced pork sample was homogenized with 90 mL sterile 0.9% (w/v) normal saline (NS), and a shaker was used for 30 min to prepare a 1:10 sample suspension. Additional 10-fold dilutions were prepared with sterile NS. Pour-plate method, using plate count agar (PCA, Merck) incubated at 37°C for 48 h, was used to determine the total bacterial count. Microbial colonies were counted and expressed as lg cfu (colony forming units) per gram of pork meat.

2.5. Sensory evaluation

The sensory characteristics of stewed-pork sample were evaluated by a trained panel of seven selected members from the laboratory faculty and graduate students who had prior experience on meat evaluation. On each specific day, samples were cooked individually in a microwave oven (MM823ESJ-PA, Midea, China) at full power (700 W) for 5 min and presented to the panellists immediately. Panellists scored for sensory qualities, especially colour, off-odour, and overall acceptability successively, using a 9-point hedonic scale. Sensory evaluation was carried out in individual booths under controlled conditions. Water was provided for cleaning the palate between samples.

For colour, the scale points were: excellent, 9; very good, 8; good, 7; acceptable, 6; poor, 5; for overall acceptance: extremely strong, 9 and dislike extremely, 1; for off-odour: extremely strong, 9 and not strong at all, 1.

2.6. Data analysis

All analysis were ran in triplicate for each replicate (n = 2 × 3). Results were expressed as mean ± standard error of mean (SEM). Statistical assessment was carried out with the programe system of SPSS (Version 16.0 for Windows). The results were analysed using one-way analysis of variance (ANOVA). Comparison of treatment means was based on Duncan’s multiple range test. Differences were considered significant at the p < 0.05 level.

3. Results and discussion

3.1. pH changes during storage with different treatments

The initial pH values of the control and samples with various treatments were 5.92 and 5.47–5.89, indicating that the addition of GOG and CHI slightly decreased the initial pH in stewed-pork (Fig. 1). pH values of all the samples gradually increased during storage at 4°C for 12 days. All treatments inhibited the pH increasing to some extent during the storage period. Treated with Mix1 had the best effect which ultimately only reached pH of 5.63 compared with the control (CON) of 7.62. There was no significant difference between the samples treated with Mix2 and CHI0.5 (p > 0.05) which made the final pH around 5.80. GOG5 and GOG10 treatments had the worst effect on pH retaining but the values were also lower 1.07 and 1.27 compared with the control, respectively. CHI treatment made changes of pH values more stable than GOG during stewed-pork storage.

The rise of pH was due to an increase in volatile bases produced. Endogenous or microbial enzymes utilise low molecular compounds such as amino acids present in meat samples and induce the decomposition of alkaline ammonia, e.g., trimethylamine and ammonia (Masniyom, Benjakul, & Visessanguan, 2002). The lower pH in stewed-pork with CHI and GOG treatments postulated to be due to the presence of antimicrobial ingredients in natural spice extracts and the inhibitory effect of chitosan on the growth and reproduction of spoilage microorganisms which metabolizing basic nitrogen compounds. Acetic acid which was solvent of chitosan also influenced the pH value.

3.2. TVB-N changes during storage with different treatments

Total volatile basic nitrogen (TVB-N), which is mainly composed of ammonia and primary, secondary and tertiary amines, is widely used as an indicator of meat spoilage (Fan et al., 2009). TVB-N values of stewed-pork during refrigerated storage were gradually increased (Fig. 2). The increasing order of TVB-N value with different treatments at 12th day were: Mix1 (20.2 mg N/100 g) < CHI1 (24.1 mg N/100 g) < CHI0.5 (27.0 mg N/100 g) < Mix2 (28.6 mg N/100 g) < GOG10 (31.5 mg N/100 g) < GOG5 (32.9 mg N/100 g) < CON (41.0 mg N/100 g). Value of control sample was significantly higher than the CHI and GOG treated samples (p < 0.05). Preserved solutions contained chitosan (Mix1, CHI1, CHI0.5, Mix2) had better effect of TVB-N inhibition than GOG. Jeon, Kamil, and Shahidi (2002) also observed a 26–51% reduction of TVB-N value in chitosan-coated cod and herring fillets stored at 4°C for 12 days. The dose of GOG had no significant influence on the inhibition of TVB-N (p > 0.05).

Increase of TVB-N value is related to the activity of endogenous enzymes and spoilage bacteria (Ruiz-Capillas & Moral, 2005).
Anti-bacterial chitosan and GOG solution may have the effect on inhibiting the microbial decomposition of pork proteins. TVB-N value increased during storage could explain the pH rising mentioned above.

3.3. 

POV changes during storage with different treatments

Lipid oxidation causes undesirable rancid off-flavours and potentially toxic products, leading to the qualitative deterioration. Peroxide value (POV) is the primary products of lipid oxidation which is generated by oxygen attacking on the double bond in fatty acids. Therefore, it seemed reasonable to determine the concentration of peroxide in meat samples to clarify the extent of oxidation (Donald, 1998).

The initial POV value of the control sample (CON) was 3.0 meq/kg lipid and increased to 6.8 meq/kg lipid after 12 days storage, significantly higher than other treatments (p < 0.05). The value of the 6th day (5.8 meq/kg lipid) had exceeded the state maximum limit (5.0 meq/kg lipid) in CON sample (Fig. 3). GOG treatment made stewed-pork a stronger resistance to oxidation compared with CHI treatment. Mix1 and GOG10 treatments had the lowest POV value with the storage time extending. There was no significant difference between the samples treated with Mix2 and GOG5 (p > 0.05). The results showed that GOG solution played a major role on the lipid oxidation retarding in this experiment. Research has been shown that gingerol in ginger is endowed with strong antioxidant action both in vivo and in vitro (Ali, Blunden, Tanira, & Nemmar, 2008). Flavonoid and diallyl sulfide, presenting in onion and garlic, respectively, also markedly inhibited lipid peroxidation (Fanelli, Castro, de Toranzo, & Castro, 1998; Ioku et al., 2001).

On the other hand, values of POV fluctuated during storage in samples from CHI0.5 and GOG10 treatments. Peroxide, as the primary product of lipid oxidation, is very reactive and actually decreases during the storage of lipid-containing foods (Juntachote, Berghofer, Siebenhandl, & Bauer, 2007). Therefore, its content depends on the balance between formation and degradation (Saguy & Dana, 2003).

3.4. 

TBA changes during storage with different treatments

TBA values represent the content of secondary lipid oxidation products, mainly aldehydes, which contribute to off-flavours in oxidised meat and meat matrix. TBA is a good indicator to assess lipid oxidation because of its relatively simple measurement and good correlation with the sensory quality of foods (Donald, 1998). The effect of different treatments on TBA value of stewed-pork over 12 days of refrigerated storage is shown in Fig. 4.

3.5. Total bacterial count changes during storage with different treatments

Total bacterial count of the control sample (CON) was approximately 2.4 lg cfu g⁻¹ on the initial day, and increased to 8.5 lg cfu g⁻¹ after 12 days storage (Fig. 5). The value was reached to 6.6 lg cfu g⁻¹ on the 6th day which already exceeded the acceptable limit (6.0 lg cfu g⁻¹) proposed by many studies (Jeon et al.,...
2002). Hence, the shelf life of the CON sample is estimated as about 6 days, based on microbiological data.

Total bacterial count of GOG10 treated samples exceeded 6.0 lg cfu g⁻¹ on day 9 and the value reached to 7.5 lg cfu g⁻¹ on the 12th day, which resulted in 1 log reduction compared with the CON. GOG5 treated samples reached the value of 7.9 lg cfu g⁻¹ on the 12th day which showed slightly worse effect on anti-microbial growth than GOG10 treated ones. CH11 treated samples reached the acceptable limit on day 12, indicating a significantly delayed microbial spoilage (p < 0.05) than GOG treatment. Mix1 and Mix2 treatments had the lowest total bacterial count during storage which were lower than acceptable limit even after 12 days cold storage (reached 4.9 cfu g⁻¹ and 5.6 lg cfu g⁻¹, respectively). Thus, a microbiological shelf life extension of 5–6 days was achieved for Mix treated samples compared with the control. Results also showed that CHI had better antimicrobial capacity than GOG, and CHI combined with GOG had possible synergistic effect on microbial inhibition.

Benkeblia (2004) has researched that sulfur compounds presenting in garlic and onion could inhibit the gram-positive bacteria and gram-negative bacteria. Plant extracts possess potential as natural agents for food preservation with antimicrobial compounds (Kim, Kim, Kim, Whang, & Suh, 2008). The mode of antimicrobial activity of chitosan might be due to its interaction with cell wall and membranes components, resulting in increased leakage of cell material from tissue and permeability of the membranes, or due to its water-binging capacity and inhibition of various enzymes (Helander, Nurmiho-Lassila, Ahvenainen, Rhoades, & Røller, 2001). Chitosan also has the ability to absorb nutrients of microorganisms and thus inhibit their growth (Knorr, 1991).

3.6. Sensory changes during storage with different treatments

Results in Table 1 revealed the score changes of colour, off-odour and overall acceptance of stewed-pork with different treatments during refrigerated storage. Judged by the surface appearance, the bright colour of stewed-pork changed to brown and different treatments had no significant difference (p > 0.05). The colour changes of meat have been explained by the formation of carboxy form of haem pigments either carboxymyoglobin or carboxyhaemoglobin (Millar, Moss, & Stevenson, 2000).

Stewed-pork was rich in meat flavour immediately after cooking treatment. Score of off-odour was gradually increased with storage extending and characteristic undesirable odour appeared intensely due to oxidation of polysaturated fatty acids. Treatments with GOG and/or CHI resulted in reduction of off-odour scores to some extent, especially Mix2 and GOG5 treatments which also gave aroma flavour of spice extracts. However, preserved solution with high concentration of GOG (GOG10, Mix1) made a masking of meaty flavour in the later.

The overall acceptance of food by consumers determines the future of that food in the market. Therefore, increasing the consumer acceptance of food processed with new technologies will accelerate their market share in food industry. Different treatments (GOG and/or CHI) resulted in significantly (p < 0.05) higher overall acceptance scores than the control (CON) at the end of storage. Stewed-pork treated with CHI0.5, CHI1, GOG10 and Mix1 had no significant difference (p > 0.05) on the changes of overall acceptance scores and high concentration GOG treated samples (GOG10, Mix1) revealed strong spicy flavour of ginger, onion and garlic with storage time extending. Mix2 treated samples were mostly accepted by panellists might be attributed to oxidative and microbial stability with possible synergistic effect of GOG and CHI. Proper concentration of GOG giving stewed-pork a desired aroma flavour of spice extracts was also an important factor.

4. Conclusions

Chitosan (CHI), aqueous extract of ginger, onion and garlic (GOG) had antioxidative and antimicrobial activity on stewed-pork...
matrix in this study. Stewed-pork immersed in CHI and/or GOG solutions could reduce the values of pH, TVB-N, POV, TBA and total bacterial count during 12 days of refrigerated storage. Chitosan revealed better antibacteria but weaker antioxidation than GOG. CHI combined GOG had possible synergistic effect on antioxidation and microbial inhibition for stewed-pork preservation. Specifically, 5% aqueous extract of ginger, onion and garlic–0.5% chitosan (Mix2) could be used as natural antioxidants in cooked pork products.

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