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Effects of Sorghum Grain Tannin Addition in Refrigerated Pork Meat against Lipid Oxidation

Lei Zang[1], Xinping Jin[1], Xiaopei Liu[1], Dongdong Wang[1], Ying Xu[1], Shanshan Li[1], Nan Xue[1], Rongrong Liang[2], Huawen Zhang[3], Hailian Wang[3], Shubing Liu[1], Yuye Wu[1,*]

[1]National Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Tai'an, China
[2]School of Food Science and Engineering, Shandong Agricultural University, Tai'an, China
[3]Crop Research Institute, Shandong Academy of Agricultural Sciences, Jinan, China
*Corresponding author: yywu@sdau.edu.cn

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Abstract As one of the favorite foods, lipid oxidation of pork meat has always been a problem faced by meat industry. Meat producers typically add synthetic antioxidants to mitigate lipid oxidation in pork. With the growth of health awareness and pursuit of natural products in recent years, there is a growing demand to develop natural antioxidants. The aim of this work was to extract tannins from sorghum grain and theninvestigate its effect on lipid oxidation of chilled pork. The tannins extracted from sorghum grain contained three monomers (15.17 mg/g for catechin, 9.74 mg/g for epicatechin and 17.2 mg/g for epicatechin gallate, individually) and exhibited higher antioxidant capacity than rutin in vitro. The addition of 0.2% tannin on pork meat significantly inhibited the increase of pH value, TBA value, TVB-N and total number of colonies (by 2.4%, 64%, 35%, 7.5%, respectively) and maintained the desired red color of chilled pork during refrigerated storage of 10 days. The antioxidant effect of 0.2% tannin treatment was better than that of BHT. Further, higher sensory scores were observed for the tannin-incorporated samples. The addition of 0.2% tannin significantly inhibited the lipid oxidation of pork, slowed down the meat color change, and prolonged the shelf life of pork while maintaining the sensorial characteristics intact. This study indicates the feasibility of sorghum tannin as a natural antioxidant for pork preservation, further boosting the commercial value of sorghum.

Keywords: *sorghum tannin, natural antioxidant, meat preservation, lipid oxidation*

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1. Introduction

Meat provides high quality protein, minerals, vitamins and many other micronutrients and is a highly nutritious food source [1]. Among them, pork is popular among consumers because of its delicate taste and soft texture, and it has become one of the main meats for daily consumption. However, lipid oxidation of pork meat and meat products is considered as the main issues of meat and meat products' deterioration, leading to discoloration, unpleasant smell, poor taste, and even loss of nutrients. Therefore, mitigating oxidation of pork product has always been one of the most important challenges for meat producers [2,3].

Antioxidants have long been added to meat to effectively mitigate the adverse effects of oxidation, most of which are chemically synthesized (e.g., butylhydroxyanisole, butylhydroene, tert-butylhydroquinone) [4,5]. However, due to the potential adverse effects of synthetic antioxidants on human health, the demand for safe and effective natural antioxidant alternatives is increasing [6]. The development and utilization of natural sources of antioxidants have been the trend of development in this field [7]. To seek natural antioxidant, considerable research on plant extracts has been engaged and showed positive effects in sensory quality and safety of processed products [5,8-16].

The antioxidant activities of the plant natural extracts have been identified mainly from high level of bioactive phenolic compounds with ability to scavenging free radicals [17]. The tannins also known as condensed tannins formationed with catechin and epicatechin are a class of polyphenolic compounds [18]. Tannins are naturally found in various plants such as grape seed, tea, legumes, berries and cereals [19,20]. Studies have showed that tannins have multiple functions such as antimicrobial activity, antioxidant capacity and UV protection etc. Based on a comparative study, antioxidant capacity in tannin ranked highest among a range of polyphenols [21,22]. It has been reported that synthetic tannin can be used as antioxidants to combat lipid oxidation and maintain meat quality for chicken, fish, and beef during storage [23,24]. Tannins can also be consumed in daily

life and diets rich in tannins such as grape seed are beneficial to human health due to its immunomodulatory and antioxidant, anticancer, anti-inflammatory, cardioprotective, and UV-protective functions [25,26]. In a study, persimmon tannin from peel showed antidiabetic properties [27]. With increasing concern over the food safety, tannin extracts from plants such as Quebracho Colorado Wood and oak gall nuts have been evaluated on meat quality attributes [28].

Generally, Berries and fruits are the best sources of tannins. However, sorghum has been reported to be harboring the highest tannin content in certain varieties with black tasta [29]. Sorghum condensed tannins are mainly composed of oligomers or polymers of flavane-3-alcohol and flavane-3, 4-diol, which have many functions including resistance to pathogens and predators, reduce digestion efficiency by binding proteins, avoid uv radiation and reduce oxidative stress [30,31]. Studies have shown that the antioxidant capacity of tannins in sorghum bran is nearly 30 times higher than that of grapes [32], in addition, tannins in sorghum are 15-30 times higher than simple phenolic compounds in terms of free radical scavenging capacity [22]. Therefore, sorghum tannin is of special concern as an important source of antioxidant.

With a trend towards utilizing natural antioxidants as replacements for synthetic ones, tannins can be isolated as food additives and natural antioxidants. Therefore, this study extracted tannin from sorghum grain, investigated its structure, antioxidant activity in vitro, and then added to pork meat as a contribution to the potential use of this food sourced antioxidant in meat industrial application. Exploitation of tannin from sorghum can be a sustainable source of dietary supplements and functional ingredients.

2. Materials and Methods

2.1. Tannin Extraction, Purification and Identification

Six genotypes with different color of sorghum grains (Figure 1) were selected for tannin extraction analysis, including Is-2803(Is28, brown), GanzaoShua (Gzs, white), Dahong Pao (Dhp, red), Niangao Liang (Ngl, cherry red), Heigao Liang(Hgl, black), Tx430 (yellow), which were all provided by Shandong Academy of Agricultural Science (Jinan, China).

Sorghum seeds were first dried to constant weight at room temperature and grounded to fine powder by ball mill. Then 1g powder were added to 10 mL 70% acetone water, and incubate in water bath at 56℃ for 2.5 h. The extract was centrifuged at 6000 rpm for 10 min. The supernatant was steamed under vacuum at 40℃ to obtain the aqueous solution of crude extract. Crude extract was prepared by vacuum freeze-drying of crude extract aqueous solution at -85℃. The crude extract aqueous solution was extracted 5 times with ethyl acetate 1:2(V/V). The upper organic phase was steamed at 40℃ to dry, and then tannin aqueous solution was obtained by adding distilled water. Tannin powder was prepared by vacuum freeze-drying of tannin aqueous solution at -85℃ and then stored at -20℃ for use. The purified sorghum tannin was completely dissolved in methanol, thoroughly mixed, filtered by 0.45μm microporous filter membrane, and analyzed.

Analysis conditions were as follows: Shim-pack GIST C18 column (2.1x50 nm, 2μm), mobile phase consisted of acetonitrile (A) -0.1% formic acid (B) flow rate was 1 mL/min, detection wavelength was 278nm, column temperature was 30°C, sample size was 2μL, gradient elution. Gradient elution (0-8 min, 15%-35%A; 8-10min, 35%-60%A; 10-20 min, 60%A; 20-21min, 60%-15%A; 21-25min, 15%A) 0.005g of catechin, epicatechin, epicallocatechin and epicatechin gallate were accurately weighed, respectively and diluted to 5mL with methanol to prepare 1 mg/mL mixed standard reserve solution. The standard reserve solution was diluted with methanol step by step and the standard working series curves were configured. The concentrations were 0.01, 0.02, 0.05, 0.1 and 0.15 mg/mL, respectively. After being shaken well, the filtrate was filtered by 0.45μm microporous filter membrane, and 2μL filtrate was sampled for analysis. The peak area of each standard substance was measured, and the standard curve of its concentration was drawn.

2.2. Antioxidant Capacity Analysis of Sorghum Tannin in Vitro

2.2.1. DPPH Free Radical Scavenging Ability

The 1.1-diphenyl-2-picrylhydrazyl (DPPH) assay was performed as described by Wei et al., [33] with slight modification. Briefly, 1mL volume of sorghum tannin extract methanolic solution was added to 3mL of methanolic DPPH (0.2mmol/L), vortexed, and kept at room temperature in the dark for 30 min.The absorbance was measured at 518 nm using a spectrophotometer (UV-2550, Shimadzu Corporation, Japan), and a blank was used with 1mL methanol and 3 mL DPPH mixed. Assays were performed in triplicate and averaged. The IC50, is the centration giving 50% inhibition of DPPH, was read off a graph of I% (percentage inhibition) versus extract concentration. Vc and Rutin(Solarbio Company, China) were measured as positive control in the study.

The percentage of inhibition of DPPH (I%) was calculated using the following equation:

$$I\% = \frac{A_0 - A}{A_0} \times 100\%$$

A_0 is the absorbance of the standard solution.

2.2.2. Ferric Reducing Antioxidant Power (FRAP)

Fe^{3+} reducing power was determined using the method described by Zhang and Wang [34], with minor modifications. 1 mL sample, 2.5 mL phosphate buffer (0.2 mol/L, pH=6.6) and 2.5 mL 1% potassium ferricyanide were added to the centrifuge tube, and the mixture was thoroughly mixed, and put in the 50℃ water bath for 20 min. The reaction was terminated by adding 2.5 mL of 10% trichloroacetic acid (TCA) and centrifuged at 6000 rpm for 10 min. 2.5 mL supernatant, 2.5 mL deionized water, and 0.5 mL 0.1% ferric trichloride solution were added to a test tube and allowed to stand at room temperature for 10 min before absorbance was measured at 700 nm. Each set of experiments was repeated three times. The calculation formula of reducing power

expressed by absorbance:

$$Fe^{3+}\text{reducing power} = A - A_0$$

A is the absorbance after the reaction of the sample, and A_0 is the absorbance after the reaction of deionized water.

2.3. The Application of Sorghum Grain Tannin in Pork Meat

Fresh pork tenderloin was selected and placed at 4℃ to reduce the center temperature to 0-4℃. After disinfection, fat and fascia were removed, and the meat samples were cut into cuboids (3 cm×3 cm×1.5 cm), 25 g per piece. The processed meat samples were randomly divided into 3 groups, with 3 replicates in each group. The following solution was added to the surface of each group: (1) control group (deionized water); (2) 0.1% tannin extract; (3) 0.2% tannin extract; (4) 0.1% BHT (Butylated hydroxytoluene); (5) 0.2% TP (Green Tea Polyphenols). Let the samples stand for 5 min, then drain and wrap in plastic wrap and store at 4℃. Meat color, pH, thiobarbituric acid reactants (TBA) value, volatile base nitrogen(TVB-N) value were measured on day 0, 2, 4, 6, 8, and 10 and averaged in triplicate for each set of experiments, to compare the effects of different concentrations of sorghum tannin and other preservative on refrigerated pork.

2.3.1. Color Measurement

The method of Fan et al., [16] was used to determine the meat color. The white board and black tube of the color difference meter were first corrected by X-Rite SP62 color difference meter, and the meat color brightness (L*), redness (a*) and yellowness (b*) of the pork surface were measured after the correction. The surface of each treated sample was measured at six different positions every 2 days.

2.3.2. pH Measurement

The pH values were determined directly using a portable pH meter (SenvenGo, Mettler-Toledo, Switzerland) calibrated in pH 4.00 and 7.00 buffers, and the probe inserted into the center of patties. Each sample was measured at least three times.

2.3.3. Lipid Oxidation

Thiobarbituric acid reactants (TBA) value was determined according to the method of Fan et al. [16] with minor modifications. Four grams samples were randomly collected from the raw pork (tendons and fat were trimmed), and homogenized with 20 mL of distilled water with a homogenizer (Ultra-Turrax T18 homogenizer, T18, IKA, Germany) for 1 min (homogenized for 30 s rested for 30 s and then homogenized for 30 s). Then, 20 mL of 10% (w/v) TCA solution was added, vortexed, and filtered through Whatman NO.1 filter paper (Whatman International Ltd., Maidstone, England). Then the filtrate (4 mL) was obtained and 1 mL of 60 mM thiobarbituric acid solution was added and mixed. Then the mixtures were incubated at 80℃ for 90 min in a water bath. After incubation, it was taken out and cooled to room temperature. Then 250 μL mixture was transferred to a 96-well plate, and the absorbance at 532 nm was determined spectrophotometrically (TU 1901; Purkinje General, Beijing, China) against a blank containing all reagents (2 mL of distilled water, 2 mL of 10% TCA and 1 mL of 60 mM TBA reagent). TBA values were calculated using the standard curve (obtained from solutions of 1, 1, 3, 3-tetraethoxypropane at known concentrations). The results were expressed as mg /100g samples.

$$\text{TBA value}\left(\text{mg}/100\text{g}\right) = \frac{A_{532} - A_{600}}{155} \times \frac{1}{10} \times 72.6 \times 100$$

A_{532} and A_{600} is the absorbance value of meat samples at 532 nm and 600 nm.

2.3.4. TVB-N

The TVB-N concentration was determined according to the Chinese National Food safety standard methods GB 5009.228–2016. Briefly, Samples were taken from the pork frozen at 4℃ until analyzed. A 10 g sample was homogenized using an Ultrapure T18 homogenizer (T18; IKA, Germany) in 50 mL of distilled water at 11000 rpm for 30 s, and then 25 mL of distilled water was added. The homogenate equilibrated for 30 min at room temperature (20℃ ~ 25℃) and was subsequently transferred to a distillation tube. After 1 g of magnesium oxide was added to the distillation tube and the sample, the distillation tube was connected to the automatic kjeldahl apparatus (K - 355, Buchi, Switzerland) according to the instructions. The volatile basic nitrogen was removed by steam and was absorbed using a boric acid solution (20 g/L). Volatile base nitrogen content in the sample was calculated as:

$$\text{TVB} - \text{N}\left(\text{mg}/100\text{g}\right) = \frac{\left(V_1 - V_2\right) \times \text{C} \times 14}{\text{m}} \times 100\%$$

V_1 denotes the volume of the hydrochloric acid (mL) consumed by tested sample; V_2 denotes the volume of the hydrochloric acid (mL) consumed by blank sample; C denotes the actual concentration of hydrochloric acid (mol/L); 14 denotes molar mass of nitrogen; m denotes sample weight (g).

2.3.5. Total Number of Bacterial Colonies

Total number of bacterial colonies was analyzed using the method described in GB 4789.2-2016 "Determination of the Total Number of Colonies in Food Microbiology Inspection". The 10 g of raw surface samples were randomly collected and transferred aseptically to a stomacher bag (BagPage®, Interscience, St Nom, France) containing 90 mL of sterile tryptone salt solution at 0.85%, and tapped with a tapping homogenizer for 2 min. A 10-fold dilution series was prepared to perform microbial analysis. Diluted samples were cultured in plate count agar (PCA, LandBridge Co., Ltd., Beijing, China) and incubated at 37℃ for 48 h to determine TVC (log CFU/g).

$$\text{Total number of bacterial colonies}\left(\lg cfu/g\right) = \frac{\sum C}{\left(n_1 + n_2\right)d}$$

In the equation, ∑C is the total number of plates, n_1 is the number of plates containing high concentration samples, n_2 is the number of plates with low sample concentration, and d is the first dilution ratio.

2.3.6. Sensory Evaluation

To evaluate the effect of tannin addition on sensory characteristics of pork meat, the meat samples were sliced to pieces 0.8-1.0 cm wide and labeled individually with random numbers and served to panelists in separate booths.

Panel members were recruited from students in the National Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University. 10 panelists were 20-35 years old (5 female, 5 male). Panel members were given verbal instructions before they evaluated the products. All requirements for the achievement of sensory tests were complied with according to the Chinese Standard GB 2707-2016 "Fresh (Frozen) livestock and Poultry Products", following the general guidelines intended for sensory analysis of meat products.

The sensory attributes of meat samples including color, odor, elastic, viscosity were evaluated on day 0, 2, 4, 6, 8, 10. A 9-point hedonic scale was used to determine the four sensory attributes: 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely.

3. Results

3.1. Tannin Contents, Purification, and Identification

The tannin content in sorghum grains can vary greatly among different genotypes and colors. In this study, six sorghum genotypes with different grain color (brown, white, red, cherry red, black and yellow) were investigated for tannin extraction. The results showed that the tannin content of the six sorghum genotypes ranged from 0 to 370 mg/100g with brown genotype (Dhp) ranks the highest amounts (Figure 1). Then sorghum variety "Dhp" was selected for the tannin extraction for subsequent experiments.

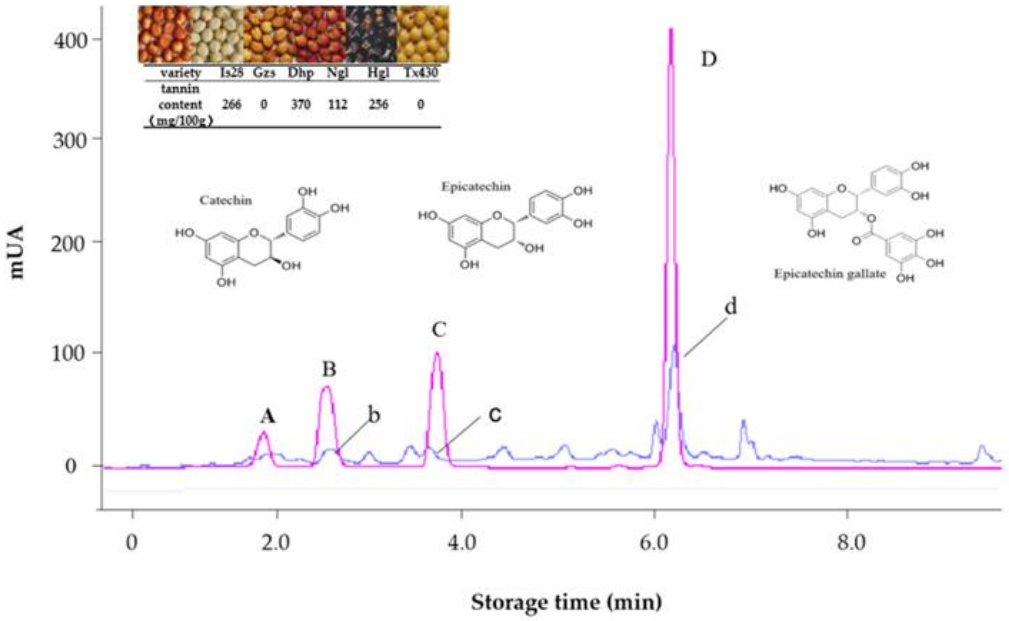

Figure 1. Comparative chromatogram of standard and sorghum tannin. (A) Epigallocatechin; (B) Catechin; (C) Epicatechin; (D) Epicatechin gallate; (b) Catechin in the sample; (c) Epicatechin in the sample; (d) Epicatechin gallate in the sample. Red, mixed standard; Blue, sorghum tannin extract. The sorghum seed pictures in the figure are Is-2803 (Is28, brown), GanzaoShua (Gzs, white), Dahong Pao (Dhp, red), Niangao Liang (Ngl, cherry red), Heigao Liang (Hgl, black) and Tx430 (yellow) from left to right. The inserted table shows the tannin content of the six sorghum varieties

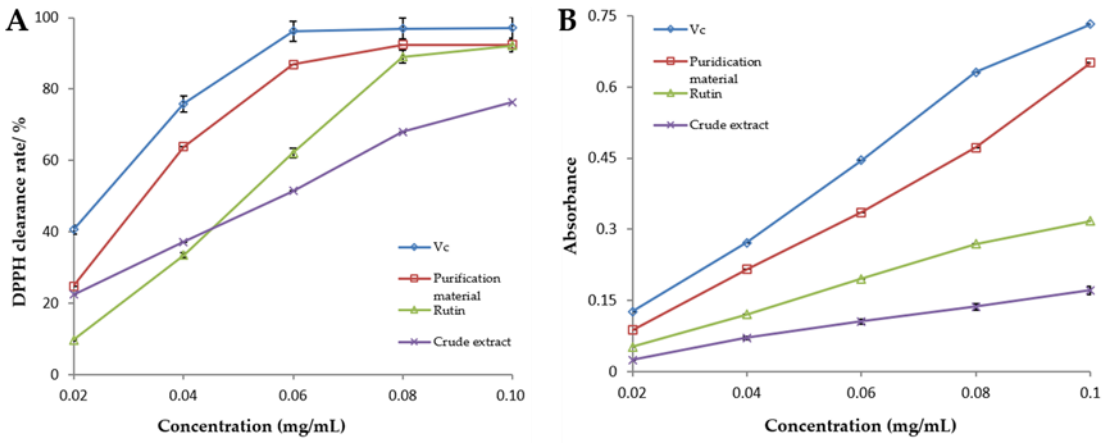

Figure 2. Antioxidant capacities of sorghum tannin extract assayed by DPPH and FRAP. (A) DPPH radical scavenging rate; (B) Fe^{3+} reducing power

Generally, the tannin crude extract contains polysaccharides and other impurities that can resist oxidation. Therefore, the crude tannin extract was further purified by liquid phase extraction based on methods [35]. In order to identify the specific components and absolute content of the purified sorghum tannin monomer, ultra-high-performance liquid chromatography (UPLC) was used in this study. There are 4 standard substances used, namely, epigallocatechin (EGC), catechin (C), epicatechin (EC), and epicatechin gallate (ECG). Through UPLC analysis, it was found that the purified sorghum tannins contained C, EC, ECG three types of tannin monomers (Figure 1). Another standard ingredient, EGC, was not found in the tannin purification extracted. The contents of the three monomers in tannin purification were 15.17 mg/g for catechin, 9.74 mg/g for EC and 17.2 mg/g for ECG, individually. According to the chromatographic analysis results, the sorghum tannin extraction has been shown other unknown monomer components due to the limitations of the standard added (Figure 1).

3.2. Antioxidant Capacities

Tannin extract of sorghum grain has been determined for in vitro antioxidant and free radical scavenging activities by DPPH, and FRAP assays, two methods with different principles. Rutin, one of the most important flavonoids and Vitamin C were chosen as references of natural extract in this study because their antioxidant capacities have been well demonstrated and commercialized. The antioxidant capacities of tannin extract with different concentrations were shown in Figure 2. In the range of 0.02-0.1 mg/mL, the higher the concentration, the stronger the antioxidant capacities. The antioxidant capacities of four tested samples (tannin extract purified, tannin crude extract, rutin, Vc) were consistent in the order Vc> tannin extract purified>rutin> tannin crude extract by DPPH and FRAP. Compared with tannin crude extract, the average antioxidant capacity of tannin extract after purification elevated 29% by DPPH radical scavenging activities and 71% by ferric reducing ability respectively, which may be due to some metabolites (carbohydrates) being removed and tannin content got further concentrated via purification process. The tannin extract after purified showed 20% DPPH radical scavenging activities and 46% ferric reducing ability higher than rutin. These above findings suggested that sorghum tannin extract in the current study exhibited high antioxidant capacities in vitro.

3.3. Lipid Oxidation

Generally, microbial growth and lipid oxidation are two major causes that incur meat products freshness loss and shelf-life shorten [35,36]. Historically, synthetic antioxidants such as BHA (Butyl hydroxyl anisol), BHT (Butylated hydroxytoluene) and gallates have been successfully prevent lipid oxidation in meat industry [37]. In the recent years, meat industry and consumers are demanding antioxidants from natural sources due to the negative health consequences and adverse toxicological reports of some synthetic compounds. Tannins from sorghum grain have been proved to be having high antioxidant capacities in intro, and in this study, it was incorporated to pork to investigate the effect on meat lipid oxidation. It may afford the meat industry an opportunity to develop novel meat products with enhanced nutritional and health benefits, improved shelf-life and quality. The effects of tannin addition and time of storage at 4℃ on lipid oxidation related parameters are presented in Table 1. The pH, TBA and TVB-N values and total number of bacterial colonies of all treatments exhibited an upward trend as days of storage increased. However, of the four parameters measured, compared to pork added with 0 tannin, tannin's inclusion affected TBA and TVB-N values greatly (with 64% and 35% reduction), pH and bacterial colonies lightly (with 2.4% and 7.5% reduction). When considering the effects of tannin level on pork, during the whole storage time at 4℃, as the concentration of tannin increased, TBA and TVB-N values decreased accordingly. Pork formulated with 0.2% tannin further decreased TBA and TVB-N values compared to 0.1% tannin. Tannin addition in meat product affected total number of bacterial colonies not obviously as TBA and TVB-N, only with 7.5% reduction compared to 0% tannin. Overall, tannin addition (especially 0.2% level) can greatly reduce the production of MDA and basic nitrogenous substance during storage at 4℃, thus can work as additives on meat products to prolong shelf life and preserve product quality.

3.4. Effects of Sorghum Tannin Extract on Color of Pork

Discoloration is one of the easily detectable appearances that indicate meat product deterioration. In the present study, pork meat with different level of tannin addition and control has been taken photos on day 0-10 during storage at 4℃ (Figure 3). Based on the pictures of all treatments, visual discs after meat pork were formulated (day 0) and following daily during the next 10 days of storage. The L*, a * and b * values of meat pork showed a decreasing trend with the storage time at 4℃, while 0.1% and 0.2% tannin addition did not affect the L* and b * values compared to control ($P< 0.05$) (Table 2). The effects of tannin addition on a* values began on day 6 of storage, as addition level increased, a* values were higher than control. On day 8 and 10, a* values of pork meat added with 0.2% tannin showed 25.6% and 52.3% higher than 0 tannin addition. Overall, 0.2% tannin addition was effective in maintaining red color in pork based on a * value measurement and visual detection.

3.5. Comparison of Antioxidant Capacities of Sorghum Tannin, BHT and TP on Pork Meat

BHT and TP (Green Tea Polyphenols) have been demonstrated to be effective antioxidants that can extend shelf-life of meat products as synthetic and natural resourced respectively [16]. In the present study, BHT, TP and sorghum tannin extract were incorporated in pork meat separately during storage at 4℃. By measuring TBA and TVB-N values, the effects of sorghum tannin, TP, and BHT on lipid oxidation of pork were compared. From the

results shown in Figure 4, the control samples had overall higher values than other three treatments (BHT, TP, and Tannin extract) by TBA and TVB-N measurement from 4-10 day of storage, which reached to 0.41 mg/100g TBA and 31.06 mg/100g TVB-N, respectively, on 10 day. While, there was not big difference among BHT, TP and tannin treatments except on 10 day, the BHT was significantly higher than TP and tannin extract by TBA value. For TVB-N value, 0.2% TP had the lowest values within the three treatments. These results indicated that the application of the three kinds of antioxidants reduced lipid oxidation on meat. Among the three antioxidants, TP and tannin extract had the similar TBA values and showed better antioxidant capacities than BHT.

Table 1. Effects of sorghum tannin extract on lipid stability parameters of pork

	Tannin (%)	Days at storage					
		day 0	day 2	day 4	day 6	day 8	day 10
pH	Control (None)	6.18^{aA}±0.01	6.26^{aA}±0.01	6.3^{aA}±0.01	6.38^{aA}±0.01	6.49^{aA}±0.02	6.6^{aA}±0.01
	0.1	6.17^{aA}±0.01	6.25^{aA}±0.01	6.28^{bB}±0.01	6.34^{bB}±0.01	6.4^{bB}±0.01	6.5^{bB}±0.02
	0.2	6.18^{aA}±0.02	6.2^{bB}±0.02	6.22^{cC}±0.02	6.25^{cC}±0.01	6.27^{cC}±0.01	6.34^{cC}±0.01
TBA values (mg/100g)	Control (None)	0.03^{aA}±0.01	0.06^{aA}±0.01	0.15^{aA}±0.01	0.24^{aA}±0.01	0.32^{aA}±0.031	0.49^{aA}±0.06
	0.1	0.03^{aA}±0.01	0.05^{aA}±0.01	0.11^{bB}±0.01	0.13^{bB}±0.01	0.16^{bB}±0.013	0.29^{bB}±0.01
	0.2	0.03^{aA}±0.01	0.03^{bA}±0.01	0.07^{cC}±0.01	0.10^{cC}±0.01	0.09^{cC}±0.01	0.14^{cC}±0.01
TVB-N values (mg/100g)	Control (None)	9.47^{aA}±0.20	11.32^{aA}±0.13	16.60^{aA}±0.27	19.84^{aA}±0.32	23.45^{aA}±0.20	31.06^{aA}±1.31
	0.1	9.47^{aA}±0.20	10.21^{bA}±0.13	12.21^{bB}±0.07	15.13^{bB}±0.20	17.44^{bB}±0.13	27.02^{bA}±0.13
	0.2	9.47^{aA}±0.20	9.54^{cB}±0.07	10.57^{cC}±0.20	12.00^{cC}±0.07	13.93^{cC}±0.07	20.89^{cB}±0.46
Total number of bacterial colonies (lg CFU/g)	Control (None)	3.18^{aA}±0.39	4.5aA±0.10	4.86^{aA}±0.10	5.85^{aA}±0.15	6.33^{aA}±0.26	6.4^{aA}±0.20
	0.1	3.18^{aA}±0.39	4.35^{aA}±0.14	4.58^{bA}±0.20	5.31^{bB}±0.45	6.11^{bA}±0.35	6.3^{aA}±0.20
	0.2	3.18^{aA}±0.39	4.2^{bA}±0.23	4.28^{cB}±0.20	5.29^{bB}±0.50	5.98^{bA}±0.15	6.11^{bA}±0.15

* A, B Means with different superscripts are significantly different within days of preservation at 4℃ (P<0.01). a, b, c Means with different superscripts are significantly different within tannin extract level (P<0.05).

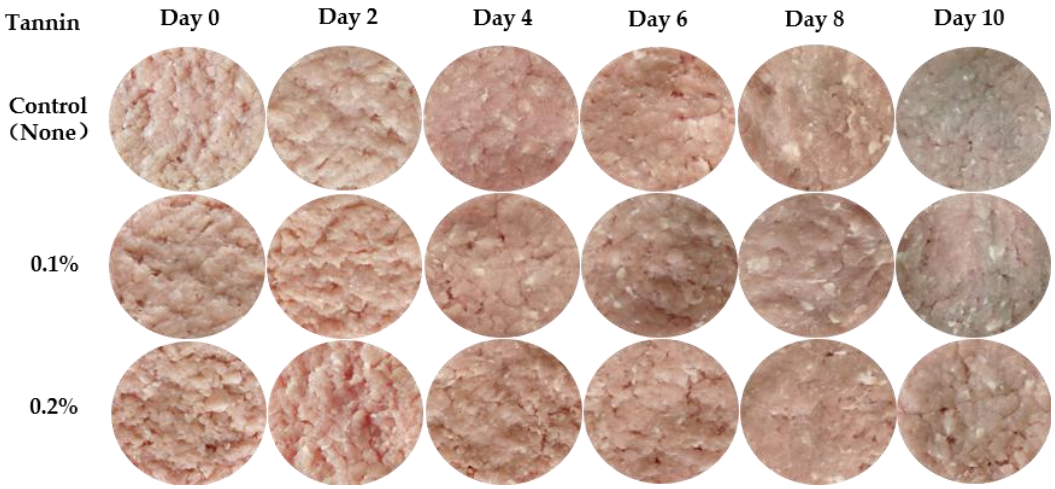

Figure 3. Photos of pork meat added with different tannin during storage at 4℃

Table 2. Effects of tannin addition on color parameters of pork meat

	Tannin (%)	Days at storage					
		0	2	4	6	8	10
L* value	Control (None)	55.50^{aA}±2.33	56.08^{aA}±0.94	55.28^{aA}±1.88	53.13^{bA}±2.17	56.46^{aA}±1.21	55.13^{aA}±0.82
	0.1	56.09^{aA}±2.43	56.66^{aA}±1.75	55.96^{aA}±0.54	55.83^{aA}±2.77	55.79^{aA}±1.52	55.30^{aA}±0.89
	0.2	55.88^{aA}±1.14	56.75^{aA}±1.45	56.01^{aA}±1.34	55.75^{aA}±2.05	55.81^{aA}±1.16	55.65^{aA}±0.47
a* value	Control (None)	4.66^{aA}±0.90	4.27^{abAB}±1.24	3.34^{bB}±1.00	1.62^{cC}±0.54	1.09^{cdCD}±0.40	0.44^{dD}±0.29
	0.1	4.62^{aA}±0.57	4.01^{aA}±0.77	3.25^{bB}±0.87	1.82^{cC}±0.73	1.15^{dC}±0.54	0.51^{eC}±0.26
	0.2	4.65^{aA}±0.70	4.36^{bA}±0.72	3.21^{cB}±0.50	1.85^{cBC}±0.83	1.37^{dCD}±0.37	0.67^{eD}±0.28
b* value	Control (None)	15.17^{aA}±1.15	14.90^{bAB}±1.56	14.31^{bcBC}±0.57	13.94^{bcBC}±1.47	13.58^{bcBC}±0.83	13.10^{cC}±0.74
	0.1	15.62^{aA}±1.08	14.76^{bAB}±1.09	14.33^{bcC}±1.05	14.04^{bcC}±0.52	13.86^{bcC}±0.72	13.44^{cC}±0.78
	0.2	15.18^{aA}±1.07	14.93^{aA}±0.59	14.79^{aA}±1.37	14.24^{aA}±0.80	14.14^{aA}±0.42	14.09^{aA}±0.46

* A, B Means with different superscripts are significantly different within days of preservation at 4 ℃ (P<0.05). a, b, c Means with different superscripts are significantly different within tannin extract level (P<0.01).

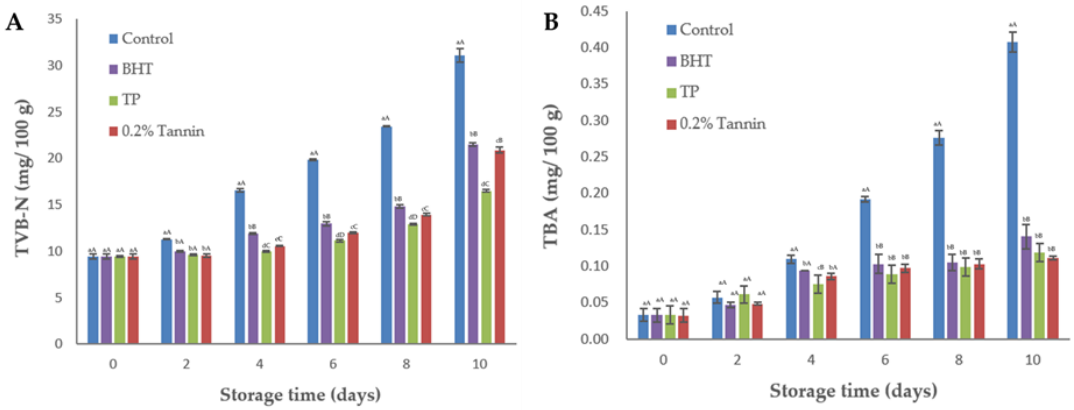

Figure 4. Comparison of antioxidant capacities of tannin extract, BHT and TP on pork during storage at 4℃. (A) TVB-N; (B) TBA. A, B Means with different superscripts are significantly different within days of preservation at 4 degree (P<0.05). a, b, c Means with different superscripts are significantly different within tannin extract level (P<0.01)

Table 3. Effects of tannin addition on sensory characteristics of pork meat

Sensory characteristics	Treatments	Storage time/d					
		0	2	4	6	8	10
Color	Control	9.51^{aA}±0.14	8.94^{aA}±0.21	7.46^{aB}±0.22	6.18^{aB}±0.36	4.98^{aC}±0.33	3.22^{aD}±0.52
	BHT	9.44^{aA}±0.36	9.03^{aA}±0.13	7.74^{aB}±0.66	6.36^{aB}±0.39	4.94^{aC}±0.19	3.45^{aC}±0.82
	TP	9.45^{aA}±0.11	9.11^{aA}±0.26	8.14^{aA}±0.58	7.28^{aB}±0.51	5.81^{bC}±0.21	4.54^{cC}±0.21
	0.2% Tannin	9.57^{aA}±0.04	9.14^{aA}±0.33	7.95^{aA}±0.25	7.20^{aB}±0.44	5.65^{bC}±0.15	4.14^{bC}±0.31
Odor	Control	9.33^{aA}±0.23	9.08^{aA}±0.21	7.94^{aA}±0.36	4.25^{aB}±0.36	2.75^{aC}±0.16	1.51^{aC}±0.34
	BHT	9.46^{aA}±0.34	9.11^{aA}±0.17	8.56^{aA}±0.35	6.16^{bB}±0.55	4.78^{bC}±0.36	2.61^{bD}±0.31
	TP	9.46^{aA}±0.34	9.03^{aA}±0.12	8.18^{aA}±0.49	6.97^{bB}±0.46	5.59^{bB}±0.51	4.15^{cC}±0.19
	0.2% Tannin	9.48^{aA}±0.25	9.14^{aA}±0.22	8.39^{aA}±0.16	6.78^{bB}±0.36	5.27^{bB}±0.19	4.35^{bC}±0.31
Elasticity	Control	9.30^{aA}±0.27	8.23^{aA}±0.16	6.99^{aB}±0.55	4.56^{aC}±0.58	2.64^{aD}±0.31	1.65^{aD}±0.14
	BHT	9.24^{aA}±0.15	8.16^{aA}±0.35	7.26^{aB}±0.67	5.86^{bC}±0.14	4.15^{bC}±0.51	3.16^{bD}±0.64
	TP	9.36^{aA}±0.13	8.27^{aA}±0.38	7.17^{aB}±0.56	6.06^{bB}±0.39	5.16^{bC}±0.15	4.22^{cC}±0.65
	0.2% Tannin	9.26^{aA}±0.24	8.43^{aA}±032	7.13^{aB}±0.73	5.77^{bC}±0.58	4.64^{bC}±0.55	3.97^{cD}±0.14
Viscosity	Control	9.31^{aA}±0.25	7.91^{aB}±0.35	6.46^{aB}±0.85	4.44^{aC}±0.66	2.19^{aD}±0.32	1.29^{aD}±0.47
	BHT	9.25^{aA}±0.15	7.92^{aA}±0.22	6.65^{aB}±0.14	5.25^{bB}±0.54	4.23^{bC}±0.39	2.56^{bD}±0.58
	TP	9.32^{aA}±0.35	8.34^{aA}±0.85	7.16^{aB}±0.38	6.19^{bB}±0.15	4.91^{cC}±0.52	3.57^{dC}±0.51
	0.2% Tannin	9.44^{aA}±0.14	8.17^{aA}±0.66	7.34^{aB}±0.36	5.85^{bC}±0.11	4.11^{C}7±0.64	2.94^{cD}±0.11

* A, B Means with different superscripts are significantly different within days of preservation at 4℃ (P<0.05). a, b, c Means with different superscripts are significantly different within the treatments (P<0.01).

3.6. Sensory Evaluation Results

Sensory attributes of pork meat treated with 0.2% tannin, BHT and TP are tabulated in Table 3, which display that 0.2% tannin treatment and storage time impacted the sensory attributes independently (P<0.05). All the treatments presented a trend of decreased overall sensory score over the storage period with lower values observed in control compared to the pork meat containing 0.2% tannin, BHT or TP after 6 days of preservation at 4℃. A lower off-flavor development was observed in the 0.2% tannin treated samples over the storage period compared to the control, BHT and TP treated samples (P>0.05). The results signify that 0.2% tannin used did not pose severe negative impacts on the physical and sensory properties of pork meat.

4. Conclusions

Sorghum tannin extract contains three major compounds of catechin (C), epicatechin (EC), and epicatechin gallate (ECG) usually found in tannin. Sorghum tannin extract exhibits better antioxidant capacities in vitro than Rutin by DPPH and FRAP assays and delays the process of lipid oxidation of pork under refrigerated conditions. Pork meat treated with 0.2% sorghum grain tannin is the most effective at slowing oxidation production while maintaining the desired red color and sensory characteristics within 10-day refrigeration period. Our results show that 0.2% sorghum grain tannin rivals TP in effectiveness and outperforms BHT treatment. This study confirms the feasibility of sorghum tannin as a natural antioxidant for pork preservation.

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