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RESEARCH ARTICLE

Effect of an Antioxidant from Bamboo Leaves Combined with Tea Polyphenol on Biogenic Amine Accumulation and Lipid Oxidation in Pork Sausages

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Abstract The effect of an antioxidant from bamboo leaves (AOB) combined with tea polyphenol (TP) on biogenic amine accumulation and lipid oxidation in pork sausages during storage was investigated. The peroxide (POV) and the 2-thiobarbituric acid (TBA) values were determined for 8 biogenic amines. Treatment with AOB combined with TP showed a significant ($p<0.05$) antioxidative effect with lower POV and TBA values, and suppression of accumulation of the total level of biogenic amines, and the individual biogenic amines tryptamine, putrescine, cadaverine, histamine, and tyramine, compared to control sausages. Treatment with AOB combined with TP is a promising candidate for retardation of lipid oxidation and prevention of biogenic amine formation in pork sausages.

Keywords: bamboo leave, tea polyphenol, biogenic amine, lipid oxidation, pork sausage

Introduction

Sausage is a favorite traditional processed meat product with a unique cured meat flavor (1). It is popular in many parts of the world and present in different cultural diets because of convenience, variety, and economy (2). Sausage results from biochemical, microbiological, physical, and sensorial changes that occur in a meat mixture during ripening under defined temperature conditions. Traditionally, sausage manufacturing technologies across cultures have been similar with sausage products made of pork or beef that is chopped and thoroughly mixed with seasonings, such as hot chili, pepper powder, spice paprika, onion, salt, rice wine, sugar, monosodium glutamate, and ginger. After stuffing the meat mixture into natural casings, usually the cleaned small intestine of pigs, sausages are dried at room temperature (3).

Due to a high protein content and complex microbial populations in sausage, proteolysis plays an important role in biochemical changes in sausage during storage. This process influences both texture and flavor development through formation of several low molecular weight (Mw) compounds, mainly peptides, amino acids, aldehydes, organic acids, and amines, which are important flavor compounds and precursors of flavor compounds (4). Biogenic amines, which are a concern for food safety, are basic nitrogenous compounds formed mainly during microbial decarboxylation of amino acids, or by enzymes present in sausgae. Accumulation of biogenic amines may result from growth of active microbial populations, acidification, and proteolysis that generate free amino acids in sausage (5). However, biogenic amines have been reported as toxic, and high amounts of biogenic amine consumption may cause hypotension (in the case of histamine, putrescine, and cadaverine) or hypertension (in the case of tyramine), nausea, headache, rash, dizziness, cardiac palpitation, intracerebral hemorrhage, and even death (6). Furthermore, some biogenic amines can react with nitrite to form carcinogenic nitrosamines. Therefore, measures to suppress accumulation of biogenic amines in sausage during storage are worthwhile.

Lipid oxidation, which is an important quality index for sausage, contributes to the reaction of unsaturated fats with

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oxygen that is usually influenced by heat, light, and pro-oxidant compounds found in salt and sodium lactate (7). Developmet of a high degree of lipid oxidation has been regarded as a serious problem during holding and storage of sausage. Therefore, enhancement of oxidative stability is also necessary for maintainance of the quality and safety of sausage products.

Tea polyphenol (TP), which is mainly found in green tea, has beneficial antibacterial and antioxidative activities, plays an important role in protein precipitation and enzyme inhibition, and also exhibits strong free radical scavenging activities in both aqueous-phase and lipid-phase assay systems. In some cases, tea polyphenols are 5× more effective than vitamin C and E (8). TP is widely used as a preservative and as an antioxidant in the food industry, especially for preservation of manufactured meat products. Antioxidant compounds extracted from bamboo leaves (AOB) are capable of blocking the chain reactions of lipid auto-oxidation, chelating transient state metal ions, and acting simultaneously as both primary and secondary antioxidants (9). The main functional components of AOB are flavonoids, phenolic acids, and lactones. Additionally, AOB are acceptable for addition to puffed foods, and dairy and meat products, according to the Ministry of Health, China.

There have been only a few studies of the use of AOB combined with TP on biogenic amine levels and lipid oxidation in pork sausage during storage. Thus, the objective of this study was to examine the effects of AOB combined with TP on levels of biogenic amines and lipid oxidation in pork sausage during storage. Examination was based on peroxide and TBA values, on levels of the biogenic amines tryptamine, spermidine, tyramine, histamine, putrescine, cadaverine, spermine, and 2-phenylethylamine, and on the level of total biogenic amines.

Materials and Methods

Sausage sample preparation Fresh boneless pork ham and pork backfat were purchased from a local meat market in Chengdu, China in November of 2013. Lean tissue and pork backfat were ground by meat grinder (JR-100D; HUADUO, Henan, China) fitted with a 10 mm plate. Approximately 70% lean tissue and 30% pork backfat were mixed thoroughly with non-meat ingredients, including 2% salt, 2.5% rice wine, 0.5% monosodium glutamate, 0.5% sugar, and 0.1% pepper powder. Two different pork sausage treatments used were (1) control sausages made using a basic formulation), and (2) sausages prepared using the basic formulation with addition of 0.02% tea polyphenol (95%) (Sichuan Leshan Tea Technology & Develepment Co., Ltd., Sichuan, China), and 0.02% antioxidant from bamboo leaves (90%) (Sichuan Leshan Tea Technology & Develepment Co., Ltd.). Then, the meat mixtures were stuffed into natural pork casings, which were previously soaked in water. Raw sausages were manually tied at an approximate 15 cm interval and dried at 50°C in an air oven for 8 h, then stored at 20°C in water bath. Three sausages from each treatment group were randomly selected every 7 days as samples for analysis.

Determination of the peroxide value The lipid fraction was extracted with vacuum suction machine (L400-P3; SIKE, Shanghai, China) from sausage samples using a 2:1 (v/v) chlorofrom/methanol solvent, then the peroxide value (POV) was determined according to the AOAC method (10).

Determination of the TBA content The TBA content was determined colorimetrically following the method of Porkony and Dieffenbancher as described by Kirk and Sawyer (11). The method is based on spectrophotometric quantitation of a pink complex formed after reaction of a molecule of malondialdehyde (MDA) with 2 molecules of 2-thiobarbituric acid. TBA values were expressed as mg of MDA/kg of pork sausage.

Determination of biogenic amine levels Levels of biogenic amines were estimated as described by Hong *et al*. (12) with modification. Quantification of 8 biogenic amines was carried out using a reverse phase HPLC apparatus (Agilent 1100 series; Agilent, Santa Clara, CA, USA) equipped with a CAPCEL pak ODS C18 column (4.0×100 mm, 3-μm), and a multiwavelength UV detector. Ammonium acetate (0.1 M, solvent A) and acetonitrile (solvent B) were used as mobile phases. Elution was carried out using a gradient of 50% B for 0 min, 90% B for 20 min, and 50% B for 40 min. Individual biogenic amines were identified on the basis of retention times compared with standard solutions. A BAS sample (5 μL) was injected at a flow rate of 1.2 mL/min, and the peak was detected at 254 nm.

Statistical analysis All analyses were run in triplicate. Data were analyzed using the general linear model (GLM) of the Statistical Analysis System (SAS Ins., Cary, NC, USA). Duncan's multiple range tests was used to determine differences between means at a significance level of $p<0.05$.

Results and Discussion

Antioxidative effects of AOB combined with TP in pork sausage during storage Lipid oxidation is a major quality

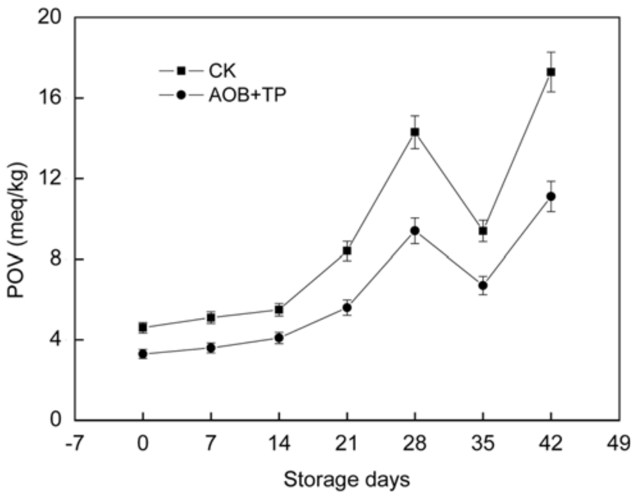

Fig. 1. Changes in POV value of pork sausages during storage. CK, control; AOB+TP, antioxidant from bamboo leaves combined with tea polyphenol treatment

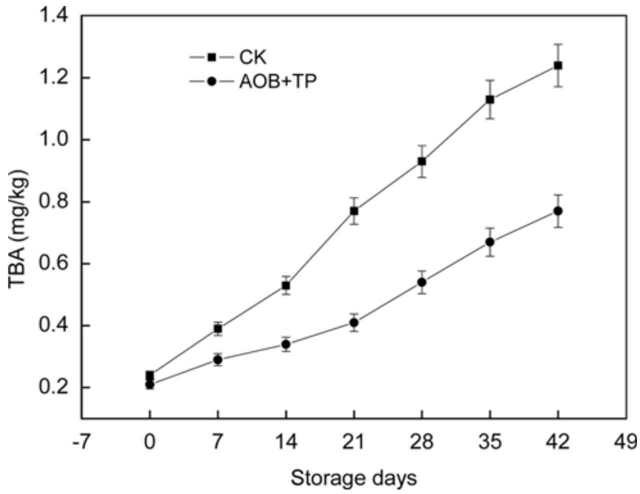

Fig. 2. Changes in TBA value of pork sausages during storage. CK, control; AOB+TP, antioxidant from bamboo leaves combined with tea polyphenol treatment

concern for pork sausage as it usually leads to development of oxidative rancidity. The peroxide (POV) value and the 2-thiobarbituric (TBA) value are major chemical indices of oxidative rancidity. The POV value is a measurement of the level of the primary product of lipid oxidation while the TBA value is a measurement of the level of the secondary product (13).

Changes in POV and TBA values of pork sausage during storage are shown in Fig. 1 and 2. POV values of all samples increased during the early storage period, then declined. POV value changes during storage were consistent with the mechanism of lipid oxidation, which is a dynamic 3-stage process. During the initial stage of oxidation, the formation rate of hydroperoxides exceeded the decomposition rate, but this situation was reversed during later oxidation stages. When the hydroperoxide decomposition rate was similar to the hydroperoxide formation rate, the POV value was constant during the last stage of oxidation, from the 35^{th} to the 42^{nd} storage day (14). POV values of pork sausages supplemented with AOB and TP were significantly ($p<0.05$) lower than for control sausages during the storage period (Fig. 1). At the end of the storage period, the POV values of treated and control sausages were 11.1 and 17.3 meq/kg, respectively, neither of which exceeded the maximal permissible limit of 20 meq/kg for human consumption (15).

The initial TBA values of treated and control sausages were 0.21 and 0.24 mg MDA/kg (Fig. 2). These values increased to 1.24 and 0.77 mg MDA/kg at the end of storage, respectively. The increase in TBA values during storage was attributed to partial dehydration of pork sausage and to increased oxidation of unsaturated fatty acids (16). Moreover, TBA values of treated sausages were significantly ($p<0.05$) lower than corresponding values for control sausages during the storage period, showing that AOB and TP inhibited lipid oxidation in pork sausages. Ladikos and Lougovois (17) reported that the rate and degree of lipid oxidation are related to levels of unsaturated fats and temperature, time, oxygen exposure, and removal of oxygen. Natural antioxidants in meat products can retard the rate and degree of lipid oxidation during storage (18).

Effects of AOB combined with TP in pork sausage during storage Biogenic amines are basic nitrogenous compounds produced mainly by microbial decarboxylation of amino acids, with the exception of physiological polyamines. High levels of amines can be found in fermented sausages derived from raw materials with high protein contents, and accumulation of biogenic amines in sausage is related to the quality of raw materials, effects of starter cultures, and additive and processing conditions (19). Therefore, the level of biogenic amines in sauasge has been used as a quality index of good manufacturing practice. Furthermore, ingredients and additives used in dry sausage formulation are important factors that modulate biogenic amine formation (20).

Changes in the concentrations of biogenic amines present in pork sausage during storage are shown in Table 1. Eight biogenic amines were detected in sausages. The dominant biogenic amines were tyramine, tryptamine, and putrescine, followed by cadaverine and histamine, in agreement with González-Fernández *et al*. (21), who reported that tyramine and putrescine were quantitatively the principal amines in chorizo. Parente *et al*. (22) also observed that tyramine, putrescine, and cadaverine were the main biogenic amines in some dry sausages produced in southern Italy.

The initial amounts of tyramine in treated and the control

Table 1. Changes in concentrations of biogenic amines present in pork sausage during storage

Bas$^{1)}$ (mg/kg)	Treatment	Storage days						
		0	7	14	21	28	35	42
TRM	CON	9.12±0.37$^{2)b}$	145.23±7.78^{g}	87.89±4.87^{g}	75.67±3.89^{e}	68.12±4.01^{d}	78.76±3.12^{de}	82.12±3.09^{e}
	AOB+TP	8.45±0.24^{b}	89.12±5.12^{f}	45.23±2.11^{e}	22.17±1.21^{b}	10.10±0.43^{a}	31.98±1.38^{b}	29.09±1.52^{b}
2-PHE	CON	3.67±0.12^{a}	15.76±0.67^{bc}	27.64±1.67^{c}	23.78±1.55^{b}	19.90±0.95^{ab}	26.56±1.87^{b}	37.09±2.19^{c}
	AOB+TP	4.12±0.16^{a}	13.45±0.51^{b}	30.56±1.01^{d}	37.46±2.32^{c}	17.87±1.12^{ab}	21.34±1.04^{ab}	25.89±1.93^{b}
PUT	CON	16.78±0.44^{c}	25.67±1.12^{d}	41.78±2.42^{e}	64.99±3.32^{d}	85.56±3.90^{e}	89.32±3.13^{e}	75.87±2.99^{e}
	AOB+TP	9.98±0.15^{b}	12.78±0.78^{b}	23.89±0.99^{c}	39.74±1.59^{c}	55.36±2.65^{d}	48.92±2.28^{c}	31.97±1.56^{c}
CAD	CON	7.78±0.23^{ab}	16.89±0.87^{bc}	69.63±1.85^{f}	138.34±4.12	99.34±3.87^{e}	63.67±1.77^{d}	41.67±1.27^{cd}
	AOB+TP	ND$^{3)}$	4.56±0.19^{a}	29.97±1.31^{cd}	55.67±2.34^{d}	42.9±1.98^{cd}	19.90±1.24^{ab}	8.98±0.53^{a}
HIM	CON	ND	65.89±3.21^{e}	96.98±2.11^{g}	125.60±6.77^{f}	87.45±2.48^{e}	56.98±2.90^{cd}	29.65±1.95^{b}
	AOB+TP	ND	23.65±1.01^{d}	54.98±0.98^{e}	77.24±2.13^{e}	45.87±0.78^{cd}	29.01±1.31^{b}	10.56±0.47^{a}
TYM	CON	13.65±0.36^{c}	28.65±0.99^{d}	35.98±1.52^{d}	42.87±1.98^{d}	59.45±2.34^{d}	74.98±3.01^{de}	87.43±3.48^{e}
	AOB+TP	9.45±0.23^{b}	16.98±0.66^{bc}	22.89±1.02^{c}	29.06±1.67^{bc}	36.87±1.89^{c}	41.98±2.61^{c}	49.06±3.26^{d}
SPD	CON	7.98±0.26^{ab}	12.98±0.43^{b}	12.56±0.55^{a}	10.87±0.56^{a}	11.98±0.74^{a}	14.89±0.49^{a}	12.98±0.56^{a}
	AOB+TP	10.76±0.36^{b}	13.87±0.38^{b}	14.76±0.51^{a}	12.98±0.45^{a}	13.76±0.49^{a}	12.54±0.31^{a}	10.88±0.23^{a}
SPM	CON	21.76±0.77^{c}	25.56±0.94^{d}	18.98±0.86^{b}	23.75±1.12^{b}	27.87±1.09^{bc}	23.87±1.22^{b}	28.87±1.54^{b}
	AOB+TP	19.76±0.67^{c}	23.98±1.12^{d}	16.98±0.52^{ab}	23.98±1.15^{b}	29.78±1.19^{bc}	26.97±0.99^{b}	20.98±1.61^{b}
Total	CON	80.74±3.37^{e}	336.63±9.34^{i}	391.44±12.98^{i}	505.87±23.91^{h}	459.67±18.75^{g}	429.03±15.89^{g}	395.68±12.45^{g}
	AOB+TP	62.52±1.82^{d}	198.39±5.87^{h}	239.26±7.75^{h}	298.3±14.67^{g}	252.51±2.11f	232.64±1.97^{f}	187.41±5.77^{f}

$^{1)}$BAS, biogenic amines; TRM, tryptamine; 2-PHE; 2-phenylethylamine; TYM, tyramine; HIM, histamine; PUT, putrescine; CAD, cadaverine; SPM, spermine; SPD, spermidine; Total, total level of biogenic amines
$^{2)}$The values are expressed as mean±SD (n=3); $^{a\text{-}i}$means followed by different letters in the same column are significantly different (p<0.05).
$^{3)}$ND, not detected

sausages (13.65 and 9.45 mg/kg, respectively) increased to 87.43 and 49.06 mg/kg at the end of storage, respectively. Accumulation of these aromatic biogenic amines is caused by decarboxylases produced by lactic acid bacteria, particularly *Enterococcus* spp. and *Lactobacillus* spp., even when strains of other genera contribute to production (23). The concentrations of these biogenic amines were slightly lower in treated sausage, perhaps due to a mild decarboxylase activity influenced by AOB and TP during storage.

Substantial increases in putrescine and cadacerine concentrations were observed on days 21 and 35 in this study, followed by decreased values by the end of storage. The immediate presence of putrescine in sausages after stuffing indicated that the putrescine source was a raw material used for sausage manufacture (24). Compared with control sausages, treated sausages showed significantly (p<0.05) reduced accumulations of putrescine and cadacerine by more than 50 and 80%, respectively. The Enterobacteriaceae are recognized as microorganisms with high lysine-decarboxylas and ornithine-decarboxylase activities, in relation to production of cadaverine and putrescine (25). Ikonić *et al*. (26) reported that concentrations of putrescine and cadacerine can be used as chemical indicators of raw material quality and/or manufacturing and hygiene practices as accumulation is associated with the activities of contaminant bacteria. Therefore, reductions in putrescine and cadacerine levels in treated sausages can be attributed to the inhibitory effects of AOB and TP on growth of wild amine producing bacteria, particularly the Enterobacteriaceae.

Tryptamine is another dominant biogenic amine present in pork sausage. Ikonić *et al*. (26) reported that the dominant amine in Petrovská klobása was tryptamine (24.3-150 mg/kg of dry material), in agreement with Candogan *et al*. (27) who reported tryptamine at concentrations varying from 20.8 to 131.2 mg/kg of dry material in fermented beef sausage during storage. In this study, there was a significant (p<0.05) increase in the tryptamine level from the beginning to day 7 of storage, with a significant (p<0.05) decrease by the end of storage on day 42 day for both treated and control sausages. Compared with control sausages, significant (p<0.05) changes in the tryptamine content occurred in treated sausages. The concentration of tryptamine was lower in treated sausages with a reduction of approximately 65%.

Histamine is the most important biogenic amine from a toxicological point of view, causing urticaria, hypotension, headache, flushing, abdominal cramps, chemical intoxication, and other health problems. The potential toxicity of histamine can be enhanced by other biogenic amines, such as putrescine and cadaverine. Nout (28) pointed out that the the allowable level of histamine is 100 mg/kg in sausage products. In this study, no initial histamine was detected in sausages, indicating a good quality of fresh boneless pork used in sausage preparation. The concentration of histamine rose sharply in both treated and control sausages during the first 21 days of storage, then decreased to 29.65 and 10.56 mg/kg, respectively, at the end of the storage period, neither of which exceeded the allowable level of 100 mg/kg. The sausage treatment significantly (p<0.05) reduced the accumulation of histamine by nearly 80%, compared with controls.

2-Phenylethylamine, which is usually a minor biogenic amine in sausage products, is always present at a high level when tyramine is present, perhaps because microorganisms with a strong tyrosine-decarboxylase activity also have a moderate capacity for decarboxylation of phenylalanine. The initial amount of 2-phenylethylamine in treated pork sausages was 3.67 mg/kg, which increased to 37.09 mg/kg at the end of storage. 2-Phenylethylamine levels in Spanish dry-cured "chorizo" sausage reported by Ruiz-Capillas *et al*. (29) were low (<20 mg/kg) after 74 days of storage (29), consistent with Paulsen and Bauer (30) who found that levels of this monoamine were low, rarely above 50 mg/kg in fermented sausages. However, no significant (p>0.05) difference in the 2-phenylethylamine content was found between control and treated sausages.

Spermidine and spermine occur naturally in pork. They are not products of enzymatic decarboxylation by microorganisms. In this study, concentrations of spermidine and spermine in both treated and control sausages remained stable during storage, ranging from 8 to 15 mg/kg and 20 to 30 mg/kg, respectively. No significant (p>0.05) difference in the concentrations of spermidine and spermine were found between treated and control sausages during storage.

The initial total biogenic amine contents of treated and the control sausages were 80.74 and 62.52 mg/kg. (Table 1). These values increased to 395.68 and 187.41 mg/kg at the end of storage, respectively. According to Nout (28), a level of 1,000 mg/kg in sausage products elicits toxicity in humans. In this study, the total biogenic amine contents of sausages were lower than recommended hazardous levels, indicating good manufacturing practice for pork sausage. Furthermore, the level of biogenic amines in treated sausages was significantly (p<0.05) lower than for control sausages, reaching a 53% reduction.

Additives used in dry sausage formulation are important factors that modulate biogenic amine formation (20). A 53% reduction in the total amine content of treated sausages, and 65, 58, 78, 64, and 44% reductions in tryptamine, putrescine, cadaverine, histamine, and tyramine contents, respectively, were achieved. Based on changes in concentrations of biogenic amines between treated and control sausages during storage, the type and total content of biogenic amines were affected by additioon of AOB combined with TP during sausage preparation. AOB and TP acted as antimicrobial agents for inhibition of both bacterial growth and decarboxylase-positive spoilage activities.

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Disclosure The authors declare no conflict of interest.

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