PAPER

EFFECTS OF GARLIC EXTRACT ON COLOR, LIPID OXIDATION AND OXIDATIVE BREAKDOWN PRODUCTS IN RAW GROUND BEEF DURING REFRIGERATED STORAGE

XINZHUANG ZHANG, QINGXIANG MENG, LIWEN HE, LIPING ZHAO and LIPING REN*
State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193,China
*Corresponding author: Tel. +86 10 62733799, Fax +86 10 62829099,
email: renlp@cau.edu.cn

ABSTRACT

The study aims to investigate the effects of garlic extracts on color, lipid oxidation, and oxidative breakdown products in raw ground beef during refrigerated storage. The two treatments were: control group (C, with no addition) and experiment group (D, 50 mg garlic extracts added to 100 g beef). Adding garlic extracts significant increased a* value ($P_A \leq 0.05$), and significant decreased TBARS and PV values ($P_A \leq 0.05$). The pH and –SH value of D group had a decreasing tendency (P_A=0.0522) and an increasing tendency (P_A=0.0636) respectively compared to C group. Garlic extracts protected phospholipids, fatty acids and polypeptides from oxidation. The results indicate that garlic extracts have the antioxidant activity, helping maintain the meat color, inhibiting lipid oxidation and protein degradation of raw ground beef during refrigerated storage.

- Keywords: garlic extracts, color, lipid oxidation, oxidative breakdown products, raw ground beef -

INTRODUCTION

Lipid oxidation is one of the primary mechanisms of quality deterioration in meat and meat products. The adverse changes in quality are manifested in flavor, color, texture and nutritive value, and the possible production of toxic compounds (CLAUDIA *et al.*, 2014). Beef and its products are rich in protein and lipids, which make them most suitable for consumer. However, beef contains high level of unsaturated fatty acids which are prone to oxidation (TICHIVANGANA and MORRISSEY, 1985). To prevent or reduce lipid oxidation, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), trihydroxybutyrophenone (THBP), propyl gallate (PG), nordihydroguaiaretic acid (NDGA) and ethoxyquin have been applied in meat products (TRINDADEA *et al.* 2014). However, their application had been restricted because of their potential health risks and toxicity (SUN and FUKUHARA, 1997). Natural antioxidants can be used as alternatives to the synthetic antioxidants because of their safety and equivalent or greater effect on inhibition of lipid oxidation (ANDREW *et al.*, 2014).

Garlic (*Allium sativum*) has been a favorite additive to enhance the flavor of food as well as herbal medicine for many years in various cultures. It is well known that garlic has antimicrobial, antiprotozoal, antimutagenic, antiplatelet and antihyperlipidemic properties. Garlic holds a unique position for therapeutic potential due to the antioxidant activity by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes, and increasing glutathione in the cells. Numerous studies have demonstrated that garlic exhibits cardioprotective (RAHMAN and LOWE, 2006), liver-protective (WANG *et al.*, 1998), beneficial effects in diseases such as ischemic-reperfusion arrhythmias and infarction (RIETZ *et al.*, 1993), ischemic heart disease (ARORA *et al.*, 1981), hypertension (FOUSHEE *et al.*, 1982), hyperlipidemia (ERNST *et al.*, 1985), as well as prevent the processes of cancer (TANAKA *et al.*, 2006) and aging (LI *et al.*, 2012).

A number of researchers reported that garlic and different garlic extracts have antioxidant activity contributed by organosulfur compounds, flavonoids and phenolic compounds (BOREK, 2001; OTUNOLA and AFOLAYAN, 2013). RAHMAN *et al.* (2012) researched antioxidant properties of raw garlic extract using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging methods. CAO *et al.* (2013) had studied the effects of garlic on quality and shelf life of stewed-pork during refrigerated storage. However, less attention has been assigned to garlic extracts used as an antioxidant in beef, especially in identifying the oxidative breakdown products so far. The objective of the present study was to determine the effect of garlic extracts on pH, color stability, lipid oxidation, and oxidative breakdown products of raw ground beef during refrigerated storage in order to provide a scientific basis for using garlic extracts as natural antioxidants to maintain the meat quality, extend shelf-life and prevent economic loss.

MATERIALS AND METHODS

Materials

Garlic extracts (10:1 of garlic: garlic extracts, three percent allicin content) was purchased from Yuanshen Bio-Tech Ltd. (Xian, China). Glutathione were obtained from Sigma Aldrich Inc. (St. Louis, MO, USA). Ethanol, chloroform, methanol, ammonium thiocyanate, iron (II) chloride, trichloroacetic acid (TCA), thiobarbituric acid (TBA), 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB), cumene hydroperoxide, tetraethoxypropane and other reagent were 'AnalaR' grade from China Medicine (Group) Beijing Chemical Reagent Corporation (Beijing, China). Water for ultra-performance liquid chromatography quadrupole time of flight (UPLC-QTOF) was purified with a Milli-Q Gradient A10 system (Millipore, Beijing, China). Formic acid and acetonitrile were HPLC-grade (Fisher Scientific, New Jersey, USA).

Three Simmental crossed cattle (620±15kg, 18 months) were slaughtered at a local commercial abattoir (Jinweifuren Co. Ltd., Beijing, China). Transport, slaughtering, or invasive procedures on live animals involving in this study were handled in strict accordance to the guidelines approved by the Animal Welfare Committee of China Agricultural University (Permit Number: DK1008). After ageing at 4°C for 72 h, the *longissimus dorsi* (LD) were excised from 12^{th} and 13^{th} rib of left half side carcass. The muscles were minced twice through a 5 mm plate of meat mincer (model JYS-A800, Joyoung, China) after removing the connective tissue and visible fat. The contents of moisture, protein and fat of the ground beef were 73.14%, 22.26%, and 2.83% respectively.

Treatments

The meat sample of each cattle was formed into two patties (100 g portions) using a meat former and assigned to the following two treatments:: control group (C, 100 g ground beef with no addition) and experiment group (D, 50 mg garlic extracts added to 100 g ground beef). To eliminate the influence of microorganism and ensure thorough mixed, garlic extracts dissolved in 10 mL of a distilled water and ethanol mixture (1:1, v/v) and then mixed with the muscles of the experiment group. The same volume of distilled water and ethanol mixture (with no added ingredients) was added to the control group.

Meat samples were put into a constant temperature incubator (MJX-320, Jiangnan, Ningbo, China) at 4 °C for 13 days. Meat samples (three replicates) were collected in 1.5 mL centrifuge tube at storage times of 1, 3, 5, 7, 10, 13 days and were frozen rapidly in liquid nitrogen for subsequent analysis.

Analysis of pH and color

A pH meter (pH Spear, Eutech Instruments, USA) was used to measure the pH of the ground beef. The color of the ground beef was determined by portable colorimeter (CR400/410, Minolta, Japan). The specifications of the colorimeter are light source: pulsed xenon lamp; Illuminant: C, D65; illumination area: Φ8/Φ11; Inter instrument agreement: ΔE*ab within 0.6; repeatability: within ΔE*ab0.07 standard deviation. The color results were calculated based on L*, a*, b* (lightness, redness, and yellowness respectively) in the CIELAB space. A white plate (CIE L*= 97.83, a*=-0.43, b*=1.98) was used for calibration.

Analysis of lipid oxidation

Lipid oxidation was evaluated by the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) according to the method of RICHARDS *et al.* (2003) (RICHARDS and DETTMANN, 2003) and free thiol groups (-SH) was analyzed following the method proposed by EGELANDSDAL *et al.* (2011) with minor modifications.

Determination of PV: Approximately 0.3 g of sample was homogenized for 30 s with 5 mL of cold chloroform/methanol (1:1) in a 50 mL glass tube using a refiner (FJ-200, Jintan, China). Subsequently, the glass tube was rinsed for 30 s again with 5 mL of cold chloroform/methanol (1:1). The homogenate and wash solution were then combined in a 25 mL glass test tube. Adding 3.08 mL of 0.5% NaCl then mixed for 10 s with a vortex (G560E, Scientific Industries Inc., New York, USA). Next, the mixture was centrifuged at 1821g (DL-6000B, Shanghai Anting, China) for 6 min at 4°C. Two milliliters of the lower chloroform layer was removed and transferred to a tube by a glass syringe before 1.3 mL chloroform/methanol (1:1) was added to the 2 mL sample. Then 25 μL of 3.94 mol/L ammonium thiocyanate and 25 μL of 18 mmol/L iron (II) chloride were added to the tube, mixing thoroughly after each addition. Finally, the sample was incubated at room temperature for 20 min and the absorbance at 500 nm was measured using a spectrophotometer (UV1102, Shanghai Tianmei, China). A standard curve was constructed using cumene hydroperoxide and the PV in the sample was expressed as μmol/kg.

Determination of TBARS: mixing 50% trichloroacetic acid (TCA) with 1.3 % thiobarbituric acid (TBA) on the day of use and then heating to 65°C. Approximately 0.12 g of the muscle sample was added to 1.2 mL the TCA - TBA mixture and mixed via a vortex (G560E, Scientific Industries Inc., New York, USA) and then heated at 45°C for 60 min using a vapor-bathing constant temperature vibrator (HQ45, Chinese Academy Of Sciences, Wuhan, China) before the sample was centrifuged at 12,000 g for 5 min (Mikro 200R, Hettich, Germany). The absorbance of the supernatants at 532 nm was determined. A standard curve was prepared using tetraethoxypropane, and the concentration of TBARS in the samples was expressed as μmol/kg.

Determination of -SH: approximately 0.1 g of the muscle sample was weighed into a 10 mL centrifuge tube then 1700 μL phosphate-buffered saline (pH=8.2) and 100 μL DTNB were added. The mixture was heated at 45 °C away from light for 60 min using a vapor-bathing constant temperature vibrator (MJX-320, Jingbo Jiangnan, China). Eight milliliters of methanol was added to the tube which was vortexed for 30 s before centrifuging at 1821g for 20 min at 4°C. Subsequently, 1 mL supernate was transferred to a new centrifuge tube, diluted with 3 mL methanol and then the absorbance was measured at 412 nm. Glutathione was used for generating a standard curve and concentration of -SH in samples was expressed as mmol/kg.

Analysis of oxidative breakdown products

Sample preparation: 50 mg muscle sample from each treatment at 13 days was weighed into a 2 mL Eppendorf tube. The first step: 1.5 mL cold water/methanol (1:1) and 0.5 g 1 mm Zirmil ceramic beads were added to the tube and then mixed thoroughly using a tissue homogenizer (Precellys 24, Bertin, France) for two cycles at 6500 Hz, 40 s for each cycle. The mixture was centrifuged at 12,000 g (Mikro 200R) for 10 min at 4°C. Supernate (400 μL) was collected in a new Eppendorf tube and stored at 4°C for further use. The second Step: the residual sediment from step one was extracted by 1.5 mL cold chloroform/methanol (3:1) again with the same procedure of homogenization and centrifugation and the same volume of supernate collected in the Eppendorf tube. The mixed samples from two steps, were then concentrated in a centrifugal concentration meter (ZLS-1, Herexi, Hunan, China). Water/methanol (9:1; 120 μL) was used to dissolve the samples with vortex oscillation for 40 s. Supernate (100 μL) was collected in a lining tube for subsequent UPLC-QTOF analysis.

UPLC analysis: The substances in the 6 μL extracted sample were separated using a UPLC system (Acquity UPLC/XEVO G2 Q ToF, Waters) after being loaded onto a high-resolution and high-

performance UPLC BEH C18 column (1.7 μm, 2.1 mm × 50 mm, Waters). The column temperature was 50 °C and the flow rate was 0.3 mL/min with solvent A (water + 0.1 % formic acid) and solvent B (acetonitrile + 0.1% formic acid). The elution program consist of 98:2 (A: B) for 1 min, increasing gradually to 100 % B by 16 min, then 100 % B held for 2 min, a ramp to 98:2 (A: B) within 10 s, and returned to 98:2 (A: B) for 2 min (re-equilibration of the column) before the loading of the next sample.

Q-TOF MS conditions: The separated components from the UPLC were subsequently analyzed by quadrupole time of flight mass spectrometry (Q-TOF MS) equipment (Acquity UPLC/XEVO G2 Q ToF, Waters) operated using a negative electrode for electrospray ionization. The settings were as following: data acquired in a full scan mode (mass: charge ratio (m/z) 50-1200) at a rate of 2 spectra/s; capillary and sampling cone voltages at 2500 and 35 V, respectively; desolvation temperature at 350°C; desolvation gas flow at 720 L/h; cone gas flow at 50 L/h; source temperature at 105°C. All gas paths used nitrogen and data were collected using Masslynx 4.1 data management software (Waters, USA). The final data was expressed by the ID number, relative m/z values, retention times and ion intensity.

STATISTICAL ANALYSIS

This experiment was assigned two factors with two treatments and seven storage time levels.s. The mixed model procedure of SAS 9.0 (SAS Institute Inc.) was used to analyze the effects of garlic extracts and storage time. The following statistical model was used for analysis:

$$y_{ik} = \mu + \alpha_i + \beta_k + (\alpha\beta)_{ik} + e_{ik}$$

where y_{ik} is an observed value for TBARS, PV, -SH, pH, L*, a*, and b*, taken from sample receiving treatment i at time k;μis the overall mean; α_i is the fixed effect of treatment i; β_k is the fixed effect of time k; $(\alpha\beta)_{ik}$ is the interaction between treatment and storage time; and e_{ik} is the residual value.

The variation tendency of pH and color, lipid oxidation with different storage times was analyzed by the contrast model of SAS 9.0. The means that were significantly different were analyzed using the t-test and different storage times were analyzed using Duncan's multiple comparison tests. A level of $P \leq 0.05$ was considered significant and $0.05 < P < 0.1$ was considered tendency.

Differences in oxidative breakdown products among treatments were analyzed with principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) using SIMCA-P software (Umetrics, Ume, Sweden). The different oxidative breakdown products between two treatments were analyzed in terms of ion intensity using the t-test. Metabolites that met the criterion of $P \leq 0.05$ of the t-test and a fold change > 1.5 were selected as different oxidative breakdown products whose formulae were searched in metlin database.

Table 1 - The effects of garlic extracts and storage time on physical meat quality, lipid oxidation in raw ground beef during refrigerated storage.

Items		TBARS	PV	-SH	pH	L*	a*	b*
Treatments	C	22.41^{a}	46.56^{a}	6.62	5.86	51.22	13.50^{b}	14.07^{b}
	D	19.59^{b}	30.22^{b}	7.10	5.85	51.35	14.49^{a}	14.59^{a}
	SEM	0.6121	9.7158	0.3015	0.0027	0.2020	0.2086	0.1422
Times	1	10.25^{c}	23.78^{b}	7.16^{ab}	5.91^{a}	51.05^{b}	18.05^{a}	14.71^{a}
	3	13.50^{b}	28.94^{b}	8.14^{a}	5.84^{b}	50.13^{c}	15.68^{b}	13.52^{b}
	5	26.92^{a}	42.11^{ab}	7.23^{ab}	5.84^{b}	51.96^{a}	15.44^{b}	14.76^{a}
	7	24.20^{a}	69.32^{a}	7.12^{ab}	5.83^{b}	51.99^{a}	13.98^{c}	15.02^{a}
	10	26.07^{a}	44.82^{ab}	6.03^{bc}	5.82^{c}	51.01^{b}	10.18^{d}	13.55^{b}
	13	25.08^{a}	21.39^{b}	5.45^{c}	5.91^{a}	51.57^{ab}	10.65^{d}	14.43^{a}
	SEM	0.6121	9.7158	0.3015	0.0027	0.2020	0.2086	0.1422
P_A		<0.001	0.0500	0.0636	0.0522	0.4248	<0.001	0.0001
P_B	L	<0.001	0.5139	<.0001	0.1356	0.0123	<0.001	0.5835
	Q	<0.001	0.0085	0.0238	<.0001	0.0852	0.9871	0.8118
P_{AB}		0.0383	0.9684	0.1200	0.0035	0.5571	0.0034	0.0152

Means in the same column with different superscripts are significantly different (P <0.05).C-control group, D-garlic extracts group. P_A is the P value of treatments; P_B is the P value of storage times; L stands for the linear effects of storage times; Q stands for the quadratic effects of storage times. P_{AB} is the P value interaction between treatments and storage times.

RESULTS

The effects of garlic extracts and storage time on physical meat quality, lipid oxidation in raw ground beef during refrigerated storage

According to Table 1, L* and a* value presented an increasing and decreasing linear change respectively (P_BL<0.05), TBARS, PV, -SH, and pH had a quadratic change over storage time (P_BQ<0.05). More details, TBARS value significant increased in the first five storage days (P_B<0.05), and then no significant changes in the residual storage time. PV and -SH value were firstly increased and then decreased with the highest value at seventh day and third day respectively of storage time. On the contrary, pH was firstly decreased and then increased with the lowest value at tenth day of storage time, ranging from 5.82 to 5.91. However, storage time had neither linear nor quadratic effect on b* value in raw ground beef during refrigerated storage.

As shown in Table 1, Adding garlic extracts could significant increased ($P_A \leq 0.05$) a* and b* values. The pH of D group had a decreasing tendency compared to C group (P_A=0.0522). There was no significantly different on L* value between treatments (P_A=0.4248). TBARS and PV values of D group were significant lower ($P_A \leq 0.05$), and –SH tended to increase (P_A=0.0636) than C group in raw ground beef during refrigerated storage.

Because table 1 could not show the effects of garlic extracts on every storage time, the significant indicators a*, PV, and TBARS values ($P_A \leq 0.05$) influenced by garlic extracts were further analyzed on every storage time. Fig. 1 showed that a* value was significant increased following the addition of garlic extracts on days 3, 7 and 13 during refrigerated storage relative to the control($P < 0.05$). Adding garlic extracts had a tendency to reduce the PV values on days 5 and 13 ($0.05<P < 0.1$), and significantly reduced the PV value on 10 days of storage time as compared to control ($P < 0.05$) (Fig. 2). Fig. 3 showed that a decreasing tendency of TBARS value in E group was found compared to C group at the storage time 3, 7, and 10 days ($0.05<P < 0.1$). The TBARS value was significantly ($P < 0.05$) lower in D group than C group at the end of storage time.

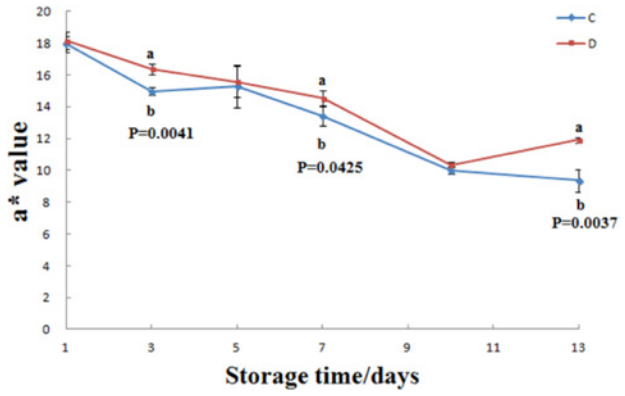

Fig. 1 - Effects of garlic extract on a* value at different storage time of raw ground beef.

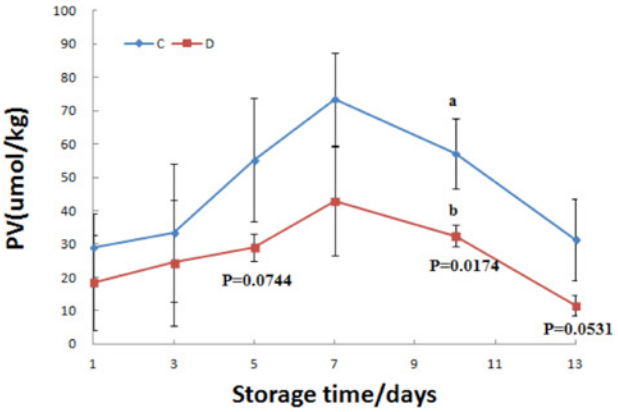

Fig. 2 - Effects of garlic extract on PV at different storage time of raw ground beef.

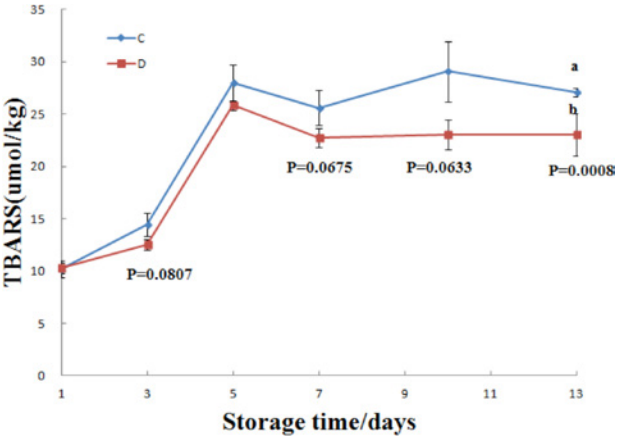

Fig. 3 - Effects of garlic extract on TBARS at different storage time of raw ground beef.

Effects of garlic extract on oxidative breakdown products

PCA score plot of different treatments at the 13 days storage time of raw ground beef was shown in Fig. 4. These results show an easily visible separation of two different groups C and D marked with black and red colors. Fig. 5 showed the loading scores plot from OPLS-DA between C group and D group. Each numbered point represented one breakdown product, which the further away from the center, the more likely that breakdown products between two groups are different.

According to the results from OPLS-DA analysis and original data, the different breakdown products were confirmed between C and D group and shown in Table 2. There were total nine different breakdown products significantly higher in D group than C group ($P < 0.05$) and could been divided into three categories, including phospholipids, fatty acids and polypeptides. PC was belongs to phospholipids. Fatty acids contained malic acid, 16-hydroxy-9E-hexadecenoic acid and 9-hydroxy-10E-octadecen-12-ynoic acid. Polypeptides were made up with Gln-Ile-Asn-Leu, Arg-Pro-Lys-Arg, Met-His-Gln –Asn, Thr-Lys-Lys-Thr, and Gly-Arg-Cys.

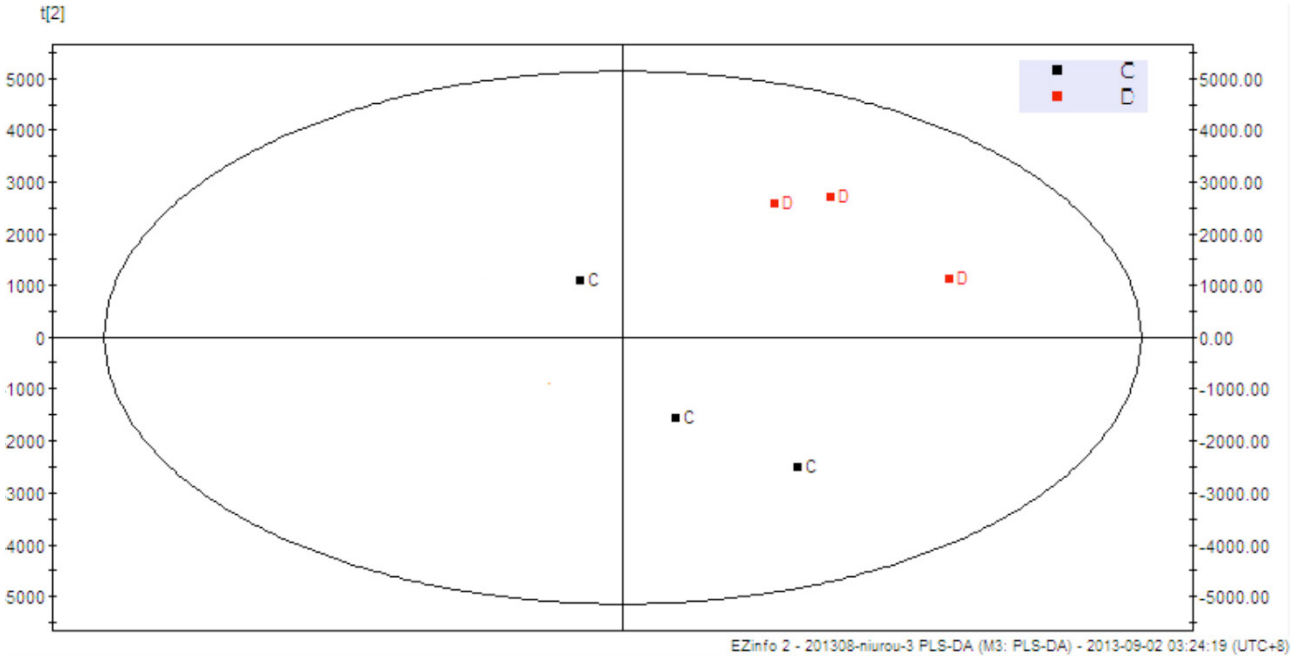

Fig. 4 - PCA score plot of different treatments at the end of storage time in raw ground beef.

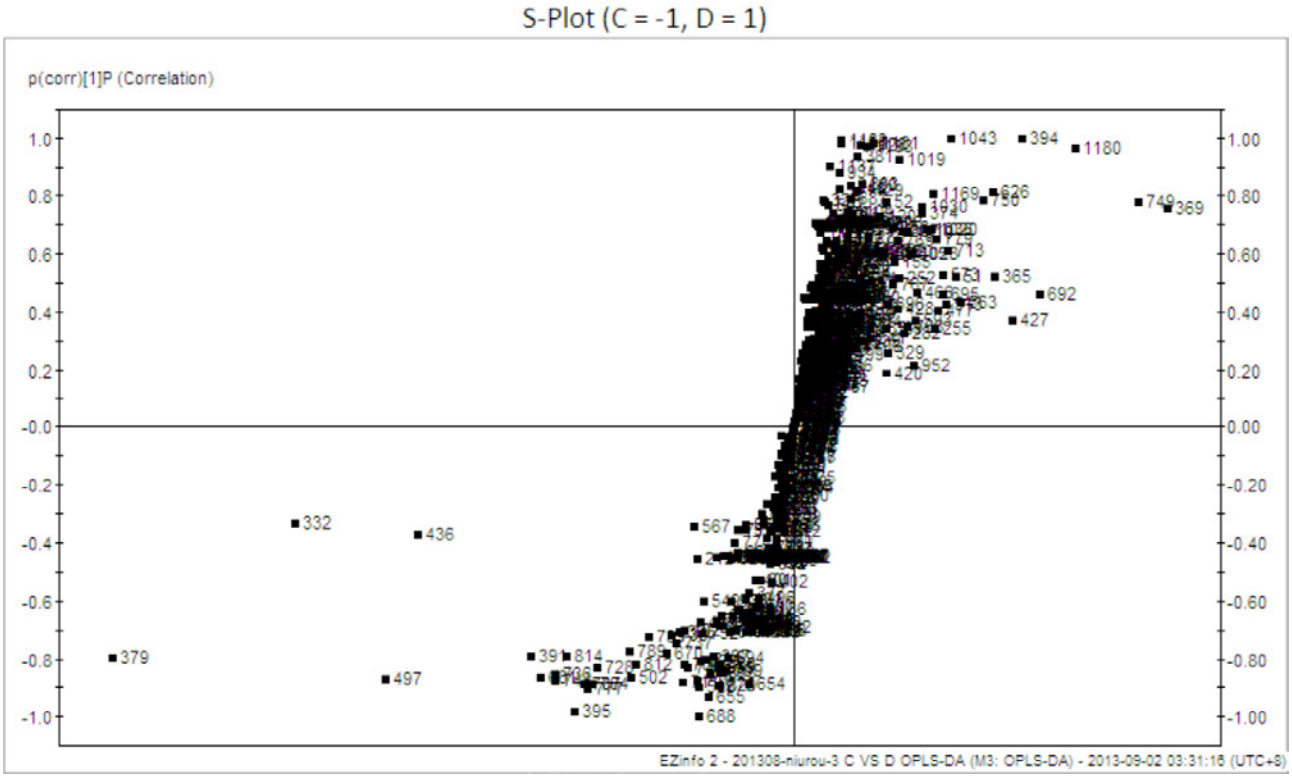

Fig. 5 - Loading scores plot from OPLS-DA between C group and D group at the end of storage time in raw ground beef.

Table 2 - Different breakdown products between C and D treatments at the end of storage time (n=3).

Products	Formula	[M-H] calculated	[M-H] observed	Masserror	*P*	Fold change
PC(15:1(9Z)/0:0)	$C_{23}H_{46}NO_7P$	478.2939	478.2930	1.88	0.0099	+∞
Malic acid	$C_4H_6O_5$	133.0142	133.0142	0	<0.001	+∞
16-hydroxy-9E-hexadecenoic acid	$C_{16}H_{30}O_3$	269.2122	269.2119	1.11	0.0034	10.54
9-hydroxy-10E-octadecen-12-ynoic acid	$C_{18}H_{30}O_3$	293.2122	293.2110	4.09	0.0166	2.35
Gln- Ile-Asn-Leu	$C_{21}H_{38}N_6O_7$	485.2729	485.2773	9.07	0.0047	+∞
Arg-Pro-Lys-Arg	$C_{23}H_{45}N_{11}O_5$	554.3532	554.3462	12.63	0.0094	+∞
Met-His-Gln-Asn	$C_{20}H_{32}N_8O_7S$	527.2042	527.2073	5.88	0.0159	+∞
Thr-Lys-Lys-Thr	$C_{20}H_{40}N_6O_7$	475.2886	475.2886	0	0.0196	+∞
Gly-Arg-Cys	$C_{11}H_{22}N_6O_4S_1$	333.1351	333.1349	0.60	0.0494	+∞

$[M-H]^-$, protonated molecular ion; Mass error expressed as ppm.
Fold change, which was based on the original data, was defined as the fold difference in the observed concentrations between C and D groups. A positive number for a fold change indicates that the value of D group was greater than the C group, whereas the opposite is indicated by a negative number.

DISCUSSIONS

pH and color

The pH of fresh meat is an important indicator to determine the freshness of meat, which can be influenced by the accumulation of organic acids and amines during refrigerated storage. Organic acids, produced by gram positive bacteria and released by the decomposition of lipids oxidative, decrease the pH of meats, whereas the rise of pH was due to the decomposition of alkaline ammonia, which induced by gram negative bacteria (LEFEBVRE *et al.*, 1994). The pH value with a quadratic change over storage time in this study because the result of the interaction of these two effects. The results pH value was inconsistent with CAO *et al.* (2013) found that all the samples gradually increased in stewed-pork during storage at 4°C for 12 days (CAO *et al.*, 2013). The reason of different results was mainly caused by adding ethanol to eliminate the influence of microorganism in the present study.

The color of foods is one of the major determinants of its appeal to consumers and consequently, sales of the product. The color of meat and meat products is influenced by the percentage of red appeared deoxymyoglobin, metmyoglobin and oxymyoglobin in muscle, which would predispose the meat to a faster browning rate (HUNT *et al.*, 1999). The result of decreased a* value over storage time in the present study highly agreed with FERNANDEZ-LOPEZ *et al.* (2005) (FERNANDEZ-LOPEZ *et al.*, 2005) mainly was caused by the formation of metmyoglobin during storage. Several authors have studied the effect of different antioxidants on the color of meat and meat products and have reported that the antioxidants could retard the decrease of a * values during storage (FERNANDEZ-LOPEZ *et al.*, 2005; SANCHEZ-ESCALANTE *et al.*, 2001; KIM *et al.*, 2013). In agreement with these studies, garlic extracts also could retard the decrease of a * values compared to the control group during storage. Garlic extracts could stabilize the redness in raw ground beef during refrigerated storage due to its antioxidant properties preventing the oxidation of oxymyoglobin.

Lipid oxidation

Lipid oxidation is a critical and undesirable phenomenon for meat and meat productions since the undesirable rancid off-flavours and potentially toxic products, leading to the qualitative deterioration. Peroxides are the primary products of lipid oxidation which is generated by oxygen attacking on the double bond in fatty acids. Therefore, peroxide value (PV) is usually as an indicator to clarify the extent of oxidation (MOTTRAM, 1998). Peroxide is very reactive and actually degraded into secondary oxidation products during the storage of lipid-containing foods (JUNTACHOTE *et al.*, 2007). Therefore, PV value increased and thereafter decreased during storage in present study was caused by the peroxide formation and degradation. Similar results of PV fluctuated over storage time were reported by TEETS *et al* (2008) (TEETS and WERE, 2008).

The TBARS value is an index of secondary lipid oxidation and can be used to monitor lipid oxidation in meat samples during storage (QIN *et al.*, 2013). The secondary lipid oxidation products mainly aldehydes (or carbonyls), which contribute to off-flavors in oxidized meat and meat products, result from the degradation of lipid hydroperoxides formed during the oxidation process of polyunsaturated fatty acids(FERNANDEZ *et al.*, 1997). XIE *et al.* (2012) reported that the TBARS value was increased with the storage time at 4°C for 3 days in beef of five cattle breeds (XIE *et al.*, 2012). The results of this study showed that a similar trend, which the TBARS value significant increased in the first five storage days.

CAO *et al* (2013) found that stewed-pork treated with extracts of ginger, onion, garlic had lower PV and TBARS value compared to the control. PARK *et al.* (2010) reported that all extrac-

tion solvents of garlic could reduce the TBARS value in fresh pork patties during refrigerated storage (PARK and CHIN, 2010). SALLAM *et al* (2004) observed that garlic extracts had a marked effect in reducing PV and TBARS values of chicken sausage (SALLAM *et al.*, 2004). In agreement with earlier studies, the same results of decreased PV and TBARS values in the present study indicate that garlic extracts are effective at delaying lipid peroxidation in raw ground beef during refrigerated storage. According to YIN *et al.* (2003), four garlic-derived organosulfur compounds, diallyl sulfide (DAS), diallyl disulfide (DADS), s -ethyl cysteine (SEC), n-acetyl cysteine (NAC), significantly delayed oxymyoglobin and lipid oxidations in ground beef (YIN and CHENG, 2003). OTUNOLA *et al.* (2013) found that garlic extracts have high flavonoids and phenolics contents and high antioxidant activities (OTUNOLA and AFOLAYAN, 2013). Therefore, the ability of garlic extracts to inhibit lipid oxidation is probably related to their antioxidant activity contribute to organosulfur compounds, flavonoids and phenolics compounds.

Free thiol (-SH) was used to stabilize primary oxidation products (SISTA *et al.*, 2000). The increase of -SH value at the first three storage days was probably caused by oxidized glutathione (GSSH) being transformed into glutathione (GSH) under the action of glutathione reductase. On the contrast, transforming hydrogen peroxide into water under the action of glutathion peroxidase results in the decrease of –SH value after three days during storage time. The decrease –SH value in beef over storage time was observed by SULLIVANA *et al.* (2012) (ZAKRYS-WALIWANDER *et al.*, 2012).

GSH is considered an abundant antioxidant within the cell and is essential for regulation of intracellular redox status (IZIGOV *et al.*, 2011). Allicin is formed by alliin enzymatically modified under the action of alliinase in garlic (OKADA *et al.*, 2005). According to the study of KIM *et al.* (1997), Allicin is the main antioxidative component of freshly crushed garlic cloves (KIM *et al.*, 1997). HOREV AZARIA *et al.* (2009) reported that Allicin could directly raising glutathione content in the cell and indirectly increasing glutathione by allicin derivatives such as S-allylmercaptoglu tathione and S-allylmerc aptocysteine (HOREV-AZARIA *et al.*, 2009). Therefore, the existence of Allicin could explain the results that adding gailic extracts increase the –SH value.

Breakdown products

In the present study, garlic extracts could protect the phospholipids, unsaturated fatty acids and polypeptides from oxidation. Phospholipids, which constitute the lipid bilayer defining the outer confines of a cell, are the primary substrates for lipid oxidation and membrane components in close contact with the catalysts of lipid oxidation, which are located in the aqueous phase of the muscle cell (PULFER and MURPHY, 2003). Skeletal muscle was susceptible to oxidative due to the membrane lipid systems that were high in unsaturated fatty acids (CHAN and DECKER, 1994) (CHAN and DECKER, 1994). XIE *et al.* (2012) found that higher unsaturated fatty acids content in Qinchuan cattle lead to more easily lipid oxidation (XIE *et al.*, 2012). In addition, GRUEN *et al.* (2001) revealed that increase of oxidation can enhance protein degradation (GRUNE *et al.*, 2001). Therefore, the results indicate that garlic extracts could help to stabilize the muscle membrane and reduce the degradation of fat and protein due to its antioxidant.

CONCLUSIONS

The results of this study clearly revealed that the effects of garlic extract on color, lipid oxidation and oxidative breakdown products of raw ground beef during refrigerated storage. The results of higher a * value and lower PV, TBARS values indicate that garlic extracts have the antioxidant activity, helping maintain the beef color, inhibiting lipid oxidation. In addition, our experiments provide new and important information regarding the effects of garlic extracts on inhibiting lipid oxidation and protein degradation through protecting phospholipids, unsaturated fatty acids and polypeptides in raw ground beef during refrigerated storage. Overall, garlic extracts could be used as natural antioxidants to maintain the meat quality, extend shelf-life and prevent economic loss.

ACKNOWLEDGEMENTS

This study was supported by grant from the National Natural Science Foundation of China (No. 31172231) and Innovation Special Doctoral Research. Liping Ren designed this study. Xinzhuang Zhang assigned the study and collected test data, interpreted the results and drafted the manuscript. Qingxiang Meng reviewed the manuscript. Liwen He and Liping Zhao helped measuring some indicators in this study.

REFERENCES

Andrew B. F., Peter O. F. and Voster M. 2014. Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. Food Res Int. 64:171-181.

Arora R.C., Arora S. and Gupta R.K. 1981. The long-term use of garlic in ischemic-heart-disease - an appraisal. Atherosclerosis. 40: 175-179.

Borek C. 2001. Antioxidant health effects of aged garlic extract. J Nutr. 1313:1010-1015.

Cao Y.M., Gu W.G., Zhang J.J., Chu Y., Ye X.Q., Hu Y.Q. and Chen J.C. 2013. Effects of chitosan, aqueous extract of ginger, onion and garlic on quality and shelf life of stewed-pork during refrigerated storage. Food Chem. 141: 1655-1660.

Chan K.M. and Decker E.A. 1994. Endogenous skeletal-muscle antioxidants. Crit Rev Food Sci. 34: 403-426.

Claudia C., Rocío Á., Michael O., Denis P.D., Sean Ó.G. and Frank J.M. 2014. Effect of an active packaging with citrus

extract on lipid oxidation and sensory quality of cooked turkey meat. Meat Sci. 96: 1171-1176.

Egelandsdal B. Ren L.P., Kathirvel P., Gong Y.S., Greaser M.L. and Richards M.P. 2011. Addition of partly reduced bovine serum albumin to a metmyoglobin-fortified washed cod system gives reduced formation of lipid oxidation products and increased degradation of proteins. LWT-Food Science and Technology. 44: 1005-1011.

Ernst E., Weihmayr T. and Matrai A. 1985. Garlic and blood-lipids. Brit Med J. 291: 139.

Fernandez J., Perezalvarez J.A. and Fernandezlopez J.A. 1997. Thiobarbituric acid test for monitoring lipid oxidation in meat. Food Chem .59: 345-353.

Fernandez-lopez J., Zhi N., Aleson-carbonell L., Perez-alvarez J.A. and Kuri V. 2005. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. Meat Sci. 69: 371-380.

Foushee D.B., Ruffin J. and Banerjee U. 1982. Garlic as a natural agent for the treatment of hypertension - a preliminary-report. Cytobios. 34: 145-152..

Grune T., Klotz L.O., Gieche J., Rudeck M. and Sies H. 2001. Protein oxidation and proteolysis by the nonradical oxidants singlet oxygen or peroxynitrite. Free Radical Bio Med. 30: 1243-1253.

Horev-azaria L., Eliav S., Izigov N., Pri-chen S., Mirelman D., Miron T., Rabinkov A., Wilchek M., Jacob-hirsch J., Amariglio N. and Savion N. 2009. Allicin up-regulates cellular glutathione level in vascular endothelial cells. Eur J Nutr: 48, 67-74.

Hunt M.C., Sorheim O. and Slinde E. 1999. Color and heat denaturation of myoglobin forms in ground beef. J Food Sci. 64: 847-851.

Izigov N., Farzam N. and Savion N. 2011. S-allylmercapto-N-acetylcysteine up-regulates cellular glutathione and protects vascular endothelial cells from oxidative stress. Free Radical Bio Med. 50: 1131-1139.

Juntachote T., Berghofer E., Siebenhandl S. and Bauer F. 2007. The effect of dried galangal powder and its ethanolic extracts on oxidative stability in cooked ground pork. LWT-Food Sci Technol. 40: 324-330.

Kim H.W., Choi Y.S., Choi J.H., Kim H.Y., Hwang K.E., Song D.H., Lee S.Y., Lee M.A. and Kim C.J. 2013. Antioxidant effects of soy sauce on color stability and lipid oxidation of raw beef patties during cold storage. Meat Sci. 95: 641-646.

Kim S.M., Kubota K. and Kobayashi A. 1997. Antioxidative activity of sulfur-containing flavor compounds in garlic. Biosci Biotech Bioch. 61: 1482-1485.

Lefebvre N., Thibault C., Charbonneau R. and Piette J.1994. Improvement of shelf-life and wholesomeness of ground-beef by irradiation.2.Chemical-analysis and sensory evaluation. Meat Sci. 36: 371-380.

Li X.H., Li C.Y., Lu J.M., Tian R.B. and Wei J. 2012. Allicin ameliorates cognitive deficits ageing-induced learning and memory deficits through enhancing of Nrf2 antioxidant signaling pathways. Neurosci Lett. 514: 46-50.

Mottram D.S.1998. Flavour formation in meat and meat products: a review. Food Chem. 62: 415-424.

Okada Y., Tanaka K., Fujita I., Sato E. and Okajima H. 2005. Antioxidant activity of thiosulfinates derived from garlic. Redox Rep. 10: 96-102.

Otunola G.A. and Afolayan A.J. 2013. Evaluation of the polyphenolic contents and some antioxidant properties of aqueous extracts of Garlic, Ginger, Cayenne Pepper and their mixture. Journal of Applied Botany and Food Quality. 86: 66-70.

Park S.Y. and Chin K.B. 2010. Evaluation of pre-heating and extraction solvents in antioxidant and antimicrobial activities of garlic, and their application in fresh pork patties. Int J Food Sci Tech. 45: 365-373.

Pulfer M. and Murphy R.C.2003. Electrospray mass spectrometry of phospholipids. Mass Spectrom Rev. 22: 332-364.

Qin Y.Y., Zhang Z.H., Li L., Xiong W., Shi J.Y., Zhao T.R. and Fan J. 2013. Antioxidant effect of pomegranate rind powder extract, pomegranate juice, and pomegranate seed powder extract as antioxidants in raw ground pork meat. Food science and biotechnology. 22: 1063-1069.

Rahman K. and Lowe G.M. 2006. Garlic and cardiovascular disease: A critical review. J Nutr. 136: 736-740.

Rahman M.M., Fazlic V. and Saad N.W. 2012. Antioxidant properties of raw garlic (Allium sativum) extract. International Food Research Journal.19:589-591

Richards M.P. and Dettmann M.A. 2003. Comparative analysis of different hemoglobins: Autoxidation, reaction with peroxide, and lipid oxidation. J Agr Food Chem. 51: 3886-3891.

Rietz B., Isensee H., Strobach H., Makdessi S. and Jacob R. 1993. Cardioprotective actions of wild garlic (allium-ursinum) in ischemia and reperfusion. Mol Cell Biochem. 119: 143-150.

Sallam K.I., Ishloroshi M. and Samejima K. 2004. Antioxidant and antimicrobial effects of garlic in chicken sausage. LWT-Food Sci Technol. 37:849-855.

Sanchez-escalante A., Djenane D., Torrescano G., Beltran J.A. and Roncales P. 2001. The effects of ascorbic acid, taurine, carnosine and rosemary powder on colour and lipid stability of beef patties packaged in modified atmosphere. Meat Sci. 58: 421-429.

Sista R.V., Erickson M.C. and Shewfelt R.L. 2000. Lipid oxidation in a chicken muscle model system: Oxidative response of lipid classes to iron ascorbate or methemoglobin catalysis. J Agr Food Chem. 48: 1421-1426.

Sun B.X. and Fukuhara M. 1997. Effects of co-administration of butylated hydroxytoluene, butylated hydroxyanisole and flavonoids on the activation of mutagens and drug-metabolizing enzymes in mice. Toxicology. 122: 61-72.

Tanaka S., Haruma K., Yoshihara M., Kajiyama G., Kira K., Amagase H. and Chayama K. 2006. Aged garlic extract has potential suppressive effect on colorectal adenomas in humans. J. Nutr. 136: 821-826.

Teets A.S. and Were L.M. 2008. Inhibition of lipid oxidation in refrigerated and frozen salted raw minced chicken breasts with electron beam irradiated almond skin powder. Meat Sci. 80: 1326-1332.

Tichivangana J.Z. and Morrissey P.A. 1985. Metmyoglobin and inorganic metals as pro-oxidants in raw and cooked muscle systems. Meat Sci. 15: 107-116.

Trindade R.A., Mancini F. J., Villavicencio A.L.C.H. 2014. Natural antioxidants protecting irradiated beef burgers from lipid oxidation. LWT-Food Sci Technol. 43:98-104.

Wang B.H., Zuzel K.A., Rahman K. and Billington D. 1998. Protective effects of aged garlic extract against bromobenzene toxicity to precision cut rat liver slices. Toxicology 126:213-222.

Xie X.X., Meng Q.X., Cui Z.L. and Ren L.P. 2012. Effect of Cattle Breed on Meat Quality, Muscle Fiber Characteristics, Lipid Oxidation and Fatty Acids in China. Asian Austral. J. Anim. 25: 824-831.

Yin M.C. and Cheng W.S. 2003. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. Meat Sci. 63: 23-28.

Zakrys-waliwander P.I., O'sullivan M.G, O'neill E.E. and Kerry J.P. 2012. The effects of high oxygen modified atmosphere packaging on protein oxidation of bovine M. longissimus dorsi muscle during chilled storage. Food Chem. 131: 527-532.

Paper Received October 24, 2014 Accepted June 25, 205