Air frying combined with grape seed extract inhibits N^{ε}-carboxymethyllysine and N^{ε}-carboxyethyllysine by controlling oxidation and glycosylation

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ABSTRACT Advanced glycation end products (**AGE**), compounds formed in meat at the advanced stage of Maillard reaction, are easily exposed to thermal processing. Improving cooking condition and adding antioxidants are 2 common ways for AGE reduction. The present work compared the inhibition of grape seed extract (**GSE**) on levels of free and protein-bound N^{ε}-carboxymethyllysine (**CML**) and N^{ε}-carboxyethyllysine (**CEL**) in chicken breast under deep-frying and air-frying conditions. Efficiency of 5 concentrations of GSE (0.0, 0.2, 0.5, 0.8, and 1.0 g/kg) in retarding oxidation, glyoxal (**GO**), methylglyoxal (**MGO**), lysine (**Lys**), Maillard reaction degree (A_{294}, A_{420}), and Shiff's base were tested. Results showed that 0.5 g/kg GSE before heating significantly ($P < 0.05$) reduced AGE in fried breast chicken, whereas excessive supplementation of GSE (0.8 and 1 g/kg) was reverse. Air frying was found significantly ($P < 0.05$) better than deep frying to reduce the precursor substances (GO, MGO, and Lys) of AGE. In conclusion, GSE-derived polyphenols exhibited different inhibitory effects on oxidation and glycosylation at different concentrations. We found that 0.5 g/kg of GSE combined with air frying was the best recommendation for inhibiting CML and CEL.

Key words: heat treatment, antioxidant extract, advanced glycation end products

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INTRODUCTION

High levels of advanced glycation end products (**AGE**) are easily exposed to thermal processing in fat and protein-rich meat products because of the strong Maillard reaction and oxidation reactions occurred at thermal processing (Yu et al., 2016). N^{ε}-carboxymethyllysine (**CML**) and N^{ε}-carboxyethyllysine (**CEL**) are 2 typical AGE formed at the advanced stage of Maillard reaction with nonfluorescent characteristic (Prasad et al., 2013). Briefly, AGE can be divided into free and protein-bound forms because of their different bioavailability and particle size during digestion (Sheng et al., 2018). Accumulation of AGE has been indicated to aggravate oxidative stress, inflammation and structural tissue damage leading to chronic diseases (Khan et al., 2020). Therefore, regulating the levels of AGE in food is of great significance for keeping human health and preventing diseases (Zhu et al., 2020). For AGE formation, thermal processing is the main pathway. For instance, levels of CML and CEL in meat products treated by boiling, braising, deep frying, roasting has been widely reported (Wang and Xiong, 2005; Roldan et al., 2015; Sun et al., 2015). These thermal treatments promoted AGE formation by active α-dicarbonyl compounds (such as methylglyoxal (**MGO**), glyoxal (**GO**)) and reactive oxygen radicals accumulation which result from oxidation and Maillard reaction (Degen et al., 2012; Sheng et al., 2018). Frying is extensively used in the family kitchen as well as commercial fast food industry for its high ability to make the food tastier and more delicious (Teruel et al., 2015). However, many reports suggested that frying at higher cooking temperature produced higher levels of CML and CEL in meat products (Chen and Scott Smith, 2015; Roldan

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et al., 2015; Yu et al., 2016; Ortiz et al., 2018). Therefore, changing the frying methods is quite meaningful to decrease AGE contents in fried meat products.

Air frying aimed to produce a "fried product" by belching hot air around the food material instead of infiltrating it into hot oil (Teruel et al., 2015). Moreover, compared with deep frying, air frying not only greatly reduced the lipid oxidation, but also the amount of oil used in air frying showed an 80% decrease (Andres et al., 2013; Heredia et al., 2014). However, there are little reports comparing the AGE inhibitory ability of air frying with deep frying, as well as the mechanism underlying in AGE inhibition during air frying is unclear.

In addition to improving cooking method to reduce the level of AGE, adding plant extracts rich in polyphenols is another commonly used and effective way. Grape seed extracts (**GSE**) is a by-product of grape wine and juice production, which contain 5 to 8% polyphenols including catechin, epicatechin, gallic acid, and procyanidins (Chedea et al., 2010). The polyphenols of GSE possessed a strong antioxidant activity as well as scavenging free radicals scavenging ability. Wang et al. (2018) found that the application of GSE in Chinese traditional meat products improved its oxidation stability during storage as well as reduced the level of harmful substances. Hence, GSE is suitable inhibit to AGE formation.

However, the influence of deep frying and air frying on the levels of free and protein-bound CML and CEL in chicken meat is still unclear. On the other hand, as per our information, there is no comparing report available on the free and protein-bound AGE inhibition by GSE addition during chicken air frying. Accordingly, the purpose of the present study is to 1) investigate the inhibitory effects of different concentration of GSE on AGE, Maillard reaction and oxidation during different concentration of GSE addition during deep frying and air frying, 2) illustrate the main influencing factor under these 2 frying processes by principal component analysis (**PCA**), 3) explore the possible mechanism of air frying combined with GSE polyphenol on inhibition of CML and CEL.

MATERIALS AND METHODS

Chemicals and Materials

Thirty-three frozen chicken breasts, soybean oil, caramel, and spices were purchased from Nanjing supermarket (Jiangsu province in China). Commercial grape seed extract (Grajfnol JF-NATURAL Co., Ltd., China. Procyanidins(95%)) 5,5′-dithiobis, (99% **DTNB**), 2,4-dinitrophenylhydrazine, trichloroacetic acid (**TCA**), thiobarbituric acid (**TBA**), guanidine hydrochloride, 1,1,3,3-tetraethoxypropane, sodium tetraborate decahydrate, and ethylenediaminetetraacetic acid disodium salt were analytically pure. Chicken CML/CEL double-antibody enzyme-linked immune assay (**ELISA**) kits were purchased from Nanjing Maibo Reagent Co., Ltd. The solid-phase extraction columns Oasis MCX cartridge (60 mg/3 mL, 30 μm) were obtained from Waters Corporation (Milford, MA).

Samples Preparation

Frozen chicken breasts were taken out from the -20°C refrigerator and completely thawed at 4°C. Thirty-six chicken breasts were divided into 2 groups, one is the deep frying group, and another one is the air frying group. As shown in Figure 1, to make the surface color and induce strong Maillard reaction occurred at chicken, we first prepared a sugar solution (caramel: water = 4:6, w: v). Then, GSE was added to the sugar solution with the concentration at 0.0, 0.2, 0.5, 0.8, and 1.0 g/kg, respectively. Next, GSE-sugar solution was evenly smeared on the surface of chicken breast (10 mL/100 g meat). Finally, the prepared chicken breasts were put into the air-fryer (Philips, made in China) for air frying and a pan for deep frying separately. The temperature for both frying procedures was 180°C, and the time was 3 min. Peanut oil (Purchased at Suguo Supermarket in Nanjing, Jiangsu, China) was used for deep frying. The sugar-only without GSE smeared raw meat was taken as control. The fried meats of different treatment groups (T_0 (0.0 g/kg GSE), $T_{0.2}$ (0.2 g/kg GSE), $T_{0.5}$ (0.5 g/kg GSE), $T_{0.8}$ (0.8 g/kg GSE), and T_1 (1 g/kg GSE)) and control groups were pulverized to measure the indicators, and each group was repeated 3 times.

Microscopy

Chicken breast was cut into 0.5 × 0.5 × 1.0 cm strips in accordance with the direction of the muscle fibers. The strips were quickly frozen with liquid nitrogen and cut into 10 μm slices. In accordance with the method of hematoxylin and eosin staining kit (Beyotime, Product number: C 0105, China), the color finished slices were placed on a microscope slide. Then slices were observed at room temperature using an optical microscopy Scope (Carl Zeiss, Dresden, Germany). Microphotographs were taken using a 40 × objective lens.

Determination of Maillard Reaction

The absorbance at 294 and 420 nm are usually used to indicate the amount of reaction products in the early and last stage of Maillard reaction (Onorato et al., 2000). About 1 g of chicken breast and 9 mL of phosphate buffer solution (pH 7.2) were accurately added into a polyethylene (**PE**) tube and the absorbance (294 and 420 nm) of the supernatant were determination by microplate reader (M2e IKA, Germany).

Measurement of AGE

The samples of free and protein-bound CML and CEL were separated and prepared in accordance with the method of Sun et al. (2016) and Niu et al. (2017) with slight modification. Briefly, the main preparation methods of free CML and CEL was as follows: 1 g of chicken breast and precooled 5% TCA were put into 10 mL PE tube and homogenized twice at 10,000 r/min (IKA-Ultra-Turrax T-25 mixer, Braun), then it was centrifuged at 8,000 r/min for 5 min (Allegra 64R high-speed frozen centrifuge,

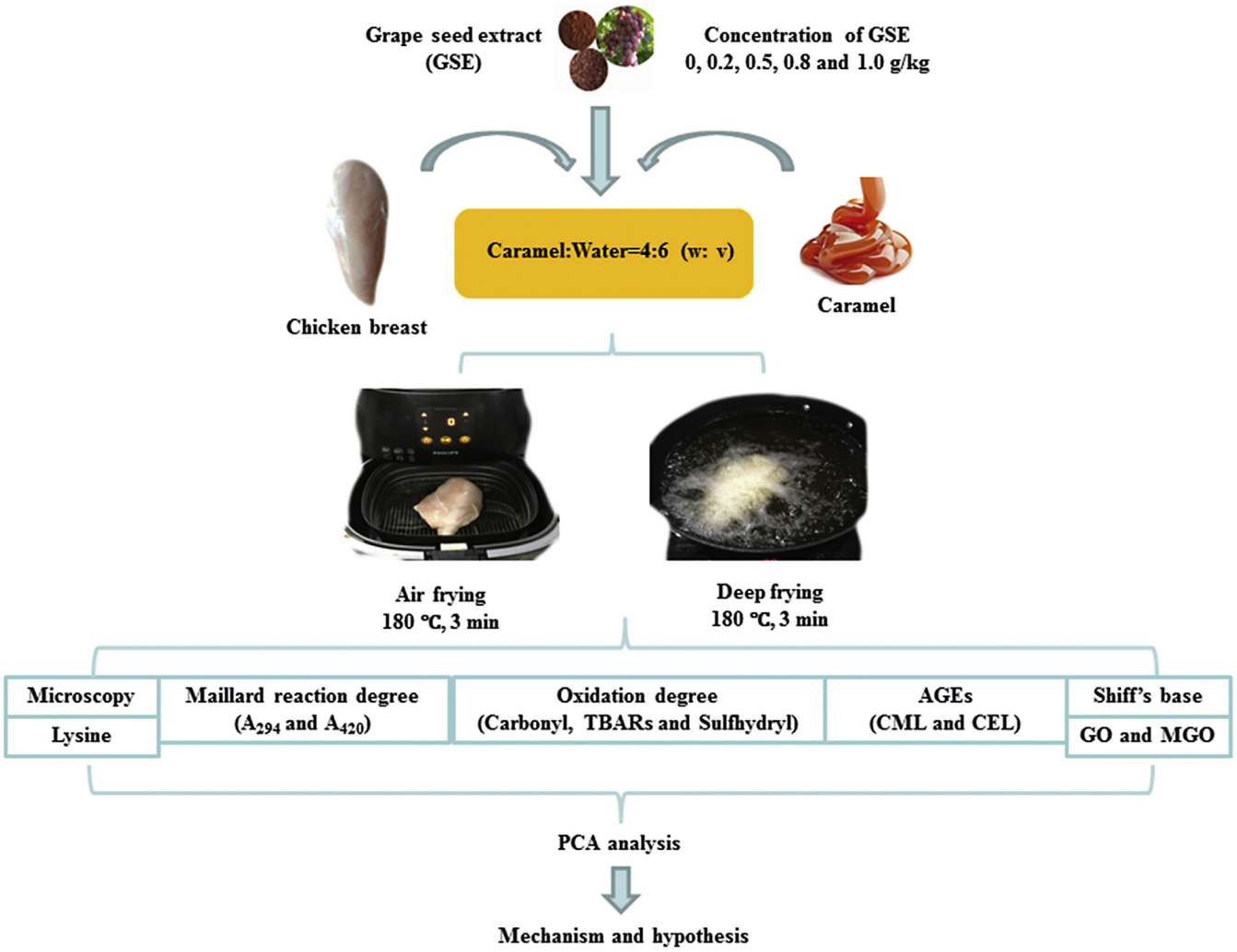

Figure 1. Sample preparation and organizational framework.

Beckman Coulter Inc, CA). The supernatant was shaken for 1 min with 10 mL of n-hexane and the process was repeated 3 times. The upper layer of fat was discarded and 5 mL of the liquid was added into the MCX solid phase extraction cartridge for further purification and ELISA analysis (Zhu et al., 2019).

The main preparation methods of the protein-bound CML and CEL as follows: 0.4 g of chicken breast meat, sodium borate buffer solution, and sodium borohydride were added for reduction reaction overnight. Then, TCA (5 mL, 20%) and n-hexane was added and centrifuged at 1,000 r/min for 30 min. The lower layer precipitate was placed in a pressure-resistant bottle with the addition of 3 mL of hydrochloric acid and was acidified at 110°C for 24 h. The acid solution was diluted to 8 mL, and 3 mL of the liquid was added into MCX solid-phase extraction cartridge for further purified and ELISA analysis (Zhu et al., 2019).

Measurement of Carbonyl

For the measurement of carbonyl, the method of Liu et al. (2000) with slight modification was used. In accordance with the color reaction of 2,4-dinitro phenylhydrazine (10 mmol/L) and guanidine hydrochloride (6 M), the solution supernatant was collected and absorbance was determined at 370 nm. The final carbonyl content was checked as nmol/mg protein.

Determination of Sulfhydryl and Thiobarbituric Acid–Reactive Substances (TBARs)

The total and active sulfhydryl determination methods were appropriately modified according to Xue et al. (2017). About 1 g sample and 9 mL phosphate buffer solution were added into a PE tube and centrifuged at 5,000 r/min homogenized for 30 s (4°C). The active and total sulfhydryl determination was conducted by the color reaction of DTNB (10 mmol DTNB dissolved in 20 mmol KH_2PO_4) and urea (8 M). The solution absorbance was measured at 412 nm and final results were expressed as μmol/mg protein. The determination of fat oxidation (**TBARs**) was carried out in accordance with the method of Utrera et al. (2014) with minor modifications. The sample solution absorbance was measured at 532 nm and the results were expressed as mg MDA/kg of meat.

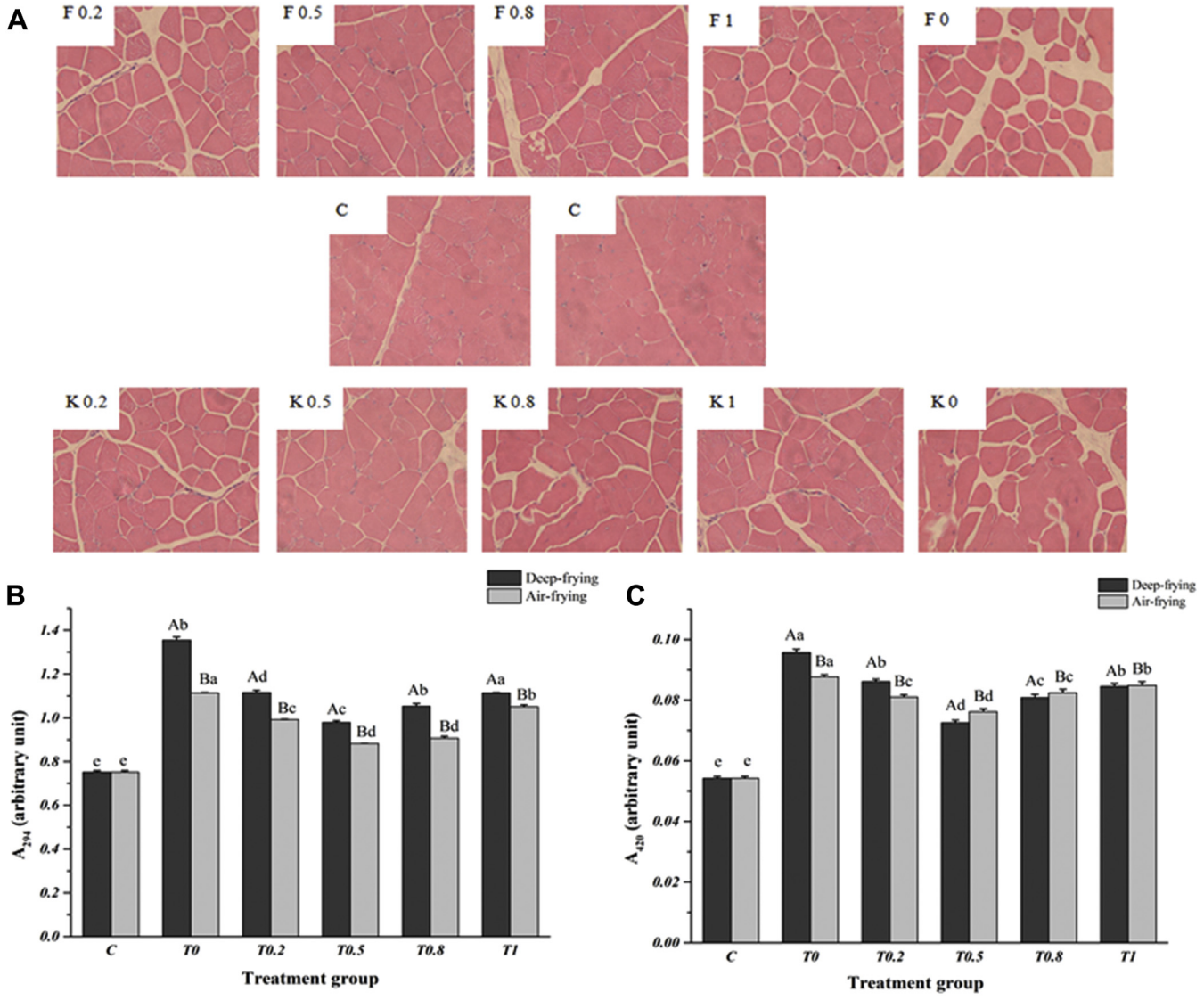

Figure 2. (A) Light micrographs of transversal sections in pectoralis major muscle of broiler breast meat under deep frying and air frying treatments. Magnification: 400 × . Changes of A_{294} (B) and A_{420} (C). Note: F refers to deep frying, K refers to air frying. 0, 0.2, 0.5, 0.8, and 1 refer to the different GSE concentrations, respectively. T_0 (0.0 g/kg GSE), $T_{0.2}$ (0.2 g/kg GSE), $T_{0.5}$ (0.5 g/kg GSE), $T_{0.8}$ (0.8 g/kg GSE), and T_1 (1 g/kg GSE) refer to treatment groups, respectively. C refers to control. Different capital letters (A, B) in the same treatment group with different frying methods were significant ($P < 0.05$). Different lowercase letters ($^{a\text{-}e}$) between different treatments with the same frying method were significant ($P < 0.05$), n = 3. Abbreviation: GSE, grape seed extract.

Determination of GO, MGO, Lys, and Shiff's Base

The determination of GO and MGO was based on the method of Sawicki et al. (1962) and Gilbert and Brandt (1975) with minor modification. The absorbance value at 233 nm was used to indicate the content of GO. Briefly, 0.5 mL of sample solution, 1 mL of 1.5 g/L sodium acetate solution, and 2 mL of 2 g/L hydroxylamine hydrochloride were added into a tube. The absorbance value was measured at 233 nm after heating in water at 60°C for 30 min. The MGO was measured by 0.5% TBA colorimetric, and the content of MGO was calculated by $6.45*(A_{532} - A_{600})-0.56*A_{450}$, of which 6.45 and 0.56 were conversion factors.

The Lys was measured in accordance with the method of Church et al. (1983) and Guan et al. (2006) with slightly modification. In details, 0.146 g of Lys was mixed with A solution (0.004 g OPA +1 mL methanol + 3 mL distilled water) and B solution (100 mmol sodium borate + 20% SDS + 100 μL mercaptoethanol) to a concentration of 0.25-2 mmol/L standard solution. The absorbance was checked at 340 nm and a standard curve was plotted. The concentration of lysine in the sample solution was calculated, and the final results were expressed as mg/g of meat.

During meat frying, the protein combined with lipid peroxidation will results in the fluorescent pigments formation, which is the precursors of the Maillard products named Shiff's base. The Schiff's base was determined in accordance with the method of Gatellier et al. (2010) described with small modification. It was measured by using an extraction in dichloromethane/ethanol mixture and the fluorescence was measured at 420-430 nm after excitation at 360 nm.

Table 1. Content of free and protein-bound CML and CEL by deep frying and air frying.

Treatment group	Protein-bound CML	Free CML	Protein-bound CEL	Free CEL
F_0	136,093.33 ± 1,240.86^{A,a}	13,579.50 ± 187.35^{A,a}	3,985.74 ± 38.18^{A,a}	405.06 ± 5.39^{A,a}
$F_{0.2}$	124,146.66 ± 2,207.11^{A,b}	12,658.50 ± 180.67^{A,b}	2,913.20 ± 28.17^{A,b}	363.52 ± 4.78^{A,b}
$F_{0.5}$	108,546.67 ± 1,935.49^{A,c}	11,376.00 ± 122.33^{A,c}	2,183.36 ± 17.91^{A,c}	351.79 ± 2.97^{A,c}
$F_{0.8}$	114,013.33 ± 880.30^{A,d}	9,942.00 ± 79.27^{A,d}	2,737.39 ± 22.38^{A,d}	325.69 ± 4.12^{A,d}
F_1	119,840.00 ± 1,604.49^{A,e}	10,885.50 ± 82.11^{A,e}	3,132.35 ± 43.78^{A,e}	320.56 ± 3.10^{A,d}
K_0	105,133.33 ± 1,179.38^{B,a}	9,223.50 ± 119.42^{B,a}	2,443.32 ± 21.05^{B,a}	310.06 ± 4.05^{B,a}
$K_{0.2}$	97,320.00 ± 1,446.65^{B,b}	7,878.00 ± 96.75^{B,b}	2,214.11 ± 15.56^{B,b}	284.52 ± 2.23^{B,b}
$K_{0.5}$	90,240.00 ± 1,393.13^{B,c}	6,792.00 ± 72.89^{B,c}	2,049.47 ± 21.01^{B,c}	251.32 ± 3.38^{B,c}
$K_{0.8}$	93,893.33 ± 800.33^{B,d}	5,463.00 ± 64.89^{B,d}	2,119.08 ± 17.93^{B,d}	230.34 ± 1.84^{B,d}
K_1	101,266.67 ± 1,226.59^{B,e}	5,526.00 ± 41.24^{B,d}	2,289.59 ± 24.09^{B,e}	222.67 ± 1.76^{B,e}
C	28,893.33 ± 608.71	2,307.00 ± 32.56	1,213.69 ± 27.09	160.53 ± 1.28

F refers to deep frying, K refers to air frying. 0, 0.2, 0.5, 0.8 and 1 refer to the different GSE concentrations, respectively. C refers to control. Different capital letters (A, B) in the same treatment group with different frying methods were significant ($P < 0.05$). Different uppercase letters (^{A,B}) in the same column indicate significant differences ($P < 0.05$), and different lowercase letters ($^{a\sim e}$) in the same treatment group indicate significant differences ($P < 0.05$), unit: ng/g meat, n = 3.

Abbreviations: CML, N^{ε}-carboxymethyllysine; CEL, N^{ε}-carboxyethyllysine; GSE, grape seed extract.

Statistics Analysis

All experiments were determined with 3 repetition ($n = 3$), and expressed as mean ± SD. SAS analysis software (SAS software research institute, version 8.1) was used for statistical analysis of data, one-way ANOVA method was used for analysis of variance and Duncan's multiple range test was used to compare the differences between mean value. $P < 0.05$ indicated a significant difference in the results. SIMCA-P 12.0 (UMETRICS, Umea, Sweden) software was used for the PCA was used.

RESULTS AND DISCUSSION

Microscopic Picture of Muscle Fiber

Morphological observations revealed that F_0 (deep frying, 0.0 g/kg GSE) and K_0 (air frying, 0.0 g/kg GSE) showed loose muscle fiber bundles, large intracellular gaps, and shrunken muscle fiber, whereas the raw muscle exhibited tight bundles of muscle fibers filling the endomysial space. Compared with K_0 and F_0, deep frying and air frying with GSE added showed similar regularity, which indicated that both $K_{0.5}$ (air frying, 0.5 g/kg GSE) and $F_{0.5}$ (deep frying, 0.5 g/kg GSE) displayed the best properties for protecting the muscle fiber shapes (Figure 2 A). Microscopic picture of muscle fibers indicated that a series of complex mass transfer processes were happened during frying, the muscle contents were let out and evaporation of water loss was caused (Teruel et al., 2015). As a result, the integrity of muscle fiber was destroyed and oxidation stability was decreased. It has been reported that air frying can improve oxidation stability and maintain the tissue integrity compared with deep frying (Sansano et al., 2015). When GSE was added during frying, the tissue morphology was significantly improved when compared with the no GSE added frying group because of the prominent anti-oxidation ability of GSE (Wang et al., 2018).

Maillard Reaction and Levels of AGE

The degree of Maillard reaction can reflect the content of AGE to some extent. As markers of the early and last stage of Maillard reaction, browning intensity (A_{294} and A_{420}) is the easiest measurable methods for the direct visual reckon (Yu et al., 2017). As shown in Figures 2B and 2C, compared with the control group, the values of A_{294} and A_{420} in the treatment group increased significantly ($P < 0.05$), which indicated the occurrence of Maillard reaction and AGE generation. With the increase of GSE concentration to certain level (0.5 g/kg GSE addition), the Maillard reaction was inhibited in both deep frying and air frying, but, when the concentration exceeded 0.5 g/kg, an upsurge in the intensity of Maillard reaction was observed. Deep frying exhibited higher intensity of Maillard reaction than air frying. These findings could be explained by the mechanism of air frying and GSE characteristic. Because air frying was based on hot air as heat transfer medium, and 80% less oil was used as compared with deep frying. Thus, the Maillard reaction induced by lipid oxidation pathway also reduced (Andres et al., 2013). Moreover, moderate amount of GSE addition also inhibited Maillard reaction due to the antioxidants effects (Weber et al., 2007). However, the excessive amount of GSE addition promoted Maillard reaction may because the GSE itself had many amino acids and other substances that could promote Maillard reaction (Chedea et al., 2010).

The content of free and protein-bound CML and CEL were shown in Table 1. For F_0, the content of free and protein-bound CML and CEL increased as compared with control (free CML: 488.62%, protein-bound CML: 371.02%, free CEL: 152.32%, protein-bound CEL: 228.40%). Adding 4 concentrations of GSE during deep frying have effectively reduced the AGE content compared with F_0 (free CML: 8.17–32.27%, protein-bound CML: 11.14–25.70%, free CEL: 16.98–34.56%,

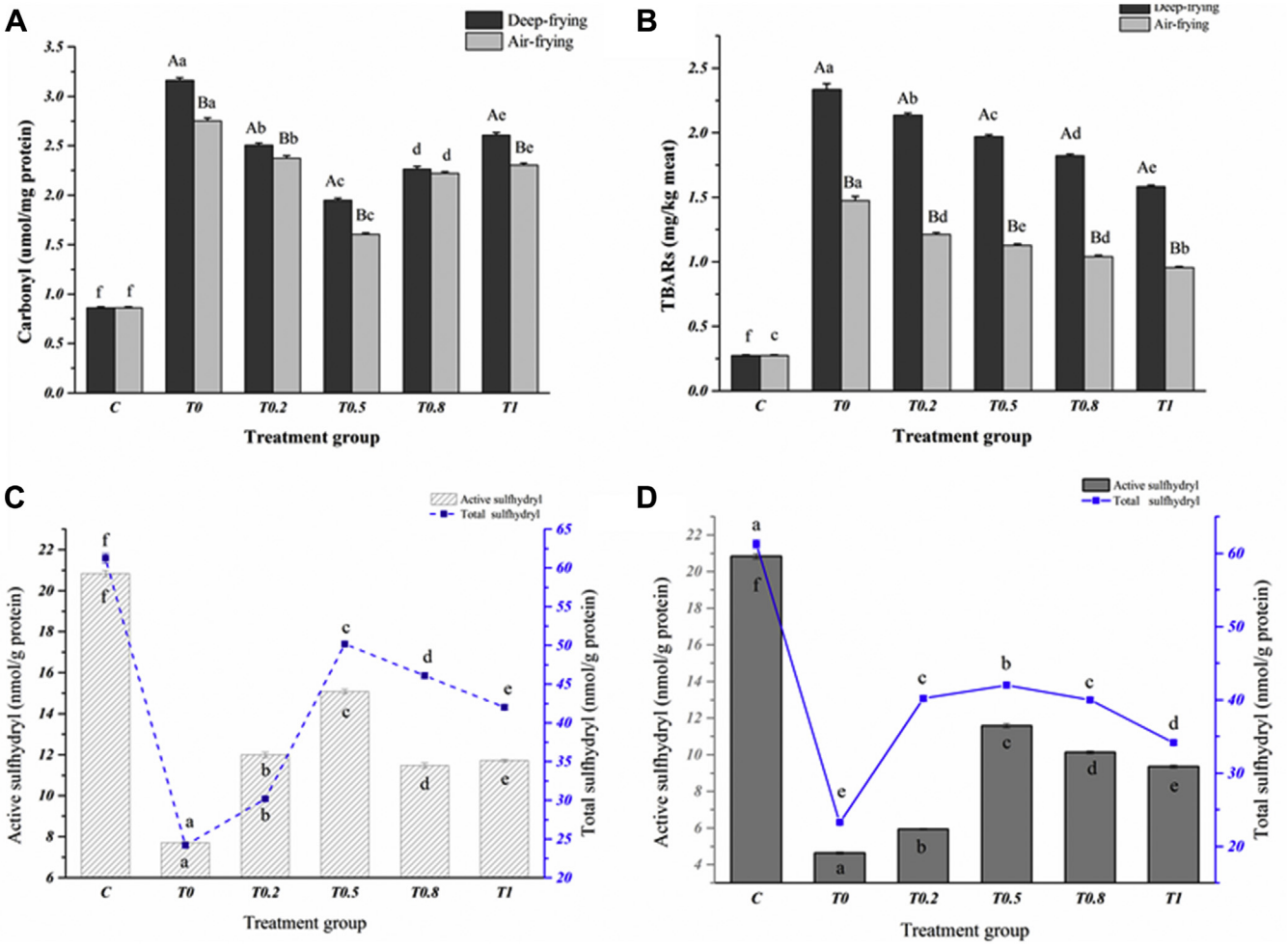

Figure 3. Changes of carbonyl (A), TBARs (B), sulfhydryl of air frying (C), sulfhydryl of deep frying (D) in chicken breast under deep frying and air frying. Note: T_0 (0.0 g/kg GSE), $T_{0.2}$ (0.2 g/kg GSE), $T_{0.5}$ (0.5 g/kg GSE), $T_{0.8}$ (0.8 g/kg GSE), and T_1 (1 g/kg GSE) refer to treatment groups, respectively. C refers to control. Different capital letters (A, B) in the same treatment group with different frying methods were significant ($P < 0.05$). Different lowercase letters ($^{a\text{-}e}$) between different treatments with the same frying method were significant ($P < 0.05$), n = 3. Abbreviations: GSE, grape seed extract; TBARs, thiobarbituric acid–reactive substances.

protein-bound CEL: 30.79–65.02%). Similarly, the content of free and protein-bound CML and CEL in K_0 also increased as compared with control, but the level was less than F_0 (free CML: 299.80%, protein-bound CML: 263.87%, free CEL: 93.14%, protein-bound CEL: 101.31%). On the other hand, the addition of 4 concentrations of GSE during air frying have efficiently reduced the AGE content compared with K_0 (free CML: 19.45–54.37%, protein-bound CML: 5.07–19.53%, free CEL: 17.08–58.44%, protein-bound CEL: 12.50–32.03%). Thus, air frying combined with GSE addition could significantly reduce the content of free and protein-bound CML and CEL. The optimal content of GSE addition during deep frying for free CML, protein-bound CML, free CEL, and protein-bound CEL was 0.8 g/kg, 0.5 g/kg, 0.5 g/kg, and 1.0 g/kg, respectively. The optimal content of GSE addition during air frying was consistent with deep frying expect for protein-bound CEL (0.5 g/kg). Our findings revealed the unanimous consequence that adding GSE was an effective method to suppress AGE (Addai, 2010; Nowshehri et al., 2015).

Oxidation Analysis

Lipid oxidation was evaluated by the TBARs value and protein oxidation was expressed by carbonyl and sulfhydryl value. As shown in Figure 3, compared with the control group, the values of TBARs and carbonyl in the treatment group increased significantly ($P < 0.05$). For treatment group, both air frying and deep frying showed a similar trend. With the increase of GSE addition during air frying and deep frying, the carbonyl values were lowest at $T_{0.5}$, which indicated the strongest capacity of antioxidation (Figure 3A). With the increase in the concentration of GSE, a trend with decreasing amount of TBARs was observed (Figure 3B), whereas the inhibitory effect was better ($P < 0.05$) in air frying as compared with deep frying. Our results were similar with the outcomes of Mielnik et al. (2006), which exhibited efficiency of 4 concentrations of GSE (0.0, 0.4, 0.8, and 1.6 g/kg) promoting an excellent oxidation stability during heart treatment and storage. Hence, lipid oxidation could be prevented by the GSE and it showed a concentration-dependent

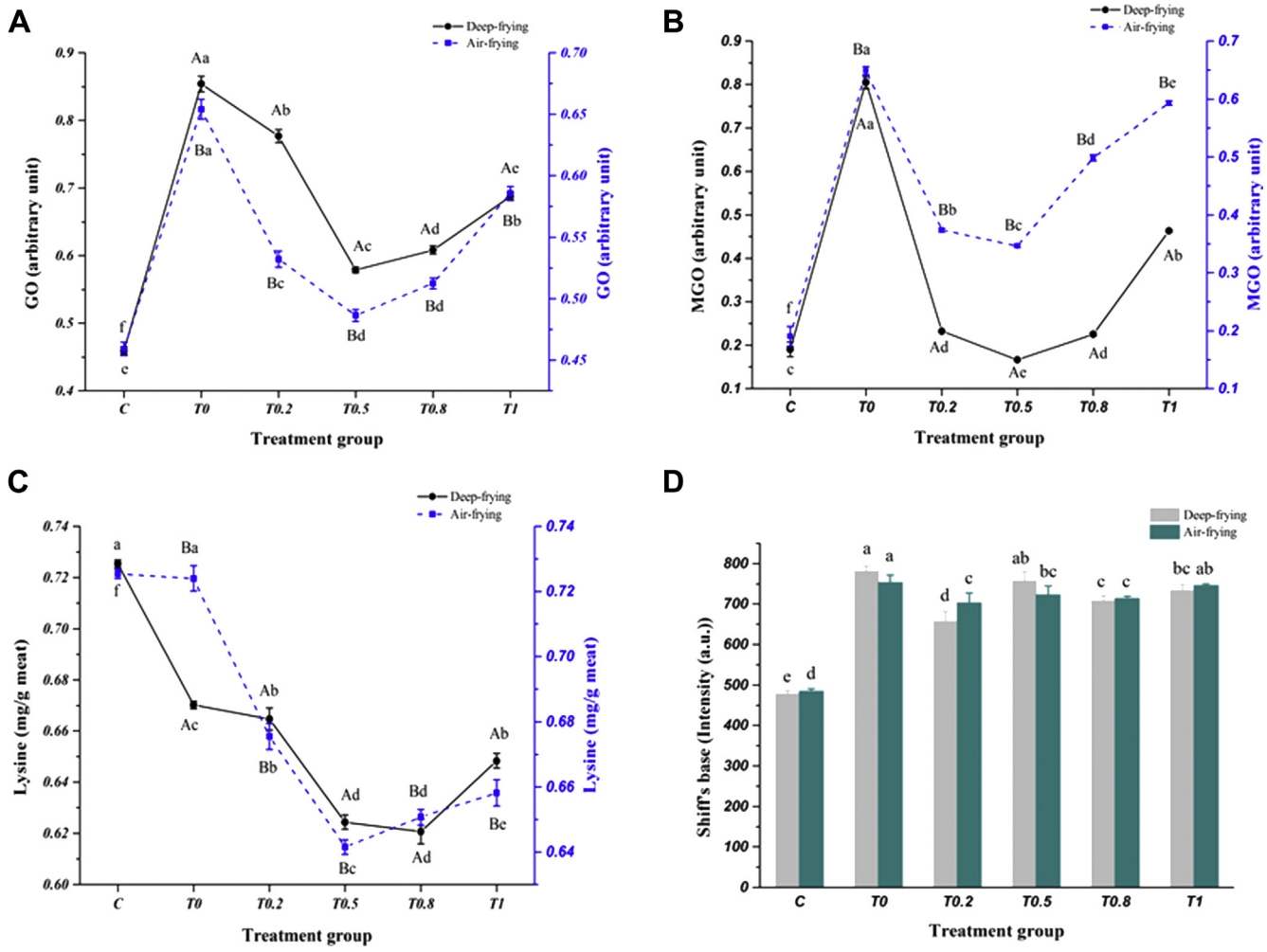

Figure 4. Changes of GO (A), MGO (B), lysine (CC_3), Shiff's base (D) in chicken breast under deep frying and air frying. Note: T_0 (0.0 g/kg GSE), $T_{0.2}$ (0.2 g/kg GSE), $T_{0.5}$ (0.5 g/kg GSE), $T_{0.8}$ (0.8 g/kg GSE), and T_1 (1 g/kg GSE) refer to treatment groups, respectively. C refers to control. Different capital letters (A, B) in the same treatment group with different frying methods were significant ($P < 0.05$). Different lowercase letters ($^{a-e}$) between different treatments with the same frying method were significant ($P < 0.05$), n = 3. Abbreviations: MGO, methylglyoxal; GO, glyoxal; GSE, grape seed extract.

trend (Mielnik et al., 2006) in both air frying and deep frying methods.

Another marker of protein oxidation in meat and meat products is the loss sulfhydryl group (Wen et al., 2019). The reduction of total and active sulfhydryl contents was shown in Figures 3C and 3D. The content of sulfhydryl showed a significant reduction in treated groups ($P < 0.05$) compared with control. For treatment groups, the total and active sulfhydryl value was highest at $T_{0.5}$, which indicated the best antioxidation at this level. The reason why the sulfhydryl increased from T_0 (0 g/kg GSE addition) to $T_{0.5}$, and decreased from $T_{0.5}$ to T_1 (1 g/kg GSE addition) was the changes in intraprotein and interprotein disulfide bonds and mixed-disulfides (Stadtman, 1990). The degree of protein oxidation was inhibited when the GSE addition was 0.2 g/kg to 0.5 g/kg for the intersulfhydryl radical scavenging by polyphenols (Brannan and Mah, 2007). However, the protein oxidation was promoted when the GSE addition was 0.5 g/kg to 1.0 g/kg for the intrasulfhydryl radical generation (Wen et al., 2019).

Precursors of AGE and the Interaction Between Maillard Reaction and Oxidation

Free and protein-bound CML and CEL is mainly formed by the precursors including GO, MGO, and Lys (Singh et al., 2001). The effect of GO and MGO formation during frying was shown in Figures 4A and 4B. The values of GO and MGO showed a decreasing trend from T_0 to $T_{0.5}$, whereas an increasing trend was observed from $T_{0.5}$ to T_1. Thus, it was found that the 0.5 g/kg concentration was the best level of GSE addition which avoided GO and MGO generation. Meanwhile, AGE can be largely formed by α-dicarbonyl compounds reacting with Lys (Zhu et al., 2018). In accordance with the results shown in Figure 4C, it also concluded that $T_{0.5}$ exhibited the best effect on the inhibition of Lys formation, which indicated the ideal concentration for cutting off the AGE generation. These findings were found consistent with the antioxidation analysis above.

Maillard reaction is the key pathway for AGE formation. More importantly, the oxidation occurred during

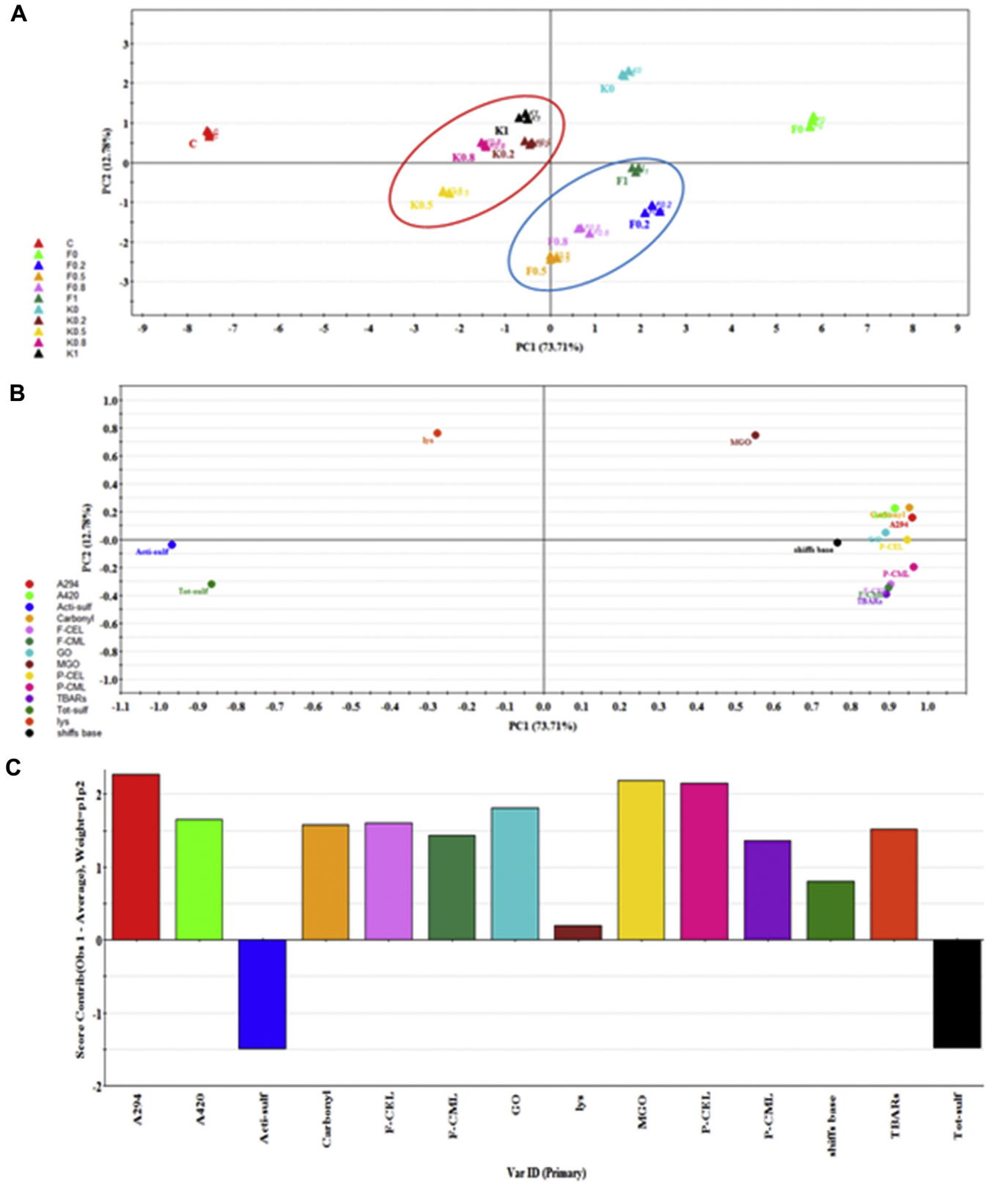

Figure 5. Scatter plot (A), loading plot (B), and contribution rate graph (C) of PCA analysis. Structures of proanthocyanidins in GSE adapted from the study by Xie and Chen (2013) (D). Mechanism hypothesis of catechin inhibit AGE under different oxidation and glycation conditions (E). Abbreviations: AGE, advanced glycation end products; GSE, grape seed extract.

thermal processing would also influence the AGE formation. The reaction mechanism of AGE formation by Maillard reaction is mainly because of the dicarbonyl compounds formation at Shiff's base and Amadori Rearrangement Product stage (Thornalley, 2005; Baskara et al., 2017). So, the Schiff's base could reflect the interaction between Maillard reaction and oxidation on the influence of AGE formation (Sobral et al., 2018).

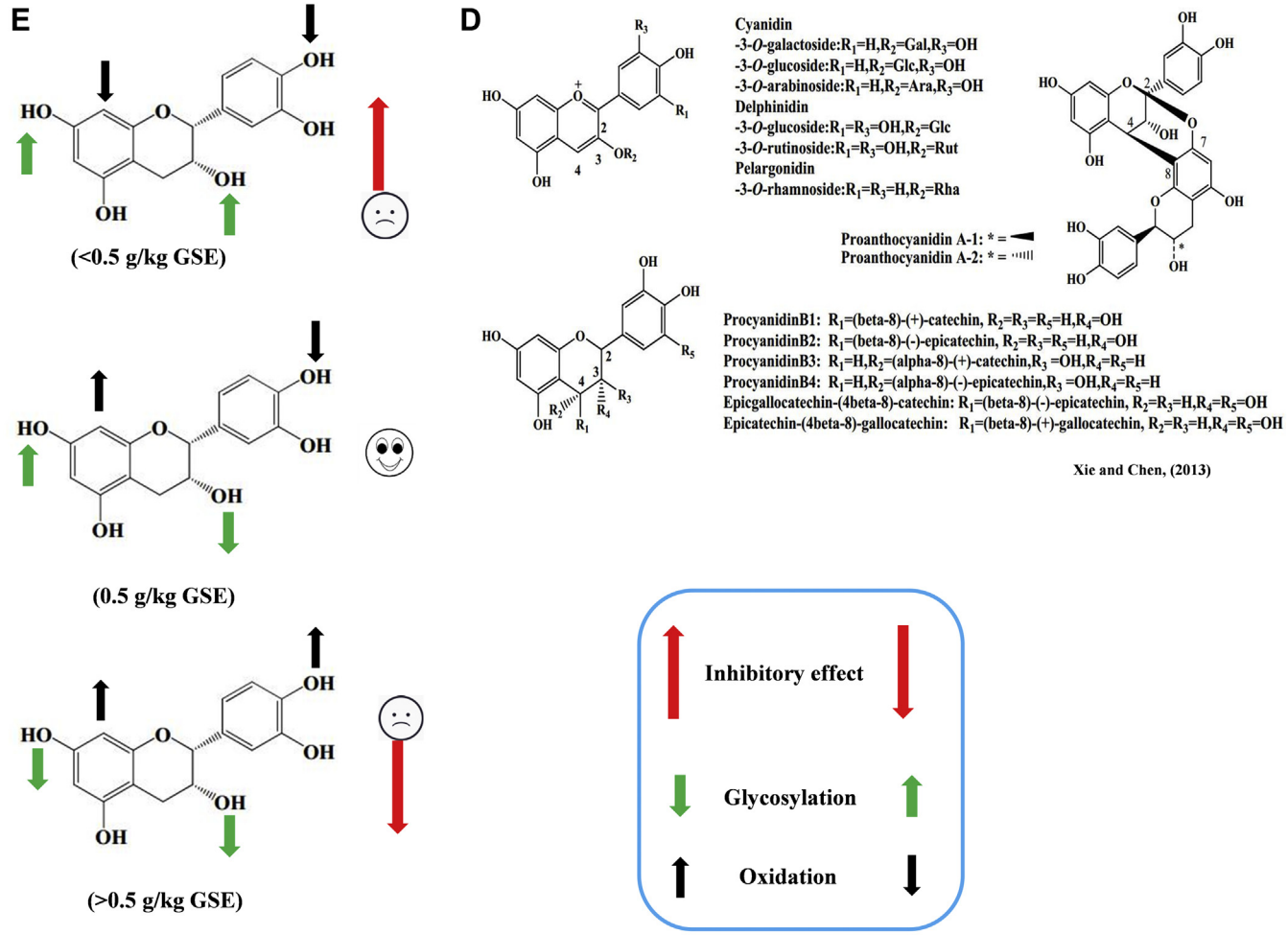

Figure 5. Continued

As shown in Figure 4D, our finding revealed that the interaction between Maillard reaction and oxidation increased significantly ($P < 0.05$) with GSE addition during chicken breast air frying and deep frying. However, there was no significant difference in the Shiff's base value between air frying and deep frying methods in different GSE treatments group ($P < 0.05$).

PCA Analysis

The PCA models were suitable for 2 principal components for analysis, as can be seen in Figures 5A–5C. Score plot (Figure 5A) was visualized in accordance with the trends of samples from treatment groups, and the involvement of the different AGE in each group was used to be revealed by loadings (Figure 5B) and contribution plots (Figure 5C). Our results indicated that PC_1 (73.71%) and PC_2 (12.78%) were suitable for analysis by using PCA models. Score plots showed that air frying and deep frying could be split apart, regardless of the inter and intra groups, K_0 as well as F_0 were far from the GSE added group, respectively, which indicated an important influence one air frying and deep frying when GSE was added.

The loading plots revealed that the A_{294}, A_{420}, free and protein-bound CML and CEL, GO, TBARs, carbonyl, and Shiff's base were all on the right of the plot, which were very close to F_0 indicating a high amount of AGE formation. Contrarily, the 0.2, 0.5, 0.8, 1.0 g/kg GSE addition of air frying and deep frying groups were grouped 2 together at a distance from this indicator, suggesting the excellent AGE inhibition effects. These findings verified the results in Table 1 discussed. At the same, the sulfhydryl was found on the left side of the plot, which was close to the control group, suggesting a major impact of protein oxidation on chicken raw material and there were biological differences in the AGE formation (Niu et al., 2017). Furthermore, the Lys and MGO were on the top of the plot, which was close to the K_0 and air frying group, indicating the importance of precursor influences on the AGE formation during air frying and GSE restraining.

The contribution plots exhibited that the average contribution was 1.114. When the contribution rate was bigger than the average contribution rate, it was considered to have a significant impact on the principal component. So, the A_{294} (contribution rate: 2.277), A_{420} (contribution rate: 1.648), free CML (contribution rate: 1.436), protein-bound CML (contribution rate: 1.365), free CEL (contribution rate: 1.606), protein-bound CEL (contribution rate: 2.151), GO (contribution rate: 1.806), MGO (contribution rate: 2.188), TBARs (contribution rate: 1.516), and carbonyl (contribution rate: 1.584) were significantly

influenced the air frying and deep frying by the 4 concentrations of GSE in both air frying and deep frying. Thus, these results and analysis verified the conclusion that adding GSE during air frying and deep frying taking an important role on the AGE formation, Maillard reaction and oxidation, but the interaction between Maillard reaction and oxidation was not significant.

As we know, polyphenols play an important role in scavenging free radical and antioxidation. Xie and Chen (2013) reviewed the main polyphenol antioxidants of GSE. As shown in Figure 5D, GSE are rich in proanthocyanidins. Procyanidins are oligomeric compounds formed from catechin and epicatechin molecules which are members of the proanthocyanidins, and they can be classified into 2 types in accordance with the number of bonds between the adjacent units (Peng et al., 2010). It is reported that structure of polyphenols also play an important role during inhibition of AGE (Xie and Chen, 2013). Catechin (one of the most abundant polyphenols) being monomers of flavan-3-ols is abundantly available in many natural plants. The inhibitory effects of catechins on CML and CEL formation also have been investigated several times (Wu and Yen, 2005; Lee et al., 2008). More important, as reported by Lee et al. (2008), the inhibitory activity of AGE was decreased because of catechins glycosylation. In addition, Jiao et al. (2019) investigated the catechins derived from green tea on CML and CEL inhibition. It was found that catechins not always induce AGE under different thermal processing, but the mechanisms by which catechins can promote AGE formation is unclear. One possible reason was illustrated by Chen et al. (2019) using molecular docking study. It was found that different catechins structures ((+)-catechin (**CC**) and (−)-epicatechin (**EC**)) exhibited different oxidation free radical clearance and enzyme interaction.

Based on the studies reported and our experimental results, we concluded that air frying combined with different doses of grape seed extract results in different AGE inhibitory effects. As shown in Figure 5E, with the increase in GSE concentration, both Maillard reaction (as shown in Figures 2B and 2C) and oxidation decreased (as shown in Figure 3). So, in this stage, oxidation combined with glycosylation could increase the AGE inhibitory effects of catechin. When GSE concentration was >0.5 g/kg, oxidation combined with glycosylation could decrease the AGE inhibitory effects of catechin because of the Maillard reaction and oxidation products accumulation. When GSE concentration was 0.5 g/kg, the AGE inhibitory effect of catechin reached to the best. Finally, different concentrations of GSE combined with air frying created a different oxidative and glycosylated environment for catechin, which in turn influenced its inhibitory effect on AGE.

CONCLUSIONS

First, air frying combined with 0.5 g/kg GSE showed a preferable effect on Maillard reaction (A_{294} and A_{420}), superior oxidation stability (lipid and protein oxidation), complete fiber shapes, prominent inhibitory effects of precursors (GO, MGO, and Lys) and extraordinary AGE inhibition as compared with deep frying. Second, PCA analysis revealed that adding GSE showed significant influence on both air frying and deep frying. Air frying combined with GSE can inhibit AGE formation by controlling oxidation and glycosylation reaction. The addition of 0.5 g/kg GSE was the best recommended level for CML and CEL reduction during air frying chicken.

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DISCLOSURES

The authors declare no competing financial of interest.

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