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Formation of AGEs in *Penaeus vannamei* fried with high oleic acid sunflower oil

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ABSTRACT

Here, we investigated the effects of frying process on the formation of advanced glycation end products (AGEs) in shrimps using *Penaeus vannamei* as the raw material. The results showed that the oil, malondialdehyde, fluorescent AGEs, carboxymethyl lysine (CML), methylglyoxal hydroimidazolone (MG-H1) and the outer layer carboxyethyl lysine (CEL) content was higher in the fried shrimps than that in the raw unfried shrimps. The outer layer CML, CEL and inner CEL, MG-H1 values all reached the maximum after the first batch of frying (22.43 mg/kg, 304.24 mg/kg, 83.76 mg/kg, and 169.42 mg/kg respectively). However, fluorescent AGEs and MG-H1 of the outer layer reached the maximum after the fifth and fourth batches of frying (1230.0 AU/g and 341.63 mg/kg). Malondialdehyde, fluorescent AGEs, CML, MG-H1, and CEL concentration in the fried shrimps firstly increased and then decreased to stabilization with more frying batches, with higher content in the outer layer of fried shrimps.

Introduction

Penaeus vannamei is one of the most famous shrimp species in the world for high nutritional value and great economic value (Rui et al., 2022). However, because of the high moisture and protein content in fresh shrimps, they are perishable and are usually processed using various cooking methods to extend their shelf life (Sharifimehr et al., 2019). Deep-frying is an economical and convenient cooking method that extends the shelf life of food (Bou et al., 2012).

Frying is usually performed at high temperatures (180–220 °C), where the proteins, carbohydrates, water, and vitamins in these foods are more prone to participate in a variety of chemical reactions including Maillard, caramelization, and lipid oxidation reactions (Bou et al., 2012). However, while these reactions add color and flavor to food, they also generate food safety hazards (Yang et al., 2021). The presence of AGEs in foods with high fat or protein content has been reported to be high, and previous studies in our laboratory also found that fried shrimps or fish produced large amounts of AGEs (Ruike et al., 2022; Wu et al., 2022). AGEs are a series of structurally complex covalent adducts generated from glucose or other reducing sugars and terminal free amino groups of lipids, proteins, peptides, and amino acids *via* the Maillard reaction pathway (Lu et al., 2022). Their production can be roughly divided into three stages: (1) free amino groups of proteins, lipids, nucleic acids, and other substances are condensed with carbonyl groups of reducing sugars to produce Schiff bases, and Schiff bases are destabilized and rearranged to produce Amadori rearrangement products (ARPs). (2) ARPs undergo dehydration, cyclization, oxidation, and rearrangement to generate dicarbonyl compounds with high reactivity, such as 3-Deoxyglucosone (3-DG), methylglyoxal (MGO), and glyoxal (GO). (3) Dicarbonyl compounds react with functional groups of amino acid (*i.e.*, lysine and arginine) to generate different types of AGEs. In addition, lipid oxidation products are also an important source of carbonyl compounds (Yang et al., 2021). However, in the food and catering industries, frying oil is often used repeatedly in the production of fried foods, which leads to the oil degradation, accompanied by a decrease in non-polar compounds and an increase in polar compounds (Lirong et al., 2019). Oxidation is the main chemical reaction that contributes to the deterioration of frying oil quality. Under the high temperature conditions of frying, both Maillard reactions and lipid oxidation occurred simultaneously (Chao et al. 2009). Oxidation

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parameters such as acid value, total polar material (TPM), conjugated dienoic acid (CDA) and *p*-anisidine value (*p*-AV) changed (Song et al., 2017). These greatly increased the content of carbonyl compounds in frying oils and promoted lipid oxidation in foods, which in turn affected the formation of AGEs. Diets high in AGEs have been shown to be strongly associated with the development of cardiovascular and Alzheimer's disease (Qingyi et al., 2018). Therefore, it is necessary for us to investigate the production of these AGEs in an effort to reduce their formation in fried foods and contribute to safe and healthy food production.

Based on the current mechanism of AGEs formation, we speculated that the change in frying oil quality during the processing of fried foods may affect the intermediate products required during AGEs generation, thus affecting the specific AGEs produced. It was shown that the presence of polyunsaturated fatty acids was positively correlated with the oxidative degradation of different oils (Rr et al., 2021). High oleic sunflower oil has 75%-90% oleic acid content and satisfactory frying stability (Kim et al., 2015). At present, the generation of AGEs in fried shrimps prepared with palm oil has been investigated in the previous report (Zhao et al., 2022). Thereafter, the aim of this study was to investigate the variation in quality and AGE content of fried shrimps cooked with high oleic sunflower oil during different heating times. The chromaticity, moisture, oil, malondialdehyde, fluorescent AGEs, and non-fluorescent AGEs (CML, CEL, and MG-H1) contents were examined to systematically analyze the trends of quality and AGEs under different frying batches.

Materials and methods

Materials and chemicals

Penaeus vannamei and high oleic acid sunflower oil (Zhongliang Co., Ltd. China) were purchased from Huazhong Agricultural University market (Wuhan, Hubei, China). The oil contained 46.34 mg/100 g of VE, 78.46% oleic acid and 14.3% linoleic acid. TCA, thiobarbituric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, chloroform, acetone, disodium hydrogen borate, sodium dihydrogen borate, sodium hydroxide, sodium borohydride, hydrochloric acid, methanol (chromatographic grade), ammonia (chromatographic grade) were acquired from Sinopharm Chemical Reagent Co.

Sample preparation

Shrimps were thawed overnight at 4 °C. The defrosted samples were washed, deheaded, peeled, deveined and tailed. The cutting depth was around 90% of ventral section.

6 L of high oleic sunflower oil was added to a deep fryer (Model DF-6L, Guangdong, China) and the frying test was performed at 180 ± 5 ℃. Frying was carried out continuously for 2 d (12 h/d), with 1 batch of shrimps added every 3 h for 120 s. The blank control was raw shrimp. The solid-to-liquid ratio of 1:16 (kg:L) was set (Table 1). Besides, the outer layer of shrimp was the surface part, which was in direct contact with frying oil, and the inner layer was the interior part of the samples.

Table 1
The ratio of material to liquid for deep-frying process.

Frying Batch	Volume of oil(L)	Number of shrimps
1	5.7	20
2	5.4	19
3	5.1	18
4	4.8	17
5	4.5	16
6	4.2	15
7	3.9	14
8	3.6	13
9	3.3	12

Determination of chromaticity

The color of the sample was measured by Chroma Meter CR-400 optical sensors (Konica Minolta Inc., Osaka, Japan). Each sample was measured six times. Working conditions: color spot diameter of 10 mm, the standard vertebral white plate as a standard sample. L* (brightness), a* (red (+)/green (-)), b* (yellow (+)/blue (-)) and △E* values (color difference) were measured on both sides of the second abdominal segment of the shrimps. L_0*, a_0* and b_0* for fresh unfried shrimps chromaticity. The calculation formula is as follows:

$$\Delta E^* = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2}$$

Determination of moisture and oil content

Moisture and oil content were determined according to AOAC official methods 950.46 and 960.39 (AOAC, 2004), respectively.

Determination of malondialdehyde content

The thiobarbituric acid active substance was determined by N_2S spectrophotometer (Jinko Co., St. Shanghai, China) according to the previous study (Jiang et al., 2021). The results were expressed as malondialdehyde (MDA) content (mg MDA/kg sample).

Determination of the content of fluorescent AGEs

The fluorescent AGEs content was based on the method of previous report (Jiang et al., 2021; Wu et al., 2022) with slight modifications. After the fried shrimps were well crushed, 2 g of the sample was accurately weighed and added to 18 mL of phosphate buffer (50 mmol/L, pH 7.4) and stirred at 37 °C for 1 h. The filtrate was filtered and collected by centrifugation at 7741 × g (Avanti J-26 XP Centrifuge, Beckman Coulter, Fullerton, CA, USA) for 5 min, and the fluorescence values were determined by fluorescence spectrophotometer with F-4600 spectrofluorometer (Shanghai Zhuohao Laboratory Equipment Co., Ltd., Shanghai, China). The instrument parameters were set to excitation wavelength 345 nm, emission wavelength 425 nm, slit width Ex/Em = 5 nm/5 nm, voltage 700 V, and response time 0.5 s. The fluorescence intensity at an emission wavelength of 425 nm (maximum) was recorded for three replicates of each sample, and the values were expressed in AU/g samples.

Determination of the content of non-fluorescent AGEs

The non-fluorescent AGEs content were determined according to (Ruike et al., 2022). The 100 mg lyophilized sample was dissolved in 3 mL chloroform-acetone (1:3) and centrifuged at 5000 × g for 15 min. The centrifuged sample was concentrated to dryness through nitrogen blowing (LB-K200 nitrogen purifier, Shanghai Zikis Electromechanical Equipment Co., Ltd., Shanghai, China), and the above operation was repeated once. After blowing dry, the sample was reacted with 2 mL of 0.2 M borate buffer (pH 9.2) and 0.4 mL of 0.2 M sodium borohydride solution at 4 ℃ for 8 h. Then 4 mL of 6 mol/L HCl was added for hydrolysis at 110 ℃ for 24 h. 1 mL of the hydrolysate was concentrated by nitrogen blowing to near dryness and then re-dissolved with 3 mL of ultrapure water. Then the filtrate was passed through an MCX solid phase extraction column, and the eluate was collected and concentrated by nitrogen blowing to near dryness and then dissolved in 2 mL of ultrapure water. Finally, the solution was filtered (0.22 μm nylon) and subjected to chromatographic separation.

The contents of CML, CEL and MG-H1 in shrimps were determined by ultra-performance liquid chromatography (UPLC) (Waters, Milford, USA) and Xevo TQ MS was used for multiple reactions monitoring. The chromatographic column was a BEH Amide (100 mm × 2.1 mm, 1.7 μm) with a column temperature of 35 ℃, the mobile phase A was a mixture of

0.25 mol/L ammonium acetate and 1% formic acid, and the mobile phase B was acetonitrile with a sample volume of 3 μL. The separation was performed using a linear gradient from A to B at a flow rate of 0.3 mL/min as follows: 0–1 min, 0–15% A, 1–6.5 min, 15–35% A, 6.5–9 min, 35% A, 9–10 min, 35–15% A. The ion source was electric ion spray, the mass spectrometer was in positive ion scan mode, the detection mode was MRM, the dryer temperature was 350 °C, the drying gas flow rate was 10 L/min, the nebulizer pressure was 20 psi, the capillary voltage was 4000 V, and the fragmentation voltage was 135 V. MRM specific ion pairs m/z 205 → m/z 84, m/z 219 → m/z 84 and m/z 229 → m/z 70 were used for the quantification of CML, CEL and MG-H1, respectively. The linear range of the calibration curve was 0.01–0.2 μg/mL, and the quantification of samples was achieved by measuring the peak area ratio compared with the external standard curve.

Data processing

All experiments were performed in triplicate. The results were presented as mean values and standard deviations (SD). The test data were processed with Origin 9.1 and analyzed by ANOVA and Duncan's test ($p < 0.05$) using SPSS 23 and expressed as "mean ± standard deviation".

Results and discussion

Changes in the quality of deep-fried shrimps

Chromaticity

Chromaticity is an important quality indicator in fried food products known to affect consumer acceptance and reflect the physical and chemical reactions during the frying process. These reactions include the various Maillard and caramelization reactions to generate the specific coloration in fried foods (Albert et al., 2009). Given this, it is unsurprising that an increase in the number of fry batches resulted in distinct changes in the brightness, L*, of fried shrimps (Table 2), with this value first increasing and then decreasing with prolonged frying time. L* value reflects the key parameter of frying quality and is mainly related to the non-enzymatic browning reaction (Dueik et al., 2010). During deep frying, the high temperature of the oil produces Maillard and caramelization reactions. The product will be dehydrated at high temperatures to produce the typical golden surface (Albert et al., 2009). Therefore, the L* value of shrimps increased gradually at the beginning of frying, reaching a maximum value at batch 6. However, as the heating time extended, the Maillard browning reaction, involving the caramelization of sugar and the oxidation of the samples also increased (Lili et al., 2021), resulting in the release of more brown pigment, which leads to a decrease in L* values (Zongshuai et al., 2019).

Table 2
Effect of deep-frying on the chromaticity of shrimp.

Frying batch	L*	a*	b*	ΔE*
Raw	44.66 ± 0.96^{f}	−1.09 ± 0.36^{c}	−1.02 ± 0.45^{c}	0
1	53.46 ± 2.07^{e}	10.05 ± 0.54^{a}	23.75 ± 2.67^{b}	28.62 ± 2.90^{c}
2	56.14 ± 0.92^{d}	11.05 ± 1.29^{a}	26.74 ± 1.62^{ab}	32.45 ± 1.80^{b}
3	58.10 ± 1.44^{cd}	11.01 ± 1.89^{a}	25.69 ± 4.36^{ab}	32.36 ± 4.44^{b}
4	61.75 ± 1.22^{b}	11.85 ± 2.00^{a}	27.60 ± 2.39^{a}	35.84 ± 2.53^{ab}
5	58.60 ± 0.64^{c}	10.66 ± 3.04^{a}	28.00 ± 4.37^{a}	34.43 ± 4.39^{ab}
6	65.00 ± 1.88^{a}	5.24 ± 1.27^{b}	29.13 ± 1.93^{a}	37.00 ± 1.82^{a}
7	61.73 ± 1.86^{b}	7.41 ± 1.52^{b}	28.37 ± 1.56^{a}	35.08 ± 2.44^{ab}
8	61.64 ± 2.07^{b}	5.31 ± 1.33^{b}	27.62 ± 0.91^{a}	33.95 ± 1.28^{ab}
9	61.16 ± 1.61^{b}	6.68 ± 1.29^{b}	26.39 ± 2.05^{ab}	33.01 ± 2.01^{b}

Note: L*, brightness, a*, red (+) / green (-), b*, yellow (+) / blue (-), ΔE*, color difference. Different lowercase letters indicated significant differences among different batches ($p < 0.05$).

In addition, batches 1 to 5 had significantly higher a *values than batches 6 to 9. The a* value indicates the degree of redness and greenness in samples, which depends on the content of fat-soluble pigment astaxanthin in shrimp (Koomyart et al., 2017). Deep-frying induces oxidation and rancidity of oil, increasing the overall free radical content of the sample and promoting the oxidation of astaxanthin, which causes shrimp to turn red (Xianfeng et al., 2015). The decrease in a* recorded for later batches may be the result of astaxanthin of shrimps migrated into the oil. The b* value is the yellow-blue value and its positive value represents the yellowness. The ΔE* values represents the overall color difference between the sample and the reference. b* and ΔE* values increase and then decrease and reach a maximum in the sixth batch. However, both of them showed non-significant changes, indicating that the heating time of oil had no significant effect on the b* and ΔE* values of fried shrimps.

Moisture and oil content

Moisture loss and oil absorption are the most important changes during deep frying, with Fig. 1(a) clearly demonstrating the changes in moisture associated with different frying batches. These data revealed that the moisture content of the outer and inner layers of the fried shrimps were significantly lower than that in the raw unfried shrimps, and that the outer layer was significantly less moist than the inner layer. This is because the oil temperature is much higher than the boiling point of water, so the water in the fried shrimp evaporates a lot and the moisture content is reduced (Liu et al., 2015). Meanwhile, the outer layer is directly in contact with the frying oil during the process, contributing to its higher temperature and faster water evaporation.

Fig. 1 (b) showed that the oil content of the fried shrimps were significantly higher than that in the unfried raw shrimps, and the outer layer was also significantly higher than the inner layers. This maybe a direct result of cooking, with the water evaporation leaving many holes in the surface layers of the foodstuff, allowing the frying oil to penetrate into the products (Oluka 2007; Liu et al., 2015).

There are two modes of mass transfer during frying: one in which moisture and soluble components escape from the interior of the food to the surface and the other in which moisture evaporates from the surface of the fried product, while oil is transferred to the products. The steam leaves voids that allow oil to penetrate, therefore, the amount of oil absorbed depends primarily on the moisture content of the fried raw material (Zhang et al., 2020). The moisture content of fried shrimps were significantly negatively correlated with the oil content (Table 3). The correlation coefficients of -0.972^{**} and -0.941^{**} for the outer and inner layers, respectively, indicating that the lower the moisture loss in the product during the frying process, the lower the oil absorption of the product (Oluka 2007). Based on the results, we can see that the moisture content of the outer layer was the lowest in the fifth batch, while the oil content of the outer layer was the highest. With the increase of frying batches, the moisture in the outer layer first decreased and then increased, and the moisture in the inner layer decreased first and then increased slightly to stabilized, but the overall change was not so much. At the same time, the oil content of the outer layer firstly increased and then decreased to stabilized, while the internal oil content did not change much. This is likely because high temperatures caused the shrimp shells to dehydrate and harden, reducing the gap forcing the internal moisture to move outwards, thus decreasing oil uptake and increasing viscosity, eventually reducing the heat transfer coefficient of the frying process (Yanan et al., 2018).

Malondialdehyde content

Malondialdehyde is characterized by the presence of two–three

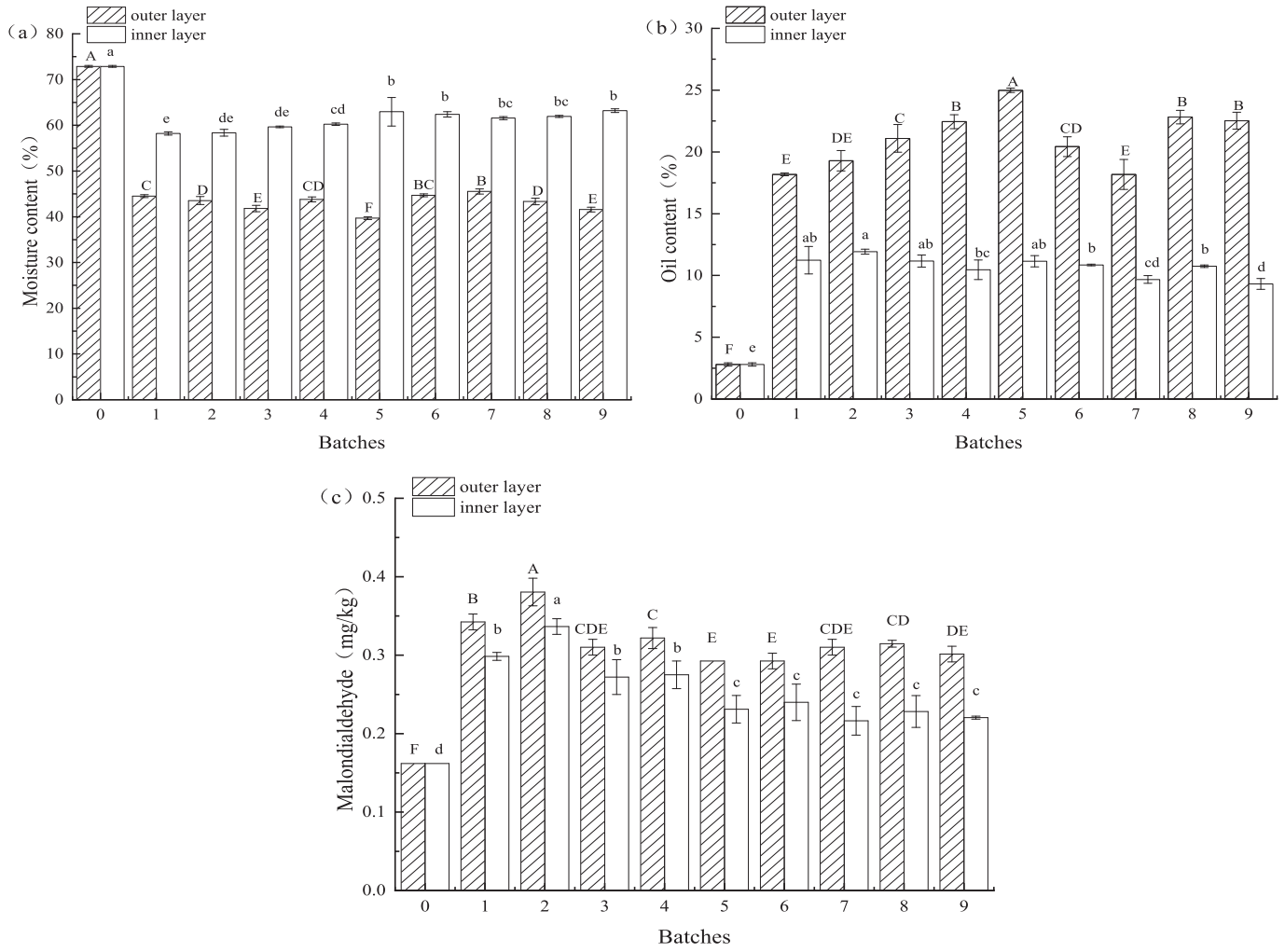

Fig. 1. Changes in the quality of deep-fried shrimps. (a) Moisture. (b) Oil content. (c) Malondialdehyde content.

carbon aldehyde groups and is primarily produced by the oxidative decomposition of fatty acids containing more than two unsaturated double bonds. This compound is an important end product of lipid oxidation decomposition, and its content reflects the degree of lipid oxidation (Koh and Surh, 2015). At high temperatures, lipids (especially fatty acids) are oxidized to primary lipid oxidation products, which are further oxidized to ROOH. These primary lipid oxidation products are subsequently broken down into secondary lipid oxidation products. Lipid oxidation products accumulated in frying oil are added to fried foods. Polar oxidation products (e.g., MDA) in frying oil are more readily absorbed into the surface and inner layers of fried foods (Koh and Surh, 2015).

As shown in Fig. 1(c), there was a significant increase in malondialdehyde content in both the outer and inner layers of the cooked shrimps when compared to that in raw starting material, while the oil content of *Penaeus vannamei* was very low and most of the oil was absorbed during cooking. Therefore, malondialdehyde content in shrimp is an indicator of absorbed oil content. In addition, we noted that an increase in the number of the frying batch induced an initial increase in malondialdehyde content, but this gradually decreased to stabilization over time. The results also showed that the malondialdehyde content of the outer layer of fried shrimps were higher. This may be related to the higher oil content and temperature of the outer layer leading to more serious lipid oxidation. Subsequently, the correlation coefficients of oil and malondialdehyde contents for the outer and inner layers of the fried shrimp were 0.689* and 0.687*, respectively (Table 3). This indicated that malondialdehyde content and oil were significantly correlated. In addition, we noted that the malondialdehyde content showed a decreasing trend with a continuous increase in frying batch, which may be due to the reaction between some malondialdehyde and proteins. Because malondialdehyde is known for its high reactivity, especially with protein side chains at high temperatures, which may result in protein cross-linking and oxidative modification (Adams et al., 2008). It was also found that the malondialdehyde content eventually stabilized, probably because it reached reaction equilibrium (Jiang et al., 2020).

Changes in the content of AGEs in deep-fried shrimps

Fluorescent AGEs

Some AGEs have characteristic fluorescence which allows for their easy quantification, with most Maillard reactions producing fluorescence at Ex = 340–370 nm and Em = 420–470 nm. Evaluation and quantification of the intensity of these signals is considered to be an important indicator of the level of protein modification by AGEs (Corsini et al., 2005; Matiacevich et al., 2005). Fig. 2(a) clearly showed that there was a significant accumulation of fluorescent AGEs in deep-fried shrimp. The contents of fluorescent AGEs in the outer layer were higher than those in the inner layer, and reached a maximum value of 1230.0 AU/g in the fifth batch. This was due to the direct contact of the outer layer with the frying oil. Also, the high processing temperature of 180 °C resulted in a more intense Maillard reaction and lipid oxidation

Table 3
Correlation analysis of moisture, malondialdehyde, oil and AGEs in fried shrimps.

		Moisture		Oil		Malondialdehyde		Fluorescent AGEs		CML		CEL		MG-H1	
		KO	KI	KO	KI	KO	KI	KO	KI	KO	KI	KO	KI	KO	KI
Moisture	KO	1													
	KI	0.860**	1												
Oil	KO	−0.972**	−0.752*	1											
	KI	−0.946**	−0.941**	0.886**	1										
Malondialdehyde	KO	−0.800**	−0.948**	0.698*	0.889**	1									
	KI	−0.447	−0.779**	0.332	0.687*	0.776**	1								
Fluorescent AGEs	KO	−0.978**	−0.873**	0.951**	0.920**	0.825**	0.475	1							
	KI	−0.806**	−0.955**	0.701*	0.893**	0.878**	0.817**	0.851**	1						
CML	KO	−0.826**	−0.972**	0.729*	0.910**	0.859**	0.774**	0.834**	0.951**	1					
	KI	−0.581	−0.791**	0.486	0.673*	0.613	0.730*	0.642*	0.882**	0.881**	1				
CEL	KO	−0.514	−0.794**	0.392	0.668*	0.712*	0.758*	0.516	0.749*	0.812**	0.684*	1			
	KI	0.409	0.057	−0.504	−0.225	−0.099	0.394	−0.367	0.061	0.012	0.228	0.439	1		
MG-H1	KO	-0.928**	−0.948**	00.870**	0.948**	0.902**	0.666*	0.929**	0.886**	0.928**	0.721*	0.692*	−0.170	1	
	KI	-0.831**	−0.947**	0.735*	0.914**	0.857**	0.804**	0.834**	0.946**	0.961**	0.839**	0.823**	0.127	0.940**	1

Note: ** indicates significant correlation at the 0.01 level (bilateral), *Indicates significant correlation at the 0.05 level (bilateral), KO and KI indicate the outer and inner layers, respectively.

compared to the inner layer. Thereafter, the substrates produced in the lipid oxidation and Maillard reaction promoted the formation of fluorescent AGEs (Chunping et al., 2019). We also noticed that the concentration of fluorescent AGEs firstly increased in the outer and inner layers of fried shrimps, and then decreased to the stabilization from batch 5. These trends were similar to that of malondialdehyde, with correlation coefficients shown in Table 3. This observation may be explained by the thought that the oxidation of oils provides the carbonyl donor required for the Maillard reaction (Hu et al., 2017), which promotes the formation of AGEs.

Non-fluorescent AGEs

Usually, frying was carried out at high temperatures. Repeated use of frying oil promoted the degradation of frying oil and greatly increased the carbonyl compounds in frying oil. These in turn facilitated lipid oxidation in meat product and generated large amounts of reactive oxygen radicals, which would further accelerate the Maillard reaction (a key pathway for the formation of AGEs) to produce di-carbonyl compounds, such as MGO and GO. Consequently, the di-carbonyl compounds could reacted with functional groups of lysine or arginine to produce different AGEs (Zongshuai et al., 2019). CML is widely used as a marker product for AGEs (Tavares et al., 2018), and its level is usually used to evaluate the level of total AGEs in food systems. CEL, a homolog of CML, is formed by the reaction of lysine residues in proteins with methylglyoxal, propyl phosphate, and other sugars (Ahmed et al., 1997; Nomi et al., 2016). Dicarbonyl methylglyoxal (MG) reacts with arginine residues to form late glycosylation end products, including MG-H1 (Ito et al., 2016). Thus, MG-H1 can also be used as an alternative for AGEs evaluation when the food is deficient in lysine and rich in arginine (Ahmed et al., 2002).

As shown in Fig. 2(b-d), the content of non-fluorescent AGEs (CML, MG-H1 and outer layer CEL) increased significantly after deep-frying, except for the inner layer CEL. Meanwhile the outer layer was higher than the inner layer. It was also observed that the outer layer CML reached a maximum value of 22.43 mg/kg at batch 1, which was 6.21 times higher than that of unfried raw shrimps. The inner layer CML reached a maximum value of 11.74 mg/kg at batch 3, which was 3.25 times higher than that of unfried raw shrimps. Meanwhile, the outer layer MG-H1 reached a maximum value of 341.63 mg/kg at batch 4, which was 11.7 times that of the unfried raw shrimps. The inner layer MG-H1 reached a maximum value of 169.42 mg/kg at batch 1, which was 5.99 times that of the unfried raw shrimps. The CEL content of the outer layer reached a maximum value of 304.24 mg/kg at batch 1, which was 4.01 times higher than that of the unfried raw shrimps. However, the internal CEL content in the cooked product appeared to be lower compared to the second batch of raw shrimp and increased with increasing batches of frying, but these differences were not significant. These changes could be related to the reduction of moisture in shrimps and the protection of the shell, subsequently retarding the lipid oxidation and the Maillard reaction.

The correlation coefficients of CML, CEL, MG-H1, and malondialdehyde were shown in Table 3, and all of them were significantly correlated with malondialdehyde except for the inner CEL. The formation of CML, CEL and MG-H1 was related with lysine and arginine. However, malondialdehyde produced through lipid oxidation could reacted with a large amount of lysine and protein residues in the system (Niu et al., 2019), resulting in a decrease in CML, MG-H1 and CEL content. It may also be due to the fact that the unsaturated oils are more likely to degrade and produce small molecules of acids under high temperature conditions, and that the acidic conditions slowed down the formation of AGEs (Baldensperger et al., 2018).

Base on previous studies in our laboratory, it was found that the AGEs content in fried shrimps prepared with high oleic sunflower oil was largely lower than that of palm oil (Zhao et al., 2022). This may be related to the quality of frying oil, since most of the oil in fried shrimps came from frying oil. Also, oils with high oleic acid content provided

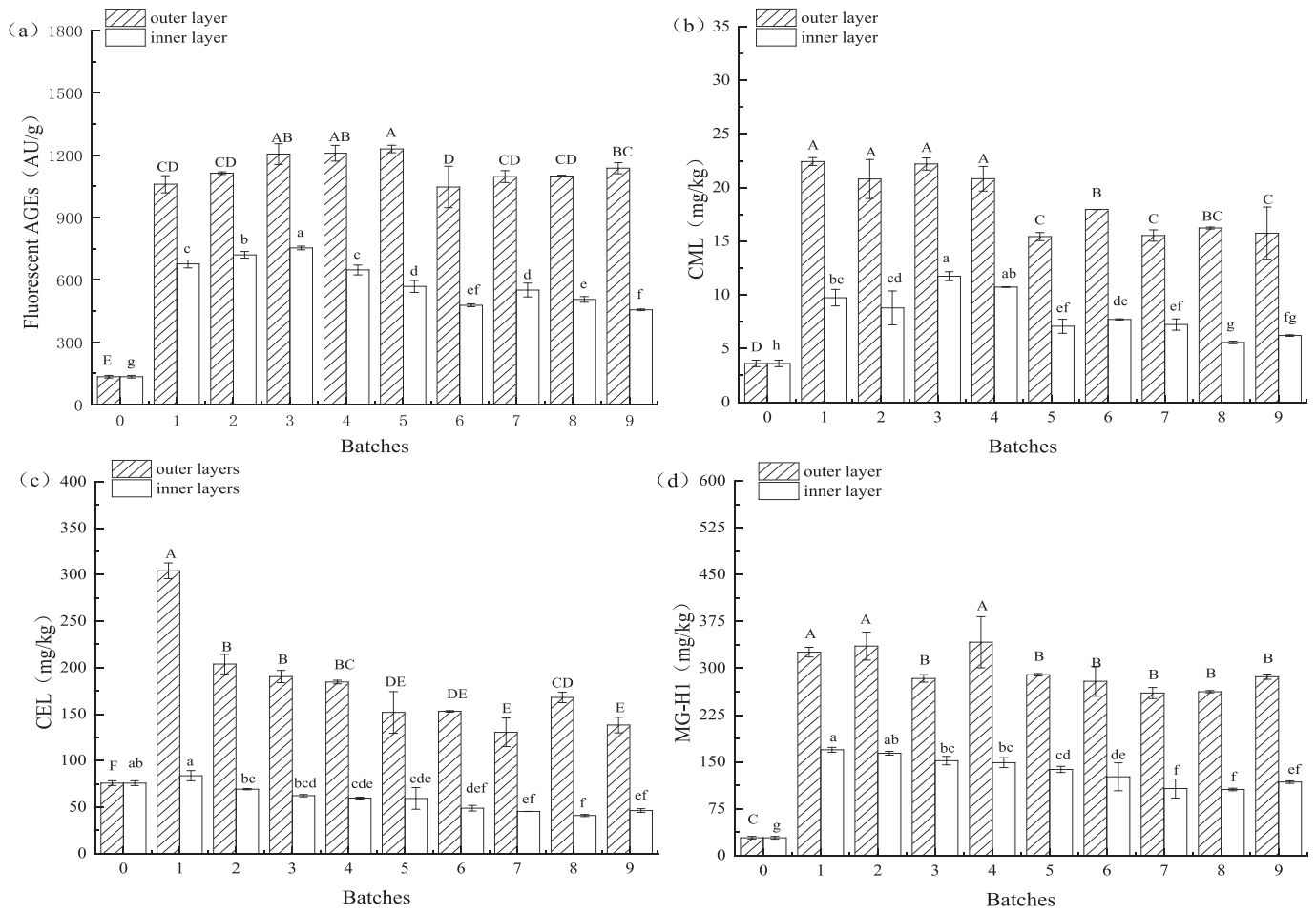

Fig. 2. Changes in the content of AGEs in deep-fried shrimps. (a) Content of fluorescent AGEs. (b) CML content. (c) CEL content. (d) MG-H1 content. Note: Different lowercase letters indicate significant differences between the internal samples of fried shrimps ($p < 0.05$); different capital letters indicate significant differences between the outer samples of fried shrimps ($p < 0.05$).

better frying performance and product sensory satisfaction compared to palm oil (Aladedunye and Przybylski, 2013). Additionally, high oleic sunflower oil used in this study contained 46.34 mg/100 g VE. VE is an endogenous antioxidant commonly used in frying oils to retard the oxidation of frying oils, thus slowing the oxidation of shrimps fried and inhibiting the increase of AGEs content.

Conclusions

In summary, this study investigated the basic physicochemical indexes and the production of AGEs in different frying batches of *Penaeus vannamei*. It was found that the AGEs in fried shrimps firstly increased with the addition of frying batches, and it was higher in the outer layer. Also, the content of AGEs in fried shrimp was greater compared to raw material, except for the inner CEL. Additionally, the AGEs content decreased to stabilization at the sixth batch. It is encouraged that the shell of shrimps were kept for reducing oil absorption and improving product quality during frying. The present study provided some data support for investigating the formation and control measures of AGEs during deep-frying procedure. Furthermore, the safety evaluation of the frying foods could be comprehensively explored through combining the physicochemical indexes of the products and the amount of AGEs generated during different frying times.

CRediT authorship contribution statement

Jiao Mo: Conceptualization, Methodology, Writing – original draft, Formal analysis. **Yuanyuan Zhao:** Investigation, Validation. **Runlin Wu:** Visualization. **Benlun Hu:** Data curation. **Caihua Jia:** Supervision, Resources, Writing – review & editing. **Jianhua Rong:** . **Ru Liu:** . **Siming Zhao:** Software.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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