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The inhibition effect and mechanism of typical hydrocolloids on the formation of heterocyclic amines: A study based on quantum chemical computation analysis

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ABSTRACT

Hydrocolloids, as thickeners, have been receiving increasing attention from researchers. Although they exhibit significant free radical quenching abilities, which demonstrate potential heterocyclic amines (HAs) inhibitory capabilities by blocking the free radical pathway, the inhibitory effect and mechanism are still unclear. This study investigated the effects of three typical hydrocolloids (alginic acid, chitosan, and carrageenan) on both free and bound HAs in fried meatballs, along with their mechanisms of free radical quenching using density functional theory. The result showed that all three hydrocolloids can effectively inhibit the generation of HAs. The maximum inhibition rate reached 33.33% for free HAs and 23.18% for bound HAs. Phenylacetaldehyde, glyoxal and methyl glyoxal, were significantly inhibited, indicating that hydrocolloids alleviated the production of HAs by inhibiting the generation of intermediates. At moment, three hydrocolloids effectively inhibited the generation of total free radicals. Frontier orbital and density functional theory analysis revealed that carrageenan had the lowest HOMO-LUMO energy gaps, ionization potential, highest nucleophilic index, chemical potential, and was more likely to react with free radicals. The results of this study indicate that three hydrocolloids can effectively inhibit HAs and provide theoretical support for their applications in food processing and safety.

1. Introduction

Heterocyclic amines (HAs), as one of the common pyrolysis products of foods with high protein content, are harmful polycyclic compounds to the human body (Barzegar et al., 2019). HAs have strong mutagenicity and are one of the important triggers of cancer in people's diet. As early as the 1990s, 2-amino-3-methyl-6-phenylimidazoline [4,5-f] - quinoline (IQ) was marked as Class 2 A, and 2-amino-3,4-dimethyl-imidazole [4, 5-f] - quinone (MeIQ), 2-amino-1-methyl-6-phenylimidazole [4,5-b] - pyridine (PhIP) was marked as Class 2 B carcinogen (Felton and Knize, 1990; Sugimura et al., 2004). High carcinogenic HAs are commonly present in daily life and seriously affects people's health. For example, HAs are found in high-temperature processed meat products and smoke, it enter the body through diet, drinking water and breathing. (Barzegar et al., 2019). Research has shown that as the intake of HAs increases, the production of DNA-HAs adducts becomes more significant, which will further enhance the expression of cancer in tissues and organs with metabolic functions such as the liver, colon, and prostate (Le Marchand, 2021; Nohmi and Watanabe, 2021). Moreover, research has indicated relationship between HAs and various types of cancer. Notably, a significant positive correlation was identified between the consumption of 2-amino-3,4-dimethylimidazo [4,5-f]quinoline (MeIQ) and the likelihood of developing colorectal adenomas. Additionally, a link was observed between the intake of overall HAs and the risk of prostate cancer. (Iwasaki and Tsugane, 2021).

Currently, studying the effective inhibition of the HAs is important for human health (Barzegar et al., 2019). The reduction of cooking temperature and the use of cooking methods that produce low HAs are widely recommended. Cooking methods such as baking and frying are not recommended because they produce more HAs. (ur Rahman et al., 2014). But the feasibility of lowering cooking temperature in practical life is relatively low. Consumers are more inclined to choose food that has been treated with high temperatures, as high temperatures give food better sensory and flavor quality (Wang et al., 2023). At the same time, the use of certain additives in the diet can also effectively reduce the production of Has. For example, some plant extracts and spice can inhibit the production of HAs, which achieve the goal of a healthy diet to a certain extent (Lee et al., 2020; Q. Wang et al., 2019). However,

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research also indicates that the stability of some plant extracts is poor during high-temperature processing. For example, curcumin shows HAs inhibitory capabilities, but it is unstable during the heating process and prone to degradation (Chen et al., 2014; Xue et al., 2022a,b). The efficiency of some plant extracts' in inhibiting HAs is low, and they can also have a certain adverse impact on the flavor and color of grilled meat products (Gibis and Weiss, 2012; Li et al., 2023).

Hydrocolloid received more and more attention from researchers in recent years (Zang et al., 2024). It is a natural thickener and can form gel under certain conditions. Therefore, it is often used to thicken the viscosity of solutions (Yemenicioğlu et al., 2020). With the further development of food safety and health, hydrocolloids have also been studied and applied in food safety, especially in inhibiting harmful chemicals in food (Zhang et al., 2021). Wang et al. has shown that hydrocolloids have been proven to have good inhibit effects for advanced glycation end products (AGEs) in chemicals model system and baked goods (S. Wang et al., 2022). Wang et al. also evaluated the inhibition of six hydrocolloids on the formation of AGEs in foods, results showed that carrageenan had the most significant effect (S. Wang et al., 2023). Xu et al. found that hydrocolloids including alginic acid, pectin, carboxymethyl cellulose sodium salt and chitosan can effectively inhibited the amount of N-(carboxymethyl) lysine (CML) and N-(carboxyethyl) lysine (CEL) in chemical model and fish patties (Xu et al., 2022). The carcinogenic acrylamide and 5-hydroxymethylfurfural produced in french fries was effectively inhibited by coating formed by hydrocolloid like chitosan (Huang et al., 2022; Z. Wang et al., 2023). Moreover, previous study also confirmed that hydrocolloids were effective inhibitors for PHIP formation in roast meat (Yang et al., 2021).

Numerous studies have shown that the production mechanism of HAs (such as IQ, MeIQ, and PhIP) is relatively clear (Dong et al., 2020; Zamora and Hidalgo, 2020). At present, two widely studied approaches are as follows: (1) Maillard reaction approach: Strecker degradation products, such as pyridine and pyrazine, can form precursors of HAs. (2) Free radical pathway: Alkylpyridine radical combines with Creatinine to form IQ and MeIQ, in which dialkylpyrazine radical is the precursor of MeIQx. Free radicals can also promote the production of active carbonyls, such as acetaldehyde, propionaldehyde, butyraldehyde, acetaldehyde and methylglyoxal, which are important intermediates of Has (Dong et al., 2020). Comprehensive analysis reveals that free radicals play a crucial role in the production of Has (Khan et al., 2022). If exogenous inhibitors can quench free radicals, theoretically they can effectively inhibit the production of HAs.

Hydrocolloids are widely used as thickeners in the food industry. It is worth noting that research has found that hydrocolloids have significant antioxidant and free radical quenching abilities. However, the ability of hydrocolloids to inhibit HAs in fried meatballs, and pathways based on free radical pathways have not been reported. This limits the further application of hydrocolloids in the field of food safety, and further exploration is needed. Based on the above analysis, this experiment aims to study the influence of three hydrocolloids: alginic acid, chitosan, and carrageenan on the content of free and bound HAs in fried meat balls. At the same time, the possible ways to reduce HAs were further explored, which involved the production of free radicals and the changes of intermediates. In addition, the mechanism of quenching free radicals by hydrocolloids was explored through frontline orbitals and conceptual density functional calculations. In this study, we systematically investigated the ability of hydrocolloids to inhibit HAs in fried meatballs and deeply probed the potential mechanism from the perspective of free radical quenching using electron paramagnetic resonance (EPR) and quantum chemical calculations, which provides theoretical support for the application of hydrocolloids in the field of food safety.

2. Materials and methods

2.1. Reagents and chemicals

Standards of PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-b]-pyridine; MeIQ, 2-amino-3,4-dimethyl-imidazo [4,5-f]-quinoline; IQx, 2-amino-3-methyl-imidazo [4,5-f]-quinoxaline; 4,8-DiMeIQx, 2-amino-3,4,8-trimethyl-imidazo [4,5-f]-quinoxaline; Harman, 1-methyl-9H-pyrido [3,4-b]indole, and other compounds of interest were obtained from Santa Cruz Biotechnology, Inc. (California, USA). The purity of the above standards is 98%. Methanol, acetonitrile, and formic acid, etc. were purchased from Thermo Fisher Scientific, Inc. (Massachusetts, USA). Oasis solid phase extraction column (3 cc/60 mg) were purchased from Waters, Inc. (Florida, USA). Hydrocolloids (analytical grade) were purchased from Aladdin Reagent Ltd (Shanghai, China). The meat was obtained from a local market (Wuxi, China).

2.2. Cooking methods of fried meatballs

Before thawing at 4 °C (24 h), pork was stored at −1 °C. Other materials were kept in a refrigerator at 4 °C before cooking. Ground pork was mixed with alginic acid, chitosan, or carrageenan (0.1%, 1.0%, 5.0%) respectively. Meatballs (weight 15 ± 0.1 g, diameter 9.0 ± 0.2 mm) were produced by hand. Each meatball (control group and group added with hydrocolloids) was fried in a deep fryer (180 °C) for 3 min. Remove the excess oil from the fried meatballs, cool them to room temperature, and then store them in vacuum packing at −18 °C.

2.3. Qualitative and quantitative extraction and determination of HAs

The determination of free HAs was based on literature methods (Y. Xu et al., 2022; Xue et al., 2022). First, 40 mL NaOH (3 M) was fully mixed with 2 g of fried meat samples; Next, 20 mL of ethyl acetate was added to the solution and sample was processed with ultrasound (40 kHz, 40 min). Then, the above solution was centrifuged (3000 g, 10 min) to obtain the supernatant. 20 mL of ethyl acetate was added to the centrifugal precipitate and the above steps were repeated to enhance the extraction process. The supernatant was concentrated to 20 mL. 100 μL of HCl (2 M) was added before solid-phase extraction (Quan et al., 2022).

Regarding the acquisition of bound state HAs: the precipitate after centrifugation mentioned above contains bound state HAs. precipitate was thoroughly mixed with an equal volume of hydrochloric acid (6 M). then sample was applied at 110 °C for 24 h. Finally, the mixed solution was filtered and diluted to 100 mL before solid-phase extraction (Quan et al., 2022).

Waters Acquisition UPLC system and triple quadrupole mass spectrometer (Waters, USA) werer used for analysis of HAs. ACQUITY UPLC BEH C18 inverted column (2.1 mm × 100 mm, 1.7 μ m) was choosed. The gradient elution process required the configuration of a binary mobile phase consisting of acetonitrile (A) and 0.1% formic acid (B). The procedure was as follows: 0–0.1 min 4% A; 0.1–12 min, 4%–20% A; 12–14 min, 20%–100% A; 14–17 min, 100%–4% A. Flow rate was 0.3 mL/min, injection volume was 5 μL.

Multiple reaction monitoring scanning settings was selected for the mass spectrometry detection process. The specific parameter settings were as follows: the capillary voltage was set to 3.5 kV; The ion source temperature is set to 120 °C; The specified temperature for solvent removal is 350 °C; The flow rate of the conical gas flow with nitrogen as input was set to 60 L/h; Flow rate of the desolvent gas (nitrogen) was 650 L/h. The data was collected using Waters MassLynx 4.1 software.

2.4. Identification of free radicals in fried meatballs

In order to determine the content of free radicals in the sample, 100 mg of fried meat ball samples from the control group and the group

Table 1
Mitigative capacity of hydrocolloids on generation free and bound heterocyclic amines in meat.

Free HAs (ng/g)	Control	0.1% Alginic acid	1.0% Alginic acid	5.0% Alginic acid	0.1% Chitosan	1.0% Chitosan	5.0% Chitosan	0.1% Carrageenan	1.0% Carrageenan	5.0% Carrageenan
MeIQx	1.00 ± 0.03^{a}	0.94 ± 0.01^{ab}	0.98 ± 0.05^{a}	0.86 ± 0.05 (14%)^{b}	0.94 ± 0.01^{ab}	0.98 ± 0.05^{a}	0.84 ± 0.03 (15%)^{b}	0.76 ± 0.02 (24%)^{c}	0.61 ± 0.03 (39%)^{d}	0.55 ± 0.04 (45%)^{d}
PhIP	1.66 ± 0.02^{a}	1.56 ± 0.07^{ab}	1.57 ± 0.02^{ab}	1.50 ± 0.10^{ab}	1.46 ± 0.09 (12.05%)^{b}	1.49 ± 0.03 (10.24%)^{b}	1.35 ± 0.02 (14.46%)^{bc}	1.26 ± 0.01 (24.1%)^{cd}	1.21 ± 0.00 (27.11%)^{de}	1.08 ± 0.00 (34.94%)^{e}
7,8-DiMeIQx	0.86 ± 0.03^{a}	0.82 ± 0.01^{ab}	0.69 ± 0.00 (19.77%)^{c}	0.62 ± 0.05 (27.91%)^{c}	0.85 ± 0.02^{ab}	0.80 ± 0.01^{ab}	0.66 ± 0.01 (10.47%)^{b}	0.82 ± 0.01^{ab}	0.61 ± 0.04 (29.07%)^{c}	0.52 ± 0.01 (39.53%)^{d}
Norharman	4.30 ± 0.05^{a}	4.27 ± 0.03^{a}	4.27 ± 0.06^{a}	3.76 ± 0.05 (12.56%)^{c}	4.13 ± 0.05 (3.95%)^{b}	3.72 ± 0.01 (13.49%)^{c}	3.41 ± 0.29 (14.19%)^{c}	4.24 ± 0.01^{ab}	3.71 ± 0.00 (13.72%)^{c}	3.07 ± 0.06 (28.6%)^{d}
Harman	1.56 ± 0.01^{a}	1.52 ± 0.01^{a}	1.48 ± 0.01^{ab}	1.35 ± 0.03 (13.46%)^{d}	1.53 ± 0.03^{a}	1.44 ± 0.02 (7.69%)^{bc}	1.27 ± 0.03 (12.18%)^{cd}	1.52 ± 0.01^{a}	1.3 ± 0.05 (16.67%)^{d}	1.08 ± 0.03 (30.77%)^{e}
AαC	0.13 ± 0.01^{a}	0.12 ± 0.00^{ab}	0.11 ± 0.01 (15.38%)^{b}	0.11 ± 0.01 (15.38%)^{b}	0.13 ± 0.01^{a}	0.11 ± 0.01 (15.38%)^{b}	0.06 ± 0.01 (15.38%)^{b}	0.12 ± 0.01^{ab}	0.11 ± 0.00^{ab}	0.04 ± 0.01 (69.23%)^{c}
Total	9.51	9.23	9.10	8.20 (13.77%)	9.04	8.54 (10.2%)	8.21 (13.67%)	8.72 (8.31%)	7.55 (20.61%)	6.34 (33.33%)
Bound HAs (ng/g)										
PhIP	6.22 ± 0.37^{a}	6.27 ± 0.10^{a}	6.36 ± 0.10^{a}	4.85 ± 0.12 (22.03%)^{cd}	6.11 ± 0.26^{ab}	5.43 ± 0.74^{abc}	5.13 ± 0.44 (17.52%)^{bcd}	6.31 ± 0.14^{a}	5.55 ± 0.21^{abc}	4.34 ± 0.07 (30.23%)^{d}
7,8-DiMeIQx	5.11 ± 0.24^{a}	4.99 ± 0.23^{a}	3.89 ± 0.18 (23.87%)^{bc}	3.60 ± 0.12 (29.55%)^{bc}	4.99 ± 0.23^{a}	4.35 ± 0.29^{ab}	3.60 ± 0.12 (29.55%)^{bc}	5.05 ± 0.41^{a}	3.25 ± 0.23 (36.4%)^{c}	2.03 ± 0.29 (60.27%)^{d}
Norharmane	328.57 ± 6.26^{a}	328.75 ± 3.34^{a}	329.26 ± 1.26^{a}	307.05 ± 6.10 (6.55%)^{b}	328.98 ± 2.46^{a}	325.92 ± 8.26^{a}	299.84 ± 5.57 (8.74%)^{bc}	328.82 ± 0.95^{a}	287.75 ± 5.41 (12.42%)^{c}	251.60 ± 7.08 (23.43%)^{d}
Harmane	443.93 ± 10.12^{a}	440.59 ± 1.07^{a}	389.66 ± 1.51 (12.22%)^{b}	382.16 ± 4.18 (13.91%)^{b}	446.67 ± 5.71^{a}	393.95 ± 5.80 (11.26%)^{b}	375.37 ± 2.20 (15.44%)^{b}	442.44 ± 9.70^{a}	381.66 ± 8.72 (14.03%)^{b}	342.58 ± 5.29 (22.83%)^{c}
1,5,6-TMIP	4.30 ± 0.12^{ab}	4.34 ± 0.02^{a}	4.02 ± 0.12 (6.51%)^{bcd}	3.57 ± 0.14 (16.98%)^{e}	4.06 ± 0.16^{abc}	3.74 ± 0.02 (13.02%)^{de}	3.74 ± 0.02 (13.02%)^{de}	4.27 ± 0.05^{ab}	3.81 ± 0.10 (11.4%)^{ced}	3.64 ± 0.07 (15.35%)^{e}
MeAaC	39.35 ± 0.51^{ab}	39.54 ± 0.47^{ab}	35.20 ± 0.44 (10.55%)^{c}	32.90 ± 0.28 (16.39%)^{d}	40.40 ± 0.40^{a}	39.68 ± 0.47^{a}	38.36 ± 0.45 (2.52%)^{b}	39.52 ± 0.40^{ab}	38.40 ± 0.16 (2.41%)^{b}	32.97 ± 0.21 (16.21%)^{d}
MeIQx	2.07 ± 0.12^{abc}	2.16 ± 0.12^{ab}	2.37 ± 0.05^{a}	2.09 ± 0.14 (-0.97%)^{abc}	2.18 ± 0.09^{ab}	1.93 ± 0.03 (6.76%)^{bc}	1.81 ± 0.19 (12.56%)^{cd}	1.95 ± 0.09 (5.8%)^{bc}	1.58 ± 0.09 (23.67%)^{de}	1.46 ± 0.07 (29.47%)^{e}
AaC	1.77 ± 0.10^{a}	1.70 ± 0.03^{a}	0.84 ± 0.61 (52.54%)^{b}	\	1.69 ± 0.07^{a}	1.30 ± 0.14^{ab}	\	1.63 ± 0.04^{a}	\	\
Total	831.32	828.34	771.60 (7.18%)	736.22 (11.44%)	835.08	776.30 (6.62%)	727.85 (12.45%)	829.99	722 (13.15%)	638.62 (23.18%)

[A] Data was expressed as mean ± standard deviation, n = 3.
[B] Different letters in the same line indicated significant differences ($p < 0.05$).

added with alginic acid, chitosan and carrageenan were obtained and added to NMR tube. Method for EPR was as follow: central field, 3360 G; Scanning width, 100 G; Microwave power, 20 mW; Modulation amplitude, 1.0 G; Scanning time, 60 s (Xue et al., 2022).

2.5. *Density functional studies of hydrocolloids*

In current study, hydrocolloids were studied theoretically by DFT approach (Xue et al., 2022). All quantum chemical calculations were done by DFT approach with ORCA 5 program. B3LYP/def2-TZVP basis was used for all computations such as geometric structure optimization and electronic structure calculation. On this basis, the molecular surface electrostatic potential, HOMO-LUMO energy, and conceptual density functional parameters are obtained. Images were analyzed and drawn with VMD and Multiwfn 3.6 software.

2.6. *Identification of intermediates of HAs*

The content of phenylacetaldehyde in fried meat balls was determined by literature methods (Y. Xu et al., 2022; Xue et al., 2022). 30 mL ethyl acetate was used as the extractant, mixed with the fried meat ball sample, and were ultrasonic treated for 30 min. After waiting for clarification and precipitation, the ethyl acetate phase of the target layer was collected. Repeated the above step three times. Next, diluted the ethyl acetate to a volume of 25 mL. PEG-20 M column was selected for GC analysis. The column flow rate was 3 mL/min, the hydrogen flow rate was 46 mL/min, the split ratio is 3:1, and the injection volume is 0.8 μL.

The determination for GO and MGO was as follows: 25 mL of water was added to 5 g of sample; After ultrasonic treatment for 30 min, sample was centrifuged at a speed of 5000 g for 10 min 400 μL supernatant was mixed thoroughly with 200 μL 5 mM O-Phenylenediamine and stored at 4 °C for 12 h. Then, the acquisition UPLC TQD coupled triple quadrupole mass spectrometry was used for analysis. Instrument parameters can be found in literature.

2.7. *Methodology validation*

In the experiment, 17 HAs standard samples were dissolved with methanol to obtain the experimental standard solution. When evaluating the linear range and R^2, the experiment diluted the standard solution to 14 different concentrations. The limit of detection (LOD) was the concentration of standard solution corresponding to SNR≥3, and the limit of quantification (LOQ) is the concentration of standard solution corresponding to SNR≥10. Standard solution was added to control group sample, next, the experiment repeated the preprocessing methods shown in sections 2.3 and 2.4 in order to obtain data and further calculate the recovery rate and precision. The recovery rate was calculated by [(HAs content in spiked sample – HAs content in control sample)/theoretical spiked quantity] × 100%. The precision was calculated by the [(standard deviation/mean) × 100%].

2.8. *Statistical analysis*

The experiments and measurements were repeated for three times, and the data presentation method for the research results was the mean ± standard deviation. The specific chromatographic analysis of HAs of

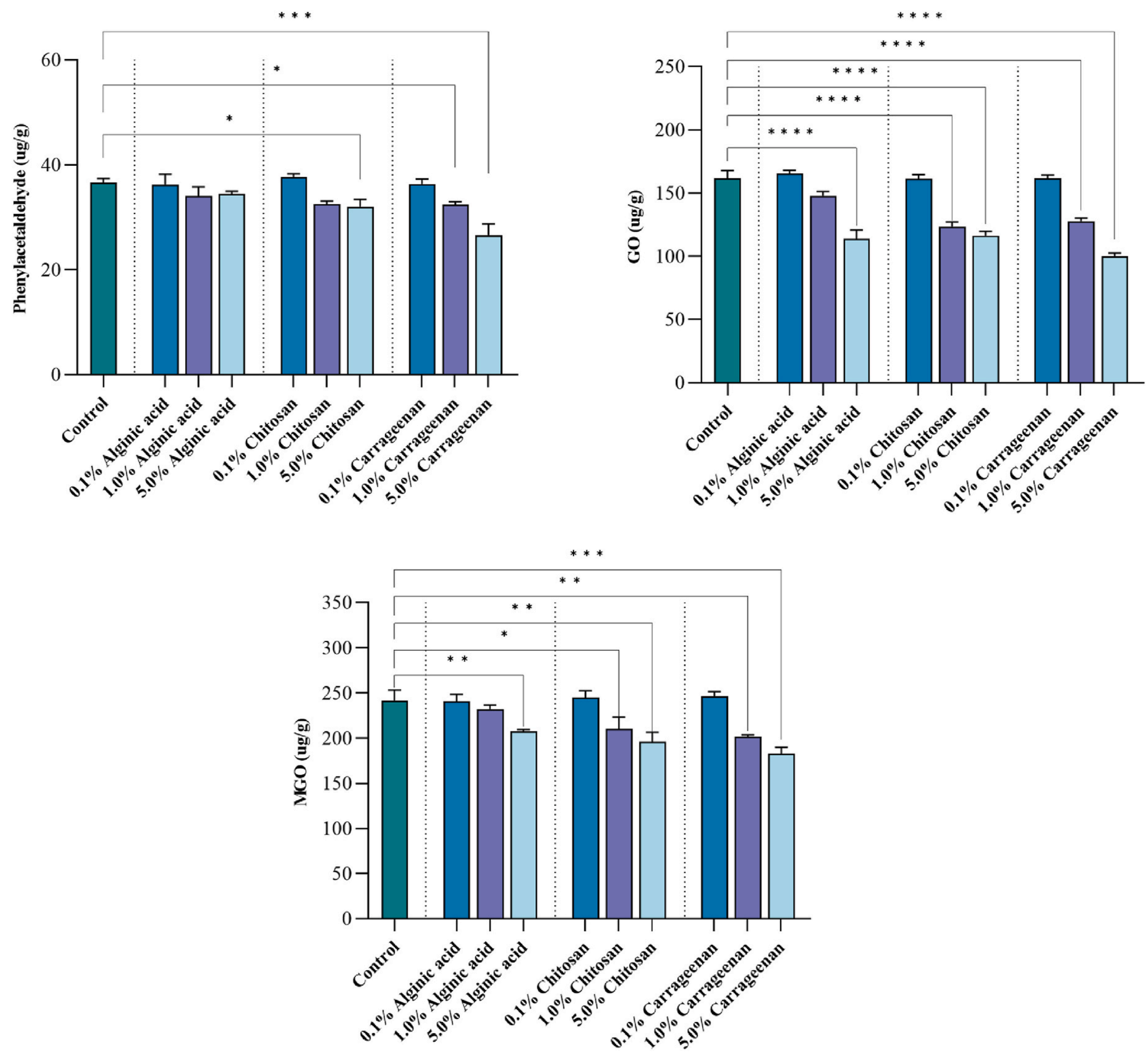

Fig. 1. Mitigative capacity of hydrocolloids on intermediate (phenylacetaldehyde, GO, and MGO) of heterocyclic amines. Asterisk in the histogram indicated significant differences compared with others ($p < 0.05$).

the target sample was carried out using MassLynx v4.1 software. The differences between samples were detected through the least significant difference test, with a level of $p = 0.05$. Meanwhile, the statistical software Statistix 9.0 further interpreted the differences between the above results. The relevant charts in this study were drawn using GraphPad 8.0 software.

3. Results and discussion

3.1. Methodological study for determination of HAs

The UPLC-MS/MS chromatogram of the standard substance (including 17 types of HAs) is shown in Supplemental Fig. 1. The chromatographic peak of the target separated successfully. As shown in Supplemental Table 1, the recovery rate of HAs (free and bound HAs) was between 70% and 110%, and the RSD value was less than 18%. R^2 was higher than 0.99. This detection method had high recovery rate and high precision. The LOD was 0.01–1.62 ng/mL, and the LOQ was 0.06–5.37 ng/mL. This method had high reliability and was consistent with existing studies. After preliminary verification, this method can effectively determine the types and quantities of HAs.

3.2. Inhibition of free and bound HA in fried meatballs by alginic acid, chitosan and carrageenan

As shown in Table 1, the total free HAs concentration is 9.51 ng/g, the three most abundant HAs were norharmane, harmane, and PhIP, with values of 4.30 ± 0.05 ng/g, 1.56 ± 0.01 ng/g, and 1.66 ± 0.02 ng/g, respectively, similar to the results of Chen et al. and Xue et al. (Chen et al., 2017; Xue et al., 2020). Alginic acid, chitosan and carrageenan had obvious inhibitory effects on the production of HAs at different concentrations. The inhibitory effect of 5.0% alginic acid on total free HAs was 13.77%, the inhibitory rates of 1.0% and 5.0% chitosan on total free HAs were 10.2% and 13.67%, respectively. The inhibitory effects of 0.1%, 1.0% and 5.0% carrageenan on total free HAs were 8.31%, 20.61% and 33.33%, respectively.

Table 1 shows the effect of alginic acid, chitosan, and carrageenan on the formation of bound HAs in fried meat ball. The total bound HAs content is 831.32 ng/g. The highest concentrations of norharmane and harmane were found (443.93 ± 10.12 ng/g and 328.57 ± 6.26 ng/g, respectively), which is similar to previous studies. When adding 1.0% and 5.0% alginic acid, the total bound HAs content was inhibited by 7.18% and 11.44%, respectively. When 1.0% and 5.0% chitosan were added, the inhibition rates of total bound HAs were 6.62% and 12.45%, respectively. When 1.0% and 5.0% carrageenan were added, the

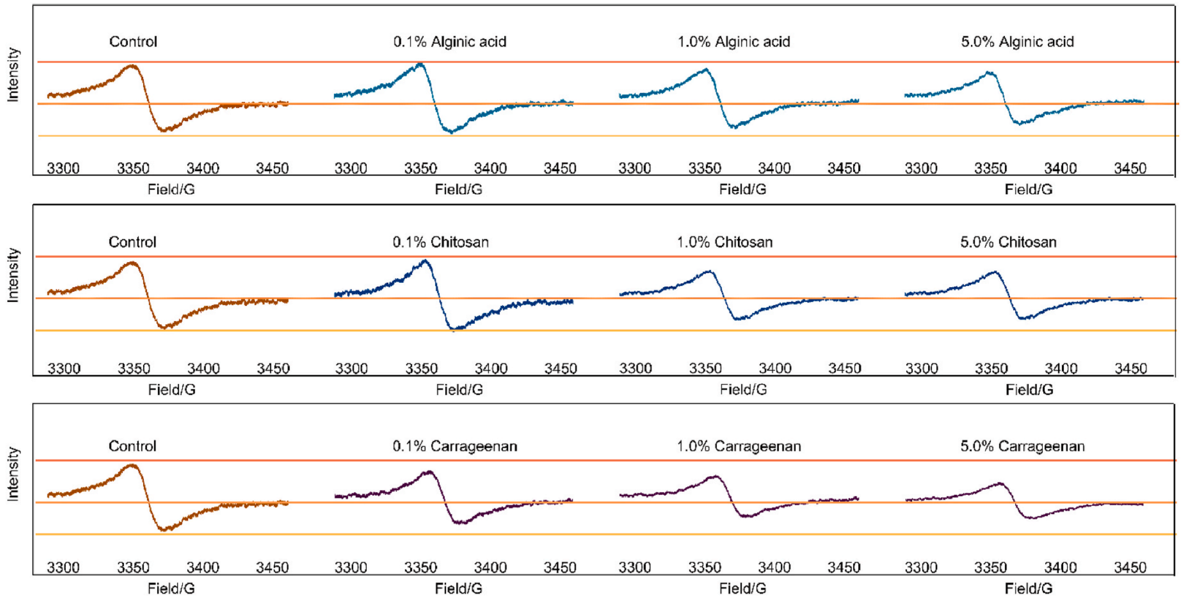

Fig. 2. Mitigative capacity of alginic acid, chitosan, and carrageenan on free radicals measured by electron paramagnetic resonance in meat.

inhibition rates of total bound HAs were 13.15% and 23.18% respectively. Alginic acid, chitosan and carrageenan can significantly inhibit the two most abundant HAs (harmane and norharmane), thus showing the ability to reduce total HAs. The inhibitory effects of 5.0% alginic acid on norharman and harmane were 13.91% and 6.55%, respectively. The inhibitory effects of 5.0% chitosan on norharman and harmane were 15.44% and 8.47%, respectively. The inhibitory effects of 5.0% carrageenan on norharman and harmane were 22.83% and 23.43%, respectively. Alginic acid, chitosan and carrageenan also inhibited pyridine, quinoline and quinoxaline.

3.3. Inhibit effects of alginic acid, chitosan, and carrageenan on the intermediates generation of HAs in fried meatballs

PhIP can be produced by heating phenylalanine or other amino acids and creatinine; The phenylalanine pathway is the most explored pathway (Zamora et al., 2014). Researchers found that phenylalanine was degraded to form phenylacetaldehyde in Strecker reaction, phenylacetaldehyde was condensed and dehydrated with creatinine, and then reacted with another molecule phenylalanine and creatinine, finally forming PhIP (Zamora et al., 2013; Zamora et al., 2014). Therefore, the content of phenylacetaldehyde in meat and the effects of alginic acid, chitosan, and carrageenan on phenylacetaldehyde were determined. Fig. 1 shows the effect of alginic acid, chitosan, and carrageenan on the content of phenylacetaldehyde in fried meatball. The results showed that the content of phenylacetaldehyde in fried meat ball added with alginic acid, chitosan and carrageenan (control group 36.59 ± 0.60 μg/g) decreased to 34.08 ± 1.22, 32.03 ± 0.96 and 26.57 ± 1.52 μg/g, respectively.

Active aldehydes were considered by researchers to significantly promote the production of hazardous substances, like Has (Dong et al., 2020; Kanzler and Haase, 2019). We further measured the content of GO and MGO of groups added with alginic acid, chitosan, and carrageenan, as shown in Fig. 1. Alginate, chitosan, and carrageenan can interfere with the generation of GO and MGO significantly. The GO content in the control group was 161.72 ± 4.24 μg/g. It decreased to 147.79 ± 2.42 μg/g and 113.87 ± 4.85 μg/g, respectively after the 1.0% and 5.0% alginic acid were added. Similarly, GO was also inhibited in the 1.0% and 5.0% chitosan groups, with values of 123.56 ± 2.42 μg/g and 116.29 ± 2.42 μg/g, respectively. The GO content in 1.0% and 5.0% carrageenan groups were also decreased, with values of 127.8 ± 1.82 μg/g and 99.94 ± 1.82 μg/g, respectively. MGO content was also significantly affected by alginic acid, chitosan, and carrageenan. The MGO content in the control group was 241.73 ± 8.22 μg/g. The MGO amount of fried meatballs in 5.0% alginic acid group was 207.67 ± 1.41 μg/g. The MGO amount of fried meatballs in 1.0% and 5.0% chitosan groups was 210.25 ± 9.16 μg/g and 196.16 ± 7.28 μg/g. The MGO amount of fried meatballs added with 1.0% and 5.0% carrageenan groups was 201.79 ± 1.17 μg/g and 182.77 ± 5.17 μg/g.

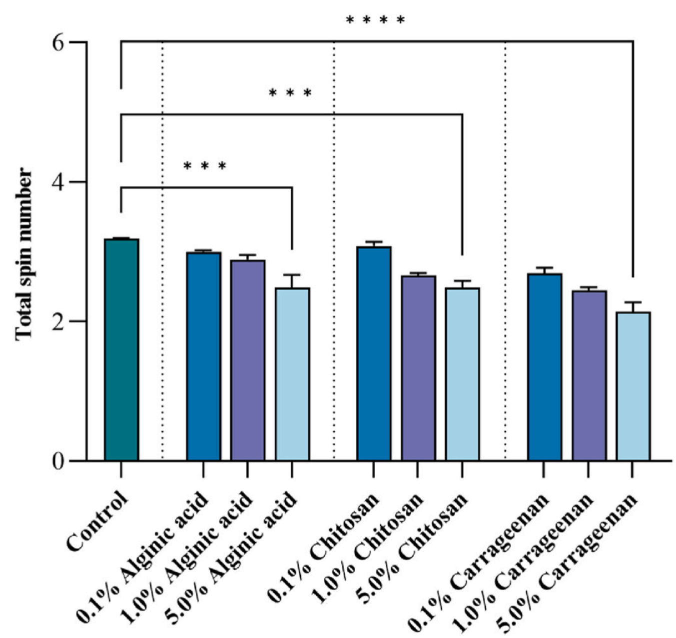

Fig. 3. Mitigative capacity of alginic acid, chitosan, and carrageenan on total spin number in meat. Asterisk in the histogram indicated significant differences compared with others ($p < 0.05$).

As intermediates of Maillard reaction, GO and MGO had high reactivity, and their formation was closely related to free radicals (Lund and Ray, 2017). It had been pointed out that in the Maillard reaction model system, OH• can catalyze the generation of GO and MGO. Previous studies had mentioned that hydrogen peroxide promotes GO production

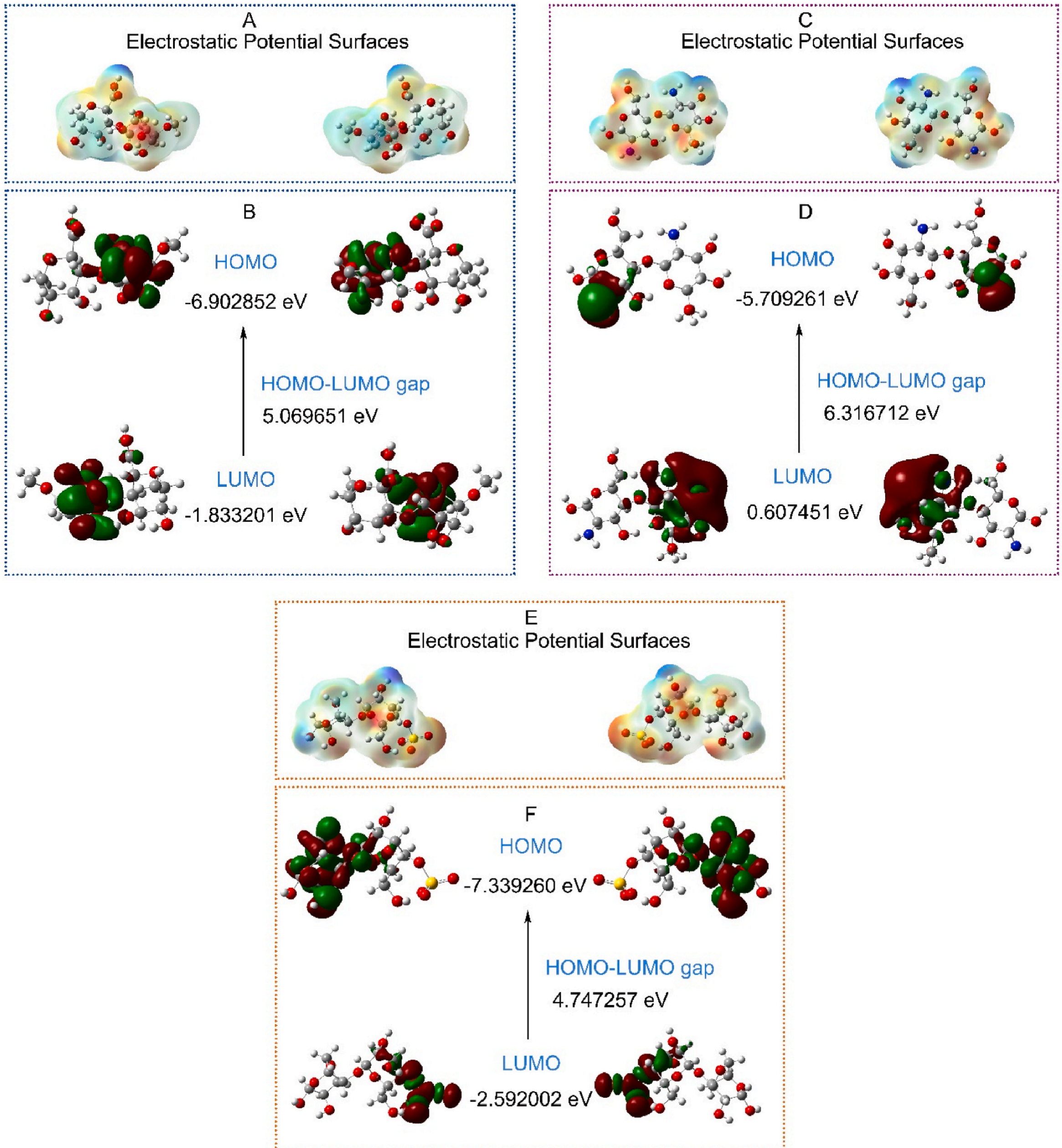

Fig. 4. Molecular electrostatic potential and frontier molecular orbital analysis of alginic acid, chitosan, and carrageenan.

through the hydroxyl radical mediated pathway (Thornalley, Langborg and Minhas, 1999). Thus, we further speculate that alginic acid, chitosan, and carrageenan may inhibit the content of GO and MGO by inhibiting free radicals.

3.4. *Capacity of alginic acid, chitosan, and carrageenan to mitigate formation of free radicals in fried meatballs*

The EPR spectrum of free radicals in fried meatballs added with alginic acid, chitosan, and carrageenan in this experiment is shown in Fig. 2. Compared with the control group, the amplitude of the detection spectrogram corresponding to the fried meatballs added with alginic acid, chitosan, and carrageenan showed a decreasing trend, which was most obvious in the samples added with 5.0% alginic acid, 5.0% chitosan, and 5.0% carrageenan. The total spin number of the experimental group of fried meatballs added with alginic acid, chitosan, and carrageenan is shown in Fig. 3. The total spin number showed a negative correlation trend with the increase of the concentration of alginate, chitosan, and carrageenan. Compared with a spin number of $3.19 \pm 0.01 \times 10^{14}$ in the control group, the spin numbers in the 5% alginic acid, chitosan, and carrageenan group were only $2.48 \pm 0.13 \times 10^{14}$, $2.14 \pm 0.1 \times 10^{14}$, and $2.48 \pm 0.07 \times 10^{14}$, respectively. This phenomenon is consistent with the phenomenon in the EPR spectrum, suggesting that the addition of hydrocolloids can significantly inhibit the production of free radicals. Previous studies have also found that antioxidant substances of plant origin, such as curcumin, significantly inhibited HAs and also significantly reduced the total spin number in meat (Xue et al., 2022a,b).

3.5. *Density functional study of hydrocolloids*

The MEP of the three colloidal molecules at the B3LYP/Def2-TZVP level is shown in Fig. 4. Alginate contains negative electrostatic potential, distributed around the oxygen atom. Chitosan contains some

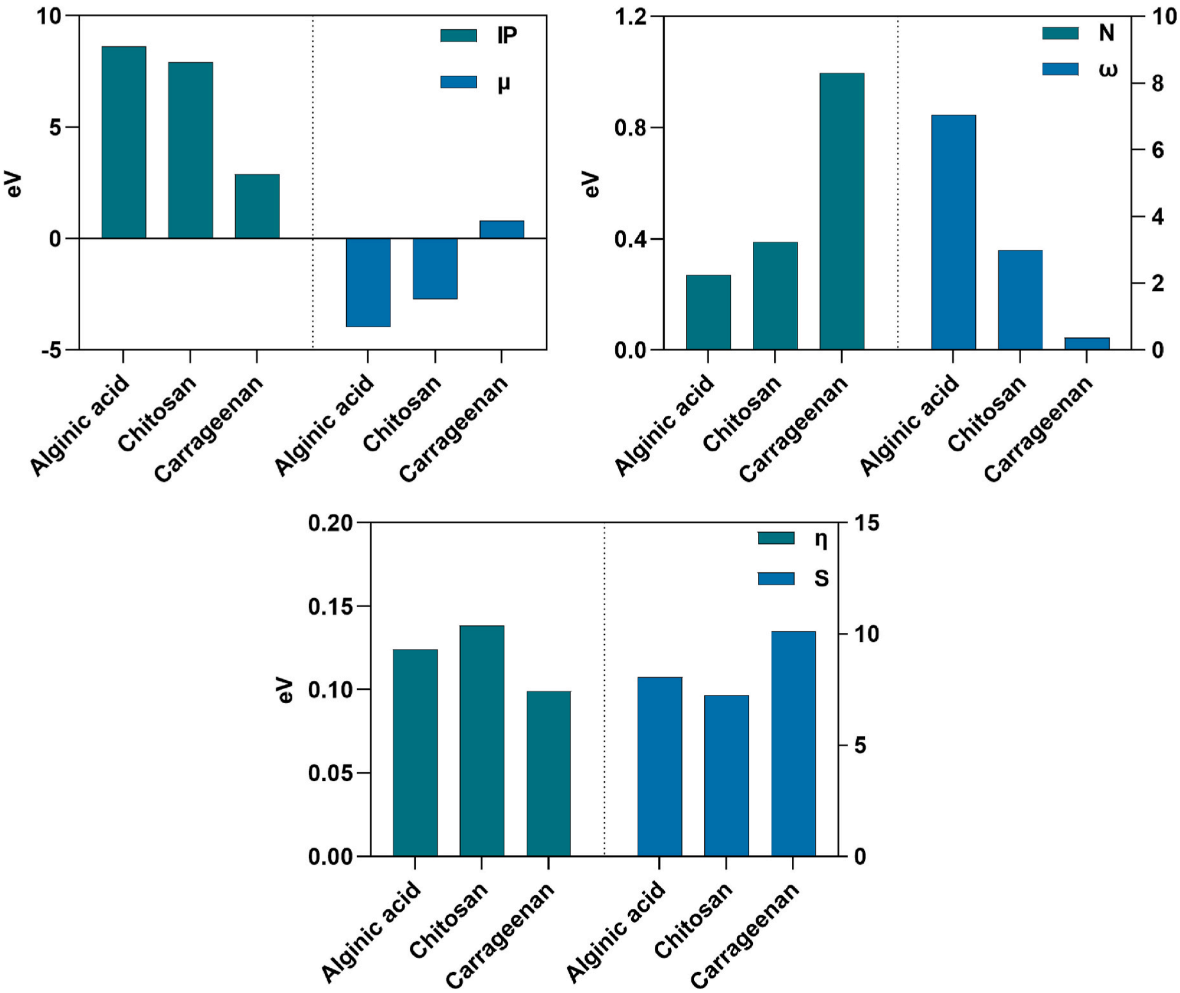

Fig. 5. Conceptual density functional analysis of alginic acid, chitosan, and carrageenan. IP, ionization potential; μ, chemical potential; N, nucleophilic index; ω, electrophilic index; η, hardness; S, softness.

negative electrostatic potentials, distributed around the oxygen atom and nitrogen atoms, and the rest positive. Carrageenan contains more negative electrostatic potentials, distributed around the Sulfur atom and oxygen atom, while the rest has a positive electrostatic potential. Having different electrical sites leads to differences in the physicochemical properties of the molecules.

The HOMO, LUMO and HOMO-LUMO energy gaps of the three hydrocolloids are shown in Fig. 5. The HOMO-LUMO energy gaps of the three hydrocolloids are 5.069651, 6.316712 and 4.747257 eV. Thus, the possible reactivity order is carrageenan > chitosan > alginate. This is consistent with the ability of the three molecules to inhibit heterocyclic amines and free radicals. In a study by Veselinović et al. it was found that 7,8-Dihydroxy-4-phenyl coumarin has the highest antioxidant potential as it has the lowest HOMO-LUMO energy gaps value, indicating that the substance has the lowest energy barrier required to quench free radicals (Veselinović et al., 2014).

Conceptual density functional theory, which is applicable to the quantum mechanical analysis of multi-electron structures, is an important branch of DFT, giving a relatively accurate description of chemical concepts that were previously known but relatively vague (D. Zhang et al., 2011). The global reactivity indices measured in this study include: ionization potential (IP), electron affinity potential (EA), chemical potential (μ), electronegativity (χ), hardness (η), softness (S), electrophilicity index (ω), and nucleophilic index (Farrokhnia, 2020).

The ionization potentials of alginate, chitosan and carrageenan were 8.6195, 7.9133 and 2.8979 eV respectively, with carrageenan having the lowest ionization potential, suggesting that the easier the outermost electrons of carrageenan are to detach, the higher the chemical reactivity and therefore the easier the reaction is to occur. Farrokhnia et al. showed that antioxidants with lower IP are more easily ionized and the rate of electron transfer between free radicals and antioxidants is easier. In his study it was found that substance number 3 (meroterpenoid isolated from brown alga of the genus Sargassum) had the lowest IP and the strongest antioxidant capacity (Farrokhnia, 2020).

The hardness of the three colloidal molecules is similar and is positive, indicating a high resistance to the electron cloud distortion of the chemical system under micro-interference. The softness reflects the distribution of electron clouds around the molecule and reflects the reactivity of the compound, with chitosan having the lowest softness value and carrageenan having the highest softness value, indicating that carrageenan is the most active. In the study of Farrokhnia et al. it was found that the order of softness in a series of molecules was $1 > 2 > 3 > 4$, suggesting that molecule 1 is more favorable for reaction during quenching of free radicals (Farrokhnia, 2020).

A positive chemical potential means that the molecule has high-energy bonds or functional groups that are more reactive and therefore more likely to react with other substances, especially free radicals. Conversely, if a molecule has a negative chemical potential, it means that the molecule is capable of low energy within the molecule, has more stable chemical bonds and needs to absorb energy in order to react with other substances, in contact with electron-deficient substances such as free radicals, these molecules are usually more difficult to react with free radicals. Of the three colloidal molecules, only carrageenan has a positive chemical potential, indicating that carrageenan has the optimum reactivity. Carrageenan has the lowest electrophilic index ω and the highest nucleophilic index, indicating that the easier the reaction between carrageenan and some free radicals (containing electron-deficient parts).

4. Conclusion

In this study, various dose (0.1%, 1.0%, 5.0%) of alginic acid, chitosan, and carrageenan were used as health and safety additives in the process of fried meatballs, to inhibit the production of free and bound HAs. Compared with the control group, the experimental group added 5.0% carrageenan showed the most obvious inhibition of free HAs (inhibition rate 33.33%) and bound HAs (inhibition rate 23.18%). Furthermore, this study found that phenylacetaldehyde, glyoxal and methyl glyoxal, three pivotal aldehydes during heating process, were significantly inhibited, showing that hydrocolloids reduced the generation of total HAs by alleviate intermediates. Total free radicals were detected, and found to be inhibited effectively after addition of hydrocolloids, which means that hydrocolloids can reduce the total HAs by inhibit generation of free radicals and aldehydes intermediate. Frontier orbital and concept density functional theory analysis revealed that carrageenan had the lowest HOMO-LUMO energy gaps, ionization potential, highest nucleophilic index, chemical potential, and possess the highest reactivity with free radicals. Food hydrocolloids demonstrate efficient inhibition of HAs and further structure-activity relationships need to be explored for screening or synthesis of more potent hydrocolloids molecules. Compounding of hydrocolloids based on the different inhibition mechanism of various substances to develop efficient and economical formulations is worth investigating. Development of combinatorial strategies for simultaneous inhibition of multiple hazardous products needs to be further explored.

CRediT authorship contribution statement

Tiantian Huang: Investigation, Writing – original draft, Formal analysis, Funding acquisition, Methodology. **Pin Jiang:** Validation, Writing & reviewing draft. **Tao Li:** Validation, Writing & reviewing draft. **Guangyu Li:** Data curation, Software. **Yuyu He:** Validation, Software. **Yuezheng Kuang:** Project administration, Supervision. **Yijie Wang:** Project administration, Supervision.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100862.

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