The conformational structural change of β-lactoglobulin via acrolein treatment reduced the allergenicity

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A B S T R A C T

β-lactoglobulin (BLG) is a major allergen of milk. Since lipid peroxidation such as acrolein commonly exists during milk processing, it is necessary to evaluate its influence on BLG structure and potential allergenicity. The structure of acrolein-treated BLG was detected using SDS-PAGE, fluorescence, ultraviolet spectrum (UV), circular dichroism (CD) and LC-MS-MS, and the potential allergenicity was assessed by in vitro and in vivo assays. Results showed that acrolein could cause structural changes by BLG aggregation, which decreased the IgE binding capacity. Further, the release of mediators and cytokines decreased with acrolein treatment in RBL-2H3 cells. Mice showed lower allergenicity by the levels of BLG-specific antibody and the release of histamine and mMCP-1. These results explained that acrolein-induced BLG aggregation could damage the allergic epitopes and decrease the allergenicity of BLG in milk. The study will provide a new aspect to explore the natural phenomenon of allergen changes during food processing.

1. Introduction

Food allergy is an important food safety issue that affects the health of about 8% children and 5% adults (Sicherer & Sampson, 2014). Food allergy causes different allergic symptoms such as skin, respiratory tract and gastrointestinal tract, and in severe case, causes systemic anaphylactic reactions (Takac’s et al., 2014). Milk allergy is one of the most widespread allergic diseases that cause a series of allergic reactions. The prevalence of milk allergy is globally 2–3% (Savage, Sicherer, & Wood, 2016), however, about 50%–90% of children with childhood-onset milk allergy acquire tolerance by school age, children with severe milk allergy that have high milk allergy specific immunoglobulin E (sIgE) levels and a history of anaphylaxis often have a persistent milk allergy (Wood, Sicherer, & Vickery, 2013). Current research reports that different processing methods could decrease the risk of milk allergy (Golkar, Milani, & Vastijevic, 2019; Bavaro, Angelis, Barni, Pilolli, & Monaci, 2019). However, chemical reactions could inevitably occur during food processing (German, 1999). The mechanism that affects food allergy is still not clear. Therefore, it will be necessary to explore the mechanism of allergic changes in milk from the perspective of chemical reactions.

Milk contains a large amount of unsaturated fatty acids, which could form various secondary products by peroxidation during processing and preservation (Bianchi, Zortea, Cazzarotto, Machado, & Macedo, 2018). These secondary products contain reactive aldehyde, which can cross-link with amino acid residues and induce changes in protein structure and functional properties including allergenic characteristics. Acrolein (CH2=CH-CHO) was considered as one of the main secondary products of unsaturated fatty acids by lipid peroxidation in food system. In order to illustrate the changes in the properties of food allergens, it is necessary to understand the interaction between the allergens and the representative secondary product-acrolein.

β-lactoglobulin (BLG), an 18.4 kDa allergic protein in milk, could cause allergic reactions intensively (Wal, 2002). The potential allergenicity of BLG depends on its allergic epitopes, which has been identified in BLG (Sélo et al., 1999; Clement, Boquet, Frobert, Hervé & Grassi, 2002). In order to explore the mechanism of BLG allergenic changes during milk processing, it’s necessary to explain the interaction between BLG and acrolein. Some research showed that acrolein could modify the structural characteristics of protein and affect the functional properties (Li, Wang, Zhou, Qu & Li, 2014; Feng et al., 2019). Therefore, we hypothesized that acrolein could cause the structural transformation of proteins, resulting in their allergenicity changes during in vitro and in vivo studies.

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In this study, we treated BLG with acrolein and analyzed the structural changes following acrolein interaction with the allergen. In addition, the potential allergenicity of acrolein-treated BLG was assessed by immunological methods and RBL-2H3 cell model in vitro. Moreover, an established mouse model was used to evaluate the allergenicity of BLG after acrolein treatment in vivo. This research will provide information about the allergenic properties of milk protein following lipid peroxidation during food processing.

2. Materials and methods

2.1. Materials

BLG (protein content >90%), acrolein, bovine serum albumin (BSA), 3,3′,5,5′-tetrakisylbenzidine (TMB) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The precision marker and the enhanced chemiluminescent (ECL) kit were bought from Thermo Fisher Scientific Inc. (Waltham, MA, USA). ELISA kits for histamine, prostaglandin D2, cysteinyl leukotriene, IL-4, IL-13 and mouse mast-cell protease-1 (mMCP-1) were obtained from R&D Systems (Minneapolis, MN, USA).

2.2. Human sera

Sera from milk allergic patients were supplied by Qingdao Eighth People’s Hospital (the medical ethics committee no is QD-EPH-201910283568, Qingdao, China). The specific IgE concentration was evaluated using the ImmunoCAP system (Phadia AB, Uppala, Sweden). The specific IgE levels of allergic patient sera above 0.35 kU/L were selected for performing western blot and ELISA experiments. After written consent were obtained from the patients, their sera were used in the research.

2.3. Preparation of samples

Acrolein-treated BLGs were prepared by the following method. The purified BLG (10 mg mL⁻¹) were dissolved in 10 mmol/L phosphate buffer solution (pH 7.4) and then were added in acrolein solution with the same volume at 25°C for 24 h. The concentration of acrolein was finally set at 0.01, 0.1, 1, and 10 mmol/L, respectively, whereas, the untreated BLG was considered a control. After the reaction, free acrolein was removed from the resulting solution by dialysis for 72 h at 4°C.

2.4. Protein SDS-PAGE

SDS-PAGE was performed according to the research of Laemmli (1970) with a few modifications. The treated BLG solutions were heated for 7 min before loading to the 12% prefabricated gels. Gels were carried out in an electrophoresis system cell at 120 V and separated bands were stained with Coomassie Brilliant Blue R-250. The gel was then de-stained with deionized water and the rotein bands were imaged with ChemiDoc XR5 Imaging System (Bio-Rad Laboratories, Hercules, CA, USA).

2.5. Western blot

The IgE binding capacity of acrolein-treated BLGs was further analyzed using western blotting technology. The gel of SDS-PAGE was transferred to the polyvinylidene fluoride (PVDF) membrane by iBlot Transfer Device. Afterward, immunological detections were performed by iBind Device. The primary antibody (human sera 1:4, v/v) and secondary antibody (anti-human IgE 1:2000, v/v) were diluted to 2 mL using iBind solution, respectively. The membrane was incubated with the primary antibody and secondary antibody for 4 h in total. At last, the membrane was detected by Tanon-4200SF gel imaging system with ECL in order to observe the IgE binding capacity of BLG.

2.6. Competitive inhibition ELISA (ciELISA)

ciELISA was performed as previously described by Lasekan and Nayak (2016). The purified BLG solution was coated onto ELISA plates at 4°C overnight. After washing, the plates were blocked with PBST containing 1% BSA at 37°C for 2 h. Moreover, 50 L pre-incubated mixture containing the different concentrations of all the samples (from 0.025 to 100 μg/mL) and human sera (1:20 with 0.5% BSA in PBST) was added to the plates and then incubated for 1 h at 37°C. After washing with PBST, the secondary antibody (HRP-conjugated goat anti-human IgE, 1:2000, v/v) was added and incubated for 2 h at 37°C. At last, all the samples were reacted with 50 μL TMB followed by the addition of 2 M H₂SO₄ to terminate the reaction. The absorbance value was detected using an automated ELISA plate reader (BioTek Instruments, Inc.) at 450 nm. The result of inhibition rate was analyzed through the following formula:Inhibition rate (%) = ((OD no inhibitor − OD inhibitor)/(OD no inhibitor)) × 100%

2.7. Fluorescence intensity

As per the techniques defined by Ahmed et al. (2018), the concentrations of all the samples were adjusted to 0.5 mg mL⁻¹ in PBS. The intrinsic fluorescence intensities of BLG and acrolein-treated BLGs were determined by RF-5301PC fluorescence spectrometer at 25°C (Shimadzu, Kyoto, Japan).

2.8. UV absorption spectrum

Different treated BLGs were adjusted to 0.5 mg/mL in PBS and the wavelength was recorded from 230 to 330 nm via UV-1101 Device (Techcomp, Shanghai, China).

2.9. CD spectroscopy for secondary structure

To evaluate the secondary structure of BLG and acrolein-BLG, a JASCO J-815CD spectropolarimeter (Spectroscopic Co. Inc., Japan) was used to detect the acrolein-treated BLGs (0.5 mg/mL). The operating parameters were: 190 to 260 nm spectral range; 1.0-nm bandwidth; 100-nm/min scan rate and 0.25-s interval.

2.10. LC-MS/MS analysis

The result of LC-MS/MS was analyzed according to our previous method (Lv, Lin, Li, Song, Ling & Wang, 2014). After the SDS-PAGE, the target proteins were sliced and kept in Eppendorf (EP) tubes at 4°C. The samples were sent to Beijing Protein Innovation R & D Center Co. Ltd. (China) for identification. High-performance liquid chromatography (HPLC) was performed to separate the peptide samples, using an Eksigent Nanoflex chipHPLC system (Eksigent, Dublin, CA, USA). The peptide samples were separated using a reversed-phase chipHPLC column (75 μm x 150 mm) at a flow rate of 300 nL/min. Mobile phase A contains deionized water having 0.1% (v/v) formic acid and mobile phase B contains acetonitrile with 0.1% (v/v) formic acid. A 40-minute gradient condition was applied, where mobile phase B was increased linearly from 3% to 40% within 30 min and further to 80% for 5 min, and then finally returned to 3% for re-equilibration. All the peptides eluted from the chipHPLC column at a particular elution time were analyzed by mass spectrometry at a resolution of 60,000.

2.11. RBL-2H3 cell assay

RBL-2H3 cells were obtained from the Chinese Academy of Sciences, and the cells were cultured according to the method of Song et al. (2016). The release of β-hexosaminidase was measured with BLG and Acrolein-BLG (1 mmol/L acrolein) for 6 h, as a model of mouse serum IgE-mediated mast cell allergic reaction. All the samples were then...
The release of histamine, cysteinyl leukotriene, prostaglandin D2, IL-4 and IL-13 was measured using ELISA kits.

2.12. Sensitization to BLG by intraperitoneal injection

Female BALB/c mice (5 weeks old, specific pathogen-free) were approved by Shanghai Laboratory Animal Center. The animals were raised in an air-conditioned room and allowed free access to tap water and food (without milk protein).

The mice model of allergy is carried out according to the method previously described (Ma, Lozano-Ojalvo, Chen, Lopez-Fandiño & Molina, 2015) with some modifications. For sensitization, intraperitoneal injection of mice was carried with 200 μg BLG and (1 mmol/L acrolein) Acrolein-BLG (n = 10) adsorbed in alum adjuvant (2 mg ml⁻¹) on days 7, 14, 21 and 28, as described in Fig. 5A. After sensitization stage, mice were distributed into three groups, namely; PBS, BLG and Acrolein-BLG in order to observe the changes in allergenicity. On days 31, blood samples of each samples were collected after sacrifice. Sera were obtained from the blood after centrifugation and were stored at 20 °C until analyzed. The level of BLG-specific IgE and IgG1 in serum was determined by indirect ELISA, while the concentrations of histamine and mMCP-1 were measured using sandwich ELISA kits.

2.13. Statistical analysis

All the data were analyzed by Prism 8.0.2 software. Results were exhibited as mean ± SD. Differences were determined by ANOVA with SPSS Statistics (Chicago, USA) and the P-values ≤ 0.05 were considered significantly different.

3. Results

3.1. SDS-PAGE

The bands of SDS-PAGE analysis showed that BLG aggregation occurred with acrolein treatment, as presented in Fig. 1-A. The native band of BLG was about 18 kDa, which became aggregated with increasing acrolein concentrations. As compared with native BLG, the aggregation bands were found at ~54 kDa and above after 1 and 10 mmol/L acrolein treatment. Moreover, no changes occurred when BLG was treated with acrolein concentrations below 0.1 mmol/L.

3.2. Western blot

The result of western blot showed that the IgE binding capacity of BLG significantly decreased following treatment with the high concentration of acrolein. However, the aggregates could still be visualized when BLG was modified with 1 and 10 mmol/L acrolein treatment. Moreover, no changes occurred when BLG was treated with acrolein concentrations below 0.1 mmol/L.

3.3. Inhibition ELISA

To determine whether acrolein treatment induced further changes in the immunogenic properties of BLG, the IgE binding capacity of acrolein-treated BLGs was analyzed using human sera, as shown in Fig. 1-C. The IC₅₀ value showed large differences for acrolein treatment with different concentrations. Compared with the purified BLG, the IC₅₀ value increased from 1.26 μg/mL to 3.16, 16.56, 46.8, and 76.2 μg/mL when the acrolein concentrations were 0.01, 0.1, 1 and 10 mmol/L, respectively. The results of ciELISA indicated that high concentrations of acrolein treatment had great influence on decreasing the IgE binding capacity of BLG.
3.4. Effect of acrolein treatment on the structural properties of BLG

Intrinsic fluorescence spectroscopy reveals the tertiary structural characteristics of the protein. Fig. 2-A shows the intrinsic fluorescence intensities of treated BLG, where the maximum emission spectrum of BLG was ~335 nm. The maximum fluorescence intensity was lower for acrolein-treated BLGs than native BLG. Moreover, the maximum emission wavelength of acrolein-treated BLGs showed a blue shift from 335 to 333 nm after modification. In addition, as compared with native BLG, 10 mmol/L acrolein treated BLGs showed a minimum intrinsic fluorescence intensity (about 19.4%).

UV absorption was also utilized to infer structural changes of proteins. The maximum UV absorption peak of BLG appeared at ~278 nm. As shown in Fig. 2-B, the UV absorption of acrolein-treated BLGs increased, and a blue shift in the maximum absorbance peak occurred after 1 and 10 mmol/L acrolein treatment, respectively.

The CD spectra for different treated BLG are presented in Fig. 2-C. BLG spectra showed a positive peak (~196 nm) and two negative peaks (~212 and ~223 nm). Moreover, as compared with native BLG, 10 mmol/L acrolein treated BLGs showed a minimum intrinsic fluorescence intensity (about 19.4%).

3.5. Oxidation sites analysis by LC-MS-MS

The proteomics technology was utilized to analyze the oxidation sites of BLG modified with acrolein treatment. After SDS-PAGE (Fig. 1-A), the target BLG bands were sliced and then analyzed by LC-MS/MS. Database retrieved that the amino groups including lysine (Lys), histidine (His) and phenylalanine (Phe) of the peptide chains were modified to form adducts with increasing molecular weight of 38 Da following acrolein treatment (Table 1). Moreover, \(\alpha\)-methylionine (Met) residues also were found to form adducts with increasing molecular weight of 16 Da, which was considered as [O] addition through reactive oxygen species. In the database, 13 peptides were found to be modified with acrolein oxidation.

LC-MS-MS results are listed in Fig. 3 A–C. The modified sites for some of acrolein and [O] adducts were observed on amino groups of the peptide chains. \(\text{138Lys-Ala-Leu-Lys-Ala-Leu-Arg-Leu149}\) showed acrolein adduct formation on the Lys\textsuperscript{141} and His\textsuperscript{145} residues (Fig. 3A). In addition, [O] adduction also was found on Met residue of the same peptide chain. The peptide chain- \(\text{141Lys-Val-Leu-Val-Leu-Asp-Thr-Asp-Tyr-Lys-Tyr152}\) was found to form adducts on Lys\textsuperscript{149}, Lys\textsuperscript{150} after acrolein treatment (Fig. 3B). \(\text{148Arg-Leu-Ser-Phe-Asn-Pro-Thr-Gln-Leu-Glu-Glu-Gln-Cys-His-Ile162}\) showed acrolein adduct formation on the Phe\textsuperscript{151} residue (Fig. 3C).

3.6. Effect of acrolein treated BLGs on RBL-2H3 cells assay

RBL-2H3 cells are mainly assayed as an IgE-mediated type I allergic-reaction mast cell model. Thus, RBL-2H3 cells were used to evaluate the potential allergenicity of treated BLG. The release of \(\beta\)-hexosaminidase decreased by 55.1% in cells exposed to 1 mmol/L acrolein treated BLG (Acrolein-BLG) as compared with BLG alone (Fig. 4 A). In addition, Acrolein-BLG also caused a substantial reduction in the content of histamine (46.7%) (Fig. 4A). The release of cysteinyl leukotriene and prostaglandin D\textsubscript{2} also showed alike tendencies (Fig. 4 C and D).

RBL-2H3 cells can produce cytokines such as IL-4 and IL-13, which could have a pathologic effect on IgE-mediated allergic symptoms including atopic dermatitis, atopic rhinitis and asthma. Acrolein-BLG showed significant (\(P < 0.01\)) decrease in the release of IL-4 (49.01%) and IL-13 (46.9%) (Fig. 4 E and F).

3.7. Sensitization potentials following intraperitoneal exposure to acrolein treated BLGs

BALB/c mice model was carried out to test the allergenicity of Acrolein-BLG after i.p. injection (Fig. 5A). The exposure to Acrolein-BLG showed lower levels of BLG-specific IgE and IgG1, which was 2- to 3-fold lower with Acrolein-BLG than BLG alone (Fig. 5B and 5C). The serum histamine and mMCPI-I release were significantly (\(P < 0.01\)) decreased with Acrolein-BLG (Fig. 5D and E).
4. Discussion

Oxidation could cause structural changes in BLG upon acrolein treatment, which eventually influenced the allergenicity. The bands in SDS-PAGE were used to observe the structural changes of BLG upon oxidation. Oxidation proceeds and BLG aggregation occurred with increasing concentrations of acrolein ranging from 1 to 10 mmol/L. β-mercaptoethanol was added to the sample buffer prior to loading the samples to the gel in order to break the protein disulphide bonds. Thus acrolein and amino acid residues of BLG are linked via non-disulphide covalent linkages. Accordingly, these results indicated that oxidation caused aggregation of BLG via non-disulphide covalent bonds. The result of SDS-PAGE by oxidation reaction of BLG with acrolein was consistent with the previous study (Wu, Wu, & Hua, 2010). The possible reason for the increase in molecular weight of the product may be due to the binding of more amino acid chains to the protein (Iwan et al., 2011).

Protein aggregation through oxidation reaction can alter the potential BLG allergenicity of milk. Here we observed that IgE binding capacity was significantly (P < 0.05) decreased after the acrolein concentration reached 1 and 10 mmol/L. The possible reason was that protein aggregation could cause structural modification and the reduction of IgE binding capacity. The previous research reported that the crab arginine kinase aggregation by enzymatic cross-linking also mitigated the IgE binding capacity (Fei, Liu, & Chen, 2016). Further alterations occurred in the amino acids of BLG might have damaged certain epitopes, leading to the loss of IgE binding capacity. This phenomenon was also confirmed by western blot and cELISA assays, demonstrating a reduction in the IgE binding capacity of BLG with the increasing concentrations of acrolein, which eventually altered the molecular properties of BLG. Moreover, the aggregates could illustrate that the epitopes of 1 and 10 mmol/L acrolein-treated BLGs were still kept and active.

The intrinsic fluorescence spectra of proteins that result from the emission spectrum of amino acid residues, mainly Tryptophan (Trp), Tyrosine (Tyr) and Phe are considered. Upon treatment of BLG with acrolein, the maximum fluorescence intensity declined and emission spectra shifted towards the lower wavelength. With blue-shifted emission spectra and reduced maximum intensity, it was postulated that these amino acid residues of BLG were covered inside the BLG upon oxidation. These results of the fluorescence from acrolein-treated BLGs suggested that acrolein treatment altered the tertiary structure which caused aggregation. Wu et al. (2018) reported that the intrinsic fluorescence intensity of BLG decreases by covalent conjugation with dietary polyphenols.

Further exposure of specific amino acid residues on the surface of the BLG molecule caused an increase in UV absorption after BLG oxidation. The UV absorption spectra of protein are mainly dependent on Trp and Tyr residues in the side chain groups (Tong et al., 2012). The results indicated that specific amino acids such as Trp and Tyr residues might be exposed following acrolein treatment. A blue shift indicated that the polarity of the protein structure was adjusted upon oxidation owing to broken peptide bonds. These modifications in the conformational structure of BLG followed by oxidation may affect the IgE binding capacity.

Far-ultraviolet CD spectroscopy was used to analyze the secondary structure of BLG. The secondary structure of protein mainly includes α-helix, β-turn, β-sheet, and a random structure similar to the measurements given by Li et al. (2007). The presence of β-sheet structure was confirmed by the indicated positive peak at ~196 nm in the spectra. Two negative peaks appeared at ~212 nm and ~223 nm symbolized the presence of α-helix. Spectral intensity of acrolein-treated BLG steadily declined with the increasing concentrations of acrolein. The result indicated that α-helix and β-sheet of secondary structure were reduced. The possible reason was that the original van der Waals interactions were disrupted via amino acid residues combining with acrolein by oxidation (McClain, Garnon & Oakley, 2002). The secondary structure of BLG was rearranged, thus it was postulated that acrolein treatment might have disrupted the BLG conformational epitopes. The secondary structure may also get interrupted due to the denaturation of proteins, owing to BLG aggregation (Shin, Han & Ahn, 2015).

The sites of certain amino acids (Lys, His and Phe) could be modified with acrolein treatment by LC-MS-MS, which caused changes in the protein secondary and tertiary structures. Therefore, the functional properties of BLG might be affected. The previous study has shown that oxidation could occur on Lys and His residues (Zhao, Chen, Zhu, & Xiong, 2012). Moreover, the modified Lys, His and Phe residues in the side chain groups are on the allergic epitopes of BLG (Li, Gao, He, Wu, & Chen, 2014). Therefore, the identification of the modified sites by oxidation, accompanied by the analysis of epitope, could be a better option to explain the changes of BLG immunogenicity during food processing and preservation.

IgE-mediated degranulation in RBL-2H3 cells was used as the model to evaluate the allergenic potential of acrolein-treated BLGs upon oxidation by assessing mediators related to allergic symptoms as degranulation biomarkers. The results demonstrated that acrolein-treated BLGs significantly (P < 0.05) reduced the release of mediators and cytokines associated with degranulation of RBL-2H3 cells. This can be explained in a way that the altered IgE epitopes of BLG upon oxidation by acrolein could hinder the basophils degranulation. This was in agreement with a previous study in which the treatment of tropomyosin with malonaldehyde decreased degranulation (Song, Li, Gao, Pavase, & Lin, 2016).

The oxidation of acrolein treatment affected the potential allergenicity of BLG in mouse studies. This is exhibited by the decrease of BLG-specific IgG1 and IgE in the immunogenic response of acrolein-treated BLG. Furthermore, acrolein-treated BLG decreased the capacity to
Fig. 3. (A) MS/MS spectrum for a peptide containing Lys and His from acrolein treated BLG. Acrolein adduction (*, +38) were observed on the Lys and His side-chain group in Lys-Ala-Leu-Lys-Ala-Leu-Pro-Met-His-Ile-Arg-Leu. (Acrolein-modified residue underlined). (B) MS/MS spectrum for a peptide containing Lys from acrolein treated BLG. Acrolein adduction (*, +38) was observed on the Lys side-chain group in Lys-Val-Leu-Val-Leu-Asp-Thr-Asp-Tyr-Lys-Lys-Tyr. (Acrolein-modified residue underlined). (C) MS/MS spectrum for a peptide containing Phe from acrolein treated BLG. Acrolein adduction (*, +38) was observed on the Phe side-chain group in Arg-Leu-Ser-Phe-Asn-Pro-Thr-Gln-Leu-Glu-Glu-Gln-Cys-His-Ile. (Acrolein-modified residue underlined).
induce the release of mMCP-1 and histamine in mice sera. Therefore, the potential allergenicity of BLG was reduced after acrolein treatment. Zhang et al. (2020) reported that the allergenicity of ovalbumin decreased after quercetin treatment. In addition, one research showed that enzymatic interaction of crab allergen decreased the IgE/IgG1 levels in mice serum (Liu et al., 2017). The possible explanation is that the allergic epitope of BLG was covered or destroyed by the chemical modification, which could change the immunological properties of BLG. Both the in vivo and in vitro research indicated that the potential allergenicity of BLG decreased by acrolein treatment.

In conclusion, the oxidation of BLG with increasing the level of acrolein resulted in the changes of fluorescence intensity and UV absorption spectra. Further acrolein treatment affected the primary and secondary protein structure resulting in the oxidation of the sites, gradual loss of α-helix and backbone fragmentation. Non-disulphide covalent bonds lead to high molecular weight aggregates. Acrolein and amino acids interactions might have modified the BLG conformational structure, thus disrupting its allergenic epitopes. The IgE binding capacity of BLG decreased with the increasing level of acrolein. Moreover, the data revealed that oxidized BLG by acrolein treatment reduced the release of mediator from RBL-2H3 cells, supported by the data of mice study. Our study highlighted that structural alterations induced by acrolein treatment of BLG declined its allergenic potential during processing and preservation.

Fig. 4. Effects of acrolein (1 mmol/L) treated BLG in IgE-mediated allergic responses in RBL-2H3 cells sensitized with mouse sera IgE. A, The release of β-hexosaminidase; B, The release of histamine; C, The release of cysteinyl leukotriene; D, The release of prostaglandin D2; E, The release of IL-4; F, The release of IL-13. *p < 0.05, **p < 0.01. The data represent the mean ± SD from three independent experiments.
CRediT authorship contribution statement

Liangtao Lv: Investigation, Validation, Writing - original draft, Writing - review & editing. Xin Qu: Investigation, Methodology, Validation. Ni Yang: Resources, Supervision. Ishfaq Ahmed: Supervision, Writing - review & editing.

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.

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Appendix A. Supplementary data

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