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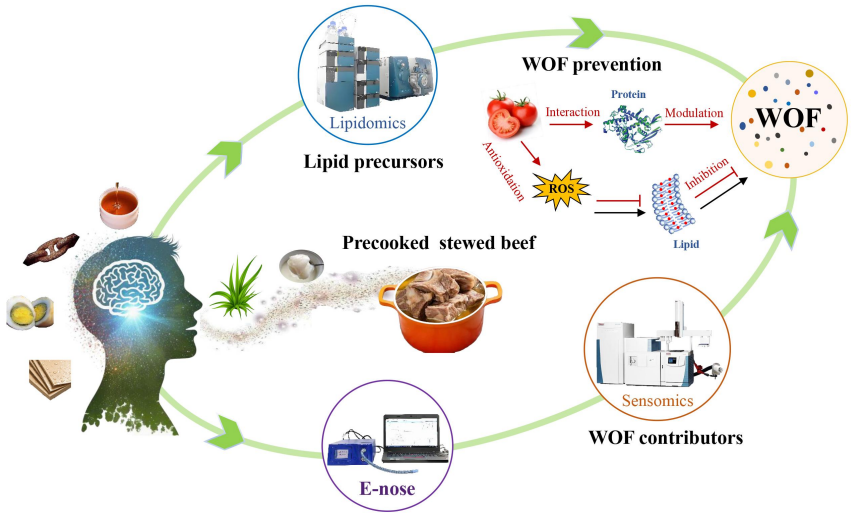
Formation and prevention of warmed-over flavor in precooked Chinese stewed beef dishes



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UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

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Junmei LIU

Formation et prévention d'un goût de réchauffé dans les plats de bœuf mijotés chinois précuits

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Abstract

Junmei LIU (2025). "Formation and prevention of warmed-over flavor in precooked Chinese stewed beef dishes" (PhD Dissertation in English).
Gembloux, Belgium, Gembloux Agro-Bio Tech, University of Liege.
202 pages, 30 figures, 7 tables.

Summary:

Stewed beef, a famous Chinese dish, contains beef and other auxiliary ingredients like vegetables and spices. It is highly popular for its attractive flavor and high nutritional value. In China, the precooked Chinese dish (PCD) sector is expanding rapidly. Precooked Chinese stewed beef (PCSB), a representative PCD, has been widely developed because its cooking procedure is more stable and simpler than other Chinese cooking methods such as stir-frying and pan-frying. Most of PCSB dishes are refrigerated and need to be reheated before consumption. Nevertheless, even after a short period of refrigeration within their shelf life, they still develop a characteristic warmed-over flavor (WOF), an off-flavor that diminishes consumer acceptance. Simultaneously, the meaty aroma of PCSB gradually weakens, affecting its sensory quality. The development of WOF is inextricably associated with lipids. On the one hand, an increase in secondary lipid oxidation products is primarily responsible for WOF formation. On the other hand, the lipophilic nature of these odorants allows them to dissolve in lipids and to be gradually released during processing or consumption. Therefore, it is very important to characterize the key aroma-active compounds contributing to WOF in PCSB and identify the lipid markers as potential precursors of WOF, aiming to maintain and improve the flavor quality of PCSB dishes. The objective of the present research was to investigate and confirm the key aroma-active compounds contributing to WOF and the differential lipid markers as potential precursors of WOF, elucidating the pathways involved in WOF formation in PCSB.

Firstly, a sensomics approach was used to characterize the key aroma-active compounds contributing to WOF in reheated PCSB. 36 odorants were identified, and based on flavor dilution factors, odor activity values, aroma recombination, and omission test, hexanal, (E,E)-2,4-decadienal, (E,E)-2,4-nonadienal, pentanal, decanal, octanal, heptanal, (E)-2-octenal, (E)-2-undecenal, 1-octen-3-ol, and (E)-2-nonenal, mainly derived from lipid oxidation were characterized as the key aroma-active compounds contributing to the formation of WOF. In particular, 3-(methylthio)propanal, which was positively correlated with meaty aroma, was also associated with an overall increase in WOF. Thus, these odorants were elected as potential markers of WOF in PCSB. In summary, the WOF in PCSB could be attributed to an overall increase in lipid oxidation products and a decrease in the odorants with desirable aromas. The characterization of WOF in PCSB will aid in the flavor quality control of PCSB dishes.

Secondly, lipidomics was used to investigate the role of lipids in the formation and development of WOF in PCSB. A total of 1236 lipids were detected in cooked and reheated stewed beef. Triacyclglycerols (TGs), notably TG(18:0/18:1/18:1) and TG(16:0/18:1/18:1), were considered key lipids associated with predominant odorants. Among 153 differential lipids (VIP > 1, $P < 0.05$), phosphatidylserine (PS)(18:0/18:2) and PS(16:0/17:2) were identified as potential markers for distinguishing all samples. A total of 142 differential lipids were significantly correlated with the predominant odorants, with ether-bonded phosphatidylethanolamines (ePEs), particularly PE(P-18:0/18:2), serving as crucial precursors in WOF formation. Furthermore, lysophosphatidylcholine (LPC)(20:3) and phosphatidylcholine (PC)(16:0/18:1) notably facilitated WOF development. The results provide a theoretical basis for flavor correction in precooked Chinese stewed beef dishes.

Finally, the role of tomatoes in mitigating WOF in PCSB was elucidated through analyses of aroma profiles, lipid composition, protein secondary structure, and sensory attributes. Adding tomatoes at 25%, 50%, 75%, and 100% of the raw beef weight significantly inhibited lipid oxidation, reducing losses in phospholipids (e.g., PE, LPC, and PC) and TG, thereby reducing WOF development. Tomato-originated 2-isobutylthiazole exhibited a potent flavor endowment effect on PCSB, with its endowment rate increasing from 602.64% to 2860.10%, thus masking the WOF in PCSB. Tomato additions also altered the secondary structure of beef protein, potentially affecting WOF retention in PCSB. Sensory evaluation revealed that the 75% tomato group achieved the highest overall acceptability, balancing meaty and tomato-like aromas while significantly reducing the WOF. This research offers a viable strategy for reducing WOF in PCSB, thereby enhancing its commercial appeal and consumer acceptance.

In conclusion, the present study has identified lipid oxidation as the primary driver of WOF. Eleven key aroma-active compounds, including hexanal, (E,E)-2,4-decadienal, (E,E)-2,4-nonadienal, pentanal, decanal, octanal, heptanal, (E)-2-octenal, (E)-2-undecenal, 1-octen-3-ol, and (E)-2-nonenal, were confirmed as critical contributors to WOF in the reheated PCSB using sensomics approach. Lipid oxidation was confirmed as the primary driver of WOF. Lipidomics analysis revealed the importance of e-PEs, which are key contributors to WOF formation. Additionally, the study demonstrated the potential of tomato additives to mitigate WOF by inhibiting lipid oxidation and enhancing sensory quality. Specifically, the tomato-originated compound 2-isobutylthiazole played a major role in flavor enhancement, thus masking WOF and improving the sensory quality of the dish. These results provide valuable insights into the molecular mechanisms behind flavor changes in PCSB and offer practical solutions for improving the flavor stability of stewed beef dishes, ensuring better quality control in the production of precooked meat products.

Key words: precooked Chinese stewed beef, warmed-over flavor, key aroma-active compounds, lipidomics, precursors, prevention

Résumé

Junmei Liu (2025). "Formation et prévention d'un goût de réchauffé dans les plats de bœuf mijotés chinois précuits" (thèse de doctorat en anglais).

Gembloux, Belgique, Gembloux Agro-Bio Tech, Université de Liège.

202 pages, 30 figures, 7 tableaux.

Résumé:

Le bœuf mijoté, un plat chinois célèbre, contient du bœuf et d'autres ingrédients auxiliaires comme des légumes et des épices. Il est particulièrement apprécié pour sa richesse nutritionnelle et sa saveur attrayante. En Chine, l'industrie des plats chinois précuits se développe rapidement. Les plats de bœuf mijotés précuits, plats chinois précuits typiques, sont désormais largement développés en raison de leurs processus de cuisson plus stables et plus simples par rapport aux méthodes de cuisson chinoises telles que la friture et la poêle. La plupart des plats de bœuf mijotés précuits sont réfrigérés et doivent être réchauffés avant d'être consommés. Néanmoins, même après une courte période de réfrigération lors de leur conservation, ils développent toujours une saveur particulière de réchauffé (WOF), qui est considérée comme une saveur désagréable qui affecte l'acceptation du consommateur. Parallèlement, l'arôme carné des PCSB tend à s'atténuer progressivement, ce qui compromet leur qualité sensorielle. Le développement de WOF est inextricablement lié aux lipides. D'une part, des produits d'oxydation lipidique secondaires, principalement des aldéhydes, augmentent. D'autre part, la nature lipophile de ces odorants leur permet de se dissoudre dans les lipides et d'être libérés durant la consommation. Par conséquent, il est très important de caractériser les principaux composés aromatiques actifs contribuant au WOF dans le bœuf mijoté chinois réchauffé et d'identifier les marqueurs lipidiques comme précurseurs potentiels du WOF, dans le but de maintenir et d'améliorer la qualité gustative des plats de bœuf mijoté précuits. L'objectif de la présente étude était d'étudier et de confirmer les principaux composés aromatiques actifs contribuant au WOF et les marqueurs lipidiques différentiels comme précurseurs potentiels du WOF, en élucidant les voies impliquées dans la formation du WOF dans le bœuf mijoté précuit.

Tout d'abord, l'approche sensomique a été utilisée pour caractériser les principaux composés aromatiques actifs contribuant au WOF du bœuf mijoté précuit réchauffé. Trente-six odorants ont été identifiés et, sur la base de facteurs de dilution de la saveur, de valeurs d'activité olfactive, de recombinaison d'arôme et de test d'omission, l'hexanal, le (E,E)-2,4-décadiénal, le (E,E)-2,4-nonadiénal, le pentanal, le décanal, l'octanal, l'heptanal, le (E)-2-octénal, le (E)-2-undécénal, le 1-octène-3-ol et le (E)-2-nonénal, principalement dérivés de l'oxydation des lipides, ont été caractérisés comme les odorants clés contribuant à la formation de WOF. En particulier, le 3-(méthylthio)propanal, qui était positivement corrélé à l'arôme de

viande, était impliqué dans une augmentation globale du WOF. Ainsi, ces odorants ont été élus comme marqueurs potentiels du WOF dans le bœuf mijoté précuit réchauffé. En résumé, le WOF du bœuf mijoté précuit pourrait être attribué à une augmentation globale des produits d'oxydation des lipides et à une diminution des odorants aux arômes désirables. La caractérisation du WOF dans le bœuf mijoté précuit aidera au contrôle de la qualité gustative des plats de bœuf mijoté précuits.

Deuxièmement, la lipidomique a été utilisée pour étudier le rôle des lipides dans la formation et le développement du WOF dans le bœuf mijoté précuit. Au total, 1236 lipides ont été détectés dans le bœuf mijoté cuit et réchauffé. Les triadylglycérols (TG), notamment le TG(18:0/18:1/18:1) et le TG(16:0/18:1/18:1), ont été considérés comme des lipides clés associés aux odorants prédominants. Parmi 153 lipides différentiels (VIP > 1, P < 0,05), la phosphatidylsérine (PS)(18:0/18:2) et la PS (16:0/17:2) ont été identifiées comme des marqueurs potentiels permettant de distinguer tous les échantillons. Au total, 142 lipides différentiels étaient significativement corrélés aux odorants prédominants, les phosphatidyléthanolamines à liaison éther *(*ePE*)*, en particulier la PE(P-18:0/18:2), servant de précurseurs cruciaux dans la formation du WOF. De plus, la lysophosphatidylcholine (LPC)(20:3) et la phosphatidylcholine (PC)(16:0/18:1) ont particulièrement facilité le développement du WOF. Les résultats fournissent une base théorique pour la correction de la saveur dans les plats de bœuf mijotés chinois précuits.

Enfin, le rôle des tomates dans l'atténuation du goût réchauffé (WOF) dans le bœuf mijoté précuit a été élucidé grâce à des analyses des profils aromatiques, de la composition lipidique, de la structure secondaire des protéines et des attributs sensoriels. L'ajout de tomates à 25 %, 50 %, 75 % et 100 % du poids de bœuf cru a inhibé de manière significative l'oxydation des lipides, réduisant les pertes de phospholipides (par exemple, PE, LPC et PC) et de TG, diminuant ainsi le développement de WOF. Le 2-isobutylthiazole originaire de la tomate a montré un puissant effet de dotation en saveur sur le bœuf mijoté précuit, son taux d'apport passant de 602,64 % à 2860,10 %, masquant ainsi le WOF dans le bœuf mijoté précuit. L'ajout de tomates a également modifié la structure secondaire des protéines de bœuf, affectant potentiellement la rétention du WOF dans le bœuf mijoté précuit. L'évaluation sensorielle a révélé que le groupe à 75 % de tomates a obtenu l'acceptabilité globale la plus élevée, équilibrant les arômes de viande et de tomate tout en réduisant considérablement le WOF. Cette recherche propose une stratégie rationnelle pour réduire le WOF dans le bœuf mijoté précuit, améliorant ainsi son attrait commercial et l'acceptation des consommateurs.

En conclusion, la présente étude a identifié l'oxydation des lipides comme le principal moteur du WOF. Onze composés aromatiques clés, dont l'hexanal, le (E,E)-2,4-décadiénal, le (E,E)-2,4-nonadiénal, le pentanal, le décanal, l'octanal, l'heptanal, le (E)-2-octénal, le (E)-2-undécénal, le 1-octène-3-ol et le (E)-2-nonénal, ont été confirmés comme contributeurs essentiels au WOF dans le bœuf mijoté précuit réchauffé à l'aide de l'approche sensomique. L'oxydation des lipides a été confirmée comme le principal moteur du WOF. L'analyse lipidomique a révélé l'importance des phosphatidyléthanolamines à liaison éther (e-PE), qui sont des

contributeurs clés à la formation du WOF. De plus, l'étude a démontré le potentiel d'additifs à base de tomates pour atténuer le WOF en inhibant l'oxydation des lipides et en améliorant la qualité sensorielle. Plus précisément, le composé 2-isobutylthiazole dérivé de la tomate a joué un rôle majeur dans l'amélioration de la saveur, masquant ainsi le WOF et améliorant la qualité sensorielle du plat. Ces résultats fournissent des informations précieuses sur les mécanismes moléculaires à l'origine des changements de saveur dans le bœuf mijoté précuit et offrent des solutions pratiques pour améliorer la stabilité de la saveur des plats de bœuf mijoté, garantissant un meilleur contrôle de la qualité dans la production d'aliments précuits à base de viande.

Mots clés: bœuf mijoté précuit, saveur réchauffée, composés aromatiques clés actifs, lipidomique, précurseurs, prévention

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Junmei Liu

07/06/2025 in Gembloux, Belgium

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List of Abbreviations

WOF	Warmed-over flavor
CSB	Chinese stewed beef
PCSB	Precooked Chinese stewed beef
PCSBT	Precooked Chinese stewed beef with tomato
PCD	Precooked Chinese dish
TOFE	Target-oriented flavor editing
MDA	Malondialdehyde
TBARS	Thiobarbituric acid reactive substance
LPO	Lipid peroxide content
HPLC	High performance liquid chromatography
HS-GC-IMS	Headspace-gas chromatography-ion mobility spectrometry
GC-O	Gas chromatography-
GC-MS	Gas chromatography-mass spectrometry
UPLC-ESI-MS/MS	Ultra-high performance liquid chromatography coupled with electrospray ionization mass spectrometry
FT-IR	Fourier transform infrared
E-nose	Electronic nose
APA	Sensory aroma profile analysis
HS-SPME	Headspace solid-phase microextraction
DVB/CAR/PDMS	Divinylbenzene/carboxen/polydimethylsiloxane
SAFE	Solvent-assisted flavor evaporation
RI	Retention indice
DFA	Detection frequency analysis
DF	Detection frequency
AEDA	Aroma extract dilution analysis
FD	Flavor dilution
OAV	Odor activity value
ERV	Endowment rate value
ANOVA	One-way analysis of variance
OPLS-DA	Orthogonal partial least squares discrimination analysis
PLS-DA	Partial least squares discrimination analysis
VIP	Variable importance in the projection
PCA	Principal component analysis
LDA	Linear discriminant analysis
PL	Phospholipid
GP	Glycerophospholipid
GL	Glycerolipid
SP	Sphingolipid
FA	Fatty acyl ester
ST	Sterol lipid
PR	Isoprenoid lipid
CAR	Acylcarnitine

CE	Cholesterol ester
Cer	Ceramide
CerP	Ceramide-1-phosphate
Cho	Cholesterol
CoQ	Coenzyme Q
DG	Diacylglycerol
DG-O	Alkyl-diacylglycerol
DGDG	Digalactosyl diacylglycerol
FFA	Free Fat Acid
HexCer	Glycosphingolipids
LPC	Lys phosphatidylcholine
LPC-O	Alkyl-Lys phosphatidylcholine
LPE	Lys phosphatidylethanolamine
LPE-P	Plasmalogen-Lys phosphatidylethanolamine
LPG	Lys phosphatidylglycerol
LPI	Lys phosphatidylinositol
LPS	Lys phosphatidylserine
MG	Monoacylglycerol
MGDG	Mon galactosyl diacylglycerol
PA	Phosphatidic acid
PC	Phosphatidylcholine
PC-O	Alkyl-phosphatidylcholine
PE	Phosphatidylethanolamine
PE-O	Alkyl-phosphatidylethanolamine
PE-P	Plasmalogen-Lys phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PMeOH	Phosphatidyl methanol
PS	Phosphatidylserine
SM	Sphingomyelin
SPH	Sphingosine
TG	Triacylglycerol
BA	Bile Acid
LPA	Lysophosphatidic acid
FA	Fatty acid
ePE	Ether phospholipid
PUFA	Polyunsaturated fatty acid
UFA	Unsaturated fatty acid
SFA	Saturated fatty acid

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Chapter I General introduction

1.1. Context and objectives

1.1.1. Context

Stewed beef, a famous Chinese dish, contains beef and other auxiliary ingredients like vegetables and spices. It is particularly popular for its rich nutrition and attractive flavor. In Chinese culinary culture, the practice of combining meat and vegetables, known as hun-su pairing, reflects both philosophical and nutritional considerations. Rooted in the concept of yin-yang balance, meats are regarded as "yang" (warm) and vegetables as "yin" (cool), and their combination is believed to support health through dietary harmony. This pairing also offers nutritional complementarity: meats provide high-quality protein and micronutrients such as iron and vitamin B12, while vegetables contribute dietary fiber, antioxidants, and phytosterols that modulate lipid metabolism. Furthermore, to preserve the natural flavor and nutritional quality of ingredients, it is customary to add vegetables later in the cooking process, particularly in stewed dishes, so that their color, texture, and heat-sensitive nutrients are better retained.

In parallel with these culinary traditions, food consumption patterns in China have evolved significantly in recent years. A growing number of consumers, particularly in urban areas, are turning to ready-to-eat and ready-to-heat meals, influenced by time constraints, changing family structures, and reduced home cooking frequency. In addition, the long-standing cultural preference for hot meals over cold dishes continues to shape consumer expectations. Unlike Western countries where cold-served foods such as sandwiches and salads are widely accepted, most Chinese consumers still associate warmth with freshness and comfort, making reheating an essential step before consumption.

Precooked Chinese stewed beef (PCSB) dishes, typical Chinese stewed dishes, are now widely developed owing to their more stable and simpler cooking processes compared with Chinese cooking methods such as stir-frying and pan-frying. Most of the PCSB dishes are refrigerated and need to be reheated before eating. Nevertheless, even after a short period of refrigeration within their shelf-life, they still develop a particular warmed-over flavor (WOF), which is considered an off-flavor that affects consumer acceptance (Tims & Watts, 1958). Simultaneously, the meaty aroma of PCSB gradually weakens, affecting its sensory quality (O'Sullivan et al., 2003). Tims and Watts (1958) were the first to introduce the concept of WOF, and described it as an oxidized aroma such as rancid and stale. Subsequently, researchers described WOF using aroma profile evaluations, identifying descriptors such as wet cardboard, linseed oil, paint, sour, hard-boiled egg, and fatty notes (An et al., 2022; Lage et al., 2012). According to Tims and Watts (1958), the heat processing of uncured meats caused lipid oxidation, resulting in the loss of palatability during later storage. Thereafter, numerous studies have reported that WOF is mainly caused by lipid oxidation, as the development of WOF was related to lipid oxidation products (Pegg et al., 2014; Ruenger et al., 1978; Zhang et al., 2022). O'Sullivan et al. (2003) found that hexanal, 1-octen-3-ol, 2-pentylfuran, octanal, pentanal, and nonanal were associated with sensory data in cooked samples of two pork muscles using GC-MS.

These compounds proved to be valid indicators of lipid oxidation. An et al. (2022) identified (E,E)-2,4-heptadienal, heptanal, (E)-2-octenal, octanal, (E)-2-nonenal, nonanal, (E)-2-decenal, decanal, and (E,E)-2,4-decadienal as the key odorants responsible for the warmed-over flavor (WOF) in surimi gels, using aroma extract dilution analysis (AEDA), aroma recombination, and omission tests. In addition to lipid oxidation, protein degradation also contributes to WOF by causing the loss of desirable aroma compounds (Pegg et al., 2014; Zhang et al., 2022). Previous studies have revealed that WOF may result not only from the formation of undesirable volatiles but also from the loss of favorable odorants associated with meaty notes. In particular, the decline of certain furanones and sulfur-containing compounds with meaty aromas in meat products has been linked to increased WOF (An et al., 2022). Kerler and Grosch (1996) found that the reduction of 4,5-dimethyl-3-hydroxy-2(5H)-furanone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone, both derived from the Maillard reaction and associated with meaty flavor, contributed to WOF in precooked beef. However, An et al. (2022) also reported the presence of some Maillard reaction-derived sulfur-containing compounds in surimi gels, such as benzothiazole, 2-methoxybenzenethiol, and 2-furfurylthiol, which presented floral, coffee-like, onion-like, and warmed-over notes. Additionally, some nitrogen-containing compounds, such as 2-propylpyridine and 2,6-dimethylpyrazine, exhibited potato-like and cardboard-like notes and were suggested to have a positive impact on WOF development. This was likely due to differences in both the concentrations and odor characteristics of these compounds between meat products and surimi gels. Nonetheless, the odorants responsible for the generation of the WOF in PCSB remain unclear.

WOF development is inextricably associated with lipids. On one hand, as aforementioned, increase in secondary lipid oxidation products has been reported as the main cause of WOF formation in meat products (Konopka & Grosch, 1991). Nevertheless, few studies have investigated the specific lipid markers for the formation of WOF. On the other hand, the lipophilic nature of these odorants allows them to be dissolved in lipids and be gradually released during processing or consumption (Guo et al., 2022). Consumers' perceptions of WOF are considerably influenced by the release and retention of these compounds in the food matrix (Wang et al., 2023). However, the lipid fingerprints of PCSB are not clear and the key lipid molecules potentially associated with key aroma compounds in PCSB have not been reported. Therefore, it is crucial to clarify the lipid fingerprints of reheated PCSB.

WOF mainly results from lipid oxidation, leading to the accumulation of aroma compounds such as hexanal, (E,E)-2,4-decadienal, and (E)-2-octenal, which contribute to WOF that diminish consumer acceptability (Chen et al., 2024). With the growing consumer demand for natural properties and clean-label characteristics of precooked dishes, the study of plant-based antioxidants in mitigating lipid oxidation in cooked meat products has gained importance (Dang et al., 2024). Various plant-based antioxidants, such as perilla juice, ginger juice (Dang et al., 2024), and essential oils from winter savory (*Satureja montana* L.) (Jokanović et al.,

2020), have been shown to reduce and mask WOF, offering a promising alternative to synthetic antioxidants. Notably, tomatoes, a widely used culinary ingredient globally, possess a synergistic antioxidant system composed of lycopene, β-carotene, vitamins C and E, which exhibit strong antioxidant properties (Skiepko et al., 2016). Stewed beef with tomato, widely recognized across various culinary traditions, is an essential component of the PCD industry. Nevertheless, the impact of tomato incorporation on lipid molecular changes and WOF mitigation in PCSB remains unclear. Moreover, the tomato-originated aroma compounds endow the dish with a distinctive tomato-like aroma. Whereas, the specific tomato-originated compounds with flavor endowment effect and their flavor endowment rate remain unknown. These uncertainties directly contribute to the absence of standardized industrial formulations that optimally balance WOF suppression (via lipid stabilization) with flavor authenticity (avoiding sensory dominance by tomato notes). Furthermore, WOF perception is closely linked to its retention and release within the meat matrix. Substantial evidence suggests that proteins, particularly myofibrillar proteins, serve as ideal binding matrices for aroma compounds (Chen et al., 2024). The flavor endowment process through which the aroma compounds from tomatoes contribute to the unique flavor of stewed beef involves diffusion, capillary adsorption, and non-covalent binding to proteins (Qiang et al., 2025). Additionally, lycopene has been reported to interact hydrophobically with catfish myosin, altering its structural properties (Zhao et al., 2023). These interactions might modulate beef protein conformations and consequently influence WOF retention. At present, the impact of tomatoes on the structural properties of beef proteins and their subsequent effects on WOF binding remain largely unexplored. It is hypothesized that tomatoes could effectively reduce WOF formation while enhancing the overall flavor profile of PCSB. Therefore, it is crucial to elucidate the underlying mechanisms of tomatoes in mitigating WOF in PCSB.

1.1.2. Objectives

The general objective of the present study is to clarify the patterns of variation in the WOF of the PCSB during cooking-refrigeration-reheating and clarify the mechanism of ingredient interaction between beef and other ingredients during stewing to suppress the WOF. This work aims to provide information for off-flavor correction, which may be used to maintain and improve the flavor quality of PCSB dishes. In details:

(1) To characterize the key aroma-active compounds contributing to WOF in reheated PCSB using a sensomics approach and elucidate the changes in the aroma profiles of PCSB during cooking-refrigeration-reheating;

(2) To obtain comprehensive information about the lipid fingerprints of PCSB, identify the differential lipid markers as potential precursors of WOF, and determine the major lipids responsible for binding WOF in reheated PCSB;

(3) To characterize the flavor endowment properties of tomato-originated aroma compounds and their WOF-masking effects, evaluate the regulatory impact of protein structural changes and tomato-originated aroma compounds on the release of

WOF, and determine the optimal tomato addition level through comprehensive analysis of WOF variation patterns.

1.2. Structure of the thesis

This thesis structure is presented as follows: Chapter I: General introduction; Chapter II: Literature review on warmed-over flavor formation and prevention; Chapter III: Characterization of key aroma-active compounds contributing to warmed-over flavor in PCSB; Chapter IV: Elucidation of potential lipid precursors and formation pathways for the WOF in PCSB; Chapter V: Ingredient interaction mechanisms: The role of tomato in mitigating warmed-over flavor in PCSB with tomato; Chapter VI: General discussion; Chapter VII: Conclusion and perspectives.

1.3. Research roadmap and outline

1.3.1. Research roadmap

The research roadmap is displayed in **Fig. 1-1**.

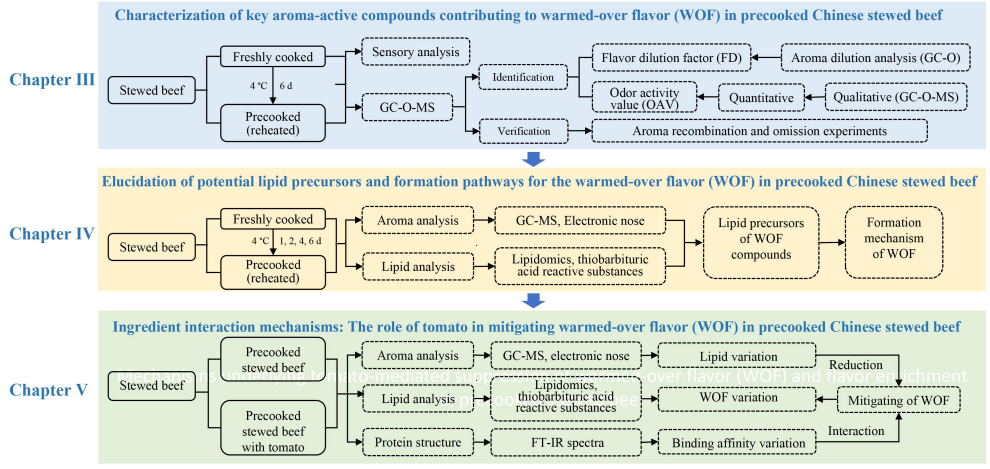

Fig. 1-1 Research roadmap

1.3.2. Outline

In chapter II, the literature review synthesizes the current knowledge regarding the research on WOF in PCSB. Aroma compounds contributing to WOF, formation pathways of WOF in precooked dishes, and potential strategies for WOF mitigation are summarized. The analytical techniques employed to assess WOF, including aroma extraction, enrichment, analysis, and identification are outlined. Additionally, applications of lipidomics in food science are summarized.

In chapter III, the key odorants contributing to the WOF of reheated PCSB were characterized using a sensomics approach. Freshly cooked stewed beef samples were

refrigerated at 4 °C for 6 days to develop the WOF, then reheated at 100 °C for 10 min in a water bath. Odor attributes of cooked and reheated stewed beef were selected by trained panelists using sensory aroma profile analysis. Aroma compounds were extracted by solid-phase microextraction (SPME) and solvent-assisted flavor evaporation (SAFE). Aroma-active compounds were identified by detection frequency analysis (DFA) using a Q Exactive GC-Orbitrap-MS system. The quantitative analysis of aroma-active compounds was achieved by constructing external standard curves using an artificial odorless matrix with various concentrations of authentic flavor standards. Key aroma compounds verified by aroma recombination and omission experiments. Finally, key aroma-active compounds of cooked and reheated stewed beef were obtained, the changes in the aroma profiles of PCSB during cooking-refrigeration-reheating were elucidated.

In chapter IV, based on chapter III, the information about the lipid profiles were analyzed by lipidomics. Freshly cooked stewed beef samples were refrigerated at 4 °C for 0, 1, 2, 4, 6 days, then reheated at 100 °C for 10 min in a water bath. The lipid profiles of cooked and reheated samples were analyzed by using UPLC-ESI-MS/MS. Aroma profiles were analyzed by GC-MS. The differential lipid markers as potential precursors of WOF were obtained from multivariate statistical analysis, including orthogonal partial least squares discrimination analysis (OPLS-DA), correlation analysis.

In chapter V, stewed beef with tomato (25%, 50%, 75%, and 100%, w/w of raw meat) and stewed beef samples were refrigerated at 4 °C for 6 days, then reheated at 100 °C for 10 min in a water bath. PCSB and PCSBT were then used for further analyses. The impact of varying tomato addition levels on the WOF in PCSB and the mechanisms through which tomatoes influenced WOF were elucidated through the analyses of aroma profiles, lipid composition, protein secondary structure, and sensory attributes.

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2

Chapter II Literature review on formation and prevention of WOF in precooked meat products

Short overview of chapter II

In this chapter, the literature review synthesizes current knowledge regarding the research on WOF in PCSB. Aroma compounds contributing to WOF, formation pathways of WOF in precooked dishes, and potential strategies for WOF mitigation are reviewed. And the analytical techniques employed to assess WOF, including aroma extraction, enrichment, analysis, and identification are outlined. Additionally, applications of lipidomics in food science are summarized.

2.1. Introduction

Prefabricated dishes, also known as prepared dishes, are defined as finished or semi-finished culinary products that have undergone preliminary processing (full or partial cooking) by manufacturers. These products can be stored under ambient or refrigerated conditions and require only minimal preparation, such as reheating, before consumption (Wang et al., 2024b). These convenient products have gained popularity as modern lifestyles become increasingly fast-paced, with consumers valuing time-saving meal options. Industrial production of traditional cuisines, such as Chinese dishes, has accelerated in response to rising demand and is supported by advances in food processing and distribution networks. Prefabricated dishes offer consistent quality and reduce the burden of home cooking, thereby holding significant socio-economic value. The world market for these products is forecast to reach USD 350 billion by 2027, growing at a strong compound annual growth rate of 6.5% from 2021 to 2027, driven by higher disposable incomes and a preference for convenience. The Asia-Pacific region currently dominates the market, owing to large populations, urbanization, and cultural acceptance in countries like China and India, while North America and Europe also represent substantial markets due to their established food service industries and widespread use of convenience foods. Geographically, production and innovation in prefabricated dishes are concentrated in East Asia, with many leading manufacturers based in China, although the trend has become globally pervasive (DiMarket, 2025).

The currently developed prepared food products can be categorized into four types based on processing level and consumption method (Yu et al., 2022): (1) Read-to-eat food (RTE): RTE refers to products that require no further preparation and can be consumed directly upon opening the package, like RTE ham or cooked meats. (2) Heated foods: These are often fully cooked and only need to be reheated prior to serving, including prepackaged dishes like precooked stewed beef, spicy chicken, etc. (3) Ready-to-cook foods: These usually consist of partially processed ingredients, some of which may have been blanched or lightly fried, and are generally stored refrigerated or at ambient temperature. They can be directly transferred to the cooking pot, though seasoning is usually added during final preparation. Yangzhou-style pork patties are a typical example. (4) Paired foods: These involve basic physical processing such as washing and cutting. These often include fresh meat cuts or vegetables and are intended to shorten preparation time while preserving the need for full cooking and seasoning. Currently, the industrial production of prepared foods predominantly focuses on meat-based dishes, with vegetable-based options playing a supplementary role. Proteins and lipids, as the major constituents of meat, contribute significantly to their structural and sensory properties. Compared to plant-based ingredients, meat exhibits greater stability in terms of color and texture during processing and storage, particularly under high-temperature conditions such as steaming, frying, and sterilization. In addition, the Maillard reaction occurring during thermal processing enhances the development of characteristic and desirable aroma compounds, which plays a key role in flavor formation (Mottram, 1998). Consequently, the standardization of meat-based dish production is more readily achievable, making it the preferred choice for large-scale

industrial manufacturing. Precooked meat dishes, typical heated foods, stored at low temperatures generally require a secondary heating process prior to consumption; however, this reheating step can adversely affect their flavor and quality. Specifically, after undergoing heating, refrigeration, and subsequent reheating, meat products tend to develop an off-flavor known as WOF (Pegg et al., 2014).

The concept of WOF was first introduced by Tims and Watts (1958) to describe the undesirable off-flavor that develops when cooked meat products are refrigerated and subsequently reheated. They characterized this off-flavor as resembling "rancid," "stale," or "cardboard-like" odors. Subsequent sensory studies have provided further descriptions of WOF. However, it was not until Johnson and Civille (1986) established a specialized terminology system for WOF that researchers began systematically defining its sensory characteristics. For instance, Byrne et al. (2001) further described WOF with attributes such as "linseed oil-like," "stale," "wet cardboard-like," "painty," "putrid," "bitter," and "sour." A standardized sensory evaluation system for WOF was developed by (Byrne et al., 1999; Byrne et al., 2001), which included 15–25 sensory descriptors. This framework has since been adopted and refined by various researchers (An et al., 2022; O'Sullivan et al., 2003) for different meat products. Studies have shown that freshly cooked meat exhibits prominent meaty and species-specific aromas, such as characteristic pork flavor. However, as storage time extends, these desirable meat notes gradually diminish, while undesirable flavors such as rancid, linseed oil-like, sulfurous, wet cardboard-like, and bitter notes become increasingly pronounced.

It has been well established that the primary cause of WOF is the autoxidation of phospholipids (Zhang et al., 2022a). Meat lipids are composed of both intramuscular and intermuscular fat, with phospholipids being particularly rich in highly oxidizable polyunsaturated fatty acids (PUFAs). When meat is subjected to grinding, chopping, or thermal processing, cell membranes are disrupted, releasing intracellular components and exposing PUFAs to oxidative conditions (Pegg et al., 2014). Additionally, the heating process facilitates the release of catalytically active iron ions from myoglobin and other heme-containing proteins, which, in turn, accelerate phospholipid oxidation (Igene & Pearson, 1979). Unlike the slow lipid oxidation that occurs in raw meat, WOF formation is rapid and pronounced, making it a distinct phenomenon. This off-flavor emerges swiftly, typically within 48 hours of refrigerated storage followed by reheating, leading to a significant deterioration in product flavor (Pearson & Gray, 1983). Consumers generally reject products exhibiting WOF, with most readily perceiving this off-flavor (Angelo et al., 1987). Beyond its undesirable sensory impact, WOF is associated with the formation of a variety of lipid oxidation byproducts, including free radicals, hydroperoxides, and secondary compounds such as aldehydes, ketones, and malondialdehyde (MDA). Among these, aldehydes, particularly alkanals, 2-alkenals, 2,4-alkadienals, and 4-hydroxyalkenals, are of special toxicological interest due to their high reactivity and diffusibility. These compounds can migrate from their site of formation and covalently bind to proteins and nucleic acids, thereby acting as secondary toxic messengers (Eckl & Bresgen, 2017). As a result, WOF has emerged as a potential

challenge for a variety of reheated meat products offered by the food service industry, thereby impeding the industrialization of precooked dishes. With the growing market demand for such products, WOF has become a major concern within the meat industry. Consequently, both researchers and producers are increasingly focusing on elucidating the formation mechanisms of WOF and developing effective control strategies.

This review synthesizes current knowledge regarding the principal aroma-active compounds and formation pathways of WOF in prepared dishes, the analytical techniques employed to assess WOF, and potential strategies for its mitigation.

2.2. Advances in research on WOF in PCSB

Beef is a highly popular protein source worldwide due to its rich content of proteins, vitamins, and minerals, as well as relatively low fat and cholesterol levels. According to data from the Food and Agriculture Organization (FAO) and the United States Department of Agriculture (USDA), global beef production reached approximately 64 million tonnes in 2023, with consumption steadily rising. Notably, China's beef consumption reached 10.27 million tons, reflecting a 4% year-on-year growth, ranking second worldwide. Beef's versatility in cooking methods, including grilling, frying, stir-frying, and stewing, caters to diverse culinary preferences. Among these, stewed beef is a traditional and widely popular dish with numerous regional variations worldwide, reflecting each region's unique culinary traditions and flavor preferences. Despite differences in recipes, the core feature of stewed beef is a prolonged slow simmering process that renders the meat tender, succulent, and richly flavored. Stewing not only enhances the meat's sensory attributes and reduces muscle fiber toughness, but also makes it more easily digestible. In China, there is a wide variety of stewed beef dishes, including soy-braised beef, clear stewed beef, and herbal beef soup. Each of these dishes has deep roots in traditional culinary culture and has become an enduring classic in Chinese cuisine. Furthermore, compared with pan-frying, deep-frying, or stir-frying, stewing is a more stable and easily controlled cooking process, which makes it particularly important in China's prepared foods industry. However, during the industrial production and marketing of PCSB, numerous challenges have emerged. Among these challenges, the development of WOF is a major factor that compromises flavor quality and thus requires urgent attention.

Considerable research has been devoted to identifying factors contributing to WOF, elucidating the mechanisms underlying its formation in precooked beef products, and devising effective control strategies, all aimed at enhancing product flavor and advancing the PCSB industry. Poste et al. (1996) employed 2-thiobarbituric acid (TBA) value and sensory aroma scores as indics of WOF development in precooked beef. (Angelo et al., 1987) employed gas chromatography–mass spectrometry (GC–MS) combined with an external closed injection device to analyze volatile flavor compounds in reheated beef. They found that compounds associated with lipid oxidation were critical contributors to WOF formation. Notably, hexanal, 2,3-octanedione, and the total amount of volatile compounds showed a highly significant correlation with sensory scores and TBA

values. Furthermore, many of the volatile compounds identified in WOF-affected beef samples were also detected in the distillates from the TBA assay. Konopka and Grosch (1991) extended this work by using sensory evaluation in conjunction with gas chromatography-olfactometry–mass spectrometry (GC-O-MS) via solvent-assisted flavor evaporation and aroma extract dilution analysis (SAFE-AEDA) technique to identify the key odorants responsible for WOF in reheated precooked beef. Their findings highlighted compounds such as hexanal, 1-octen-3-one, both (E)- and (Z)-2-octenals, (Z)-2-nonenal, (E,E)-2,4-nonenal, and trans-4,5-epoxy-(E)-2-decenal the principal contributors to WOF. Among these, trans-4,5-epoxy-(E)-2-decenal exhibited an exceptionally high flavor dilution (FD) factor, suggesting that it is a particularly important marker of WOF development. Kerler and Grosch (1996) further investigated the formation mechanisms of WOF in precooked beef patties by combining sensory analysis with GC-O-MS, incorporating SAFE, AEDA, stable isotope dilution analysis (SIDA), and model experiments. They revealed that WOF results primarily from the loss of desirable odorants, such as 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 3-hydroxy-4,5-dimethyl-2(5H)-furanone, and the accumulation of lipid oxidation products, notably hexanal and trans-4,5-epoxy-2-(E)-decenal. Igene and Pearson (1979) investigated the role of triglycerides (TG) and phospholipids (PL) in the development of WOF in cooked beef. By incorporating TG, total lipids, total PL, phosphatidylcholine (PC), and phosphatidylethanolamine (PE) into a lipid-free muscle fiber model. Results indicated that PE is the predominant factor influencing WOF formation, while TG only contributed to WOF in the presence of PL, and PC exhibited no significant impact.

The factors influencing WOF formation in precooked beef and corresponding control measures have also been extensively studied. Yang et al. (2002) compared grass-fed and grain-fed beef during storage, revealing that grass-fed beef, with higher α-tocopherol and linolenic acid contents alongside lower linoleic acid levels, exhibited greater resistance to lipid oxidation, thereby reducing the risk of WOF. Lepper-Blilie et al. (2014) demonstrated that cooking beef in sealed cooking bags could mitigate WOF upon reheating. Gros et al. (2006) assessed the effects of different cooking methods, microwave, microwave/convection combination, and oven roasting, on WOF formation in beef patties. Their results indicated that oven roasting effectively delayed WOF development in refrigerated beef patties. Jayathilakan et al. (2007) explored the effects of natural antioxidants (e.g., Maillard reaction products, ascorbic acid, clove, and cinnamon) and synthetic antioxidants (e.g., tert-butylhydroquinone, butylated hydroxyanisole, and propyl gallate) on WOF and non-heme iron release during storage, noting that the antioxidant efficacies were in the order: MRPs > clove > ascorbic acid > cinnamon for natural antioxidants, and TBHQ > BHA > PG for synthetic ones. Moreover, nitrites have been shown to delay lipid oxidation and suppress WOF in roasted beef (Cheng & Ockerman, 1998). Mann et al. (1989) demonstrated that phosphoric acid treatment significantly reduces both WOF scores and TBA values in reheated reconstituted beef. Nunez de Gonzalez et al. (2008) reported that the incorporation of 2.5% fresh or dried plum

concentrate into precooked beef effectively diminishes lipid oxidation (as evidenced by lower TBARS values) and reduced WOF development. Cheng and Ockerman (2013) further observed that electrical stimulation induces changes in amino acids and other compounds, ultimately lowering the manifestation of desirable aromas in reheated beef. Parvin et al. (2020) found that the addition of 0.02% nutmeg extract to microwave-reheated frozen beef meatballs significantly ($P < 0.05$) reduced TBARS values, cooking loss, and WOF. To date, the specific aroma compounds associated with WOF in PCSB remain unidentified, and its precursor markers have not been clearly defined. This research gap hinders the development of targeted interventions to effectively control WOF in PCSB.

2.3. Aroma compounds contributing to WOF

The formation of WOF is primarily attributed to the secondary oxidation products of lipids. During lipid oxidation, free radical-mediated chain reactions lead to the formation of lipid hydroperoxides and conjugated dienes (or trienes). These primary oxidation products are highly unstable and readily decompose into a series of secondary oxidation compounds, including aldehydes, alcohols, ketones, carboxylic acids, and epoxides. These volatile compounds, often characterized by low sensory thresholds, are closely associated with the emergence of undesirable “cardboard-like” and “painty” off-flavors in meat products during storage. Hexanal and 2,3-octanedione were identified to be correlated to the WOF in cooked beef using GC-MS combined with sensory analysis (Angelo et al., 1987). Similarly, pentanal, hexanal, heptanal, 2,3-octanedione, and nonanal were characterized as key WOF markers in cooked lamb by direct GC with Tenax trapping (Lamikanra & Dupuy, 1990). Additionally, these compounds increased after six days of storage at 4 °C. Konopka and Grosch (1991) further reported hexanal, (Z)-2-nonenal, trans-4,5-epoxy-(E)-2-decenal, both (E)- and (Z)-2-octenals, 1-octen-3-one, and (E,E)-2,4-nonenal were key indictors to WOF development in boiled beef. Kerler and Grosch (1996) investigated the impact of refrigeration and reheating on the accumulation of lipid oxidation products in boiled beef, identifying the increased levels of n-hexanal and trans-4,5-epoxy-(E)-2-decenal as critical determinants of WOF formation. In a subsequent study, Kerscher and Grosch (1997) performed odor activity value (OAV) analyses on reheated chicken and found that the increase in hexanal and (E,E)-2,4-decenal closely paralleled the intensification of WOF. Research by O'Sullivan et al. (2003) indicated a significant correlation between sensory perception of WOF in cooked pork and the presence of hexanal, pentanal, 1-octen-3-ol, octanal, 2-pentylfuran, and nonanal, suggesting these compounds as reliable indicators of lipid oxidation. Tikk et al. (2008) further confirmed that in chilled and reheated pork, hexanal, pentanal, pentanol, and nonanal concentrations increased in direct relation to WOF intensity. Additionally, (E)-2-octenal, (E)-2-decenal, 1-octen-3-ol, and (E,E)-2,4-decadienal were identified as critical WOF markers in precooked pork products, all exhibiting OAVs exceeding 1 (Zhang et al., 2022b). Additionally, Xu et al. (2023a), investigating the impact of microwave and steam heating on beef flavor, identified (Z)-2-heptenal, tridecanal, tetradecanal, pentadecanal, and hexadecanal as the predominant WOF-related compounds.

Moreover, WOF compounds in surimi gel were characterized by An et al. (2022), results showed heptanal, (E,E)-2,4-heptadienal, (E)-2-octenal, octanal, (E)-2-nonenal, nonanal, (E)-2-decenal, (E,E)-2,4-decenal, decanal, and 2,3-pentanedione were contributed to WOF formation in surimi. The above researches suggest that aldehydes with carbon chain lengths ranging from C_3 to C_{12} play a pivotal role in both WOF development and lipid oxidation during the storage of cooked meat products (Pegg et al., 2014). Ullrich and Grosch (1987) highlighted that these aldehydes, characterized by rapid formation during lipid autoxidation and exceptionally low flavor thresholds, exert a pronounced influence on WOF perception.

2.4. Mechanisms of WOF formation

2.4.1. Lipid oxidation pathway in WOF formation

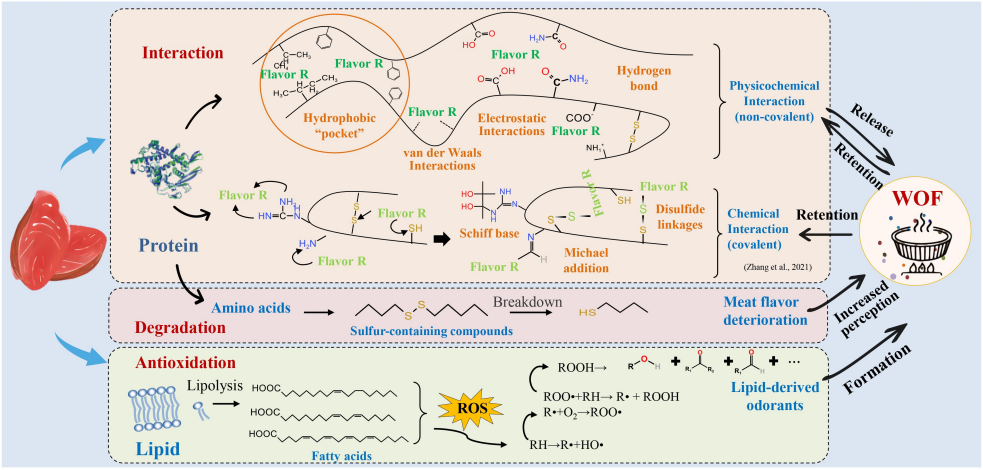

Fig. 2-1 Mechanism of WOF formation

Lipid oxidation in meat products is initiated during cooking and persists throughout storage. Thermal degradation of lipids generates free fatty acids (FFAs), with linoleic acid, oleic acid, and arachidonic acid serving as primary precursors for volatile compounds formed through lipid autoxidation (Ladikos & Lougovois, 1990). The thermal oxidation of fatty acids (FAs) is typically initiated by reactive oxygen species (ROS), such as hydroxyl radicals (•OH) or singlet oxygen, which abstract hydrogen atoms from the allylic positions of unsaturated fatty acids (UFAs), thereby generating lipid radicals. These radicals propagate through chain reactions, undergoing structural transformations such as radical coupling, atomic transfer, decomposition, or double-bond rearrangement, leading to conjugated diene formation (Dinh et al., 2021). As oxidation progresses, lipid radicals react with oxygen, forming hydroperoxides as primary oxidation products. Although hydroperoxides are odorless, their weak O–O bonds and thermal sensitivity make

them unstable and prone to decomposition into alkoxyl and hydroxyl radicals. These radicals further degrade, generating a variety of volatile and non-volatile secondary oxidation products, including aldehydes, ketones, carboxylic acids, alcohols, lactones, furans, and epoxides, which contribute to the aroma of cooked meat (Frankel, 1980; Mottram, 1998). This stage of the reaction is commonly referred to as chain termination. Even in refrigerated storage, lipid hydrolysis continues, releasing FFAs that undergo further oxidation through free radical reactions, a process known as autoxidation. This reaction may be catalyzed by singlet oxygen, metal ions, heme compounds, ultraviolet radiation, or certain enzymes (Pegg et al., 2014). Furthermore, the disruption of muscle membranes during cooking releases free iron, further promoting lipid oxidation (Drumm & Spanier, 2002). Both thermal oxidation and autoxidation follow radical initiation, propagation, and termination mechanisms, though the rate of thermal oxidation is significantly faster (Choe & Min, 2007; Dinh et al., 2021). Among oxidation products, short-chain aldehydes, ketones, and alcohols, including pentanal, hexanal, (E)-2-octenal, (E,E)-2,4-decadienal, and 1-octen-3-ol, are notable for their low odor thresholds. In moderate amounts, these compounds enhance meat flavor, but excessive accumulation leads to undesirable off-flavors such as “green,” “fatty,” “rancid,” “cardboard-like,” or “pungent” notes, which become more pronounced with extended storage (Pegg et al., 2014). Hexanal, a primary oxidation product of linoleic and arachidonic acids, is widely recognized as a key WOF marker (Merlo et al., 2021). Additionally, the oxidation of oleic and linoleic acids produces 2-alkenals, intensifying oxidative off-flavors (Elmore et al., 1999). The main differences between autoxidation and thermal oxidation of meat products during processing lie in oxygen availability and temperature. During thermal oxidation, optimal oxygen levels (Choe & Min, 2007) and high cooking temperatures (Wasserman, 1972) accelerate the conversion of short-chain aldehydes, ketones, and alcohols into organic acids and esters. Lipid peroxides also degrade, forming oxygen-containing heterocyclic compounds such as cyclic carboxylic acids and lactones. Saturated fatty acids (SFAs), when exposed to high temperatures, break down into long-chain alkanes, aldehydes, and lactones, while the reduction of short-chain and unsaturated aldehydes and alcohols improves the desirable aroma of cooked meat while reducing volatility (Dinh et al., 2021). During cooking, lipid oxidation products influence flavor and interact with Maillard reaction intermediates, forming alkylthiazoles, alkylpyrazines, and alkylpyridines, which contribute to roasted and meaty aromas (Mottram, 1998). Notably, such interactions do not occur during the auto-oxidation process in storage (Dinh et al., 2021). Furthermore, UFAs, especially those on the meat surface, are highly susceptible to oxidation and may polymerize, forming oxygen-rich dimers and polymers. PUFAs are particularly prone to cyclization, and extensive polymerization of UFAs and PUFAs reduces the formation of volatile compounds, thereby endowing cooked meat with a more desirable flavor profile (Dinh et al., 2021; Mottram, 1998). However, lipid thermal oxidation products, such as dimers and cyclic compounds, may undergo further oxidation during storage, ultimately decomposing into off-flavor compounds that intensify WOF (Dinh et al., 2021). These off-notes predominantly accumulate during refrigeration and are

released upon reheating, enhancing the perception of WOF. A study by Huang et al. (2024) demonstrated that in surimi gel products, WOF primarily develops during cold storage. Refrigeration and subsequent reheating accelerate the loss of UFAs, while repeated freezing and reheating cycles result in a relative increase in SFAs. In summary, autoxidation is the central mechanism behind WOF formation, with lipid hydrolysis acting as a key driving factor in this oxidative process.

If the product is packaged in transparent materials and exposed to light, particular attention should be paid to quality deterioration and discoloration caused by photooxidation. Unlike autoxidation, photooxidation requires both light and photosensitizers, and it proceeds via two rapid pathways: (1) direct excitation of photosensitizers to generate free radicals (Type I), and (2) energy transfer leading to the formation of singlet oxygen (Type II) (Dominguez et al., 2019). Both routes result in the rapid formation of lipid hydroperoxides, with reaction rates significantly higher than those of autoxidation.

2.4.2. Protein degradation pathway in WOF formation

Beyond lipid oxidation, protein degradation significantly contributes to WOF. Aroma compounds in meat products are generally categorized as either enhancers of the desirable meaty aroma or as non-contributors. Notably, non-cyclic sulfur compounds, as well as aromatic and non-aromatic heterocyclic compounds and lactones, are recognized as primary Maillard reaction products that are fundamental to the desirable meat aroma (Elmore & Mottram, 2006; Khan et al., 2015). Precursors such as cysteine and methionine undergo degradation to yield a range of sulfur-containing compounds including thiophenes, thiazoles, and others (Drumm & Spanier, 1991). Typically characterized by extremely low odor thresholds, these sulfurous compounds are essential for enhancing meaty aroma. However, during refrigeration, the breakdown of certain sulfur-containing heterocycles contributes to WOF, resulting in a gradual attenuation of the inherent meaty aroma while off-notes from lipid oxidation become more pronounced, potentially obscuring the perception of desirable odorants (Angelo et al., 1990). Kerler and Grosch (1997) observed that a reduction in 2-furfurylthiol intensifies the perception of WOF in reheated chicken. Likewise, Kerler and Grosch (1996) confirmed that, in boiled beef, the formation of WOF during refrigeration and subsequent reheating is closely linked to the loss of desirable aroma compounds, specifically 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 3-hydroxy-4,5-dimethyl-2(5H)-furanone. Moreover, certain low-molecular-weight sulfur compounds, cyclic or aliphatic, accumulate significantly during storage. At low concentrations, these compounds may contribute to the meaty aroma; however, at higher levels they can impart strong sulfurous odors, thereby contributing to off-flavor formation. Additionally, some sulfur-containing molecules are capable of undergoing cross-linking reactions with proteins or lipids, resulting in further sensory deterioration characterized by sulfurous off-notes. An et al. (2022) demonstrated that WOF in surimi-based gel products is driven not only by lipid oxidation but also by protein degradation, which leads to an increase in nitrogen- and sulfur-containing compounds such as 2-propylpyridine, 2,6-dimethylpyrazine, benzothiazole, 2-furanmethanethiol, and

2-methoxybenzenethiol (An et al., 2022; Zhang et al., 2023). In meat products, a decrease in sulfur compounds tends to promote WOF, whereas in surimi gels, WOF is linked to their accumulation. This difference may be due to variations in both the levels and flavor characteristics of these compounds. Drumm and Spanier (1991) observed that during refrigeration, the levels of thiols such as 1,1-ethanedithiol, 1-(methylthio)ethanethiol, and 2-furanmethanethiol increased in cooked beef, whereas dimethyl trisulfide declined ($P < 0.05$). Lipid radicals facilitate sulfur compound degradation, such as dimethyl trisulfide, while promoting the formation of other sulfur volatiles, including 2-furanmethanethiol ($P < 0.05$). Byrne et al. (2002) conducted sensory analysis and found WOF closely linked to "rancid" and "sulfur/rubber-like" attributes, along with a reduction in the typical "meaty" aroma of chicken. Early in refrigeration (day 1), key thiol-based aroma compounds diminished while dimethyl trisulfide increased. As storage continued (day 4), progressive lipid oxidation reduced oxygen availability, causing partial conversion of sulfur compounds to thiols, leading to an overall reduction in sulfur volatiles. However, in later stages, thiols and sulfur compounds re-enter radical reactions, decreasing their concents, while sulfur radicals react with lipids and proteins, intensifying the "sulfur/rubber" odor ($P < 0.05$) and significantly diminishing the desirable cooked chicken aroma ($P < 0.05$). Some small sulfur-containing compounds also undergo cross-linking with proteins or lipids, forming additional off-flavors, a mechanism regarded as a contributor to WOF formation (Byrne et al., 2002). During the early storage period, WOF-related off-flavors may be initially masked due to limited oxidation-derived volatiles. However, as storage time extends, WOF intensity increases, making undesirable aromas more perceptible (Pegg et al., 2014).

Another factor contributing to WOF is peptide breakdown through enzymatic activity. Studies have shown that even after heat treatment and subsequent refrigeration, some meat enzymes remain active, breaking down proteins into small amino acids. Some sulfur-containing amino acids undergo further degradation, breaking disulfide bonds and forming volatile derivatives. These derivatives interact with other flavor precursors, leading to chemical transformations that enhance WOF development (Chen et al., 2024; Pegg et al., 2014).

2.4.3. Flavor-proteins interactions

The intensity of WOF is closely linked to its perception, which is primarily determined by the composition of aroma compounds and their release or retention within the food matrix (Zhang et al., 2021). Studies indicate that muscle proteins, such as myofibrillar proteins (including myosin and other matrix proteins), serve as effective binding matrices for volatile compounds, facilitating interactions with aldehydes, ketones, alcohols, esters, furans, and pyrazines (Wang et al., 2023b). Proteins can bind aroma compounds either reversibly or irreversibly. Aldehydes, particularly unsaturated ones, thiols, and certain furans, exhibit strong reactivity with proteins. Some odorants form covalent bonds with protein side chains, such as lysine-lysine and amine-carbonyl interactions, leading to irreversible binding that reduces the availability of key aroma compounds, thereby amplifying off-flavors

(Zhang et al., 2021). Covalently bound compounds remain trapped, whereas non-reacted fractions may be retained within the protein network, affecting overall flavor perception (Reineccius, 2022). These interactions influence both aroma retention and food quality deterioration. Sulfur compounds interact with proteins through covalent and non-covalent forces. Non-covalent interactions, primarily driven by hydrophobic forces, are reversible, enabling aroma compounds to be released under specific conditions (Zhang et al., 2021). Typically, unfolded or moderately denatured proteins offer multiple binding sites for aroma compounds (Wang & Arntfield, 2016).

Factors such as temperature, pH, ionic strength, and oxidation can significantly alter protein structure and, consequently, flavor-binding capacity (Zhang et al., 2021). For example, Xu et al. (2019) found that short-term treatment at 90 °C (< 10 min) increases the surface hydrophobicity and sulfhydryl content of myofibrillar proteins (MP), facilitating secondary structure unfolding and improving aldehyde binding. However, prolonged heating (> 10 min) leads to protein refolding and aggregation, reducing hydrophobic interactions and decreasing flavor-binding capacity. Similarly, Yang et al. (2017) demonstrated that pH variations significantly influence the surface hydrophobicity and secondary structure of duck myofibrillar proteins, thereby affecting their adsorption of alcohols, aldehydes, ketones, and esters. As pH increases from 5.0 to 8.0, protein unfolding decreases along with hydrophobicity, exposing Schiff base and electrostatic sites that enhance aldehyde and ester binding while reducing ketone adsorption. Salt concentration also affects protein stability and intermolecular interactions, altering flavor-binding capacity. Higher concentrations of non-chaotropic salts stabilize hydrophobic regions, enhancing protein-ketone interactions (e.g., 2-hexanone, 2-heptanone, and 2-octanone), whereas chaotropic salts disrupt these interactions, lowering binding affinity. Yu et al. (2024) demonstrated that increasing salt concentration from 0.6% to 2.4% alters protein secondary structure, reducing α-helix content while increasing β-sheet structures, which enhances MP binding capacity for furan volatiles. Oxidation by free radicals and lipid hydroperoxides modifies protein structure, impacting its ability to bind volatiles (Domínguez et al., 2019). Zhang et al. (2021) reported that oxidation-induced structural changes influenced protein binding capacity. Certain natural antioxidants also alter protein-flavor interactions. Zhao et al. (2023) found that lycopene binds hydrophobically to myosin, modifying its secondary structure. Similarly, Huang et al. (2022b) showed that mulberry polyphenols promote myofibrillar protein unfolding and aggregation, shifting α-helix structures to β-turn formations, which influences flavor binding. Huang et al. (2022a) reported that rosmarinic acid (RA), carnosic acid (CA), and carnosol (CS) compete for MP hydrophobic sites, promoting the release of fishy odor compounds.

Other factors, including mild oxidation (Shen et al., 2020), enzymatic hydrolysis (Zhao et al., 2020), microwave treatment (Han et al., 2021), high-pressure processing (Yang et al., 2018), and macromolecular interactions within the food matrix (Martins et al., 2010), also influence protein-flavor interactions. In conclusion, protein structure and its interaction with aroma compounds play a vital

role in WOF development and perception. Protein denaturation, oxidation, enzymatic activity, and environmental factors regulate binding affinities, shaping the final sensory attributes of food.

2.5. WOF regulation strategies

Currently, the mitigation of warmed-over flavor (WOF) in prepared dishes primarily relies on limiting lipid oxidation and protein degradation throughout processing, storage, and reheating stages (Chen et al., 2024).

Thiobarbituric acid reactive substances (TBARS) value is widely used as a representative index to monitor lipid oxidation, serving as an indirect indicator of WOF intensity. A marked increase in TBARS values often correlates with the accumulation of secondary lipid oxidation products, which are known to impair flavor quality (Cheng & Ockerman, 2013; Lepper-Blilie et al., 2014; Parvin et al., 2020; Yang et al., 2002). In addition to TBARS, the presence and concentration of specific aroma compounds have been extensively recognized as effective markers for assessing the degree of WOF formation. Compounds such as pentanal, hexanal, heptanal, octanal, nonanal, pentanol, 1-octen-3-ol, 2,3-octanedione, 2-pentylfuran, and 2-heptanone, which are typical products of lipid oxidation, significantly increase during WOF development (Konopka & Grosch, 1991; O'Sullivan et al., 2003; Tikk et al., 2008; Zang et al., 2020).

The effectiveness of WOF regulation strategies is thus commonly assessed by monitoring the reduction in TBARS levels and the inhibition or modulation of key odorant markers. Given the significant impact of WOF on the quality of precooked meat dishes, this review provides a systematic summary of both current and potential approaches for its control, with the aim of improving flavor stability during storage and reheating.

2.5.1. Antioxidant

Antioxidants in food production can be broadly classified into natural antioxidants (including those derived from plants) and synthetic antioxidants.

2.5.1.1. Natural antioxidants

Natural antioxidants are primarily derived from plant polyphenols, which are abundant in fruits, vegetables, nuts, seeds, leaves, roots, and bark. Their mechanisms include free radical scavenging, metal chelation, and singlet oxygen quenching (Jayathilakan et al., 2007). For example, vitamin E (VE) acts as both a radical scavenger and a singlet oxygen quencher (Yang & Min, 1994), while ascorbic acid functions mainly as an oxygen scavenger to delay lipid oxidation (Jadhav et al., 1995). Other natural sources include pepper extracts, which effectively inhibit off-flavor formation in meat (Emrick et al., 2005), and rosemary extract, which demonstrates strong antioxidant properties and is more effective in suppressing WOF in cooked pork patties compared to grape skin, green tea, and coffee extracts (Nissen et al., 2004). Additionally, plum extracts at a 2.5% addition level have been shown to significantly reduce lipid oxidation and WOF in precooked beef (de Gonzalez et al., 2008). Nutmeg extract at 0.02% can lower oxidation rates and WOF

development in reheated beef meatballs (Parvin et al., 2020). Clove essential oil improves antioxidant performance and reduces oxidation-induced off-flavors in beef sausages (Gamil Sedki et al., 2020). Winter savory (*Satureja montana* L.) markedly decreases TBARS in precooked pork chops, thus suppressing WOF (Jokanović et al., 2020). Powders from *Moringa oleifera* roots and leaves effectively slow WOF development in cooked pork mince during refrigeration (Lungu et al., 2022). Moreover, perilla juice (PJ) and ginger juice (GJ) have been found to reduce lipid and protein oxidation in surimi gels, lowering the levels of heptanal and decanal by more than 30% and decreasing compounds associated with cardboard and rubber aromas by over 50% (An et al., 2024). Polysaccharides have shown potential as bioactive compounds with antioxidative properties through free radical scavenging, lipid peroxidation inhibition, and biomembrane protection (Liu et al., 2018). Their mechanisms primarily involve superoxide radical scavenging, redox potential modulation, and metal chelation (Chen et al., 2024). For instance, extracts from *Ostrea rivularis Gould* is significantly enhance total antioxidant capacity (TAOC) and reduce malondialdehyde (MDA) formation (Li et al., 2015), while resveratrol exhibits effective DPPH•, ABTS•$^{+}$, and O_2•$^{-}$ scavenging activity while chelating Fe^{2+} to inhibit lipid peroxidation, making it suitable for both food and pharmaceutical applications (Gülçin, 2010). In summary, antioxidants play a crucial role in controlling WOF. Although many plant polyphenols, terpenoids, and flavonoids act as effective antioxidants at appropriate levels, they may exhibit pro-oxidant effects at high concentrations due to redox cycling (Palozza et al., 2003). Thus, careful selection and controlled application of antioxidants are essential in food processing to enhance the stability and shelf life of precooked dishes.

2.5.1.2. Synthetic antioxidants

Synthetic antioxidants widely employed in the meat industry include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) (Ito et al., 1986). Jayathilakan et al. (2007) demonstrated that at a concentration of 0.02%, these antioxidants effectively inhibited lipid oxidation in lamb, beef, and pork during cooking and refrigeration, while also reducing the release of non-heme iron. Their antioxidative effectiveness ranked as TBHQ > BHA > PG. However, concerns over the potential toxicological risks of synthetic antioxidants have emerged in recent years. Studies suggest that BHA and BHT may be associated with gastrointestinal disorders and food allergies (Ribeiro et al., 2019; Silva et al., 2009). As consumer awareness of health risks increases, the food industry is shifting towards natural alternatives.

2.5.2. Functional additives

During cooking and storage of meat products, the release of free iron accelerates lipid oxidation. Negatively charged phospholipids bind to free iron, promoting localized oxidation, which can lead to WOF within 24 h of refrigeration. Mann et al. (1989) found that phosphate treatment significantly reduced WOF intensity and TBARS values in reheated restructured beef. Furthermore, phytates, by chelating iron ions, markedly diminish WOF formation, and multivalent cations can slow

WOF progression through competitive displacement of iron from phospholipids (Graf & Panter, 1991). Researches have shown that adding nitrites significantly lowers TBARS values in cooked beef and chicken after 48 hours of refrigeration, reducing TBARS by 50% in beef and chicken, and by up to 80% in pork, accompanied by sensory evaluations indicating a reduction in WOF (Fooladi, 1979). In a phospholipid model system, 25 mM Ca^{2+} inhibited malondialdehyde formation by 50% (Graf & Panter, 1991). The antioxidant mechanism of nitrites likely involves three aspects: (1) forming stable complexes with heme to suppress the release of non-heme iron and its catalytic effect on lipid oxidation; (2) directly binding Fe^{2+} released during heme degradation, thereby reducing its oxidative potential; and (3) stabilizing unsaturated lipids within the cell membrane to prevent oxidation (Igene et al., 1985). Although nitrite curing effectively reduces WOF, its use has declined due to potential health risks, with the food industry increasingly favoring natural antioxidants (Ito et al., 1986; Ribeiro et al., 2019; Silva et al., 2009).

2.5.3. Reduction of WOF retention through flavor-protein interactions

The perception of WOF is influenced not only by the concentration and thresholds of the volatile compounds but also by their interactions within the food matrix, particularly their binding to proteins (Wang et al., 2023b). Proteins can adsorb aroma compounds through specific molecular interactions and subsequently release them under environmental changes such as variations in temperature, pH, or oxidation state. These interactions are regulated by multiple factors, including inherent protein conformational changes and the presence of external additives like salt ions and phenolic compounds (Huang et al., 2022a; Shen et al., 2019). Moreover, natural antioxidants not only inhibit lipid oxidation but also induce conformational changes in proteins, affecting the binding sites for flavor molecules and thereby modulating their retention and release. For example, rosmarinic acid (RA), carnosic acid (CA), and carnosol (CS) can form complexes with myofibrillar proteins via hydrophobic interactions, reducing the available binding sites for off-flavor compounds (Huang et al., 2022a). In addition, phenolic compounds may compete for these hydrophobic sites, promoting the release of unwanted aromas. Variation of pH also influences protein adsorption of volatile compounds by altering the microenvironment of amino acid residues, modifying protein hydrophobicity, and inducing secondary structure rearrangement (Yang et al., 2017). These factors collectively determine the binding and release dynamics of WOF within the food system, ultimately impacting sensory perception.

2.5.4. Masking techniques

Masking is a traditional approach to flavor modulation, typically involving smoking, the addition of strongly flavored seasonings (e.g., spices), or the use of barrier films to mitigate off-flavors in meat products (Ren et al., 2024). Jokanović et al. (2020) reported that adding winter savory (*Satureja montana* L.) not only reduced TBARS values in precooked pork chops but also masked WOF-associated off-flavors through its inherent aromatic compounds, thereby improving product

flavor. Similarly, onion and ginger extracts have been utilized to suppress undesirable flavors in meat by-products. Luo et al. (2022) demonstrated that this approach significantly decreased the concentrations of hexanal, 1-nonanol, 2-octanone, octanal, and 1-octen-3-ol in treated products, likely due to interactions between spice compounds and off-flavor molecules, which reduced their sensory perception. Lepper-Blilie et al. (2014) further found that using high-barrier oxygen oven bags during reheating preserved desirable attributes such as “rich broth” and “fatty” while effectively suppressing oxidation-related off-notes. An et al. (2024) observed that yeast extracts with high concentrations of pyrazines and esters mask WOF in surimi gel, improving its overall flavor profile. Similarly, Dang et al. (2024) demonstrated that the distinct aromas of perilla juice and ginger juice effectively masked WOF in surimi-based products, enhancing their sensory characteristics. Collectively, masking techniques balance flavor profiles within the food matrix, reducing the sensory impact of WOF and improving the palatability of meat and surimi-based products.

2.5.5. Supercritical carbon dioxide for WOF removal

Supercritical carbon dioxide (SC-CO_2) refers to CO_2 above its critical point (7.38 MPa, 31.06 °C), where it exhibits both the low viscosity and high diffusivity of a gas, and the high density and solvation power of a liquid. This dual characteristic allows SC-CO_2 to selectively extract non-polar compounds. In addition, its low cost, non-toxicity, and non-flammability, along with the moderate temperatures employed in SC-CO_2 extraction, make it particularly suitable for food applications. Previous studies have shown that SC-CO_2 can effectively remove off-odor compounds from foods. Thongwong et al. (1999) demonstrated that SC-CO_2 significantly reduced volatile compounds associated with WOF in precooked meat, with a 73.5% reduction in hexanal at 30 MPa and a 60.3% reduction at 10.3 MPa. More recently, Abril et al. (2023) applied SC-CO_2 for deodorizing pork liver, successfully eliminating key off-flavor compounds such as 1-octen-3-ol, 1-nonanol, and (E,E)-2,4-heptadienal. However, research on the use of SC-CO_2 for removing volatile off-odors in precooked meat dishes remains limited. Further studies are needed to assess its techno-economic feasibility, optimize processing parameters, and improve its potential for industrial-scale application. Despite current challenges, SC-CO_2 remains a promising, efficient, and environmentally friendly technology for deodorization.

2.5.6. Raw material selection

The choice of raw materials significantly influences off-flavor formation, as the inherent antioxidant content can effectively reduce WOF, while materials rich in pro-oxidants may accelerate its development. Research indicates that feeding practices significantly influence the oxidative stability of meat products. Yang et al. (2002) found that beef from cattle primarily fed on forage exhibited significantly lower levels of off-flavor during storage compared to grain-fed counterparts. This effect is likely attributed to the higher VE content in forage-fed beef, which enhances antioxidative capacity. Similarly, Higgins et al. (1999) reported that

supplementing turkey feed with α-tocopheryl acetate effectively reduced WOF development during refrigerated storage. O'Sullivan et al. (2003) further confirmed that dietary VE supplementation lowered WOF, whereas iron supplementation accelerated its formation. Beyond feeding strategies, pre-slaughter handling also influences WOF development. Byrne et al. (2001) demonstrated that pre-slaughter stimulation could mitigate the accumulation of off-flavors during storage. Due to higher phospholipid and iron content, red poultry muscle exhibits a greater susceptibility to WOF compared to white poultry muscle (Mielche & Bertelsen, 1994). Additionally, the oxidative stability of different muscle types varies significantly. Wu and Sheldon (1988) observed that during cold storage, the outer layer of roasted turkey rolls developed off-flavors more rapidly than the inner portion, likely due to its higher phospholipid and iron content. Pegg et al. (2014) and Jayathilakan et al. (2007) reported that the oxidative stability of different meat types follows the order: fish > poultry > pork > beef > lamb. This variation is primarily influenced by muscle lipid composition, phospholipid content, and myoglobin levels. In summary, feeding strategies, pre-slaughter handling, muscle type, and the intrinsic balance between antioxidative and pro-oxidative components all play essential roles in WOF formation. Selecting appropriate raw materials and optimizing feeding and processing conditions can enhance flavor stability and minimize the development of off-flavors in meat products.

2.5.7. Processing technologies

Sous-vide (SV) is a low-temperature vacuum cooking method that involves sealing food in plastic bags prior to cooking and then subjecting it to prolonged heating at controlled, mild temperatures. Owing to its ability to preserve food texture, nutritional value, and flavor, SV is regarded as one of the most promising methods for developing high-quality food products (Chen et al., 2024; Latoch et al., 2023). Studies have demonstrated that compared to roasting lamb patties, SV significantly reduces TBARS values, thereby diminishing the occurrence of WOF (Ortuño et al., 2021).

Cold plasma (CP) is an active medium composed of excited atoms, molecules, ions, and free radicals, including electrons, charged particles, ground-state or excited gas molecules, and ultraviolet radiation (Gavahian et al., 2018). Due to its abundance of high-energy reactive species, CP effectively eliminates spoilage microorganisms, extending meat shelf life. However, during CP treatment, if oxygen (O_2) and nitrogen (N_2) are present, highly reactive oxygen (ROS) and nitrogen (RNS) radicals may be generated, which can promote lipid oxidation on the surface of uncooked meat. This leads to the formation of both volatile and non-volatile secondary oxidation products such as alcohols, aldehydes, carbonyls, furans, and hydrocarbons (Bak & Paulsen, 2023; Gavahian et al., 2018). The accumulation of these oxidation products may exacerbate WOF, thereby deteriorating the flavor quality of meat. In contrast, CP treatment in cooked meat has shown promising results in suppressing off-flavors. For example, Hui et al. (2023) found that CP-assisted curing significantly reduced lipid oxidation–induced off-odors in superheated steam–roasted beef ($P < 0.05$). Hence, optimizing CP parameters and

combining this method with complementary technologies is essential to enhance its flavor control efficacy and industrial applicability.

2.5.8. Packaging strategies

Research has clearly indicated that the development of WOF is closely related to the availability of oxygen. Both vacuum packaging (VP) and modified atmosphere packaging (MAP) have proven effective in inhibiting the progression of WOF in cooked meat products (Stapelfeldt et al., 1993). Whereas, their suitability depends on product characteristics. VP is ideal for sliced meat but less practical for RTE meals containing meat juices or rice, where deep vacuum processing may not be feasible. In such cases, MAP serves as a viable alternative, allowing better control over packaging conditions and delaying WOF onset (Stapelfeldt et al., 1993). Packaging material and gas composition should be optimized to maintain residual oxygen levels below the critical threshold for WOF development (Mielche & Bertelsen, 1994; Stapelfeldt et al., 1993).

Active packaging (AP) has gained increasing attention in the meat industry, particularly with the incorporation of natural antioxidants. Extracts and essential oils from plant sources and grain by-products have been utilized in AP to extend shelf life and reduce oxidative changes (Ribeiro et al., 2019). For instance, José M. Lorenzo et al. (2014) found that storing foal meat at 2 °C under an 80% O_2/20% CO_2 atmosphere with an oregano oil-infused active film effectively lowered lipid and protein oxidation. More recent research has focused on developing single- or multi-layer packaging films using nanoparticles, polysaccharides, and essential oil emulsions. Shen et al. (2022) reported that chitosan-curcumin nanoparticles effectively inhibited lipid oxidation in pork, while Hamzaoui et al. (2020) demonstrated that green algae polysaccharides significantly reduced lipid oxidation in refrigerated beef sausages. These findings highlight the potential of antioxidant-infused active packaging for improving shelf stability and flavor retention in meat products.

2.5.9. Low-temperature storage methods

Conventional low-temperature storage methods include refrigeration (0−4 °C) and freezing (−18 to −40 °C). While refrigeration preserves food quality, its shelf life is limited. Freezing extends storage duration but increases drip loss upon thawing and has higher energy demands (Cao et al., 2023). To address these limitations, ice-temperature storage (ITS) and micro-freezing storage (MFS) have gained attention as novel preservation techniques.

ITS involves storing food at temperatures between 0 °C and its initial freezing point, a range lower than standard refrigeration but above complete freezing. Compared to conventional refrigeration, ITS extends shelf life while better preserving quality and has been widely applied in fresh produce and seafood preservation (Cao et al., 2023). Ji et al. (2021) found that that chicken breast stored at -1.5 °C exhibited a significantly slower decline in muscle solubility, total sulfhydryl content, and soluble sulfhydryl levels compared to samples stored at 4 °C, indicating that ITS effectively delays protein oxidation and degradation. MFS, also

known as partial freezing or supercooled refrigeration, maintains food at 1−2 °C below its freezing point, subjecting it to mild freezing conditions. This method inhibits microbial growth while minimizing structural damage to muscle tissues, thereby extending the shelf life of meat products (Cao et al., 2023). Cao et al. (2023) compared traditional refrigeration, freezing, and modern low-temperature storage methods (ITS and MFS), results showed that freezing provided the longest shelf life, while ITS best preserved product quality within its storage duration. Additionally, MFS exhibited superior protection against myofibrillar protein oxidation and microstructural damage.

2.5.10. Reheating methods

Precooked dishes typically require reheating or secondary cooking before consumption, a process that often exacerbates lipid oxidation and contributes significantly to WOF formation. Currently, common reheating methods for precooked dishes include direct flame reheating, steam reheating, water bath reheating, and microwave reheating (Song et al., 2022). Zhang Kaihua et al. (2018) found significant differences in the effects of various reheating methods on lipid oxidation and key WOF flavor compounds. Steam reheating, while producing the highest level of oxidation, results in relatively minor changes in flavor; conversely, pasteurization-style reheating (90 °C water bath for 15 min) yields slightly lower oxidation levels yet significantly increases the concentration of key WOF compounds. High-temperature reheating (121 °C for 15 min) led to lower levels of lipid oxidation compared to both pasteurization and steam reheating but still elevated WOF-related volatiles. Conversely, microwave reheating significantly reduced the concentration of WOF markers, demonstrating potential in mitigating WOF. Li et al. (2023) further investigated the effects of different reheating methods, microwave (MR), steam (SR), open flame (OR), and boiling (BR), on the edible quality, lipid oxidation, and flavor characteristics of a stewed beef and potato dish. The results revealed that, except for microwave reheating, steam, open flame, and boiling methods significantly increased TBARS values, with open flame reheating yielding the highest TBARS levels and being the least effective at inhibiting WOF. However, in terms of flavor and color, open flame reheating enhanced the sensory qualities of both beef and potatoes more than the other methods. Additionally, Ping et al. (2024) compared microwave, steam, and water bath reheating for ceramic pot–sealed meat (CPSM) and found that microwave reheating best preserved the original aroma and taste of CPSM, achieving the highest sensory scores while effectively reducing WOF-related volatiles. In contrast, water bath reheating produced the highest levels of off-flavor compounds.

In conclusion, the choice of reheating method significantly influences both WOF development and the overall flavor quality of precooked dishes. Appropriately controlled microwave reheating conditions can reduce WOF formation while preserving the sensory integrity of food. Further optimization of microwave heating protocols could enhance the quality and consumer appeal of ready-to-eat meals.

2.6. Analytical techniques for aroma compounds in meat products

The flavor of meat and its products is marked by trace levels of compounds that are both diverse and complex. Consequently, researchers have developed a comprehensive analytical framework to systematically elucidate the aroma compounds present in these products.

2.6.1. Extraction techniques for aroma compounds

The extraction of aroma components is a fundamental step in flavor analysis and is crucial for ensuring the accuracy of results. Selecting an extraction method that faithfully represents the authentic aroma composition of meat products is essential. Currently, the primary extraction techniques for aroma compounds in thermally sensitive meat products mainly include solid-phase microextraction (SPME*)*, headspace sampling (HS), solvent-assisted flavor evaporation (SAFE*)*, and supercritical fluid extraction (SFE*)*.

SPME combines sampling, extraction, concentration, and injection into one solvent-free, environmentally friendly technique. This technique effectively minimizes alterations to the original aroma profile. However, different fiber coatings, owing to their varying polarities, may lead to a selective extraction of compounds, potentially limiting the representativeness of the aroma profile (Gu et al., 2025).

HS, encompassing static (SHS) and dynamic (DHS) forms, directly collects volatile compounds from the space above the sample. By avoiding artifacts from thermal concentration. HS is particularly effective in enriching highly volatile substances. This approach has been successfully applied to study WOF-related volatiles in precooked pork (Zang et al., 2020).

SAFE consists of a small-scale distillation unit coupled with a high-vacuum pump, designed to eliminate high-boiling and less-volatile components from solvent extracts. Conducted under low-temperature and high-vacuum conditions with liquid nitrogen-cooled trapping, this method effectively prevents the formation of WOF while minimizing interference from high-boiling pigments and other matrix-derived impurities. SAFE has proven to be highly effective in extracting medium- and low-volatility compounds and has been successfully applied in studies on duck broth (Pu et al., 2022), dry-cured ham (Chen et al., 2023), and sauced beef (Wang et al., 2023a).

SFE employs supercritical CO_2, whose solvent properties can be tuned by adjusting temperature and pressure, to efficiently extract aroma compounds from meat products. SC-CO_2 offers mild operating conditions, short extraction times, low toxicity, and high sustainability. The process parameters can be optimized according to the physicochemical properties of the target compounds, enhancing extraction efficiency and compound specificity (Moreira et al., 2023).

2.6.2. Aroma-active compound screening

Meat matrices contain numerous aroma compounds, yet only a few critically influence the overall aroma. These pivotal substances are known as aroma-active compounds (Acree & Barnard, 1994; Wang et al., 2023a). Gas Chromatography-Olfactometry (GC-O) utilizes the human nose as a highly sensitive detector, enabling direct evaluation of the odor characteristics and intensities of individual volatiles. Based on experimental principles, GC-O methods include frequency detection methods, dilution to threshold methods and direct intensity methods (Brattoli et al., 2013).

Detection frequency methods is a simple but efficient GC-O approach that does not require specialized training for sensory assessors. Typically, at least three panelists simultaneously sniff an undiluted aroma extract, noting the retention time (Rt) and odor descriptors of each compound. The frequency detected for each compound was summarized. An aromagram is then constructed, with retention index (RI) as the x-axis and detection frequency (DF) as the y-axis. Peak height in the DF spectrum reflects the number of assessors who detected a given compound, independent of odor intensity. A higher detection frequency indicates a stronger contribution to the overall aroma profiles (Yu & Chen, 2010). This method has been effectively used in analyzing key aroma compounds in mangrove crab (*Scylla serrata*) (Yu & Chen, 2010) and cantaloupe (Pang et al., 2012).

Direct intensity analysis is a GC-O technique that evaluates the contribution of aroma compounds based on their perceived odor intensity. Known as the OSME technique, it utilizes a computerized 0-16-point scale to continuously record changes in odor intensity and corresponding sensory attributes (Brattoli et al., 2013; Fu et al., 2002). The resulting OSME spectrum closely resembles the chromatographic signal obtained via flame ionization detection (FID). In the OSME spectrum, peak height is directly proportional to a compound's impact on overall aroma, with higher peaks indicating greater odor significance (Delahunty et al., 2006). Since this method requires assessors with highly developed olfactory sensitivity and extensive sensory training, its application in food aroma research is relatively limited, a few applications have been reported, such as cashew juice (Garruti et al., 2006), fermented bamboo shoots (Fu et al., 2002), and fish sauce (Pham et al., 2008). However, due to its precision in quantifying aroma contributions, the OSME technique remains valuable for high-precision food flavor analysis.

Dilution to threshold methods is a widely used technique involving serially diluting the aroma extract and analyzing it via GC-O to determine the detection threshold and contribution of each compound. A sensory panel (typically 8—12 evaluators) records the time of odor detection, quality, and intensity. Commonly used methods in this category are aroma extract dilution analysis (AEDA) (Ullrich & Grosch, 1987) and charm analysis (Acree & Barnard, 1994). In AEDA, the highest dilution at which at least one panelist can still detect an odor, referred to as the flavor dilution (FD) factor, reflects the compound's aroma contribution. Higher FD values indicate a greater impact (Van Ruth, 2001). Charm analysis employs random dilution while recording odor duration and characteristics to construct a

spectrum that quantitatively relates retention indices (RI) to dilution levels. This approach has been successfully applied to the identification of key aroma compounds in soy-sauce-marinated beef (Wang et al., 2023a), dry-cured ham (Chen et al., 2023), and duck broth (Pu et al., 2022), among other products.

2.6.3. Key aroma-active compound screening and identification

2.6.3.1. Evaluation of odor activity

Odor activity value (OAV) is a quantitative measure that indicates the contribution of a specific compound to the overall aroma in a food matrix. It is calculated as follows:

$$OAV = \frac{C}{T}$$

Where C represents the concentration of the aroma compound in the food matrix, and T denotes its odor threshold. Generally, OAV > 1 suggests that a compound significantly contribute to the aroma profile, with higher values indicating a greater contribution (Wang et al., 2023a).

This approach has been widely applied in identifying key aroma compounds in meat products (Chen et al., 2023; Kerler & Grosch, 1996), tea (Yang et al., 2022), and edible oils (Xu et al., 2023b), providing a quantitative basis for food flavor analysis.

2.6.3.2. Quantitative analysis of aroma-active compounds

The accuracy of OAV assessments depends on the precise quantification of the aroma compounds present in the food. For instance, Pang et al. (2012) employed selective ion monitoring (SIM) in mass spectrometry and an internal standard method to enhance detection sensitivity. However, using a single internal standard may compromise the accuracy when the target compounds differ considerably from the standard. In contrast, the stable isotope dilution assay (SIDA) involves adding a known amount of a stable isotope labeled target compounds into the sample. This ensures that both the analyte and the internal standard undergo identical extraction, purification, and detection processes, thereby achieving highly accurate and sensitive quantification. SIDA is particularly well-suited for the precise measurement of aroma compounds in complex food matrices (Wang et al., 2024a). This technique has been successfully employed in quantifying aroma compounds in pork (Fischer et al., 2014), offering a reliable analytical approach for food flavor chemistry research.

2.6.3.3. Verification of key aroma-active compounds

Traditional methods for identifying key aroma-active compounds primarily rely on gas chromatography (GC) to separate and analyze individual volatiles. However, this approach may overlook interactions between aroma compounds and their binding effects with non-volatile matrix components, potentially affecting the

accuracy of qualitative assessments. Therefore, verification of identified key compounds is essential.

Aroma recombination studies and omission experiments are widely recognized as effective validation techniques. These methods utilize precise quantification data to construct aroma recombinant models that mimic the aroma profile of the actual food product. By comparing the sensory attributes of the complete model, an omission model *(E*xcluding specific compounds), and the original food sample, researchers can validate the contribution of key aroma-active compounds to the overall flavor profile. This approach has been widely adopted in the aroma analysis of soy-sauce-marinated beef (Wang et al., 2023a), dry-cured ham (Chen et al., 2023), and duck broth (Pu et al., 2022), etc., providing a robust methodological framework for food flavor chemistry research.

2.7. Analytical techniques for lipids in meat products

Lipids, as one of the fundamental biomolecules, serve as essential nutrients and play multiple physiological roles in mammalian cellular functions. Lipid oxidation is a primary contributor to the development of WOF in precooked meat dishes. Therefore, understanding and controlling lipid oxidation is crucial for maintaining precooked meat dishes. The concept of lipidomics was first introduced by Han and Gross (2003) as a high-throughput approach to systematically analyze the lipid composition and expression changes in biological systems. Currently, lipid analysis employs two main strategies: targeted analysis and untargeted analysis. Targeted analysis focuses on the precise detection and quantification of specific lipid classes to study their roles within biological systems. Untargeted analysis involves an unbiased, comprehensive examination of all lipid types in a sample to reveal the dynamic changes and biological functions of the lipid family. Lipidomics is invaluable for elucidating the dynamic behavior of lipid molecules in biological systems. It is widely used to investigate lipid metabolism, cell signaling, and changes in food quality, thereby uncovering underlying biological mechanisms. As a specialized branch of omics studies, lipidomics expands analytical capabilities, providing new insights into the extensive lipid molecular family and enhancing the understanding of lipid functions across life sciences and food sciences (Cartoni Mancinelli et al., 2022).

In the field of lipid research, the LIPID MAPS® Structure Database (LMSD) stands as one of the most extensive public lipid databases, compiling structural and annotation information of biologically relevant lipids. According to LMSD classifications, lipids are divided into eight major categories: fatty acyls (FA), glycerolipids (GL), glycerophospholipids (GP), sphingolipids (SP), sterol lipids (ST), prenol lipids (PR), saccharolipids (SL), and polyketides (PK). As of March 2025, LMSD has catalogued 49,512 distinct lipid structures, serving as a vital resource for lipidomics research.

Lipidomics analysis primarily employs two technical approaches: shotgun techniques, in this method, samples are directly introduced into the ion source for high-resolution MS or tandem mass spectrometry (MS/MS) without prior chromatographic separation. Identification is based on the mass, headgroup, or fatty

acyl chain characteristics of the lipids; separation techniques, this approach involves chromatographic separation of lipids before detection with high-resolution MS or MS/MS, thereby enhancing identification accuracy and resolution (Triebl et al., 2017). Each method has its advantages: shotgun techniques are well-suited for high-throughput lipid screening, while separation techniques offer superior analytical precision. Researchers can select the appropriate method based on specific experimental needs.

As a crucial tool in food lipid research, lipidomics generates extensive multidimensional datasets, necessitating sophisticated data processing and statistical analysis. As a subdivision of metabolomics, lipidomics shares similar analytical methodologies, often requiring dimensionality reduction to extract key information. Chemometric tools play a central role in lipidomics data analysis, typically divided into univariate and multivariate analyses. Univariate methods, such as fold-change analysis, T-tests, and analysis of variance (ANOVA), assess significant differences between groups under specific variables. Multivariate analysis includes both unsupervised techniques, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), and supervised techniques, such as partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (LDA), orthogonal PLS-DA (OPLS-DA), random forest (RF), and support vector machines (SVM).

Lipidomics has been widely applied to the complex analysis of meat lipids to elucidate lipid composition, nutritional value, and their roles in flavor formation. This technique enables comprehensive profiling of meat lipids, revealing differences under various processing conditions or among different breeds, and helps identify potential lipid biomarkers for optimizing flavor and quality (Liu et al., 2022; Wu et al., 2024). Lipids are critical precursors for meat flavor development. During hydrolysis and oxidation, lipids generate a variety of volatile flavor compounds, significantly influencing meat aroma. In recent years, lipidomics techniques have been employed to investigate lipid composition and flavor characteristics across different meat types. For example, Li et al. (2021) used untargeted lipidomics combined with volatile flavor analysis to characterize the lipid profiles and volatile compounds in Beijing Heiliu and Laiwu Chinese black pork, revealing significant differences in potential lipid markers that provide scientific support for the identification of Chinese black pork. Liu et al. (2022) applied UPLC-ESI-MS/MS combined with flavoromics to study key lipids associated with aroma compound formation in roasted mutton, identifying 61 out of 488 differential lipids that may be closely linked to flavor development, particularly PC and PE. Zhou et al. (2023) employed UPLC-Q-TOF-MS/MS and GC-MS to analyze the formation mechanisms of aroma compounds in crayfish meat under three different thermal treatments, boiling (BO), air-frying (AF), and a combination of boiling and air-frying (BO-AF), and found that processing conditions affect the conversion of key lipids in crayfish, thereby determining the composition of volatile flavor compounds.

In summary, lipidomics provides a robust tool for precise analysis of meat lipids and flavor optimization, advancing research in meat quality evaluation, flavor control, and product development.

2.8. Reference

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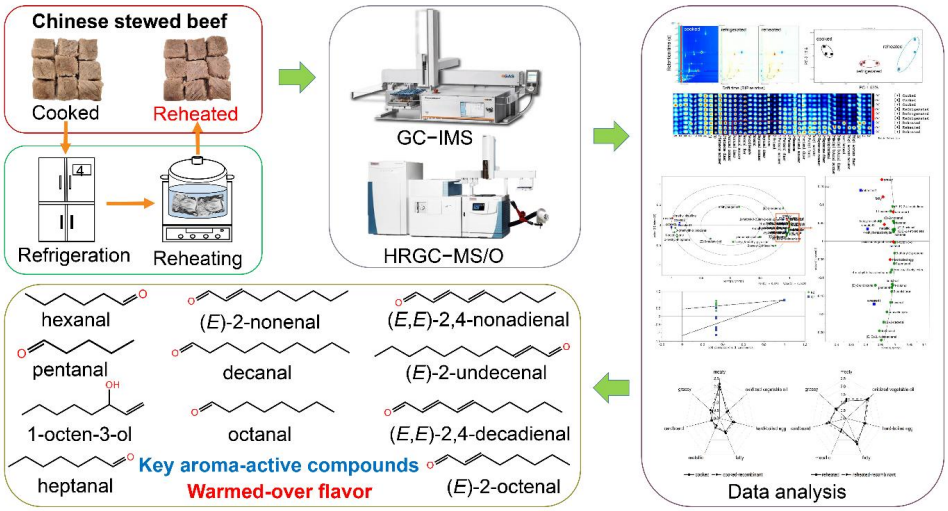
3

Chapter III Characterization of key aroma-active compounds contributing to warmed-over flavor in precooked Chinese stewed beef

Short overview of chapter III

Based on the literature review in chapter II, the presence of WOF has become a critical bottleneck for the PCSB industry. Nonetheless, the odorants responsible for the generation of the WOF in PCSB remain unclear. Sensomics is a systematic analytical approach for the precise identification of key aroma-active compounds in food. Therefore, in this chapter, we characterized the key aroma-active compounds contributing to WOF in PCSB using a sensomics approach and elucidated the changes in the aroma profiles of PCSB during cooking-refrigeration-reheating.



Graphical abstract: Characterization of key aroma-active compounds contributing to warmed-over flavor in PCSB

The work from this chapter was published in *Food Chemistry: X*, as can be retrieved by:

Liu, J., Deng, S., Wang, J., Huang, F., Han, D., Xu, Y., Yang, P., Zhang, C., & Blecker, C. (2024). Comparison and elucidation of the changes in the key odorants of precooked stewed beef during cooking-refrigeration-reheating. *Food Chemistry: X*, 23, Article 101654. https://doi.org/10.1016/j.fochx.2024.101654.

Abstract

The key odorants contributing to the warmed-over flavor (WOF) of reheated PCSB were characterized using a sensomics approach. Overall, 36 odorants were identified, and based on flavor dilution factors, odor activity values, aroma recombination, and omission test, 11 compounds mainly derived from lipid oxidation were characterized as the key odorants contributing to the formation of WOF. In particular, 3-(methylthio)propanal, which was positively correlated with meaty aroma, was implicated in an overall increase in WOF. Thus, these odorants were elected as potential markers of WOF in the reheated PCSB. In summary, the WOF of the PCSB could be attributed to an overall increase in lipid oxidation products and a decrease in the odorants with desirable aromas. The characterization of WOF in PCSB will aid in the flavor quality control of PCSB dishes.

Key words: Precooked stewed beef, warmed-over flavor, key aroma-active compounds, sensomics approach, lipid oxidation, interaction

3.1. Introduction

Stewed beef, a famous Chinese dish, contains beef and other auxiliary ingredients like vegetables and spices. It is particularly popular for its rich nutrition and attractive flavor. In China, the PCD industry is growing rapidly. PCSB dishes, typical Chinese stewed dishes, are now widely developed owing to their more stable and simpler cooking processes compared with Chinese cooking methods such as stir-frying and pan-frying. Most of the PCSB dishes are refrigerated and need to be reheated before eating. Nevertheless, even after a short period of refrigeration within their shelf-life, they still develops a particular warmed-over flavor (WOF), which is considered an off-flavor that affects consumer acceptance (Tims & Watts, 1958). Simultaneously, the meaty attribute of PCSB gradually weakens, affecting their sensory quality (O'Sullivan et al., 2003).

Tims and Watts (1958) were the first to introduce the concept of WOF, and described it as an oxidized aroma such as rancid or stale. Subsequently, researchers described WOF using aroma profile evaluations, identifying descriptors such as wet cardboard, linseed oil, paint, sour, hard-boiled egg, and fatty notes (An et al., 2022; Lage et al., 2012). According to Tims and Watts (1958), the heat processing of uncured meats caused lipid oxidation, resulting in the loss of palatability during later storage. Thereafter, numerous studies have reported that WOF is mainly caused by lipid oxidation, as the development of WOF was related to lipid oxidation products (Pegg et al., 2014; Ruenger et al., 1978). O'Sullivan et al. (2003) found that hexanal, 1-octen-3-ol, 2-pentylfuran, octanal, pentanal, and nonanal were associated with sensory data in cooked samples of two pork muscles using GC-MS. These compounds proved to be valid indicators of lipid oxidation. Recently, (E,E)-2,4-heptadienal, heptanal, (E)-2-octenal, octanal, (E)-2-nonenal, nonanal, (E)-2-decenal, decanal, (E,E)-2,4-decadienal, and 2,3-pentanedione were selected as the key odorants of the WOF in surimi gels by An et al. (2022) using aroma extract dilution analysis (AEDA), aroma recombination, and omission test.

In addition to lipid oxidation, protein degradation contributes to WOF by causing the loss of desirable aroma (Pegg et al., 2014; Zhang et al., 2022). Previous studies have revealed that WOF might be caused by a loss of desirable odorants, which are related to meaty notes attributable to 4,5-dimethyl-3-hydroxy-2(5H)-furanone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, 2-propylpyridine, 2,6-dimethylpyrazine, benzothiazole, 2-furfurylthiol, and 2-methoxybenzenethiol (An et al., 2022; Kerler & Grosch, 1996). In addition, WOF varies in different animal species, processing methods, and heating temperatures, to name a few. Heating is the leading cause of oxidation in meat (Khan et al., 2015). Unlike most meat products, Chinese stewed beef products have unique production processes. They are produced via a three-stage thermal process, including blanching (20 °C -100 °C), boiling (100 °C), and braising (without heating, with the temperature reduced from 100 °C to approximately 75 °C). Nonetheless, the odorants responsible for the generation of the WOF in PCSB remain unclear. The objectives of the present work were to (1) characterize the key aroma-active compounds contributing to WOF in reheated PCSB using a sensomics

approach and (2) elucidate the changes in the aroma profiles of PCSB during cooking-refrigeration-reheating. The study will provide information for off-flavor correction using the target-oriented flavor editing (TOFE) technology proposed by (Wang et al., 2023a) and (Wang et al., 2023a), which may be used to maintain and improve the flavor quality of PCSB dishes.

3.2. Materials and methods

3.2.1. Preparation of stewed beef

Thirty-six batches of beef chuck (seventy-two pieces) weighing 2.3 ± 0.2 kg per piece were obtained from 48-month-old Simmental steers at Hebei Fucheng Wufeng Food Co., Ltd. (Hebei, China). All animals had the same genetic background and were fed the same diet (complete formula feed). Raw beef chucks were vacuum-packed in the factory and transported to the laboratory via cold-chain logistics. Thirty-six batches of chucks were randomly divided into three groups, with twelve animals in each group. All visible fat was removed, and beef chucks were washed, and cut into approximately 2 cm × 2 cm × 1.5 cm blocks. The raw beef blocks (500 g) were added to 1500 g of water, heated to boil, kept for 3 min, and then drained for use. These drained blocks were first added to 1500 g of water, heated to boil, and held for 5 min using an induction heater (Midea Group Co., Ltd., Guangdong, China) at 1200 W. The heat was subsequently reduced to 600 W and 6 g (1.2% per raw beef*)* of salt was added. The samples were then simmered for 45 min, maintaining a faint boil. Following that, the heat was turned off and the samples were braised for 20 min. Subsequently, the samples were drained, divided into aluminum foil bags, vacuum-packed, cooled with running water, and then refrigerated at 4 °C for 6 days to develop the WOF. For reheating, the refrigerated stewed beef was reheated at 100 °C for 10 min in a water bath using the induction heater. Microbial counts were in the acceptable range (< 10^4 CFU/g) (National Food Safety Standard, 2017). The stewed beef samples were assigned into three sets: Group 1-Cooked: freshly cooked stewed beef. Group 2-Refrigerated: PCSB refrigerated for six days. Group 3-Reheated: PCSB refrigerated for 6 days and then reheated. All samples (except those used for sensory aroma profile analysis) were immediately cut into approximately 0.5 cm^3 cubes, frozen in liquid nitrogen for 5 min, and ground into a fine powder using a blender, and then stored at −80 °C for use. All analyses were completed within one week.

3.2.2. Lipid oxidation

Thiobarbituric acid reactive substance (TBARS) values were used to assess the levels of lipid oxidation according to the method described by Wang and Xiong (2005) with some modifications. Stewed beef powder sample (2g) was mixed with 3 mL of 1% thiobarbituric acid (TBA) solution. Subsequently, 17 mL of 2% trichloroacetic acid (TCA) solution (pre-cooled at 4 °C) was added. The mixture was vortexed thoroughly and then heated in a water bath at 90°C for 40 min. After heating, the mixture was rapidly cooled in an ice bath. An equal volume of chloroform was added, followed by homogenization for 30 s. The homogenate was then centrifuged at 3000 × g for 10 min. The absorbance of the supernatant was

measured at 532 nm. The data were given in mg malondialdehyde (MDA) per kg. TBARS content was calculated using the formula:

TBARS (mg MDA/kg) = A_{532} / m × 9.48

Where A_{532} is the absorbance at 532 nm, m is the weight of the meat sample (in grams), and 9.48 is a constant.

3.2.3. Aroma profiles characterized by headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS)

The aroma profiles of cooked, refrigerated, and reheated samples were analyzed using an HS-GC-IMS device (FlavorSpec®, Gesellschaft für Analytische Sensorsysteme mBH, G.A.S., Dortmund, Germany), equipped with an automatic sampling device (CTC Analytics AG, Zwingen, Switzerland). Stewed beef powder samples (2 g each) were quickly placed into a 20 mL glass vial. The samples in headspace vials were heated at 50 °C with an oscillation rate of 500 rpm for 20 min. The detection conditions and identification methods were adopted from Wang et al. (2021).

3.2.4. Sensory aroma profile analysis (APA)

Sensory evaluation was performed by 12 trained panelists (8 females and 4 males aged 22-45) according to the methods described by Yang et al. (2022). They received an extra three hours of training to recognize and define the descriptive terms of cooked and reheated stewed beef. Possible aroma terms listed in **Table 3-1** were provided by previous studies (Lage et al., 2012). Seven odor attributes (meaty, grassy, cardboard, metallic, fatty, hard-boiled egg, and oxidized vegetable oil) were selected, and their intensities were rated on a scale from 0 (not perceivable) to 3 (very high intensity) on the scale steps of 0.5. The final score for each aroma attribute was determined based on the scores of all assessors. The sensory analysis was performed in a sensory laboratory with individual booths at 20 ± 2 °C. Most importantly, participation was voluntary for all participants. We confirmed that every participant's rights and privacy were appropriately protected while the study was being conducted. We guaranteed that all samples were nontoxic, and harmless to the human body. The participants were fully informed of the requirements and risks of the study, and they gave their consent. We also promised to keep all details on the evaluators confidential. All data obtained through this evaluation were used only for the identification of key aroma-active compounds on the aspects of sensory studies.

Table 3-1 Sensory descriptive terms with definitions developed for the evaluation of cooked and reheated Chinese stewed beef.

Descriptor	Definition with reference material
meaty	freshly boiled beef lean
oxidized vegetable oil	soybean oil heated for 5 min at 198 °C

Descriptor	Definition with reference material
hard-boiled egg	boiled egg in boiling water for 20 min
fatty	roast beef fat in the oven for 5 min at 150 °C
metallic	metal products (metal key)
cardboard	shredded wet cardboard
grassy	grass
linseed oil	warmed linseed oil
nutty	crushed fresh hazel nuts
roasted	oven cooked beef steaks with surface browning

3.2.5. Extraction of aroma compounds

3.2.5.1. Solid-phase microextraction (SPME)

Stewed beef powder samples (2 g each), 1 μL 1, 2-dichlorobenzene (0.653 μg/μL), and 1 μL 2-methyl-3-heptanone (0.408 μg/μL) were placed into a 20 mL glass vial. The vial was stored at 50 °C for 20 min. Then, a 50/30 μm DVB/CAR/PDMS fiber was inserted into the headspace to adsorb for 30 min, followed by desorption at the injector port (250 °C) of the Q Exactive GC-Orbitrap-MS-O system for 5 min.

3.2.5.2. Solvent-assisted flavor evaporation (SAFE)

Stewed beef powder samples (50 g each), 0.653 μg/μL of 1, 2-dichlorobenzene (30 μL), 0.408 μg/μL of 2-methyl-3-heptanone (50 μL), and dichloromethane (150 mL) were mixed in a Teflon bottle, shaken at 120 rpm at 4 °C in an incubator shaker (Tianjin Honour Instrument Co., Ltd., Tianjin, China) for 8 h, and extracted as described by Sun et al. (2021).

3.2.6. Identification of aroma-active compounds of the cooked and reheated stewed beef

3.2.6.1. Q Exactive GC-Orbitrap-MS-O analysis

Aroma compounds in all samples were identified by a Q Exactive GC-Orbitrap-MS system (Trace 1310 GC System, TSQ9000 MSD, Thermo Scientific, Bremen, Germany) equipped with an olfactometer detector ODP4 (Gerstel, Inc., Linthicum, MD, U.S.A.). VF-WAXms (60 m × 0.25 mm, 0.25 μm) and DB-5 capillary columns (30 m × 0.25 mm, 0.25 μm) were used to separate the odorants. The temperature program of the VF-WAXms column was 40 °C for 2 min, increased at 4 °C/min to 230 °C, and held for 5 min. The final column temperature of the DB-5 column was 250 °C for 5 min. The flow rate of the helium carrier gas (99.999% purity) was 1.5 mL/min. MS conditions: electron impact (EI) energy; 70 eV, ion source temperature, 230 °C; MS source temperature, 280 °C. The ODP temperature was 250 °C.

The compounds were identified based on odor attributes (O), retention indices (RIs), mass spectra (MS), and data obtained from authentic reference standards

(STDs). The RI values were calculated using the retention times of a series of n-alkanes (C7-C40).

3.2.6.2. Detection frequency analysis (DFA)

DFA was used to obtain odor patterns of the cooked and reheated stewed beef as described by Pang et al. (2012). The detection frequency (DF) for an odor with the same retention index and a similar description was summed. Any odorant at the sniffing port with DF ≥ 2 was considered to have aroma potential activity, regarded as an aroma-active compound (Pang et al., 2012).

3.2.7. AEDA

AEDA was used to acquire a preliminary concept of which odorants should be significant for the overall aroma. The SAFE extracts were diluted stepwise by 1 + 1 (v + v) with dichloromethane and analyzed by Q Exactive GC-Orbitrap-MS-O system. The results are represented as the flavor dilution (FD) factor of the maximum dilution for the perceived odor. AEDA was performed on the VF-WAXms column as more odorants were detected by VF-WAXms than DB-5.

3.2.8. Quantitation of the aroma-active compounds and calculation of odor activity values (OAVs)

As matrix effects were not considered in the DFA analysis, the aroma intensity of the aroma-active compounds detected at the sniffing port does not necessarily indicate the importance of a single aroma compound. Thus, quantitation and OAVs determination of odorants need to be analyzed (Yang et al., 2022). According to Sun et al. (2021), the quantitative analysis of aroma-active compounds was achieved by constructing external standard curves using an artificial odorless matrix with various concentrations of authentic flavor standards. The standard solutions were prepared by diluting the corresponding stock standard solution with dichloromethane. Certain concentrations of authentic flavor standards containing internal standards were added to the artificial odorless matrix extracted by SAFE, and then detected using the Q Exactive GC-Orbitrap-MS-O system in the selected ion monitoring mode. The linear regression equation for each compound was determined by plotting the ratio of the peak area of the target compound to that of the internal standard against the corresponding concentration. All analyses were repeated in triplicate.

To obtain the odorless matrix, the cooked and reheated stewed beef samples were frozen with liquid nitrogen, ground into fine powder, extracted with dichloromethane, and filtered. The filtrates were subjected to high vacuum distillation using SAFE to eliminate all aroma compounds until nothing was detected by the Q Exactive GC-Orbitrap-MS-O system. Finally, the filtration residues were dried to remove the solvent and form an odorless powder (Sun et al., 2021).

The contribution of a single aroma compound can be considered by its OAV (Yang et al., 2022). The OAVs were calculated by dividing the concentration of

each odorant by its threshold in water found in literature or detected in the present study.

3.2.9. Aroma recombination and omission experiments

To confirm that the key aroma compounds were correctly identified and quantitated, the cooked-recombinant model (odorants with OAVs ≥ 1 and FD ≥ 4) and the reheated-recombinant model (odorants with OAVs ≥ 1 and FD ≥ 8) were constructed according to the method described by Sun et al. (2021). The contribution of individual odorants to the overall aroma was assessed through omission experiments, which were conducted by removing individual odorants from the respective recombinant models. The flavor similarity between the recombinant model and the original sample, as well as between the recombinant model and each omission model, was evaluated using a triangle test.

3.2.10. Statistical analysis

All experiments were conducted in triplicate. Analysis of variance (ANOVA) for statistical analysis were conducted using the Statistical Program of Social Science (SPSS 25.0, Chicago, IL, USA) software. Statistically significant differences between groups were determined using Duncan's multiple range test at $P < 0.05$. A completely randomized design was employed. The orthogonal partial least squares discrimination analysis (OPLS-DA) was performed using SMICA 14.1 software (Umetrics, UmeUmeå, Sweden). The radar chart, column chart, and heatmap were drawn using Origin 2021 (OriginLab, Northampton, Massachusetts, USA).

3.3. Results and discussion

3.3.1. Changes in TBARS in the PCSB during refrigeration-reheating

TBARS values can reflect the degree of lipid oxidation (Xia et al., 2009). The TBARS value of the PCSB significantly increased after refrigeration-reheating ($P \leq 0.05$). There was no significant difference between refrigerated and reheated samples ($P > 0.05$) (**Fig. 3-1**). This finding indicated that refrigeration-reheating induced significant lipid oxidation, which is recognized as often concomitant with the deterioration of flavor quality.

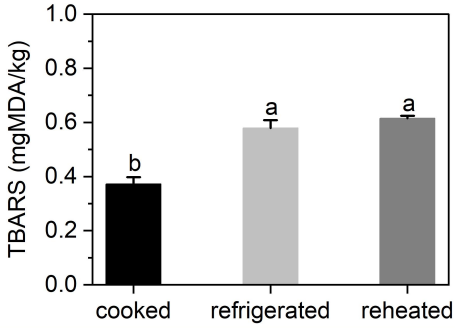


Fig. 3-1 Thiobarbituric acid reactive substances (TBARS) value variation of Chinese stewed beef during cooking-refrigeration-reheating (different lowercase letters show significant differences at $P \leq 0.05$)

3.3.2. Changes of odorants in the PCSB during cooking-refrigeration-reheating by HS-GC-IMS

A total of 40 signal peaks were detected using HS-GC-IMS, and 28 typical aroma compounds were successfully identified via GC-IMS Library searches. The observed 40 peaks were analyzed using a fingerprint plot (**Fig. 3-2**A), including 13 aldehydes, 7 ketones, 4 alcohols, 3 esters, 1 furan, and 12 undefined compounds. **Fig. 3-2**A shows the intensities of each odorant. The signal intensities of most aldehydes in refrigerated and reheated samples were higher compared with those in cooked samples. Almost no new signals or spots were generated, suggesting that almost no new compounds were detected in the PCSB after refrigeration and reheating. Moreover, except for two compounds (2-butanone monomer and pentanal monomer), 26 odorants mainly produced via lipid oxidation (Merlo et al., 2021; Wang et al., 2021), such as hexanal, heptanal, 2-heptanone, and 2-pentylfuran, increased in intensity after refrigeration and reheating, suggesting an increasing trend of lipid oxidation. Additionally, 3-methylbutanal and 2-methylbutanal are Strecker aldehydes with malt and cocoa odors, respectively. According to a previous research from Zamora et al. (2008), some carbonyls produced by lipid peroxidation, such as ketodienes and alkadienals, have been proven to facilitate the breakdown of specific amino acids to produce the corresponding Strecker aldehydes through Strecker-type reactions, which might lead to increased levels of 3-methylbutanal and 2-methylbutanal following refrigeration and reheating. Their increasing intensity suggested that Strecker degradation might be another reason for the aroma variation of PCSB after refrigeration and reheating. The differences in aroma compounds among the three samples were also visually distinguished by the two-dimensional differential topographic plots (**Fig. 3-2**B). Compared with the cooked samples, the signal intensities of most of the aromas were higher following refrigeration and reheating. Furthermore, PCA was conducted to inspect the clustering of the samples. As shown in **Fig. 3-2**C, the cumulative variance contribution was 68%. The PCA model can be regarded as the separation model (Wang et al., 2021). Three samples were separated in the distribution map, suggesting that after refrigeration and reheating, the aroma profiles of the PCSB considerably varied. The cooked samples were clustered in the upper left plot, the reheated samples were mainly located in the upper right plot, and the refrigerated samples were clustered in the bottom middle area closer to the reheated samples (**Fig. 3-2**C). The result demonstrated that the odorants of the cooked and reheated samples considerably differed. The differences between the refrigerated and reheated samples were smaller compared with the differences between the cooked and refrigerated samples (**Fig. 3-2**A, C), confirming the TBARS results. Thus, to characterize the indicators contributing to the WOF of reheated PCSB, the reheated and cooked stewed beef samples (control group) were further investigated in subsequent studies.

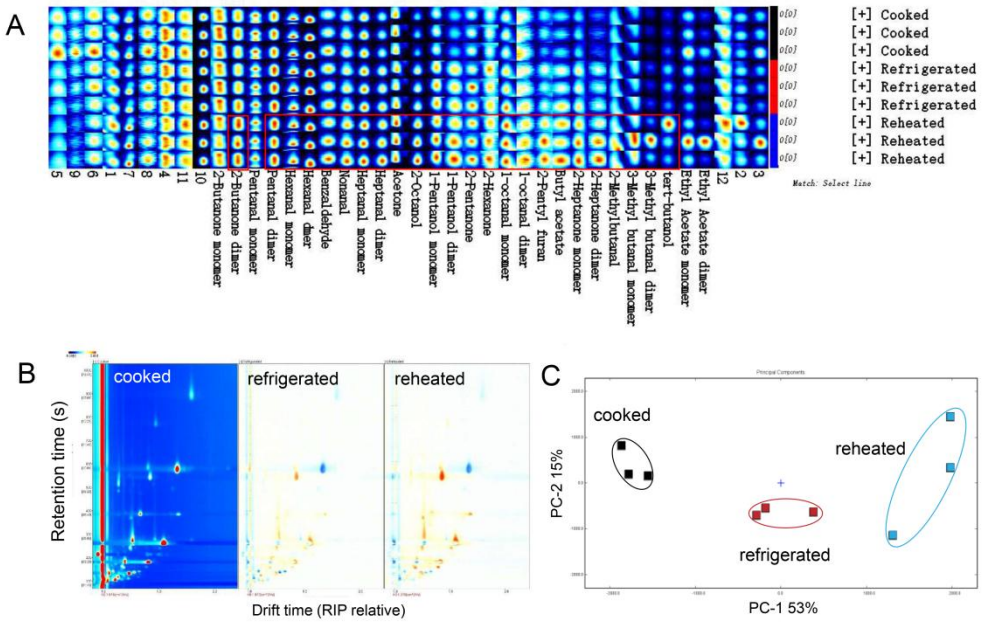


Fig. 3-2 Fingerprint map of aroma compounds(A), two-dimensional differential map of HS−GC−IMS spectra (B), and principal component analysis based on the peak intensities of aroma compounds detected by HS−GC−IMS (C).

3.3.3. APA

Sensory evaluation results of the cooked and reheated samples are shown in **Fig. 3-3**, illustrating that the aroma profiles of the two samples were considerably different. The cooked samples had an intensely meaty odor. After refrigeration-reheating, the PCSB presented a strong WOF, manifesting as weaker intensities of meaty note, and considerably increased fatty and oxidized vegetable oil aroma. Moreover, the grassy, hard-boiled egg, metallic, and cardboard-like aroma significantly increased.

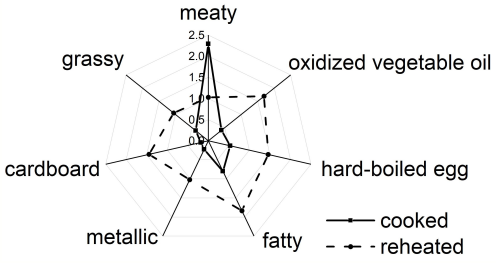


Fig. 3-3 Aroma profiles of cooked (solid line) and reheated (broken line) Chinese stewed beef

3.3.4. Composition of aroma-active compounds in cooked and reheated stewed beef

Overall, 36 active compounds were detected in the cooked and reheated samples, including 15 aldehydes, 6 alcohols, 6 benzene-containing compounds, 5 sulfur-containing compounds, 1 nitrogen-containing compound, 1 ketone, 1 ester, and 1 furan. No additional odorants were detected in the reheated samples, confirming the HS-GC-IMS result (**Fig. 3-2**A, B). These compounds exhibited FD factors between 1 and 64 (**Table 3-2**).

Hexanal (no. 4; grassy), heptanal (no. 7; fatty), octanal (no. 10; fresh), nonanal (no. 16; fresh), (E)-2-octenal (no. 17; fatty, herbal), (E,E)-2,4-heptadienal (no. 20; fatty), (E,E)-2,4-nonadienal (no. 26; fatty), and (E,E)-2,4-decadienal (no. 31; fatty) had FD factors of ≥ 8 in the reheated samples. Nos. 4, 7, 10, 16, 17, 26, 31, and 25 (fatty) were presented at FD factors of ≥ 4 in the cooked samples. Nos. 7, 10, 16, and 31 can be generated from oleic acid, and no. 4 is an oxidation product of linoleic and arachidonic acids (Merlo et al., 2021). The oxidation of oleic and linoleic acid can produce 2-alkenals (Elmore et al., 1999). Aldehydes were the dominant odorants in the cooked and reheated samples. They have a strong aroma and a lower threshold, contributing significantly to meat aroma (Wang et al., 2021).

In the cooked samples, 1-hexanol (no. 13; herbal, fatty), (Z)-3-hexen-1-ol (no. 14; fatty), and 1-octen-3-ol (no. 18; mushroom-like, oily) showed FD factors of ≥ 4. Nos. 14 and 18 had FD factors of ≥ 8 in the reheated samples. No. 18 can be generated from n-6 fatty acids (Elmore & Mottram, 2006). No. 14, a typical green leaf volatile, has been identified as a key odorant in some plant products (Tamura et al., 2000; Yang et al., 2022). It probably originated from grass and grain fed to the steers.

Formic acid octyl ester (no. 24; fruity) had an FD of 8 in reheated samples. Nevertheless, it has a high odor threshold and might contribute less to the overall aroma profile of PCSB. In the reheated samples, the FD factor of 1-octen-3-one (no. 11; mushroom), which may be derived from omega-3 fatty acids (Lee et al., 2001), was 16. In addition, the FD factor of 5-methyl-2-ethylpyrazine (no. 15; coffee-like) was 8 in the reheated samples. The carbohydrate degradation and Strecker products, such as amino acids or ammonia, may generate pyrazines (Liu et al., 2019). The thermal degradation of lipids and the Maillard reaction are the main reactions for the formation of aroma compounds in cooked meats (Khan et al., 2015).

3-Phenyl-2-propenal (no. 35; spice-like) had the highest FD of 32 and 64 in cooked and reheated samples, respectively. Benzyl alcohol (no. 32; phenolic), methyleugenol (no. 34; waxy), and benzaldehyde (no. 22; bitter almond) had FD factors of ≥ 4 in cooked samples. Nos. 32 and 34 had FD factors of ≥ 8 in reheated samples. Nos. 34 and 35 might be developed in the stomachs of ruminants that consume green feed (Gąsior et al., 2021; Khan et al., 2015). Benzaldehyde can be derived from the Strecker degradation of phenylalanine or the α-linolenic acid degradation (Elmore & Mottram, 2006; Feng et al., 2021).

Table 3-2 Comparison of FD factors of the aroma-active compounds in cooked and reheated Chinese stewed beef

No.	Aroma-active compound	Odor quality	RI		FD factors		Extraction method
			WAX	DB-5	Cooked	Reheated	
1	Pentanal	Fermented	983	701	2	1	SAFE/SPME
2	2-Methyl-3-buten-2-ol	Oily	1034		1	1	SAFE
3	Dimethyl disulfide	Sulfurous, cooked cabbage	1081	745	2	1	SAFE/SPME
4	Hexanal	Grassy, fatty	1087	800	4	8	SAFE/SPME
5	2-Methyl-thiophene	Roasted	1100		1	1	SAFE
6	1-Butanol	Sweet, whiskey	1142		2	4	SAFE
7	Heptanal	Fatty	1191	906	4	8	SAFE/SPME
8	2-Pentylfuran	Green	1236	991	1	2	SAFE/SPME
9	1-Pentanol	Oily, sweet, balsamic	1248	770	1	4	SAFE/SPME
10	Octanal	Fresh , fatty	1295		16	16	SAFE
11	1-Octen-3-one	Mushroom-like	1306		2	16	SAFE
12	(E)-2-Heptenal	Fatty, oily	1333	957	1	1	SAFE/SPME
13	1-Hexanol	Herbal, nutty	1351	874	4	1	SAFE/SPME
14	(Z)-3-Hexen-1-ol	Herbal, fatty	1371		4	8	SAFE
15	5-Methyl-2-ethylpyrazine	Coffee-like, nutty	1391		1	8	SAFE
16	Nonanal	Green, fatty	1400	1094	32	16	SAFE/SPME
17	(E)-2-Octenal	Fatty, herbal	1439	1061	8	8	SAFE/SPME

No.	Aroma-active compound	Odor quality	RI		FD factors		Extraction method
			WAX	DB-5	Cooked	Reheated	
18	1-Octen-3-ol	Mushroom-like	1447	982	16	32	SAFE/SPME
19	3-(Methylthio)propanal	Potato-like, meaty	1467	902	2	1	SAFE/SPME
20	(E,E)-2,4-Heptadienal	Fatty	1474		1	8	SAFE
21	Decanal	Orange-like, fresh	1505	1195	2	4	SAFE/SPME
22	benzaldehyde	Bitter almond	1540	954	4	4	SAFE/SPME
23	(E)-2-Nonenal	Cardboard, nutty	1545	1155	1	4	SAFE/SPME
24	Formic acid octyl ester	Fruity, orange-like	1557		1	8	SAFE
25	(E)-2-Decenal	Fatty	1653		8	4	SAFE
26	(E,E)-2,4-Nonadienal	Fatty	1672	1223	4	8	SAFE/SPME
27	Dodecanal	Soapy, waxy	1716	1407	1	1	SAFE/SPME
28	4-Ethyl benzaldehyde	Almond, bitter	1725		1	1	SAFE
29	(E)-2-Undecenal	Soapy, metallic	1761		1	4	SAFE
30	2-Acetyl-2-thiazoline	Cooked rice-like	1777		4	4	SAFE
31	(E,E)-2,4-Decadienal	Fatty, nutty	1822	1327	4	8	SAFE/SPME
32	Benzyl alcohol	Phenolic	1888		16	8	SAFE
33	Phenethyl alcohol	Floral	1925		1	4	SAFE
34	Methyleugenol	Waxy	2018		4	8	SAFE
35	3-Phenyl-2-propenal	Spice-like	2040		32	64	SAFE

No.	Aroma-active compound	Odor quality	RI		FD factors		Extraction method
			WAX	DB-5	Cooked	Reheated	
36	4-Methyl-5-thiazoleethanol	Fatty	2323		4	1	SAFE

In the cooked samples, 2-acetyl-2-thiazoline (no. 30; cooked rice) and 4-methyl-5-thiazoleethanol (no. 36; fatty) had an FD factor of 4. Sulfur-containing compounds can be generated from the Maillard reaction and amino acid and thiamine degradation (Khan et al., 2015; Wang et al., 2021). They are considered important odorants contributing to the meaty aroma (Khan et al., 2015). Odorant no. 30 had the highest FD of 2048 in roasted duck (Straßer & Schieberle, 2014). No. 36 was reportedly the major aroma compound in pork broth (Zhao et al., 2017).

A comparison of aroma-active compounds suggested that the odorants detected in the sniffing port in the two samples had similar categories and amounts, indicating that desirable and undesirable odorants were generated by the same aroma compounds. The differences were reflected in their intensities (**Table 3-2**), such as nos. 4, 7, 26 (grassy and fatty) had increased FD factors from cooked to reheated samples, confirming the sensory results of the APA. Most of these odorants were reported to be the main contributors to the WOF in meat products (Konopka & Grosch, 1991; Pegg et al., 2014; Ruenger et al., 1978). In addition, 16 odorants in the reheated samples and only 7 in the cooked samples had FD factors of ≥ 8, showing a larger number of odorants with higher FD in the reheated samples than in the cooked samples (**Table 3-2**). The FD factors of three sulfur-containing compounds (nos. 3, 19, and 36) decreased from cooked to reheated samples, which might be the crucial factor for the reduced meaty attribute in the reheated samples (**Fig. 3-3**).

3.3.5. Quantitation of the aroma-active compounds and calculation of OAVs.

The concentrations and OAVs of aroma-active compounds are presented in **Table 3-3**. The highest concentration was measured for hexanal (no. 4) in the cooked (246.17 μg/kg) and reheated samples (1070.33 μg/kg), followed by nos. 14 and 1. Meanwhile, nos. 7, 16, 18, 10, and 17 had relatively high concentrations. Nevertheless, high concentrations do not consequently imply a significant influence on the overall aroma of food.

To exhibit the contribution of a single aroma compound to the overall aroma, the OAVs were calculated. Overall, 16 identical compounds showed OAVs ≥ 1 in the cooked and reheated samples (**Table 3-3**). These compounds might have a significant contribution to the overall aroma of PCSB. By far, the OAV of hexanal is the highest (cooked, 246; reheated, 1070). It has been identified as a key odorant and a typical indicator of the WOF development in meat products (Konopka & Grosch, 1991; Liu et al., 2020). Kerler and Grosch (1996) reported similar results, revealing that the highest concentration of no. 4 in the reheated boiled beef was nine-fold higher than that in fresh-boiled beef. Meanwhile, the OAV of no. 4 considerably increased after refrigeration-reheating, manifesting that no. 4 was the most potent odorant of reheated boiled beef. No. 31, which was responsible for the fatty aroma, showed a very low concentration (cooked, 0.03 μg/kg; reheated, 0.21 μg/kg). Nonetheless, it had OAVs (cooked, 1; reheated, 8) ≥ 1 owing to its lower threshold (0.027 μg/kg). In a previous study, no. 31 was identified as a contributor to WOF in

precooked pork (Zang et al., 2019). Among odorants with an OAV of ≥ 1, although pentanal (no. 1) had a lower FD factor (cooked, 1; reheated, 2), its OAV was greater than 1 because of higher concentration (cooked, 31.57 μg/kg; reheated, 102.38 μg/kg) and lower threshold (9 μg/kg). Due to lower concentrations or higher threshold, only one sulfur-containing compound, 3-(methylthio)propanal (methional) (no. 19), had an OAV of > 1. The lower concentrations might be due to the fact that the PCSB in this study was cooked at a much lower temperature than the roasted and fried meats, thus producing a limited amount of Maillard reaction products (Khan et al., 2015). No. 19 with a roasted potato aroma had lower concentrations (cooked, 0.74 μg/kg; reheated, 0.42 μg/kg) and FD factors (cooked, 2; reheated, 1), presenting higher OAVS (cooked, 19; reheated, 11) because of their relatively lower thresholds (0.04 μg/kg). Similarly, decanal (no. 21, orange/fresh), dodecanal (no. 27, soapy/waxy), (E)-2-undecenal (no. 29, soapy/metallic), and (E)-2-nonenal (no. 23, cardboard/nutty), which are derived from lipid oxidation, had a lower FD factor, but OAVs ≥ 1. Nevertheless, most of the odorants with an OAV of > 1 exhibited higher concentrations and FD factors. Meantime, they processed higher OAVs, covering no. 10 (cooked, 20; reheated, 179), no. 16 (cooked, 17; reheated, 76), no. 7 (cooked, 12; reheated, 25), and no. 18 (cooked, 5; reheated, 21). These aromas significantly contributed to the WOF of the reheated samples. In addition, nos. 11, 16, 26, 25, and 17 showed OAVs of ≥ 1 (Table 3) and mainly presented grassy, green, fatty, and metallic aromas. Their OAVs increased after reheating except for that of no. 19, which decreased in the reheated samples. The result was in line with the APA. These results suggested that the formation of WOF in the reheated PCSB was mainly caused by an increase in certain odorants and a decrease in certain odorants, which was similar to the findings of previous studies (An et al., 2022; Pegg et al., 2014).

Additionally, some aromas with higher FD factors showed lower OAV values. No. 35, with the highest FD, had an OAV of < 1 attributing to its relatively low concentration and high threshold, including nos. 32, 34, 24, 14, 15, 13, 30, and 22. These odorants have a minor aroma contribution because of their low OAVs (< 1).

Table 3-3 Concentrations, odor thresholds, and odor activity values (OAVs) of key aroma-active compounds in cooked and reheated Chinese stewed beef. Different uppercase letters show significant differences in concentrations of different samples in each row at $P < 0.05$.

No.	Aroma-active compound	Odor threshold in water (μg/kg)	Concentrations (μg/kg)		OAV	
			Cooked	Reheated	Cooked	Reheated
1	Pentanal	9	31.57 ± 1.14^{B}	102.38 ± 8.93^{A}	4	11
2	2-Methyl-3-buten-2-ol	51600	0.34 ± 0.01^{A}	0.33 ± 0.01^{A}	<1	<1
3	Dimethyl disulfide	1.1	0.21 ± 0.00^{A}	0.07 ± 0.02^{B}	<1	<1
4	Hexanal	1.00	246.17 ± 21.36^{B}	1070.33 ± 108.94^{A}	246	1070
5	2-Methyl-thiophene	3000	0.20 ± 0.01^{A}	0.13 ± 0.02^{B}	<1	<1
6	1-Butanol	459.20	4.31 ± 0.06^{B}	9.27 ± 0.91^{A}	<1	<1
7	Heptanal	2.8	32.99 ± 0.25^{B}	69.46 ± 3.68^{A}	12	25
8	2-Pentylfuran	6	0.59 ± 0.01^{B}	2.35 ± 0.20^{A}	<1	<1
9	1-Pentanol	4000	3.63 ± 0.01^{B}	14.95 ± 0.38^{A}	<1	<1
10	Octanal	0.1	2.01 ± 0.28^{B}	17.89 ± 0.32^{A}	20	179
11	1-Octen-3-one	0.1	1.75 ± 0.11^{A}	1.41 ± 0.01^{B}	18	14
12	(E)-2-Heptenal	13	2.95 ± 0.41^{B}	8.30 ± 1.18^{A}	<1	<1
13	1-Hexanol	5.6	0.56 ± 0.10^{B}	4.06 ± 0.70^{A}	<1	<1
14	(Z)-3-Hexen-1-ol	200	104.75 ± 1.60^{A}	97.91 ± 1.28^{B}	<1	<1

No.	Aroma-active compound	Odor threshold in water (μg/kg)	Concentrations (μg/kg)		OAV	
			Cooked	Reheated	Cooked	Reheated
15	5-Methyl-2-ethylpyrazine	1000	0.10 ± 0.02 ^{A}	0.09 ± 0.02 ^{A}	<1	<1
16	Nonanal	1.00	16.52 ± 1.36^{B}	75.63 ± 6.96^{A}	17	76
17	(E)-2-Octenal	3.00	3.89 ± 0.05^{B}	13.49 ± 0.57^{A}	1	5
18	1-Octen-3-ol	1.5	7.00± 0.14^{B}	31.85 ± 0.48^{A}	5	21
19	3-(Methylthio)propanal	0.04	0.74 ± 0.09^{A}	0.42 ± 0.02^{B}	19	11
20	(E,E)-2,4-Heptadienal	15.4	0.85 ± 0.02^{B}	2.18 ± 0.12^{A}	<1	<1
21	Decanal	2.00	2.56 ± 0.15^{B}	6.84 ± 0.21^{A}	1	3
22	benzaldehyde	41.70	26.51 ± 0.38^{B}	31.41 ± 1.02^{A}	<1	<1
23	(E)-2-Nonenal	0.08	2.93 ± 0.14^{B}	6.26 ± 0.19^{A}	37	78
24	Formic acid octyl ester	3132	1.25 ± 0.20^{B}	3.63 ± 0.23^{A}	<1	<1
25	(E)-2-Decenal	0.35	1.89 ± 0.05^{B}	2.33 ± 0.11^{A}	5	7
26	(E,E)-2,4-Nonadienal	0.100	1.11 ± 0.01^{B}	5.75 ± 0.14^{A}	11	58
27	Dodecanal	2.00	2.38 ± 0.13^{B}	4.08 ± 0.50^{A}	1	2
28	4-Ethyl benzaldehyde	123.2	0.00 ± 0.00^{B}	0.02 ± 0.00^{A}	<1	<1
29	(E)-2-Undecenal	1.40	1.83 ± 0.13^{B}	3.04 ± 0.56^{A}	1	2
30	2-Acetyl-2-thiazoline	1	0.12 ± 0.01 ^{A}	0.14 ± 0.01 ^{A}	<1	<1
31	(E,E)-2,4-Decadienal	0.027	0.03 ± 0.00^{B}	0.21 ± 0.05^{A}	1	8

No.	Aroma-active compound	Odor threshold in water (μg/kg)	Concentrations (μg/kg)		OAV	
			Cooked	Reheated	Cooked	Reheated
32	Benzyl alcohol	5500	3.76 ± 0.69^{B}	13.11 ± 2.55^{A}	<1	<1
33	Phenethyl alcohol	4000	0.57 ± 0.05^{A}	0.61 ± 0.04^{A}	<1	<1
34	Methyleugenol	1250	0.09 ± 0.01^{A}	0.10 ± 0.01^{A}	<1	<1
35	3-Phenyl-2-propenal	90	0.15 ± 0.00^{B}	0.22 ± 0.00^{A}	<1	<1
36	4-Methyl-5-thiazoleethanol	4748	0.65 ± 0.06^{B}	1.19 ± 0.04^{A}	<1	<1

3.3.6. Correlation among samples, aroma-active compounds, and sensory attributes.

To screen the effective aroma-active compounds contributing to sensory profiles, two data sets (**Table 3-3**) were analyzed by OPLS-DA, and the results are presented in **Fig. 3-4**A. The correlation biplot involving three ellipses indicated 50%, 75%, and 100% explained variance, respectively. Most variables (OAVs of the aroma-active compounds and intensities of the sensory attributes) were located within the 50%-100% ellipses. The derived OPLS-DA model with two principal components explained 99.6% of the validated variation. Excellent parameters (R^2X = 0.996, R^2Y = 0.999, and Q^2 = 0.996) indicated a good model fit and acceptable predictability. In addition, in the 200-times permutation test (**Fig. 3-4**B), the values of R^2 and Q^2 of the original points on the right were higher than the values on the left, indicating that the OPLS-DA model was not overfitting. As shown in **Fig. 3-4**A, the two samples were located on the left side of the plot, and the reheated samples were located on the opposite side, exhibiting good separation on the PC1. Methyleugenol, (Z)-3-hexen-1-ol, and 5-methyl-2-ethylpyrazine with OAVs of < 1 were located in the inner circle. These variables did not have adequate structured variation to be distinguished during processing (Li et al., 2020). The cooked sample was associated with meaty notes. The reheated sample was correlated with the other six sensory notes (grass, fatty, cardboard, metallic, oxidized vegetable oil, and hard-boiled egg), showing a strong WOF and weak meaty aroma, confirming the APA results. What's more, 3-(methylthio)propanal, dimethyl disulfide, 2-methyl-thiophene, and 1-octen-3-one, mainly sulfur-containing compounds, were positively correlated with the meaty aroma ($P < 0.05$). Other aroma-active compounds were relatively concentrated on the right side of the plot, close to the reheated samples and the other sensory attributes, except for 2-methyl-3-buten-2-ol, phenethyl alcohol, and 2-acetyl-2-thiazoline (OAVs < 1). They were positively correlated with those undesirable sensory notes (grassy, fatty, cardboard, metallic, oxidized vegetable oil, and hard-boiled egg) ($P < 0.05$) (**Fig. 3-4**A). The result was consistent with that of a previous report (Pegg et al., 2014), revealing that the intensity of the off-flavor notes was positively correlated with the content of carbonyl compounds produced via lipid oxidation. Furthermore, hexanal, octanal, nonanal, (E,E)-2,4-nonadienal, and (E)-2-nonenal had a VIP of ≥ 1, P values of < 0.05, indicating that they contributed significantly to the discrimination between the two groups. Moreover, they all exhibited higher OAVs (> 10 in cooked; > 50 in reheated), all of which increased in the reheated samples.

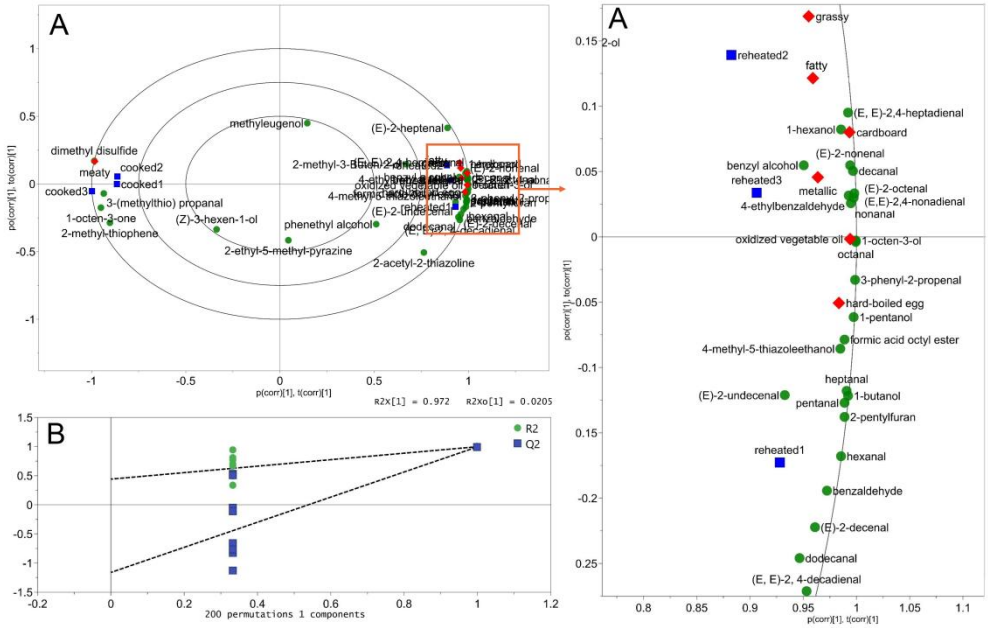


Fig. 3-4 OPLS-DA correlation bi-plot of the relationship between 36 aroma-active compounds (OAVs) (green plots) and sensory attributes (red plots) (A), OPLS-DA model permutation test plots (B)

3.3.7. Aroma recombination and omission experiments

The cooked- and reheated-recombinant models exhibited very good similarities to the original food samples (**Fig. 3-5**A, B), confirming the correct identification and quantitation of all key aroma compounds of the cooked and reheated samples. The aroma contribution of a single odorant to the overall aroma of the cooked and reheated stewed beef was assessed using omission experiments. A total of 24 and 23 aroma omission models were built for the cooked and reheated samples, respectively. As shown in **Table 3-4**, all aroma-active compounds with an OAV of < 1 exhibited no importance for the overall aroma of the cooked and reheated stewed beef. Omitting hexanal, (E)-2-decenal, pentanal, (E)-2-undecenal, octanal, 1-octen-3-ol, 3-(methylthio)propanal, (E,E)-2,4-nonadienal, 1-octen-3-one, and (E,E)-2,4-decadienal caused a significant change in the aroma of the cooked-recombinant model ($P < 0.05$), revealing that they contributed significantly to the overall aroma of the cooked stewed beef and were selected as the key aroma-active compounds. The results also showed that 11 aroma compounds, including hexanal, (E)-2-nonenal, pentanal, (E,E)-2,4-nonadienal, decanal, (E)-2-undecenal, 1-octen-3-ol, (E,E)-2,4-decadienal, octanal, (E)-2-octenal, and heptanal, were the key aroma-active compounds of reheated PCSB. They were positively correlated with oxidized vegetable oil, hard-boiled egg, fatty, metallic,

grassy, and cardboard notes (**Fig. 3-4**A). These sensory notes are closely related to the WOF based on sensory evaluation and previous studies (An et al., 2022; Lage et al., 2012).

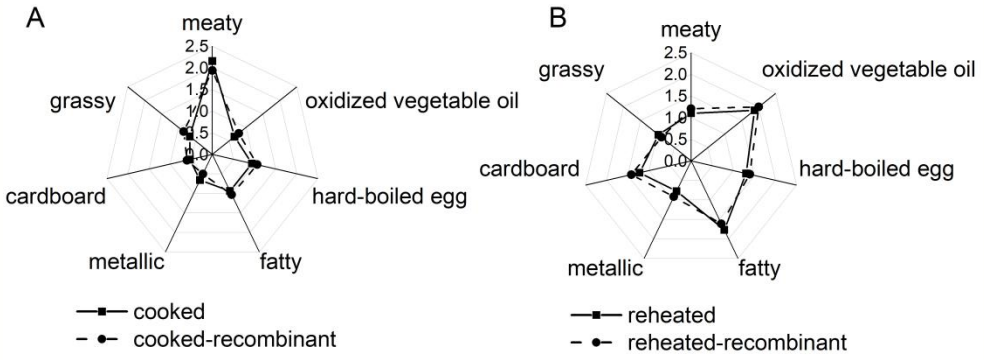


Fig. 3-5 Aroma profiles of cooked (solid line) and cooked-recombinant (broken line) (A), and aroma profiles of reheated (solid line) and reheated-recombinant (broken line) (B).

Table 3- 4 The triangle test results of each key aroma-active compounds by omission experiments in cooked and reheated Chinese stewed beef

Cooked			Reheated		
Key aroma-active compounds[a]	**n (cooked)[b]**	**Significant[c]**	**Key aroma-active compounds[a]**	**n (reheated)[b]**	**Significant[c]**
Hexanal	12	***	Hexanal	12	***
(E)-2-Decenal	11	***	(E)-2-Nonenal	9	**
Pentanal	11	***	(E,E)-2, 4-Nonadienal	9	**
(E)-2-Undecenal	10	***	Pentanal	9	**
Octanal	9	**	Decanal	9	**
3-(Methylthio)propanal	9	**	(E)-2-Undecenal	9	**
1-Octen-3-ol	9	**	1-Octen-3-ol	9	**
1-Octen-3-one	8	*	(E,E)-2, 4-Decadienal	8	*
(E,E)-2,4-Nonadienal	8	*	Octanal	8	*
(E,E)-2,4-Decadienal	8	*	Heptanal	8	*
(E)-2-Nonenal	7	NS	(E)-2-Octenal	8	*
Nonanal	7	NS	Nonanal	7	NS
Heptanal	6	NS	(E)-2-decenal	6	NS
(E)-2-Octenal	6	NS	(E,E)-2,4-Heptadienal	4	NS
Decanal	6	NS	Methyleugenol	3	NS

Dodecanal	5	NS	(Z)-3-Hexen-1-ol	3	NS
(Z)-3-hexen-1-ol	5	NS	1-Octen-3-one	3	NS
1-Hexanol	3	NS	Dodecanal	3	NS
2-Acetyl-2-thiazoline	3	NS	3-(Methylthio)propanal	2	NS
4-Methyl-5-thiazoleethanol	3	NS	Benzyl alcohol	2	NS
Benzaldehyde	3	NS	5-Methyl-2-ethylpyrazine	1	NS
Benzyl alcohol	2	NS	Formic acid octyl ester	1	NS
Methyleugenol	1	NS	3-Phenyl-2-propenal	1	NS
3-Phenyl-2-propenal	1	NS			

[a] Aroma-active compounds involved in aroma recombination experiments.

[b] Number of correct judgments from 12 assessors by the triangle test.

[c] Significance: * significant ($\alpha \leq 0.05$), ** highly significant ($\alpha \leq 0.01$), *** very highly significant ($\alpha \leq 0.001$), NS, no significant different

3.3.8. Changes in the concentrations of key aroma-active compounds of the PCSB during cooking-refrigeration-reheating

To clarify the patterns of variation in the WOF of the PCSB during the cooking-refrigeration-reheating, the aroma profiles of the refrigerated samples were detected by Q Exactive GC-Orbitrap-MS system. After 6 days of refrigeration, the concentrations of 10 key odorants were significantly increased (**Table 3-5**) ($P < 0.05$) because of the lipid autoxidation during chill storage. Notably, the increase rates decreased for pentanal, hexanal, heptanal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, and 1-octen-3-ol. This might be because on the one hand, the shorter reheating time produced limited oxidation products compared with the refrigeration period; on the other hand, reheating promoted the volatilization of the aroma compounds and increased the number of binding sites which promoted the irreversible covalent binding between aldehydes and proteins (Xu et al., 2020), thus reducing the formation rate of off-flavor substances. Unlike aldehydes, alcohols and proteins are predominantly bound via reversible noncovalent bonding (Wang & Arntfield, 2017). As a result, they tend to be released during subsequent reheating processes, which agrees with the result in our study that 1-octen-3-ol significantly increased after reheating. Nonetheless, the rate of increase in 1-octen-3-ol during reheating (71.84%) was lower than that during refrigeration (164.94%), illustrating that the WOF of the PCSB developed primarily during the refrigeration stage. Although double bonds may enhance the affinity of aroma compounds with proteins, potentially involving a “Michael addition” mechanism with the double bond (Kuhn et al., 2008) and resulting in the loss of aroma compounds, it was observed that the rate of concentration increase for (E)-2-octenal and (E)-2-nonenal was higher after reheating compared with that after refrigeration. This might be attributed to their pronounced formation induced by lipid oxidation during reheating. 3-(methylthio)propanal and 1-octen-3-one have been identified as key odorants in cooked meat (Aliani & Farmer, 2005; Kerscher & Grosch, 1997). They were positively correlated with the meaty aroma of the PCSB ($P < 0.05$) (**Fig. 3-4**A) and selected as key aroma-active compounds of the cooked stewed beef in our study. The concentration of 1-octen-3-one significantly decreased following refrigeration, subsequently increasing after reheating ($P < 0.05$). Its loss during refrigeration was probably because of its release to the environment and bonding to proteins (Wang et al., 2023a). However, ketones only form weak reversible hydrophobic contacts with proteins (Wang & Arntfield, 2016), which are weakened upon heating (Kuhn et al., 2008). Thus, after reheating, the concentration of 1-octen-3-one increased ($P < 0.05$), which also accounted for the continued lipid oxidation during reheating. Meanwhile, 1-octen-3-one was also selected as a WOF indicator for boiled beef by AEDA (Konopka & Grosch, 1991), but it was not identified as a key odorant for the formation of WOF in the reheated samples of our study. This might be due to the fact that OAV values were calculated, and the matrix effect was considered in this study. When meat is cooked, the primary reactions responsible for the development of the characteristic meat aroma are the oxidation of lipids, Maillard reaction between amino acids and sugars, Strecker reaction, and degradation of thiamine

(Elmore & Mottram, 2006; Khan et al., 2015). During cooking, the reactions occur rapidly and provide a varied profile of aromas that contribute to desirable aromas. While such reactions such as lipid oxidatin also lead to off-flavors (such as WOF) during long-term storage (Mottram, 1998) (**Fig. 3-6**). This was also corroborated in our study, where compounds such as, pentanal, hexanal, (E)-2-undecenal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, and 1-octen-3-ol, which are products of lipid oxidation, were characterized as the key aroma-active compounds of the freshly stewed beef and key contributors to the WOF of the reheated PCSB. The differences in their concentrations during cooking-refrigeration-reheating were remarkable. The concentration of 3-(methylthio)propanal decreased during cooking-refrigeration-reheating. 3-(methylthio)propanal, which is related to the Strecker degradation of methionine, serves as a valuable indicator for assessing the flavor acceptability in products (Han et al., 2021). The Maillard reaction produces heterocyclic compounds, sulfur-containing aroma compounds, furanones and their derivatives, etc., which are responsible for the desirable aroma of meat products (Elmore & Mottram, 2006; Khan et al., 2015) (**Fig. 3-6**). Thus, the loss of 3-(methylthio)propanal led to the diminished meaty attribute of the PCSB, which was also verified via the omission experiment. Furthermore, 3-(methylthio)propanal decreased after reheating at a reduced rate (36.53%) compared with that after refrigeration (16.26%), suggesting that the degradation in the desirable aroma of the PCSB occurred primarily during the refrigeration period. In addition, 3-(methylthio)propanal is a labile aldehyde that may break down into low-boiling compounds (Drumm & Spanier, 2002). Furthermore, the binding of sulfur compounds to proteins can be categorized as an irreversible covalent interaction (Zhang et al., 2021). Numerous studies have proven that heating facilitates covalent binding as well as the volatilization of flavor compounds (Kuhn et al., 2008; Wang et al., 2023a). These contributed to the reduction of 3-(methylthio)propanal. Interestingly, despite 1-octen-3-one and 3-(methylthio) propanal exhibiting notable reductions after refrigeration, their impact on the development of WOF in the PCSB differed. The decrease in the former might alleviate the lipid-oxidized aroma, whereas the reduction in the latter accentuated the WOF by lessening the desirable meaty aroma. Similarly, they both underwent changes that might be associated with matrix effects, particularly those involving proteins in the matrix. Protein is an important component in meat products responsible for flavor loss (Zhang et al., 2021). The dynamic reaction between aroma compounds and the components of food matrices is complex and related to the chemical structure and functional groups of proteins and aroma compounds, as well as the stability and chemical activity of aroma compounds, impacting the overall aroma profile of the PCSB (Zhang et al., 2021). (Wang et al., 2023a) detected the significant flavor dissipation of soy sauce–marinated beef during the air cooling stage, highlighting that the loss of the odorants might be caused by volatilization and the covalent binding of aroma compounds to proteins. In addition, (Wang et al., 2023b) pointed out that the binding ability of aroma substances to proteins differ with changing processing conditions. To date, the interaction mechanism of each key odorant to proteins during cooking-refrigeration-reheating is unclear, which will be the focus of our

future research. Based on the above results, the WOF in the reheated PCSB was attributed to hexanal, (E,E)-2,4-decadienal, (E,E)-2,4-nonadienal, pentanal, decanal, octanal, heptanal, (E)-2-octenal, (E)-2-undecenal, 1-octen-3-ol, and (E)-2-nonenal, as well as increased aldehydes, which contributed to the oxidized aroma (An et al., 2022). This finding is consistent with those of previous reports (Konopka & Grosch, 1991; O'Sullivan et al., 2003; Pegg et al., 2014; Ruenger et al., 1978; Zang et al., 2019). (E)-2-undecenal, which was considered an off-flavor aldehyde (Chen et al., 2019), was identified as a key aroma-active compound contributing to the WOF in the reheated PCSB for the first time in this study to the best of our knowledge. The significantly increased concentration of (E)-2-undecenal after reheating imparted the PCSB with a significantly enhanced metallic aroma, which confirmed the results of the APA. Moreover, our study revealed for the first time that the reduction of 3-(methylthio)propanal greatly contributed to an overall increase in WOF in the PCSB. In addition, the ratio of the total concentration of the 11 key odorants of WOF in the reheated PCSB to that amount of 3-(methylthio)propanal increased from 450.65 in the cooked samples to 1529.45 in the refrigerated samples and to 3657.45 in the reheated samples, with the increase rate of the concentration decreasing during reheating, which supported the aforementioned idea that the deterioration of the flavor quality of the PCSB occurred mainly during refrigeration.

Table 3-5 Concentration changes of key aroma-active compounds in Chinese stewed beef during cooking-refrigeration-reheating.

Key aroma-active compounds[a]	Concentrations (μg/kg)[c]			Increase rate (%)[d]	
	Cooked	Refrigerated	Reheated	Cooked - Refrigerated	Refrigerated - reheated
Pentanal	31.57 ± 1.14^{C}	59.81 ± 10.17^{B}	102.38 ± 8.93^{A}	91.90 ± 29.44^{x}	67.02 ± 8.07^{y}
Hexanal	246.17 ± 21.36^{C}	625.10 ± 47.22^{B}	1070.33 ± 108.94^{A}	155.42 ± 25.35^{x}	71.00 ± 3.63^{y}
Heptanal	32.99 ± 0.25^{C}	62.56 ± 0.79^{B}	69.46 ± 3.68^{A}	89.64 ± 0.87^{x}	10.99± 3.67^{y}
Octanal	2.01 ± 0.28^{C}	6.73 ± 0.88^{B}	17.89 ± 0.32^{A}	263.84 ± 21.14^{x}	167.71 ± 21.29^{x}
Decanal	2.56 ± 0.15^{C}	4.47 ± 0.12^{B}	6.84 ± 0.21^{A}	74.72 ± 11.71^{x}	53.10 ± 1.86^{x}
(*E*)-2-Octenal	3.89 ± 0.05^{C}	5.99 ± 0.24^{B}	13.49 ± 0.57^{A}	53.93 ± 4.40^{y}	125.23 ± 6.14^{x}
(*E*)-2-Nonenal	2.93 ± 0.14^{C}	4.01 ± 0.15^{B}	6.26 ± 0.19^{A}	36.92 ± 9.11^{y}	56.22 ± 1.29^{x}
(*E*)-2-Decenal	1.89 ± 0.05^{B}	2.28 ± 0.07^{A}	2.33 ± 0.11^{A}	20.50 ± 5.53^{x}	5.01 ± 0.67^{y}
(*E*)-2-Undecenal	1.83 ± 0.13^{C}	2.55 ± 0.03^{B}	3.04 ± 0.56^{A}	39.57 ± 8.54^{x}	27.01 ± 6.66^{x}
(*E*,*E*)-2,4-Nonadienal	1.11 ± 0.01^{C}	3.14 ± 0.06^{B}	5.75 ± 0.14^{A}	182.59 ± 7.34^{x}	83.37 ± 0.71^{y}
(*E*,*E*)-2,4-Decadienal	0.03 ± 0.00^{C}	0.09 ± 0.00^{B}	0.21 ± 0.05^{A}	194.50 ± 17.96^{x}	103.26 ± 4.63^{y}
1-Octen-3-ol	7.00 ± 0.14^{C}	18.54 ± 0.57^{B}	31.85 ± 0.48^{A}	164.94 ± 10.65^{x}	71.84 ± 2.56^{y}
1-Octen-3-one	1.75 ± 0.11^{A}	1.28 ± 0.06^{B}	1.41 ± 0.01^{B}	-26.78 ± 0.82^{y}	13.41 ± 1.14^{x}
3-(Methylthio)propanal	0.74 ± 0.09^{A}	0.50 ± 0.00^{B}	0.42 ± 0.02^{B}	-36.53 ± 0.13^{x}	-16.26 ± 2.83^{y}

Concentration ratio[e]	450.65 ± 28.10^{C}	1529.45 ± 126.94^{B}	3657.45 ± 613.30^{A}	241.33 ± 42.25^{x}	137.57 ± 20.92^{y}

[a] Key aroma-active compounds of cooked and reheated Chinese stewed beef.

[b] Significance: * significant ($\alpha \leq 0.05$), ** highly significant ($\alpha \leq 0.01$), *** very highly significant ($\alpha \leq 0.001$), NS, no significant difference. Different uppercase letters show significant differences in concentrations in each row at $P < 0.05$. Different lowercase letters show significant differences in increase rates in each row at $P < 0.05$.

[c] Concentrations of aroma-active compounds.

[d] the rate of increase in the concentration of key odorants after refrigeration and reheating.

[e] Ratio of total contents of the key aroma-active compounds contributing to WOF of reheated prepared stewed beef to the key odorants associated with meaty notes.

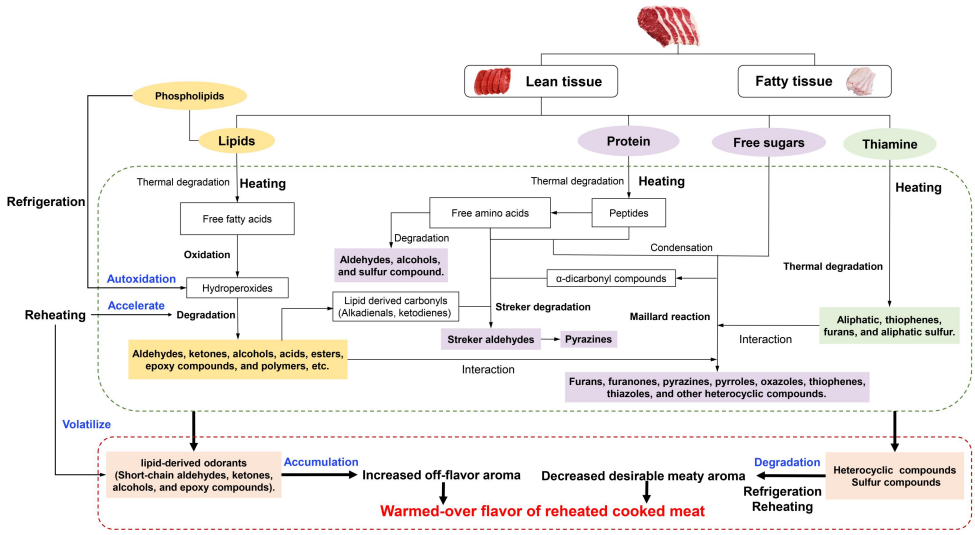


Fig. 3-6 Mechanism of formation of warmer-over flavor (WOF) in meat products.

3.3.9. Correlation between TBARS and key aroma-active compounds

(E)-2-Decenal and 1-octen-3-one, the key odorants of the cooked stewed beef, did not exhibit a significant correlation with TBARS ($P > 0.05$) (**Fig. 3-7**). Although these two substances were lipid oxidation products, were not selected as key odorants of the reheated stewed beef in the omission experiments. **Fig.3-7** shows a significantly positive correlation between TBARS and the 11 key aroma-active compounds of the reheated PCSB ($P \leq 0.05$). The outcome further validated that refrigeration and reheating promoted lipid oxidation, thus facilitating the formation of WOF. As shown in **Fig. 3-6**, refrigeration promoted lipid autoxidation of the cooked stewed beef, resulting in the formation of hydroperoxides, which are quite unstable and tend to degrade. Their degradation generated large amounts of secondary oxidation products, such as alcohols, aldehydes, and ketones. Low-molecular-weight aldehydes (C3-C12) produced by the degradation of hydroperoxides, such as pentanal, hexanal, heptanal, octanal, (E,E)-2,4-nonadienal, and (E,E)-2,4-decadienal, are of great importance as they contribute to the formation of WOF (Pegg et al., 2014). Thereafter, reheating accelerated lipid oxidation and led to the volatilization of these active off-flavor compounds, contributing to more noticeable unpleasant WOF notes (Pegg et al., 2014).

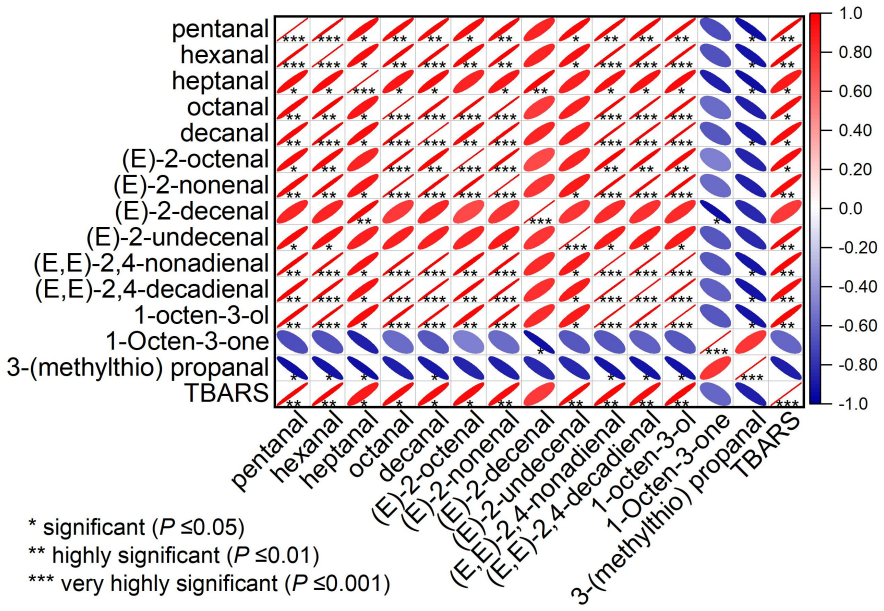


Fig. 3-7 Heat map representations of results of Pearson correlation analysis between key aroma-active compounds and TBARS.

3.4. Conclusions

The reheated PCSB exhibited a strong WOF, presenting with a diminished meaty aroma and enhanced fatty, oxidized vegetable oil, grassy, hard-boiled egg, metallic, and cardboard aroma. Hexanal, (E,E)-2,4-decadienal, (E,E)-2,4-nonadienal, pentanal, decanal, octanal, heptanal, (E)-2-octenal, (E)-2-undecenal, 1-octen-3-ol, and (E)-2-nonenal were identified as key aroma-active compounds contributing to the WOF in the reheated PCSB. Additionally, the reduction of 3-(methylthio)propanal might greatly contribute to an overall increase in WOF in the reheated PCSB. Thus, these odorants were elected as potential markers of WOF in the reheated PCSB. All in all, the increase in lipid oxidation products, interactions between odorants and protein, and volatilization of key odorants might largely affect the development of WOF. Further studies are required to reveal the mechanism of binding between the key odorants contributing to WOF and proteins during cooking-refrigeration-reheating will be taken into account, and TOFE-based off-flavor correction technologies will be combined for better handling of the flavor quality of PCSB dishes.

3.5. Reference

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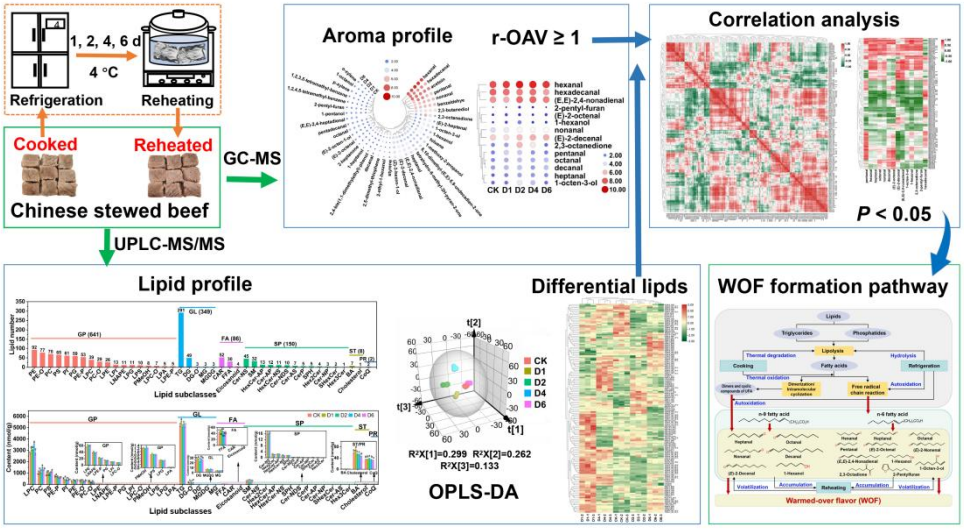
4

Chapter IV Elucidation of potential lipid precursors and formation pathways for the warmed-over flavor in precooked Chinese stewed beef

Short overview of chapter IV

In chapter III, 11 odorants were identified as key aroma-active compounds contributing to WOF in PCSB. Lipid oxidation has been proven to be the main factor causing WOF. However, changes in the lipid fingerprints of PCSB remain unclear. The specific lipid molecules involved in WOF formation and those potentially binding to key aroma compounds in PCSB remain unidentified. Hence, the objective of present study was to obtain comprehensive information about the lipid fingerprints of PCSB using lipidomics and identify the differential lipid markers as potential precursors of WOF.

Graphical abstract:



Graphical abstract: Elucidation of potential lipid precursors and formation pathways for the warmed-over flavor in PCSB

The work from this chapter was published in *Food Chemistry*, as can be retrieved by:

Liu, J., Huang, F., Han, D., Xu, Y., Shen, S., Luan, Y., Yang, P., Zhang, C., & Blecker, C. (2025). Elucidation of potential lipid precursors and formation pathways for the warmed-over flavor (WOF) in precooked Chinese stewed beef through lipid oxidation mechanisms. *Food Chemistry*, 475, Article 143294. https://doi.org/10.1016/j.foodchem.2025.143294.

Abstract

The role of lipids in the formation and development of warmed-over flavor (WOF) in PCSB was investigated using lipidomics. Fourteen predominant odorants (odor activity value ≥ 1) were identified in the reheated stewed beef. A total of 1236 lipids were detected in cooked and reheated stewed beef. TG, notably TG(18:0/18:1/18:1) and TG(16:0/18:1/18:1), were considered key lipids associated with predominant odorants. Among 153 differential lipids (VIP > 1, $P < 0.05$), PS(18:0/18:2) and PS(16:0/17:2) were identified as potential markers for distinguishing all samples. A total of 142 differential lipids were significantly correlated with the predominant odorants, with ePEs, particularly PE(P-18:0/18:2), serving as crucial precursors in WOF formation. Furthermore, LPC(20:3) and PC(16:0/18:1) notably facilitated WOF development. This study provides a theoretical basis for flavor correction in PCSB dishes.

Keywords: precooked stewed beef, warmed-over flavor, lipidomics, lipid fingerprint, precursors

4.1. Introduction

In recent years, China's precooked dish industry has developed rapidly. Stewed beef, which is a traditional Chinese dish, is widely popular. PCSB, which has a simpler preparation process and more stable quality than pan-fried, deep-fried, and stir-fried dishes, has become indispensable in the Chinese precooked dish industry. Most PCDes require reheating prior to consumption. Nevertheless, warmed-over flavor (WOF), a distinctive off-flavor, is commonly found in reheated meats that have been refrigerated for up to 48 h (Pegg et al., 2014). WOF is described as having a fatty, metallic, wet cardboard or rancid aroma, which is easily perceived by consumers and negatively affects product quality (Pegg et al., 2014). WOF development is inextricably associated with lipids. On one hand, an increase in secondary lipid oxidation products, primarily aldehydes such as pentanal, hexanal, (E)-2-octenal, and (E,E)-2,4-decadienal, has been reported as the main cause of WOF formation in meat products (An et al., 2022; Konopka & Grosch, 1991). On the other hand, the lipophilic nature of these odorants allows them to be dissolved in lipids and be released at an appropriate time (Guo et al., 2022a). Consumers' perceptions of WOF are considerably influenced by the release and retention of these compounds in the food matrix (Wang et al., 2023b). However, previous studies have primarily focused on detecting WOF through sensory evaluation, thiobarbituric acid (TBA) assessment (Fooladi, 1979; Smith et al., 2006), identification of key odorants contributing to WOF (An et al., 2022; Konopka & Grosch, 1991; Zang et al., 2019), analysis of variations in fatty acid profiles (Zhang et al., 2022) and the initial exploration of WOF formation mechanisms using reaction systems. For instance, Zhang et al. (2021) utilized solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) to investigate the volatile profiles of reaction systems containing phospholipids (PC and PE), xylose, and cysteine before and after reheating, aiming to clarify the mechanism of WOF formation. To date, lipids in reheated precooked Chinese stewed beef have been evaluated holistically rather than at the molecular level. Changes in the lipid fingerprints of reheated precooked Chinese stewed beef remain unclear. The specific lipid molecules involved in WOF formation and those potentially binding to key aroma compounds in reheated precooked Chinese stewed beef remain unidentified. This poses challenges for targeted correction of WOF in precooked Chinese stewed beef. Therefore, it is essential to clarify the lipid fingerprints of precooked Chinese stewed beef. Lipidomics allows for large-scale, comprehensive lipid studies. It has been widely employed to study meat quality and flavor, including changes in lipid profiles during cold storage (chilled/frozen) (Fang et al., 2022; Lv et al., 2023) and various processing methods such as thermal processing (Shi et al., 2019), roasting (Liu et al., 2022a), and irradiation (Zhang et al., 2023a). Lipidomics has also been combined with sensomics to investigate the key lipids responsible for generating and binding odorants in meat products (Liu et al., 2022a). Nevertheless, lipidomics methods have rarely been employed to evaluate lipid fingerprints and the formation and development of WOF in reheated precooked meat products.

In this study, aroma profiles were analyzed by GC-MS, lipid metabolic profiles in reheated precooked Chinese stewed beef were characterized by targeted lipidomics and absolute quantitative lipid technology using ultra-high performance liquid chromatography coupled with electrospray ionization mass spectrometry (UPLC-ESI-MS/MS). The aims of this study were the following: (1) to identify the predominant aroma compounds of precooked Chinese stewed beef at different storage times and elucidate their changing patterns, (2) to obtain comprehensive information about the lipid fingerprints of precooked Chinese stewed beef at different storage times, (3) to identify the differential lipid markers as potential precursors of WOF, and (4) to determine the major lipids responsible for binding WOF in reheated precooked Chinese stewed beef. These findings contribute to the elucidation of the pathways involved in WOF formation in precooked Chinese stewed beef and may offer theoretical insights into oriented-target flavor improvement.

4.2. Material and methods

4.2.1. Samples preparation

A total of eighteen 48-month-old Simmental steers were randomly selected from Hebei Fucheng Wufeng Food Co., Ltd. (Hebei, China). All animals had similar weights and genetic backgrounds and were uniformly fed a complete formula diet. These animals were randomly divided into three groups with six animals per group, serving as three biological replicates. The chuck tenders of each steers were vacuum-packed at the source after aging and transported to the laboratory under cold-chain conditions. External fat and connective tissues were eliminated. For each replicate, the chuck tenders were washed, cut into blocks measuring approximately 2 cm × 2 cm × 1.5 cm, and mixed thoroughly. The mixed blocks were added to water, brought to a boil, blanched for 3 min, and drained. The drained blocks were then added to water, heated to a boil using an induction cooker at 1200 W and maintained for 5 min. The power was then reduced to 600 W to maintain a gentle simmer and 1.2% salt (w/w of raw meat) was added. The blocks were simmered for 45 min and maintained for 20 min without heat. The samples were removed, drained, and then randomly divided into five treatment groups. The five groups were portioned into aluminium foil bags, cooled to ambient temperature under running water, and then stored at 4 °C for 0, 1, 2, 4, and 6 days, receptively. After the storage, the samples were reheated in a water bath at 100 °C for 10 min. The five treatment groups were as follows: CK (freshly cooked stewed beef), D1 (stewed beef reheated after 1 day of refrigeration), D2 (stewed beef reheated after 2 days of refrigeration), D4 (stewed beef reheated after 4 days of refrigeration), and D6 (stewed beef reheated after 6 days of refrigeration). Microbial counts were in the acceptable range ($< 10^4$ CFU/g) (National Food Safety Standard, 2017). All samples (except those used for sensory aroma profile analysis) were immediately frozen in liquid nitrogen for 5 min, ground into a fine powder using a blender, and then stored at −80 °C for use. All analyses were completed within one week.

4.2.2. Sensory aroma profile analysis (APA)

Sensory evaluation was conducted following the method described by Pang et al. (2012) involving 12 trained panelists (8 women and 4 men, aged 22 - 45 years). The panelists were selected and trained according to the Chinese national standard GB/T 16291.1-2012 (https://www.chinesestandard.net/PDF/English.amp.aspx/GBT16291.1-2012). The panelists underwent professional training to identify and define descriptive terms for cooked and reheated stewed beef in chapter III. They had one year of sensory evaluation experience. The panel identified seven odor attributes (meaty, grassy, cardboard, metallic, fatty, hard-boiled egg, and oxidized vegetable oil) to describe the aroma profile of reheated precooked Chinese stewed beef. Each attribute was rated on a 0-3 scale (0 = imperceptible and 3 = very intense) with a scale step of 0.5. The final scores for each odor attribute were determined as the average ratings from all panelists. Sensory analysis was conducted in isolated booths in a sensory laboratory at 20 °C ± 2 °C. Our institution granted ethical permission to conduct this human sensory study. The sensory panelists were entirely voluntary. We ensured that panelists' rights and privacy were fully protected throughout the study. All samples were confirmed to be non-toxic and safe for human use. Panelists were fully informed of the study's requirements and risks and provided their consent. All details regarding the sensory evaluators were kept confidential. Data from this evaluation were exclusively used to assess aroma changes in precooked Chinese stewed beef.

4.2.3. E-nose analysis

E-nose analysis was performed using commercial PEN 3.5 E-nose (Airsense Analytics, GmBH, Schwerin, Germany). The obtained powder (2 g each) was placed in a 20 mL glass vial, sealed with a PTFE-silicon stopper, and analyzed following the modified method reported by Chen et al. (2018). The samples were maintained at room temperature for 20 min before the headspace gaseous compounds were pumped into the sensor array chamber via Teflon tubing. Sensor cleaning took 180 s, automatic zero adjustment took 10 s. The internal and inlet flow rates were set to 600 mL/min. The sample detection time was 60 s. Each sample was analyzed in triplicate.

4.2.4. Lipid oxidation

Lipid oxidation was evaluated by determining TBARS values and lipid peroxide content (LPO) using the methods described by in the section 3.2.2, and Ohkawa et al. (1979) , respectively.

4.2.5. Aroma compounds analyzed by SPME-GC-MS

Two grams of stewed beef powder were quickly transferred to a 20 mL glass vial containing 2 μL of 0.204 μg/μL 2-methyl-3-heptanone internal standard solution (methanol as solvent). The vial was pre-equilibrated in a 50 °C water bath for 20 min, followed by extraction for 30 min with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (2 cm,

Supelco, Bellefonte, PA, USA). After extraction, the fibre was transferred to a GC-MS (8890-5977, Agilent Technologies, Inc., Santa Clara, CA, USA) injector port and desorbed at 250 °C for 5 min in splitless mode. The SPME extracts were separated using a DB-WAX column (30 m × 0.25 mm, 0.25 μm, Agilent Technology, USA). The column temperature was programmed to start at 40 °C then 4 °C/min to 150 °C, held for 3 min, and then 8 °C/min to 230 °C, held for 5 min. Helium carrier gas (99.999% purity) flowed at 1.0 mL/min. The quadrupole mass spectrometer scanned M/Z 30-550 in EI mode at 70 eV.

Odorant identification was based on retention indices calculated following the procedure reported by Vandendool and Kratz (1963), mass spectra (MS) comparisons with the NIST 20 library and data from authentic reference standards (STDs). The semi-quantitative analysis of the aroma compounds was conducted using an internal standard as described by Li et al. (2022), with slight modifications. The relative content (C) of each compound was calculated using formula (1). The odor activity values (OAVs) were calculated using formula (2), obtained from Li et al. (2022).

$$Cx = \frac{1000 \times C_i \times V_i \times A_x}{A_i \times m} \quad (1)$$

$$OAV_x = \frac{C_x}{T} \quad (2)$$

Where C_x represents the relative content of the target compound (μg/kg); C_i represents the concentration of the internal standard compound (μg/μL); Vi is the injection volume of the internal standard (μL); Ax represents the peak area of the target compound; Ai is the peak area of the internal standard; m is the mass of the sample (g); T denotes the sensory threshold of the target compound in a water medium (μg/kg).

Odorants with OAVs ≥ 1 were considered the predominant aroma compounds contributing to the aroma profile of stewed beef (Liu et al., 2022a).

4.2.6. Lipidomics analysis

4.2.6.1. Lipid Extraction

Lipids were extracted following the method described by Liu et al. (2022b) with slight modifications. Precooked Chinese stewed beef powder was thawed. The powder samples were thawed prior to analysis. A 20 mg portion of the sample was transferred into a 2 mL centrifuge tube, followed by the addition of 1 mL mixture containing methanol, tert-butyl methyl ether, and internal standard mixture. The mixture was homogenized using a ball mill (MM400, Retsch, Haan, Germany). After removing any insoluble residue, the supernatant was vortexed for 2 min and sonicated for 5 min. Thereafter, 200 μL of distilled water was introduced, and the sample was vortexed for an additional minute before centrifugation at 12,000 rpm for 10 min at 4 °C. A 200 μL aliquot of the resulting supernatant was collected, evaporated to dryness under a nitrogen stream, and reconstituted in 200 μL of an

acetonitrile/isopropanol solution (10:90, v/v) containing 0.1% formic acid and 10 mmol/L ammonium formate.

4.2.6.2. UPLC-ESI-MS/MS analysis

Lipid extracts were analyzed using an ExionLC AD system coupled with a QTRAP 6500 plus tandem mass spectrometer (SCIEX Corp., Framingham, USA). The analytical parameters were set as described by Liu et al. (2022b): chromatographic column, Thermo Accucore™ C30 (2.6 μm, 2.1 mm × 100 mm); solvent system, A, acetonitrile/water (60/40, v/v, 0.1% formic acid, 10 mmol/L ammonium formate), and B, acetonitrile/isopropanol (10/90, v/v, 0.1% formic acid, 10 mmol/L ammonium formate). Gradient programme (A/B, v/v): 80:20 at 0 min, 70:30 at 2 min, 40:60 at 4 min, 15:85 at 9 min, 10:90 at 14 min, 5:95 at 15.5 min, 5:95 at 17.3 min, 80:20 at 17.5 min, and 80:20 at 20 min. The flow rate was 0.35 mL/min, the column temperature was 45 °C; and the injection volume was 2 μL. The samples were ionized using ESI, mass spectrometry conditions were as follows: ESI temperature, 500 °C; ion spray voltage (IS), +5500 V (positive mode), and −4500 V (negative mode); ion source gas 1 (GS1), 45 psi; gas 2 (GS2), 55 psi; and curtain gas (CUR), 35 psi. Each ion pair in the triple quadrupole was scanned and detected using an optimized de-clustering potential (DP) and collision energy (CE).

4.2.6.3. Qualitative and quantitative lipids in PCSB

Based on the self-constructed MetWare (http://www.metware.cn/) Database, qualitative analysis was performed by assessing the retention time and parent ions of the detected compounds. Lipid quantification was performed in the multiple reaction monitoring mode using triple quadrupole mass spectrometry. The peak areas of all detected substances were integrated after acquiring lipid mass spectrometry data from various samples. The internal standard method was used for semi-quantification. The content (C) of each compound was calculated using formula (3):

$$C = \frac{0.001 \times R \times F \times c \times V}{m} \quad (3)$$

Where C is the content of target compound in the sample (nmol/g), R is the peak area ratio of the target compound to the internal standard, F is the internal standard correction factor for different compounds, c is the concentration of the internal standard (μmol/L), V is the injection volume of the sample extract (μL), and m is the mass of the sample taken (g).

4.2.7. Statistical analysis

All experiments were conducted in triplicate using a completely randomized design. Statistical analyses were conducted using analysis of variance (ANOVA) with SPSS software (v25.0, Chicago, IL, USA). Differences between the groups were analyzed using Duncan's multiple range test at a significance level of $P < 0.05$. PCA and OPLS-DA analyses were conducted using SIMCA software (v14.1; Umetrics, Umeå, Sweden). K-means clustering was conducted using R software

(www.r-project.org). Heatmaps were generated using TBtool (Chen et al., 2023), and other graphs were produced by Origin 2021 (OriginLab, Northampton, MA, USA).

4.3. Results

4.3.1. APA and E-nose analysis

Sensory evaluation indicated that prolonged refrigeration increased the WOF in PCSB, marked by a decrease in meaty aroma and an increase in fatty, oxidized vegetable oil, cardboard, hard-boiled egg, metallic, and grassy notes (**Fig. 4-1**A).

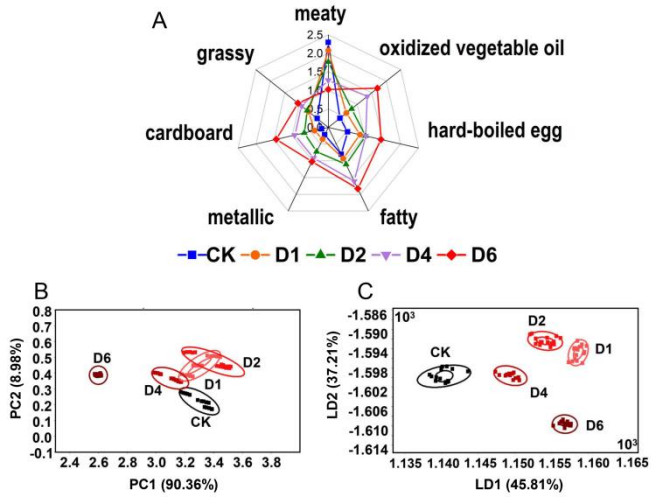


Fig. 4-1 Descriptive aroma profiles of freshly cooked (CK) and PCSB samples (D1, D2, D4, and D6) (A), principal component analysis (PCA) score plot (B), and linear discriminant analysis (LDA) score plot (C) of E-nose result.

E-nose was used to distinguish the odor characteristics of reheated stewed beef stored for different refrigeration periods. In the PCA plot (**Fig. 4-1**B), D6 was distant from CK, indicating a significant difference between them. D1, D4, and D2 followed, with D1 overlapping with both D2 and D4, and D4 slightly overlapping with CK, suggesting similar aroma profiles among these samples. No overlap was observed between the groups in the LDA plot (**Fig. 4-1**C). CK was close to D4 and D1 was near D2. D1, D2, and D6 were distant from CK. These findings indicated that the aroma profiles varied among the samples and were effectively distinguished by both PCA and LDA.

4.3.2. Lipid oxidation

Oxidative reactions occur during processing and storage of meat products. Lipid oxidation generates aldehydes that are strongly linked to off-flavors in meat products (Al-Dalali et al., 2021). Malondialdehyde (MDA) is a typical secondary product

formed during lipid peroxidation and is widely used as a marker of oxidative damage in lipids. The TBARS value reflects the overall level of substances that react with thiobarbituric acid, mainly MDA, but it may also include other aldehydes such as hexanal. Therefore, the TBARS value reflects the degree of lipid oxidation and is one of the most commonly used indicators for assessing lipid oxidation in meat (Al-Dalali et al., 2021; Xia et al., 2009). The TBARS value in CK was 0.53 mg MDA/kg and significantly increased to 1.03 mg MDA/kg in D2 (**Fig. 4-2A**), indicating a rapid decline in the quality of PCSB. This outcome is similar to that reported by Lv et al. (2023), where the TBARS value of Sanhuang chicken significantly increased after 6 days of refrigerated storage (P <0.01). A notable difference is that lipid oxidation in cooked meat occurs more rapidly, as highlighted in previous research (Pegg et al., 2014). Notably, the TBARS growth rate in D2 reached 94.34%, which was significantly higher than the 5.6% increase observed in raw Sanhuang chickens after 6 days of refrigeration (Lv et al., 2023).The TBARS of D4 and D6 significantly decreased because of a decline in MDA and other reactive aldehydes such as hexanal, as they can form adducts with lysine residues in meat proteins (Al-Dalali et al., 2021). This lipid-protein interaction is common and can occur in various forms. Lipid oxidation products such as hydroperoxides and aldehydes can interact with proteins through hydrophobic forces and hydrogen bonds. This interaction may result in the formation of covalent bonds and adducts via a Schiff base arrangement. This process could explain the decrease in TBARS value of PCSB during refrigeration (Al-Dalali et al., 2021). Rapid lipid oxidation of meat occurs during the processing of raw meat, such as grinding, cooking, and the addition of salt, especially thermal processing (Bastida et al., 2009). Thermal oxidation not only generates characteristic aromas in meat products but also promotes lipid peroxide accumulation. Heating significantly increases lipid oxidation in meat. Reheating after refrigeration accelerates the breakdown of lipid peroxides, producing alcohols, aldehydes, and ketones, which contribute to WOF (Pegg et al., 2014). This might contribute to the significant decrease in LPO observed in D1 (**Fig. 4-2**B). Cooked meat is more prone to lipid peroxidation than raw meat when stored under refrigerated conditions. Reheating significantly increases lipid oxidation in meat (Bastida et al., 2009). Lipid oxidation in cooked meat products continued after refrigeration and reheating, generating significant lipid peroxides over time, which might contribute to the increase in LPO observed in D2 and D4 ($P < 0.05$). However, lipid peroxides are unstable and readily decompose over extended refrigeration time, which may be one reason for a decrease in the LPO value in D6 ($P < 0.05$).

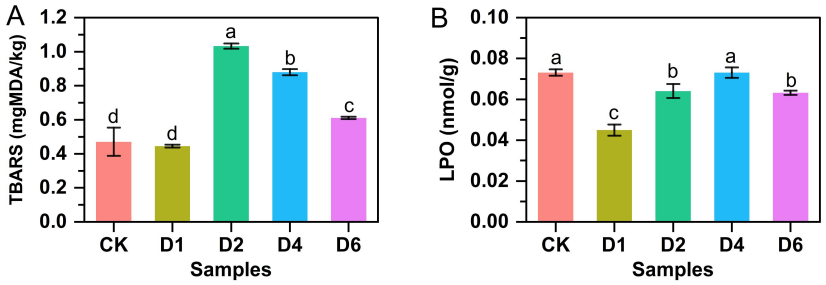


Fig. 4-2 Thiobarbituric acid reactive substances (TBARS) values (A) and lipid peroxide content (LPO) variation (B) for both freshly cooked (CK) and PCSB samples (D1, D2, D4, and D6). Data labeled with different lowercase letters indicate significant differences at $P < 0.05$.

4.3.3. Changes in predominant aroma compounds in reheated PCSB

A total of 38 aroma compounds were identified, comprising 13 aldehydes, 10 alcohols, 4 ketones, 3 heterocyclic compounds, and 8 benzenoic compounds (**Fig. 4-3**A). Among these, (E,E)-2,4-heptadienal was only detected in D2, D4, and D6. Aldehydes played a dominant role in the aroma profiles of PCSB, followed by ketones and alcohols. The aldehyde and ketone contents initially increased and then decreased with refrigeration duration, peaking in D2 (**Fig. 4-3**B). Hexanal consistently remained at its highest level during refrigeration, followed by hexadecanal, pentanal, nonanal, and benzaldehyde. As shown in **Fig. 4-3**A, the content of most aldehydes, except pentadecanal and hexadecanal, increased from CK to D2, followed by a decrease in D6. This pattern was aligned with the TBARS results, which also peaked at D2 before decreasing significantly. This phenomenon might be due to the interaction between lipid oxidation products (hydroperoxides and aldehydes) and proteins (Al-Dalali et al., 2021). Alternatively, it could result from the dissipation of flavor compounds into the environment during cold storage (Wang et al., 2023a). Secondary lipid oxidation products, particularly short-chain aldehydes (C3-C12), are the primary contributors to WOF (Pegg et al., 2014). Typically, OAV is used to assess the odor contribution of each odorant (Li et al., 2022). As shown in **Fig. 4-3**C, 14 odorants had OAVs ≥ 1 in the reheated PCSB. Except for hexadecanal, which displayed an overall decreasing trend, all other odorants showed an increase followed by a decrease in OAVs. Hexanal had the highest OAV value during chilled storage, peaking at 495 in D2. Although the concentrations of (E,E)-2,4-nonadienal (CK, 9.70; D1, 6.70; D2, 16.36; D4, 18.22; and D6, 12.64 μg/kg) were much lower than those of pentanal (CK, 54.43; D1, 160.60; D2, 145.72; D4, 69.29; and D6, 53.04 μg/kg), its lower threshold (0.1 μg/kg) resulted in a much higher OAV than pentanal. Similar outcomes were observed for (E)-2-decenal. Both are products of lipid oxidation (Elmore et al., 1999; Liu et al., 2020). (E)-2-octenal, (E,E)-2,4-nonadienal, hexanal, pentanal, heptanal, decanal, octanal, and 1-octen-3-ol were identified as the key aroma-active compounds

responsible for WOF in reheated PCSB using sensomics techniques in Chapter III. Heptanal, (E)-2-octenal, octanal, nonanal, (E)-2-decenal, and decanal were selected as the key odor-active contributors to WOF in surimi gels (An et al., 2022). Angelo et al. (2006) determined hexanal and 2,3-octanedione as the primary compounds associated with WOF development using chemical, instrumental (GC-MS), and sensory methods. Previous researches have confirmed that an increase in lipid oxidation products, primarily aldehydes, and a reduction in desirable odorants, such as furanones and sulfur-containing compounds, are the main causes of WOF in reheated meat products (An et al., 2022).

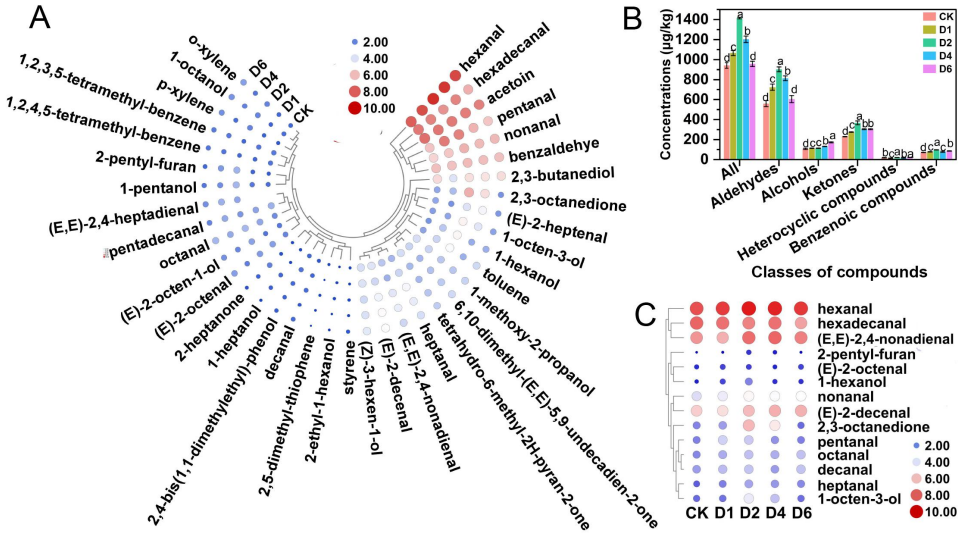


Fig. 4-3 Heat map distribution of aroma compound contents (μg/kg), with all data log-transformed (A). The color intensity, ranging from blue to red, indicates an increase in the concentration of aroma compounds. Changes in the contents of aroma compounds (data marked with different lowercase letters indicate a significant difference at $P < 0.05$) (B). Heat map distribution of odor activity values (OAVs) of predominant aroma compounds (OAV ≥ 1) in freshly cooked (CK) and PCSB samples (D1, D2, D4, and D6) (C), with all data log-transformed. The color intensity from blue to red represents an increase in the OAV of each aroma compound.

4.3.4. Overall lipidomics analysis

A total of 1236 lipid metabolites were detected across 15 samples from the five groups, encompassing six major lipid categories (**Fig. 4-4**A). Glycerophospholipids (GP) were the most numerous, with 641 species, followed by glycerolipids (GL) with 349 species, sphingolipids (SP) with 150 species, fatty acyl esters (FA) with 86 species, sterol lipids (ST) with 8 species, and isoprenoid lipids (PR) with 2 species. These lipids were categorized into 42 subclasses, including 291 triglycerides (TG),

92 phosphatidylethanolamines (PE), 77 alkyl-phosphatidylethanolamines (PE-O) with an alkyl-ether bond, 70 phosphatidylcholines (PC), 65 phosphatidylserines (PS), 61 phosphatidylinositols (PI), 59 phosphatidylglycerols (PG), and 53 plasmalogen-lysophosphatidylethanolamines (PE-P) with a vinyl-ether bond. These lipid subclasses comprised a larger proportion of lipid molecules in PCSB samples. TG, PE, PE-O, PC, PS, PI, and PG accounted for 23.54%, 7.44%, 6.23%, 5.66%, 5.26%, 4.94%, and 4.77% of the total lipid count, respectively (**Fig. 4-4**B). These accounted for over 50% of the total lipids, whereas other classes were present in smaller amounts. The type and quantity of each lipid molecule remained unchanged after reheating. Among the six major categories, GP was the most abundant, comprising approximately 28% of total lipids, followed by GL, SP, ST, and FA, with PR being the least abundant (**Fig. 4-4**C). Lysophosphatidylcholine (LPC) was the most prevalent GP, constituting approximately 39.3% of GP (CK), followed by PA, PC, PE-P, and PE. TG was the most abundant in GL, constituting 96.89% of GL, and was the most prevalent of all lipid subclasses, comprising 41.15% of the total lipids (**Fig. 4-4**D).

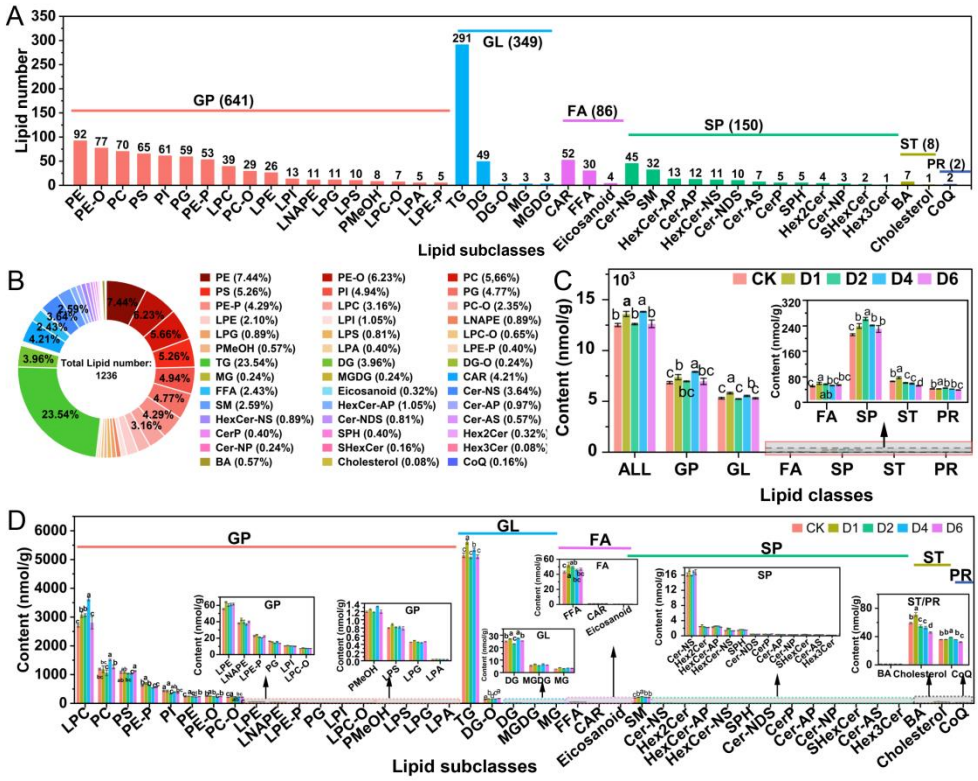


Fig. 4-4 Number of all detected lipid species (A), percentage of identified lipid species (B), changes in the contents of six major lipid categories (C), and variations in the contents of

detected lipid subclasses (D) from freshly cooked (CK) and PCSB samples (D1, D2, D4, and D6). Different lowercase letters indicate significant differences at $P < 0.05$.

To further investigate changes in lipid molecules between samples, k-means cluster analysis was performed. The lipids were classified into nine categories based on their quantity and type (**Fig. 4-5**). GP, GL, and SP were the primary lipid types in all nine clusters (**Fig. 4-4**C, **Fig. 4-5**). Clusters 3 and 4 were rich in GP and SP, whereas GL was concentrated in clusters 2 and 6 (**Fig. 4-5**). Hence, the focus was on clusters 2, 3, 4, and 6. As shown in **Fig. 4-6**, clusters 3 and 4 initially increased and then decreased, with peak values observed in D4, indicating that the lipid content first rose and then fell. A similar pattern was noted in **Fig. 4-4**C, where the GP and SP contents also exhibited an upward trend before declining, with GP peaking in D4 and SP peaking in D2. However, because the GP content was approximately 30 times greater than the SP content, changes in GP content predominated in clusters 3 and 4. Clusters 2 and 6 exhibited a similar trend, but both reached their maximum values in D1 (**Fig. 4-6**). Cluster 6 showed a slight increase in D4, followed by a continued decrease, consistent with the GL and TG trends shown in **Fig. 4-4**C and 4D. Because TG constituted approximately 97% of GL, the changes in GL were mainly driven by TG. As GP and GL together comprised approximately 97% of the total lipids, the overall lipid content mirrored the trends observed for GP and GL (**Fig. 4-4**C and 4D). These results suggested that the lipid composition of reheated stewed beef changes dynamically throughout the storage period. The significant variations among the groups may be attributed to lipid degradation, backbone cleavage, side-chain modifications, and/or lipolysis during storage.

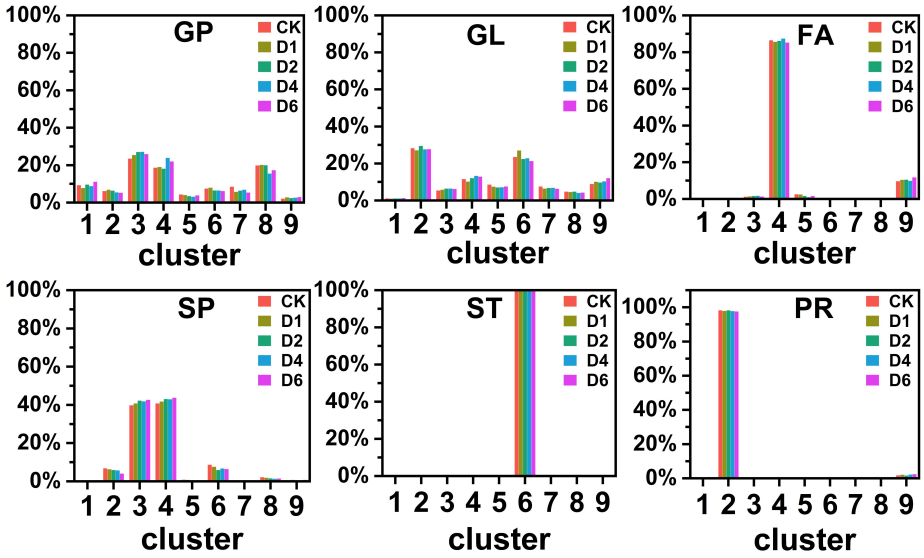


Fig. 4-5 Relative percentages of GPs, GLs, FAs, SPs, STs, and PRs in clusters.

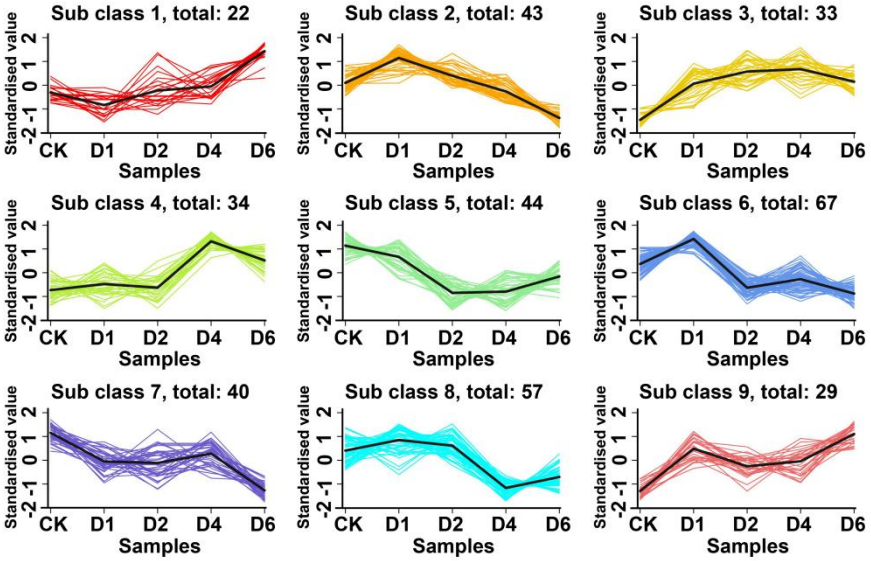


Fig. 4-6 K-means cluster analysis of different lipids from freshly cooked (CK) and PCSB samples (D1, D2, D4, and D6).

Triglycerides and phospholipids constitute the majority of intramuscular lipids in meat. The complex structure of lipids imparts these molecules with diverse physiological functions. In terms of the unsaturation of the fatty acid chains in lipids, PE-O exhibited the highest degree of unsaturated fatty acids (100.00%), followed by PE-P (99.90%), LPC (99.48%), PE (99.46%), PC-O (99.41%), SM (97.45%), PS (96.03%), PI (95.73%), and TG (95.22%) (**Fig. 4-7**). The degree of unsaturation of acyl constituents in meat lipids is the primary factor that influences the rate of meat flavor deterioration (Shahidi, 2002). PE-O possessed the highest percentage of polyunsaturated fatty acids (PUFAs) at 99.67%. PE(O-18:1/18:2), PE (O-16:1/20:4), PE(O-18:1/20:4), and PE (O-16:1/18:1) were dominant, comprising 56.04% of PE-O. PC-O, LPC, PE-P, PE, PS, and TG contained 96.48%, 92.67%, 87.39%, 86.74%, 82.2%, and 64.85% of PUFAs, respectively. Conversely, saturated fatty acids (SFAs) were more abundant in DG-O and FFA, with percentages of 98.59% and 52.67%, respectively. The PUFAs detected in PCSB were mainly side-chains conjugated with GP and TG, with a significant amount of PUFAs primarily deposited in GP. This result is consistent with the outcomes of Jia et al. (2021b). Lipids with higher proportions of unsaturated fatty acids are more susceptible to oxidation (Dinh et al., 2021; Lv et al., 2023; Shahidi, 2002). Thus, in PCSB, PE-O, PE-P, LPC, PE, PC-O, SM, PS, PI, and TG were more prone to oxidation. Lv et al. (2023) observed similar findings, showing that lipids with higher proportions of unsaturated fatty acids, such as TG, PC, PE, and ether-bonded PE (ePE), in Sanhuang chicken breast were more susceptible to oxidation during refrigeration. TG is the major energy storage substance in cells, and phospholipids are the fundamental components of cell membranes. Studies have shown that phospholipids (PL) and triglycerides (TG) are

prone to oxidation during processing because of their high unsaturated fatty acid content, which affects the flavor of meat products. In particular, PL are more susceptible to oxidation than TG owing to its higher proportion of unsaturated fatty acids and greater solubility in aqueous environments (Li et al., 2021; Zhang et al., 2023b). Furthermore, PL oxidation disrupts cell membranes, leading to the oxidation of intracellular components and the promotion of further oxidation (Jia et al., 2021a). PC and PE, the major polar phospholipid classes, are crucial for maintaining cell membrane fluidity, structure, function, and signal transduction in muscle tissues. PC, the most abundant glycerophospholipid in skeletal muscle cells, mainly has a fluidising effect (Yan et al., 2022),and PE is believed to have antioxidant activity. They contribute to membrane sclerosis and play a key role in the development of WOF in cooked meat (Igene & Pearson, 2006). Particular attention was given to ePE, and a substantial presence of ePE in stewed beef was identified, accounting for 6.82% of the total lipids (CK) and 78.9% of total PE. The observed ePE comprised 56.90% of PE-P and 22.01% of PE-O. ePE is abundant in pork (Chao et al., 2020) and is considered an endogenous antioxidant. Lv et al. (2023) found a relatively high ePE content in Sanhuang chicken breast, accounting for 3.9% of the total lipids.

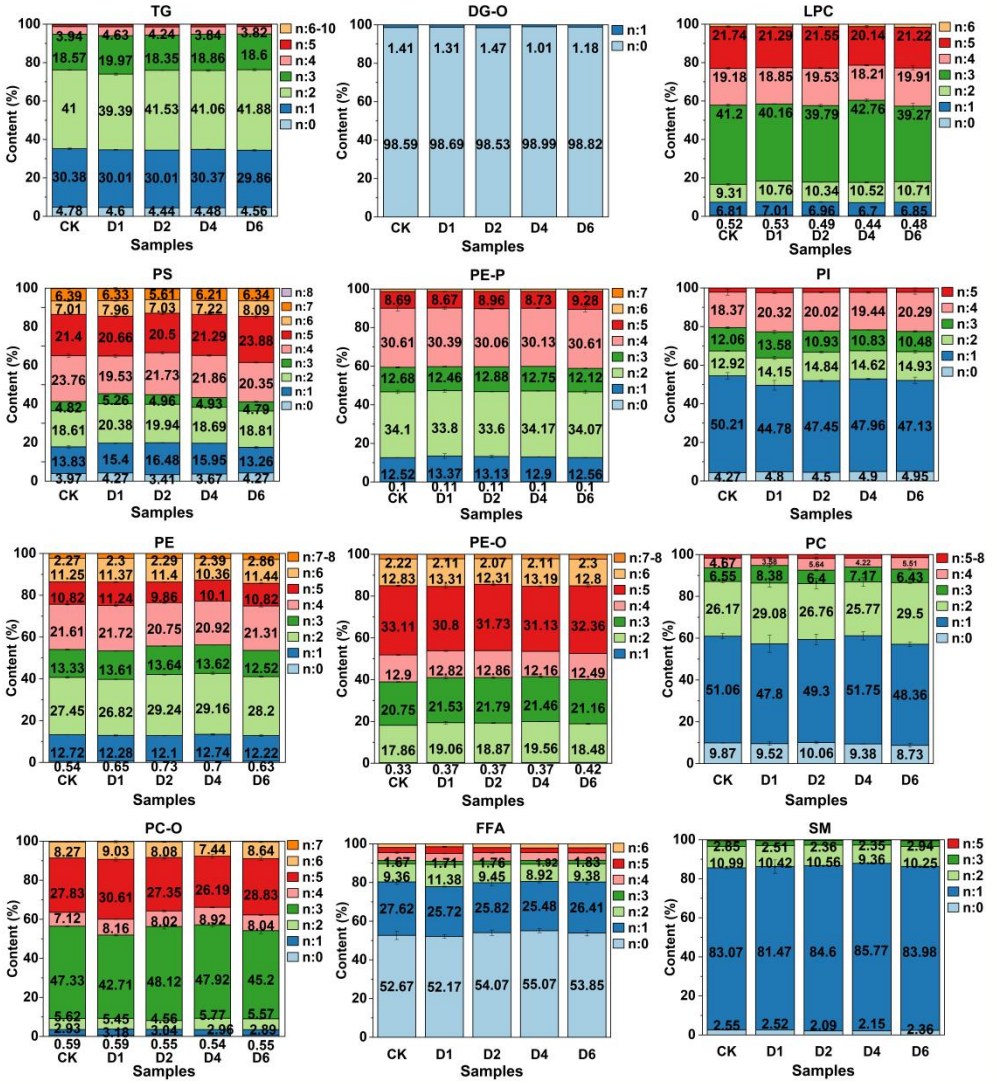


Fig. 4-7 The percentage composition of the predominant lipid molecular species (total acyl carbons: total double bonds) in total TG, DG-O, LPC, PS, PE-P, PI, PE, PE-O, PC, PC-O, FFA, and SM of freshly cooked (CK) and PCSB samples (D1, D2, D4, and D6).

4.3.5. Analysis of crucial lipids for aroma compounds binding

Lipids are considered the most effective retainers of volatiles compared to proteins and carbohydrates (Liu et al., 2022a; Wu et al., 2024). TG played a crucial role in aroma retention due to its abundance in both variety and content in roast mutton (Liu et al., 2022a). As shown in **Fig. 4-4**A and D, TG exhibited the highest levels of both species and content. Therefore, TG may significantly contribute to the retention of

WOF in PCSB. TG(18:0/18:1/18:1) (345.04-372.46 nmol/g), TG(16:0/18:1/18:1) (304.47-401.88 nmol/g), TG(16:0/18:0/18:1) (324.61-349.46 nmol/g), and TG(16:0/16:1/18:1) (290.42-333.89 nmol/g) were detected at high concentrations. In particular, TG(18:0/18:1/18:1) remained stable throughout the entire refrigeration-reheating process, showing no significant change in concentration. Similarly, TG(16:0/18:0/18:1) exhibited only a slight decrease in D6. These lipids might be crucial for the binding of the aroma compounds in PCSB. Given TG's significant effect on aroma retention, it was inferred that variations in TG content might contribute to changes in aroma compound levels. TG content in D1 significantly increased ($P < 0.05$). This increase could be attributed to reheating, which further disrupted cell membranes in the PCSB samples, leading to the release and increase in TG content (Liu et al., 2023). Concurrently, the contents of odorants in D1 significantly increased, particularly aldehydes like pentanal, hexanal, and heptanal. Li et al. (2020) found that lamb with high intramuscular lipid content had significantly higher TG levels, including TG(16:0/16:0/18:1) and TG(16:0/18:0/18:1). Castration has been shown to significantly elevate lipid levels in lamb, particularly TG, which might lead to a marked increase in odorants like hexanal and 1-octen-3-ol. The possible reason was that increased lipid content can elevate the partition coefficients of odorants in samples, leading to improved aroma retention (Liu et al., 2022a).

4.3.6. Differential lipids analysis

Unsupervised PCA and supervised OPLS-DA analyses were used to distinguish the lipid profiles of cooked and reheated stewed beef. The PCA score plot demonstrated clear clustering among the five groups (**Fig. 4-8**A). The first four principal components accounted for 77.1% of the total variance, indicating that the PCA model effectively distinguishes among the groups (Wang et al., 2021). The OPLS-DA score plot showed a better separation of the five groups (**Fig. 4-8**B). In the established OPLS-DA model, which included five principal components, the parameters R^2X, R^2Y, and Q^2 were 0.861, 0.983, and 0.933, respectively, demonstrating the model's good discriminative and predictive performance for the tested samples. A permutation test with 200 repetitions was conducted to confirm the validity and reliability of the OPLS-DA model. The OPLS-DA permutation plot (**Fig. 4-8**C) showed that $R^2 = (0.0, 0.543)$ and $Q^2 = (0.0, -0.822)$, indicating that the model was not overfitted. Collectively, the PCA and OPLS-DA models demonstrated distinct separations among the test samples, confirming significant changes in the lipid profiles of reheated stewed beef. This demonstrated that PCSB underwent remarkable alterations due to oxidation caused by chilling−reheating. P-values from the univariate analysis were combined with VIP values to further identify differential lipids. A total of 153 lipid molecules with VIP > 1 and $P < 0.05$ were selected as differential lipids and classified into the following subclasses: 50 TG, 31 PS, 16 PC, 15 LPC, 10 PE-P, 7 PI, 4 PE, 4 SM, 3 PE-O, 2 PC-O, 1 LPE, 1 DG-O, 1 CoQ, and 1 cholesterol. Differential analysis of 153 lipid molecules revealed that only PS(18:0/20:4) and PS(16:0/17:2) exhibited significant changes throughout the entire refrigeration−reheating process. **Fig. 4-8**D shows that

PS(16:0/17:2) showed a significant decrease in D1, followed by an increase in D2, a further rise in D4, and then decreased its lowest level in D6 ($P < 0.05$). PS(18:0/20:4) significantly increased in D1 and D2, decreased thereafter in D4, and increased again in D6 ($P < 0.05$) (**Fig. 4-8**E). The results suggested that among the 153 differential lipid molecules, only PS(16:0/17:2) and PS(18:0/20:4) could distinguish all stewed beef samples. Phospholipids have been demonstrated to be crucial for distinguishing meat products. PG(18:1/18:1) has been identified as a biomarker for chemical changes during lipid oxidation in non-fried roasted chicken (Liu et al., 2023). Liu et al. (2022a) identified PC(30:6) and PC(28:3) as potential biomarkers for distinguishing charcoal-grilled roasted lamb.

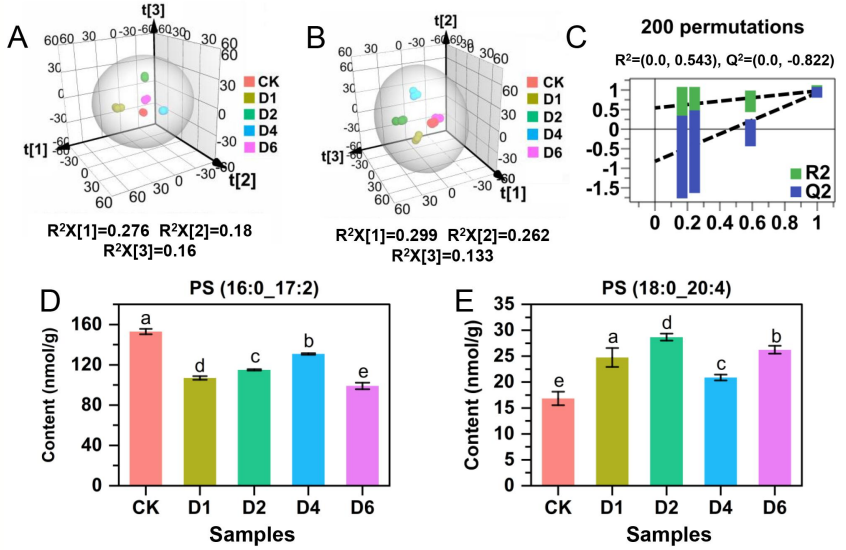


Fig. 4-8 Principal component analysis (PCA; A), partial least squares discriminant analysis (OPLS-DA; B), permutation score plots (C), and potential lipid markers (D and E) were obtained from freshly cooked (CK) and PCSB samples (D1, D2, D4, and D6) through lipidomics analysis. Data marked with different lowercase letters indicate a significant difference at $P < 0.05$.

4.3.7. Correlation between differential lipids and predominant aroma compounds

To elucidate the role of lipid degradation and oxidation in WOF formation, correlation analysis was performed using 153 differential lipid metabolites and predominant aroma compounds with OAV ≥ 1. The results revealed a strong correlation between differential lipids and predominant aroma compounds. A total of 142 lipid molecular markers were significantly associated with predominant aroma compounds ($P < 0.05$). These markers included 47 TG, 30 PS, 15 PC, 3 ePC, 16 ePE, 4 PE, 13 LPC, 6 PI, 1 LPE, 1 DG-O, 4 SM, 1 CoQ, and 1 cholesterol (**Fig. 4-9**). Phospholipids and their derivatives comprised 61.97% of all species and 56.02% of

the total content. Among them, 103 potential lipid markers showed significant negative correlations with predominant aroma compounds ($r < 0$, $P < 0.05$) (**Fig. 4-9**), suggesting that a reduction in these lipid molecules might lead to an increase in the concentrations of predominant aroma compounds. Of the 15 PC molecules, 11 exhibited negative correlations with predominant aroma compounds. For example, PC(14:0/18:1) was negatively correlated with pentanal, hexanal, 1-hexanol, heptanal, (E)-2-octenal, octanal, decanal, nonanal, 1-octen-3-ol, and 2,3-octanedione. PC(18:0/18:2) was negatively correlated with hexanal, heptanal, decanal, (E)-2-octenal, 1-octen-3-ol, 1-hexanol, 2,3-octanedione, and 2-pentyl-furan. PC(O-16:1/20:4) and PC(O-18:2/20:4) showed a negative correlation with (E)-2-decenal and (E,E)-2,4-nonadienal. The degradation of PC can produce LPC and FFA, generating aroma compounds (Liu et al., 2023). Thus, higher LPC levels might indicate an increase in aroma compounds Most LPCs were positively correlated with predominant aroma compounds, except LPC(16:1) and LPC(20:3). For instance, LPC (0:0/22:5) showed strong positive correlations with hexanal ($r = 0.820$), heptanal ($r = 0.790$), and (E,E)-2,4-nonadienal ($r = 0.771$) ($P < 0.0001$). LPC(0:0/18:1) and LPC(0:0/20:2) were positively correlated with hexanal, heptanal, (E,E)-2,4-nonadienal, 1-octen-3-ol, 2,3-octanedione, and 2-pentyl-furan. Notably, LPC(20:3) was negatively correlated with (E)-2-octenal. Twenty-three PSs exhibited negative correlations with predominant aroma compounds. For example, PS(19:0/18:2) showed strong negative correlations with hexanal, 1-hexanol, heptanal, octanal, nonanal, 1-octen-3-ol, (E,E)-2,4-nonadienal, 2,3-octanedione, and 2-pentyl-furan. Fourteen ePEs and three PEs were negatively correlated with specific predominant odorants. For instance, PE(15:1/22:4) and PE(20:4/18:0) had negative correlations with hexanal, (E)-2-decenal, (E,E)-2,4-nonadienal, 2,3-octanedione, 1-octen-3-ol, and 2-pentyl-furan. Additionally, PE(20:4/18:0) was negatively correlated with heptanal. PE(O-16:1/20:4) showed negative correlations with (E,E)-2,4-nonadienal, hexanal, heptanal, 1-octen-3-ol, 2-pentyl-furan, and 2,3-octanedione. PE(O-18:1/20:4) had negative correlations with (E)-2-decenal, and PE(O-18:1/18:2), PE(20:2/20:4), and 11 PE-Ps were negatively correlated with (E)-2-decenal and (E,E)-2,4-nonadienal. Thirty-eight TGs exhibited strong negative correlations with predominant aroma compounds ($P < 0.05$). For instance, TG(16:0/16:0/16:0) exhibited negative correlations with hexanal, octanal, heptanal, nonanal, 1-octen-3-ol, (E,E)-2,4-nonadienal, 2,3-octanedione, 1-hexanol, and 2-pentyl-furan. TG(16:0/16:1/16:1) and TG(16:0/16:1/18:1) showed negative correlations with pentanal and (E)-2-octenal. TG(16:0/16:0/18:0) was negatively correlated with all predominant odorants except for pentanal, decanal, and (E)-2-octenal. TG(16:0/16:1/17:1) had negative correlations with all predominant odorants except for (E)-2-decenal and (E,E)-2,4-nonadienal. Lv et al. (2023) demonstrated that TG molecules containing 16:0 and 18:1 fatty acids, along with PC, PE, and ePE molecules containing 18:1, 18:2, and 20:4 fatty acids, were prone to degradation during chicken breast storage. The reduction of these TG molecules likely led to an increase in these aroma compounds. TG possibly underwent oxidation and/or hydrolysis to DG during refrigeration-reheating, producing aroma compounds (Guo et al., 2022b). DG(O-19:0/16:0) showed positive correlations with

(E)-2-octenal (r = 0.517). In addition, 6 PI, 1 SM, and 1 cholesterol were negatively correlated with specific predominant aroma compounds.

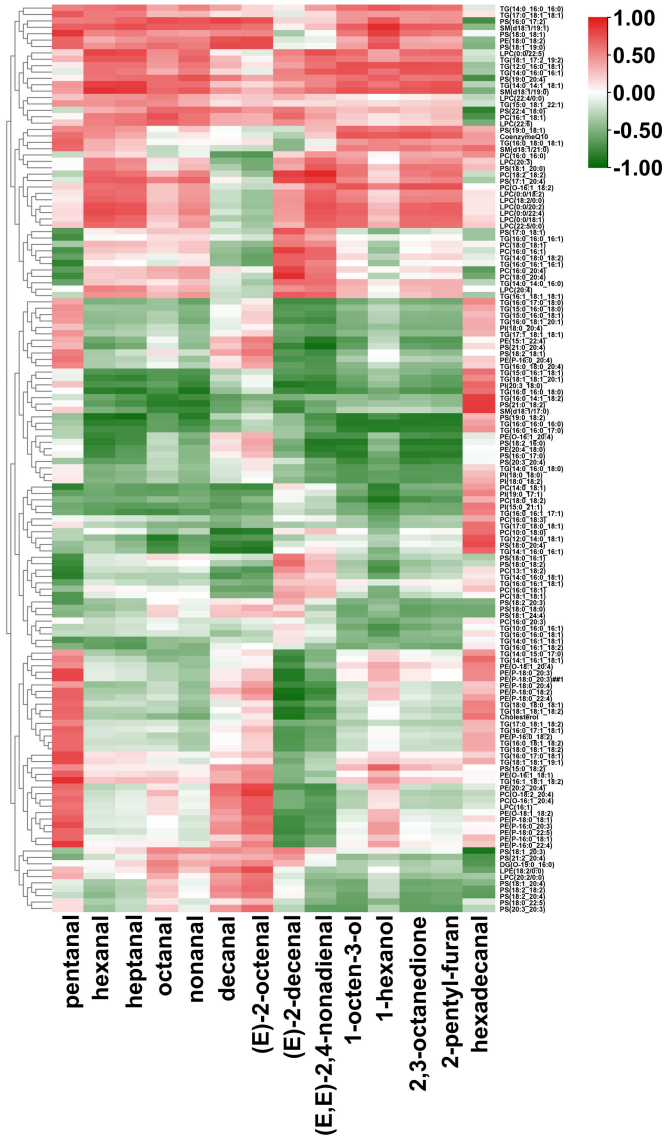


Fig. 4-9 Heat map representations of Pearson correlation analysis results between predominant aroma compounds (OAV ≥ 1) and 142 significantly correlated differential lipids ($P < 0.05$). Red units indicate positive correlations, while green units represent negative correlations.

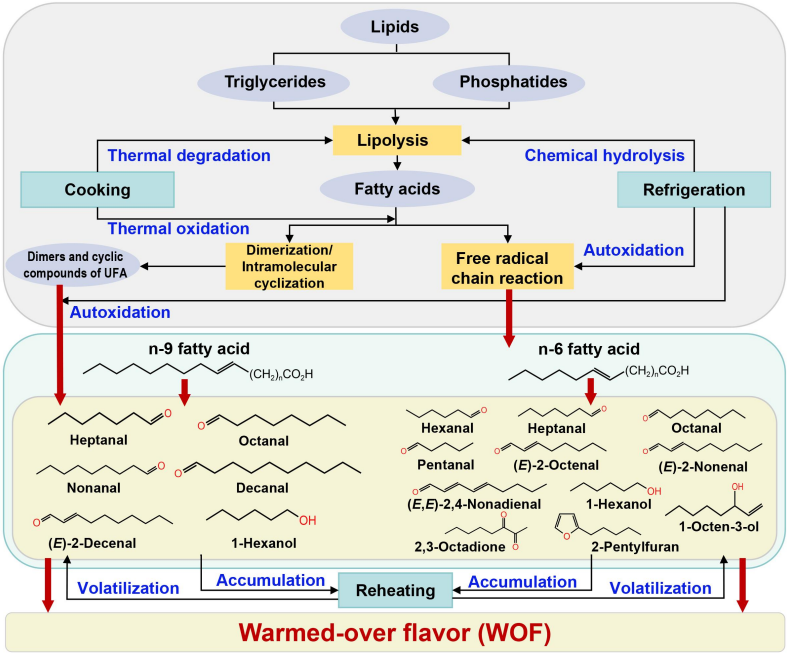


Fig. 4-10 Lipid-sourced warmed-over flavor (WOF) formation pathway.

As shown in **Fig. 4-10**, during cooking, the thermal degradation of phospholipids and triglycerides, including the above lipid molecules, resulted in the formation of FFAs that underwent various free radical chain reactions to generate hydroperoxides. The O-O bond in hydroperoxides is relatively weak and heat-sensitive, leading to their decomposition into various volatile compounds, including aldehydes (alkanal), ketones (alkanone), carboxylic acids (alkanoic acid), alcohols (alkanol), lactones, and alkyl furans, which contribute to the characteristic flavor of cooked meat (Mottram, 1998). During refrigeration, lipids continue to be hydrolyzed, generating free fatty acids that are oxidized through a free radical chain reaction, similar to thermal oxidation. This process forms short-chain aldehydes, ketones, and alcohols, which have low odor thresholds and accumulate over time, leading to off-flavors (Dinh et al., 2021). Unsaturated fatty acids, particularly on the meat surface, oxidize more rapidly, resulting in the formation of dimers and polymers with a higher oxygen content through the polymerization of unsaturated fatty acids (UFAs). Long-chain PUFAs are more prone to intramolecular cyclization. The extensive dimerization and polymerization of UFAs, as well as PUFA cyclization, result in non-volatile products, limiting PUFA involvement in generating volatile compounds with off-odors (Dinh et al., 2021; Mottram, 1998). Furthermore, high temperatures and optimal oxygen levels during cooking promote further oxidation of short-chain aldehydes, ketones, and alcohols into organic acids and esters. Lipid peroxides

polymerize into more oxygenated heterocyclic compounds, including cyclic carboxylic acids and their lactones (cyclic esters). SFAs degrade into long-chain alkanes, aldehydes, and lactones. The reduction of short-chain and unsaturated aldehydes and alcohols results in more desirable odors and lower volatility, explaining the more favorable volatile profile of cooked meat compared with autoxidation (Dinh et al., 2021; Song et al., 2011). However, lipid thermal oxidation products, such as dimers and cyclic compounds, can further decompose through autoxidation during storage, producing undesirable odorants that contribute to WOF (Dinh et al., 2021). The accumulation of these off-flavor compounds during refrigeration and their volatilization upon reheating enhance WOF development.

4.3.8. Analysis of key lipid precursors responsible for the formation of WOF

The fatty acid composition of lipid molecules is crucial for their oxidative properties. **Fig. 4-9** shows that stearic acid, palmitic acid, linoleic acid, oleic acid, and arachidonic acid were the primary fatty acids in these lipid molecules. Among the lipids significantly negatively correlated with predominant aroma compounds, phospholipids such as PE, PC, PS, PI, and LPC contained a high proportion of PUFAs, including C(18:2), C(18:3), C(20:4), C(22:5), and C(18:1), for a total of 74.76%. Notably, PE, especially ePE, was rich in PUFAs. Ether phospholipids are known to affect membrane fluidity and fusion, with their ether bonds being particularly vulnerable to cleavage by reactive oxygen species (Lv et al., 2023). **Fig. 4-3**B shows that the content of total aroma compounds, particularly aldehydes, increased remarkably in D1, D2, and D4. However, as shown in **Fig. 4-4**D, the contents of several major lipid subclasses varied differently. PC and PS showed no significant changes before D2 but increased significantly by the end of storage. LPC increased significantly before D4, and PC-O increased significantly by D2 and then stabilized. PI showed no significant change in D1, followed by a brief decrease in D2, and then a significant increase ($P < 0.05$). By contrast, PE-P, PE-O, and PE all increased in D1, followed by a significant decrease before D6 ($P < 0.05$). The temporary increase in PE-P, PE-O, and PE might result from the further disruption of cell membranes caused by reheating, which releases lipid molecules (Liu et al., 2023). Additionally, protein degradation in meat may release glycerophospholipids from their binding sites with membrane proteins, potentially leading to an increase in certain lipid types. When lipid synthesis exceeds degradation, the concentration of specific lipid species increases (Lv et al., 2023). In particular, PE(P-18:0/18:2) content was notably higher than other PE molecules, showing an initial increase in D1, followed by a significant decline ($P < 0.05$) (**Fig. 4-11**A). It presented a significant negative correlation with (E)-2-decenal ($r = -0.903$) and (E,E)-2,4-nonadienal ($r = -0.783$). PE (P-18:0_20:4) showed a trend similar to PE(P-18:0/18:2) during storage (**Fig. 4-11**B), with its content second only to PE(P-18:0/18:2). The decomposition of PE(P-18:0/18:2) and PE(P-18:0/20:4) produces FFA(18:2) and FFA(20:4). Linoleic acid oxidation produces the characteristic aldehyde, hexanal. Previous studies have reported that alkanals, 2-alkenals, and alkanols can originate from the autoxidation of linoleic acid (C18:2

n-6) and oleic acid (C18:1 n-9) (Elmore et al., 1999). (E,E)-2,4-decadienal can be derived from n-6 PUFAs, including arachidonic acids and linoleic (Liu et al., 2020; Shahidi & Abad, 2019). In addition, 1-octen-3-ol can be produced by the oxidation of linoleic and arachidonic acids. It is reported that WOF develops rapidly in cooked meat products stored within 48 h (Pegg et al., 2014). Therefore, ePE, including PE(P-18:0/18:2) and PE(P-18:0/20:4), might be key precursors for WOF formation in PCSB. Furthermore, the amino groups in PE are more reactive than the double bonds in the acyl chains, making them more susceptible to reactions with free radicals, ROS, and subsequent oxidation products (Fang et al., 2022; Pongsetkul et al., 2017). Nevertheless, few studies have linked ePE to WOF. It is still unclear whether the oxidation products of amino groups, which differ from those of acyl groups, affect acyl group oxidation and contribute to WOF formation. Consequently, our forthcoming research will focus on this area to facilitate targeted control of WOF formation and development, aiming for TOFE of PCSB (Wang et al., 2023b), thereby enhancing its edible quality. Notably, what's noteworthy is that LPC and PC contents decreased from 3622.65 and 1509.78 nmol/g in D4 to 2803.93 and 1268.34 nmol/g in D6, respectively (**Fig. 4-4**D). LPC(20:3) was the most abundant lipid molecule, with its content significantly declining from 987.61 nmol/g in D4 to 640.21 nmol/g in D6 (**Fig. 4-11**C). PC(16:0/18:1) was the most abundant PC species and the third most prevalent among all lipid molecules. Its levels significantly decreased from 568.08 nmol/g in D4 to 440.33 nmol/g in D6 (**Fig. 4-11**D). This finding suggested their critical role in WOF development. Thus, the reduction in LPC and PC over time might significantly contribute to WOF development.

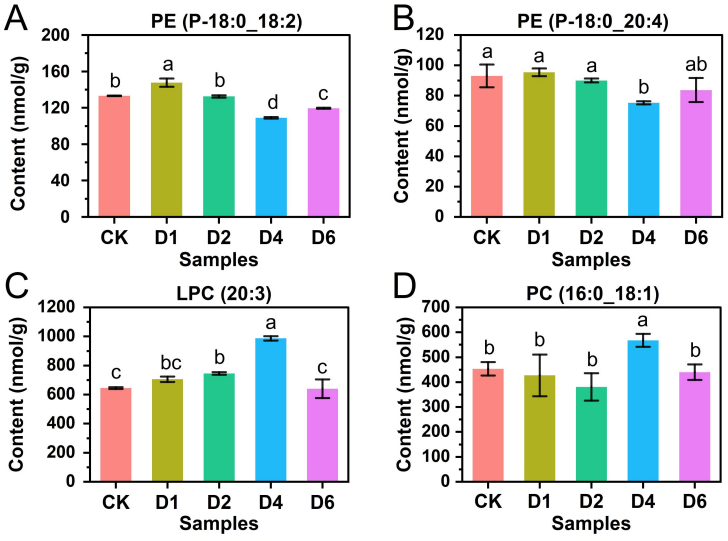


Fig. 4-11 The changes in the content of PE (P-18:0/18:2) (A), PE (P-18:0/20:4) (B), LPC (20:3) (C), and PC (16:0/18:1) (D) in freshly cooked and reheated PCSB samples. Data marked with different lowercase letters indicate a significant difference at $P < 0.05$.

TG possessed more SFAs and monounsaturated fatty acids (MUFAs), such as C(16:0), C(18:0), and C(18:1), making up 36.89% of the 103 negatively correlated potential lipids ($P < 0.05$). Moreover, TG's unsaturation exceeded 95% (**Fig. 4-7**), indicating its crucial role in forming predominant aromas in PCSB. Additionally, as shown in **Fig. 4-4**D, the TG content significantly increased in D1, likely because of further disruption of cell membranes during reheating, leading to TG release (Liu et al., 2023; Suri et al., 2019). Furthermore, GP can be converted to TG through oxidation (Guo et al., 2022b). Concurrently, aroma compounds, particularly aldehydes such as pentanal, hexanal, and heptanal, increased significantly. This could be attributed to the oxidative breakdown of lipids. The elevated TG levels in D2 might result from its oxidative degradation during storage. Lv et al. (2023) reported that TG molecules with fatty acids like C(16:0) and C(18:1) in *Sanhuang* chicken decomposed readily during refrigeration. At this stage, the total amount of aroma compounds, including aldehydes and ketones, increased significantly. The extensive oxidation of phospholipids such as PE, PE-P, and PE-O, along with the oxidation of TG, might account for this. TG content significantly increased in D4 compared with CK and D2, suggesting a slower oxidation rate relative to its production. The overall aroma compound content, particularly aldehydes, decreased remarkably but remained higher than that in the CK group. During this period, the levels of PE, PE-P, and PE-O continued to decrease. The ongoing oxidation of these lipids, along with TG, continued to produce aroma compounds. However, prolonged storage led to a decrease in aroma compounds due to their binding to proteins and subsequent dissipation (Wang et al., 2023a). TG levels significantly decreased in D6 but showed no significant difference compared with those in CK and D2, suggesting that the oxidation rate of TG might increase over time as storage quality declined. At this stage, the overall content of aroma compounds, particularly aldehydes, significantly decreased, and the total alcohol content markedly increased. On one hand, the binding of odorants to proteins and their subsequent dissipation with the extension storage possibly lead to a decrease in these compounds. On the other hand, significant degradation and oxidation of LPC, PC, and TG might result in a marked increase in certain flavor compounds. These findings demonstrated that TG played a crucial role in the formation and development of WOF in PCSB. These results were similar to Igene and Pearson (2006), who added total lipids, triglycerides, total phospholipids, PE, and PC to lipid-extracted muscle fibers to form model systems. After heating to 70 °C and storing at 4 °C for 48 h, they evaluated WOF using TBA and sensory evaluation. Results showed that total phospholipids, especially PE, were the primary contributors to WOF in cooked meat. Triglycerides were found to promote WOF development only when phospholipids (as total lipids) were present. PC had no significant impact on WOF during the first two days of storage in the model system. However, our study suggested that LPC and PC might significantly promote WOF development in stewed beef during the later storage stage (D6). Wu et al. (2024) found that PE was crucial for aroma formation in the fat portion of

bacon, with both PE and TG contributing to the aroma of the salted lean portion. Yan et al. (2022) reported that PC, PE, and TG in refrigerated hairtail were more susceptible to oxidation and hydrolysis because of their high polyunsaturated content, serving as lipid markers to differentiate fresh and refrigerated hairtail samples.

4.4. Conclusion

This study elucidated the alterations in the lipidomics profile of PCSB during refrigeration. Using UPLC-ESI-MS/MS-based lipidomics, 1236 lipids were identified across 42 subclasses, with triglycerides (TG) being the most abundant in both variety and content. TG, including TG(18:0/18:1/18:1) and TG(16:0/18:1/18:1), were primarily responsible for binding aroma compounds. Fourteen aroma compounds, including aldehydes and alcohols, were identified as the predominant aroma compounds in PCSB. PCA and OPLS-DA demonstrated that the lipidomics data effectively distinguished all stewed beef samples. PS(18:0/20:4) and PS(16:0/17:2) were determined as potential markers for distinguishing all stewed beef samples. Of the 153 differential lipids, 142 might contribute to WOF formation, with ePE, particularly PE(P-18:0/18:2), likely being crucial lipids. Additionally, LPC and PC, especially LPC(20:3) and PC(16:0/18:1), might significantly influence WOF development. Simultaneously, TG contributed to both the formation and development of WOF. In the future, target-oriented off-flavor correction technologies will be taken into account for the targeted correction of WOF in PCSB.

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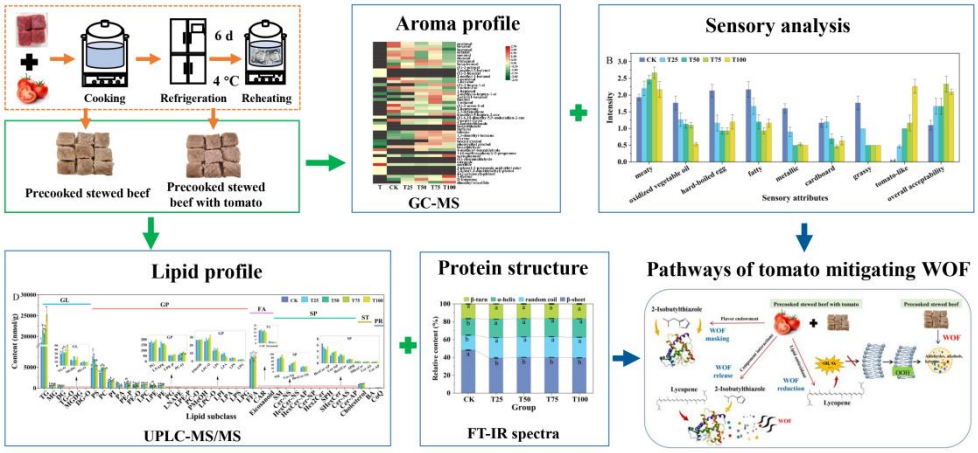
5

Chapter V Ingredient interaction mechanisms: The role of tomato in mitigating warmed-over flavor in precooked Chinese stewed beef

Short overview of chapter V

Key odorants contributing to WOF formation and crucial lipid precursors in WOF formation have been confirmed based on the result of chapter III and chapter IV. In this chapter, the role of tomato in mitigating WOF in PCSB was elucidated by analyzing aroma profiles, lipid profiles, and sensory attributes. Furthermore, WOF perception is closely linked to its retention and release within the meat matrix, particularly proteins. Thus, protein secondary structure was analyzed to evaluated the regulatory impact of protein structural changes and tomato-originated aroma compounds on the release of WOF.



Graphical abstract: Ingredient interaction mechanisms: The role of tomato in mitigating warmed-over flavor (WOF) in PCSB

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Liu, J. (2025). Mechanisms underlying tomato-mediated suppression of warmed-over flavor (WOF) and flavor enrichment in precooked stewed beef. *Food Chemistry*. (revision)

Abstract

The role of tomatoes in mitigating warmed-over flavor (WOF) in PCSB was elucidated through analyses of aroma profiles, lipid composition, protein secondary structure, and sensory attributes. Adding tomatoes at 25%, 50%, 75%, and 100% (w/w of raw meat) significantly inhibited lipid oxidation, reducing losses in phospholipids (e.g., phosphatidylethanolamine, lysophosphatidylcholine, and phosphatidylcholine) and triglycerides, thereby reducing WOF development. Tomato-originated 2-isobutylthiazole exhibited a potent flavor endowment effect on PCSB, with its endowment rate increasing from 602.64% to 2860.10%, thus masking the WOF in PCSB. Tomato additions also altered the secondary structure of beef protein, potentially affecting WOF retention in PCSB. Sensory evaluation revealed that the 75% tomato group achieved the highest overall acceptability, balancing meaty and tomato-like aromas while significantly reducing the WOF. This study offers a viable strategy for reducing WOF in PCSB, thereby enhancing its commercial appeal and consumer acceptance.

Key words: Precooked stewed beef, warmed-over flavor (WOF), lipid oxidation, natural antioxidants, flavor endowment.

5.1. Introduction

Stewed beef is a popular traditional dish in Chinese cuisine, appreciated for its rich taste, nutritional value, and easy preparation, making it a staple in China's growing precooked dish market. However, during refrigerated storage and reheating, its flavor quality often deteriorates due to the development of warmed-over flavor (WOF), a common off-note characterized by cardboard-like, oxidized vegetable oil, and metallic aromas (Chen et al., 2024; Liu et al., 2024). This deterioration is primarily attributed to lipid oxidation, which leads to the accumulation of volatile aldehydes, such as hexanal, (E,E)-2,4-decadienal, and (E)-2-octenal, which contribute to WOF that negatively impact consumer perception (Chen et al., 2024). In response to increasing demand for clean-label solutions, recent studies have explored natural antioxidants as alternatives to synthetic additives to control lipid oxidation in meat products (Dang et al., 2024). Various plant-based antioxidants, such as perilla juice, ginger juice (Dang et al., 2024), and essential oils from winter savory (*Satureja montana* L.) (Jokanović et al., 2020), have been shown to reduce and mask WOF, offering a promising alternative to synthetic antioxidants. Notably, tomatoes, a widely used culinary ingredient globally, possess a synergistic antioxidant system composed of lycopene, β-carotene, VC, and VE, which exhibit strong antioxidant properties (Skiepko et al., 2016). Stewed beef with tomato, widely recognized across various culinary traditions, is an essential component of the PCD industry. Although tomato extracts have been shown to improve oxidative stability and color in muscle foods (Luisa García et al., 2009; Østerlie & Lerfall, 2005; Sánchez - Escalante et al., 2003), most research to date has focused on physicochemical parameters, with limited insight into how tomato-originated compounds interact with the meat matrix to modulate WOF or affect aroma perception. Specifically, the impact of tomato incorporation on lipid molecular changes and WOF mitigation in PCSB remains unclear. Moreover, the tomato-originated aroma compounds endow the dish with a distinctive tomato-like aroma. Whereas, the specific tomato-originated compounds with flavor endowment effect and their flavor endowment rate remain poorly characterized. Furthermore, WOF perception is closely linked to its retention and release within the meat matrix. Substantial evidence suggests that proteins, particularly myofibrillar proteins (MPs), serve as ideal binding matrices for aroma compounds (Chen et al., 2024). According to Qiang et al. (2025), the flavor endowment effect primarily involves diffusion, capillary adsorption, and non-covalent binding to beef proteins. This raises a critical question: could these interactions also influence the retention and release of WOF within the meat matrix, thereby altering WOF perception? Additionally, while previous studies have reported that lycopene can alter the secondary structure of fish MPs (Zhao et al., 2023), little is known about its effect on the conformational changes of beef proteins of stewed beef and how such structural shifts might modulate the binding and release of aroma compounds associated with WOF. These interactions might modulate beef protein conformations and consequently influence WOF retention. At present, the impact of tomatoes on the structural properties of beef proteins and their subsequent effects on WOF binding remain largely

unexplored. It is hypothesized that tomatoes could effectively reduce WOF formation while enhancing the overall flavor profile of PCSB. Therefore, it is crucial to elucidate the underlying mechanisms of tomatoes in mitigating WOF in PCSB.

This study aims to systematically examine the effects of varying tomato addition levels on lipid oxidation, aroma profiles, protein secondary structure, and sensory characteristics in PCSB. Specific objectives include: (1) Quantifying the antioxidative effects of tomato at different inclusion levels by analyzing lipid oxidation markers and WOF-related compounds; (2) Characterizing the flavor endowment properties of tomato-originated aroma compounds and their WOF-masking effects; (3) Evaluating the regulatory impact of protein structural changes and tomato-originated aroma compounds on the release of WOF; (4) Determining the optimal tomato addition level through comprehensive analysis of WOF variation patterns. This ingredient-based regulatory mechanism not only eliminates the need for exogenous additives but also aligns with the modern consumer preference for clean-label foods, offering an innovative approach to optimizing the quality of convenience meals.

5.2. Materials and Methods

5.2.1. Sample collection and preparation

Fresh tomatoes (*Solanum lycopersicum*, cv. Provence) at full ripeness were purchased from a local supermarket in Beijing, China, and washed thoroughly. Each tomato was cut into eight equal pieces for use. Fifteen 48-month-old Simmental steers, with similar body weights and genetic backgrounds, were randomly selected from Hebei Fucheng Wufeng Food Co., Ltd. (Hebei, China). These animals were uniformly fed a complete formulated diet. The animals were randomly assigned to five treatment groups, with three animals per group. The chuck tenders of each steers were vacuum-packed at the source after aging and transported to the laboratory under cold-chain conditions. External fat and connective tissues were eliminated. The chuck tenders were washed, cut into blocks of approximately 2 cm × 2 cm × 1.5 cm. For preparation of the stewed beef with tomato, 600 g of meat blocks were added to water, brought to a boil, blanched for 3 min, and drained. The drained blocks were then added to fresh water, heated to a boil using an induction cooker set at 1200 W, and maintained for 5 min. The power was reduced to 600 W to allow a gentle simmer, and 1.2% salt (w/w of raw meat) was added. The blocks were simmered for 25 min (at 600 W), followed by the addition of tomatoes at 0%, 25%, 50%, 75%, and 100% (w/w of raw meat), respectively. The mixture was then simmered for an additional 20 min (at 600 W) and maintained for 20 min without heat. The mixtures were removed, portioned into aluminum foil bags containing 200 g of broth, vacuum-sealed, cooled to ambient temperature under running water, and stored at 4 °C for 6 days. After storage, the samples were reheated in a water bath at 100 °C for 10 min. Microbial counts were in the acceptable range (< 10^4 CFU/g) (National Food Safety Standard, 2017). The samples were divided into five groups: CK, T25, T50, T75, and T100. The CK group served as the control (no added tomato), while the T25, T50, T75, and T100 groups contained 25%, 50%, 75%, and 100% tomato, respectively. All samples (except those used for sensory aroma profile

analysis) were immediately frozen in liquid nitrogen for 5 min, ground into a fine powder using a blender, and stored at −80 °C until analysis. All analyses were completed within one week.

5.2.2. Sensory aroma profile analysis (APA)

APA was conducted following Liu et al. (2024), using a panel of 12 trained assessors (8 females and 4 males, aged 22-45 years). Panelists were trained to identify and describe aroma attributes of PCSB, both with and without tomato, in accordance with the Chinese national standard GB/T 16291.1-2012 (https://www.chinesestandard.net/PDF/English.amp.aspx/GBT16291.1-2012).
Training lasted one year, ensuring panelists could accurately assess sensory characteristics. Eight odor descriptors were identified: meaty, grassy, cardboard-like, metallic, fatty, hard-boiled egg, oxidized vegetable oil, and tomato-like. Each attribute was rated on a 0–3 scale (0 = imperceptible, 3 = extremely intense*)* with 0.5-point increments. Panelists also rated overall acceptability (0 = unacceptable, 3 = highly preferred) on the same scale. Sensory evaluations were performed in individual booths under controlled conditions 20 °C ± 2 °C. Ethical approval was granted by the research institution, and all panelists participated voluntarily after providing informed consent. Panelists' privacy was protected, and the data were solely used for aroma profile evaluation. All samples were confirmed safe for consumption.

5.2.3. Electronic nose (E-nose) analysis

Electronic nose analysis was performed using a PEN 3.5 E-nose (Win Muster Airsense Analytics, Inc., Germany) equipped with ten metal oxide sensors, as described in the section 4.2.3.

5.2.4. Aroma compound analysis

Aroma compounds were extracted using headspace solid-phase microextraction (HS-SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS) (8860-5977, Agilent Technologies, Inc., Santa Clara, CA, USA), following a modified method from the section 4.2.5. Three grams of the powdered sample were placed in a 20 mL glass vial with 2 µL of 0.016 µg/µL 1,2-dichlorobenzene (internal standard, dissolved in methanol). The sample was equilibrated in a 50 °C water bath for 10 min before being extracted using a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (2 cm, Supelco, Bellefonte, PA, USA) for 40 min. The fiber was then thermally desorbed in the GC-MS injection port at 250 °C for 5 min under a 5:1 split ratio. Separation was performed using a DB-Wax column (30 m × 0.25 mm, 0.25 µm, Agilent Technology, Inc., Santa Clara, CA, USA). Helium (≥ 99.999%) was used as the carrier gas at a flow rate of 1.0 mL/min. The temperature program was as follows: initial hold at 40 °C for 5 min, increased to 120 °C at 4 °C/min, then to 230 °C at 10 °C/min, with a final hold for 10 min. Mass spectra were acquired at 70 eV, with an m/z scan range of 30–550 amu. Compounds were identified by comparing their mass spectra with the NIST 20 library, retention indices (RI) calculated based on Vandendool and

Kratz (1963), and authenticated reference standards. Semi-quantitative analysis was performed using the internal standard method. Relative content (C) were determined according to the method described in the section 4.2.5. The odor activity value (OAV) was calculated by dividing C of each aroma compounds by its threshold value in water sourced from literature (Beldarrain et al., 2022; Giri et al., 2010; Van Gemert, 2011; Yang et al., 2021; Zhang et al., 2022; Zhu et al., 2018). Aroma compounds with OAV ≥ 1 were classified as key aroma compounds that significantly contributed to the aroma profile of PCSB. The flavor endowment rate value (ERV) was used to assess the flavor endowment efficiency of tomato-originated aroma compounds (Qiang et al., 2025) and was calculated as ERV= C_b/C_c, where C_b represents the relative content of tomato-originated compounds in PCSB and C_c refers to their relative content in tomatoes.

5.2.5. TBARS measurement

The determination of thiobarbituric acid reactive substances (TBARS) was conducted following the method of described in the section 3.2.2.

5.2.6. Lipidomics analysis

Lipids were extracted according the method described in the section 4.2.6. Lipid extracts were analyzed by an ExionLC AD system coupled with a QTRAP 6500 plus tandem mass spectrometer (SCIEX Corp., Framingham, USA). Chromatographic separation was performed using chromatographic column, Thermo Accucore™ C30 (2.6 μm, 2.1 mm × 100 mm). The solvent system consisted of A: acetonitrile/water (60:40, v/v, containing 0.1% formic acid and 10 mmol/L ammonium formate) and B: acetonitrile/isopropanol (10:90, v/v, containing 0.1% formic acid and 10 mmol/L ammonium formate). The gradient program (A/B, v/v) was as follows: 80:20 at 0 min, 70:30 at 2 min, 40:60 at 4 min, 15:85 at 9 min, 10:90 at 14 min, 5:95 at 15.5 min, held until 17.3 min, then returned to 80:20 at 17.5 min, and maintained until 20 min. The flow rate was set at 0.35 mL/min, the column temperature was 45 °C, and the injection volume was 2 μL. Ionization was achieved using electrospray ionization (ESI), with the following mass spectrometry conditions: ESI temperature of 500 °C; ion spray voltage (IS) of +5500 V (positive mode) and −4500 V (negative mode); ion source gas 1 (GS1) at 45 psi; gas 2 (GS2) at 55 psi; and curtain gas (CUR) at 35 psi. Each ion pair in the triple quadrupole was scanned and detected using optimized de-clustering potential (DP) and collision energy (CE). Specific multiple reaction monitoring (MRM) transitions were observed for metabolites eluting during each gradient interval. After data collection from lipid mass spectrometry, peak areas were integrated for all chromatographic peaks, and quantitative analysis was performed using the internal standard methodn. The content (C) of each compound was calculated as described in chapter IV.

5.2.7. Fourier transform infrared (FT-IR) analysis

FT-IR spectra were obtained using a TENSOR 27 spectrometer equipped with an MB-ATR accessory (Bruker, Ettlingen, Germany) within the spectral range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. The secondary structure content of proteins was determined using Peakfit 4.12 software (Seasolve Software, Inc., Framingham,

USA) (Xu et al., 2024). Protein secondary structures were analyzed via second-derivative spectra in the 1600–1700 cm^{-1}. region. Specific peaks were assigned to distinct structural elements: β-sheet (1611–1640 cm^{-1}), random coil (1642–1650 cm^{-1}), α-helix (1654–662 cm^{-1}), and β-turn (1665–1693 cm^{-1}).

5.2.8. Statistical analysis

All experiments followed a completely randomized design with three replicates. Group differences were assessed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at $P \leq 0.05$, using SPSS version 22.0 (IBM Corp., Chicago, IL, USA). Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed in SIMCA v14.1 (Umetrics, Umeå, Sweden). Heatmaps were generated using TBtools (Chen et al., 2023), additional graphs were created in Origin 2021 (OriginLab, Northampton, MA, USA).

5.3. Results

5.3.1. APA

APA results indicated that the CK group exhibited the highest scores for oxidized vegetable oil, hard-boiled egg, fatty, metallic, cardboard-like, and grassy aromas, indicating a strong WOF with a faint meaty note (Fig. **Fig. 5-1**). As the tomato additions increased,WOF diminished, with T75 and T100 showing almost no detectable WOF. Conversely, the tomato-like aroma intensified, with a distinct tomato-like aroma (sweet-sour and green) in T100. The CK group had the lowest score for the meaty note. From T25 to T75, the meaty aroma progressively strengthened, while in T100, it decreased. Regarding overall acceptability, T25 and T50 showed similar ratings, both characterized by noticeable WOF, weak meaty aroma, and mild tomato-like aroma, resulting in lower acceptability. In contrast, T75 displayed a stronger meaty note, a significant reduction in WOF, and a subtle tomato-like aroma, leading to the highest acceptability. T100, dominated by a strong tomato-like aroma, showed a faint meaty note and minimal WOF, yielding an acceptability score similar to T75.

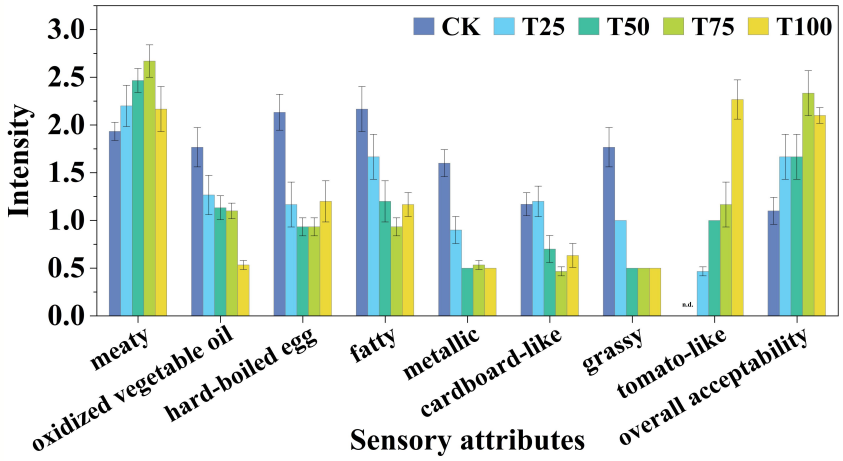

Fig. 5-1 Descriptive aroma profiles of PCSB and PCSBT samples.

5.3.2. E-nose analysis

The E-nose was employed to analyze the aroma characteristics of PCSB with varying tomato concentrations. The PCA score plot revealed that the first two principal components (PCs) explained 82.2% of the cumulative variance (**Fig. 5-2**A)), suggesting that these components captured the majority of the variation in the aroma profiles of the PCSB samples. A clear distinction in odor profiles was observed between the control (CK) and all treatment groups, with the most notable differences in the T100 group. This indicated that tomato addition significantly altered the aroma compound composition. T25 showed partial overlap with CK along PC1, suggesting that their aroma profiles were relatively similar. However, the distinct separation along PC2 indicated that the addition of 25% tomato caused only a slight change in aroma composition. Medium addition levels (T50, T75) showed a gradual divergence from CK, particularly along the PC1 axis. When compared to T25, T50 exhibited significant changes along both PC1 and PC2, suggesting that the higher tomato addition resulted in more noticeable alterations in aroma characteristics. In comparison to CK, T50 demonstrated a marked shift along PC1, indicating a substantial change in its aroma composition, while its minor displacement on PC2 implies that some aroma attributes remained partially similar to CK. T75 and T50 had similar projections on PC1, indicating overall similarity in their aroma profiles. However, T75 showed slight variation along PC2, suggesting potential enhancement or attenuation of specific aroma compounds. In contrast, T75 displayed a considerable shift from CK along both PC1 and PC2, indicating a significant change in its aroma profile. This suggested that increasing the tomato concentration to 75% further enhanced the aroma differentiation from CK. At the highest concentration, T100 was completely separated from CK and all other groups along both PC1 and PC2, indicating the most significant transformation in aroma composition. Overall, the systematic shift in aroma profiles with increasing tomato additions led to a systematic change in aroma characteristics.

5.3.3. Aroma compound analysis

A total of 36 aroma compounds were detected in the samples of CK, T25, T50, T75, and T100, including 9 aldehydes, 10 alcohols, 3 ketones, 2 heterocyclic compounds, 10 aromatic compounds, and 3 other compounds (**Table 5-1**). Aldehydes were the most abundant, comprising 72.94% of the total aroma compounds (**Fig. 5-2**B), with hexanal being the most prevalent (CK: 51.51%; T25: 42.17%; T50: 33.71%; T75: 32.70%; T100: 24.93%). A total of 21 aroma compounds were detected in tomatoes, including 2 aldehydes, 4 alcohols, 1 ketones, 3 heterocyclic compounds, and 11 aromatic compounds (**Table 5-1**, **Fig. 5-2**C). Aromatic compounds accounted for 82.96% of the total aroma compounds (**Fig. 5-2**B). Among these, anethole was the most abundant (**Fig. 5-2**C), representing 23.22%, followed by 4-methoxy-benzaldehyde (20.40%), and (E)-cinnamaldehyde (17.82%). As the tomato additions increased from 25% to 100%, significant changes in the aroma compounds of the PCSB were observed (**Table 5-1**). The total aldehyde content significantly decreased, with pentanal, hexanal, and (E)-2-octenal showing substantial reductions. Specifically, (E)-2-octenal decreased the most, from 0.93 μg/kg to 0.58 μg/kg, a reduction rate of 37.72%, followed by hexanal (34.76%) and pentanal (28.96%). Apart from hexadecanal, no significant changes were observed in other aldehydes. Alcohol content didn't show significant changes, although 1-pentanol, 1-octen-3-ol, and (E)-2-octen-1-ol significantly decreased. On the other hand, the content of 1-hexanol and 2-ethyl-1-hexanol significantly increased, while other alcohols remained unchanged. Ketone content decreased significantly, particularly 2,3-octanedione (80.30%). 2-pentyl-furan showed a significant decrease of 21.13%. In T50, aldehyde content continued to decline significantly, with pentanal, hexanal, and (E)-2-octenal showing reductions. No significant changes were observed in the contents of alcohols and ketones. In T75, no significant changes were found in the total amounts of fatty aldehydes, alcohols, and ketones. Interestingly, the contents of pentanal, octanal, nonanal, and decanal increased significantly, although they did not exceed CK levels. Notably, the content of heptanal was significantly higher than in the CK group. Among the fatty alcohols, only 1-hexanol showed a significant decrease, with no other significant changes. In T100, the contents of pentanal, hexanal, and heptanal were significantly lower than those in CK, except for tridecanal and hexadecanal. The contents of 2-heptanone and 2,3-octanedione decreased significantly, with 2,3-octanedione dropping by 44.50%, followed by heptanal (44.06%), 2-heptanone (41.18%), and hexanal (39.88%).

In the CK group, only hexanal, heptanal, octanal, nonanal, hexadecanal, and 1-octen-3-ol exhibited OAV > 1 (**Table 5-2**). The OAV variation trends of hexanal and 1-octen-3-ol were similar, showing a gradual decrease from CK to T50, followed by stable levels in T75, and the lowest values in T100 . For octanal and nonanal, the OAVs decreased from CK to T50, then increased in T75, before dropping again in T100 to a value similar to T50. Heptanal increased in T25, remained unchanged in T50 and T75, and decreased in T100 to levels comparable to CK. In addition, 2-isobutylthiazole in T100 had OAV > 1 (**Table 5-2**). Among the compounds detected in tomatoes, (Z)-3-hexen-1-ol, 2-methyl-6-hepten-1-ol, linalool,

4-methoxy-benzaldehyde, and phenylethyl alcohol were found only in T50-T100 (**Fig. 5-2**C), indicating migration from tomato to beef meat. 4-methoxy-benzaldehyde significantly decreased in T75 with no change in T100, while the migration of other compounds increased with higher tomato content, peaking at T75 (**Table 5-1**). Specifically, (Z)-3-hexen-1-ol increased from 0.37 μg/kg in T50 to 2.57 μg/kg in T75 ($P < 0.05$), then either decreased or remained unchanged in T100. However, these compounds in PCSB had OAV <1, implying minimal contribution to the PCSB. Additionally, 2-isobutylthiazole, detected only in the PCSBTsamples, showed a distinct increase from T25 to T100. Its content rose from 0.76 μg/kg in T25 to 3.59 μg/kg in T100 ($P < 0.05$), with its ERV increasing as follows: T25 (602.64%), T50 (858.25%), T75 (1188.49%), and T100 (2860.10%) (**ig. 5-2**D).

Table 5-1 Relative content (C) of aroma compounds in PCSBT.

Compounds	Aroma compounds relative content (C) (μg/kg)					
	Tomato	**CK**	**T25**	**T50**	**T75**	**T100**
Aldehydes	0.56 ± 0.09	176.83 ± 9.26^{A}	136.61 ± 1.38^{B}	106.90± 2.81^{C}	112.72 ± 4.46^{C}	77.31 ± 3.31^{D}
Pentanal	N.D.	8.69 ± 0.87^{A}	6.18 ± 0.45^{B}	4.48 ± 0.55^{C}	6.08 ± 0.94^{B}	3.84 ± 0.70^{C}
Hexanal	N.D.	124.89 ± 11.92^{A}	81.48 ± 4.09^{B}	55.19 ± 1.50^{C}	56.32 ± 1.52^{C}	33.86 ± 4.01^{D}
Heptanal	N.D.	4.19 ± 0.49^{B}	5.09 ± 0.85^{AB}	5.02 ± 0.15^{AB}	5.14 ± 0.44^{A}	2.88 ± 0.14^{C}
Octanal	N.D.	6.28 ± 1.38^{A}	5.54 ± 1.12^{A}	3.51 ± 0.65^{C}	5.30 ± 0.49^{AB}	3.62 ± 0.34^{BC}
Nonanal	N.D.	13.24 ± 1.21^{A}	12.28 ± 1.75^{AB}	8.69 ± 1.22^{C}	10.70 ± 0.37^{BC}	9.22 ± 0.93^{C}
Decanal	N.D.	0.52 ± 0.07^{A}	0.57 ± 0.07^{A}	0.41 ± 0.04^{C}	0.50 ± 0.03^{AB}	0.44 ± 0.02^{BC}
Tridecanal	N.D.	1.54 ± 0.26^{B}	1.66 ± 0.27^{B}	2.67 ± 0.42^{A}	2.44 ± 0.19^{A}	1.60 ± 0.18^{B}
Hexadecanal	N.D.	16.56 ± 1.16^{D}	22.84 ± 2.08^{BC}	26.61 ± 0.89^{A}	25.92 ± 2.84^{AB}	21.61 ± 1.05^{C}
(E)-2-Octenal	N.D.	0.93 ± 0.13^{A}	0.58 ± 0.04^{B}	0.30 ± 0.04^{C}	0.34 ± 0.04^{C}	0.24 ± 0.03^{C}
2-Methyl-2-butenal	0.10 ± 0.01	N.D.	N.D.	N.D.	N.D.	N.D.
(E)-2-Hexenal	0.08 ± 0.01	N.D.	N.D.	N.D.	N.D.	N.D.

Compounds	Aroma compounds relative content (C) (μg/kg)					
	Tomato	**CK**	**T25**	**T50**	**T75**	**T100**
Alcohols	0.63 ± 0.05	17.00 ± 0.66^{A}	15.86 ± 0.97^{AB}	14.79 ± 0.72^{B}	15.66 ± 0.64^{AB}	12.09 ± 1.15^{C}
2-Methyl-1-butanol	0.13 ± 0.03	N.D.	N.D.	N.D.	N.D.	N.D.
1-Pentanol	N.D.	2.88 ± 0.26^{A}	2.00 ± 0.21^{B}	1.72 ± 0.31^{B}	1.69 ± 0.07^{B}	1.28 ± 0.13^{C}
1-Hexanol	N.D.	2.35 ± 0.23^{B}	3.86 ± 0.23^{A}	3.74 ± 0.22^{A}	1.60 ± 0.04^{C}	1.25 ± 0.23^{C}
(Z)-3-Hexen-1-ol	0.26 ± 0.05	N.D.	N.D.	0.37 ± 0.05^{B}	2.57 ± 0.06^{A}	2.82 ± 0.32^{A}
1-Octen-3-ol	N.D.	8.36 ± 0.99^{A}	6.51 ± 0.91^{B}	4.86 ± 0.48^{C}	5.00 ± 0.50^{C}	3.06 ± 0.47^{D}
1-Heptanol	N.D.	0.87 ± 0.16^{A}	1.32 ± 0.17^{A}	1.30 ± 0.52^{A}	1.30 ± 0.12^{A}	0.96 ± 0.18^{A}
2-Methyl-6-hepten-1-ol	0.06 ± 0.02	N.D.	N.D.	0.31 ± 0.04^{B}	0.46 ± 0.06^{A}	0.52 ± 0.07^{A}
2-Ethyl-1-hexanol	N.D.	0.32 ± 0.03^{C}	0.44 ± 0.09^{B}	0.64 ± 0.05^{A}	0.75 ± 0.07^{A}	0.51 ± 0.06^{B}
Linalool	0.18 ± 0.00	N.D.	N.D.	0.25 ± 0.03^{B}	0.441 ± 0.05^{A}	0.31 ± 0.08^{B}
1-Octanol	N.D.	1.07 ± 0.18^{AB}	1.15 ± 0.11^{AB}	1.07 ± 0.20^{AB}	1.31 ± 0.11^{A}	0.88 ± 09^{B}
(E)-2-Octen-1-ol	N.D.	1.15 ± 0.18^{A}	0.57 ± 0.06^{B}	0.51 ± 0.10^{B}	0.57 ± 0.08^{B}	0.51 ± 0.06^{B}
Ketones	0.61 ± 0.05	15.11 ± 1.76^{A}	5.57 ± 0.50^{B}	4.73 ± 0.11^{B}	5.97 ± 0.47^{B}	6.17 ± 0.40^{B}
2-Heptanone	N.D.	1.13 ± 0.07^{AB}	1.29 ± 0.19^{A}	0.92 ± 0.11^{B}	0.99 ± 0.08^{B}	0.58 ± 0.05^{C}
2,3-Octanedione	N.D.	13.24 ± 1.73^{A}	2.61 ± 0.30^{B}	1.06 ± 0.18^{C}	0.81 ± 0.03^{C}	0.45 ± 0.02^{C}
6-Methyl-5-hepten-2-one	N.D.	0.74 ± 0.10^{E}	1.67 ± 0.21^{D}	2.74 ± 0.37^{C}	4.17 ± 0.55^{B}	5.14 ± 0.42^{A}
(E)-6,10-Dimethy-5,9-undecadien-2-one	0.13 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.

Compounds	Aroma compounds relative content (C) (μg/kg)					
	Tomato	**CK**	**T25**	**T50**	**T75**	**T100**
Heterocyclic compounds	0.76 ± 0.12	2.54 ± 0.43^{B}	2.76 ± 0.20^{B}	2.60 ± 0.33^{B}	3.10 ± 0.05^{B}	4.62 ± 0.37^{A}
2-Pentyl-furan	N.D.	2.54 ± 0.43^{A}	2.00 ± 0.20^{B}	1.52 ± 0.36^{BC}	1.61 ± 0.07^{B}	1.03 ± 0.12^{C}
2-Isobutylthiazole	0.18 ± 0.07	N.D.	0.76 ± 0.05^{C}	1.08 ± 0.29^{C}	1.49 ± 0.06^{B}	3.59 ± 0.37^{A}
Benzothiazole	0.57 ± 0.07	N.D.	N.D.	N.D.	N.D.	N.D.
Furfural	0.13 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.
Aromatics	12.46 ± 0.65	28.58 ± 0.85^{A}	29.63 ± 0.27^{A}	31.52 ± 1.87^{A}	31.76 ± 1.23^{A}	31.51 ± 2.85^{A}
Toluene	N.D.	3.80 ± 0.75^{C}	4.59 ± 0.95^{BC}	6.21 ± 1.08^{AB}	6.57 ± 1.04^{A}	5.74 ± 0.61^{AB}
1,3-Dimethyl-benzene	N.D.	0.54 ± 0.05^{B}	0.54 ± 0.09^{B}	0.88 ± 0.10^{A}	0.91 ± 0.11^{A}	0.87 ± 0.10^{A}
Styrene	N.D.	0.53 ± 0.03^{B}	0.50 ± 0.08^{B}	0.73 ± 0.03^{A}	0.67 ± 0.10^{A}	0.71 ± 0.04^{A}
Benzyl alcohol	0.20 ± 0.03	N.D.	N.D.	N.D.	0.58 ± 0.04^{A}	0.57 ± 0.09^{A}
Phenylethyl alcohol	0.37 ± 0.01	N.D.	N.D.	0.82 ± 0.09^{A}	0.86 ± 0.10^{A}	0.60 ± 0.11^{B}
Benzaldehyde	0.34 ± 0.03	22.51 ± 1.35^{A}	22.96 ± 1.50^{A}	19.95 ± 1.53^{A}	19.84 ± 2.14^{A}	20.50 ± 3.49^{A}
4-Methoxy-benzaldehyde	2.70 ± 0.34	N.D.	N.D.	2.06 ± 0.33^{A}	1.28 ± 0.10^{B}	1.33 ± 0.19^{B}
1-(4-Methoxyphenyl)-2-propanone	0.17 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.
Acetophenone	N.D.	0.74 ± 0.03^{B}	0.62 ± 0.04^{B}	0.74 ± 0.09^{B}	0.80 ± 0.10^{B}	0.99 ± 0.14^{A}
(E)-cinnamaldehyde	3.09 ± 0.19	N.D.	N.D.	N.D.	N.D.	N.D.

Compounds	Aroma compounds relative content (C) (μg/kg)					
	Tomato	**CK**	**T25**	**T50**	**T75**	**T100**
estragole	0.12 ± 0.01	N.D.	N.D.	N.D.	N.D.	N.D.
anethole	3.51 ± 0.40	0.46 ± 0.04^{A}	0.42 ± 0.09^{A}	0.12 ± 0.02^{C}	0.25 ± 0.06^{B}	0.20 ± 0.02^{BC}
3-phenyl-2-propenoic acid ethyl ester	0.43 ± 0.10	N.D.	N.D.	N.D.	N.D.	N.D.
2,4-bis(1,1-dimethylethyl) phenol	0.19 ± 0.05	N.D.	N.D.	N.D.	N.D.	N.D.
4-(2-propenyl)-phenol	1.77 ± 0.03	N.D.	N.D.	N.D.	N.D.	N.D.
others		2.38 ± 0.16^{C}	2.77 ± 0.31^{B}	3.22 ± 0.14^{B}	3.05 ± 0.16^{B}	4.11 ± 0.07^{A}
1-decene	N.D.	1.25 ± 0.20^{B}	1.19 ± 0.18^{B}	1.37 ± 0.15^{B}	1.46 ± 0.16^{B}	2.32 ± 0.18^{A}
D-limonene	N.D.	1.13 ± 0.09^{C}	1.32 ± 0.19^{BC}	1.61 ± 0.22^{A}	1.40 ± 0.06^{AB}	1.45 ± 0.08^{AB}
dimethyl trisulfide	N.D.	N.D.	0.26 ± 0.02^{B}	0.24 ± 0.02^{BC}	0.19 ± 0.03^{C}	0.33 ± 0.04^{A}

Table 5-2 Odor activity values (OAVs) of aroma compounds in precooked stewed beef with tomato.

Compounds	Odor activity values (OAVs)					
	Tomato	**CK**	**T25**	**T50**	**T75**	**T100**
Aldehydes						
Pentanal	N.D.	< 1	< 1	< 1	< 1	< 1
Hexanal	< 1	125	81	55	56	34
Heptanal	N.D.	1	2	2	2	1

Compounds	Odor activity values (OAVs)					
	Tomato	CK	T25	T50	T75	T100
Octanal	< 1	9	8	5	8	5
Nonanal	< 1	5	4	3	4	3
Decanal	N.D.	< 1	< 1	< 1	< 1	< 1
Tridecanal	N.D.	< 1	< 1	< 1	< 1	< 1
Hexadecanal	N.D.	1	2	2	2	2
(E)-2-Octenal	N.D.	< 1	< 1	< 1	< 1	< 1
2-Methyl-2-butenal	< 1	N.D.	N.D.	N.D.	N.D.	N.D.
(E)-2-Hexenal	< 1	N.D.	N.D.	N.D.	N.D.	N.D.
Alcohols						
2-Methyl-1-butanol	< 1					
1-Pentanol	N.D.	< 1	< 1	< 1	< 1	< 1
1-Hexanol	N.D.	< 1	< 1	< 1	< 1	< 1
(Z)-3-Hexen-1-ol	< 1	N.D.	N.D.	< 1	< 1	< 1
1-Octen-3-ol	N.D.	6	4	3	3	2
1-Heptanol	N.D.	< 1	< 1	< 1	< 1	< 1
2-Methyl-6-hepten-1-ol	< 1	N.D.	N.D.	< 1	< 1	< 1
2-Ethyl-1-hexanol	N.D.	< 1	< 1	< 1	< 1	< 1
Linalool	< 1	N.D.	N.D.	< 1	< 1	< 1

Compounds	Odor activity values (OAVs)					
	Tomato	CK	T25	T50	T75	T100
1-Octanol	N.D.	< 1	< 1	< 1	< 1	< 1
(E)-2-Octen-1-ol	N.D.	< 1	< 1	< 1	< 1	< 1
Ketones						
2-Heptanone	N.D.	< 1	< 1	< 1	< 1	< 1
2,3-Octanedione	N.D.	< 1	< 1	< 1	< 1	< 1
6-Methyl-5-hepten-2-one	< 1	< 1	< 1	< 1	< 1	< 1
(E)-6,10-Dimethy-5,9-undecadien-2-one	< 1	N.D.	N.D.	N.D.	N.D.	N.D.
Heterocyclic compounds						
2-Pentyl-furan	N.D.	< 1	< 1	< 1	< 1	< 1
2-Isobutylthiazole	< 1	N.D.	< 1	< 1	< 1	1
Benzothiazole	< 1	N.D.	N.D.	N.D.	N.D.	N.D.
Furfural	< 1	N.D.	N.D.	N.D.	N.D.	N.D.
Aromatics						
Toluene	N.D.	< 1	< 1	< 1	< 1	< 1
1,3-Dimethyl-benzene	N.D.	< 1	< 1	< 1	< 1	< 1
Styrene	N.D.	< 1	< 1	< 1	< 1	< 1
Benzyl alcohol	< 1	N.D.	N.D.	N.D.	< 1	N.D.
Phenylethyl alcohol	< 1	N.D.	N.D.	< 1	< 1	< 1

Compounds	Odor activity values (OAVs)					
	Tomato	**CK**	**T25**	**T50**	**T75**	**T100**
Benzaldehyde	< 1	< 1	< 1	< 1	< 1	< 1
4-Methoxy-benzaldehyde	< 1	N.D.	N.D.	< 1	< 1	< 1
1-(4-Methoxyphenyl)-2-propanone	71	N.D.	N.D.	N.D.	N.D.	N.D.
Acetophenone	< 1	< 1	< 1	< 1	< 1	< 1
(E)-cinnamaldehyde	< 1	N.D.	N.D.	N.D.	N.D.	N.D.
estragole	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
anethole	< 1	< 1	< 1	< 1	< 1	< 1
3-phenyl-2-propenoic acid ethyl ester	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4-bis(1,1-dimethylethyl) phenol	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4-(2-propenyl)-phenol	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
others						
1-decene	N.D.	< 1	< 1	< 1	< 1	< 1
D-limonene	N.D.	< 1	< 1	< 1	< 1	< 1
dimethyl trisulfide	N.D.	N.D.	3	2	2	3

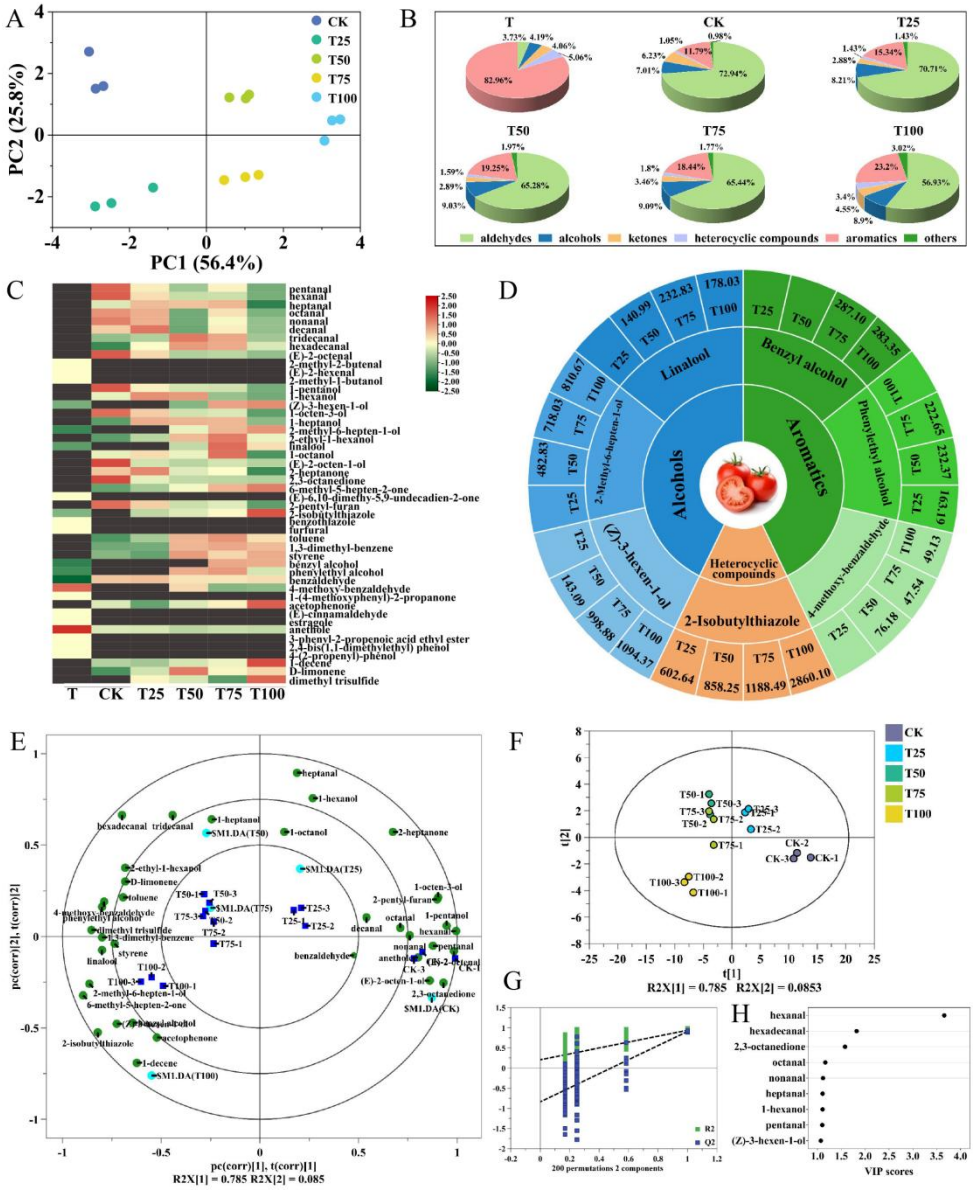

Fig. 5-2 Pincipal component analysis (PCA) score plot of electronic nose results (A), changes in aroma profiles (B), heat map distribution of aroma compound contents (μg/kg), with all row normalized scale. The color intensity, ranging from green to red, indicates an increase in the concentration of aroma compounds (C), The flavor endowment wheel of aroma compounds in tomatoes (D), partial least squares discriminant analysis (OPLS-DA; E),

principal component analysis (PCA; F), permutation score plots (G), and VIP score plot of differential aroma compounds (H) of PCSB (CK) and PCSBT samples (T25, T50, T75, and T100).

The differences in aroma compounds between samples were further analyzed using PLS-DA and PCA (**Fig. 5-2**E, F). As shown in the PCA score plot (**Fig. 5-2**F), PC1 and PC2 explained 78.50% and 8.53% of the variance, respectively, with PC1 accounting for the most of the variation. The samples' projections on PC1 diverged from CK as the tomato content increased from 25%-100%. Specifically, Compared to the other treatment groups, T25 is closest to CK, with their projection on PC1 located on the positive axis, indicating their relatively similar qualities. The projections of T50-T100 were located on the negative half-axis. T50 and T75 projections were positioned on the negative half-axis, overlapping, suggesting similar aroma profiles. Although T100's projection was close to T50 and T75, it did not overlap and was the farthest from CK, indicating a greater variation in aroma composition with higher tomato additions. This distribution trend was also reflected in the PCA score plot generated by the electronic nose. The PLS-DA biplot (**Fig. 5-2**E) that the explained variances for the three ellipses were 50%, 75%, and 100%, respectively. Most variables were within the 50%-100% ellipse. The PLS-DA model, based on five principal components, explained 96.1% of the validation variance, with excellent model parameters (R^2X = 0.961, R^2Y = 0.934, Q^2 = 0.784), confirming good fit and predictive ability. Moreover, 200 permutation tests (**Fig. 5-2**G) indicated that the PLS-DA model was not overfitted. As shown in **Fig. 5-2**E, the samples from T25-T75 were within the inner circle. CK showed strong positive correlations with several aroma compounds, including pentanal (r = 0.796), hexanal (r = 0.864), nonanal (r = 0.606), (E)-2-octenal (r = 0.869), 1-pentanol (r = 0.858), 1-octen-3-ol (r = 0.748), (E)-2-octen-1-ol (r = 0.939), 2,3-octanedione (r = 0.980), and 2-pentyl-furan (r = 0.978), all lipid oxidation products. T100 showed a strong positive correlation with 2-isobutylthiazole (r = 0.905). Based on variable importance in the projection (VIP) scores from PLS-DA analysis and the P-values from variance analysis, 11 aroma compounds were identified with VIP > 1 and $P < 0.05$ as potential markers that differentiate PCSB with varying tomato content. These aroma compounds include hexanal, hexadecanal, 2,3-octanedione, octanal, nonanal, heptana, 1-hexanol, pentanal, (Z)-3-hexen-1-ol (**Fig. 5-2**H). (Z)-3-hexen-1-ol was present only in tomatoes and PCSBT samples, while the other compounds are lipid-derived.

5.3.4. TBARS analysis

The addition of tomato (25%-100%) significantly reduced the TBARS values. However, no significant differences were observed among the T25, T50, T75, and T100 groups (**Fig. 5-3**A).

5.3.5. Lipidomics analysis

A total of 949 lipid metabolites were identified from 5 groups of 15 samples (**Fig. 5-3**B, C). Specifically, 936 lipids were identified in the CK group, 945 in T25 and T50, 941 in T75, and 944 in T100. These metabolites were classified into six major

lipid categories, with glycerophospholipids (GP) being the most abundant, comprising 506 species. The other categories included 294 glycerolipids (GL), 82 sphingolipids (SP), 62 fatty acyls (FA), 3 sterol lipids (ST), and 2 prenyl alcohol lipids (PR). These lipids were further categorized into 41 subcategories, with the most abundant lipid subcategories in the PCSB samples being triglyceride (TG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylethanolamine (ether) (PE-O), phosphatidylcholine (PC), phosphatidylinositol (PI), acylcarnitine (CAR), phosphatidylserine (PS), lysophosphatidylcholine (LPC), phosphatidylethanolamine (vinylether) (PE-P), lysophosphatidylethanolamine (LPE), ceramide (Cer-NS), and phosphatidic acid (PA). Among the six major lipid categories, the most abundant was GL (**Fig. 5-3**B), which constituted 46.06% of the total lipid content in the CK group, followed by GP at 35.17%. The remaining lipid categories included FA, ST, SP, and PR. As shown in **Fig. 5-3**B, the total lipid content of precooked stewed beef without tomato (CK) was significantly decreased compared to that in precooked stewed beef with tomato (T25, T50, T75, and T100), with no significant difference observed between the T25 to T100 groups. ST and PR showed the same trend. The GL content of CK was also significantly lower than that of T25, T50, T75, and T100. There was no significant difference among T25, T50, and T75. The GL content in T100 was significantly higher than that of the other groups. In addition, the GP content in CK was also significantly declined compared to that of T25 and T50. As illustrated in **Fig. 5-3**D, TG, the predominant GL, accounted for approximately 94% of the GL in CK and contributed to approximately 45% of the total lipid content. In the CK group, the TG level was significantly decreased compared to all tomato-added groups except T50. PS was the most abundant lipid subclass in GP, representing 32.12% in CK, followed by PC (24.02%), PI (9.7%), PE-P (6.39%), PA (6.08%), PE-O (5.53%), LPC (4.95%), LPE (3.99%), and PE (3.96%). The changes in PS and PC content followed a trend similar to that of GP (**Fig. 5-3**B). PE-P, PE-O, and LPE levels were significantly higher in T100, while PE levels in all tomato-added groups were significantly higher than that in CK group, though no significant differences were observed between the T25 to T100 groups. Free fatty acids (FFA), the second most abundant lipid after TG, showed no significant differences across all groups (**Fig. 5-3**D). These results indicated that in the precooked stewed beef without tomato addition (CK), the levels of most lipid species, including total lipids, major lipid classes, and subclasses, were significantly reduced. In contrast, the tomato-added groups (T25, T50, T75, and T100) exhibited a relatively increase in the contents of these lipids. Therefore, for clarity and consistency in the following sections, all relevant descriptions (including the analysis of unsaturation levels) will be presented in terms of increased lipid content.

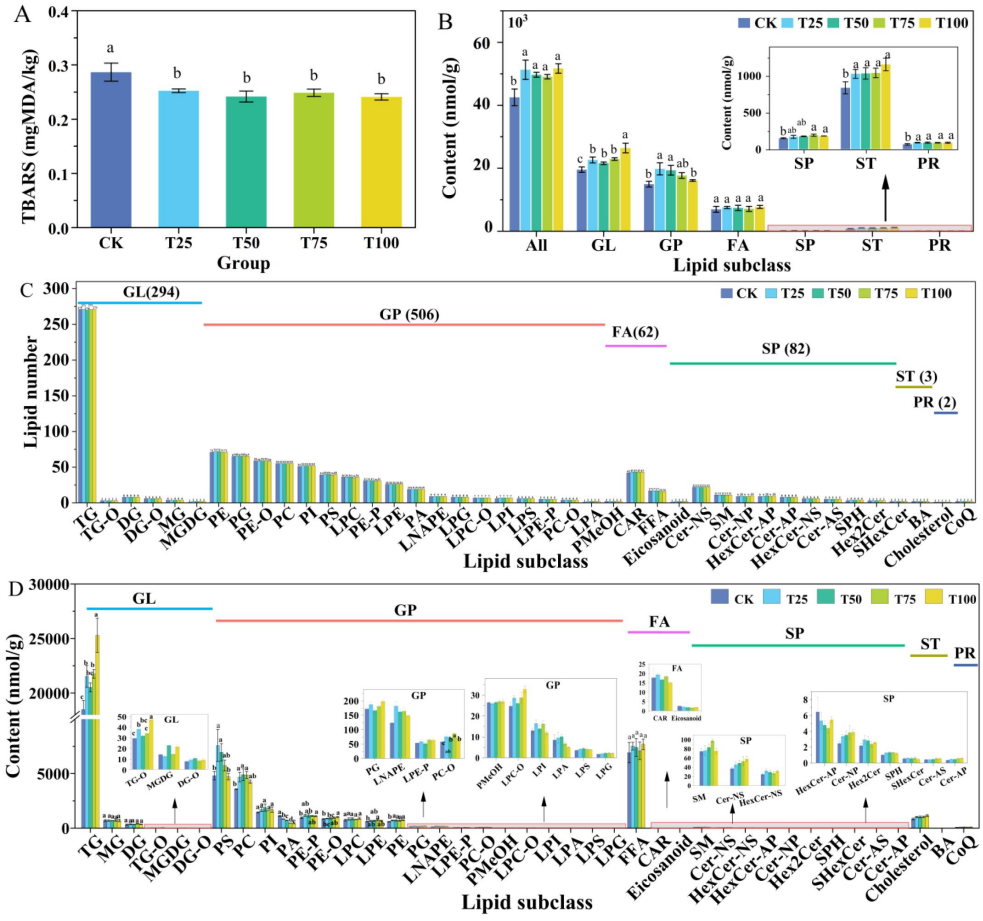

Fig. 5-3 Changes in thiobarbituric acid reactive substances (TBARS) values (A), changes in the contents of six major lipid categories (B), numbers of all detected lipid species (C), and variations in the contents of detected lipid subclasses (D) in PCSB (CK) and PCSBT (T25, T50, T75, and T100) samples. Data labeled with different lowercase letters indicate significant differences at $P < 0.05$.

Fig. 5-4 presents the unsaturation levels of fatty acid chains across several major lipid subclasses. Among these, PC-O and PA exhibited the highest degrees of unsaturation, both of which reached 100% (**Fig. 5-4**). Notably, PA also had the highest proportion of PUFAs, at 100%, with molecules predominantly containing three double bonds, which accounted for over 74%. The content of PA molecules containing more than three double bonds decreased significantly following the addition of tomato, particularly in the T75 and T100 groups. The polyunsaturated saturation rate of PC-O was also remarkably high, at 96.25%, with lipid molecules

possessing more than six double bonds comprising over 96%, primarily dominated by lipids with six double bonds (accounting for over 88%). Among these, PC(O-16:1/20:5) exhibited the highest content, representing 86.10% of the PC-O in the CK group. In the T25 to T100 groups, the content of PC(O-16:1/20:5) increased significantly, contributing substantially to the overall rise in PC-O content (**Fig. 5-3**D) and its unsaturation level (**Fig. 5-4**). The unsaturation of PE-O was also notably high, reaching 99.99%, with its polyunsaturation rate second only to that of PA at approximately 99.50% (**Fig. 5-4**). Most of these molecules contained five double bonds, followed by n:3 lipids (**Fig. 5-4**). Specifically, PE(O-18:1/18:2) (CK: 203.32 μg/kg; T25: 203.18 μg/kg; T50: 211.26 μg/kg; T75: 227.89 μg/kg; T100: 254.68 μg/kg), PE(O-18:1/20:4) (CK: 119.62 μg/kg; T25: 145.56 μg/kg; T50: 140.65 μg/kg; T75: 147.16 μg/kg; T100: 144.27 μg/kg), and PE(O-16:1/20:4) (CK: 1113.75 μg/kg; T25: 126.91 μg/kg; T50: 118.97 μg/kg; T75: 127.72 μg/kg; T100: 141.46 μg/kg) exhibited elevatedcontents, with a progressive increase from T25 to T100. These molecules accounted for approximately 50% of the PE-O content. In PE, the T100 group exhibited a significant increase in the proportions of n:0, n:1, n:2, n:3, n:4, and n:6 PE-O molecules (**Fig. 5-5**). The content of n:5 PE-O molecules was significantly higher in the T25, T75, and T100 groups (**Fig. 5-5**). In the CK group, the unsaturation rate of PE was 99.92%, with a polyunsaturated proportion of 97.66% (**Fig. 5-4**). This proportion gradually increased across the T25-T100 groups (**Fig. 5-5**). PE molecules containing four and two double bonds were more abundant (**Fig. 5-4**), with PE(20:4/18:0) exhibiting the highest content, followed by PE(18:0_18:2), which showed a significant increase in the T100 group. The contents of n:2, n:3, n:5, and n:9 PE molecules significantly increased, with n:11 PE showing a significant increase in the T75 to T100 groups, although no significant differences were found between the groups (**Fig. 5-5**). The unsaturation level of PE-P was also relatively high, at 98.58%, with a polyunsaturated rate of 95.41%. Within PE-P, n:2 lipids predominated, comprising approximately 55%, followed by n:3 lipids at approximately (**Fig. 5-4**). In the T25 group, the levels of n:3, n:4, n:5, and n:6 PE-P molecules significantly increased, whereas in T50, n:0, n:1, n:3, n:5, and n:6 PE-P molecules were significantly higher than those in the CK group (**Fig. 5-5**). At T75, n:0 and n:5 PE-P molecules were significantly higher than in the CK group, but no significant differences were observed between the T25 to T100 groups (**Fig. 5-5**). Additionally, in PC, the contents of n:2 and n:3 PC molecules significantly increased in T25, T50, and T75, while n:4, n:5, and n:6 molecules showed significant increases in T25, T50, and T75, with no significant difference among T25, T50, and T75 (**Fig. 5-5**). The contents of n:3, n:4, n:5, and n:6 TG molecules increased significantly from T25 to T100, with no significant difference among T25, T50, and T75. The content of n:1, n:2, n:3, n:4, n:5, n:6, n:7, and n:8 TGs increased significantly from T75 to T100. Overall, the levels of unsaturated lipid molecules in precooked stewed beef with tomato (T25, T50, T75, and T100) significantly higher than that in precooked stewed beef (CK), with the most significant trends observed for polyunsaturated lipid species.

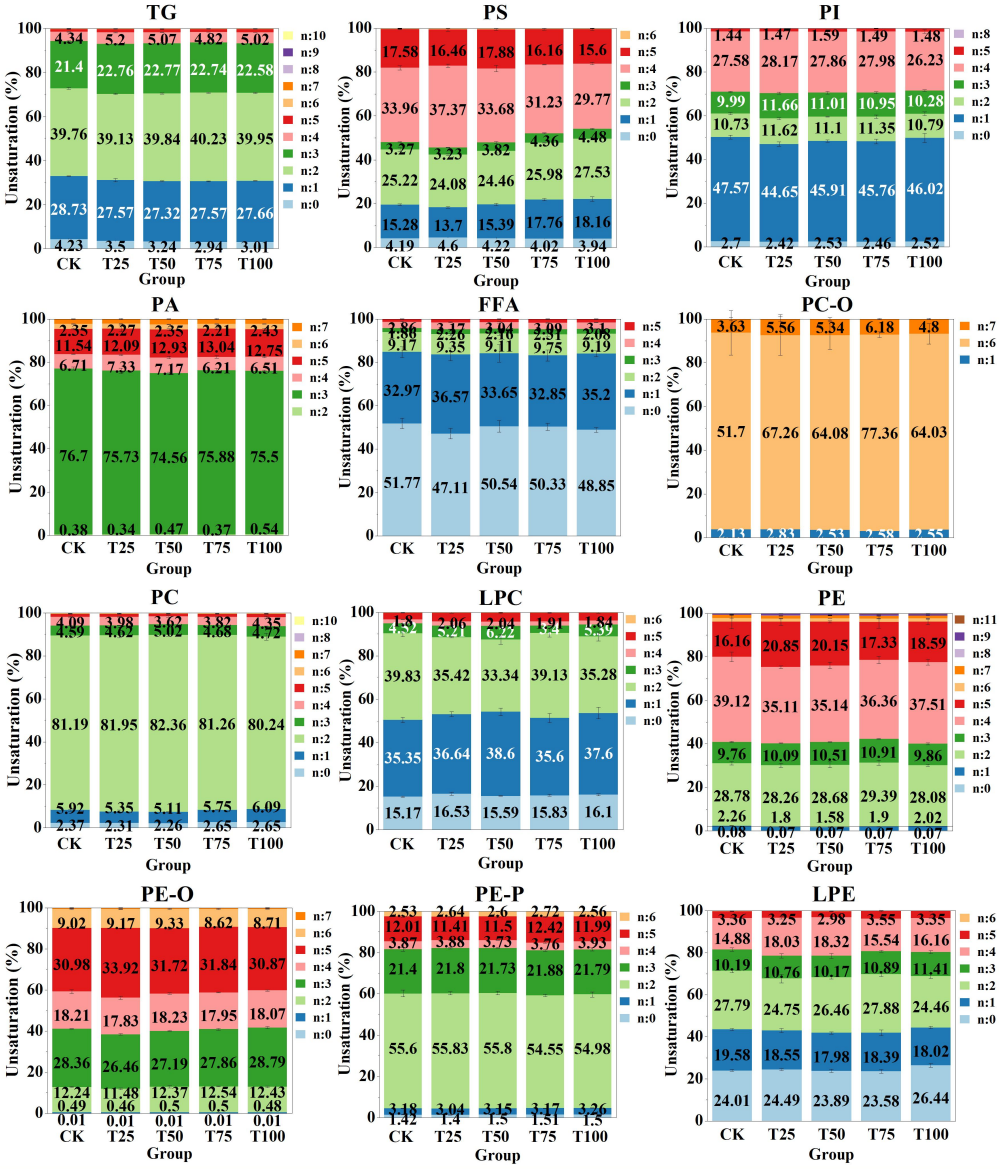

Fig. 5-4 The unsaturation composition percentage of the predominant lipid molecular species (total acyl carbons: total double bonds) in total TG, PS, PI, PA, FFA, PC-O, PC, LPC, PE, PE-O, PE-P, and LPE.

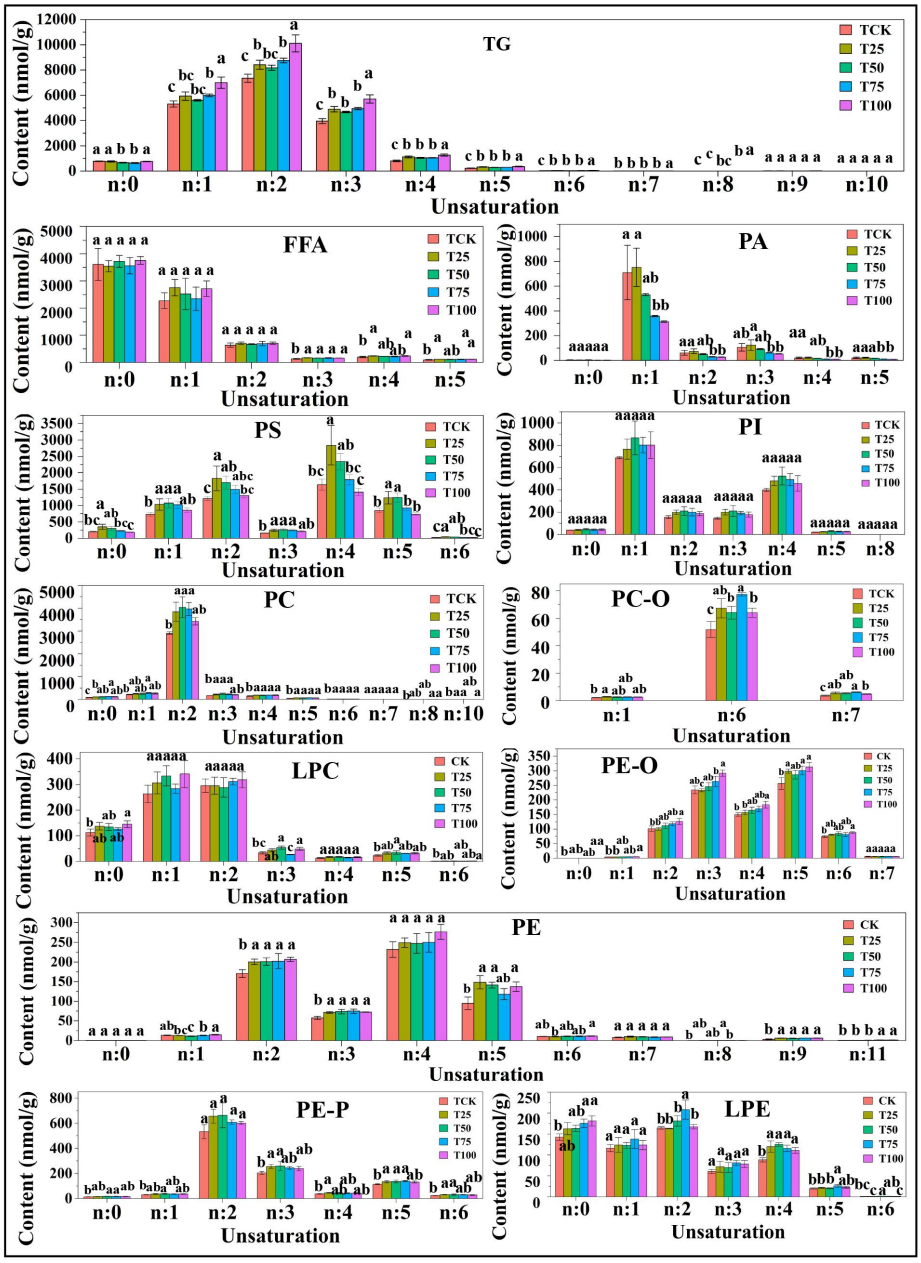

Fig. 5-5 Changes in the contents of unsaturation composition of the predominant lipid molecular species (TG, FFA, PA, PS, PI, PC, PC-O, LPC, PE-O, PE, PE-P, and LPE). Data labeled with different lowercase letters indicate significant differences at $P < 0.05$.

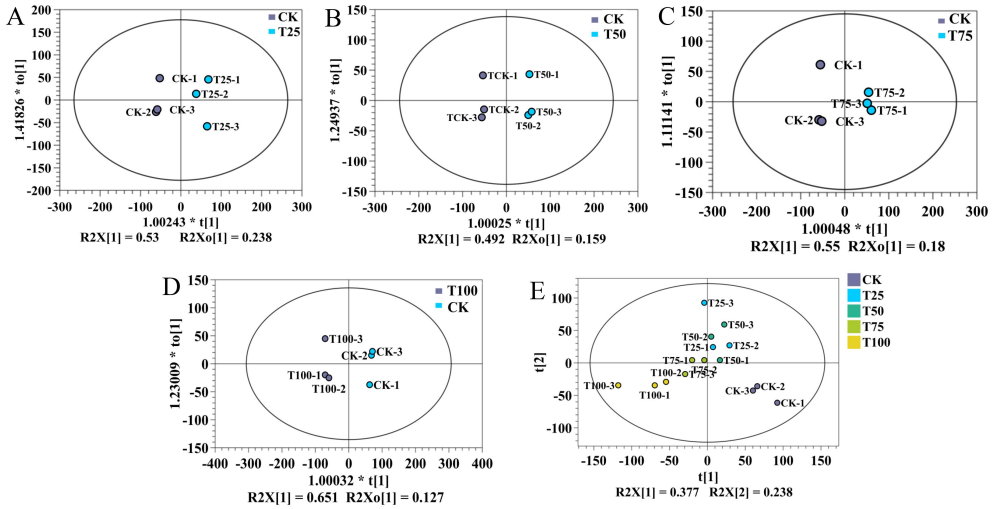

Fig. 5-6 Partial least squares discriminant analysis (OPLS-DA) score plot (CK and T25; A), OPLS-DA score plot (CK and T50; B), OPLS-DA score plot (CK and T75; C), OPLS-DA score plot (CK and T100; D), OPLS-DA score plot (CK, T25, T50, T75, and T100; E).

To investigate lipid variations across the samples, partial least squares discriminant analysis (PLS-DA) was performed. As shown in **Fig. 5-6**A-E, all groups were distinctly separated, indicating that the inclusion of 25-100% tomato significantly altered the lipid composition of the PCSB. Based on the VIP scores from PLS-DA analysis and *P*-values derived from ANOVA, lipids with VIP > 1 and $P < 0.05$ were considered differential lipids. As shown in **Fig. 5-7**A, a total of 42 differential lipids were identified between CK and T25, including 29 TG, 6 PS, 2 PC, 1 PE, 1 PE-P, 1 LNAPE, 1 FFA, and 1 DG, all of which increased from CK to T25. For the comparison between CK and T50, 49 differential lipids were identified, comprising 29 TG, 9 PS, 3 LPC, 2 LPE, 2 PC, 1 PE, 1 PE-P, 1 LNAPE, and 1 SM (**Fig. 5-7**B). Among these, TG(16:0/16:0/18:0), TG(14:0/16:0/16:0), TG(16:0/16:0/16:0), TG(16:0/17:0/18:0), and TG(16:0/16:0/17:0) decreased, while the remaining lipids increased. In the comparison between CK and T75, 75 differential lipids were detected, including 49 TG, 4 PS, 7 PC, 1 PC-O, 3 LPE, 2 PE-P, 1 LNAPE, 1 PI, 1 SM, 1 FFA, and 1 DG (**Fig. 5-7**C). Among these, TG(16:0/16:0/18:0), TG(16:0/16:0/16:0), TG(16:0/17:0/18:0), FFA(21:2), TG(14:0/16:0/16:0), LPC(20:3), and TG(16:0/16:0/17:0) decreased, while the remaining 62 lipids were upregulated. For the comparison between CK and T100, 77 differential lipids were identified, including 60 TG, 5 PS, 4 PC, 2 PE-O, 2 LPE, 1 cholesterol, 1 LNAPE, 1 FFA, and 1 DG (**Fig. 5-7**D). Among these, PS(16:0/20:1), FFA(21:2), TG(16:0/16:0/16:0), and TG(16:0/16:0/18:0) decreased, while the remaining 73 lipids were upregulated. Overall, the PLS-DA score plot (**Fig. 5-6**E*)* showed distinct separation between the CK and the T25-T100 groups, confirming significant differences in lipid composition.

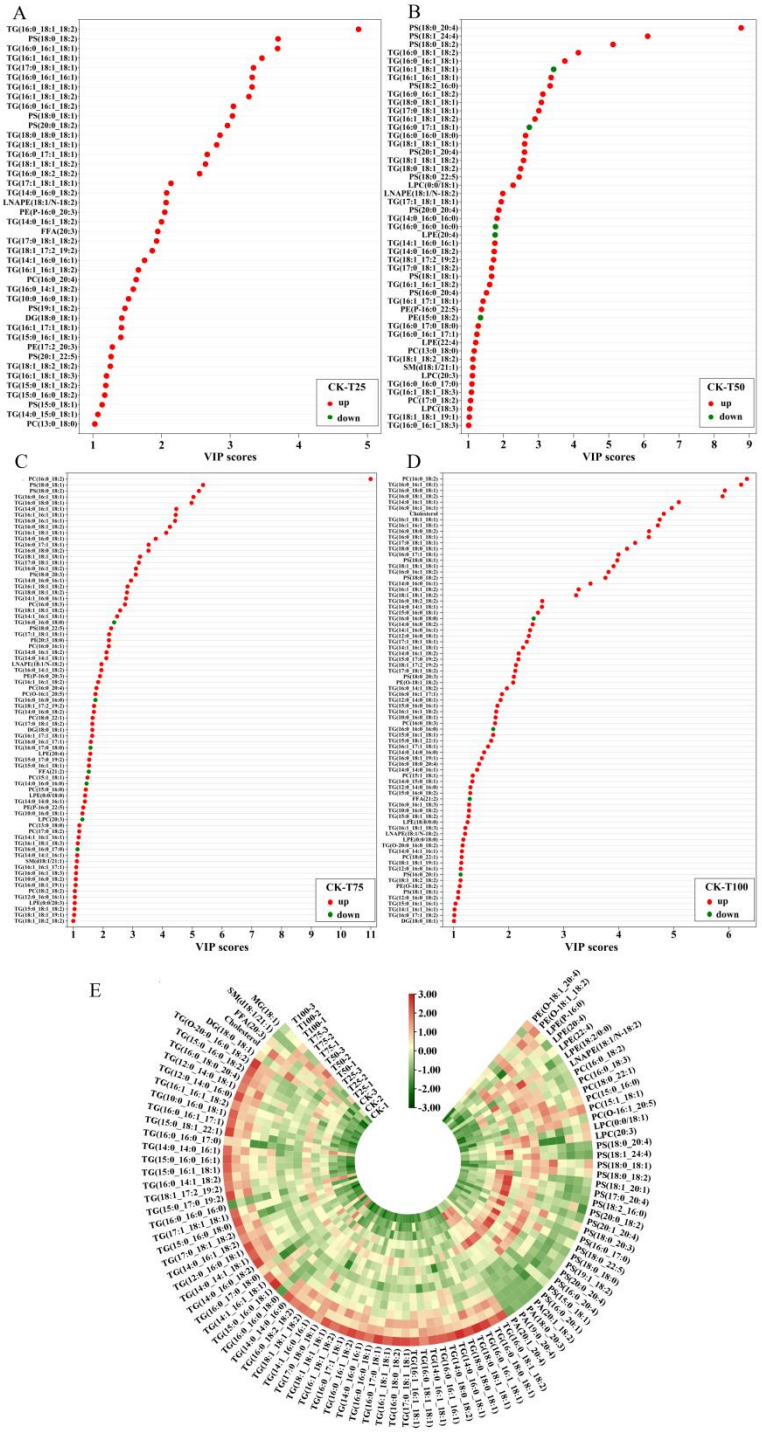
A
CK-T25
up
down
VIP scores
B
CK-T50
up
down
VIP scores
C
CK-T75
up
down
VIP scores
D
CK-T100
up
down
VIP scores
E
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00

Fig. 5-7 Variable importance in projection (VIP) score plot of differential lipids (CK and T25; A), VIP score plot of differential lipids (CK and T50; B), VIP score plot of differential lipids (CK and T75; C), IP score plot of differential lipids (CK and T100; D), and heat map distribution of differential lipids (CK, T25, T50, T75, and T100; E).

The T25 and T50 groups exhibited considerable overlap, while T75 was closer to these groups but did not overlap. T100 was positioned near T75 but showed no overlap. Based on VIP > 1 and $P < 0.05$, 96 differential lipids were identified across the five groups, including 54 TG, 18 PS, 4 PA, 2 LPC, 5 PC, 1 PC-O, 3 LPE, 1 LPE-P, 2 PE-O, and 1 LNAPE (**Fig. 5-7**E*)*. In contrast to previous results, the trends of these differential lipids varied from CK to T100.

5.3.6. FT-IR Analysis

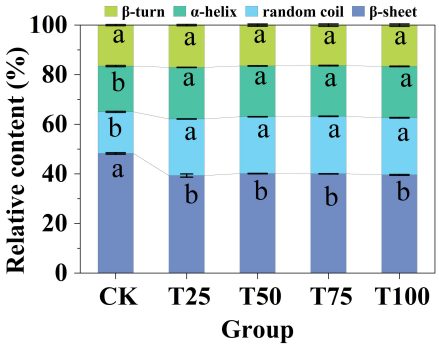

Fig. 5-8 Effect of tomato addition on secondary structure.

To investigate the effect of tomato treatment on the protein secondary structure, the Amide I band (1700–1600 cm^{-1}) was deconvoluted, and the proportions of each structural component (α-helix, β-sheet, β-turn, and random coil) were determined. As shown in the **Fig. 5-8**, the β-sheet structure was the most prevalent across all groups, followed by the random coil, α-helix, and β-turn structures. After the addition of 25%-100% tomatoes, a significant reduction in β-sheet content was observed, accompanied by an increase in random coil and α-helix proportions. However, no significant differences were found among the T25, T50, T75, and T100 groups regarding the β-sheet, random coil, and α-helix proportions.

5.4. Discussion

5.4.1. Flavor-enhancing effect of tomato on PCSB—WOF masking

A gradual decline of WOF perception, accompanied by an increase in tomato-like aroma intensity with increasing tomato addition, indicated that the sensory reduction of WOF in PCSB might, in part, be attributed to the masking effect of specific aroma compounds derived from tomatoes (**Fig. 5-1**). Among the tested groups, T75

group achieved the most optimal balance between meaty aroma, tomato-like aroma, and overall acceptability. In contrast, the tomato-like aroma predominated in the T100 group, masking the meaty aroma and resulting in a flavor profile that was dominated by tomato-like notes, with a corresponding reduction in the characteristic meaty flavors. In food processing, aroma masking is a commonly employed strategy to mitigate off-flavors, relying on the distinctive and intense aromatic attributes of masking agents to obscure undesirable volatiles. These agents are typically composed of various spices and their essential oil derivatives rich in low-threshold volatile organic compounds (VOCs), such as gingerol and shogaol from ginger, volatile sulfur compounds from garlic and onion, capsaicin from chili, and anethole from fennel. These VOCs activate olfactory receptors intensely and competitively, attenuating the sensory perception of off-notes (Xia et al., 2024). Currently, aroma masking is widely applied in ready-to-eat meals. A major limitation of this approach lies in the potential for masking agents to alter the characteristic flavor profile of the product itself (Xia et al., 2024). Tomato-originated aroma compounds imparted green and sweet-sour flavors to PCSB by introducing aroma compounds such as 2-isobutylthiazole, linalool, (Z)-3-hexen-1-ol, 2-methyl-6-hepten-1-ol, phenylethanol, and 4-methoxybenzaldehyde. 2-isobutylthiazole, a characteristic tomato-originated aroma compound, likely originating biogenetically, contributes to the strong green tomato note. It is considered a key-component of tomato aroma (Christiansen et al., 2011; Du et al., 2015). Pyne and Wick (1965) noted that the "green" note of the vegetable volatile (*Z*)-3-hexen-1-ol, derived from lipid oxidation, contributed significantly to tomato flavor. In addition, linalool and 2-phenylethanol are well known to be associated with the fruity/floral aromas in tomato puree (Baldwin et al., 2008). 2-methyl-6-hepten-1-ol, which has a green odor and has been detected in overripe tomatoes, was proposed to be formed by the reduction of 6-methyl-5-hepten-2-one, the key volatile responsible for the characteristic tomato aroma (Güler & Şekerli, 2013). 4-Methoxybenzaldehyde (also known as *p*-anisaldehyde) has a strong sweet, powdery floral aroma and is widely present in many natural plants and their essential oils (Khalid et al., 2024). Among them, 2-isobutylthiazole was the only tomato-originated aroma compound with an OAV > 1, observed exclusively in the T100 group (**Table 5-2**). The ERV of 2-isobutylthiazole in T25 reached 602.64% and increased dramatically to 2860.10% in the T100 group (**Fig. 5-2**D). In a study by Qiang et al. (2025), the flavor endowment of clove to stewed beef was analyzed, with the highest ERV of 3-carene reaching only 37.96%. Therefore, 2-isobutylthiazole might play a significant role in the flavor endowment of PCSB, contributing a green tomato-like aroma that partially masks off-flavors from lipid oxidation products (**Fig. 5-9**). Despite the fact that other tomato-originated aroma compounds, such as linalool, 2-phenylethanol, 2-methyl-6-hepten-1-ol, 4-methoxy-benzaldehyde, (*Z*)-3-hexen-1-ol exhibited OAVs < 1, their floral and tomato green notes might synergistically enhance the sensory impact of 2-isobutylthiazole. This collaboration enriched the flavor profile and effectively masked the WOF associated with lipid oxidation. While traditional aroma chemistry suggests that only compounds exceeding the threshold (OAV > 1) contribute to the overall aroma. Recent studies have shown that even sub-threshold

compounds can significantly influence sensory perception. The cooperative effect of aroma compounds enhances the complexity of the sensory experience and enriches the overall flavor profile (Lu et al., 2024). As tomato addition increased, the release and migration of tomato-originated aroma compounds, including 2-isobutylthiazole, (*Z*)-3-hexen-1-ol, 2-methyl-6-hepten-1-ol, and 4-methoxy-benzaldehyde, within the meat matrix were significantly amplified. APA results further confirmed this trend, showing a progressive intensification of the tomato-like aroma from T25 to T100 (**Fig. 5-1**), likely due to a "concentration effect", whereby increased contents amplify the impact of these aroma compounds on the overall sensory profile. Such concentration-dependent shifts have also been observed in aldehydes such as nonanal and octanal, which impart fresh and citrusy notes at low concentrations but evolve into fatty, fishy, or rancid odors at higher levels (An et al., 2024). Interestingly, these aldehydes showed decreasing trends with increasing tomato content, reinforcing the hypothesis that enhanced tomato derived aroma compounds contributed to the sensory masking of WOF. Jokanović et al. (2020) similarly reported that *Satureja montana L.* (winter savory) masked WOF in precooked pork chops through its aromatic compounds. Onion and ginger extracts have also been utilized to suppress off-odors in meat-based systems. Luo et al. (2022a) reported that such treatments significantly reduced the content of off-odor volatiles like hexanal, 1-nonanol, 2-octanone, octanal, and 1-octen-3-ol, likely due to interactions between spice-derived compounds and off-flavor compounds, thereby diminishing off-flavor sensory impact. Further evidence from An et al. (2024) demonstrated that yeast extracts rich in pyrazines and esters could mask WOF in fish gel products, improving overall flavor acceptability. Likewise, Dang et al. (2024) showed that perilla juice and ginger juice possess characteristic aromas capable of effectively masking WOF in fish paste gels.

It is noteworthy that tomato-originated volatiles exhibited varying migration behaviors. For instance, 4-methoxy-benzaldehyde and (*Z*)-3-hexen-1-ol peaked in the T75 group, whereas 2-isobutylthiazole remained stable in T25–T50 before increasing continuously from T75 onwards. This may be attributed to their physicochemical properties (Liu et al., 2021). Sun et al. (2023) also found compound-specific variations in binding affinities to MPs: anisole and carvacrol displayed stronger binding post-thermal treatment compared to eugenol and *p*-anisaldehyde. These differences suggest that protein conformational changes regulate the selective retention and release of volatiles. Nevertheless, our current understanding of the specific interaction mechanisms between proteins and both WOF-related compounds and tomato-originated volatiles remains limited. Further investigation is warranted to elucidate the molecular basis of these interactions, which may offer new insights into protein–aroma compound binding behavior and its role in flavor retention, masking, and release during heating–storage–reheating process.

5.4.2. The lipid antioxidant effect of tomato on PCSB — Reduction of WOF

The accumulation of lipid oxidation products, primarily aldehydes, is a major contributor to WOF in precooked meat products (Liu et al., 2024). Natural antioxidants have been used to mitigate WOF development. For instance, Lungu et al. (2022) demonstrated that *moringa oleifera* leaf and root powders could effectively reduce WOF formation and extend the shelf life of processed pork. Similarly, Jokanović et al. (2020) reported that the incorporating of winter savory (*Satureja montana* L.) showed notable protective effects on the oxidative stability of precooked pork chops, while the essential oils from winter savory effectively masked off-flavors associated with WOF. Additionally, Dang et al. (2024) found that perilla and ginger juice inhibited WOF formation in fish paste gels and masked these off-flavors with their distinct aromas. Elbadrawy and Sello (2016) further revealed that tomato peel extract significantly reduced peroxide, malondialdehyde (MDA), and carbonyl values in oil samples, highlighting the antioxidative potential of tomatoes. Lycopene, a carotenoid in tomatoes, plays a key role in antioxidant and provitamin activity. The hydrophobic polyene chains of carotenoids can neutralize sulfonyl radicals, quench singlet oxygen, and form stable adducts with peroxyl radicals. Studies show that lycopene scavenges superoxide radicals and inhibits lipid peroxidation (Kotíková et al., 2011; Skiepko et al., 2016). The addition of lycopene significantly reduced TBARS and carbonyl content in muscle tissue (Wang et al., 2021), consistent with our findings where TBARS values in the T25-T100 groups were significantly decreased (**Fig. 5-3**A). TBARS is a critical indicator of lipid oxidation, and its reduction suggests that tomato addition effectively inhibited lipid oxidation. Lipid analysis showed that the CK group had relatively low levels of total lipids, GL, and phospholipids (PL), indicating the reduction of lipids due to oxidation, resulting in the formation of numerous secondary oxidation products such as short-chain aldehydes, alcohols, and ketones, like hexanal, octanal, (E)-2-octenal, and 1-octen-3-ol, etc. (**Table 5-1**, **Fig. 5-9**). The PLS-DA biplot of the PCSB aroma compounds also revealed a strong positive correlation between CK and substances like pentanal, hexanal, (E)-2-octenal, 1-octen-3-ol, and 2,3-octanedione (**Fig. 5-2**E). It can be seen from **Table 5-1** that the relative contents of these compounds in CK were significantly higher than those in the T25-T100 groups. Aroma compounds with an OAV >1 contribute significantly to overall aroma profiles (Nie et al., 2023). In the CK group, aroma compounds with OAV > 1, including hexanal (grassy), pentanal (fermented), heptanal (fatty), octanal (fatty, green), 1-octen-3-ol (mushroom), were identified in our previous study as key contributors to WOF in PCSB (Liu et al., 2024). Hexanal exhibited an OAV > 100 in CK, indicating a substantial role in the WOF of PCSB. This finding aligns with APA results, which revealed that CK had distinct WOF with the most intense grassy, cardboard-like, metallic, fatty, hard-boiled egg, and oxidized vegetable oil aroma (**Fig. 5-1**).

Chapter IV has demonstrated that ether-bond phosphatidylethanolamine (e-PE), particularly PE-P, plays a key role in the formation of WOF in PCSB, while PC significantly contributes to its development. Additionally, TG have been reported to

facilitate WOF formation (Liu et al., 2025),which play a crucial role in defining the flavor profile of meat products, primarily due to their high PUFA content (**Fig. 5-4**). PUFAs are particularly prone to oxidation during processing. PL are more susceptible to oxidation than TG because of their higher PUFA content (Zhang et al., 2023). The oxidation of PL typically occurs at the unsaturated bonds, resulting in the formation of PL hydroperoxides, which break down into short- and long-chain oxidation products, contributing to WOF (Liu et al., 2024). Upon adding tomatoes, total lipids, PL, and GL were significant higher than CK (**Fig. 5-3**B), with notable increases in PE and PC content, especially PE-P, PE-O, and LPE in the T100 group (**Fig. 5-3**D). This suggested that tomatoes contributed to the stabilization of PL. Furthermore, polyunsaturated PL levels, including PE, PE-O, PE-P, and PC, were significantly elevated, indicating that tomatoes effectively protected these lipid molecules from degradation. TG was the most abundant lipid category (**Fig. 5-5**). TG content was significantly higher in the T25, T75, and T100 groups, particularly unsaturated TG molecules (n:3, n:4, n:5, n:6) (**Fig. 5-5**), which suggested that tomato additions effectively inhibited PUFA degradation in TG. FFA levels did not show significant changes (**Fig. 5-3**), indicating that tomato antioxidants reduced the breakdown of TG and PL into FFAs. Additionally, the PLS-DA score plot (**Fig. 5-6**A-E) revealed significant changes in lipid profiles, with most differential lipids (VIP > 1, $P < 0.05$) upregulated in the T25-T100 groups (**Fig. 5-5**A-D). These findings confirmed that tomatoes significantly promoted lipid accumulation in PCSB. Skiepko et al. (2016) also observed that lycopene marination increased the ratio of UFA and SFA, improving turkey meat quality. Studies have shown that tomatoes and their by-products are effective antioxidants in a variety of meat products, including patties, burgers, minced meat, cooked sausages, and dry-fermented sausages (Alves et al., 2012; Candogan, 2002; Eyiler & Oztan, 2011; Kim et al., 2011; Luisa García et al., 2009; Østerlie & Lerfall, 2005; Sánchez-Escalante et al., 2003). The advantages of incorporating tomato derivatives in the meat industry are largely attributed to their potent antioxidant capacity, enriched profile of bioactive constituents, and vivid red pigmentation. These attributes support the use of tomato-based by-products or extracts as natural alternatives to synthetic antioxidants and colorants (Rubén et al., 2020). The antioxidant components in tomatoes enhanced lipid stability by promoting UFA accumulation, such as TG, PE, PE-P, and PE-O, while simultaneously inhibiting the degradation of lipid oxidation-sensitive molecules (**Fig. 5-3D**), such as PA and FFA. This improvement in lipid stability reduced the generation of characteristic WOF-associated volatiles, notably hexanal, pentanal, and 1-octen-3-ol (**Table 5-1**), which served as differential markers (VIP > 1, $P < 0.05$) distinguishing PCSB and PCSBT (**Fig. 5-2**H). With the increasing addition of tomato, the aroma profile of the PCSB showed notable changes, particularly a significant decrease in aldehydes, such as hexanal and (E)-2-octenal (**Table 5-1**). The most pronounced alterations were observed in the T100 group. These findings were consistent with the results from principal component analysis (PCA) of the E-nose and aroma compounds, which showed a clear divergence between the T25-T100 and the CK group along the PC1

axes (**Fig. 5-2**A, E). Sensory evaluation further confirmed a reduction in WOF in the T25-T100 groups (**Fig. 5-1**).

There were no significant differences in the TBARS values among T25, T50, T75, and T100, indicating that the antioxidant effect of tomatoes reached saturation at a 25% addition, with further increases in tomato content not significantly enhancing antioxidant activity. This finding is further supported by the observation that the total lipid content did not exhibit significant differences across T25 to T100. Whereas, not all lipid subclasses showed consistent trends from T25 to T100 (**Fig. 5-7**E). Specifically, TG were significantly higher in the T100 group compared to the T25-T75 groups, while PS content was significantly higher in the T25 and T50 groups than in T100. Additionally, PC-O was significantly elevated in T75 compared to T50 and T100, while PA consistently decreased from T25 to T100 (**Fig. 5-3**D). These variations could be attributed to the interconversion of lipid molecules. PA plays a central role in lipid metabolism, acting as an intermediate in several metabolic pathways. It is a key precursor for the synthesis of PL and GL and participates in various lipid metabolic routes. PA can be converted into diacylglycerol (DAG) by phosphatidic acid phosphatase (PAP), with DAG further contributing to the synthesis of PC, PS, and other PL (Thakur et al., 2019). The increased content of PL such as PS and PC in the T25-T100 groups might be associated with the metabolism of PA into these molecules. PA can also be converted into TG via the triglyceride synthesis pathway (Thakur et al., 2019). The marked increase in TG levels from T25 to T100 further suggested that PA might be utilized in the production of GL. Furthermore, PA predominantly consist of highly unsaturated FAs, such as n:3 and n:4, which are inherently unstable. Previous research showed that in the T25-T100 groups, the content of lipid molecules containing UFAs, including PC, PE, and PE-O, increased to varying degrees (**Fig. 5-5**), which could also be attributed to the metabolic conversion of PA. The PUFAs in PA might be transferred to other lipid molecules. In the T25-T100 groups, PA might be primarily metabolized into other lipids (e.g., TG, PC, and PS), rather than oxidizing into lipid peroxides (such as MDA), as well as other short-chain aldehydes (e.g., hexanal and heptanal) and ketones (e.g., 2,3-octanedione). This reduction in PA oxidation could explain the decreased contribution of PA oxidation to the TBARS values. Zhang et al. (2023) also found that heating may lead to the conversion of PA into other lipid molecules. Consequently, these lipids may stabilize lipid oxidation through dynamic equilibrium, ensuring that total lipid content remained relatively stable across the T25, T50, T75, and T100 groups, while maintaining consistent TBARS values.

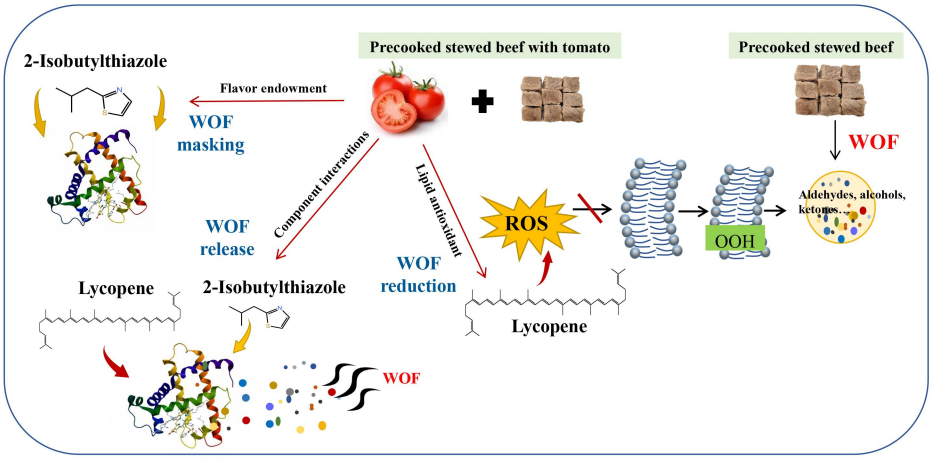

Fig. 5-9 Pathways of tomato mitigating WOF in PCSB.

5.4.3. Interaction between tomato and PCSB—Release of WOF

The lipid-derived aroma compounds in the T25-T100 groups exhibited notable changes, with a significant reduction in aldehyde compounds associated with lipid oxidation as the tomato content increased. Specifically, compounds such as hexanal, 1-octen-3-ol, octanal, and nonanal exhibiteddifferent trends in their contents (**Table 5-1**). In meat products, aroma compounds originate primarily from flavor formation and subsequent absorption mechanisms (Li et al., 2022). Muscle proteins, owing to their intrinsic affinity for flavor molecules, are considered ideal binding substrates that significantly influence the sensory attributes of meat (Zhang et al., 2021). Although food proteins are largely tasteless, they are known to bind and retain aroma compounds, an essential functional characteristic of proteins. Research has demonstrated that myofibrillar and serum proteins can effectively associate with a wide range of volatiles, including aldehydes, alcohols, ketones, sulfur- and nitrogen-containing compounds, thereby modulating their release during consumption (Guichard, 2006). Protein–flavor binding mechanisms are generally categorized as either reversible non-covalent binding via exposed hydrophobic patches and hydrogen bonds or irreversible covalent bonding through amino acid residues such as lysine, tryptophan, glutamic acid, or cysteine, which in turn modulate overall flavor perception (Dou et al., 2021). For instance, aldehydes may undergo hydrophobic interactions or form covalent bonds with amino or thiol groups, resulting in Schiff base formation or Michael addition reactions (Anantharamkrishnan et al., 2020; Anantharamkrishnan & Reineccius, 2020). Covalent protein–flavor adducts are typically resistant to volatilization during consumption, thereby reducing the availability of aroma-active compounds and altering the overall aroma profile of the product (Zhang et al., 2021). Such reactions occur rapidly during thermal processing and can continue to evolve throughout storage. The observed decrease in aldehyde levels with tomato addition may thus be

attributed to their irreversible covalent binding to protein side chains, such as aldehyde-lysine and amine-carbonyl interactions (Zhang et al., 2021). Supporting this, Wang et al. (2023) reported that (E)-2-octenal and (E,E)-2,4-decadienal can form covalent adducts with myofibrillar, sarcoplasmic, and collagen proteins, as confirmed via the detection of flavor–protein conjugate ions in mass spectrometry. Similarly, Anantharamkrishnan et al. (2020) demonstrated the formation of Schiff bases, Michael adducts, and disulfide bridges when β-lactoglobulin (BLG) was exposed to reactive volatiles such as hexanal, trans-2-hexenal, trans,trans-2,4-heptadienal, thiols, and furan derivatives. These bonds may form at different rates, resulting in dynamic changes in food aroma over time. In contrast, alcohols such as 1-menthol, geraniol, 2-pentanol, and 2,3-butanediol showed no evidence of covalent interaction with BLG. Literature suggests that ketones, alcohols, and furans primarily associate with proteins through non-covalent mechanisms (Wang & Arntfield, 2015; Wang et al., 2023).

On the other hand, the reductions observed in volatiles such as pentanal, hexanal, heptanal, octanal, nonanal, decanal, (E)-2-octenal, 1-pentanol, 1-hexanol, 1-octen-3-ol, and 1-octanol may also be explained by reversible non-covalent interactions, particularly hydrophobic binding with proteins (**Fig. 5-9**). Non-covalent interactions, including hydrophobic and hydrogen bonding, are known to influence the release and perception of aroma compounds (Reineccius, 2022; Zhang et al., 2021). Studies indicate that protein conformational changes occur upon flavor binding, which may alter hydrophobic region exposure. Enhanced hydrophobicity typically promotes stronger binding with nonpolar volatiles, thus inhibiting their release, while reduced hydrophobicity may facilitate aroma release (Guichard, 2002; Huang et al., 2022b). Thus, hydrophobic modifications in beef proteins may significantly influence their binding affinity for WOF-related compounds. The incorporation of tomatoes introduces not only tomato-originated aroma compounds but also antioxidants such as lycopene and polyphenols, which may interact with proteins through hydrophobic interactions, thereby altering their secondary structure (Sun et al., 2024; Sun et al., 2023). Zhao et al. (2023) demonstrated that lycopene binds to pangasius myosin via hydrophobic interactions, inducing conformational shifts in the protein's secondary structure. In our study, FT-IR analysis was employed to monitor secondary structure changes in beef proteins. The results revealed that tomato addition significantly modified protein conformation (**Fig. 5-8**), which might affect both the distribution and exposure of hydrophobic regions (Huang et al., 2022a). A reduction in β-sheet content is typically associated with a transition from rigid to more flexible protein structures, while an increase in random coil content indicates the exposure of disordered peptide segments, providing additional potential binding sites (Luo et al., 2022b). Concurrently, an increase in α-helix content may lead to the formation of compact hydrophobic cores, as surface hydrophobicity is often negatively correlated with α-helical content (Sun et al., 2023). However, the terminal ends of α-helices may expose hydrophobic residues, which serve as active binding sites for hydrophobic aroma compounds. These structural changes may collectively enhance the protein's selective binding capacity toward hydrophobic volatiles. Under these conditions, tomato-originated compounds

such as lycopene, polyphenols, and volatile constituents (e.g., 2-isobutylthiazole, 4-methoxy-benzaldehyde, and anethole) may compete with WOF contributors for hydrophobic binding sites on the protein, thereby facilitating the release of aldehydes, alcohols, and ketones associated with WOF (Huang et al., 2022a; Zhao et al., 2023). This competition may represent another critical mechanism underlying the observed reduction in these volatiles following tomato addition. Sun et al. (2023) found that anethole, estragole, and 4-methoxybenzaldehyde could alter the conformation of MPs through hydrophobic and hydrogen bonding. Similar findings were reported by Huang et al. (2022a), where phenolic compounds, such as rosmarinic acid, carnosic acid, and carnosol, were shown to modify myofibrillar protein structure and compete for hydrophobic binding sites, leading to the release of fishy odor compounds and significant reduction in off-odor intensity in fish products. Furthermore, Sun et al. (2024) revealed that eugenol competes with nonanal for the residue binding site, triggering conformational rearrangement and promoting the release of aldehydes. Notably, from T25 to T100, no significant differences were observed in protein secondary structure content, indicating a structural stabilization phase. During this stage, the exposure of hydrophobic regions remained relatively unchanged, suggesting that the protein's binding capacity for hydrophobic compounds had plateaued. Nevertheless, as the tomato addition level increased, competitive binding between tomato-originated constituents and lipid-derived aroma compounds intensified, leading to a progressive reduction in the contents of aldehydes, alcohols, and ketones (**Table 5-1**). This was consistent with sensory evaluations, wherein WOF perception was significantly diminished in T25–T100 samples (**Fig. 5-1**). It is worth noting that the differential volatility of individual compounds also plays a role in release dynamics, which are closely related to their physicochemical properties (Liu et al., 2021).

5.5. Conclusion

This study explored the impact of varying tomato addition levels on the WOF in PCSB. By integrating changes in protein secondary structure, lipid profiles, aroma compounds, and sensory attributes. The study elucidated the mechanisms through which tomatoes influence WOF, including the inhibition of lipid oxidation, the release of WOF, and its masking effect on flavor. The addition of tomatoes significantly suppressed lipid oxidation, thereby preserving lipid substrates and reducing oxidative losses in PL (e.g., PE, PE-P, and PC) and TGs. This, in turn, decreased the formation of aroma compounds contributing to WOF, such as pentanal, hexanal, and 1-octen-3-ol. Furthermore, tomato-originated aroma compounds, along with other constituents, might compete with WOF compounds for hydrophobic binding sites on proteins, thereby promoting the release of WOF. Notably, the characteristic tomato aroma compound 2-isobutylthiazole played a crucial role in flavor enhancement, imparting a green tomato-like aroma and a complex flavor profile to the PCSB. This created a sensory masking effect that reduced the perception of WOF and optimized the overall flavor profile. These mechanisms provide strong scientific evidence for the use of tomatoes as a natural flavor enhancer in meat products. APA results indicated that the T75 group achieved the

best balance between meaty aroma, tomato-like aroma, and WOF, followed by the T100 group. These findings suggested that an appropriate level of tomato addition not only reduced WOF, but also enhanced the characteristic tomato-like aroma, thus improving the sensory acceptance and overall flavor of meat products. However, excessive tomato addition (e.g., 100% addition) may overpower the meaty aroma, reducing its perception. This study provides both theoretical and practical support for the application of tomatoes as a natural flavoring agent in meat products. Additionally, the study highlights the importance of the interactions between proteins, lipids, and aroma compounds for flavor optimization, providing valuable insights for the development of natural, healthy seasoning technologies. Future research may further investigate the interactions between tomato-originated aroma compounds, proteins, and WOF, as well as the synergistic effects of different natural antioxidants (such as tomato and other fruit/vegetable extracts). Examining the influence of processing conditions on tomato's flavor-endowment mechanisms will offer a more comprehensive theoretical and practical foundation for food flavor modulation.

5.6. Reference

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6

Chapter VI General discussion, conclusions, and perspectives

6.1. General discussion

In Chinese cuisine, stewed beef has long been regarded as a traditional delicacy, and over the years, it has become an integral part of many people's daily diet. However, in response to the demands of modern, fast-paced lifestyles, PCSB has emerged as a convenient food option, finding its way onto numerous family tables. Most PCSB dishes are refrigerated (chilled/frozen) and require reheating before consumption. However, even within its shelf life, reheated stewed beef often develops an undesirable flavor, commonly referred to as WOF, characterized by unpleasant odors such as greasy, metallic, or cardboard-like notes. This flavor is typically described as greasy, metallic, or cardboard-like, and its presence not only diminishes the product's acceptability but also limits its application. Numerous studies have suggested that WOF primarily results from lipid oxidation, with the development of WOF being linked to lipid oxidation products such as hexanal, 1-octen-3-ol, octanal, and pentanal (Pegg et al., 2014; Ruenger et al., 2006). In addition to lipid oxidation, protein degradation leads to the loss of desirable meaty aroma, such as 4,5-dimethyl-3-hydroxy-2(5H)-furanone, 2,6-dimethylpyrazine, and benzothiazole, thus contributing to WOF (An et al., 2022; Pegg et al., 2014). However, the exact pathways through which WOF-related aroma compounds form in PCSB remain unclear, posing a challenge in the field of PCSB.

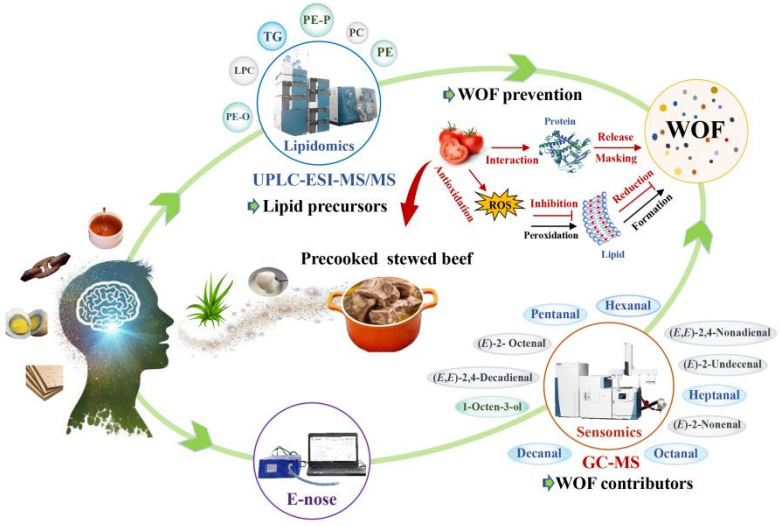

Fig. 6-1 Identification, formation, and prevention of WOF in PCSB.

Sensomics, a cutting-edge scientific concept considered to molecularize flavor entities in nature, has been successfully applied in recent years to identify key active aroma compounds in different food matrices (An et al., 2022; Chen et al., 2023). In

Chapter 3, sensomics were utilized to characterize the key aroma-active compounds responsible for WOF in PCSB after reheating and to elucidate the changes in aroma profiles during the cooking-refrigeration-reheating process. Although commercial PCSB typically contains broth, a meat-only system was employed in this study to eliminate potential interference and accurately identify the key contributors to WOF originating from the meat matrix. Through sensory descriptive analysis, we identified seven odor attributes to describe the aroma profile of freshly made and PCSB: meaty, grassy, cardboard, metallic, fatty, hard-boiled egg, and oxidized vegetable oil. APA revealed that cooked stewed beef exhibited a strong meaty aroma, whereas PCSB, after refrigeration and reheating, showed a marked increase in WOF, with a significant reduction in meaty aroma and a notable increase in grassy, cardboard, metallic, fatty, hard-boiled egg, and oxidized vegetable oil aromas (**Fig. 3-3**). Sensomics revealed 11 key aroma-active compounds in PCSB, including pentanal, hexanal, heptanal, octanal, decanal, (E)-2-nonenal, (E)-2-octenal, (E)-2-undecenal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, and 1-octen-3-ol (**Table 3-4, Fig. 6-1**). These compounds are products of lipid oxidation, such as heptanal (fatty), octanal (fresh, fatty), nonanal (green, fatty), and (E,E)-2,4-decadienal (fatty, nutty), which are derived from oleic acid oxidation. Hexanal (grassy, fatty) is a product of linoleic acid and arachidonic acid oxidation (Elmore et al., 1999). These compounds were significantly positively correlated with sensory characteristics associated with off-flavors (grassy, cardboard, metallic, fatty, hard-boiled egg, and oxidized vegetable oil) ($P < 0.05$) (**Fig. 3-4**A). Notably, heptanal, (E)-2-octenal, octanal, (E)-2-nonenal, decanal, and (E,E)-2,4-decadienal have been identified as key aroma compounds contributing to WOF in surimi gels (An et al., 2022). Hexanal and (E,E)-2,4-decadienal have also been identified as critical odorants and typical markers in the development of WOF in meat products (Konopka & Grosch, 1991; Zang et al., 2019). The content of these 11 key aroma-active compounds significantly increased after refrigeration and reheating (**Table 3-5**) and was positively correlated with TBARS, further confirming that refrigeration and reheating promoted lipid oxidation, thereby enhancing the formation of WOF. Interestingly, although the contents of these compounds increased significantly after refrigeration and reheating, the rate of increase during refrigeration was higher for most compounds, except for (E)-2-octenal and (E)-2-nonenal. This is likely due to the limited oxidation products formed during the relatively short reheating process compared to refrigeration. On the other hand, reheating promoted the volatilization of WOF and increased the number of binding sites for irreversible covalent bonding between aldehydes and proteins (Kuhn, Considine, & Singh, 2008; Wang, Yang, et al., 2023), thus reducing the formation rate of off-flavor compounds. This suggested that the formation of WOF mainly occurs during refrigeration, while reheating accelerates lipid oxidation, leading to the volatilization of WOF and a more pronounced WOF (Pegg et al., 2014). 3-(Methylthio)propanal, the only Maillard reaction product identified as a key aroma compound in freshly made stewed beef, has a roasted potato-like aroma and plays a significant role in contributing to the ideal meat flavor. It is positively correlated with the meaty note ($P < 0.05$) (**Fig. 3-4**A). 3-(Methylthio)propanal is an unstable

aldehyde that decomposes into low-boiling compounds (Drumm & Spanier, 2002). Its content significantly decreased after refrigeration and reheating, which might be a key reason for the weakening of the meaty aroma, as verified by omission experiments (**Table 3-5**). Furthermore, its rate of decrease during reheating was lower than that during refrigeration, further indicating that flavor deterioration in stewed beef primarily occurs during refrigeration. In addition, we noted that the types and quantities of identified compounds remained consistent across freshly made, refrigerated, and reheated samples. In other words, the key aroma-active compounds responsible for the noticeable WOF in reheated PCSB were present both in cooked and refrigerated stewed beef, suggesting that WOF is not the result of the formation of new compounds but rather the accumulation of lipid oxidation products during refrigeration and the cooking process. During meat cooking, the primary reactions responsible for the development of characteristic meaty aroma include lipid oxidation, Maillard reaction between amino acids and sugars, the Strecker reaction, and the degradation of thiamine (Elmore & Mottram, 2006; Khan et al., 2015). These reactions rapidly occur during cooking and contribute to the desired flavors. However, these reactions, particularly lipid oxidation, can also lead to the development of off-flavors, such as WOF, during prolonged storage (Mottram, 1998) (**Fig. 3-3**). In summary, the formation and development of WOF is primarily driven by the accumulation of lipid oxidation products, particularly aldehydes, and the reduction of desirable compounds that contribute to the meaty aroma.

The occurrence of WOF is intrinsically linked to lipids. On one hand, as previously mentioned, secondary oxidation products of lipids are the primary contributors to the formation of WOF in meat products (Pegg et al., 2014). On the other hand, compared to proteins and carbohydrates, lipids are considered the most effective carriers of volatiles (Liu et al., 2022; Wu et al., 2024). The lipophilicity of WOF compounds enables them to dissolve in lipids and be released at appropriate times (Guo et al., 2022), while consumer perceptions of WOF are largely influenced by the release and retention of these compounds within the food matrix (Wang et al., 2023b). However, the lipid fingerprint of PCSB and its changes remain unclear. The key lipid molecules involved in WOF formation, as well as the lipids potentially interacting with WOF compounds, have not yet been identified. This presents a challenge in the targeted correction of WOF in PCSB. Therefore, it is essential to elucidate the lipid fingerprint of PCSB. Lipidomics can provide a comprehensive, systematic analysis, and it has been widely used to investigate meat quality and flavor, including changes in lipid profiles during refrigeration (chilling/frozen) processes (Fang et al., 2022; Lv et al., 2023). When combined with flavoromics, lipidomics can also be applied to study the lipid fingerprints associated with the formation and binding of aroma compounds in meat products (Liu et al., 2022). To gain a thorough understanding of the lipid fingerprint of PCSB and identify the potential precursor lipids involved in WOF formation, as well as the key lipids associated with WOF, in chapter IV, a targeted lipidomics and flavoromic analysis were used to investigate the lipid profiles and WOF variations during different refrigeration stages of PCSB. Interestingly, during refrigeration, most of the fatty

aldehydes, especially short-chain aldehydes (C_3-C_{12}) such as hexanal, heptanal, and nonanal, which are also major contributors to WOF, did not exhibit a continuous increase with prolonged storage but instead followed a trend of first increasing and then decreasing, reaching a peak on D2 of refrigeration (**Fig. 4-3A, B**). This trend is consistent with the changes in TBARS, which also peaked on D2 and then significantly decreased **(Fig. 4-2A**). This phenomenon might be due to interactions between lipid oxidation products (hydroperoxides and aldehydes) and proteins (Al-Dalali et al., 2021), and it was also possible that flavor compounds volatilized into the surrounding environment during refrigeration (Wang et al., 2023a). Analysis of lipid profiles revealed dynamic changes in the lipid composition of reheated stewed beef throughout the storage process. Significant differences between groups may be attributed to lipid degradation, chain cleavage, side-chain modifications, and/or lipolysis during storage. Among all lipid subclasses, TG was the most abundant in both types and quantities (**Fig. 4-4A, D**), which were considered important lipids for retaining WOF, consistent with the conclusion by (Liu et al., 2022), where TG, as the most abundant lipid subclass in roasted lamb, was identified as a key lipid responsible for preserving lamb aroma. The fatty acid composition of lipid molecules is critical to their oxidative properties. Studies have shown that the degree of unsaturation in acyl components of meat lipids is a major factor affecting the rate of flavor deterioration, with lipids containing a higher proportion of UFAs being more prone to oxidation (Dinh et al., 2021; Lv et al., 2023; Shahidi, 2002). In PCSB, PUFAs were primarily found in the side chains of GP and TG, with a large proportion of PUFAs deposited in GP. In terms of unsaturation, PE-O had the highest UFA content, followed by PE-P, LPC, and PC-O (**Fig. 4-7**). Regarding polyunsaturation, PE-O contained the highest level of PUFAs, followed by PC-O, LPC, and PE-P (**Fig. 4-7**). Notably, PE, particularly ePE, is rich in PUFAs. The amines in PE are more reactive than the double bonds in acyl chains, making them more likely to react with free radicals, reactive oxygen species (ROS), and subsequent oxidation products (Fang et al., 2022; Pongsetkul et al., 2017). Ether PLs are known to influence membrane fluidity and fusion, with their ether bonds being especially susceptible to cleavage by reactive oxygen species (Lv et al., 2023). In particular, the content of PE(P-18:0/18:2) was significantly higher than other PE molecules, followed by PE(P-18:0/20:4). PE(P-18:0/18:2) increased on D1, likely due to protein degradation in meat, which may release GL from their binding sites with membrane proteins (Lv et al., 2023). PE(P-18:0/18:2) and PE(P-18:0/20:4) then significantly decreased ($P < 0.05$) (**Fig. 4-11**A, B), with decomposition products of FFA(18:2) and FFA(20:4). Linoleic acid oxidation produces characteristic aldehydes, such as hexanal. Alkanals, 2-enals, and alkanols can originate from the auto-oxidation of linoleic acid(C18:2 n-6) and oleic acid(C18:1 n-9) (Elmore et al., 1999). (E,E)-2,4-decadienal can be derived from n-6 PUFA (Liu et al., 2020; Shahidi & Abad, 2019). Additionally, 1-octen-3-ol can be produced by the oxidation of linoleic acid and arachidonic acid. These WOF compounds rapidly develop within 48 hours in stored meat products (Pegg et al., 2014). Therefore, ePE, including PE(P-18:0/18:2) and PE(P-18:0/20:4), might be key precursors involved in the formation of WOF in PCSB. Furthermore, as mentioned above, LPC and PC also

exhibited high unsaturation and significantly decreased in the later stages of storage (D6) (**Fig. 4-4D**). This suggested that the reduction of LPC and PC might significantly promote the late-stage development of WOF. In addition, the unsaturation of TG exceeds 95% (**Fig. 4-7**) and contains higher amounts of C(16:0), C(18:0), and C(18:1). Lv et al. (2023) reported that TG molecules containing fatty acids like C(16:0) and C(18:1) in yellow chickens are prone to decomposition during refrigeration. TG levels significantly increased on D1, likely due to further disruption of cellular membranes during reheating, leading to TG release (Liu et al., 2023). Studies have shown that GP can be oxidized to form TG (Guo et al., 2022). The elevated TG levels on D4 might be due to its slower oxidation rate compared to its formation rate. During this stage, the levels of PE, PE-P, and PE-O continued to decline, but the aroma compounds decreased, likely due to the irreversible binding with proteins and volatilization (Anantharamkrishnan et al., 2020). TG significantly decreased on D6, suggesting that its oxidation rate exceeded its formation rate, leading to a significant increase in alcohols. These results underscore the crucial role of TG in the formation and development of WOF in reheated PCSB. During cooking, PL and TG (including the aforementioned lipid molecules) undergo thermal degradation, generating FFAs. FFAs undergo free radical chain reactions, producing hydroperoxides. Hydroperoxides have weak and thermally sensitive O-O bonds that readily decompose into various aroma compounds, such as aldehydes (alkanal), ketones (alkanone), carboxylic acids (alkanoic acid), alcohols (alkanol), lactones, and alkyl furans. These compounds contribute to the characteristic aroma of cooked meat (Mottram, 1998). During refrigeration, lipids continue to hydrolyze, generating free fatty acids, which are oxidized through free radical chain reactions, forming short-chain aldehydes, ketones, and alcohols with low odor thresholds that accumulate over time, leading to off-flavors (Dinh et al., 2021). UFAs on the meat surface oxidize more quickly, forming oxygenated dimers and polymers through UFA polymerization. Long-chain PUFAs are more prone to intramolecular cyclization. The extensive dimerization of UFAs and cyclization of PUFAs leads to the generation of non-volatile products, limiting the participation of PUFAs in the production of aroma compounds contributing to off-flavors (Dinh et al., 2021; Mottram, 1998). Additionally, the high temperatures and appropriate oxygen levels during cooking promote the further oxidation of short-chain aldehydes, ketones, and alcohols into organic acids and esters. Lipid peroxides decompose into more oxygenated heterocyclic compounds, including cyclic carboxylic acids and their lactones (cyclic esters). SFAs degrade into long-chain alkanes, aldehydes, and lactones. These result in the reduction of short-chain unsaturated aldehydes and alcohols, making the aroma more desirable and decreasing volatility, which explains why cooked meat has more favorable aroma characteristics compared to auto-oxidation (Dinh et al., 2021; Song et al., 2011). However, lipid thermal oxidation products, such as dimers and cyclic compounds, can further decompose during storage through auto-oxidation, producing unpleasant aroma compounds that contribute to WOF. The accumulation of these off-flavor compounds during refrigeration and their volatilization upon reheating exacerbate the development of WOF.

Given the pivotal role of lipid oxidation in the formation of WOF, effectively inhibiting lipid oxidation has become a critical issue for improving the flavor of PCSB. In recent years, the use of natural antioxidants has gained increasing attention. These antioxidants not only suppress lipid oxidation but also improve overall flavor characteristics by imparting specific aromas (Dang et al., 2024). Plant-derived natural antioxidants, with their health benefits and sensory-enhancing properties, are more aligned with consumer demands. Natural antioxidants have been utilized to reduce WOF in meat products. Jokanović et al. (2020) found that the addition of winter savory (*Satureja montana* L.) significantly reduced TBARS values in precooked pork chops, slowing lipid oxidation, while the aroma compounds in the essential oils of winter savory masked the undesirable off-flavors associated with WOF. Perilla and ginger juice have been shown to prevent the formation of WOF compounds in surimi gels, masking them with their unique aromas (Dang et al., 2024). Tomatoes, one of the most widely consumed vegetables globally, are rich in natural antioxidants, particularly lycopene, VC, and VE, which are known for their significant antioxidant properties (Skiepko et al., 2016). Elbadrawy and Sello (2016) observed significant reductions in peroxide values, malondialdehyde, and carbonyl content in oil samples treated with tomato peel extract. Stewed beef with tomato is a classic Chinese dish that combines the savory flavor of beef with the tangy sweetness of tomatoes, offering a rich texture and balanced nutrition. However, due to the insufficient research on the changes in lipids and flavor characteristics of stewed beef after the addition of tomatoes, there is a lack of standardized industrial recipes. This has resulted in challenges in finding an optimal balance between enhancing lipid stability to suppress WOF and preventing the overwhelming dominance of tomato aroma in the sensory experience. In Chapter 5, utilized were lipidomics and flavoromics to study the changes in lipids and WOF variations in PCSB with varying amounts of added tomato, determining the optimal amount of tomato and comprehensively analyzing the pathways through which tomatoes regulate WOF in PCSB. The significant reduction in TBARS values of the PCSB samples with 25%-100% tomato addition (**Fig. 5-3A**) demonstrated the inhibitory effects of natural antioxidant components in tomatoes (such as lycopene, polyphenols, and VC) on lipid oxidation. From the lipid analysis, it was observed that the total lipid, PL, and GL contents significantly increased after the addition of tomatoes (**Fig. 5-3C**), while the contents of PE, PC, PE-P, PE-O, and LPE, as well as FFA, did not show significant changes. This suggests that the antioxidant effect of tomatoes plays a protective role in lipids, inhibiting the degradation of TG and PL like PE, PE-P, and PC into FFA. As observed in Chapter 4, e-PE, especially PE-P, played a key role in the formation of WOF in PCSB, while PC also played an important role in WOF development. TG had a certain promoting effect on the formation and development of WOF in PCSB. Therefore, the observed reduction in WOF after the addition of tomatoes to PCSB was further confirmed by our analysis of the aroma profiles and sensory results. The content of short-chain fatty aldehydes that contribute to WOF, such as hexanal and (E)-2-octenal, significantly decreased, with the most notable changes in the T100 group (**Table 5-1**). Both the electronic nose and PCA analysis of aroma compounds clearly showed a gradual divergence of

T25-T100 from CK, and sensory evaluation indicated varying degrees of alleviation of WOF in these groups. There were no significant differences in TBARS values between the T25-T100 groups, suggesting that tomato antioxidants effectively inhibit lipid oxidation even at lower concentrations. The antioxidant effect reached saturation at T25, as indicated by the absence of further significant improvements in antioxidant capacity with higher tomato addition. This result was also reflected in the total lipid content, which showed no significant difference. However, the lipid-derived flavor compounds exhibited changes; as the tomato content increased, the levels of aldehyde compounds associated with lipid oxidation significantly decreased. Both hexanal, 1-octen-3-ol, and aldehydes such as octanal and nonanal exhibited distinct variation trends (**Table 5-1**), possibly due to the interactions between aroma compounds and the food matrix. The reduction of these compounds might, on one hand, be attributed to their covalent interaction with protein side chains, including aldehyde-lysine and amine-carbonyl interactions, which are typically irreversible (Zhang et al., 2021). On the other hand, it might be related to non-covalent interactions, primarily hydrophobic interactions, as most protein-flavor interactions are due to reversible non-covalent bonding (Zhang et al., 2021). Lycopene may bind to proteins via hydrophobic interactions, altering the protein's secondary structure (Zhao et al., 2023), which could affect the hydrophobic regions of the protein responsible for WOF release. Furthermore, tomato-originated aroma compounds, such as 2-isobutylthiazole and fennel aldehyde, might compete for hydrophobic binding sites on proteins, promoting the release of WOF-contributing compounds and enhancing the sensory contribution of tomato-like aroma (Sun et al., 2023). This was also perceptible in the sensory evaluations, where WOF in the T25-T100 groups progressively weakened (**Table 5-1**). Additionally, the reduction in WOF sensory contribution might be due to the masking effect of tomato-originated aroma compounds. While endowing a distinct tomato-like aroma to the stewed beef, tomato-like aroma also masked the perception of certain WOF compounds through competitive interactions. 2-isobutylthiazole, the only aroma compound from tomatoes in stewed beef with an OAV > 1, showed a remarkable ERV of 602.64% in T25, which increased to 2860.10% in T100. Qiang et al. (2025) found that the aroma compound with the highest ERV in stewed beef, 3-carene, only had an ERV of 37.96%. The significant endowment of tomato-like aroma in PCSB, particularly from 2-isobutylthiazole, contributed to the overall tomato leaf (green) aroma, which masked some of the WOF from lipid oxidation products. This was reflected in sensory evaluations, where the tomato aroma gradually intensified and WOF progressively diminished (**Fig. 5-1**). In T75, WOF was almost imperceptible, and the optimal balance between meat flavor, tomato aroma, and overall acceptability was achieved. In contrast, the T100 group, dominated by tomato aroma, masked some of the meat flavor, resulting in a flavor profile dominated by tomato, with diminished meat characteristics (**Fig. 5-1**). In conclusion, the regulation of WOF in PCSB by tomatoes most likely occurs through three main pathways (**Fig. 6-1**): (1) by inhibiting lipid oxidation, reducing the formation of secondary oxidation products, i.e., aroma compounds that contribute to WOF; (2) through interactions with food ingredients, mainly by modifying the secondary structure of proteins via

the effective components in tomatoes (aroma compounds, lycopene, polyphenols, etc.), and promoting the release of WOF via competition for hydrophobic binding sites; (3) by masking the perception of WOF through the flavor endowment provided by tomatoes in the PCSB. This ingredient-based regulatory mechanism not only eliminates the need for external additives but also aligns with contemporary consumer preferences for clean-label products, offering an innovative strategy for optimizing the quality of convenience foods.

The regulation of WOF in PCSB, particularly through the incorporation of natural plants like tomatoes, represents a significant advancement in the enhancement of meat-based convenience foods. Lipid oxidation and its byproducts are central to the formation of WOF. Whereas, it is evident that the interactions among lipids, proteins, and antioxidants offer a multifaceted approach to stabilizing flavor. The results from lipidomics and sensomics analyses emphasize the necessity of understanding not only the oxidative mechanisms but also the complex interactions through which antioxidants, like lycopene, polyphenols, and vitamins in tomatoes, interact with the meat matrix to inhibit the formation of undesirable aroma compounds.

This research reveals that tomatoes contribute to flavor enhancement not only by reducing lipid oxidation but also by influencing the flavor profile through intricate molecular interactions. These interactions between tomato-originated compounds and the food matrix are significant as they show how natural ingredients can adjust the flavor profile without overwhelming the inherent characteristics of the meat. The ability of tomatoes to reduce the perception of WOF, particularly through distinctive aroma compounds, represents an innovative approach to flavor modulation. By competing for hydrophobic binding sites on proteins, these aroma compounds effectively reduce the sensory impact of off-flavors, enhancing the overall sensory experience of the product. This work further broadens the application of natural antioxidants in the formulation of convenience foods. With growing consumer demand for cleaner labels and natural, health-conscious products, the reliance on synthetic preservatives and additives is facing increasing scrutiny. This research not only meets these demands but also provides a scientifically supported framework for incorporating natural ingredients to improve food quality while preserving flavor and nutritional value. By focusing on lipidomics and sensomics profiles, this study addresses a key challenge in food science: how to optimize flavor preservation in products subjected to long-term storage and reheating. Moreover, the insights from this study can be applied beyond PCSB to other meat products susceptible to lipid oxidation and off-flavor development, such as precooked meats, sausages, and ready-to-eat meals. The use of natural antioxidants like tomatoes could be extended across a variety of products, potentially providing new opportunities for creating healthier and more sustainable convenience foods. The lipidomics strategy employed in this research provides the food industry with a precise tool for controlling lipid oxidation and enhancing product stability, thereby improving both food quality and consumer satisfaction. The findings also highlight the need for further exploration into the long-term effects of incorporating antioxidants into various meat products. While the short-term results are promising, further research on how these natural compounds perform over extended storage periods and under

various conditions will be essential for fully understanding their potential. Additionally, examining the synergistic effects of different antioxidants and their interactions with other preservation techniques, such as modified atmosphere packaging or refrigeration, could enhance the efficacy of this approach. In conclusion, this study demonstrates how modern food science can harness natural ingredients to address long-standing issues like WOF. By integrating lipidomics, sensomics, and sensory analysis, the research provides deeper insights into the factors contributing to WOF in PCSB and uncovers natural solutions for enhancing meat product flavor and quality. The use of tomatoes as a natural antioxidant and flavor modulator offers a promising alternative to synthetic additives, providing a cleaner, healthier approach to enhancing food quality while meeting contemporary consumer preferences. This research sets the stage for future advancements in developing high-quality, shelf-stable convenience foods, allowing consumers to enjoy traditional dishes without undesirable off-flavors. Further exploration of natural ingredients, combined with advanced scientific techniques, will lead to the development of more sustainable and flavorful food products that meet the evolving demands of both the food industry and consumers.

Furthermore, the findings of this study also offer valuable insights for mitigating WOF in PCSB through targeted modifications in processing techniques and formulations. One effective approach could be selecting raw materials with more stable lipid compositions, such as leaner cuts of meat. Additionally, modifying the lipid composition through dietary changes in livestock could enhance the stability of lipids in the final product. Moreover, employing cooking techniques like sous-vide (Ortuño, Mateo, Rodríguez-Estrada, & Bañón, 2021) or low-temperature slow cooking may prevent excessive lipid oxidation during storage and reheating. By applying these strategies, based on lipidomics and sensomics insights, it's possible to enhance the flavor stability and overall quality of PCSB, benefiting both product appeal and consumer satisfaction.

6.2. Conclusions

To summarize, the PCSB presented a strong WOF after refrigeration-reheating, manifesting as weaker intensities of meaty note, and increased fatty and oxidized vegetable oil, the grassy, hard-boiled egg, metallic, and cardboard-like aroma. The key aroma-active compounds contributing to the WOF of reheated PCSB were characterized using a sensomics approach. Overall, 36 odorants were identified, and based on flavor dilution factors, odor activity values, aroma recombination, and omission test. Hexanal, (E,E)-2,4-decadienal, (E,E)-2,4-nonadienal, pentanal, decanal, octanal, heptanal, (E)-2-octenal, (E)-2-undecenal, 1-octen-3-ol, and (E)-2-nonenal, mainly derived from lipid oxidation, were characterized as the key odorants contributing to the WOF formation. Additionally, the reduction of 3-(methylthio)propanal, which was correlated with meaty aroma, might greatly contribute to an overall increase in WOF. Thus, these odorants were elected as potential markers of WOF in the reheated PCSB. In summary, the WOF of the

PCSB could be attributed to an overall increase in lipid oxidation products and a decrease in the odorants with desirable aromas.

It is well-established that the formation of WOF is mainly attributed to lipid oxidation. Thus, the alterations of lipid profiles in PCSB was investigated using UPLC-ESI-MS/MS-based lipidomics. 1236 lipids were identified across 42 subclasses, with TG being the most abundant in both variety and content. TG, including TG(18:0/18:1/18:1) and TG(16:0/18:1/18:1), were primarily responsible for binding aroma compounds. Among 153 differential lipids (VIP > 1, $P < 0.05$), PS(18:0/20:4) and PS(16:0/17:2) were determined as potential markers for distinguishing all stewed beef samples. A total of 142 differential lipids were significantly correlated with the predominant odorants of PCSB, which might contribute to WOF formation, with ePE, particularly PE(P-18:0/18:2), likely being crucial lipids. Additionally, LPC and PC, especially LPC(20:3) and PC(16:0_18:1), might significantly influence WOF development. Simultaneously, TG contributed to both the formation and development of WOF. The findings provide information for the further WOF correction technologies.

Furthermore, to mitigate WOF in PCSB while meeting consumer demand for clean-label products, the role of tomatoes, an ingredient abundant in natural antioxidants, in reducing WOF in PCSB was explored from the perspective of ingredient interactions. Analyses of aroma profiles, lipid composition. Results showed that the addition of tomatoes significantly suppressed lipid oxidation, thereby preserving lipid substrates and reducing oxidative losses in PL (e.g., PE, PE-P, and PC) and TGs. This, in turn, decreased the formation of aroma compounds contributing to WOF, such as pentanal, hexanal, and 1-octen-3-ol. Moreover, tomato additions altered the secondary structure of beef protein, modifying their binding affinity for aroma compounds, thus enhancing the protein's selective binding capacity. Components from the tomato, such as lycopene, polyphenols, and aroma compounds (e.g., 2-isobutylthiazole), might form hydrophobic interactions with the protein, competing for binding sites with WOF. This likely promoted the release of WOF and enhanced the sensory contribution of the tomato-like aroma. Notably, the characteristic tomato-originated aroma compound 2-isobutylthiazole played a crucial role in flavor endowment, endowing a green tomato-like aroma to the PCSB. This created a sensory masking effect that reduced the perception of WOF. The study elucidated the mechanisms through which tomatoes influenced WOF, including the inhibition of lipid oxidation, the release of WOF, and its masking effect on flavor. Additionally, sensory evaluation revealed that the 75% tomato group achieved the highest overall acceptability, balancing meaty and tomato-like aromas while significantly reducing the WOF. These findings suggested that an appropriate level of tomato addition not only reduced WOF, but also enhanced the characteristic tomato-like aroma, thus improving the sensory acceptance and overall flavor of PCSB. However, the tomato-like aroma in PCSB with excessive tomato addition (e.g., 100% addition) may overpower the meaty aroma, reducing its perception. The outcomes provide both theoretical and practical support for the application of tomatoes as a natural flavoring agent in meat products.

6.3. Perspectives

This study systematically elucidated the critical role of lipid oxidation in the development of WOF in PCSB dishes and preliminarily explored the effects of tomato addition on protein conformational dynamics, highlighting how these protein structural alterations influence WOF formation and regulation. Nevertheless, several scientific questions remain unanswered, presenting significant opportunities for further investigation.

(1) Despite extensive research on interactions between aroma compounds and proteins (Chen et al., 2025; Sun et al., 2023), the molecular mechanisms underlying the binding (both covalent and non-covalent) of WOF-related compounds generated from lipid oxidation in PCSB require deeper exploration. Future studies should employ computational modeling approaches, such as molecular docking and molecular dynamics simulations, alongside advanced mass spectrometry techniques, to systematically investigate the binding sites, interaction patterns, and affinity strengths of key WOF markers (e.g., hexanal, (E,E)-2,4-decadienal, pentanal, octanal, heptanal, (E)-2-octenal, 1-octen-3-ol, and 3-(methylthio)propanal). Furthermore, clarifying how protein structural changes, particularly in secondary structures and aggregation states, influence aroma retention and release during heating, storage, and reheating processes is critical for managing WOF.

(2) Given the growing consumer preference for clean-label products, there remains considerable potential in developing natural, plant-derived antioxidants. These natural antioxidants, abundant in bioactive components such as polyphenols and carotenoids, effectively inhibit lipid oxidation and reduce WOF generation. However, some natural antioxidants may exhibit pro-oxidative activity (Palozza et al., 2003), complicating their effects on lipids and proteins. Thus, future studies should further clarify the dual oxidative and antioxidative mechanisms of these active compounds in food matrices.

(3) Previous research has predominantly focused on polyphenol-protein interactions (Huang et al., 2022a; Huang et al., 2022b; Khan et al., 2025; Zhou et al., 2025), whereas studies on other bioactive compounds like carotenoids remain limited. These natural antioxidants may induce changes in protein structures, subsequently influencing the retention and release of WOF compounds. Consequently, systematic research on the effects of diverse antioxidants and their bioactive compounds on protein structural dynamics and subsequent impacts on WOF retention and release will provide valuable insights for the selection of appropriate natural antioxidants. Furthermore, natural plant additives typically possess distinctive aromas that can mask undesirable flavors or enhance sensory attributes in food products. This flavor endowment effect fundamentally arises from interactions between these aroma compounds and meat proteins (Qiang et al., 2025), which might alter protein conformation and influencing their binding affinity for aroma compounds, thus affecting flavor retention and release in PCSB dishes (Reineccius, 2022; Shen et al., 2019). Moreover, competitive binding interactions may occur between aroma compounds originated from natural plant additives and

WOF compounds for protein binding sites, especially following changes in protein affinity. Thus, future research should prioritize detailed investigations into the complex interactions among endowed odorants originated from natural plant additives, proteins, and WOF compounds, developing a comprehensive mechanistic model that elucidates the "endowed odorants-protein-WOF" interactions.

(4) Additionally, this study identified ePE as potentially critical in WOF formation. Ether phospholipids are known to affect membrane fluidity and fusion, with their ether bonds being particularly vulnerable to cleavage by reactive oxygen species (Lv et al., 2023). Nevertheless, few studies have linked ePE to WOF. It is still unclear whether the oxidation products of amino groups, which differ from those of acyl groups, affect acyl group oxidation and contribute to WOF formation. Further dedicated investigations into ePE and its oxidative products will facilitate the development of targeted strategies for precise modulation of WOF.

In conclusion, future studies should comprehensively elucidate the mechanistic roles of natural antioxidants, lipids, and proteins in WOF formation and release, establishing an integrated framework from molecular understanding to practical industrial applications. Such efforts will significantly enhance flavor quality and market competitiveness of prepared dishes.

6.4. References

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Appendix-Publications

1. Articles

Liu, J., Deng, S., Wang, J., Huang, F., Han, D., Xu, Y., . . . Blecker, C. (2024). Comparison and elucidation of the changes in the key odorants of precooked stewed beef during cooking-refrigeration-reheating. Food Chemistry: X, 23, Article 101654. (Chapter III)

Liu, J., Huang, F., Han, D., Xu, Y., Shen, S., Luan, Y., . . . Blecker, C. (2025). Elucidation of potential lipid precursors and formation pathways for the warmed-over flavor (WOF) in precooked Chinese stewed beef through lipid oxidation mechanisms. Food Chemistry, 475, Article 143294. (Chapter IV)

Liu, J., (2025). Mechanisms underlying tomato-mediated suppression of warmed-over flavor (WOF) and flavor enrichment in precooked stewed beef (revision) (Chapter V)

Liu, J., Yuan, S., Han, D., Liu, J., Zhao, L., & Wu, J. (2023). Effects of CO_2-assisted high-pressure processing on microbiological and physicochemical properties of Chinese spiced beef. Innovative Food Science & Emerging Technologies, 84, Article 103261.

Deng, S., **Liu, J.**, Han, D., Yang, X., Liu, H., Zhang, C., & Blecker, C. (2024). Synchronous fluorescence detection of nitrite in meat products based on dual-emitting dye@MOF and its portable hydrogel test kit. Journal of Hazardous Materials, 463, 132898.

Wang, J., Yang, P., **Liu, J.**, Yang, W., Qiang, Y., Jia, W., Han, D., Zhang, C., Purcaro, G., & Fauconnier, M. L. (2023). Study of the flavor dissipation mechanism of soy-sauce-marinated beef using flavor matrices. Food Chemistry, 437(Pt 1), 137890.

Liu, J., (2025). The role of Chinese star anise in modulating warmed-over flavor (WOF) in precooked Chinese stewed beef: Insights from flavor interaction. Food Research International (under review)

2. Patents

Zhang, C., **Liu, J.**, Han, D., Yang, P., Li, X. Analytical method for biomarker of warmed–over of reheated precooked stewed beef. CN202310841617.5 (in Chinese)

3. Flash presentations and posters

FFPSI ·2024 International Forum on Food Flavor Perception Science and Innovative Technology (CHINA): **Poster Presentation** of own project, **1st author** entitled "Comparison and elucidation of the changes in the key odorants of precooked stewed beef during cooking-refrigeration-reheating"

The 4th International Flavor and Fragrance Conference (New Zealand): **Poster Presentation** of own project, **1st author** entitled "Comparison and elucidation of the

changes in the key odorants of precooked stewed beef during cooking-refrigeration-reheating"

2023 Asia-Pacific Congress of Meat Science and Technology (CHINA): **Poster Presentation** of own project, **1st author** entitled "Comparison and elucidation of the changes in the key odorants of precooked stewed beef during cooking-refrigeration-reheating"