



UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E
TECNOLOGIA DE ALIMENTOS

VIVIANE MARIA DE SOUSA FONTES

**AROMATIZANTE CÁRNEO OBTIDO DE HIDROLISADO
PROTEICO DE VÍSCERAS CAPRINAS**

JOÃO PESSOA - PB

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Dissertação apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal da Paraíba como requisito para obtenção do título de Mestre em Ciência e Tecnologia de Alimentos.

Orientadora: Prof^a. Dra. Marta Suely Madruga

JOÃO PESSOA – PB

2023

Catálogo na publicação
Seção de Catalogação e Classificação

F683a Fontes, Viviane Maria de Sousa.

Aromatizante cárneo obtido de hidrolisado proteico de vísceras caprinas / Viviane Maria de Sousa Fontes. - João Pessoa, 2023.
39 f. : il.

Orientação: Marta Suely Madruga.
Dissertação (Mestrado) - UFPB/CT.

1. Aroma cárneo. 2. Compostos voláteis. 3. Aromatizante. 4. Alimentos. 5. PH. 6. Via de formação. 7. Reação de Maillard. I. Madruga, Marta Suely. II. Título.

UFPB/BC

CDU 641:543.2(043)



ATA DE DEFESA DE DISSERTAÇÃO

MESTRADO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Ao primeiro dia do mês de setembro do ano de dois mil e vinte e três, na Sala de Estudos do PPGCTA e por videoconferência (<https://meet.google.com/eeh-zrqw-inw>), às 14h00, reuniu-se a Banca Examinadora composta pela Profa. Dra. Marta Suely Madruga, orientadora do trabalho e presidente da Banca, Profa. Dra. Taliana Kênia Alencar Bezerra (Membro Interno/UFPB) e a Profa. Dra. Maria Teresa Bertoldo Pacheco (Membro Externo/ITAL). A reunião teve por objetivo julgar o trabalho da estudante **Viviane Maria de Sousa Fontes**, matrícula nº 20221006184, sob o Título “**Aromatizante cárneo obtido de hidrolisado proteico de vísceras caprinas**”. Os trabalhos foram abertos pela Profa. Dra. Marta Suely Madruga. A seguir foi dada a palavra a estudante para apresentação do trabalho. Cada Examinadora arguiu a mestranda, com tempos iguais de arguição e resposta. Terminadas as arguições, procedeu-se ao julgamento do trabalho, concluindo a Banca Examinadora por sua **APROVAÇÃO**. Atendidas as exigências da Resolução nº 15/2019/CONSEPE que regulamentam o Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, e nada mais havendo a tratar, foi lavrada a presente Ata, que vai assinada pelos membros da Banca Examinadora e pela mestranda.

João Pessoa, 01 de setembro de 2023.

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RESUMO

O aroma é uma das características mais impactantes em alimentos que direciona a aceitação de um produto pelo consumidor. Neste estudo, um hidrolisado proteico de vísceras caprinas adicionado de xilose, cisteína e tiamina foi utilizado na produção de aromatizantes, em condições de pH 4,0 e 6,0. A matéria-prima (mistura de vísceras caprinas - VC), hidrolisado (H) e aromatizantes (F4, F6) foram avaliados físico-quimicamente e quanto ao perfil volátil. VC tinha um conteúdo de proteína de 19,61%, sob a qual foi obtido um hidrolisado com grau de hidrólise de 26,49%. O hidrolisado forneceu lipídios e aminoácidos, precursores dos compostos voláteis formados nos aromatizantes via degradação lipídica, reação de Maillard, degradação de Strecker e de interações entre essas vias. Nas amostras VC, H, F4 e F6 foram identificados um total de 117 compostos voláteis, distribuídos em 15 classes químicas. Os hidrocarbonetos foram os voláteis predominantes em VC. Características do aroma cárneo foram identificadas no hidrolisado, providas por pirazinas e aldeído de Strecker. Nos aromatizantes F4 e F6 foram identificados 96 compostos voláteis, sendo 20 identificados em F4, 15 em F6 e 61 em ambos os aromatizantes, com maioria dos voláteis formados via degradação lipídica. Produtos da reação de Maillard com forte aroma cárneo e torrado, como 2-methyl-3-furanthiol, 2-furfurylthiol e bis(2-methyl-3-furyl) disulfide foram identificados apenas no aromatizante elaborado em pH 4; enquanto o obtido em pH 6 foi superior em concentração em quase todos os demais compostos, o que indica que o aromatizante obtido neste pH pode ter uma menor característica cárnea, mas intensas nuances de doce, gorduroso e carne caprina. Notavelmente, este estudo trás esclarecimentos sobre a rota que pode ser utilizada para obtenção de um aromatizante cárneo.

Palavras-chave: aroma cárneo; compostos voláteis; aromatizante; ingrediente; pH; via de formação; reação de Maillard.

ABSTRACT

Aroma is one of the most impactful characteristics of foods, guiding consumer preference for a product. In this study, a protein hydrolysate from goat viscera, combined with xylose, cysteine, and thiamine, was used to produce flavorings under pH conditions of 4.0 and 6.0. The raw material (a mixture of goat viscera - VC), hydrolysate (H), and flavorings (F4, F6) were evaluated for their physicochemical properties and volatile profiles. The VC had a protein content of 19.61%, which yielded a hydrolysate with a degree of hydrolysis of 26.49%. The hydrolysate contained lipids and amino acids, which are precursors to volatile compounds formed in flavorings through lipid manipulation, Maillard reactions, Strecker degradation, and interactions between these pathways. In the VC, H, F4, and F6 samples, a total of 117 volatile compounds were identified, distributed across 15 chemical classes. Hydrocarbons were the predominant volatiles in VC. The hydrolysate featured meat aroma characteristics, provided by pyrazines and Strecker aldehydes. In flavorings F4 and F6, 96 volatile compounds were identified: 20 unique to F4, 15 unique to F6, and 61 present in both, with most volatiles formed via lipid degradation. Compounds indicative of Maillard reactions, which contribute a strong meaty and roasted aroma, such as 2-methyl-3-furanthiol, 2-furfurylthiol, and bis(2-methyl-3-furyl) disulfide, were identified only in the flavoring made at pH 4. Meanwhile, the flavoring produced at pH 6 had higher concentrations of almost all other compounds, suggesting it may have less meaty characteristics but more intense sweet, fatty, and goat meat nuances. Notably, this study clarifies the pathway for obtaining meat flavoring from goat viscera.

Keywords: meat flavor; volatile compounds; flavoring; ingredient; pH; formation pathway; Maillard reaction.

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ARTIGO - “Thiamine, cysteine, and xylose added to the Maillard reaction of goat protein hydrolysate potentiates the formation of meat flavoring compounds”

Fig. 1. Analysis of the main components of the volatile compounds that impact meat aroma, sugars, and amino acids in the goat protein hydrolysate and flavorings. F6, flavoring at pH 6; F4, flavoring at pH 4; H, goat protein hydrolysate.

Fig. 2. Heat map generated based on volatile compounds of flavorings and model systems. F6, flavoring at pH 6; F4, flavoring at pH 4; H, goat protein hydrolysate; M4, model system at pH 4; M6, model system at pH 6.

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1 INTRODUÇÃO

A utilização eficiente dos subprodutos gerados no processo de abate de animais continua a ser um desafio ao setor produtivo que ainda não se encontra consolidado. Este tema é de suma importância para a indústria cárnea, tendo em vista que a sua resolução pode contribuir significativamente para melhorar a eficiência do setor produtivo. Através do processamento e aproveitamento adequado desses subprodutos é possível agregar valor, aumentar a estabilidade em relação à vida útil dos resíduos e, ainda, contribuir com a sustentabilidade econômica e ambiental da indústria, integrando o volume produzido na cadeia circular de produção. Estudos mostraram a potencialidade de aproveitamento dos subprodutos animais para diversos fins, a exemplo, na produção de novas moléculas ou ingredientes alimentícios com capacidade de melhorar as características sensoriais e as propriedades tecnológicas e bioativas de produtos já existentes (QUEIROZ et al., 2017b; ZOU et al., 2021; CORDEIRO et al., 2022a).

Por constituírem excelente fonte de proteínas, lipídeos, ácidos graxos, minerais e vitaminas, as vísceras caprinas foram pesquisadas por Queiroz et al. (2017b) e Cordeiro et al. (2022a) e utilizadas para obtenção de biomoléculas em hidrolisados proteicos utilizando via enzimática. Nestes estudos observou-se que os hidrolisados apresentaram alto conteúdo proteico (18%). Foi reportado a predominância dos aminoácidos glicina, lisina, ácido glutâmico e ácido aspártico, os quais são precursores dos compostos aromáticos impactantes do aroma cárneo, gerados via reação de Maillard e degradação térmica lipídica, demonstrando assim o potencial dos hidrolisados de vísceras caprinas para aplicação como agentes aromatizantes ou intensificadores de sabor (QUEIROZ et al., 2017b; CORDEIRO et al., 2022a).

O sabor cárneo pode ser conferido ou intensificado nos alimentos através do uso de aromatizantes que são largamente comercializados e tem em sua composição majoritariamente ingredientes sintéticos. A conscientização da população transforma a indústria de alimentos que utiliza demasiadamente tal recurso para ter no alimento o sabor característico desejado e exige através de novas regulamentações a redução e/ou informação desses ingredientes em prol da saúde da população (BRASIL, 2020; FOOD (PROMOTION AND PLACEMENT) (ENGLAND) REGULATIONS, 2021; PRECEDENCE RESEARCH, 2022).

Em função da temperatura e do pH, durante a hidrólise as proteases liberam peptídeos de baixo peso molecular e aminoácidos livres, que junto aos compostos voláteis formados no meio, serão responsáveis pelo desenvolvimento de diferentes perfis aromáticos de acordo com

o substrato e enzimas utilizados e condições a que são submetidos. Cordeiro et al. (2020) reportaram que a aplicação de tratamento térmico aos hidrolisados caprinos favorece a formação de compostos aromáticos como 2,5-dimethylpyrazine e 2-acetylthiazole, os quais estão presentes no aroma de torrado e carne assada, sendo estes formados principalmente pela reação de Maillard.

Estudos tem evidenciado que o aroma cárneo pode ser modulado pela indução da reação de Maillard utilizando os precursores de hidrolisados proteicos de subprodutos e suplementos como cisteína (aminoácido sulfurado), xilose e tiamina. A cisteína, aminoácido sulfurado, destaca-se por seu envolvimento na formação de compostos voláteis com baixo limiar de detecção e aroma característico cárneo, torrado e de café (CARDINAL et al., 2020; GAO et al., 2020; LI; LIU, 2021). A xilose, pentose com maior reatividade quando comparada a hexose, trata-se de um excelente precursor do aroma cárneo (CHIANG et al., 2022). Enquanto a tiamina (vitamina B1), é uma vitamina hidrossolúvel naturalmente presentes em carnes, e que, mesmo em baixas concentrações, é associada à formação de compostos de característico aroma cárneo, como 2-furfurylthiol e 2-methyl-3-furanthiol (CERNY e BRIFFOD, 2007).

Dada a crescente demanda por ingredientes menos sintéticos, produtos e alimentos mais saudáveis e a demanda ambiental de melhor aproveitamento de subprodutos do abate, justifica-se esta pesquisa, considerando-se que a utilização de hidrolisados proteicos de vísceras caprinas na produção de aromatizantes resultará em inovação no setor produtivo, associado a ganho para a indústria de alimentos.

2 REFERENCIAL TEÓRICO

2.1 AROMATIZANTES

As características sensoriais dos alimentos dizem respeito aos atributos que podem ser percebidos pelo ser humano através dos receptores nasal e oral, discriminadamente, a aparência, sabor, aroma e textura (SILVA et al., 2012). Durante o processamento, os alimentos estão sujeitos a alterações físico-químicas que podem levar a redução de tais características (COTRIM et al., 2021) e os aditivos alimentares aromatizantes têm sido utilizados ao longo de décadas como uma ferramenta eficiente para garantir que as características sensoriais esperadas e encontradas pelo consumidor sejam aquelas idealizadas pelo produtor. Tal aditivo, é definido como a substância ou mistura de substâncias capaz de melhorar, configurar ou intensificar o sabor e aroma nos alimentos (BRASIL, 2022). Os aromatizantes podem ser formados por substâncias que conferem sabor (gosto e aroma) ou apenas por uma substância com compostos voláteis que conferem o aroma, e sua aplicação dependerá da intenção requerida para o produto.

De acordo com a resolução da diretoria colegiada (RDC) nº 725, de 1º de julho de 2022, os aromatizantes alimentares podem ser classificados quanto a forma de obtenção como: naturais, artificiais, sintéticos, de reação, de reação ou transformação, extratos, óleos essenciais e substâncias aromatizantes, previamente listados e assegurados por organizações internacionais como a Food and Drug Administration (FDA), Joint FAO/WHO Expert Committee on Food Additives (JECFA), Flavor Extract Manufacturers Association (FEMA) e União Europeia (BRASIL, 2022).

O aromatizante é natural quando obtido de processos físicos, enzimáticos ou microbiológicos a partir de matérias-primas de origem vegetal ou animal. O artificial trata-se da substância química obtida por síntese que ainda foi identificada em produtos de origem animal, vegetal ou microbiana, em seu estado bruto ou transformado para o consumo humano. Denomina-se aromatizante sintético o composto químico obtido por processo químico, podendo ser idêntico ao natural ou artificial. Quando obtido através de tratamento térmico similar a cocção de alimentos, a partir do processamento conjunto de ingredientes ou alimentos com nitrogênio amínico e açúcar redutor, denomina-se aromatizante de reação; sendo de reação natural quando obtido exclusivamente de matérias-primas ou ingredientes naturais, e sintético, quando pelo menos uma matéria-prima ou um ingrediente é sintético (BRASIL, 2022).

Outra categoria abordada, na literatura, é o aromatizante de processo térmico, que se trata do aromatizante alimentício produzido pelo aquecimento de dois ou mais ingredientes

precursores sob condições controladas, onde a principal reação é a reação de Maillard (GUO et al., 2010). Os precursores são os componentes não voláteis presentes em um meio que são utilizados nas reações de transformação em produtos voláteis e não voláteis para conferir sabor. As fontes de precursores geralmente são hidrolisados proteicos, utilizados pela indústria para obtenção de aromatizantes cárneos (LIESKE e KONRAD, 1994; FOOD AND DRUG ADMINISTRATION, 2023).

2.2 REDUÇÃO DE PROTEÍNAS A COMPOSTOS MAIS REATIVOS

A hidrólise caracteriza-se pela clivagem das ligações peptídicas das proteínas através de métodos químicos, térmicos ou enzimáticos, sendo as hidrólises química e térmica as menos utilizadas devido à baixa eficiência e aos produtos gerados terem uma baixa qualidade nutricional, devido ao uso de altas temperaturas e os reagentes serem tóxicos. Logo, a enzimólise é o método mais desejado e aplicado pela pesquisa e indústria farmacêutica e de alimentos, por ser segura e controlável (GUO et al., 2010; AGUILAR; SATO, 2018).

Durante a hidrólise, para cada ligação peptídica é liberado um grupo carboxila e um grupamento amino. De acordo com o avanço da reação, ficam dispersos no meio peptídeos de diferentes pesos moleculares e aminoácidos constituintes da matriz proteica, que na sua forma íntegra encontravam-se inativos e, através da hidrólise, são liberados e podem ser fisiologicamente ativos e funcionais. As características biotecnológicas do hidrolisado final dependem do grau de hidrólise atingido pela enzima utilizada no processo e da sequência aminoacídica dos peptídeos. O grau de hidrólise é expresso em percentual e consiste na fração de ligações peptídicas clivadas, tal parâmetro é aumentado quando as enzimas endógenas da matriz proteica são previamente inativadas, o que inviabiliza a competição com as enzimas adicionadas pelo sítio das proteínas (CORDEIRO et al., 2020).

A capacidade da protease clivar a proteína pelo no interior da molécula, categoriza a enzima como endopeptidase, quando pela extremidade, exopeptidase. A Alcalase (*Bacillus licheniformis*) é um exemplo de protease bacteriana de alta eficiência, que cliva preferencialmente ligações peptídicas com cadeia lateral hidrofóbica na porção C-terminal. Por ter uma menor especificidade, esta enzima tem um elevado potencial hidrolítico e tem sido aplicada na produção de precursores aromáticos (CORDEIRO et al., 2022a).

Esforços tem demonstrado que a hidrólise de proteínas é uma técnica altamente funcional para melhorar as atividades biotecnológicas das proteínas de subprodutos do abate

(LAFARGA e HEYS, 2014; QUEIROZ et al., 2017b; CORDEIRO et al., 2022a), fornecendo novas vias de aproveitamento para tais produtos de baixo valor agregado.

No abate de caprinos, as vísceras são um subproduto gerado, utilizadas no Nordeste brasileiro no preparo de tradicionais pratos culinários como a buchada, panelada e sarapatel, configurando-se uma alternativa de proteínas de alto valor biológico e uma fonte de minerais e vitaminas (QUEIROZ et al., 2013). Em outros lugares do mundo, o sangue, aparas de carnes e vísceras de pequenos ruminantes são utilizados para compor produtos como carvourmas na Grécia (ARVANITOYANNIS et al., 2000), morcilla de burgos na Espanha (SANTOS et al., 2003) e Chouriço Alentejano, Chouriço Mouro, Morcela de Assar, linguiças e patês em Portugal (LEITE et al., 2015; TEIXEIRA et al., 2019). Pesquisas no Brasil evidenciam a potencialidade de transformação dos subprodutos caprinos e ovinos para obtenção de buchada (MADRUGA et al., 2003; COSTA et al., 2005; MADRUGA et al., 2007; QUEIROZ et al., 2013), patês (DALMÁS et al., 2011), chouriço (SILVA et al., 2013) e sarapatel (BRASIL et al., 2014).

A abordagem limitada da literatura quanto às vísceras caprinas indica a necessidade de aprofundamento no tema. Alguns estudos anteriores iniciaram a agregação de valor às vísceras caprinas ao caracterizá-las e desenvolverem precursores aromáticos para utilização pela indústria de alimentos (QUEIROZ et al., 2017b; CORDEIRO et al., 2020, 2022a). Segundo Queiroz et al. (2017a) as vísceras caprinas (fígado, coração e pulmão) apresentam um elevado teor proteico (18%), todos os aminoácidos essenciais, um excelente perfil de ácidos graxos e características biotecnológicas que possibilitam a produção de novos alimentos, como hidrolisados proteicos.

O tratamento térmico de hidrolisado de vísceras caprinas levou a formação de compostos voláteis através da degradação lipídica e da RM, onde por meio desta foram obtidos compostos heterocíclicos como pirazinas, tiazóis e tiofenos, e não heterocíclicos, como aldeído de Strecker, contribuindo consideravelmente para a formação do aroma de carne assada e nozes no hidrolisado (CORDEIRO et al., 2020). Queiroz et al. (2017b) e Cordeiro et al. (2020) sugerem o aprofundamento no estudo das vísceras caprinas para desenvolvimento de novas formulações de aromatizantes, o que forneceria uma alternativa de processamento que acarretaria maior valor para tal subproduto.

2.3 VIAS DE FORMAÇÃO DO AROMA CÁRNEO

A aceitação de um alimento pelo consumidor é direcionada principalmente pelo sabor, o qual detém um papel fundamental na indústria de alimentos. Compreender as rotas que

formam os compostos de impacto para o aroma de carne cozida ou assada são fundamentais para indução destes compostos *in vitro*.

A formação do sabor em produtos cárneos depende da presença dos precursores, nomeadamente, os lipídios, aminoácidos, açúcares redutores, nucleotídeos e vitaminas, envolvidos na formação do sabor cárneo, cozido, assado e salgado através de reações como a reação de Maillard (RM), degradação de lipídios e reações lipídios-Maillard durante o tratamento térmico. O sabor cárneo só é formado quando a carne é submetida a um aquecimento, quando crua tem pouco ou nenhum sabor, normalmente um sabor de sangue. Na RM, dentre os compostos formados, destacam-se as pirazinas, oxazoles, tiofenos, tiazóis, piridinas, tiazolinas e outros compostos heterocíclicos devido aos seus característicos aromas cárneos. Outro resultado dessa reação é o escurecimento não enzimático conferido pelos pigmentos marrons, como as melanoidinas, que assim como na formação de sabor, são resultados da condensação de um grupo carbonila (açúcares redutores, aldeídos ou cetonas) e um grupamento amina ou compostos nitrogenados (aminoácidos, peptídeos e proteínas) sob ação do calor ou durante a estocagem (HODGE, 1953; MADRUGA e MOTTRAM, 1998; MOTTRAM, 1998).

Tiamina e compostos sulfurados também são precursores solúveis que contribuem para o aroma cárneo quando diretamente degradados pelo aquecimento ou quando envolvidos na RM, onde sua relevância dá-se devido ao baixo limiar de detecção dos compostos gerados. A tiamina é uma vitamina hidrossolúvel de fácil degradação térmica sob condições neutras e alcalinas e contribui, principalmente, para formação de compostos heterocíclicos como furanos, tiofenos, compostos sulfurados alifáticos e tiazóis, que, posteriormente, podem ser transformados em compostos com maior impacto para o aroma cárneo. Na degradação térmica de aminoácidos sulfurados, como a cisteína, ocorrem reações de descarboxilação e deaminação que levam a formação de aminas, aldeídos, sulfeto de hidrogênio e compostos benzênicos, que podem reagir com compostos de outras vias, como furanonas, furfurais e dicarbonílicos e formarem, tióis, tiazóis e derivados, que tem um singular aroma cárneo (SUN et al., 2022).

No aquecimento de matrizes cárneas também ocorre a reação de degradação de lipídios, formando diversos compostos diretamente aromáticos ou outros que podem adquirir aroma ao reagirem com intermediários na RM, os quais também contribuirão para o aroma cárneo. É através do aquecimento dos tecidos gordurosos da carne que a característica da espécie será formada no aroma da carne, e assim uma carne bovina terá uma característica aromática distinta de uma carne caprina. Na ausência ou em baixo conteúdo de gordura, o sabor cárneo não distingue a espécie (MOTTRAM, 1998). Os principais produtos odorantes da degradação

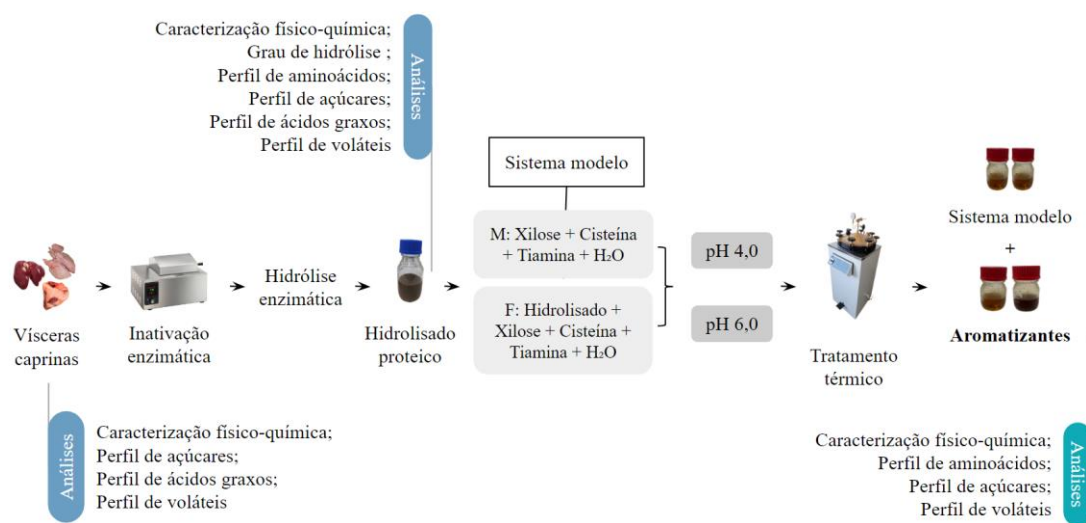
lipídica são compostos aldeídos, cetonas, álcoois, ácidos, lactonas, ésteres e furanos, dos quais os aldeídos são a classe majoritária presente em carnes cozidas, independente da espécie. Nonanal, (E)-2-octenal, (E)-2-nonenal, (E,E)-2,4-decadienal, 1-octen-3-ol, hexanal, 1-penten-3-ol, 2-ethylfuran, benzaldehyde, 2-ethylbenzaldehyde e (Z)-6-nonenal foram reportados como os principais odorantes em carne caprina cozida e grelhada, associados à degradação de ácidos graxos (MADRUGA et al., 2013; QI et al., 2022).

3 MATERIAL E MÉTODOS

3.1 PLANEJAMENTO DA PESQUISA

O desenvolvimento deste estudo foi realizado em dois estágios: no primeiro foi obtido o hidrolisado proteico de uma mistura de vísceras caprinas (fígado, coração e pulmão) utilizando a enzima Alcalase®. O segundo estágio envolveu estudos voltados à potencialização do hidrolisado como agente aromatizante, pela formação de novos compostos através indução da reação de Maillard no hidrolisado proteico suplementado com aminoácido sulfurado (cisteína), açúcar redutor (xilose) e vitamina do complexo B (tiamina-B1). O planejamento da pesquisa foi detalhado na Figura 1.

Figura 1. Delineamento experimental da pesquisa



3.2 MATERIAL

As vísceras foram obtidas de caprinos da raça Saanen com peso vivo médio de 30-35 kg, idade média de 7-10 meses, abatidos utilizando insensibilização por choque (abate humanitário) no Abatedouro Escola do Centro de Ciências Humanas Sociais e Agrárias da Universidade Federal da Paraíba (UFPB) da cidade de Bananeiras (Paraíba, Nordeste do Brasil). Após coleta, as vísceras foram armazenadas sob refrigeração ($5\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$) em cooler térmico.

para posterior transporte para o Laboratório de Análises Químicas de Alimentos do Centro de Tecnologia da UFPB, localizado na cidade de João Pessoa (Paraíba, Brasil).

3.2.1 Produção do hidrolisado de vísceras caprinas

Pré-tratamento

As vísceras caprinas (fígado, coração e pulmão) foram limpas para remoção das aparas externas aos órgãos, pesadas na proporção 1:1:1, trituradas e homogeneizadas em processador (Philco, Manaus, Brasil). A mistura de vísceras foi acondicionada em sacos plásticos à vácuo e armazenada sob congelamento ($-15\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$) para uso em até 120 dias.

A mistura de vísceras (**VC**) triturada e homogeneizada foi submetida à inativação das enzimas endógenas para inibição de competição pelo sítio ativo das proteínas durante a hidrólise. Com o banho-maria, previamente aquecido a $90\text{ }^{\circ}\text{C}$, as amostras foram adicionadas em tubos Falcon de 50 mL com tampa, os quais foram imersos no banho até cobrir o nível de amostra e mantidos a $90\text{ }^{\circ}\text{C}$ por 15 min. Ao fim do tratamento térmico, as amostras foram resfriadas em banho de gelo, seguindo as recomendações de Cordeiro et al. (2022a).

Para obtenção do hidrolisado proteico foi utilizada a protease Alcalase® (*Bacillus licheniformis*) (Novozymes Latino Americana Ltda, Paraná, Brasil), baseado no estudo prévio de Cordeiro et al. (2022a). Todos os reagentes utilizados neste estudo foram de grau analítico.

Hidrólise enzimática

Em um reator encamisado com sistema de aquecimento foi adicionado à mistura de vísceras caprinas água ultrapura na proporção 1:2 (p:v), ao qual foi acoplado uma bureta preenchida com NaOH 0,5 M para controle do pH. Ao atingir a temperatura ($50\text{ }^{\circ}\text{C}$) e o pH (8,0) desejados, a Alcalase em proporção enzima substrato de 5% foi adicionada e o sistema de agitação iniciado (3600 rpm). O tempo de reação foi de 240 min e ao final da hidrólise foi realizada a inativação da enzima Alcalase com aquecimento a $90\text{ }^{\circ}\text{C}$ por 15 min seguido de resfriamento em banho de gelo. O processo de hidrólise seguiu os procedimentos descritos por Cordeiro et al. (2022a). O hidrolisado proteico (**H**) obtido era líquido.

Grau de hidrólise (GH)

O grau de hidrólise (GH) foi determinado através da Equação 1, onde o consumo de base (NaOH) é associado à acidificação do meio devido a liberação de aminoácidos ao longo da hidrólise, no qual para que a enzima se mantenha efetiva o pH deve permanecer constante através da adição de NaOH 0,5 mol/L:

$$GH (\%) = B * N_b * \left(\frac{1}{\alpha}\right) * \left(\frac{1}{m}\right) * \left(\frac{1}{h_{total}}\right) * 100 \quad \text{Equação 1}$$

Logo, GH (%) é o grau de hidrólise; B é o consumo de base em mL; N_b é a concentração da base; $1/\alpha$ é média de grau de dissociação do grupo α -NH₂; $\alpha = 0,5267$; m é a massa de proteína na fração de amostra em solução (g); e h_{total} é o número total de ligações peptídicas da matriz proteica dado para carnes pela constante 7,6 (ADLER-NISSEN, 1986).

3.2.2 Produção do aromatizante

Vinte mililitros do hidrolisado proteico de vísceras caprinas foram transferidos para frascos rosqueáveis de 100 mL ao qual foi adicionado 0,1 g de cisteína, 1,0 g de xilose e 0,5 g de tiamina, segundo formulação adotada por Guo et al. (2010), com modificações. O pH do meio foi ajustado aos valores de 4,0 (**F4**) e 6,0 (**F6**) utilizando HCl 1 M baseado no estudo realizado por Li e Liu (2021), e o volume foi ajustado até 50 mL com água destilada. As soluções foram homogeneizadas e submetidas a tratamento térmico em autoclave a 121 °C por 1 hora (Cordeiro et al. 2020). Ao final do aquecimento os frascos foram resfriados em banho de gelo até atingir temperatura ambiente e em seguida submetidos à análise de perfil de voláteis. Os aromatizantes obtidos eram líquidos e foram armazenados sob congelamento em tubos Falcon de 50 mL, por um período não superior a 8 dias enquanto foram realizadas outras as análises (composição centesimal, cor, pH e perfil de açúcares e aminoácidos).

3.2.3 Sistemas modelos da Reação de Maillard

Com o objetivo de observar a contribuição da suplementação de cisteína, xilose e tiamina no perfil volátil do aromatizante, foram elaborados sistemas modelos constituídos por M4 (xilose, cisteína e tiamina em pH 4) e M6 (xilose, cisteína e tiamina em pH 6). Em frascos rosqueáveis de 100 mL foi adicionado 0,1 g de cisteína, 1,0 g de xilose, 0,5 g de tiamina e 20 mL de água destilada. O pH do meio foi ajustado aos valores de 4,0 e 6,0 utilizando NaOH 1 M, e o volume foi ajustado até 50 mL com água destilada. As soluções foram homogeneizadas

e aquecidas em autoclave a 121 °C por 1 hora. Após a reação, os frascos foram resfriados em banho de gelo até atingir temperatura ambiente.

3.3 ANÁLISES FÍSICO-QUÍMICAS

3.3.1 Análises físico-químicas

O conteúdo de umidade, cinzas e proteínas (fator de conversão 6,25) de todas as amostras foi determinado utilizando a metodologia descrita nos itens nº 926.07B, 923.03 e 930.25, respectivamente, da AOAC (2010). O conteúdo de gordura foi determinado conforme Folch, Less e Stanley (1957), enquanto os carboidratos foram determinados por diferença. O pH do hidrolisado, aromatizantes e sistemas modelo foi mensurado utilizando um pHmetro digital de bancada (Modelo 0400AS, Quimis, Diadema, São Paulo), previamente calibrado com soluções tampão (pH 4 e 7).

3.3.2 Perfil de aminoácidos livres

Os aminoácidos livres foram determinados nos hidrolisados e aromatizantes baseado na metodologia de Dai et al. (2014) e Araya et al. (2021) utilizando um sistema de cromatografia líquida Agilent 1260 Infinity LC (Agilent Technologies, Santa Clara, Califórnia, EUA) acoplado a um detector de arranjo de diodos (DAD) (modelo G1315D).

Para obtenção do extrato, 1 mL da amostra foi diluída para 20 mL de volume final com HCl 0,1 M em um balão volumétrico. A solução foi transferida para um erlenmeyer de 100 mL, o qual foi mantido fechado sob agitação em orbital por 60 min a 30 °C. Ao final da extração, a amostra foi filtrada em filtro de seringa de nylon de 0,45 µm de porosidade (Millex Millipore, Barueri, SP, Brasil) e uma alíquota de 50 µL foi diluída com tampão borato pH 9,8, derivatizada com ortoftaldeído (OPA) (Sigma-Aldrich, St. Louis, MO, USA) e inserida no sistema de injeção automático (20 µL).

A separação foi realizada utilizando uma coluna Zorbax Eclipse Plus RP-C18 (100 × 4,6 mm, 3,5 µm) e uma pré-coluna Zorbax C18 (12,6 × 4,6 mm, 5 µm) (Zorbax, EUA) em um fluxo constante de 1,5 mL/min a 40 °C. Duas fases móveis foram utilizadas na separação: (A) tampão borato pH 7,8; (B) acetonitrila:metanol:água (45:45:10, v:v:v), e (C) 100% metanol. O gradiente de eluição foi 0-1,9 min, 100% (A); 18,1 min-18,6 min, 42% (A) 58% (B); 22,3 min,

30% (A) 70% (B); 22,4-26,0 min, 100% (C); 26,1-28,0 min, 100% (A). Os aminoácidos foram detectados a 338 nm e quantificados por meio de curvas de calibração de padrões externos ($R^2 > 0,995$). O limite de detecção (LD) e quantificação (LQ) para todos os compostos avaliados foram $LD < 0,289$ mg/L e $LQ < 1,091$ mg/L. Os dados foram processados no software OpenLAB CDS ChemStation Edition. Os resultados foram expressos em mg de aminoácido/100g de amostra.

3.3.3 Perfil de açúcares

Extratos solúveis foram obtidos através da pesagem de 2 g de amostra (mistura de vísceras, hidrolisado, aromatizante) com diluição final de 10 mL em água ultrapura. A solução de vísceras foi triturada em ultraturrax (IKA Works, Wilmington, EUA) a 3600 rpm por 10 min, seguida de centrifugação (Solab, Piracicaba, Brasil) a 3500 rpm sob temperatura ambiente por 10 min e posterior filtração em papel filtro qualitativo. Hidrolisado e aromatizantes diluídos foram centrifugados por 10 min e filtrados em papel filtro. A seguir, as amostras foram filtradas em filtro de seringa de 0,45 μ m de porosidade e injetadas em um sistema de cromatografia líquida Agilent, modelo VARIAN (VARIAN, Waters, CA, EUA) acoplado a um detector de índice de refração (RID) (modelo 356 LC-RID). O software de processamento utilizado foi o GALAXIE Chromatography Data System. A coluna de separação utilizada foi uma Agilent Hi-Plex Ca (7,7 x 300 mm, 8 μ m), aquecida a 85 °C. O volume de injeção da amostra foi 20 μ L com sistema de solvente isocrático, no qual utilizou-se água ultrapura. O tempo de corrida foi de 30 min a um fluxo de 0,6 mL min⁻¹ (BALL E LLOYD, 2011).

A identificação foi realizada por comparação com padrões externos (Sigma-Aldrich, St. Louis, MO, USA), a partir dos quais realizou-se curvas de calibração para quantificação dos açúcares ($R^2_{\text{glicose}} = 0,9998$, $R^2_{\text{frutose}} = 0,9993$, $R^2_{\text{manose}} = 0,9999$, $R^2_{\text{ribose}} = 0,9992$, $R^2_{\text{xilose}} = 0,9993$, $R^2_{\text{maltose}} = 0,9991$). Os resultados foram expressos em mg/100g de amostra.

3.3.4 Perfil de ácidos graxos

A transesterificação direta dos ácidos graxos presentes na gordura da mistura de vísceras caprinas e hidrolisado foi realizada conforme descrito por Hartmann e Lago (1973). A identificação e quantificação dos ésteres de ácidos graxos foram realizadas utilizando um sistema de cromatografia gasosa (FID VARIAN) acoplado a uma coluna (SUPELCO,

Bellefonte, EUA) com dimensões de 100 m x 0,25 mm x 0,20 μ m de espessura, com detector de ionização de chama (DIC). Foi utilizado o hélio como gás de arraste (vazão de 1 mL.min⁻¹). As condições do CG foram: temperatura do injetor 250 °C; a temperatura inicial do forno de 40 °C por 2 minutos, aumentando-se 10 °C min⁻¹ até atingir 180 °C, permanecendo por 30 minutos, com posterior aumento de 10 °C min⁻¹ até atingir 240 °C, no qual permaneceu por 34 min, com um tempo total de corrida de 86 min; a temperatura do detector foi de 260 °C. Fluxo dos gases auxiliares foram: Hélio 25 mL.min⁻¹, Hidrogênio 30 mL min⁻¹, Ar sintético: 300 mL min⁻¹. Alíquotas de 1,0 μ L do extrato esterificado foram injetadas em injetor tipo Split/Splitless (Split 1:100). Os cromatogramas foram registrados em software tipo Galaxie Chromatography Data System. Os ácidos graxos foram identificados por comparação dos tempos de retenção dos ésteres metílicos das amostras com padrões Supelco ME19-Kit (Fatty Acid Methyl Esters C₄-C₂₄) e a semiquantificação foi realizada pela relação da área do padrão interno C21 (1 mg mL⁻¹) em hexano (adicionado a cada amostra) com a área integrada de cada composto do perfil. Os resultados foram expressos em mg/100g de amostra.

3.3.5 Perfil de compostos voláteis

A análise de voláteis foi realizada em H, F4, F6 e nos sistemas modelo (M4 e M6) em duas colunas de polaridades distintas, observando-se que a mistura de vísceras foi analisada apenas na coluna VF-5MS (apolar).

Para a extração de voláteis utilizou-se a técnica de microextração em fase sólida Headspace (HS-SPME) com dispositivo SPME (Supelco, Bellefonte, EUA), segundo metodologia adaptada de Cordeiro et al. (2022a). Uma alíquota de 2 mL de hidrolisado/aromatizante ou 2 g da mistura de vísceras e 1 μ L do padrão interno 1,2-diclorobenzeno em metanol (50 μ g.mL⁻¹) foram transferidos para um frasco de vidro de 20 mL e imediatamente fechado com tampa com septo revestido de Teflon.

Os voláteis foram extraídos a 50 °C por 10 min (equilíbrio). Em seguida, a fibra de Divinilbenzeno/Carboxeno/Polidimetilsiloxano (DVB/CAR/PDMS) 50/30 μ m foi exposta ao headspace por 30 min de adsorção sob agitação. A fibra utilizada foi condicionada de acordo com as especificações do fabricante antes do procedimento de extração. As análises cromatográficas foram realizadas utilizando Cromatógrafo Gasoso 7890B (Agilent Technologies 5977B, Little Falls, DE, EUA) equipado com colunas VF-5MS (30 m x 0,25 mm, 0,25 μ m) e HP-INNOWAX (60 m x 0,25 mm, 0,25 μ m), acoplado a espectrômetro de massa nas condições da metodologia de Sun et al. (2014): temperatura inicial do forno de 40 °C por 3

min, seguida de 5 °C min⁻¹ até 120 °C e 10 °C min⁻¹ até 230 °C, na qual permaneceu por 5 min, por um tempo total de execução de 35 min. A temperatura do injetor foi fixada em 250 °C. O hélio foi utilizado como gás de arraste em uma vazão de 1,0 mL min⁻¹ no sistema de injeção splitless.

A temperatura da linha de transferência foi de 250 °C. O espectrômetro de massa foi operado no modo de impacto eletrônico (70 eV) e a faixa de varredura de massa foi de 50 a 400 m/z a 4,44 scan/s. Os compostos foram identificados usando o banco de dados da biblioteca NIST (2014) (National Institute of Standards and Technology, USA) combinado com o espectro de massa e índice de retenção linear.

O índice de retenção linear (LRI) de cada composto foi calculado por meio dos tempos de retenção de uma série homóloga de n-alcanos C₆-C₂₀. A semiquantificação dos voláteis foi calculada extrapolando a área do padrão interno 1,2-diclorobenzeno (adicionado a cada amostra) para a área integrada de cada composto do perfil e os dados foram expressos em µg/kg de mistura de vísceras e µg/L de hidrolisado/aromatizante.

3.4 ANÁLISES ESTATÍSTICAS

Os experimentos e análises foram realizados com amostragem em triplicata. Os dados obtidos como resultado foram expressos como média, nas quais F4 e F6 foram comparadas entre si pelo teste t-student ao nível de significância de 5% ($p < 0,05$) na aplicação XLSTAT (versão 2014.5.03, Addinsoft, Nova York, EUA).

As amostras H, F4 e F6 foram submetidas a uma análise de componentes principais (PCA), enquanto H, F4, F6, M4 e M6 foram analisadas quanto a um agrupamento hierárquico, ambas análises realizadas no software XLSTAT. A padronização dos dados dos compostos voláteis, aminoácidos e açúcares foi realizada no software MATLAB (The Mathworks, Inc. versão 7.70.0.499, Natick, MA, R2010a).

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4 RESULTADOS E DISCUSSÃO

Os resultados obtidos nesta pesquisa estão apresentados a seguir em formato de artigo, em atendimento a Norma Complementar nº 03/2011 do PPGCTA.

Artigo: “Thiamine, cysteine, and xylose added to the Maillard reaction of goat protein hydrolysate potentiates the formation of meat flavoring compounds”

DOI: <https://doi.org/10.1016/j.foodchem.2024.138398>

(Publicado no periódico: Food Chemistry. Fator de impacto: 8.8 - Qualis Capes A1)



Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Thiamine, cysteine and xylose added to the Maillard reaction of goat protein hydrolysate potentiates the formation of meat flavoring compounds

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ARTICLE INFO

Keywords:

Meat aroma
Odorants
Volatile profiling
Ruminant viscera
Slaughter by-products
New ingredient

ABSTRACT

A protein hydrolysate of goat viscera added with xylose, cysteine, and thiamine under different pH was used to prepare a meat flavoring. Goat viscera hydrolysate and flavoring were subjected to analysis of physicochemical characteristics, amino acid profile, sugars, fatty acids, and volatile profile. Meat aroma characteristics were initiated in the hydrolysate, in which Strecker's pyrazines and aldehydes were identified, which also had fatty acids and amino acids available for the formation of 96 volatile compounds in the flavorings via lipid manipulation, Maillard occurrence, Strecker manipulation and interactions among these means. Maillard reaction products with intense meat aroma, such as 2-methyl-3-furanthiol, 2-furfurylthiol and bis(2-methyl-3-furyl) disulfide were isolated only in the flavoring at pH 4. In contrast, the flavoring at pH 6 showed a higher concentration than all the other compounds, providing a lower meat characteristic, but an intense sweet, fatty and goat aroma.

1. Introduction

The preference and acceptance of food is due mainly by its aroma, given by volatile compounds that are released from the food to the saliva, then diluted in the air space of the oral cavity and, then perceived by the olfactory receptors of the nasal cavity (Li et al., 2022). In the emerging involvement of consumers with nutrition and health, a more natural food, aligned with healthiness, but which is still capable of providing the expected flavor and guaranteeing the pleasure of consumption, is more likely to be accepted by the consumer (Balco and Gracia, 2022). Thus, reconciling these demands may pose a new challenge for the food industry, given the expectation that by 2030 the global market of food flavors should reach around US\$ 26 billion (Precedence Research, 2022).

In the industrial food production process, the use of additives that confer, intensify, or improve the flavor and food aroma are widespread.

Flavorings are used to correct processes that may lead to a flavor reduction; extend the sensory characteristics of the product in the shelves or even give the food a characteristic idealized by the consumer. Whether in meat products, snacks, sauces, soups, pâtés, supplements or animal ration, meat flavorings are widely used by the industry, and they ensure that the product are attractive and close to the natural meat flavor (Precedence Research, 2022). Salt (NaCl) is the main and oldest flavor enhancer in meat products. Due to global demand, already established in countries in Europe (Finland, United Kingdom, and Australia) and Asia (Japan), its consumption has seen a reduced, consequently, the use of alternative ingredients to maintain the flavor in meat products is required, especially when the use chloride substitutes for NaCl leads to increased bitterness, metallic taste, and abnormal texture (Jia et al., 2024).

In the last decades, researches have successfully sought to identify and quantify the volatile compounds that make the characteristic meat

Abbreviations: GV, goat viscera mixture; H, goat protein hydrolysate; DH, degree of hydrolysis; F4, flavoring at pH 4; F6, flavoring at pH 6; M4, model system at pH 4; M6, model system at pH 6; MR, Maillard reaction; MFT, 2-methyl-3-furanthiol; FFT, 2-furfurylthiol; MFT-MFT, bis(2-methyl-3-furyl)disulfide.

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<https://doi.org/10.1016/j.foodchem.2024.138398>

Received 31 August 2023; Received in revised form 16 December 2023; Accepted 6 January 2024

Available online 12 January 2024

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aroma. As well as describing the ways and formation conditions of these aromatic compounds, the most impactful being those containing sulfur, nitrogen, and oxygen in their composition. In general, they come from the thermal degradation of fatty acids, vitamins, and sugars, from the Maillard reaction and Strecker degradation of amino acids, or even from the reaction between products derived from these means, and these volatile compounds can be strongly influenced by pH (Madruga and Mottram, 1995; Madruga et al., 2010; Guo et al., 2010; Cardinal et al., 2020; Li and Liu, 2021). From this perspective, the notable transformations of by-products from the slaughter of animals into hydrolysates have been highlighted. Due to the high content of precursors such as amino acids and lipids, when subjected to heat treatment they create volatile compounds capable of conferring or contributing to the meat aroma (Cordeiro et al., 2020; Gao et al., 2020; Li and Liu, 2021; Cunha et al., 2023). The product of this type of processing is characterized as a process/thermal reaction flavoring (Guo et al., 2010), whose application of by-product hydrolysates is still limited.

Thus, considering the demand for less synthetic ingredients and foods, with better use and sustainability of slaughter by-products, the present study aimed to produce meat flavoring from the hydrolysate of goat viscera supplemented with xylose, cysteine, and thiamine, estimating the effect of pH in the formation means of its aromatic potential.

2. Material and methods

2.1. Materials

Viscera was obtained from Saanen goats with an average weight of 30–35 kg, average age of 7–10 months, slaughtered using shock stunning (humane slaughter) at the Slaughterhouse School of the Center for Social and Agrarian Human Sciences of the Federal University of Paraíba (UFPB) from the city of Bananeiras (Paraíba, Northeast Brazil). To obtain the protein hydrolysate, the protease Alcalase® (*Bacillus licheniformis*) (Novozymes Latino Americana Ltda, Paraná, Brazil) was used. All reagents used in this study were analytical grade.

2.2. Production of goat viscera hydrolysate

2.2.1. Pre-treatment

Viscera (liver, heart, and lung) were cleaned to remove external trimmings from the organs, weighed in a 1:1:1 ratio, crushed and homogenized in a processor (Philco, Manaus, Brazil). The goat viscera mixture (GV) was packed in vacuum plastic bags and stored under freezing ($-15\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$) for up to 120 days until ending experiment and analysis. The GV was crushed and homogenized and submitted to the inactivation of endogenous enzymes to inhibit competition for the active site of the proteins during hydrolysis ($90\text{ }^{\circ}\text{C}$ for 15 min in a water bath). At the end of the heat treatment, the samples were cooled in an ice bath, following the recommendations by Cordeiro et al. (2022).

2.2.2. Enzymatic hydrolysis

In a jacketed reactor with a heating system, ultrapure water was added to the GV in a 1:2 (w: v) ratio, to which a burette filled with 0.5 M NaOH was attached to control the pH. The proteolytic reaction was carried out using Alcalase, as previously demonstrated by Queiroz et al. (2017). Upon reaching the desired temperature ($50\text{ }^{\circ}\text{C}$) and pH (8.0), Alcalase was added in an enzyme-substrate ratio of 0.8% (g/100 g) according to previous optimization by Queiroz et al. (2017) and the stirring system started (3600 rpm). The reaction time was 240 min and at the end of the hydrolysis the enzyme was inactivated by heating at $90\text{ }^{\circ}\text{C}$ for 15 min followed by cooling in an ice bath. The goat protein hydrolysate (H) obtained had a liquid consistency (Cordeiro et al., 2022).

2.2.3. Degree of hydrolysis

The degree of hydrolysis (DH) was determined through Eq. (1), where the pH was adjusted with 0.5 mol/L NaOH:

Eq. (1). Calculation to determine degree of hydrolysis

$$\text{DH}(\%) = B \times N_b \times (1/\alpha) \times (1/m) \times (1/h_{\text{total}}) \times 100 \quad (1)$$

where DH (%) is the degree of hydrolysis; B is the base consumption in mL; N_b is the base concentration; $1/\alpha$ is the average degree of dissociation of the $\alpha\text{-NH}_2$ group; $\alpha = 0.5267$; m is the protein mass in the sample fraction in the solution (g); and h_{total} is the total number of peptide bonds in the protein matrix given for meat by the constant 7.6 (Adler-Nissen, 1986).

2.2.4. Maillard reaction

Twenty milliliters of goat viscera protein hydrolysate were transferred to 100 mL screw-on flasks to which 0.1 g of cysteine, 1.0 g of xylose and 0.5 g of thiamine were added, according to the formulation adopted by Guo et al. (2010), with modifications. The pH of the medium was adjusted to values of 4.0 (F4) and 6.0 (F6) using 1 M HCl based on the study by Li and Liu (2021), and the volume was adjusted to 50 mL with distilled water. The solutions were homogenized and subjected to thermal treatment in an autoclave at $121\text{ }^{\circ}\text{C}$ for 60 min (Cordeiro et al., 2020). After finished heating, the flasks were cooled in an ice bath until they reached room temperature and then subjected to volatile profile analysis. The flavorings obtained were liquid and stored under freezing in 50 mL Falcon tubes, for a period not exceeding 8 days while other analyzes were carried out (centesimal composition, color, pH and profile of sugars and amino acids).

2.2.5. Model systems of the Maillard reaction

In order to observe the contribution cysteine, xylose and thiamine supplementation in the volatile flavoring profile, model systems were elaborated - M4 (xylose, cysteine and thiamine at pH 4) and M6 (xylose, cysteine and thiamine at pH 6). In 100 mL screw-on bottles, 0.1 g of cysteine, 1.0 g of xylose, 0.5 g of thiamine and 20 mL of distilled water were added. The pH of the medium was adjusted to values of 4.0 and 6.0 using 1 M NaOH, and the volume was adjusted to 50 mL with distilled water. The solutions were homogenized and heated in an autoclave at $121\text{ }^{\circ}\text{C}$ for 60 min. After the reaction, the flasks were cooled in an ice bath until reaching room temperature.

2.3. Physical-chemical analysis

2.3.1. Proximate composition and pH

For all samples in the study, the moisture, ash and protein content (conversion factor 6.25) were determined using the methodology described in items n° 926.07B, 923.03 and 930.25, respectively, of the AOAC (2010). The fat content was determined according to Folch, Less and Stanley (1957), while the carbohydrate content was determined by difference. The pH was measured using a benchtop digital pH meter (Model 0400AS, Quimis, Diadema, São Paulo), previously calibrated with buffer solutions (pH 4 and 7).

2.3.2. Free amino acid profile

The free amino acids of the hydrolysate and flavorings were determined based on the Dai et al. (2014) and Araya et al. (2021) methodology using an Agilent 1260 Infinity LC liquid chromatography system (Agilent Technologies, Santa Clara, California, USA) coupled to a diode array detector (DAD) (model G1315D).

To obtain the extract, 1 mL of the sample was diluted to 20 mL of final volume with 0.1 M HCl in a volumetric flask. The solution was transferred to a 100 mL Erlenmeyer, which was kept closed under orbital stirring for 60 min at $30\text{ }^{\circ}\text{C}$. At the end of the extraction, the sample was filtered through a $0.45\text{ }\mu\text{m}$ porosity nylon syringe filter (Millex Millipore, Barueri, SP, Brazil) and a 50 μL aliquot was diluted with borate buffer pH 9.8, derivatized with orthophthalaldehyde (OPA) (Sigma-Aldrich, St. Louis, MO, USA) and inserted into the automatic injection system (20 μL).

Separation was performed using a Zorbax Eclipse Plus RP-C18 column (100 × 4.6 mm, 3.5 µm) and a Zorbax C18 precolumn (12.6 × 4.6 mm, 5 µm) (Zorbax, USA) of according to the previous methodology by Araya et al. (2021). Amino acids were detected at 338 nm and quantified using external standard calibration curves ($R^2 > 0.995$). The detection limits (LD) and quantification (LQ) for all evaluated compounds were LD < 0.289 mg/L and LQ < 1.091 mg.L⁻¹. Data were processed using OpenLAB CDS ChemStation Edition software.

2.3.3. Sugars profile

Soluble extracts were obtained by weighing 2 g of sample (mixture of viscera, hydrolysate, flavoring) with a final dilution of 10 mL in ultrapure water. The viscera solution was ground in an ultraturax (IKA Works, Wilmington, USA) for 10 min, followed by centrifugation (Solab, Piracicaba, Brazil) at 2607 g at room temperature for 10 min and subsequent filtration on qualitative filter paper. Hydrolyzed and diluted flavorings were centrifuged for 10 min and filtered through filter paper. Next, the samples were filtered through a 0.45 µm porosity syringe filter and injected into an Agilent liquid chromatography system, model VARIAN (VARIAN, Waters, CA, USA) coupled to a refractive index detector (RID) (model 356 LC-RID). The processing software used was the GALAXIE Chromatography Data System. The separation column used was an Agilent Hi-Plex Ca (7.7 × 300 mm, 8 µm), heated to 85 °C. The injection volume of the sample was 20 µL with an isocratic solvent system, in which ultrapure water was used. The run time was 30 min at a flow rate of 0.6 mL.min⁻¹ (Ball and Lloyd, 2011). Identification was performed by comparison with external standards (Sigma-Aldrich, St. Louis, MO, USA), from which calibration curves were created to quantify the sugars ($R^2 > 0.9992$).

2.3.4. Fatty acids profile

The direct transesterification of the fatty acids presents in the fat from the goat viscera mixture and hydrolysate was carried out as described by Hartmann and Lago (1973). The identification and quantification of fatty acid esters were carried out using a gas chromatography system (FID VARIAN) coupled to a column (SUPELCO, Bellefonte, USA) measuring 100 m × 0.25 mm × 0.20 µm thick, with flame ionization detector (FID). The chromatograms were recorded in software like Galaxie Chromatography Data System. Fatty acids were identified by comparing the retention times of the methyl esters in the samples with Supelco ME19-Kit standards (Fatty Acid Methyl Esters C₄-C₂₄) and semi quantification was performed using the area ratio of the internal standard C₂₁ (1 mg.mL⁻¹) in hexane (added to each sample) with the integrated area of each compound in the profile.

2.3.5. GC-MS

The analysis of volatile compounds was carried out in H, F4, F6 and in the model systems (M4 and M6) in two columns of different polarities, observing that the viscera mixture was analyzed only in the VF-5MS column (non-polar).

For the extraction of volatiles, the Headspace solid-phase micro-extraction technique (HS-SPME) was used with a SPME device (Supelco, Bellefonte, USA), according to the methodology adapted from Cordeiro et al. (2022). A 2 mL aliquot of hydrolysate/flavoring or 2 g of the viscera mixture and 1 µL of the internal standard 1,2-dichlorobenzene in methanol (50 µg.mL⁻¹) were transferred to a 20 mL glass vial and immediately closed with Teflon-coated septum cap.

Volatiles were extracted at 50 °C for 10 min (equilibrium). Then, the 50/30 µm Divinylbenzene/Carboxene/Polydimethylsiloxane (DVB/CAR/PDMS) fiber was exposed to headspace for 30 min of adsorption under agitation. The fiber used was conditioned according to the manufacturer's specifications before the extraction procedure. Chromatographic analyzes were performed using Gas Chromatograph 7890B (Agilent Technologies 5977B, Little Falls, DE, USA) equipped with VF-5MS columns (30 m × 0.25 mm, 0.25 µm) and HP-INNOWAX (60 m × 0.25 mm, 0.25 µm), coupled to a mass spectrometer under the

Table 1

Physical-chemical characterization and profile of sugars and amino acids present in the mixture of viscera, goat protein hydrolysate and flavorings.

Parameter	¹ GV	² H	³ F4	⁴ F6
<i>Proximate Composition⁵ (g/100 g)</i>				
Protein	79.93 ± 0.40	72.01 ± 0.69	65.14 ± 0.34 ^a	61.92 ± 0.16 ^b
Moisture ⁶	75.08 ± 0.51	89.85 ± 0.03	94.13 ± 0.02 ^a	93.87 ± 0.01 ^b
Lipids	22.09 ± 0.39	15.25 ± 0.72	9.07 ± 0.65 ^a	8.71 ± 0.25 ^a
Carbohydrates	2.40 ± 1.73	2.32 ± 0.37	18.03 ± 1.63 ^a	20.08 ± 3.68 ^a
Ash	4.43 ± 0.13	10.55 ± 0.23	7.16 ± 0.32 ^a	7.45 ± 0.41 ^a
<i>Sugars (mg/100 g)</i>				
Glucose	1691.95 ± 37.31	465.74 ± 5.09	165.05 ± 4.21 ^a	180.82 ± 2.82 ^b
Mannose	72.89 ± 0.33	18.30 ± 3.59	nd	nd
Fructose	240.02 ± 0.42	115.28 ± 4.85	80.27 ± 9.21 ^a	131.33 ± 7.77 ^b
Maltose	117.55 ± 3.25	42.55 ± 4.91	50.96 ± 0.27 ^a	57.89 ± 3.90 ^b
Ribose	19.87 ± 0.83	9.21 ± 1.77	11.14 ± 0.64 ^a	19.09 ± 0.93 ^b
⁷ Xylose	na	na	2258.23 ± 70.68 ^a	1998.66 ± 13.68 ^b
<i>Free amino acids (mg/100 g)</i>				
Aspartic acid	na	21.94 ± 1.14	8.40 ± 0.29 ^a	6.31 ± 1.23 ^b
Glutamic acid	na	91.27 ± 1.91	9.97 ± 0.24 ^a	13.59 ± 0.38 ^b
Asparagine	na	19.80 ± 0.77	2.32 ± 0.05 ^a	2.92 ± 0.00 ^b
Serine	na	28.28 ± 1.87	6.73 ± 0.09 ^a	5.88 ± 1.89 ^b
Glutamine	na	14.99 ± 0.61	^a < LOD	0.46 ± 0.00
Histidine	na	16.43 ± 0.57	3.75 ± 0.10 ^a	2.77 ± 0.41 ^b
Glycine	na	46.64 ± 3.86	13.01 ± 0.09 ^a	9.44 ± 3.06 ^b
Threonine	na	20.81 ± 0.07	4.46 ± 0.06 ^a	3.88 ± 0.30 ^b
Arginine	na	32.54 ± 0.06	5.53 ± 0.05 ^a	5.23 ± 0.38 ^a
Alanine	na	84.42 ± 4.18	19.47 ± 0.35 ^a	13.28 ± 7.15 ^a
Tyrosine	na	26.57 ± 0.30	5.30 ± 0.09 ^a	4.14 ± 1.63 ^a
Cysteine	na	33.57 ± 0.76	16.76 ± 12.03 ^a	5.61 ± 0.00 ^a
Valine + Methionine	na	40.36 ± 0.45	5.82 ± 0.10 ^a	4.73 ± 1.53 ^a
Tryptophan	na	24.87 ± 0.27	8.65 ± 0.02 ^a	5.28 ± 3.41 ^a
Phenylalanine	na	44.77 ± 0.45	6.52 ± 0.14 ^a	5.01 ± 1.69 ^a
Isoleucine	na	22.06 ± 1.44	3.75 ± 0.01 ^a	3.37 ± 0.73 ^a
Leucine	na	20.72 ± 0.00	<LOD	<LOD
Lysine	na	145.21 ± 4.66	26.58 ± 0.03 ^a	17.59 ± 10.23 ^a

Results are expressed as mean ± standard deviation (n = 3). Means followed by equal letters do not differ from each other by the *t*-student test at the 5 % significance level. Note: "nd", not detected; na, not applicable.

¹GV, goat viscera mixture; ²H, goat protein hydrolysate; ³F4, flavoring pH 4,0; ⁴F6, flavoring pH 6,0; ⁵ Proximate composition on a dry basis; ⁶ Moisture on a wet basis. ⁷ Xylose added as a supplement. ^a LOD, limit of detection; <LOD (leucine) = 1,358 mg/L; <LOD (glutamine) = 1,816 mg/L.

Table 2

Volatile compounds identified in the mixture of goat viscera, hydrolysate, and flavorings.

Identification	LRI	Characteristic odor	Concentration (µg/L)			
			^a GV	H	F4	F6
Lipid degradation products						
<i>Acids (4)</i>						
2-Ethylcaproic acid	1126		nd	nd	1,40 ± 0,14A	1,29 ± 0,06A
Caproic acid	1850	fatty, goat	nd	nd	12,41 ± 1,02B	152,78 ± 14,59A
Heptanoic acid	1957	rancid, sour, cheese	nd	nd	2,49 ± 0,48	nd
Octanoic acid	>2000	greasy, rancid, oily	nd	nd	2,06 ± 0,44	nd
Total concentration					18,36 ± 2,08B	154,07 ± 14,65A
<i>Alcohols (11)</i>						
2-Methyl-1-buten-3-ol	<800	earthy, oily	0,48 ± 0,13	0,61 ± 0,27C	32,98 ± 2,60B	78,26 ± 3,95A
3-Methylcyclohexanol	951	rum, fermented	nd	nd	nd	0,45 ± 0,02
2-Ethyl-1-hexanol	1030	citrus, oily, sweet	0,25 ± 0,01	nd	1,45 ± 0,00	nd
2,6-Dimethylcyclohexanol	1108		1,96 ± 0,47	3,46 ± 0,07B	1,51 ± 0,05C	6,33 ± 0,46A
Cyclododecanol	1576		nd	nd	4,65 ± 0,68B	7,82 ± 0,78A
1-Pentanol	1250	pungent bread	0,48 ± 0,06	0,06 ± 0,01A	0,08 ± 0,04A	nd
1-Hexanol	1350	fermented, oily	nd	nd	nd	2,83 ± 0,49
1-Octanol	1071	green, citrus, pungent	nd	0,30 ± 0,05B	0,91 ± 0,05A	nd
1-Octen-3-ol	981	earthy oily chicken	1,27 ± 0,10	0,53 ± 0,29C	2,38 ± 0,27B	8,96 ± 0,86A
Octenol	1445	green watermelon	nd	0,30 ± 0,01	nd	nd
(E)-2-Dodecen-1-ol	1481	oily, greasy	nd	nd	0,34 ± 0,03B	0,58 ± 0,02A
Total concentration			4,44 ± 0,77	5,26 ± 0,70C	44,30 ± 3,72B	105,23 ± 6,58A
<i>Aldehydes (25)</i>						
Pentanal	979	fermented bread	nd	0,03 ± 0,00C	0,39 ± 0,11B	11,44 ± 0,21A
Hexanal	800	green, greasy	0,69 ± 0,03	0,48 ± 0,03C	5,85 ± 0,62B	8,40 ± 0,81A
Heptanal	902	fresh, fatty	0,21 ± 0,03	0,46 ± 0,03C	1,65 ± 0,20B	3,21 ± 0,26A
Octanal	1294	pungent, citrus	nd	0,04 ± 0,01B	0,50 ± 0,03B	20,50 ± 0,76A
Nonanal	1104	waxy, citrus	1,83 ± 0,37	3,40 ± 0,18C	20,88 ± 2,42A	37,25 ± 2,73B
Decanal	1206	sweet, pungent, waxy	nd	0,14 ± 0,03B	2,53 ± 0,26A	2,92 ± 0,29A
Undecanal	1308	pungent, waxy	nd	nd	0,92 ± 0,04B	1,13 ± 0,12A
Tridecanal	1510	fresh, pungent	nd	nd	1,53 ± 0,05B	2,29 ± 0,02A
Tetradecanal	1612	greasy, creamy	0,91 ± 0,06	nd	14,60 ± 2,69B	24,32 ± 2,28A
Pentadecanal	1715		nd	nd	4,90 ± 0,60B	7,91 ± 0,67A
Hexadecanal	1817		1,16 ± 0,09	nd	33,01 ± 3,68B	58,67 ± 4,45A
2-Phenyl-2-butenal	1277	sweet, peel, bean	nd	nd	0,69 ± 0,03B	1,42 ± 0,15A
(E)-2-Octenal	1060	greasy, herbs	nd	nd	1,82 ± 0,16B	5,68 ± 0,28A
(Z)-2-Nonenal	1161	greasy, green	nd	nd	2,54 ± 0,17A	4,46 ± 0,30B
(E)-2-Decenal	1267	greasy, earthy	nd	nd	0,86 ± 0,09B	1,57 ± 0,07A
(E,E)-2,4-Decadienal	1319	greasy chicken	nd	nd	0,50 ± 0,03	nd
(E)-2-Undecenal	1367	fresh, fruity	nd	nd	0,69 ± 0,02B	1,01 ± 0,03A
(Z)-7-Hexadecenal	1798		nd	nd	0,14 ± 0,01B	0,20 ± 0,01A
13-Methyltetradecanal	1678		nd	nd	3,08 ± 0,37B	5,99 ± 0,54A
Benzaldehyde	960	oily, almond	nd	29,35 ± 9,70B	69,80 ± 2,85A	68,88 ± 3,49A
4-Methylbenzaldehyde	1681		nd	nd	1,19 ± 0,36B	31,93 ± 2,84A
Benzacetaldehyde	1044	sweet, fermented	nd	nd	7,99 ± 1,40B	28,24 ± 2,21A
2-Hydroxy-3-methylbenzaldehyde	1133		nd	nd	0,54 ± 0,06	nd
4-(1-Methylethyl)benzaldehyde	1233		nd	nd	0,72 ± 0,08	nd
1-Formyl-2,6,6-trimethyl-1-cyclohexene	1224	tropical, herb	1,62 ± 0,03	0,86 ± 0,19	nd	nd
Total concentration			6,42 ± 0,61	34,76 ± 10,17C	177,34 ± 16,33B	327,42 ± 22,52A
<i>Ketones (19)</i>						
2-Butanone	908	fruity, camphor	nd	nd	nd	1,88 ± 0,39
4-Heptanone	872	fruity, cheese, sweet	1,12 ± 0,28	nd	nd	nd
2-Heptanone	891	fruity, spicy, sweet	0,39 ± 0,03	1,09 ± 0,14B	0,55 ± 0,00C	1,92 ± 0,12A
2-Octanone	1289	earthy, herbs	nd	0,23 ± 0,07B	0,41 ± 0,15B	19,90 ± 3,81A
3-Octanone	988	fresh, sweet	0,47 ± 0,05	nd	nd	nd
2-Nonanone	1093	fresh, sweet, herbs	nd	1,38 ± 0,23B	0,83 ± 0,20B	3,91 ± 0,34A
2-Decanone	1193	floral, greasy	nd	3,61 ± 0,02B	2,66 ± 0,11C	7,80 ± 0,18A
2-Undecanone	1295	fruity, fatty	nd	0,29 ± 0,04	nd	nd
2-Pentadecanone	1699	greasy, spicy	nd	nd	0,35 ± 0,02B	0,63 ± 0,01A
2,3-Pentanedione	1062	buttery, toasted	nd	nd	1,21 ± 0,17B	5,65 ± 1,25A
2,3-Octanedione	985	asparagus, coriander	0,35 ± 0,02	1,14 ± 0,32C	2,78 ± 0,16B	5,74 ± 0,44A
2-Methyl-6-heptanone	956		0,84 ± 0,04	nd	nd	nd
Cyclohexanone	893	minty, acetone	1,74 ± 0,08	nd	nd	nd
2-Furyl methyl ketone	913	sweet, almond, cocoa	nd	nd	nd	2,44 ± 0,00
6-Methyl-5-heptene-2-one	1344	fruity, musty	nd	nd	nd	3,73 ± 0,20
1,1,3-Trimethyl-2-cyclohexanone	1035		0,38 ± 0,01	nd	nd	nd
2,6,11-Trimethyldodecane	1277		0,29 ± 0,01	nd	nd	nd
Methyl phenyl ketone	1066	sweet, coumarin	nd	nd	1,10 ± 0,22	nd
4,5,7,7a-Tetrahydro-4,4,7a-trimethyl-2(6H) benzofuranone	1542	ripe, fruity	nd	0,17 ± 0,04	nd	nd
Total concentration			5,58 ± 0,52	7,91 ± 0,86B	9,89 ± 1,03B	53,60 ± 6,74A
<i>Furans (11)</i>						
Furan	808		nd	nd	0,13 ± 0,02B	4,20 ± 0,97A

(continued on next page)

Table 2 (continued)

Identification	LRI	² Characteristic odor	¹ Concentration (µg/L)			
			^a GV	H	F4	F6
Furfural	845	sweet, woody, baked	nd	nd	1891,84 ± 166,08B	3188,44 ± 36,86A
2-(Hydroxymethyl)furan	859		nd	nd	2,50 ± 0,12B	11,55 ± 0,94A
2-Methylfuran [®]	876	ethereal, chocolate	nd	nd	0,52 ± 0,34B	15,14 ± 0,35A
2-Pentylfuran	992	fruity, earthy, bean	nd	3,43 ± 0,12B	6,57 ± 0,97B	62,72 ± 7,38A
2-Hexylfuran	1331		nd	nd	0,25 ± 0,07	nd
2-Acetylfuran	1522	almond, sweet, nut	nd	nd	nd	40,50 ± 0,69
2,2-Bifuran	1039		nd	nd	2,87 ± 0,00B	6,46 ± 0,40A
2-Allylfuran	1213		nd	nd	nd	1,25 ± 0,16
3-Phenylfuran	1224		nd	nd	3,61 ± 0,34B	14,90 ± 0,88A
(E)-2-(2-Pentenyl)furan	1303		nd	nd	1,18 ± 0,62B	23,82 ± 1,69A
Total concentration				3,43 ± 0,12C	1909,47 ± 168,56B	3368,99 ± 50,32A
<i>Hydrocarbons (14)</i>						
1-Ethyl-2-methylbenzene	979		0,54 ± 0,03	nd	nd	nd
1,3,5-Trimethylbenzene	967		1,13 ± 0,17	nd	nd	nd
1,2,4-Trimethylbenzene	992		4,84 ± 0,65	nd	nd	nd
2-Ethyl-1,4-dimethylbenzene	1076		0,34 ± 0,02	nd	nd	nd
4-Ethyl-1,2-dimethylbenzene	1079		0,41 ± 0,04	nd	nd	nd
2-Methyldecane	1063		0,96 ± 0,02	nd	nd	nd
2-Methylundecane	1164		0,12 ± 0,02	nd	nd	nd
3-Methylundecane	1170		0,22 ± 0,04	nd	nd	nd
2,6-Dimethylundecane	1214		0,68 ± 0,18	nd	nd	nd
Dodecane	1199		0,54 ± 0,12	nd	nd	nd
Tridecane	1299		0,21 ± 0,04	nd	nd	nd
Tetradecane	1399		0,40 ± 0,07	nd	0,59 ± 0,04B	2,23 ± 0,18A
Hexadecane	1597		nd	nd	nd	0,35 ± 0,02
1-Pentadecene	1544		nd	nd	1,23 ± 0,55	nd
Total concentration			10,39 ± 1,40		1,82 ± 0,59A	2,57 ± 0,20A
<i>Phenols (2)</i>						
3-Methoxythiophenol	1244		nd	nd	6,36 ± 0,05	nd
2,4-Bis(1,1-dimethylethyl) phenol	1512		0,30 ± 0,02	nd	0,48 ± 0,03B	0,42 ± 0,02A
Total concentration			0,30 ± 0,02		6,84 ± 0,08A	0,42 ± 0,03B
<i>Ester (1)</i>						
Hexyl acetate	1015	fruity, sweet	nd	nd	0,96 ± 0,00	nd
Total concentration			nd	nd	0,96 ± 0,01	nd
Maillard reaction products						
<i>Aldehydes de Strecker (4)</i>						
2-Methylpentanal	<800	fruity, green	nd	nd	6,08 ± 1,57A	2,98 ± 0,19B
2-Methylpropanal	820	fresh, pungent	nd	nd	0,29 ± 0,14B	13,38 ± 2,62A
2-methyl-butanal	923	cocoa, coffee, nuts	nd	nd	0,45 ± 0,10B	23,29 ± 1,92A
3-Methylbutanal	925	chocolate, greasy	nd	0,03 ± 0,00B	9,25 ± 1,84B	372,38 ± 34,08A
Total concentration			nd	0,03 ± 0,01B	16,07 ± 3,65B	412,03 ± 38,81A
<i>Thiols and sulfides (7)</i>						
Methyl disulfide	1080	sulphurous, malty	nd	nd	nd	0,77 ± 0,10
Methional	907	potato, earthy	nd	nd	0,77 ± 0,06B	2,01 ± 0,01A
2-Furfurylthiol [®]	910	toasted, burnt	nd	nd	0,77 ± 0,08	nd
2-Methyl-3-furanthiol [®]	867	sulphurous, meaty, chicken	nd	nd	0,93 ± 0,15	nd
2-Furfuryl methyl disulfide	1213	coffee, sulphurous, fleshy	nd	nd	nd	2,05 ± 0,29
Bis(2-methyl-3-furyl)disulfide	1537	sulphurous, fleshy, roasted	nd	nd	1,45 ± 0,05	nd
2-furfuryl 2-methyl-3-furyl disulfide	1641	sulphurous, fleshy	nd	nd	0,04 ± 0,00	nd
Total concentration					3,96 ± 0,34B	4,83 ± 0,40A
<i>Thiazóis (6)</i>						
Thiazole	1262	fish, nut, fleshy	nd	nd	0,04 ± 0,00B	4,31 ± 0,13A
4-Methylthiazole	819	nut, green, vegetable	nd	nd	nd	0,41 ± 0,02
2-Acetylthiazole	1017	nut, popcorn, toasted	nd	nd	1,06 ± 0,00B	3,46 ± 0,04A
4-Methyl-5-ethylthiazole	1026	nutty, green, earthy	nd	nd	0,53 ± 0,01B	23,00 ± 3,45A
4-Methyl-5-hydroxyethylthiazole	1285	greasy, cooked	nd	nd	47,14 ± 2,81B	337,05 ± 6,73A
5-Ethenyl-4-methylthiazole	1542	moldy, nut	nd	nd	nd	47,48 ± 12,73
Total concentration					48,77 ± 2,82B	415,71 ± 23,10A
<i>Thiophenes (5)</i>						
3-Methylthiophene [®]	<800	greasy wine	nd	nd	0,88 ± 0,06B	1,25 ± 0,05A
2-Acetyl-5-methylthiophene	1155	sweet, spicy	nd	nd	1,01 ± 0,12	nd
2-Formyl-3-methylthiophene	1122	saffron, camphor	nd	nd	2,97 ± 0,33B	6,15 ± 0,03A
2-Formyl-2,3-dihydro-thiophene	1079		nd	nd	1,37 ± 0,32B	4,96 ± 0,35A
3-Thiophenecarboxaldehyde	1000		nd	nd	5,68 ± 0,00B	9,26 ± 1,26A
Total concentration					11,91 ± 0,85B	21,62 ± 1,69A
<i>Pyrazines (4)</i>						
2-Methylpyrazine	1277	nut, cocoa, roasted	nd	0,03 ± 0,01B	nd	12,65 ± 0,41A
2-Propylpyrazine	1431	green, vegetable, nut	nd	nd	nd	19,97 ± 1,81
2,5-Dimethylpyrazine	913	cocoa, roasted, fleshy	nd	2,34 ± 0,13B	0,64 ± 0,26B	32,22 ± 3,78A
2-Methyl-5-isopropylpyrazine	1055	coffee, nut, earthy	nd	1,23 ± 0,01	nd	nd

(continued on next page)

Table 2 (continued)

Identification	LRI	² Characteristic odor	¹ Concentration (µg/L)			
			*GV	H	F4	F6
Total concentration				3,60 ± 0,15B	0,64 ± 0,26B	64,84 ± 6,00A
<i>Pyrrrole (1)</i>						
1-Furfurylpyrrole	1184	green, fruity, coffee	nd	nd	nd	5,41 ± 0,06
Total concentration						5,41 ± 0,07
<i>Others</i>						
<i>Terpenes (3)</i>						
o-Cymene	1277		nd	nd	0,07 ± 0,04	nd
6,10-dimethyl-(E)-5,9-undecadien-2-one	1455		nd	nd	0,35 ± 0,01	nd
β-Ionone	1490	woody, sweet	1,08 ± 0,16	2,02 ± 0,15B	1,78 ± 0,19B	5,15 ± 0,46A
Total concentration			1,08 ± 0,17	2,02 ± 0,16B	2,20 ± 0,24B	5,15 ± 0,47A

Results are expressed as mean ± standard deviation (n = 3). Means on the same line with different letters were significantly different (p < 0,05). Note: nd, not detected. Compounds identified in the polar column were added to the profile in a complementary way to the identification in the non-polar column, these added values are expressed in italics. More details are listed in [Supplementary Table S2](#).

¹ GV, goat viscera mixture; H, goat protein hydrolysate; F4, flavoring at pH 4.0; F6, flavoring at pH 6.0. * GV is expressed as µg/kg.

² Characteristic odor obtained from <https://www.thegoodscentscompany.com>.

Thermal degradation product of thiamine.

conditions of the methodology by [Sun et al. \(2014\)](#): initial oven temperature of 40 °C for 3 min, followed by 5 °C min⁻¹ to 120 °C and 10 °C min⁻¹ to 230 °C, where it remained for 5 min, for a while 35 min run total. The injector temperature was set at 250 °C. Helium was used as carrier gas at a flow rate of 1.0 mL min⁻¹ in the splitless injection system.

The transfer line temperature was 250 °C. The mass spectrometer was operated in electronic impact mode (70 eV) and the mass scan range was 50 to 400 m/z at 4.44 scan/s. Compounds were identified using the NIST library database (2014) (National Institute of Standards and Technology, USA) combined with mass spectrum and linear retention index.

The linear retention index (LRI) of each compound was calculated from the retention times of a homologous series of C₆-C₂₀ n-alkanes. The semi-quantification of volatiles was calculated by extrapolating the area of the internal standard 1,2-dichlorobenzene (added to each sample) to the integrated area of each compound in the profile and the data were expressed in µg/kg of viscera mixture and µg/L of hydrolysate/flavoring.

2.4. Data analysis

The experiments and analyzes were carried out with triplicate sampling. The data obtained as result of volatile compounds were expressed as mean, in which H, F4 and F6 were compared through ANOVA by Tukey's test at a significance level of 5 % (p < 0.05) in the XLSTAT application (version 2014.5.03, Addinsoft, New York, USA). The profile of fatty acids, sugars and amino acids of F4 and F6 were compared between samples by t-student test at 5 % significance level in XLSTAT.

Principal component analysis (PCA) and hierarchical clustering were applied to samples H, F4, F6, M4 and M6 using the XLSTAT software, while the standardization of data on volatile compounds, amino acids and sugars was performed using MATLAB software (The Mathworks, Inc. version 7.70.0.499, Natick, MA, R2010a).

3. Results and discussion

3.1. Physical-chemical characterization: Viscera and hydrolysate

The data obtained from the chemical composition (on a dry basis) revealed that the goat viscera mixture (GV) had a high protein content of 79.93 %, while for the hydrolysate (H) it was 72.01 % ([Table 1](#)). A low content of lipids (22.09 %), ashes (4.43 %) and carbohydrates (2.40 %) were also observed in the mixture of goat viscera. The protein content of the hydrolysate was 72.01 %, while the lipid content was 15.25 %. The low content of carbohydrates and sulfur amino acids ([Table 1](#)) in the hydrolysate confirmed the need to add the chosen amino acid in the production of the flavoring.

The hydrolysate obtained from the mixture of goat viscera reached a degree of hydrolysis of 26.49 %, close to the values found in hydrolysates of goat viscera by [Queiroz et al. \(2017\)](#) and [Cordeiro et al. \(2022\)](#), with degrees of hydrolysis ranging from 17.63 to 26.74 %, respectively. The magnitude of this hydrolysis was expected for the enzyme, which despite its low specificity, has a greater and more intense reaction kinetics on goat viscera, as previously demonstrated by [Queiroz et al. \(2017\)](#). This enzymolysis results in an increase in the amount of nitrogen available due to the released amino acids and peptides and has a greater impact on the formation of volatile compounds, which allows the hydrolysate to be used to produce flavorings ([Queiroz et al., 2017](#), [Cordeiro et al., 2022](#)).

3.2. Volatile profile: Mixture of viscera and protein hydrolysate

In the four analyzed samples, mixture of goat viscera (GV), goat protein hydrolysate (H) and flavorings (F4 and F6), a total of 117 volatile compounds were identified ([Table S2, supplementary data](#)), distributed in 15 chemical classes ([Table 2](#)). Of this amount, 60 compounds were identified in the non-polar column, 29 in the polar column and 28 in both columns. Eighty-seven volatile compounds were formed via lipid degradation, consisting of 25 aldehydes, 19 ketones, 14 hydrocarbons, 11 alcohols, 11 furans, 4 acids, 2 phenols and 1 ester. 27 compounds derived from the Maillard reaction, from the interaction of the MRI product with lipid degradation and thiamine degradation, were also identified, distributed in the classes of thiols (7), thiazoles (6), thiophenes (5), Strecker aldehydes (4), pyrazines (4) and pyrroles (1). In sum, 3 terpenes from animal feed were also found.

As expected, the predominance of volatile compounds identified in the mixture of goat viscera were hydrocarbons (12), which do not have a characteristic aroma and have a high detection threshold, thus not contributing to the composition of the desired meat aroma; followed by ketones (8), aldehydes (6) and alcohols (5) and terpene (1). Except for hydrocarbons, the main compounds found in the viscera, have aromatic notes of acetone, herbs, waxy and, citrus, which were present in low concentration, configuring little or no aromatic characteristics to the sample.

New and relevant compounds for meat flavor were formed with the protein hydrolysate production. An increase in the total concentration of volatile compounds produced was observed when comparing the profile of the viscera mixture (28.2 µg.kg⁻¹) and the hydrolysate (56.9 µg.L⁻¹). This increase can be related to the oxidation reaction of fatty acids present in the lipid fraction of GV, resulting from mild heating during the hydrolysis process, in which compounds such as benzaldehyde, decanal, octanal, 2-nonanone, 2-decanone, 2-octanone and 2-undecanone were formed, along with the intensification of other volatiles

such as: 2-heptanone, 2,3-nonanal, hexanal and heptanal. The higher molecular weight ketones, 2-nonanone (fresh, sweet and herbal notes), 2-decanone (floral, fatty) and 2,3-octanedione (asparagus and coriander), present in this hydrolysate, were previously reported by Madruga et al. (2009) as contributors to the aroma of lamb meat.

Of the twenty-seven volatile compounds identified, four were formed via the Maillard reaction, 3 pyrazines and 1 Strecker aldehyde; the remaining 23 volatiles were identified as: aldehydes (8), ketones (7), alcohols (6), furan (1) and terpene (1), thus presenting a similar characteristic to the protein hydrolysate of goat viscera by Cordeiro et al. (2020), HT, in which 26 were identified. Thus, the characteristic of the protein hydrolysate obtained in the present study was oxidized and fatty, which can be associated with the aroma of the species, strongly expressed by the aldehyde compounds, predominant in the sample, thus indicating that the intermediate product still did not contain a sufficiently meaty volatile profile, but that it demonstrated a positive potential for the formation of impacting compounds for the meaty aroma.

3.3. Products of the Maillard reaction of flavorings

The formation and concentration of volatile compounds that make the meat aroma depend on the presence and concentration of the precursors as well as the conditions of the applied treatment, such as time, temperature, and pH, involving lipid oxidation reactions, degradation of vitamins and the Maillard reaction (Li and Liu, 2021; Cunha et al., 2023).

It is possible that the protonation of the amino group of amino acids is important (Guo et al., 2010), which leads to the hypothesis that pH conditions can direct the formation of volatile compounds and influence the interaction between amino groups and carbonyls. At a more acidic pH, there are more hydrogen ions in the medium (and vice versa); which makes the amino group more protonated (linked to H ions) and, consequently, less reactive in the interaction with carbonyls in the Maillard reaction. The more acidic condition induces 1,2-enolization of Amadori compounds in which furfural is intensified and, as the reaction progresses, leads to the formation of sulfur-substituted furans. The less acidic pH induces enolization-2,3, via which the formation of dicarbonyl compounds, thiophenes and pyrazines is more pronounced. This occurs because in a higher pH condition the amino groups are not protonated and, consequently, more reactive; which favors the condensation of amino acids or ammonia with dicarbonyl compounds that lead to the formation of the aforementioned compounds (Madruga et al., 1995; Mottram, 1998).

Xylose-cysteine RM products were obtained by Guo et al. (2010) in hydrolysates heated to 160 °C and pH 4.0, leading to the formation of an aroma like cooked and roasted meat, resulting from compounds with low detection threshold. In this study, the lowest temperature of 121 °C for 60 min under different pH (4.0 and 6.0) was applied in the elaboration of flavorings supplemented with 1 g of xylose, 0.1 g of cysteine and 0.5 g of thiamine, with the aim of increasing the volatile composition and obtaining meat, sweet, roasted, and toasted aromas, mainly through the Maillard reaction and lipid degradation. Studies have demonstrated that meat aroma can be modulated by inducing the Maillard reaction using precursors of protein hydrolysates from by-products and supplements such as cysteine, xylose, and thiamine. Cysteine, a sulfur amino acid, stands out for its involvement in the formation of volatile compounds with a low detection threshold and characteristic aroma of meat, roast, and coffee (Cardinal et al., 2020; Gao et al., 2020; Li and Liu, 2021). Xylose has demonstrated greater reactivity compared to hexose, making it an excellent precursor of meat aroma (Cunha et al., 2023). Meanwhile, thiamine (vitamin B1) is a water-soluble vitamin naturally present in meat, and even in low concentrations, it is associated with the formation of compounds such as 2-furfurylthiol and 2-methyl-3-furanthiol, due to its high susceptibility to thermal degradation (Cerny and Briffod, 2007; Begum et al., 2019).

The hydrolysate supplementation by these raw materials provided

complexity and diversity in the volatile composition of the flavorings produced, since in a previous hydrolysate of autoclaved goat viscera and without supplementation, only 35 volatile compounds were formed (Cordeiro et al., 2020). In the flavorings produced from the hydrolysate of goat viscera, 96 volatile compounds were identified, and for the flavoring prepared at pH 4, 20 specific volatile compounds were identified. For the flavoring prepared at pH 6, only 15 different volatile compounds were formed and 61 were present in both flavorings. Despite the volatile composition of both having compounds in common, there is a significant difference in terms of concentration, with a higher concentration in the pH 6 flavoring (4,943.98 $\mu\text{g.L}^{-1}$) compared to the pH 4 flavoring (2,252.53 $\mu\text{g.L}^{-1}$). It is important to emphasize that the concentration is not always a parameter proportional to the aromatic impact, since there are compounds that have very low detection thresholds and manage to configure unique characteristics for the product.

As shown in Table 2, a total of 66 volatile compounds were formed in flavorings through the thermal degradation of lipids, identified as acids (4), alcohols (10), aldehydes (24), ketones (11), furans (11), hydrocarbons (3), phenols (2) and ester (1); and twenty-six formed via MR, grouped as Strecker's aldehydes (4), sulfur compounds (7), thiazoles (6), thiophenes (5), pyrazines (3) and pyrrole (1).

3.3.1. Aldehydes

Among the volatiles formed via lipid degradation, aldehydes were the predominant class in quantity at both pH, demonstrating a significant difference ($p < 0.05$) in the total concentration, with the F6 flavoring having the highest concentration. Benzaldehyde was the most abundant in both flavorings and there was no significant difference regarding its concentration. Its production can occur through oxidation or photochemical degradation of toluene or other hydrocarbons (Cordeiro et al., 2022).

The unsaturated aldehydes, more sensitive to oxidation, such as (E)-2-octenal and (E,E)-2,4-decadienal, corresponded to approximately 5 % of the total concentration of the class. During the flavoring production process, unsaturated aldehydes may have been consumed and involved in reactions with amino acids, products of the Maillard reaction, such as H_2S and NH_3 , or oxidized. (E,E)-2,4-decadienal, present only in F4, is an impacting compound for the aroma of cooked meat (Madruga et al., 2009) and can be generated from the oxidation of n-3 unsaturated fatty acids (Qi et al., 2022), contributing with aromatic notes of fatty and chicken.

The concentration of saturated aldehydes differed between samples, in which hexanal, pentanal, heptanal and nonanal was increased by at least 10x from hydrolysate to flavoring. The presence of these oxidized lipids is essential for reactions with the products of the Maillard reaction and for the synthesis of impacting compounds such as thiazoles, pyrazines and thiophenes (Yang et al., 2015). Hexanal, resulting from the oxidation of linoleic, linolenic and arachidonic acids, present in all samples, can give aromatic notes of green, fresh and fatty, presented in higher concentration in flavoring F6 (8.5 $\mu\text{g.L}^{-1}$) in comparison with flavoring F4 (5.85 $\mu\text{g.L}^{-1}$); given that its detection threshold is 5 $\mu\text{g.L}^{-1}$, this compound may have contributed positively to the aroma, since at low concentrations it can lead to a pleasant aroma in meat products, while high concentrations contribute to a rancid aroma (Wang et al., 2022), to the point of being used as an indicator of aromatic rancidity in meat (Al-Dalali et al., 2022).

3.3.2. Alcohols

Aliphatic alcohols found in flavorings ranged from 44.30 $\mu\text{g.L}^{-1}$ in F4 to 105.23 $\mu\text{g.L}^{-1}$ in F6; which can be metabolites of lipid degradation, since aliphatic aldehydes can be reduced to their respective alcohols by reducing agents in the environment, such as the present ones 1-hexanol, 1-heptanol and 1-octanol. Alcohols can also be RM intermediates, although the oxidation of polyunsaturated fatty acids is the main route of formation of these compounds (Al-Dalali et al., 2022). The highest

concentration alcohols were 2-methyl-1-buten-3-ol and 1-octen-3-ol, both significantly higher in F6; which were present in the previous stages of the process (GV and H). The presence of 1-octen-3-ol, with earthy, green, oily, vegetable and chicken characteristics, is associated with the degradation of linoleic and arachidonic fatty acids and may contribute to the overall meat aroma (Cordeiro et al., 2020).

3.3.3. Furans

Considering the concentration, furans were the main class of volatiles, mainly formed via lipid degradation, differing significantly between treatments F4 and F6. Furfural, mainly derived from the dehydration of xylose, was the predominant compound, and the pH 6 favored its formation, leading to a concentration of 3188.44 $\mu\text{g.L}^{-1}$, much higher than F4, with 1891.84 $\mu\text{g.L}^{-1}$. This result goes against a finding in a pork by-product flavoring supplemented with xylose (Li and Liu, 2021), where the acidic pH may have increased the formation of furfural as a consequence of the reduced interaction between reducing sugars and amino acids. Another route of furfural formation is through the enolization of Amadori compounds, under acidic conditions and high temperatures. In later stages, furfural can react with more reactive compounds such as amines, amino acids, aldehydes, hydrogen sulfides and ammonia, leading to the formation of compounds with a characteristic meat aroma (Mottram, 1998). 2-pentylfuran, responsible for fruity, green and earthy nuances, was another prominent compound in F6, contributing 62.72 $\mu\text{g.L}^{-1}$ to the flavoring, ten times higher than that found in F4. The synthesis of this compound takes place by thermal oxidation or auto-oxidation of linoleic acid, and its behavior at pH 6 corroborates the result found by Li and Liu (2021), in which the increase in 2-pentylfuran is also favored by the higher pH.

3.3.4. Ketones

Ketones were the second largest group of lipid-derived compounds, being dominant in F6, with 53.60 $\mu\text{g.L}^{-1}$ total, mainly due to compounds such as 2-octanone, 2-nonanone, 2-decanone, 2,3-octanedione, 2,3-pentanedione and 6-methyl-5-heptene-2-one. Despite having a high detection threshold, which leads to a low contribution to the aroma (Madruga et al., 2010), in gas chromatography/olfactometric analysis, the compounds 2-nonanone, 2,3-octanedione and 6-methyl-5-heptene-2-one have been reported as compounds of high aromatic intensity (≥ 3) in cooked goat meat (Qi et al., 2022).

3.3.5. Acids

Acids have shown little contribution to the overall aroma of flavorings due to their high detection thresholds (Gao et al., 2020; Sohail et al., 2022). The higher pH led to a significant difference in the total acid concentration between F4 and F6, attributed to the presence of hexanoic acid (caproic acid), with 12.41 $\mu\text{g.L}^{-1}$ in F4 and 154.07 $\mu\text{g.L}^{-1}$ on F6. In addition to imparting a fatty and caprine aroma, this acid is responsible for highlighting the flavor of the species (Madruga et al., 2010), but this would not be possible due to the concentrations being below the detection threshold – 890 $\mu\text{g.L}^{-1}$ (Sohail et al., 2022).

3.3.6. Thiazoles

Xylose, cysteine, and thiamine are used as precursors to obtain flavorings because the products of their interaction have a characteristic meat, roasted, toasted, and sulfured aroma, and have a low detection threshold and high aromatic impact (Cerny, 2007; Guo et al., 2010; Gao et al., 2020; Li and Liu, 2021; Begum et al., 2019). In this study, it was observed that the pH conditions directed the formation and concentration of volatile compounds formed via the Maillard reaction (Table 2), as suggested by Madruga and Mottram (1995), in which the formation of meat aroma could be dependent on of the pH. Among the 26 compounds formed via MR, six compounds were dominant, considering the concentration and detection threshold: 3-methylbutanal, 2-methyl-3-furanthiol (MFT), 2-furfurylthiol (FFT), bis(2-methyl-3-furyl)disulfide (MFT-MFT), 4-methyl-5-hydroxyethylthiazole and 2,5-

dimethylpyrazine. Sulfur compounds, pyrazines, thiazoles and thiophenes are the main contributors to the aroma of roasted and cooked meat.

Thiazoles were the odorants present in the highest total concentration in the MR products, significantly higher in F6 (415.71 $\mu\text{g.L}^{-1}$) when compared to F4 (48.77 $\mu\text{g.L}^{-1}$). The major thiazole isolate was 4-methyl-5-hydroxyethylthiazole, a primary compound formed exclusively from the thermal degradation of thiamine in MR (Güntert et al., 1994; Cerny and Briffod, 2007). Except for 4-methylthiazole and 5-ethenyl-4-methylthiazole, which were not identified in F4, it is possible to indicate that the more acidic pH did not favor the formation of thiazoles. In Cerny and Briffod (2007) the formation of 4-methyl-5-hydroxyethylthiazole is not influenced by pH, whereas in this study it appears significantly higher in F6, incorporating to this, fatty, cooked and meaty notes; the justification for the formation of this type of compound is given by the fact that thiamine has a chemical structure composed of a thiazole (4-methyl-5-2-hydroxyethylthiazole) and a pyrimidine fraction (2-methyl-4-amino-5-hydroxymethylpyrimidine) (Güntert et al., 1994).

Nutty, popcorn and roasted notes conferred by 2-acetylthiazole were attributed to the F6 aroma, since the concentration presented in F4 is below the detection threshold of this compound, 3 $\mu\text{g.L}^{-1}$ (Sohail et al., 2022). Its formation occurs from the condensation between a dicarbonyl and cysteine, through a decarboxylation, followed by the nucleophilic addition of a thiol group and subsequent elimination of hydrogen to consequently obtain the compound (Zhao et al., 2019). The thiazole and 4-methylthiazole compounds may not have contributed to the meat aroma, since their concentrations are low when compared to their high detection thresholds, 38 $\mu\text{g.L}^{-1}$ and 55 $\mu\text{g.L}^{-1}$, respectively (Sohail et al., 2022).

3.3.7. Thiols and sulfides

In addition to the above, thermal degradation of thiamine is also reported to produce compounds such as 2-methylfuran, 2-methylthiophene and 2-methyl-3-furanthiol (MFT), as well as may be involved in the production of 2-furfurylthiol (FFT). The formation of 2-methylfuran was not favored by the most acidic condition, with a concentration of only 0.52 $\mu\text{g.L}^{-1}$, while pH 6 led to the formation of 15.14 $\mu\text{g.L}^{-1}$, in which its production is associated with the reduction of furfural. 3-methylthiophene, a homologue of 2-methylthiophene, well reported in the literature, can be formed by the transformation of 2-methylfuran in the presence of H_2S (Zhao et al., 2019). The presence of thiamine seems to be fundamental to enhance the formation of impacting sulfur compounds, such as MFT, since only the cysteine/xylose system would lead to the formation of a lower concentration of compounds (Cerny and Bifford, 2007).

Unlike most volatile compounds, the lower pH favored the formation of MFT, FFT and bis(2-methyl-3-furyl) disulfide, while their presence was not detected in F6. Such compounds are extremely impactful for meat aroma, since their detection thresholds vary from 0.000032 $\mu\text{g.L}^{-1}$ to 0.13 $\mu\text{g.L}^{-1}$ (Sohail et al., 2022). In F4 a significant amount of MFT was detected – 0.93 $\mu\text{g.L}^{-1}$. Given the Amadori rearrangement between xylose and cysteine, there is formation of an intermediate that can be decomposed to dicarbonyls, furanones and furfurals, which react with H_2S (Strecker degradation product of cysteine) for subsequent formation of thiols, such as MFT (Mottram, 1998); which, when oxidized, forms a corresponding disulfide, MFT-MFT, present in F4 with 1.45 $\mu\text{g.L}^{-1}$.

The occurrence of furfural with H_2S can also lead to the production of FFT, formed in 0.77 $\mu\text{g.L}^{-1}$ in F4. The pH difference seems to have been crucial for the formation of these compounds, where a more acidic condition would be more propitious for their formation, as observed by Madruga and Mottram (1995) and Gao et al. (2020).

Methional, a sulfur present in higher concentrations in F6, can be produced by thermal manipulation of the amino acid methionine (Güntert et al., 1994; Li and Liu, 2021). It has already been reported as having an impact on the aroma of cooked meat and identified in the profile of cooked goat meat (Madruga et al., 2009).

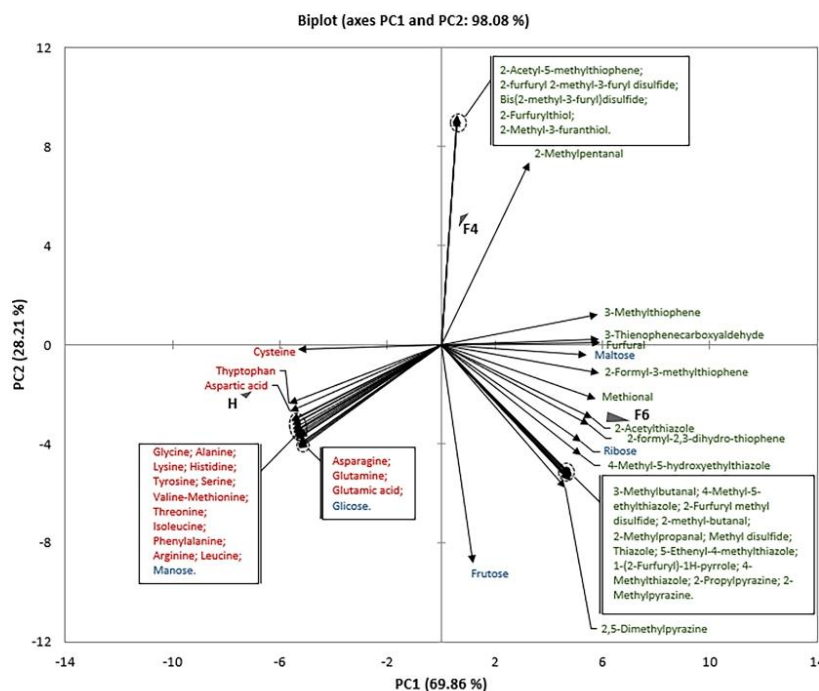


Fig. 1. Analysis of the main components of the volatile compounds that impact meat aroma, sugars, and amino acids in the goat protein hydrolysate and flavorings. F6, flavoring at pH 6; F4, flavoring at pH 4; H, goat protein hydrolysate.

3.3.8. Strecker's aldehydes

Produced through the Strecker degradation, 3-methylbutanal is an important intermediate in the Maillard reaction (RM), originating from the degradation of the amino acids valine and leucine. Its formation started in the hydrolysate, in which it was poorly developed, presenting in a concentration very close to its detection threshold, $0.2 \mu\text{g}\cdot\text{L}^{-1}$ (Gao et al., 2020). In flavorings, the higher pH favored the formation of this compound, with a total of $372.38 \mu\text{g}\cdot\text{L}^{-1}$, while the more acidic pH led to the formation of only $9.25 \mu\text{g}\cdot\text{L}^{-1}$. Due to the low detection threshold, the chocolatey and fatty aromas imparted by 3-methylbutanal can be easily noticed. In previous works, 3-methylbutanal and 2-methylbutanal were part of the aromatic profile of hydrolysates and cooked goat meat (Cordeiro et al., 2022, Madruga et al., 2009, 2010) and sheep (Zhan et al., 2017). The degradation of the valine amino acid can also be the source of the formation of 2-methylpropanal (VARLET et al., 2007). These aldehydes are formed when an amino acid is decarboxylated and deaminated, at the same time that the dicarbonyl compounds, derived from RM, are converted into aminoalcohols or aminoketones. This amino acid being cysteine, in Strecker degradation it can be converted to ammonia, acetaldehyde or hydrogen sulfide. Thiophenes, thiazoles, pyrazines, pyrroles, furans and oxazoles are examples of classes with compounds with a characteristic meat aroma that can be generated by the interaction of dicarbonyl compounds with Strecker degradation products (Mottram, 1996).

3.3.9. Pyrazines

Pyrazines are nitrogenous heterocyclic compounds that contribute to the aroma of cooked meat, usually produced under high temperatures through MRI and Strecker degradation by different routes and precursors, such as α -aminocarbonyls, generated in Strecker degradation

from the reaction between amino acids and dicarbonyl compounds (butanedione, glyoxal, ethylglyoxal and pyruvaldehyde) (Yu and Zhang, 2010). During heat treatment, these types of precursors react with other compounds to produce pyrazines, which have a characteristic roasted, cooked, and nutty odor. In this study, the higher pH favored the formation of pyrazines in F6, as expected, since low pH can inhibit the formation of these compounds due to acidic conditions (Madruga and Mottram, 1995, Yu and Zhang, 2010). 2,5-dimethylpyrazine has a cocoa aroma, roasted and fleshy, and has a detection threshold of $1.75 \mu\text{g}\cdot\text{L}^{-1}$, suggesting that such characteristics may have an impact on F6. The alkyl pyrazines 2-propylpyrazine and 2-methylpyrazine were isolated only in F6 and impart nutty and roasted characteristics; 2-methylpyrazine was reported as a key compound in the reaction of ascorbic acid, threonine, and serine, increasing significantly with increasing pH (Yu and Zhang, 2010).

3.3.10. Thiophenes

Total thiophene concentration was significantly higher in F6 ($21.62 \mu\text{g}\cdot\text{L}^{-1}$) than in F4 ($11.92 \mu\text{g}\cdot\text{L}^{-1}$). These compounds are reported in cooked meats (Zhao et al., 2019; Sohail et al., 2022) and considered important for the construction of meat aroma; formed by the interaction of degradation products of sugars and H_2S , or by the thermal degradation of sulfur amino acids (Farmer, 1994). 3-thiophenecarboxaldehyde and 2-formyl-3-methylthiophene were the two main thiophenes found in flavorings.

3.3.11. Pyrrole

The reaction between H_2S , NH_3 and furans may also have led to the formation of the nitrogenous compound 1-furfurylpyrrole, the only pyrrole present and isolated only in F6. Its mechanism of action may

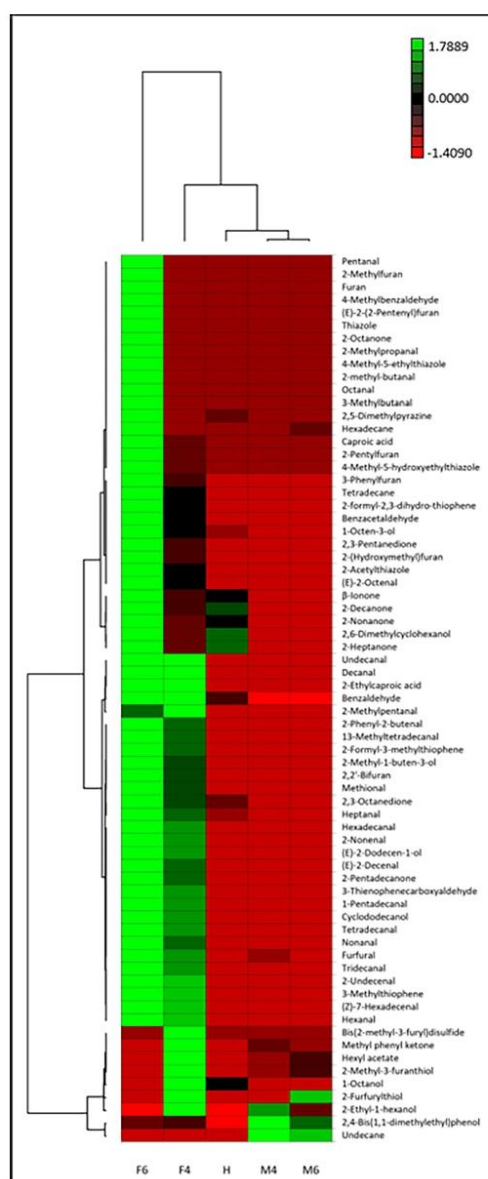


Fig. 2. Heat map generated based on volatile compounds of flavorings and model systems. F6, flavoring at pH 6; F4, flavoring at pH 4; H, goat protein hydrolysate; M4, model system at pH 4; M6, model system at pH 6.

involve the cyclization of condensation products formed from amino acids and 1,2-dicarbonyl derivatives of aldose and ketose sugars (Farmer, 1994). Its green, fruity and coffee notes were important for the configuration of the F6 aroma, since its content was much higher than its detection threshold of $0.1 \mu\text{g}\cdot\text{L}^{-1}$ (Sohail et al., 2022).

3.4. Volatile compounds by principal component analysis

In order to effectively understand the effect of pH and the contribution of free amino acid precursors and sugars (Table 1) in the formation of impacting compounds for the meat aroma of flavorings considering the hydrolysate, a principal component analysis was applied, a statistical technique for dimensionality reduction, in order to transform many original variables into a smaller and representative number. Hydrolysate and flavorings are represented by triangles whose vertices correspond to the replicates. Samples present in different quadrants show different behavior. The first principal component (PC1) explained 69.9 % of the variation in the data set, while the second principal component (PC2) explained 28.2 % of the variation, reaching a cumulative variance contribution rate of 98.1 %.

As shown in Fig. 1, the constituents were distributed and isolated in three regions of the PCA plot. Separation along PC1 was associated with the volatile compounds furfural, methional, 2-acetylthiazole, 3-methylthiophene, 3-thiophenecarboxaldehyde and 2-formyl-3-methylthiophene, and the amino acids aspartic acid, histidine, glycine, alanine, tryptophan and lysine. While 2-methyl-3-furanthiol (MFT), 2-furfurylthiol (FFT), bis(2-methyl-3-furyl) disulfide (MFT-MFT), 2-furfuryl 2-methyl-3-furyl disulfide and 2-acetyl-5-methylthiophene are the compounds that showed the greatest influence on the definition of PC2. In Fig. 1, a grouping tendency of the compounds can be seen, where the sulfides FFT, MFT and their corresponding disulfide, plotted on the positive side of PC1, were strongly correlated to F4; this result indicates that the formation of these compounds, with greater impact and characterization of meat aroma and low detection threshold, is strongly influenced by pH 4. While 2-formyl-3-methylthiophene, 2-formyl-2,3-dihydro-thiophene, 2-acetylthiazole, methional, Strecker's aldehydes and the sugars maltose and ribose are strongly correlated to F6; suggesting the greater influence of these sugars on the formation of the previously mentioned volatiles and inferring that they are dependent on pH 6. The relation of these last volatiles to pH 6 can direct this pH to form a goat aroma, more characteristic of the species, rather than purely meaty. The hydrolysate, plotted on the negative side of PC1, was associated with free amino acids, indicating that its volatile contribution was strictly due to the supply of amino acid precursors.

3.5. Volatile compounds of flavorings by cluster analysis

The heat map groups similar characteristics, where the values corresponding to the concentrations of each compound in the samples are expressed in color intensities, where the greener, the greater the value and the more different from the other compounds; the more red, the lower the value and more distinct in relation to the others; and, when black, there was no differential expression.

In Fig. 2, in each line there is a volatile compound and, in the columns, the samples, both grouped by clusters. In this analysis, the volatile profile of the model systems (MS) was presented as a new categorical variable, with the objective of evaluating the volatile compounds that would be provided only by the supplements xylose, cysteine and thiamine, when treated in the same condition of flavoring production, where M4 were the supplements at pH4 and M6 – the supplements at pH 6. The volatile profile of the model systems M4 and M6 totaled only 25 compounds (data not shown), which when plotted on the heat map it was possible to infer that only the model system wasn't enough to express a characteristic profile, suggesting that its function in flavorings was to potentiate the formation of volatile compounds, and that the other components of the hydrolysate, namely amino acids, sulphured and non-sulphured (Table 1), and lipids (Table S1), they had a great contribution to the formation of volatile compounds of flavorings; as well as only the heat-treated hydrolysate (HTA) by Cordeiro et al. (2020) would lead to the formation of a lower amount of volatiles than that obtained in flavorings and with little characteristic of the meat aroma.

Clustering gathered the samples into four groups. The first group

gathered the MS, as expected, since the volatile profile of both is similar. The second cluster grouped hydrolysate (H) and the MS, the third associated F4 with H and the MS, while the fourth cluster related F6 to F4, H and the MS.

The heat map (Fig. 2) shows that the most expressive volatile compounds to distinguish the F6 flavoring were: pentanal, 2-methylfuran, furan, 4-methylbenzaldehyde, (E)-2-(2-pentenyl)furan, thiazole, 2-octanone, 2-methylpropanal, 4-methyl-5-ethylthiazole, 2-methylbutanal, 2,5-dimethylpyrazine, hexadecane, caproic acid, 2-pentylfuran, 4-methyl-5-hydroxyethylthiazole, 3-phenylfuran, tetradecane, 2-formyl-2,3-dihydro-thiophene, benzacetaldehyde, 1-octen-3-ol, 2,3-pentanedione, 2-(hydroxymethyl)furan, 2-acetylthiazole, (E)-2-octenal, β -ionone, 2-decanone, 2-nonanone, 2,6-dimethylcyclohexanol and 2-heptanone. The most significant volatile compounds for F4 were: bis (2-methyl-3-furyl)disulfide, methyl phenyl ketone, hexyl acetate, 2-methyl-3-furanthiol, 1-octanol, 2-furfurylthiol, 2-ethyl-1-hexanol. This demonstration confirms that flavoring F6 had a more intense volatile expression of the compounds as a whole, while in F4 thiols were strongly expressed.

Thus, it is possible to indicate that the volatile compounds reported as more expressive in flavoring F4 were the least expressive in F6, leading to a distinction between the aromatic profile of F4 and F6.

4. Conclusion

The present study demonstrated that the formation and concentration of volatile compounds in flavorings were impacted by the presence of supplements and hydrolysate precursors, as well as pH-dependent. The pH conditioning led to the conclusion that the more acidic pH favored the thiols, offering a flavoring with a strongly meaty profile, while the less acidic pH favored the other compounds, which, despite not directing exactly to the meaty aroma, are often found in cooked and roasted meats and contribute to their aroma, providing this flavoring with an intense sweet, fatty and goat aroma. Thus, the products obtained are configured as potential new ingredients that can provide or intensify the meat aroma in food; as well as a new approach to transforming and adding value to goat by-products.

CRedit authorship contribution statement

Viviane Maria de Sousa Fontes: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Mércia de Sousa Galvão:** Methodology. **Leila Moreira de Carvalho:** Software. **Fabian Laís do Nascimento Guedes:** Formal analysis. **Marcos dos Santos Lima:** Methodology, Software, Supervision. **Taliana Kênia Alencar Bezerra:** Visualization, Writing – review & editing. **Marta Suely Madruga:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

The authors thank the Fundação de Apoio à Pesquisa do Estado da Paraíba (The Paraíba State Foundation for the Support of Research; FAPESQ) for their financial support on Project 006/2018/PRONEX, the Conselho Nacional de Desenvolvimento Científico e Tecnológico (National Council for Scientific and Technological Development; CNPq),

and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) for scholarships provided to M. S. Madruga and V. M. S. Fontes, respectively.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2024.138398>.

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Further reading

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5 CONSIDERAÇÕES FINAIS

Mediante o estudo realizado, foi observado que as vísceras caprinas têm um elevado conteúdo proteico, sobre a qual foi possível a obtenção de um hidrolisado com um grau de hidrólise satisfatório, que disponibilizou precursores mais reativos, aminoácidos livres, para a posterior produção dos aromatizantes. O perfil lipídico das vísceras caprinas foi responsável pela formação da maioria dos compostos voláteis do hidrolisado e dos aromatizantes. O aroma do hidrolisado foi positivamente agregado com pirazinas e aldeído de Strecker. Nos aromatizantes elaborados em pH 4,0 e 6,0, utilizando o hidrolisado enriquecido com xilose, cisteína e tiamina foram identificados um total de 96 compostos voláteis, dentre eles, compostos de baixo limiar de detecção e de característico aroma cárneo, como os tióis 2-methyl-3-furanthiol e 2-furfurylthiol, os tiazóis 2-acetylthiazole e 4-methyl-5-hydroxyethylthiazole, tiofenos como 3-methylthiophene e pirazinas como 2,5-dimethylpyrazine.

A formação e a concentração dos compostos voláteis nos aromatizantes F4 e F6 foi fortemente influenciada pelo pH do meio; onde o pH mais ácido favoreceu os tióis, oferecendo um aromatizante com perfil fortemente cárneo, enquanto pH menos ácido favoreceu os demais compostos, os quais apesar de não direcionarem exatamente para o aroma cárneo, são frequentemente encontrados em carnes cozidas e assadas e contribuem para o aroma destas como um todo, e permitem que neste aromatizante também sejam encontradas notas mais doce, gorduroso, cárneo e caprino.

Para estudos futuros, sugere-se que os aromatizantes obtidos sejam avaliados quanto ao perfil sensorial, a fim de analisar a percepção dos consumidores quanto a este novo ingrediente, bem como sugere-se que seja investigado a incorporação dos mesmos a materiais que mantenham sua capacidade aromatizadora, para ter-se um ingrediente com maior estabilidade volátil para uma vida de prateleira.