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The deodorising and flavouring effect of enzymatic hydrolysis and glycation on boiled pig trotters

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Abstract: To eliminate the unpleasant odour and produce more flavour, enzymatic hydrolysis with bromelain (Bro), papain (Pap), and Bro + Pap and boiled with maltose were applied in pig trotters. In this study, Pap, Bro, and a combination of Bro + Pap were dissolved in a saline solution to treat pig trotters. Results showed that the Bro + Pap treatment produced more amino acids, and the boiled + roasted with sugar of Bro + Pap treatment could significantly reduce aldehydes (nonanal and octanal) associated with the formation of off-flavour. Additionally, it increased the content of esters (ethyl propionate, ethyl isobutyrate, ethyl isovalerate, ethyl acetate, methyl acetate, and butyl acetate) in pig trotters, resulting in a more pleasing flavour. Electronic nose signals and sensory evaluation experiments further confirmed these findings. Moreover, the deodorising and aroma-enhancing process also improved their overall eating quality.

Keywords: volatiles; off-flavour; bromelain; papain; electronic nose

Pig trotters are known to be high in protein, fat and carbohydrates as well as in calcium, phosphorus, magnesium, iron, vitamins and other beneficial ingredients (Zhou et al. 2021). However, the bloody taint of raw meat remains when the meat was cooked inappropriately (Chang et al. 2021). Therefore, its consumption is restrained, as thermal processing produces aldehydes and ketones that are the main source of off-flavour (Yu et al. 2016). Traditional processing mainly involves the use of sauce, wine and spices to mask the off-flavours, while these seasonings can vary depending on their origin and brand (Zhang et al. 2022). Therefore, it is difficult to standardise operations for large-scale industrial production.

The flavour is a crucial aspect of food quality and an important factor that influences consumer food purchasing preferences and behaviours (Begum et al. 2021). Normally, protein degradation plays a role

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in the formation of meat flavour (Cheng et al. 2023). However, protein degradation that occurs because of the ageing of meat contributes to the development of a bitter flavour (Aaslyng and Meinert 2017). Meat flavours will be affected by the enzymatic hydrolysis of meat protein through increasing amino acids and small peptides (Xu et al. 2018; Begum et al. 2021), which can further form aroma with reducing sugars via the Maillard reaction. As a result, proteolytic reactions combined with the Maillard reaction can contribute to the formation of meat flavours (Song et al. 2016; Begum et al. 2021; Li et al. 2023).

Amino acids, e.g. glutamic acid, lysine, methionine, phenylalanine, serine, tyrosine, proline, and histidine, have been considered to be important flavour enhancers in foods; their contents can increase significantly after being treated with bromelain (Bro) and papain (Pap) (Xu et al. 2020; Zhao et al. 2020; Li et al. 2023). Additionally, aldehydes and ketones react with amino acids during enzyme treatment, contributing to the production of flavour through the Maillard reaction (Khan et al. 2015; Kanzler and Haase 2020). Consequently, the enzymatic hydrolysis and Maillard reaction are typically employed to facilitate the generation of dominant aroma compounds (Han et al. 2021).

The injection of marinade is determined to be more effective in imparting flavour to the pork than the traditional application of marinade, as the marinade can be evenly distributed throughout the meat (Peñaranda et al. 2024). Han et al. demonstrated that high-temperature stewing with enzymatic degradation and the Maillard reaction exhibit the highest concentration of volatile compounds among different treatments (Han et al. 2021). However, xylose instead of caramel was used in Han's study. Aaslyng and Meinert proposed that the flavour intensity of roasted meat could be enhanced by increasing the concentration of carbohydrates in the muscle (Aaslyng and Meinert 2017).

The aim of this study is to eliminate the unpleasant odour and produce more flavour in meat through enzymatic hydrolysis and the Maillard reaction. Therefore, pig trotters were injected with Bro, Pap, and Bro + Pap and boiled via different methods. The effects of enzymatic hydrolysis on the quality of pig trotters were investigated by measuring free amino acids. An electronic nose and a sensory evaluation were employed to analyse the distinctive flavour in boiled samples. Meanwhile, headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS) was conducted to qualitatively assess the volatile flavour substances created via cooking treatments.

MATERIAL AND METHODS

Material and reagents. Pig trotters were collected from Nanjing Luoyu Trading Co., Ltd. (Nanjing, Jiangsu, China). Bro (200 units·mg^{-1}) and Pap (200 units·mg^{-1}) were purchased from Nanning Doing-Higher Bio-Tech Co., Ltd. (Nanning, Guangxi, China). O-dichlorobenzene [standard for gas chromatography (GC), > 99.8%], hexanal (97%), octanal (97%), 1-octen-3-ol (98%), undecane (≥ 98%) and dodecane (98%) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Nonanal (98%) was purchased from Nanjing Crystal Lattice Chemical Technology Co., Ltd. (Nanjing, Jiangsu, China).

Sample preparation. Bro or Pap (0.05 g; 0.02 units·g^{-1} of pig trotters) was dissolved in a saline solution (2% NaCl, 500 g) at a temperature of 50–55 °C, and the Bro + Pap group was a combination of the Bro and Pap solutions in equal proportions. Then, the defrosted pig trotters (500 g) were split evenly into half. Meanwhile, 50 g of the saline solution was injected into half of the pig trotters (250 g), while the remaining pig trotters were soaked in the saline solution. Subsequently, the trotters were tumbled in a vacuum tumbler at 6 rpm (revolutions per minute) for 30 min. Then, the mixture was transferred into a steam bag [PET/PE/PA (polyethylene terephthalate/polyethylene/polyamide)] and heated in a water bath at 55 °C for 30 min, followed by cooking at 100 °C for 30 min. Subsequently, the juices were discarded, and maltose was used to evenly coat the surface of the pig trotters. Finally, all pig trotters were roasted in an oven at 200–220 °C for 20 min. Of which, the blank samples were devoid of both enzyme and caramel, while composite enzymes samples comprised 0.025 g of Bro and Pap, respectively. All trotters were cooled to 25 °C before further analysis. Samples in each group were prepared in biological triplicates (n = 3).

Free amino acid detected by high-performance liquid chromatography (HPLC). Free amino acids were detected using HPLC (Zhao et al. 2020). For this, the samples (2 g) were mixed with 0.02 mol·L^{-1} of hydrochloric acid (10 mL) and shaken for 1 min. Subsequently, the mixture was ultrasonicated for 10 min and then left in the dark for 2 h. Afterwards, 1 mL of the reaction solution and 1 mL of 8% sulfosalicylic acid were mixed in the dark. After 2 h, 500 μL of the reaction solution was mixed with 250 μL of the acetonitrile solution of benzene isothiocyanate (0.1 mol·L^{-1}) and triethylamine (1 mol·L^{-1}). After being derived for 1 h, 2 mL of hexane was added and shaken until the layers were separated. Then, the liquid of the lower

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layer was passed through an organic filtration membrane to be analysed using the Agilent LC1200 system (Agilent Technologies, USA) with a Capcell PAK C18 SG300 column (Osaka Soda, Japan). Gradient eluent A was aqueous sodium acetate (0.1 mol·L^{-1}) and 5% acetonitrile, while eluent B was 80% acetonitrile and 20% water. Each group was measured in triplicates.

Flavour changes measured by an electronic nose. According to Zhao et al. (2020), flavour changes in pig trotters were measured using a PEN3 electronic nose (Airsense Analytics GmbH, Germany). In brief, minced pig trotters (1.5 g) were sealed in a headspace vial of 20 mL which was then heated in a water bath at 60 °C for 8 min. The sample was analysed at 1-s intervals, with a sensor cleaning time of 120 s and a zeroing time of 10 s. The analysis was conducted at a gas flow rate of 400 mL for min, and the collection gas analysis time was 120 s.

Volatile compounds identified by headspace-gas chromatography-ion mobility spectrometry. The volatile compounds were identified using HS-GC-IMS (FlavourSpec®; G.A.S., Germany), as per the procedure reported in a previous study (Zhao et al. 2020). For this, minced stewed pig trotters (2 g) were put in a headspace vial of 20 mL and incubated at 60 °C for 15 min. The headspace bottle (500 µL) was squirted into an automatic static headspace analyser of 65 °C and further separated by a capillary column of FS-SE-54 (15 m × 0.53 mm) using nitrogen (purity ≥ 99.99%, 2 mL·min^{-1}) as the carrier gas for 2 min. Afterwards, the flow rate was increased to 100 mL·min^{-1} for 20 min before stopping. The analytes were ionised in the IMS ionisation chamber at 45 °C, and the drift gas flow was maintained at 150 mL·min^{-1}. All analyses were repeated in biological triplicates, and the measured volatile compounds were identified by comparing the retention index (*RI*) and drift time (*DT*) of the standard samples in the HS-GC-IMS library.

Sensory evaluation of off-flavour. A total of ten volunteers (aged 22–26, comprising five males and five females) were recruited to assess odour sensitivity (Shokri et al. 2014). They were required to be in good health and free of olfactory disorders. In preliminary experiments, the odour descriptors were identified as off-flavour, metallic, mushroom, fatty, and grassy. Each sample (0.002 kg) was collected and placed in a disposable paper cup. Sensory evaluators then evaluated the odour intensity and acceptance of the samples on a 10-point scale (0 – odourless, 10 – strongest).

Overall sensory evaluation. A panel of ten evaluators (aged 22–26, comprising five males and five females), trained with sensory evaluation, was selected to assess pig trotters based on five criteria: tissue status, colour, texture, flavour and aftertaste by a 10-point scale. Each criterion was weighted equally at 0.2, and the weighted average score was calculated to determine the overall acceptability level.

Statistical analysis. One-way analysis of variance was used to analyse the data, which were expressed as average ± standard deviation using SPASS (version 8.0). The parametric statistical method was checked with LSD, and the data was normally distributed and the variances were equal. A significant difference was considered if $P < 0.05$. Graphs were created using GraphPad (version 9.5).

RESULTS AND DISCUSSION

Effect of enzymatic hydrolysis on the free amino-acid content of raw pig trotters. Protein hydrolysis was quantified by the amount of free amino acids; Figure 1 displays the changes in the content of free amino acids in pig trotters treated with Bro, Pap, and Bro + Pap. Following the enzyme treatment, a notable elevation in the concentration of aspartate (Asp), glycine (Gly), proline (Pro), and cysteine (Cys) was observed. The mixed enzyme treatment exhibited the highest levels of Asp, Gly, and Pro amino acids, followed by the Pap treatment. It was noteworthy that the content of Cys was also highest in the mixed enzyme treatment, while the content of Cys was higher in the Bro treatment than in the Pap treatment. The glutamate (Glu), serine (Ser), histidine (His), threonine (Thr), alanine (Ala), tyrosine (Tyr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), and lysine (Lys) contents of the mixed enzyme treatment and the Pap treatment were found to be significantly higher than those of the Bro treatment and the blank group. Notably, the concentrations of Thr, Met, Leu, and Lys were found to be elevated in the mixed enzyme treatment in comparison to the Pap treatment. Furthermore, the Tyr, Ile, and Phe contents of the Pap treatment were observed to be higher than those of the synthetase treatment. It is possible that this is due to the fact that Pap treatment exhibited higher number of specific cleavage sites on hydrophobic or aromatic amino acids (Botinestean et al. 2017; Fernández-Lucas et al. 2017). High concentrations of aspartic acid and glutamic acid were determined to be positively correlated with the formation of the flavour substance 2-pentylfuran (Cheng et al. 2023). In addition, cysteine of the sulfur-containing compound is a crucial precursor in the generation

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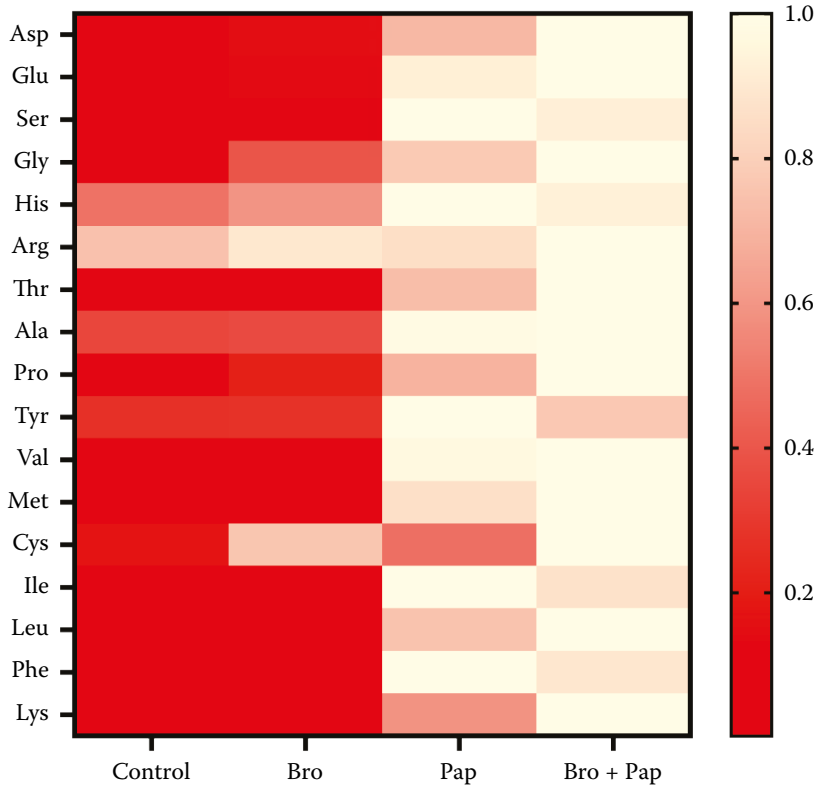

Figure 1. Amount changes in free amino acids in pig trotters after various enzyme treatments (n = 3)

Bro – bromelain; Pap – papain; Bro + Pap – combination of bromelain and papain; Asp – aspartate; Glu – glutamate; Ser – serine; Gly – glycine; His – histidine; Arg – arginine; Thr – threonine; Ala – alanine; Pro – proline; Tyr – tyrosine; Val – valine; Met – methionine; Cys – cysteine; Ile – isoleucine; Leu – leucine; Phe – phenylalanine; Lys – lysine

of meat-flavoured odorants (Wang et al. 2016; Aaslyng and Meinert 2017; Han et al. 2021). For other amino acids, leucine is noted to be conducive to the formation of furfural while glycine and valine are conducive to the formation of pyrazines (Aaslyng and Meinert 2017). It demonstrated that the treatment of mixed enzymes was more effective in producing free amino acids, which were the precursors of flavour compounds.

Effect of enzymatic hydrolysis and glycation on the electronic nose signal of pig trotters. Figure 2A reveals the volatiles and odour analysis of the electronic nose data for pig trotters subjected to different treatments, and the W1C and W3C probe signals related to aromatic volatiles were determined to be the highest in the enzymatic treatment groups. The probe signals of W1C and W3C might relate to more specific cleavage sites for aromatic amino acids by Pap (Fernández-Lucas et al. 2017). Notably, the W1C and W3C signals of the composite enzymes were stronger than those of the Pap treatment alone; this result is consistent with the results of free amino–acid contents. Among the boiled groups, the probe signals of W1W and W2W related to sulfur compounds were the strongest in the treatment via composite enzymes. This was probably related to a higher production of sulfhydryl-containing cysteines by the composite enzymes group.

As shown in Figures 2B and C, the probe signals of W1C, W3C, W1S (sensitive to methane), W1W, W2W, and W3S [reacts on high concentrations > 100 ppm, sometimes very selective (methane)] significantly increased after cooking and roasting the meat samples. Aromatic compounds associated with the W1C, W3C, and W2W signals might be more likely to be produced during roasting (Żołnierczyk and Szumny 2021; Oe et al. 2023). Interestingly, the presence or absence of sugar did not affect the W2W signal in the Pap-free group. However, Pap (boiled + roasted with sugar) and Bro + Pap (boiled + roasted with sugar) groups exhibited considerably higher W2W signals than the sugar-free group. Pap-treated samples might be more likely to produce aromatic components that were sensitive to organo-sulfides after roasting. The W1C, W3C, W1S, W1W, and W2W signals after the composite enzymes and glycation treatments

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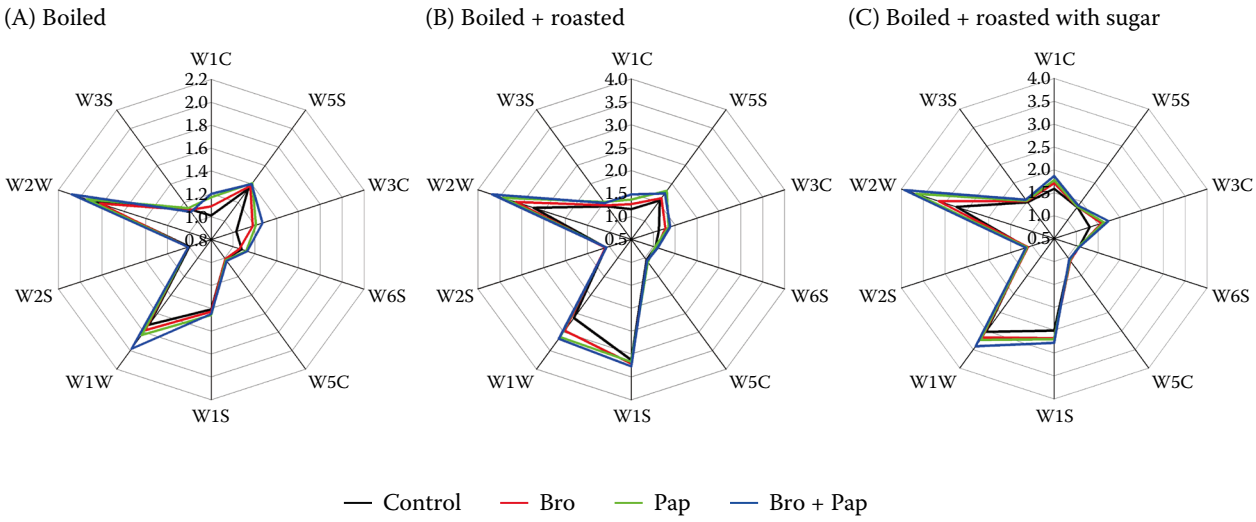

Figure 2. Volatiles and odour analysis by electronic nose for pig trotters with different treatments, including (A) cooking after various enzyme treatments, (B) roasting without sugar following various enzyme treatments, and (C) roasting with sugar following various enzyme treatments

Bro – bromelain; Pap – papain; Bro + Pap – combination of bromelain and papain; W1C – aromatic compounds; W1S – sensitive to methane; W1W – reacts on sulfur compounds; W2S – detects alcohols, partially aromatic compounds, broad range; W2W – aromatic compounds, sulfur organic compounds; W3C – ammonia, used as sensor for aromatic compounds; W3S – reacts on high concentrations > 100 ppm, sometimes very selective (methane); W5C – alkanes, aromatic compounds, less polar compounds; W5S – very sensitive, broad range sensitivity, reacts on nitrogene oxides, very sensitive with negative signal; W6S – mainly hydrogen

were the highest among all the groups, and significant differences in W2W of Figure 2C were observed among the samples, indicating the significant changes in the composition of the volatile compounds in the treatment of boiled + roasted with sugar group. Therefore, glycation and the treatment with composite enzymes could significantly enhance the aroma of pig trotters.

Effect of enzymatic hydrolysis and glycation on volatile flavour substances in pig trotters. As shown in Figure 3A, the volatile compounds showed significant differences before and after enzymatic digestion. After enzymatic hydrolysis, the contents of 2-heptanone-D, 2-pentanone-D, 2-pentanone-M, 3-octanone, 1-octen-3-ol, 3-methylbutanal, isoamyl acetate, 2-butanone-D, p-cymene, 3-methyl-1-butanol-D, 3-methyl-1-butanol-M, 2-methyl-1-propanol-D, 2-methyl-1-propanol-M, 2-butanol, styrene, ethyl pentanoate, 2-pentanone-M, 3-octanone, 3-methylbutanal, isoamyl acetate, 2-styrene, 2,3-heptanedione, acetoin-D, and acetoin-M increased significantly. Among these, 1-octen-3-ol is the most significant aroma-active alcohol, responsible for imparting the characteristic mushroom odour to meat products (Chang et al. 2021).

By contrast, the contents of methyl acetate, pentanal, heptanal, octanal and nonanal were remarkably decreased. Enzymatic hydrolysis using Bro + Pap resulted in a prominent increase in the contents of hexyl propionate, 1-butanol, (E)-2-heptenal, propanal, hexanal-D, hexanal-M, heptanal and octanal, as compared to using either Bro or Pap alone. The reduction of the (E)-2-heptenal content resulted in reduced fat of the meat (Chang et al. 2021). Meanwhile, the contents of ethylbutyl acetate, pentanal, heptanal, octanal and nonanal were considerably decreased, while that of ethyl butanoate increased. Enzymatic digestion increased the concentration of ketones and decreased the levels of heptanal, octanal and nonanal, which were long-chain aldehydes associated with off-flavour (Luo et al. 2022). The protease complex not only reduced long-chain aldehydes but also significantly reduced small-molecule aldehydes. Additionally, it increased the production of ethyl butyrate, which was considered as an important compound affording aromatic odour (Criado et al. 2019).

As demonstrated in Figure 3B, the contents of ethyl propanoate-D, ethyl acetate-D, butyl acetate-D, 3-octanone, ethanol-D, 1-propenol-M, 2-methylpropanal, and heptanal significantly increased after enzyme treatment and glycation. Conversely, the contents of 2-butanone-D, 2-pentanone-D, and 2,3-heptanedione decreased considerably as compared to the control sample. The glycation of the non-enzymatic samples

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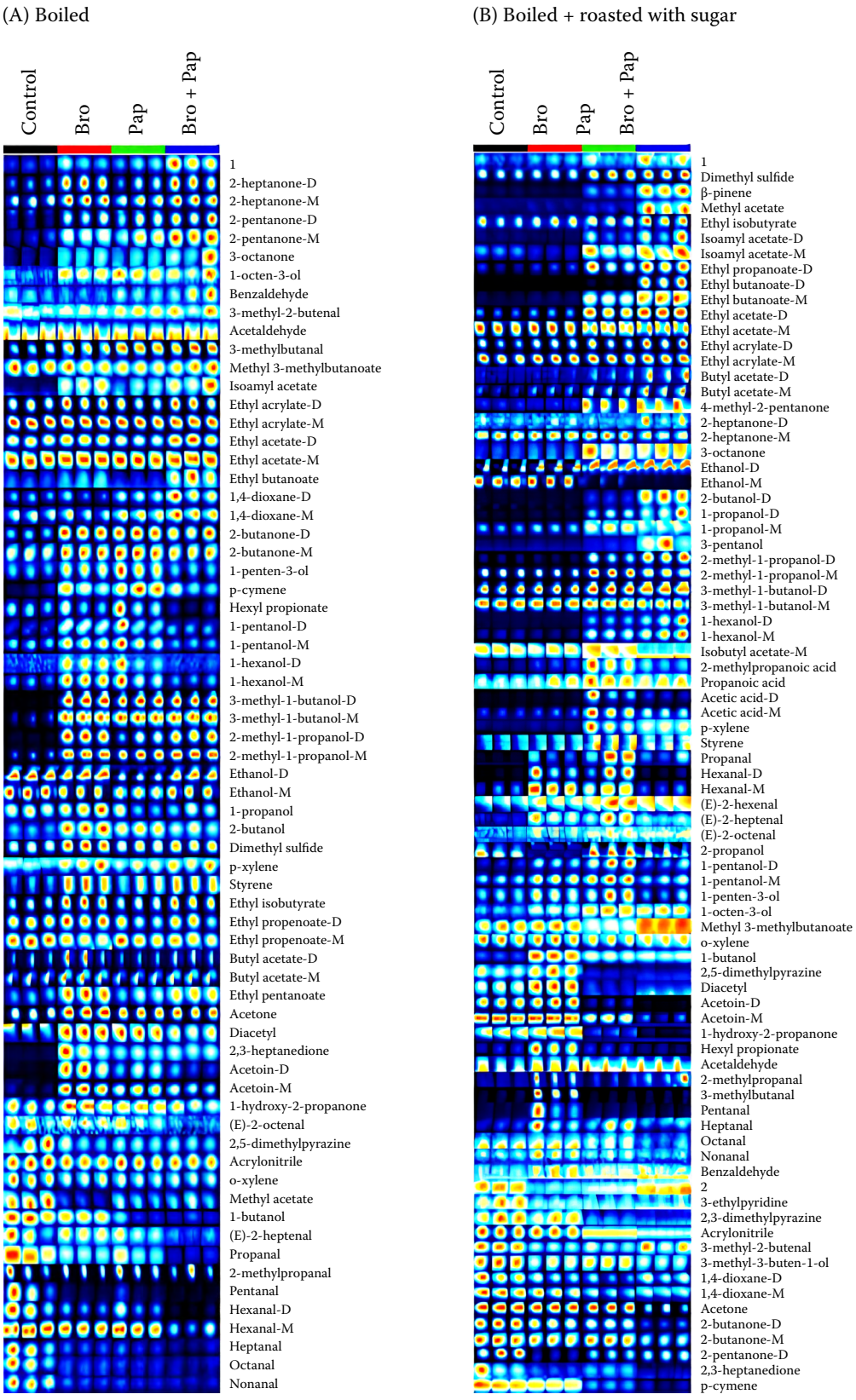

Figure 3. Changes in volatile flavour substances of pig trotters under different treatments, including (A) cooking after various enzymatic treatments and (B) roasting with sugar following various enzyme treatments

Bro – bromelain; Pap – papain; Bro + Pap – combination of bromelain and papain; 1, 2 – unknown substances

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resulted in a reduction of long-chain aldehydes such as heptanal, octanal and nonanal, which were associated with off-flavours (Aaslyng and Meinert 2017). In the glycation treatment groups, the use of composite enzymes, as compared to the use of either Bro or Pap, resulted in the production of β-pinene, methyl acetate, 2-methyl-propanoic acid, 1-pentanol-M, 1-octen-3-ol, and 1-butanol. The levels of ethyl butanoate-D, butyl acetate-D, butyl acetate-M, and 3-pentanol increased, while those of ethanol-D, hexanal-M, acetoin-M, 2,3-dimethylpyrazine, acetone, 2-butanone-D, 2,3-heptanedione, and p-cymene decreased. In brief, enzymatic treatment and glycation of boiled-roasted pig trotters reduced the levels of long-chain aldehydes (nonanal and octanal) that were associated with off-flavours. Furthermore, Bro + Pap (boiled + roasted with sugar) increased the contents of compounds associated with pleasant flavours (methyl acetate, ethyl propionate, ethyl isobutyrate, ethyl isovalerate, ethyl acetate, and butyl acetate).

Effect of enzymatic hydrolysis and glycation on the sensory evaluation of off-flavour in pig trotters after boiling-roasting. Figure 4 depicted the results of the sensory evaluation conducted on the samples subjected to distinct cooking methods. In general, boiled + roasted, resulted in a higher level of overall acceptability compared to boiling alone. This was accompanied by a notable reduction in the perception of grassy, fatty, metallic and fishy flavours, as well as a significant enhancement in the mushroom flavour. It was proposed that elevated temperatures might accelerate the decomposition of fat and reduce the fatty flavour of pig trotters (Cheng et al. 2023). Furthermore, boiled + roasted with sugar also resulted in a significant improvement in overall acceptability, with a notable reduction in grassy, fatty, metallic and fishy flavours and no discernible change in the perception of mushroom flavour.

The Maillard reaction is one of the most significant methods for the production of flavour substances in food. Aldehydes can be generated through oxidative cleavage of sugar chains and Strecker degradation during the Maillard reaction (Ramalingam et al. 2019). The kinetics of the Maillard reaction were sufficiently low at low temperatures and showed a negligible effect on the overall flavour profile (Aaslyng and

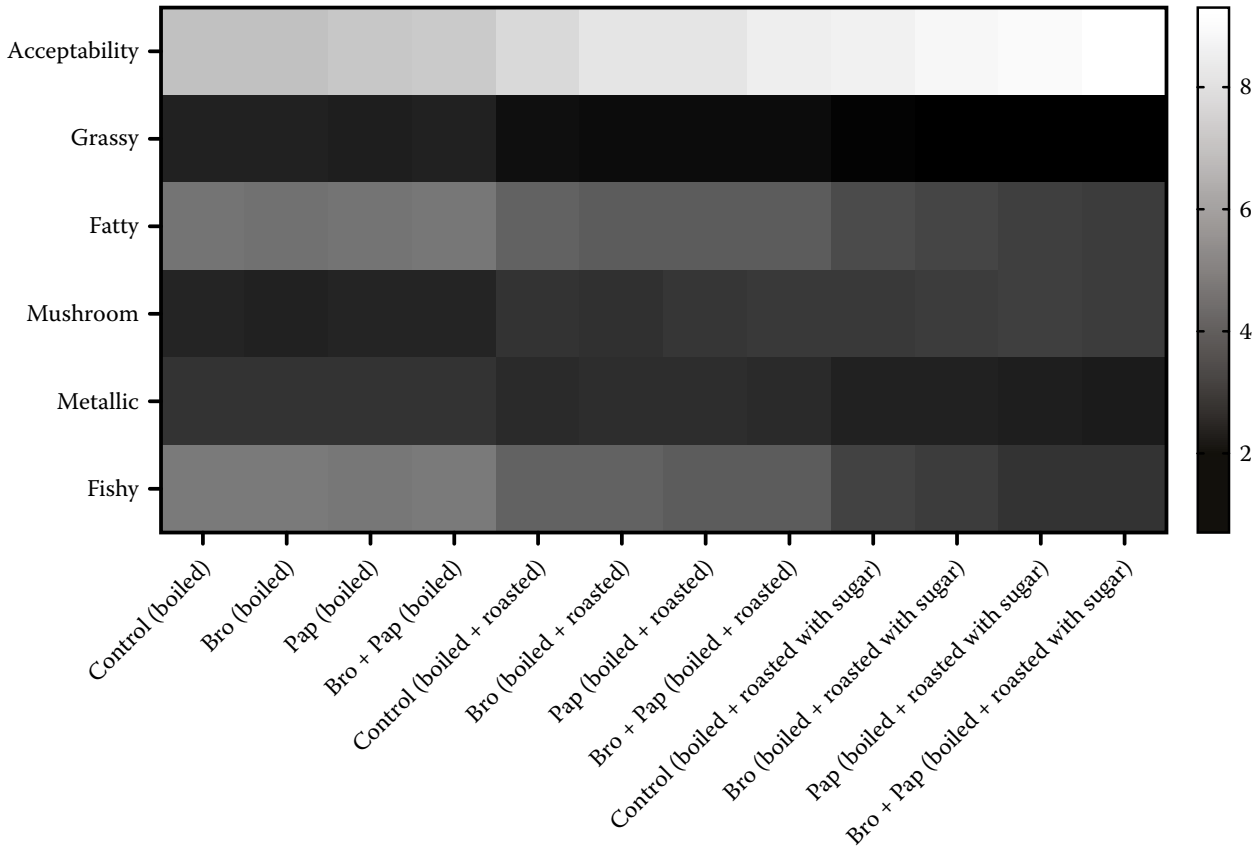

Figure 4. Sensory evaluation of odour from pig trotters after various treatments

Lighter colours mean higher scores, darker colours mean lower scores; $n = 10$; Bro – bromelain; Pap – papain; Bro + Pap – combination of bromelain and papain

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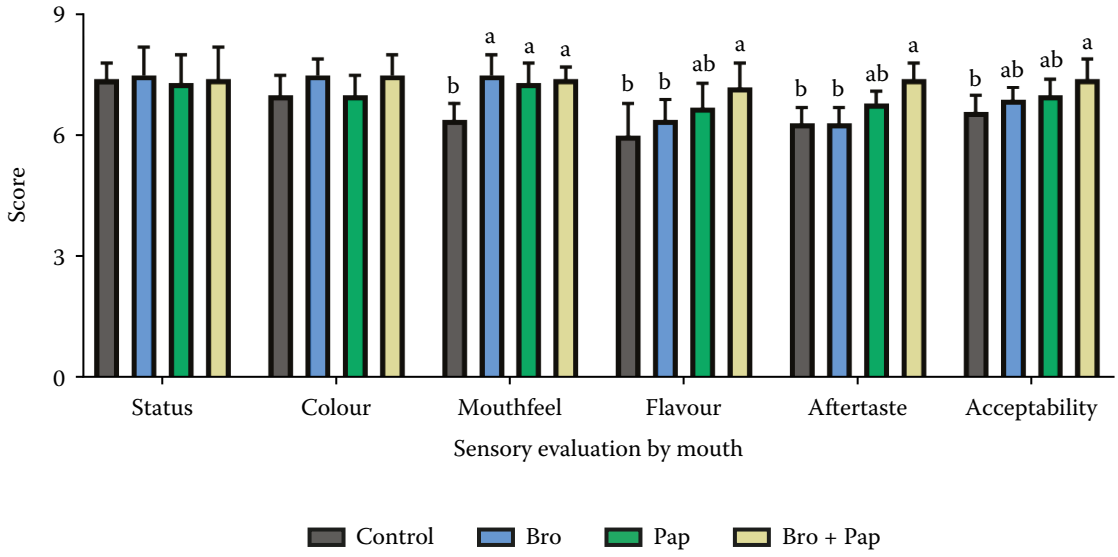

Figure 5. Overall sensory evaluation of pig trotters (boiled + roasted with sugar) after various treatments

Values represent means ± SD (statistical deviation); a, b, ab – bars with different letter mean significant difference ($P < 0.05$); $n = 10$; Bro – bromelain; Pap – papain; Bro + Pap – combination of bromelain and papain

Meinert 2017). As the temperature increased, the meat flavour became more pronounced, and higher temperatures facilitated the formation of pyrazine which is associated with the roasted meat flavour (Aaslyng and Meinert 2017). The composite enzymes method was found to be more acceptable than the single-enzyme treatment. The results demonstrate that the composite enzymes in glycation treatment had a positive impact on deodorisation.

Effect of enzymatic hydrolysis and glycation on the sensory evaluation of pig trotters. The performance of sensory evaluation is crucial in the food industry, facilitating a comprehensive assessment of product attributes and consumer perceptions of the product (Peñaranda et al. 2024). Figure 5 showed the overall sensory evaluation after enzyme treatment and glycation. The enzyme treatment resulted in a notable enhancement in the performance of mouthfeel, flavour and acceptability. Conversely, the aftertaste remained unaltered following the Bro treatment. However, Bro + Pap (boiled + roasted with sugar) exhibited notable differences in taste, flavour, aftertaste and overall acceptability when compared to the Bro (boiled + roasted with sugar) and Pap (boiled + roasted with sugar).

CONCLUSION

This study employed a composite enzymes treatment and glycation to reduce the content of long-chain aldehydes (nonanal and octanal) associated with the formation of off-flavour substances in pig trotters during the cooking process. Additionally, composite enzymes treatment increased the content of esters (methyl acetate, ethyl propionate, ethyl isobutyrate, ethyl isovalerate, ethyl acetate, and butyl acetate) in pig trotters, resulting in a more pleasing flavour. The deodorising and aroma-enhancing process also tenderised the pig trotters and improved their overall eating quality. This method was compatible with the processes of marinating, cooking and roasting of pig trotters, making it quite a potential method for industrial production.

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