

Original article

Influence of reducing sugars and protein-to-sugar ratios on volatile profile, pH, and browning intensity of Maillard-reacted nutritional yeast hydrolysateAlicia Hui Ping Theng, Raffael Osen & Jie Hong Chiang* 

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Summary This study investigated the effects of reducing sugars and protein-to-sugar ratios on generating volatile compounds from nutritional yeast hydrolysates after Maillard reaction. Nutritional yeast extract (NYE) was cleaved using Flavourzyme® at an enzyme-to-substrate ratio of 0.5% w/w, followed by heat treatment with varying concentrations of reducing sugars (i.e., hexoses: glucose and fructose and pentoses: xylose and ribose). The results showed that pentoses generate higher numbers and concentrations of volatiles than hexoses, especially furans, Strecker aldehydes, and sulphur-containing compounds such as dimethyl disulphide. However, hexoses produced higher concentrations of pyrazines. Strecker aldehydes and pyrazines served as key aroma-active compounds in meat aroma. Likewise, sulphur-containing compounds played an important role in meat aroma due to their low detection threshold. The results showed that reducing sugars significantly impacts the volatile profile of NYE, potentially influencing the formation of meaty aroma compounds. These findings demonstrate the potential of MRPs to enhance meaty flavours due to the presence of volatile compounds with characteristics of cooked meat.

Keywords Enzymatic hydrolysis, gas chromatography–mass spectrometry, Maillard reaction, nutritional yeast, reducing sugar, volatile compounds.

Introduction

Nutritional yeast extract (NYE), derived from the deactivated and dried cells of the yeast *Saccharomyces cerevisiae*, offers a readily available and inexpensive source of protein and amino acids for the food industry. Produced through enriched medium fermentation, NYE boasts a high protein content (40–60% dry weight) (Bekatorou *et al.*, 2006; Shurson, 2018; Ma *et al.*, 2023), which makes NYE a promising substrate for generating meaty flavours through the Maillard reaction.

Maillard reaction is a well-established technique in the food industry used to create complex flavours. It involves a series of reactions between proteins and reducing sugars. These reactions begin with an initial condensation, followed by a cascade of interactions between the reactants that ultimately generate aroma-active compounds (Zhang *et al.*, 2018; Raza *et al.*, 2020b). Aroma-active volatiles, such as pyrazines and sulphur-containing compounds, can also be derived from the Maillard reaction, even with plant protein

substrates, contributing to meaty aromas. The complexity of Maillard reaction arises from its dependence on numerous factors, such as temperature, pH, reaction time, water activity, and the type of protein, amino acids, or sugars involved. Slight variations in these factors can lead to unique reaction outcomes. Hence, the control of Maillard reaction can play a crucial role in shaping the flavour profile of NYE. For example, the initial phase of the Maillard reaction is critically dependent on reducing sugars, acting as precursors for flavour development and influencing reaction kinetics. Parker (2015) reported that aldopentoses are more reactive than aldohexoses and disaccharides, influencing the type of volatile compounds formed during the Maillard reaction. This highlights the crucial role of sugar selection in optimising Maillard reaction for desired flavour profiles.

Researchers have explored the impact of various reducing sugars on the Maillard reaction and resulting volatile profiles in Maillard reaction products (MRPs). Begum *et al.* (2019) and Zhang *et al.* (2019) investigated the Maillard reaction using various types of bone broth hydrolysate as starting reactants. The

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authors studied how adding reducing sugars such as glucose and xylose affected the Maillard reaction and the flavour compounds produced. In contrast, other studies focused solely on the influence of reducing sugars (e.g., glucose, fructose, galactose, and ribose) on protein precursors like casein and yeast protein (Brands & van Boekel, 2001; Raza *et al.*, 2020b). These diverse studies collectively emphasise how both starting materials and reaction conditions can significantly impact the final aroma profile of MRPs.

Despite the widespread use of yeast protein in the food industry, a critical gap remains in understanding how different reducing sugars influence the development of meaty flavours during the Maillard reaction with NYE after being hydrolysed. Nutritional yeast hydrolysates (NYH), rich in amino acids and peptides, serve as a source material for MRPs. These MRPs can be employed to enrich the flavour profile of various food products, predominantly plant-based alternatives. While NYH contributes a savoury note on their own, Maillard reaction unlocks a more intricate and nuanced flavour profile, ideal for enhancing the sensory experience of plant-based meats. Previous research has also demonstrated the potential of yeast extract in the development of meaty volatile compounds (Mahadevan & Farmer, 2006; Alim *et al.*, 2018; Wang *et al.*, 2019) and the potential between the reaction of yeast protein with various sugars in Maillard reaction to product meat-like volatiles (Lin *et al.*, 2014; Raza *et al.*, 2020a, 2020b). While the role of Maillard reaction in aroma development in yeast and other proteins has been established, understanding its specific effects on aroma development within the context of nutritional yeast is still evolving. Thus, further investigation is needed as understanding the relationships between reducing sugars and nutritional yeast during Maillard reaction would allow better strategies to be developed in the context of flavour optimisation of nutritional yeast, making them more comparable to their meaty flavour counterparts (e.g., bone broth hydrolysates). This study addresses this gap by investigating the specific impact of different reducing sugars (i.e., pentoses and hexoses) on the MRPs derived from NYH. Flavourzyme[®] was used to hydrolyse the NYE, which was then subjected to heat treatment with various reducing sugars at different concentrations. The generated MRPs were analysed for browning intensity, pH, and volatile compounds using solid-phase microextraction coupled with gas chromatography–mass spectrometry.

Materials and methods

Materials

Nutritional yeast (Bob's Red Mill, 48.17 ± 0.47% protein) was purchased from Phoon Huat Pte Ltd. (Singapore) as the protein substrate. Flavourzyme[®]

1000 L (1000 Leucine Amino Peptidase Units g⁻¹, Batch: HPN00565) was obtained from Novozymes (Bagsværd, Denmark). All reducing sugars, glucose, fructose, D-xylose, and D-ribose were bought from Phoon Huat Pte Ltd. (Singapore), The Green Earth Organic LLP (Singapore), Sigma Aldrich (Singapore), and Kimic Chemitech Pte Ltd (Singapore), respectively. Alkane standards (C7-C30) and 2-methyl-3-heptanone were procured from Sigma Aldrich (Singapore). Ultra-pure water purified using Milli-Q equipment (Millipore Corporation, Massachusetts, USA) was used. All other reagents and chemicals used were of analytical grade.

Enzymatic hydrolysis of nutritional yeast extract

The enzymatic hydrolysis of NYE was carried out in accordance with Chiang *et al.* (2022). A homogenous protein dispersion (10% w/w) was prepared by hydrating nutritional yeast flakes in ultrapure water and stirred magnetically overnight at room temperature. The dispersion was adjusted to pH 6 using 1 mol/L NaOH and mixed with Flavourzyme[®] at an enzyme-to-substrate ratio of 0.5% w/w (enzyme-to-protein weight). The hydrolysis reaction was conducted at 50 °C (based on the manufacturer's specification) using a temperature-controlled orbital shaker (Max Q6000, ThermoFisher Scientific, USA) for 2 h at 150 rpm. The enzyme was then inactivated by heating the hydrolysate in a 95°C water bath for 15 min. The NYH was stored at 4 °C for subsequent analysis.

Preparation of Maillard reaction products (MRPs)

The hydrolysate and reducing sugars (i.e., glucose [G], fructose [F], xylose [X], and ribose [R]) were mixed in different protein-to-sugar ratios of 1:0.5, 1:1 and 1:2, with the pH of the solutions being adjusted to 6.5 according to Chiang *et al.* (2022). The samples were labelled as “protein-to-sugar ratio” (e.g., G-0.5, indicating using glucose as the reducing sugar, followed by 1:0.5 protein-to-sugar ratio) in this study. The Maillard reaction of the hydrolysate was carried out in a temperature-controlled shaking water bath (BT-350D, Yihder Technology, Taiwan) at 100 °C for 2 h at 120 rpm with reference to Chiang *et al.* (2022). The NYE and hydrolysate (NYH, without adding reducing sugars) were also heat-treated to serve as controls. The resultant MRPs were cooled and kept at 4 °C until further analysis. The pH values of the MRPs were measured and recorded using a benchtop pH meter (S220, SevenCompact[™], Mettler-Toledo AG, Switzerland). The pH meter was calibrated using buffer solutions (pH 4, 7 and 9.21) before analysis.

Browning intensity analysis

The browning intensity of the samples was measured according to Chiang *et al.* (2019). The samples were

diluted to 1.25 mg/mL with ultrapure water before measuring the absorbance of the diluted hydrolysates at 420 nm (absorption) and 550 nm (turbidity correction). The browning index is determined using eq. (1):

$$\text{Browning index } (A_{420}^*) = A_{420} - A_{550} \quad (1)$$

Volatile compounds analysis

The volatiles were extracted using headspace solid-phase microextraction (HS-SPME) and were analysed by gas chromatography–mass spectrometry (GC–MS), according to Chiang *et al.* (2022) and Sun *et al.* (2014). Samples (1 g) were mixed with 1 g of sodium chloride solution (30% w/w) in a 20 mL flat-bottom headspace vial. The internal standard was 2-methyl-3-heptanone (0.0005% v/v) in methanol: water (50:50). The vial was sealed with PTFE-coated silicone septa screw caps. Before headspace solid-phase microextraction (SPME), the SPME fibre coated with 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, USA) was conditioned at 270 °C for 30 min. The fibre was then exposed in the static headspace mode for 30 min at 60 °C with an autosampler (PAL RSI 120, Agilent, USA) after the samples were equilibrated for 15 min with agitation. Volatile compounds were then dissociated at 240 °C for 5 min in spitless mode.

The volatiles of each sample were analysed by gas chromatography (GC; 7890B, Agilent, USA) equipped with a mass spectrometer (MS; 5977B, Agilent, USA). The separation analysis was carried out on a 60 m × 0.32 mm *id* × 0.5 µm ZB-WAXplus™ capillary column (Zebron, Phenomenex, USA) with helium at a 1.0 mL/min flow rate as carrier gas. The temperature oven program was as follows: the temperature was kept at 40 °C (5 min), 40–210 °C (4 °C/min), 210–240 °C (10 °C/min), and 240 °C (5 min). The transfer line towards the MS was maintained at 250 °C while the transfer line towards the quadrupole was maintained at 150 °C. The scanning speed was set at 1562 u/s, and mass spectra were fixed at 30–300 *m/z* ranges. National Institute of Standards and Technology (NIST14) database and retention indices (RI) were used to identify the volatile compounds. The concentration of the volatiles was derived by comparing the peak area of the volatiles to a standard curve established using the peak area of different concentrations of the internal standard (in ppm). Relative RI values were calculated using an *n*-alkanes series (C7–C30).

Data analysis

All experiments were carried out in triplicates and analysed with two-way multivariate variance analysis (MANOVA) using Minitab® 17 statistical software (Minitab Inc., USA). The results are expressed in mean ± standard deviation. Two-way MANOVA was

used to analyse the relationship between E/S ratios (0% and 0.5% w/w) and sugars (no sugar – NYE and NYH, G, F, X and R) and the relationship between sugars (G, F, X and R) and sugar ratios (1:0.5, 1:1 and 1:2). Principal component analysis (PCA) was conducted using Minitab® 17 statistical software to evaluate the relationships between specific volatiles and the samples. Origin 2020 software (OriginLab Corp, Massachusetts, USA) was used to plot all figures.

Results and discussion

pH and browning intensity

The extent of the Maillard reaction could be indicated by pH and browning intensity. As there was no external pH control during the heat treatment, the initial pH of 6.5 decreased due to the consumption of amino groups during Maillard reaction (Liu *et al.*, 2008). At the same time, Maillard reaction progressed to the advanced stage as the browning intensity increased by producing brown pigments, also known as melanoidins (Laroque *et al.*, 2008). Figure 1 shows the changes in pH and browning intensity with various protein-to-sugar ratios for each sugar type. The pH and browning intensity of the MRPs decreased and increased after heat treatment as the protein-to-sugar ratios increased, respectively.

The decrease in pH was attributed to the production of organic acids, where the reducing sugar degrades and reacts with proteins and amino acids under heat during Maillard reaction (Lertittikul *et al.*, 2007; Liu *et al.*, 2014). Organic acids like acetic acid could be formed from bond cleavage reactions involved in the Strecker reaction or sugar degradation, which often indicates that Maillard reaction had reacted to the final stage (Resconi *et al.*, 2013; Li *et al.*, 2021). At the same time, browning intensity serves as a potential metric to quantify the degree of Maillard reaction advancement. The increase in intensity would indicate that the reaction had progressed to the later stages of Maillard reaction, which would often be accompanied by brown pigments (Fig. 2), leading to higher browning intensity values (Laroque *et al.*, 2008).

Effects of sugar types

The pH of MRPs, when reacted with pentoses, was lower than that of hexoses (Fig. 1a), which was also reported by Chen & Kitts (2011). Pentoses are generally more reactive than hexoses due to the cyclic forms of sugars being less stable, making them react more readily with the amino compounds than hexoses (Laroque *et al.*, 2008). This would indicate that the amounts of acids produced by pentose would be higher than hexose within the same reaction time,

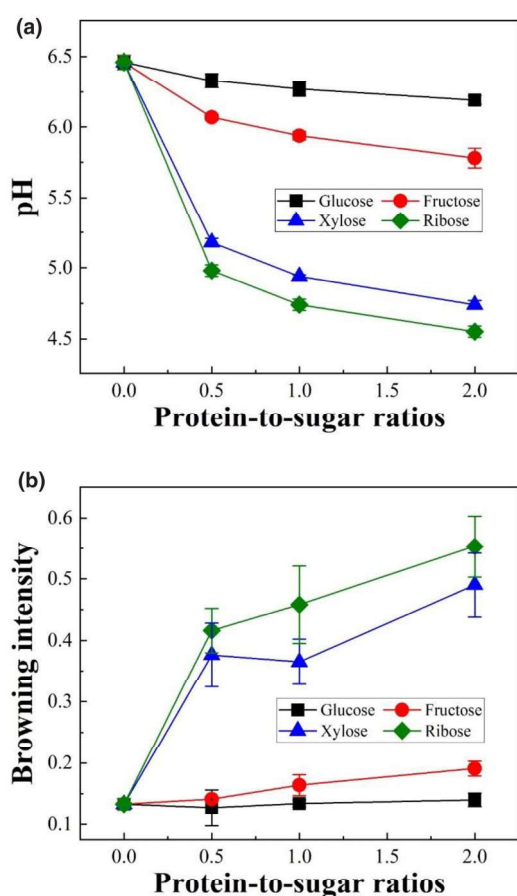


Figure 1 (a) pH and (b) browning intensity of Maillard reaction products (MRPs) at enzyme-to-substrate (E/S) ratio of 0.5% w/w, sugar types (glucose, fructose, xylose, and ribose) and protein-to-sugar (1:0.5, 1:1 and 1:2) ratios. Data are presented as the mean and standard deviation of three replicates.

which eventually caused a greater decrease in pH (Parker, 2015; Karangwa *et al.*, 2017).

Browning intensity of the MRPs was also observed to be higher in pentoses than in hexoses (Figs 1b and 2). The reactivity of pentoses could be greater than hexoses due to the higher amount of oxo form, which is the open-chain form of sugar that could promote the reaction between carbonyl and amino ends of sugar and protein, respectively (Eskin *et al.*, 2013). These are present in pentoses, allowing them to form more brown pigments, thus resulting in higher browning intensity. However, the effects of glucose and fructose on the browning intensity of MRPs were supported by different stances across various literature. Karangwa *et al.* (2017) and Kwak & Lim (2004) reported that glucose-derived MRPs had greater browning intensity than fructose-derived MRPs because glucose is an aldose. The presence of the aldehyde group in the

acyclic form of an aldose is more electrophilic than the keto group of the acyclic form of ketoses. This resulted in aldoses being more reactive than ketoses during the Maillard reaction. On the other hand, Sun *et al.* (2004) and Benjakul *et al.* (2005) reported that fructose-derived MRPs had higher browning intensity than glucose-derived MRPs, which had a similar trend as the results reported in this study. This could be due to fructose having more open-chain forms than glucose, allowing the sugar to more readily react with an amino acid to form an amino-sugar complex (Benjakul *et al.*, 2005; Phisut & Jiraporn, 2013).

It could be seen that the heat treatment of NYH with sugar types resulted in decreased pH and increased browning intensity. Hence, the reactivity of reducing sugars was observed in the following order: ribose > xylose > fructose > glucose.

Effects of protein-to-sugar ratios

The changes in protein-to-sugar ratios influenced the pH and browning intensity of the MRPs are shown in Fig. 1. Higher initial sugar concentrations likely resulted in a more extensive Maillard reaction due to an increased availability of reactants for the initial condensation between sugars and amino groups. The results aligned with Liu *et al.* (2008), who reported decreased pH values across the galactose/glycine model systems with increasing sugar concentrations. Consistent with these findings, MRPs prepared with higher protein-to-sugar ratios displayed an overall increase in browning intensity. Benjakul *et al.* (2005) reported that higher browning intensity values were observed as the sugar concentration in the initial reactants increased.

Overall results showed that sugar types and protein-to-sugar ratios influenced the pH and browning intensity of MRPs. Pentose (i.e., ribose and xylose) at a higher protein-to-sugar ratio (e.g., 1:2) led to a more pronounced decrease in pH and increased browning reaction. This observation highlights the potential for tailoring the reaction conditions to achieve desired characteristics in MRPs.

Volatile compounds

Analysis of volatile compounds in MRPs derived from different sugar types (i.e., glucose, fructose, xylose, and ribose) and protein-to-sugar ratios (e.g., 1:0.5, 1:1, and 1:2) revealed a diverse range of volatile compounds (Table 1). These volatiles can be broadly classified into the following groups.

Pyrazines

Pyrazines contribute to the roasted, meaty and nutty aroma in model meat systems and are detected at low odour thresholds. Pyrazines are associated with

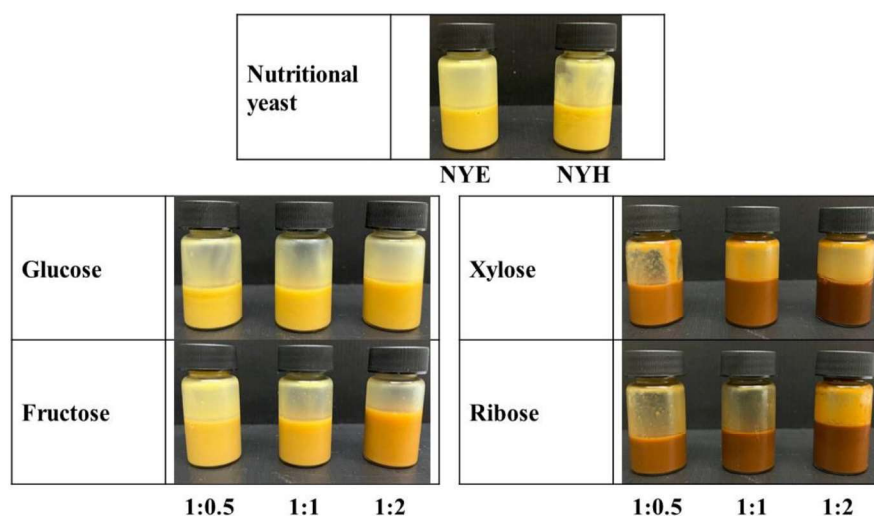


Figure 2 Visual images of nutritional yeast extract (NYE), nutritional yeast hydrolysate (NYH) at E/S ratios of 0.5% w/w, and Maillard reaction products derived from glucose, fructose, xylose, and ribose at protein-to-sugar ratios of 1:0.5, 1:1, and 1:2 (left to right).

various meat types; for instance, alkylpyrazines (e.g., methylpyrazine and 2,6-dimethylpyrazine) have been identified in chicken and lamb subjected to high-temperature cooking methods like roasting and grilling (Jayasena *et al.*, 2013; Resconi *et al.*, 2013). However, the numbers and concentrations of pyrazines detected in plant sources (e.g., flaxseed, rice bran, pea, wheat and rice) were low (Ko *et al.*, 2005; Frank *et al.*, 2016a, 2016b).

Nine pyrazines were observed in all samples (Table 1). NYE exhibited a lower amount of pyrazines (i.e., methylpyrazine, 2,6-dimethylpyrazine) than chicken bone extract, previously reported by Chiang *et al.* (2022). This difference likely arises from the lack of specific amino acids (e.g. creatine) or dipeptides (e.g., carnosine and anserine) in plant sources that influence the type and amount of pyrazines produced compared with in animal meat (Wu, 2020). The hydrolysis of NYE with Flavourzyme® did not alter pyrazine formation or concentration. Notably, some pyrazines (e.g., 2-ethyl-5-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine) were found at lower levels in NYH compared with NYE. Although enzymatic hydrolysis breaks down the proteins and peptides of NYE into smaller fragments such as di- or tri-peptides and free amino acids as flavour precursors (Purbaningrum *et al.*, 2023), this process apparently does not influence pyrazine levels in the hydrolysate. A possible explanation for the decrease in these specific pyrazines could be the preferential breakdown of precursor amino acid combinations by the enzyme (Meinlschmidt *et al.*, 2016; Cui *et al.*, 2021).

The addition of four reducing sugars to NYH during heat treatment did not significantly increase overall

pyrazine levels (Table 1). However, there was a notable exception where MRPs derived from fructose, a hexose sugar, exhibited pyrazine concentrations 3.8–7.4 times higher than NYH alone. This highlights the influence of sugar type on pyrazine formation during the Maillard reaction. Interestingly, the reducing sugars appeared to promote the generation of specific pyrazines (e.g., methylpyrazine and 2,6-dimethylpyrazine). However, the extent of this increase varied significantly, with hexoses generally leading to higher pyrazine concentrations in MRPs than in pentoses. Hexoses provide more potential carbon backbones to form complex molecules like pyrazines during the Maillard reaction. The extra carbon atom in hexoses offers more building blocks for creating these volatile compounds than pentoses (Sun *et al.*, 2023). Fructose, a ketose sugar, exists primarily in a furanose form, which is inherently more reactive than the pyranose form in glucose (an aldose sugar). The furanose structure, along with the ketose nature, potentially contributes to an increased efficiency in forming pyrazines during the Maillard reaction (Li *et al.*, 2020; Laemont & Barringer, 2023).

The type of reducing sugar added to NYH impacted the number of pyrazines present in the MRPs (Table 1). This was observed through the absence of 2-ethyl-3,5-dimethylpyrazine and 2,3,5-trimethyl-6-ethylpyrazine, and lower concentrations of ethylpyrazine and 2-ethyl-5-methylpyrazine in pentose-derived MRPs. The higher reactivity of pentoses might lead to a more rapid consumption during the initial stage of the Maillard reaction compared with hexoses (Laroque *et al.*, 2008). This could leave fewer reactive

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Table 1 Volatile compounds of heat-treated nutritional yeast extract (NYE), heat-treated nutritional yeast hydrolysate (NYH) and Maillard reaction products (derived from NYH-sugar mixtures) at enzyme-substrate (E/S) ratio of 0.5% w/w with different sugar types and ratios

Compounds	RI [†]	Relative concentration (ppm) [‡]				
		NYE	NYH	Glucose (G)		
				G-0.5	G-1	G-2
Strecker aldehydes						
2-methyl propanal	802	ND	ND	ND	ND	ND
Methional	1512	ND	ND	ND	ND	ND
Benzeneacetaldehyde	1697	ND	ND	ND	ND	ND
Aldehydes						
Heptanal	1208	0.245 ± 0.051 ^a	0.129 ± 0.028 ^b	0.137 ± 0.040 ^{bcef}	0.155 ± 0.024 ^{bce}	0.132 ± 0.048 ^{bcf}
3-methyl-2-butenal	1229	ND	ND	ND	ND	ND
Octanal	1328	0.046 ± 0.006 ^c	ND	ND	ND	ND
Nonanal	1443	0.115 ± 0.008 ^a	ND	ND	ND	ND
Benzaldehyde	1587	0.369 ± 0.098	0.311 ± 0.063	0.347 ± 0.073 ^a	0.334 ± 0.068 ^a	0.319 ± 0.054 ^a
Ketones						
2-octanone	1326	ND	0.016 ± 0.001 ^a	0.015 ± 0.001 ^a	0.015 ± 0.001 ^a	0.013 ± 0.003 ^a
1-hydroxy-2-propanone	1350	ND	ND	ND	ND	ND
Furans						
2-methyl furan	845	ND	ND	ND	ND	ND
2-(2-propenyl)furan	1235	ND	ND	ND	ND	ND
2-propyl furan	1574	ND	ND	ND	ND	ND
Furfural	1520	ND	0.016 ± 0.006 ^a	0.036 ± 0.008 ^a	0.050 ± 0.003 ^a	0.070 ± 0.004 ^{ab}
1-(2-furanyl)ethanone	1564	ND	ND	ND	ND	ND
1-(2-furanyl)-1-propanone	1632	ND	ND	ND	ND	ND
2,2'-bifuran	1650	ND	ND	ND	ND	ND
2,2'-methylenebisfuran	1658	ND	ND	ND	ND	ND
2-furanmethanol	1702	ND	0.025 ± 0.004 ^a	0.199 ± 0.000 ^c	0.353 ± 0.049 ^b	0.493 ± 0.119 ^b
1-(2-furanyl methyl)- 1H-pyrrole	1856	ND	ND	ND	ND	ND
4-(2-furanyl)- 3-buten-2-one	1922	ND	ND	ND	ND	ND
Lactones						
2(5H)-furanone	1803	ND	ND	ND	ND	ND
Sulphur-containing compounds						
Dimethyl disulphide	1074	ND	ND	ND	ND	ND
Dimethyl trisulphide	1445	ND	ND	ND	ND	ND
Thiophenes						
5-methyl-2-thiophenecarboxaldehyde	1641	ND	ND	ND	ND	ND
Pyrazines						
Methylpyrazine	1313	0.016 ± 0.002 ^a	0.031 ± 0.005 ^a	0.043 ± 0.004 ^{cd}	0.050 ± 0.001 ^{cd}	0.050 ± 0.007 ^{cd}
2,6-dimethylpyrazine	1379	0.058 ± 0.008 ^a	0.056 ± 0.006 ^a	0.057 ± 0.009 ^a	0.056 ± 0.007 ^a	0.047 ± 0.008 ^a
Ethylpyrazine	1389	0.015 ± 0.001 ^d	0.016 ± 0.002 ^d	0.012 ± 0.003 ^d	0.016 ± 0.005 ^d	0.017 ± 0.002 ^{cd}
2-ethyl-5-methylpyrazine	1452	0.042 ± 0.007 ^a	0.024 ± 0.004 ^a	0.037 ± 0.024 ^a	0.021 ± 0.003 ^a	0.018 ± 0.003 ^a
3-ethyl-2,5-dimethylpyrazine	1505	0.231 ± 0.008 ^a	0.194 ± 0.022 ^a	0.187 ± 0.038 ^a	0.211 ± 0.009 ^a	0.218 ± 0.040 ^a
2-ethyl-3,5-dimethylpyrazine	1523	0.041 ± 0.004 ^a	0.029 ± 0.003 ^a	0.027 ± 0.006 ^a	0.026 ± 0.003 ^a	0.024 ± 0.002 ^a
2-ethenyl-6-methyl pyrazine	1551	ND	ND	ND	ND	ND
2,3,5-trimethyl-6-ethylpyrazine	1573	0.032 ± 0.001 ^c	0.038 ± 0.004 ^b	0.029 ± 0.020 ^b	0.024 ± 0.005 ^b	0.031 ± 0.007 ^b
2-methyl-5-(2-propenyl)pyrazine	1616	0.037 ± 0.005 ^a	0.032 ± 0.003 ^a	0.031 ± 0.004 ^a	0.030 ± 0.005 ^a	0.028 ± 0.004 ^a
Acid						
Acetic acid	1500	ND	ND	ND	ND	ND
Propanoic acid	1603	ND	ND	ND	ND	ND
2-methylpropanoic acid	1628	0.179 ± 0.015 ^c	0.128 ± 0.014 ^c	0.196 ± 0.061 ^{ca}	0.216 ± 0.097 ^{cad}	0.171 ± 0.033 ^{cd}
Butanoic acid	1673	ND	ND	ND	ND	ND
3-methylbutanoic acid	1710	0.296 ± 0.036 ^a	0.211 ± 0.0038 ^a	0.305 ± 0.141 ^{afg}	0.362 ± 0.187 ^{af}	0.304 ± 0.028 ^{ag}
Hexanoic acid	1865	0.027 ± 0.004 ^a	0.027 ± 0.019 ^a	0.035 ± 0.009 ^a	0.036 ± 0.014 ^a	0.043 ± 0.005 ^a
Octanoic acid	2032	ND	ND	ND	ND	ND
Alcohol						
Phenylethyl alcohol	1926	0.106 ± 0.003	0.114 ± 0.011	0.123 ± 0.023 ^a	0.124 ± 0.028 ^a	0.095 ± 0.003 ^a
Pyrrole						
1H-pyrrole-2-carboxaldehyde	2017	ND	ND	ND	ND	ND
Others						
3-fluorobenzaldehyde	1668	ND	ND	ND	ND	ND
2,4-dimethylbenzaldehyde	1854	0.047 ± 0.006	ND	ND	ND	ND
Butylated hydroxytoluene	1917	0.011 ± 0.001 ^a	0.016 ± 0.002 ^a	0.020 ± 0.001 ^a	0.018 ± 0.004 ^a	0.015 ± 0.001 ^a
p-Cresol	1989	0.019 ± 0.002 ^a	0.027 ± 0.000 ^a	0.035 ± 0.004 ^a	0.033 ± 0.007 ^a	0.028 ± 0.003 ^a
Compound count		20	21	21	21	22

51 Values bearing different lowercase letters in the same row were significantly different ($P \leq 0.05$) according to 'Tukey's posthoc test'.
52 ND, Not Detected.
53 [†]Retention indices (RI) were calculated based on retention times of the standard *n*-alkanes series. RI = 30.379 Rt + 574.630, where Rt is the
54 retention time of the volatile detected in the sample.
55 [‡]Relative concentration of each compound based on internal standard (2-methyl-3-heptanone) was expressed as means and standard deviation of
56 triplicates in three decimal places.

Fructose (F)			Xylose (X)			Ribose (R)		
F-0.5	F-1	F-2	X-0.5	X-1	X-2	R-0.5	R-1	R-2
ND	ND	ND	ND	ND	ND	0.159 ± 0.001 ^b	0.192 ± 0.017 ^b	0.204 ± 0.075 ^b
ND	ND	ND	ND	ND	0.025 ± 0.001	ND	ND	ND
ND	ND	ND	ND	ND	ND	ND	0.078 ± 0.008 ^a	0.080 ± 0.006 ^a
0.126 ± 0.018 ^{cef}	0.137 ± 0.023 ^{ce}	0.144 ± 0.012 ^{cf}	0.180 ± 0.033 ^{def}	0.280 ± 0.077 ^{de}	0.201 ± 0.030 ^{df}	0.233 ± 0.011 ^{bdef}	0.245 ± 0.008 ^{bde}	0.123 ± 0.027 ^{bdf}
ND	ND	ND	ND	ND	ND	0.011 ± 0.000 ^a	0.008 ± 0.002 ^a	ND
ND	ND	ND	ND	0.020 ± 0.004 ^b	0.039 ± 0.008 ^b	0.034 ± 0.014 ^a	0.041 ± 0.008 ^a	0.029 ± 0.008 ^a
ND	ND	ND	ND	ND	ND	ND	ND	ND
0.303 ± 0.023 ^a	0.316 ± 0.079 ^a	0.308 ± 0.089 ^a	0.383 ± 0.033 ^a	0.503 ± 0.126 ^a	0.506 ± 0.039 ^a	0.338 ± 0.013 ^a	0.437 ± 0.252 ^a	0.395 ± 0.147 ^a
0.011 ± 0.001 ^a	0.013 ± 0.004 ^a	0.013 ± 0.001 ^a	ND	ND	ND	ND	ND	ND
0.019 ± 0.004 ^b	0.029 ± 0.007 ^{bc}	0.031 ± 0.006 ^c	0.130 ± 0.018 ^{ad}	0.121 ± 0.041 ^{ade}	0.045 ± 0.008 ^{ab}	0.104 ± 0.036 ^{ad}	0.068 ± 0.000 ^{ade}	0.041 ± 0.008 ^{ae}
ND	ND	ND	0.609 ± 0.131 ^{ab}	0.436 ± 0.122 ^{ab}	0.150 ± 0.014 ^a	0.493 ± 0.065 ^{ac}	0.241 ± 0.046 ^{ac}	0.065 ± 0.041 ^a
ND	ND	ND	0.008 ± 0.002 ^a	0.014 ± 0.003 ^{ab}	0.024 ± 0.001 ^b	0.016 ± 0.006 ^c	0.024 ± 0.003 ^{cd}	0.022 ± 0.001 ^d
ND	ND	ND	ND	ND	ND	0.088 ± 0.003 ^b	0.168 ± 0.017 ^a	0.178 ± 0.022 ^a
0.030 ± 0.018 ^a	0.041 ± 0.019 ^a	0.051 ± 0.014 ^{ab}	1.326 ± 0.172 ^d	8.220 ± 0.414 ^d	20.730 ± 1.239 ^{dc}	1.882 ± 0.236 ^d	10.135 ± 0.397 ^d	21.960 ± 0.822 ^{dc}
ND	ND	ND	0.079 ± 0.023 ^{ab}	0.420 ± 0.105 ^a	0.731 ± 0.019 ^a	0.228 ± 0.055 ^{ab}	0.590 ± 0.040 ^a	0.736 ± 0.033 ^a
ND	ND	ND	ND	ND	ND	0.221 ± 0.021 ^a	0.461 ± 0.036 ^a	0.393 ± 0.172 ^a
ND	ND	ND	0.028 ± 0.005 ^c	0.260 ± 0.061 ^c	1.090 ± 0.089 ^d	0.105 ± 0.018 ^b	0.728 ± 0.081 ^b	2.248 ± 0.101 ^a
ND	ND	ND	ND	ND	ND	ND	0.027 ± 0.002 ^a	0.062 ± 0.007 ^a
0.074 ± 0.012 ^{ad}	0.111 ± 0.020 ^a	0.130 ± 0.012 ^a	0.511 ± 0.020 ^{ef}	0.811 ± 0.060 ^e	0.838 ± 0.055 ^e	0.594 ± 0.052 ^{ef}	0.803 ± 0.041 ^a	0.749 ± 0.082 ^e
ND	ND	ND	ND	ND	ND	0.020 ± 0.004 ^b	0.036 ± 0.004 ^a	0.036 ± 0.001 ^a
ND	ND	ND	0.035 ± 0.008 ^a	0.074 ± 0.011 ^a	0.108 ± 0.027 ^b	0.056 ± 0.019 ^c	0.110 ± 0.021 ^c	0.207 ± 0.014 ^d
ND	ND	ND	0.055 ± 0.008 ^{ab}	0.091 ± 0.019 ^a	0.085 ± 0.009 ^a	0.056 ± 0.011 ^{ab}	0.082 ± 0.012 ^a	0.077 ± 0.008 ^a
ND	ND	ND	0.068 ± 0.011 ^a	0.070 ± 0.025 ^a	0.072 ± 0.009 ^a	0.062 ± 0.017 ^a	0.072 ± 0.006 ^a	0.076 ± 0.011 ^a
ND	ND	ND	0.113 ± 0.023 ^a	0.142 ± 0.016 ^a	0.144 ± 0.013 ^a	0.140 ± 0.019 ^b	0.172 ± 0.031 ^b	0.161 ± 0.028 ^b
0.021 ± 0.004 ^a	0.024 ± 0.006 ^a	0.022 ± 0.007 ^a	ND	ND	ND	ND	ND	ND
0.285 ± 0.012 ^{cd}	0.502 ± 0.091 ^a	0.579 ± 0.034 ^a	0.119 ± 0.003 ^c	0.111 ± 0.019 ^c	0.049 ± 0.002 ^{cd}	0.068 ± 0.004 ^{cd}	0.044 ± 0.003 ^{cd}	0.022 ± 0.001 ^d
0.940 ± 0.039 ^b	1.759 ± 0.291 ^c	2.024 ± 0.059 ^d	0.067 ± 0.010 ^a	0.060 ± 0.006 ^a	0.044 ± 0.008 ^a	0.052 ± 0.008 ^a	0.039 ± 0.003 ^a	0.026 ± 0.003 ^a
0.027 ± 0.001 ^c	0.040 ± 0.008 ^b	0.051 ± 0.005 ^a	0.013 ± 0.006 ^d	0.015 ± 0.003 ^d	0.012 ± 0.001 ^d	0.012 ± 0.001 ^d	0.008 ± 0.001 ^d	ND
0.035 ± 0.002 ^a	0.044 ± 0.003 ^a	0.040 ± 0.007 ^a	0.017 ± 0.003 ^a	0.020 ± 0.004 ^a	0.021 ± 0.004 ^a	0.018 ± 0.003 ^a	0.017 ± 0.002 ^a	0.016 ± 0.004 ^a
0.186 ± 0.025 ^a	0.256 ± 0.061 ^a	0.267 ± 0.067 ^a	0.187 ± 0.038 ^a	0.249 ± 0.048 ^a	0.229 ± 0.060 ^a	0.176 ± 0.029 ^a	0.172 ± 0.028 ^a	0.155 ± 0.017 ^a
0.025 ± 0.006 ^a	0.025 ± 0.005 ^a	0.026 ± 0.008 ^a	ND	ND	ND	ND	ND	ND
0.033 ± 0.007 ^b	0.053 ± 0.007 ^a	0.061 ± 0.014 ^a	ND	ND	ND	ND	ND	ND
0.025 ± 0.006 ^b	0.026 ± 0.004 ^b	0.028 ± 0.006 ^b	0.060 ± 0.013 ^a	ND	ND	ND	ND	ND
0.022 ± 0.003 ^a	0.021 ± 0.008 ^a	0.026 ± 0.004 ^a	0.026 ± 0.003 ^a	0.027 ± 0.006 ^a	0.029 ± 0.006 ^a	0.023 ± 0.002 ^a	0.027 ± 0.005 ^a	0.025 ± 0.004 ^a
ND	ND	ND	0.716 ± 0.061 ^a	1.216 ± 0.405 ^a	0.806 ± 0.168 ^a	0.879 ± 0.137 ^a	0.865 ± 0.055 ^a	0.661 ± 0.082 ^a
ND	ND	ND	ND	ND	ND	0.032 ± 0.004 ^a	0.041 ± 0.014 ^a	0.037 ± 0.006 ^a
0.148 ± 0.015 ^{ca}	0.230 ± 0.069 ^{cad}	0.276 ± 0.030 ^{cd}	0.883 ± 0.074 ^{ef}	1.230 ± 0.272 ^a	0.825 ± 0.008 ^f	0.878 ± 0.089 ^{ah}	0.854 ± 0.089 ^a	0.593 ± 0.042 ^h
ND	ND	ND	0.416 ± 0.315 ^{abc}	0.818 ± 0.204 ^{ab}	0.429 ± 0.068 ^{ac}	0.621 ± 0.031 ^{abc}	0.631 ± 0.041 ^{ab}	0.313 ± 0.032 ^{ac}
0.279 ± 0.044 ^{afg}	0.337 ± 0.225 ^{af}	0.473 ± 0.062 ^{ag}	1.491 ± 0.074 ^{bc}	2.022 ± 0.406 ^c	1.404 ± 0.060 ^b	1.510 ± 0.102 ^{de}	1.467 ± 0.162 ^d	0.990 ± 0.088 ^e
0.034 ± 0.010 ^a	0.052 ± 0.018 ^a	0.047 ± 0.011 ^a	0.064 ± 0.018 ^a	0.070 ± 0.016 ^a	0.066 ± 0.001 ^a	0.059 ± 0.006 ^a	0.060 ± 0.007 ^a	0.055 ± 0.005 ^a
ND	ND	ND	0.261 ± 0.141 ^a	0.315 ± 0.171 ^a	0.189 ± 0.090 ^a	0.250 ± 0.038 ^a	0.183 ± 0.151 ^a	0.207 ± 0.070 ^a
0.101 ± 0.002 ^a	0.104 ± 0.008 ^a	0.102 ± 0.014 ^a	0.101 ± 0.015 ^a	0.121 ± 0.036 ^a	0.093 ± 0.028 ^a	0.100 ± 0.010 ^a	0.115 ± 0.025 ^a	0.097 ± 0.015 ^a
ND	ND	ND	ND	ND	ND	0.052 ± 0.012 ^a	0.124 ± 0.041 ^{ac}	0.224 ± 0.015 ^c
ND	ND	ND	0.075 ± 0.003 ^b	0.096 ± 0.008 ^b	0.052 ± 0.025 ^c	0.155 ± 0.004 ^a	0.179 ± 0.017 ^a	0.079 ± 0.012 ^d
ND	ND	ND	ND	ND	ND	ND	ND	ND
0.012 ± 0.002 ^a	0.013 ± 0.001 ^a	0.013 ± 0.000 ^a	0.018 ± 0.008 ^a	0.016 ± 0.004 ^a	0.021 ± 0.001 ^a	0.015 ± 0.006 ^a	0.023 ± 0.014 ^a	0.016 ± 0.003 ^a
0.025 ± 0.002 ^a	0.030 ± 0.003 ^a	0.029 ± 0.007 ^a	0.035 ± 0.007 ^a	0.044 ± 0.006 ^a	0.033 ± 0.004 ^a	0.037 ± 0.004 ^a	0.044 ± 0.013 ^a	0.039 ± 0.011 ^a
24	24	24	32	32	32	39	40	38

intermediates available for subsequent pyrazine formation. Additionally, pentoses might be more susceptible to degradation and caramelisation that favour the formation of furans over pyrazines. This could lead to a selective generation of furans instead of pyrazines in pentose-derived MRPs (Sun *et al.*, 2023).

Within each sugar type, an overall decrease in pyrazine concentration was observed when the protein-to-sugar ratios increased (Table 1). This aligns with observations by Li *et al.* (2020), who reported a decline in pyrazine formation from defatted flaxseed meal when glucose content exceeded 15% during the Maillard reaction. These findings suggest that excessive sugar can be counterproductive for pyrazine formation. A potential explanation lies in the generation of carboxylic acids during sugar breakdown. These acids reduce the pH of the reaction system, thus hindering pyrazine formation (Davidek *et al.*, 2006). In this study, the starting protein-to-sugar ratio provided a relatively high sugar concentration, where the increasing sugar might have contributed to a decrease in pyrazine formation by lowering the reaction pH, as shown in Fig. 1a.

This study demonstrates that NYH can produce pyrazines under the Maillard reaction conditions. In contrast, other plant hydrolysates generally exhibit much lower pyrazine formation. For instance, only 2-methylpyrazine was identified in the MRPs produced using peony-seed hydrolysates. In contrast, soybean meal hydrolysate yielded just three pyrazines (i.e., 2-methylpyrazine, 2,5-dimethylpyrazine and 2,5-dimethyl-3-ethylpyrazine) (Chen *et al.*, 2018; Xia *et al.*, 2021; Sun *et al.*, 2023). On the other hand, Alim *et al.* (2020) and Raza *et al.* (2020b) identified a diverse range of pyrazines found in yeast extract. These studies collectively suggest that NYH, compared with other plant hydrolysates, is a promising source of pyrazine precursors during Maillard reaction.

Principal component analysis provided valuable insights into how sugar types influence pyrazine formation during the Maillard reaction. The score plot (Fig. 3a) visualises the samples as points in a two-dimensional space. The positions of these points reflect the similarities and differences in their pyrazine profiles. NYE appeared as a potential outlier compared with other samples. Hexoses were more dispersed, suggesting a more diverse influence on the types of pyrazines formed. Also, glucose-derived MRPs were positioned close to NYH, implying a comparable effect of glucose and no added sugar on the pyrazine profiles. In contrast, pentoses clustered in the top left corner, indicating a similar impact on pyrazine formation. As for protein-to-sugar ratios, there is an observable trend for fructose-derived MRPs; increasing protein-to-sugar ratios caused a progressively stronger (increasingly positive) association with the first (PC1) principal

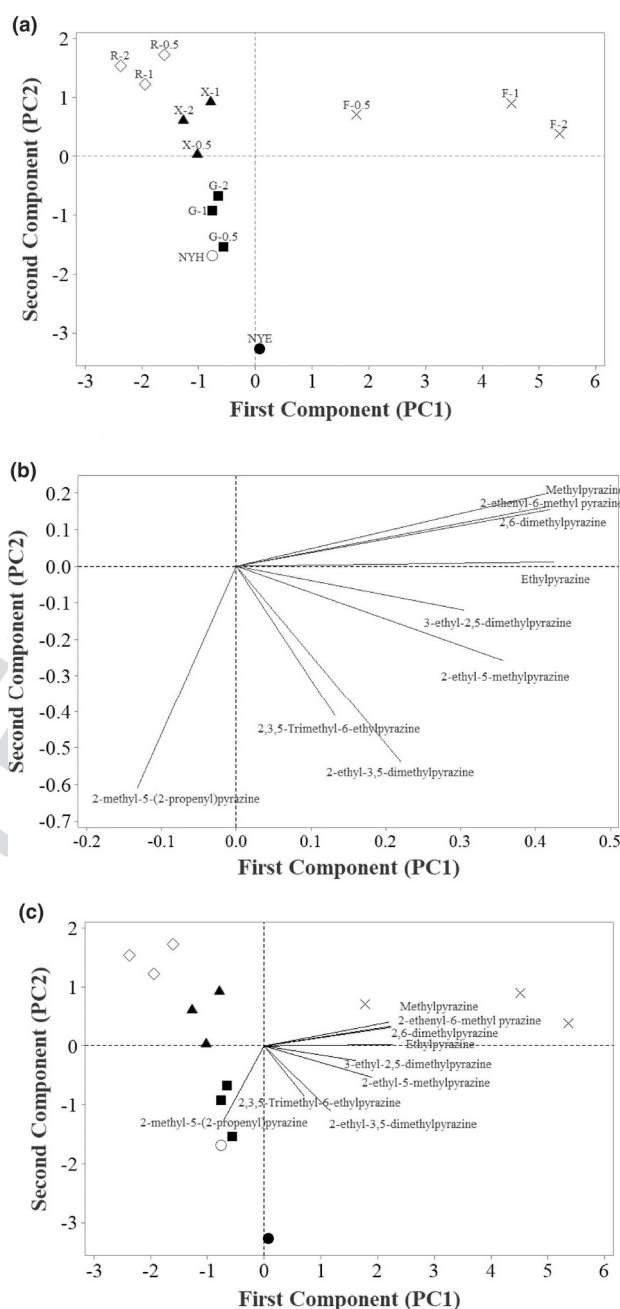


Figure 3 (a) score plot, (b) loading plot and (c) biplot for pyrazines of nutritional yeast extract (NYE), nutritional yeast hydrolysate (NYH) and Maillard reaction products (MRPs) derived from glucose (G-0.5, G-1, G-2), fructose (F-0.5, F-1, F-2), xylose (X-0.5, X-1, X-2) and ribose (R-0.5, R-1, R-2) at different protein-to-sugar ratios, generated from the principal component analysis (PCA). The following symbols represent NYE (●) and NYH (○), followed by the treatment with different reducing sugars: G (■), F (×), X (▲) and R (◇).

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component. On the other hand, a trend was not observed for the other reducing sugars.

The loading plot (Fig. 3b) depicts the pyrazines as vectors in the same 2-dimensional space, where the length and direction of each vector represent the importance of that specific pyrazine in differentiating the samples. Some alkylpyrazines (i.e., ethylpyrazine, methylpyrazine, and 2,6-dimethylpyrazine) displayed positive loadings on both PC1 and second (PC2) principal component, suggesting a strong association with these components. Additionally, these alkylpyrazines likely have a strong influence on the overall meaty profiles of the MRPs.

The biplot (Fig. 3c) combined the information from both the score and loading plots, showing the relationships between pyrazine profiles and the different samples/reaction conditions. The biplot revealed a closer relationship between pyrazine formation and hexoses, especially fructose; fructose-derived MRPs were positioned near many alkylpyrazines, suggesting a stronger link between these pyrazines and fructose compared with pentose-derived pyrazines. This might indicate distinct pathways for pyrazine formation depending on the sugar type used in the Maillard reaction.

Furans

Furans are primarily generated through the thermal degradation of sugars and are associated with caramel, sweet, and burnt aromas (Eric *et al.*, 2013; Zhang *et al.*, 2018). Furans are often associated with meat that has been thermally processed; for example, furfural has been reported to be found in grilled goat meat and cooked aged beef (Madruga *et al.*, 2009; Watanabe *et al.*, 2015). Another notable furan, 2-furanmethanol, associated with burnt meat and vitamin-like aroma, was also identified and contributed positively to the overall aroma profile in dry ham products (Pham *et al.*, 2008). Due to their low detection threshold and pleasant aroma, furans play an important role in the overall aroma profile of cooked meat (Ramírez & Cava, 2007).

A total of 11 furans were identified across all MRPs in this study. Furans were not identified in NYE, and only two furans (i.e., 2-furanmethanol and furfural) were found in trace amounts in NYH, as shown in Table 1. Enzymatic hydrolysis of NYE with Flavourzyme®, a blend of endo- and exo-peptidases, impacted the generation of furans. The enzyme breaks down proteins and peptides into smaller fragments and free amino acids, leading to a higher concentration of these smaller molecules in NYH than in NYE. While reducing sugars are generally recognised as contributors to furan formation during Maillard reaction, certain amino acids (e.g., serine and cysteine) can participate through alternative pathways (Seok *et al.*, 2015). The Strecker degradation of these amino acids generates intermediates such as acetaldehyde. Subsequently, these

intermediates can undergo aldol condensation, cyclisation, and dehydration reactions, ultimately forming furans. (Perez Locas & Yaylayan, 2004). Specifically, the furans could have further decarbonised during the hydrolysis, forming the furfural found in NYH, which was not present in NYE. Huang *et al.* (2023) reported that furfural was found in a model system that only had serine as a reactant after Maillard reaction. They proposed that furans could be generated from serine degradation during thermal treatment followed by Strecker reaction. This indicated the possibility of generating furans from only amino acids during Maillard reaction and explained the presence of furans in NYH.

The addition of sugar promoted furan formation in MRPs, which is evident from the higher concentrations and greater diversity of furans compared with NYE and NYH (Table 1). The reducing sugars significantly impacted furan formation, with pentoses favouring furan generation more than hexoses. This observation is supported by identifying more furans in pentose-derived MRPs. Specifically, another nine furans were detected in pentose-derived MRPs compared with those derived from hexoses. Volatiles such as furfural and 2-furanmethanol were also significantly higher in concentrations found in pentose-derived MRPs than in hexose-derived MRPs. Previous observations suggested pentoses are more prone to degradation and caramelisation during Maillard reaction, leading to a selective generation of furans instead of pyrazines. This was reflected in higher concentrations of furans and, conversely, lower concentrations of pyrazines found in pentose-derived MRPs compared with hexose-derived MRPs. The increased susceptibility of pentoses to degradation facilitates the generation of reactive intermediates, such as deoxyosones, which serve as a larger precursor pool for furan formation (Perez Locas & Yaylayan, 2004).

Compared within the hexoses, glucose-derived MRPs generated higher concentrations of furans than fructose-derived MRPs. Contrary to our results, literature reported that using fructose, along with single amino acids such as serine, favoured the formation of furans compared with glucose-serine mixtures (Huang *et al.*, 2023). Hu *et al.* (2018) suggested that the effects of furan formation are significantly altered by the specific amino acid combinations used in the Maillard reaction. Studies investigating model systems with single hexoses have reported that fructose promotes furan generation (Limacher *et al.*, 2008). However, contrasting results were observed when the model system was supplemented with specific amino acids, such as phenylalanine or a combination of alanine and serine. Furans can be generated through two different pathways involving different intermediates: 4-deoxyhexo-2,3-diulose and 3-deoxyhexos-2-ulose. In particular, furfural is derived from the 3-deoxyhexos-2-ulose

intermediate (Limacher *et al.*, 2008). The authors further reported that approximately 38% of furans in glucose but only 9% in fructose were generated via this pathway. This difference in pathway utilisation might explain the observed variations in furfural and overall furan content between glucose-derived MRPs and fructose-derived MRPs in literature and the observed findings in this study.

Between pentoses, ribose favoured the generation of furans compared with xylose, as higher concentrations of furans were found in ribose-derived MRPs. This could be observed in many other literature that reported higher concentrations of furans across different mediums (e.g., soybean meal, sunflower protein hydrolysate, peony seed hydrolysate) in ribose-derived MRPs than in xylose-derived MRPs (Karangwa *et al.*, 2017; Shang *et al.*, 2020; Sun *et al.*, 2023). This could be due to a higher reactivity of ribose than xylose during Maillard reaction. Compared with xylose, ribose possesses a less stable ring structure, leading to a higher proportion of acyclic forms (Laroque *et al.*, 2008). This increased presence of acyclic ribose molecules could contribute to its increased efficiency in generating furans during the Maillard reaction.

The increase in protein-to-sugar ratios led to an overall rise in furan concentration. This observation aligned with findings from other studies using different reactants, where higher sugar content resulted in increased furan formation (Van Lancker *et al.*, 2011; Guo *et al.*, 2019; Li *et al.*, 2021).

Therefore, this study demonstrated that adding reducing sugars with NYH during the Maillard reaction significantly enhanced furan formation, with pentoses being more efficient than hexoses. While furans like furfural and 2-furanmethanol have been reported in commercial yeast extracts (Alim *et al.*, 2018, 2020), no furans were detectable in NYE in this study. Furthermore, the furan concentrations observed in those studies on commercial yeast extracts were considerably higher than those found in our NYH. However, the furan concentrations in MRPs from this study were higher than those reported in the literature. This highlights the potential of using reducing sugars during Maillard reaction with NYH to generate furans with NYH.

The positions of points in the score plot (Fig. 4a) reflect the similarities and differences in the furan profiles. Hexose-derived MRPs clustered at the bottom left, indicating a similar furan profile to NYE and NYH. In contrast, pentose-derived MRPs were scattered along the plot, suggesting a more diverse influence on the types of furans formed. Ribose-derived MRPs, though scattered across the plot, showed a positive association with PC1. This association became progressively stronger (increasingly positive) as the

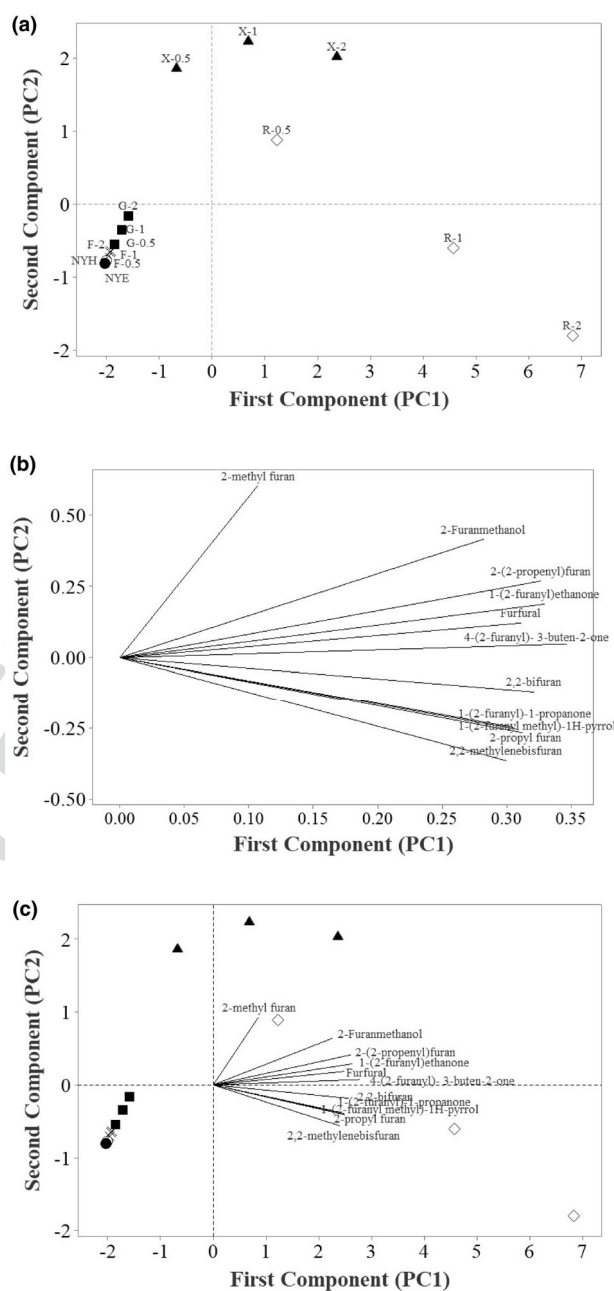


Figure 4 (a) score plot, (b) loading plot and (c) biplot for furans of nutritional yeast extract (NYE), nutritional yeast hydrolysate (NYH) and Maillard reaction products (MRPs) derived from glucose (G-0.5, G-1, G-2), fructose (F-0.5, F-1, F-2), xylose (X-0.5, X-1, X-2) and ribose (R-0.5, R-1, R-2) at different protein-to-sugar ratios, generated from the principal component analysis (PCA). The following symbols represent NYE (●) and NYH (○), followed by the treatment with different reducing sugars: G (■), F (×), X (▲) and R (◇).

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protein-to-sugar ratio increased. It was also observed that xylose-derived MRPs exhibited a progressively stronger relationship with PC1 with an increased protein-to-sugar ratio. Also, xylose-derived MRPs positioned together exhibited a strong positive association with the PC2. Similarly, the increased protein-to-sugar ratio in hexose-derived MRPs led to a stronger association with PC2. However, there was no observable change in the association with PC1 in the context of hexose-derived MRPs.

From the loading plot (Fig. 4b), several furans (i.e., 2-furanmethanol, 2-methyl furan and furfural) displayed positive loadings on PC1 and PC2. This suggests a strong association with these components and potentially indicates their significant contribution to the overall furan profile observed in the MRPs. The biplot (Fig. 4c) revealed a closer relationship between furans and pentose-derived MRPs, particularly ribose-derived MRPs. This positioning suggests a stronger correlation between ribose and furan generation during the Maillard reaction, which aligned with the higher numbers and concentrations of furans observed in ribose-derived MRPs compared with xylose-derived MRPs in Table 1.

Other contributing volatiles

Strecker aldehydes. Building on the diverse volatile profiles observed in Table 1, Strecker aldehydes strongly influenced the overall aroma profile in the MRPs. For instance, methional, known for its low odour threshold and meaty aroma, has been consistently identified as a critical volatile compound in cooked meat (Celia Resconi *et al.*, 2012; Ruan *et al.*, 2015). Frank *et al.* (2016a) and Ko *et al.* (2005) reported that methional was the most potent aroma and dominant active volatile in Korean grilled beef and Australian grilled beef when analysed by gas-chromatography olfactory (GC-O). The formation of key Strecker aldehydes, including methional (derived from methionine), 2-methylpropanal (from valine), and benzeneacetaldehyde (from phenylalanine), is directly linked to the specific amino acids present in the reaction mixture (Resconi *et al.*, 2013; Sasanam *et al.*, 2021). Strecker aldehydes have also been previously reported in plant-based MRPs. Benzeneacetaldehyde was reported to be found in MRPs derived from mushroom and peony seed hydrolysates (Chen *et al.*, 2018; Xia *et al.*, 2021). Similarly, methional and benzeneacetaldehyde have also been reported to be found in yeast extracts (Mahadevan & Farmer, 2006; Alim *et al.*, 2020; Raza *et al.*, 2020b).

Three Strecker aldehydes were identified in this study, which were only found in pentose-derived MRPs. This indicated that the reactivity of sugar, rather than the enzymatic hydrolysis of NYE, impacted the formation

of Strecker aldehydes. Similar to Arsa & Theerakulkait (2018), who did not detect Strecker aldehyde (i.e., 2-methylbutanal and 3-methylbutanal) in rice bran hydrolysates, our study also did not identify these specific Strecker aldehydes. This highlights that the formation pathway of Strecker aldehydes is more likely to be influenced by the reaction with reducing sugars during Maillard reaction.

Pentoses are more reactive than hexoses during Maillard reaction due to their higher proportion of acyclic forms than hexoses. The acyclic form readily undergoes carbonyl-amino reactions, creating precursors for Strecker aldehydes (Parker, 2015). This explained why only pentose-derived MRPs contained Strecker aldehydes. Furthermore, ribose, being more reactive than xylose due to a higher acyclic form content, facilitated a greater extent of Maillard reaction and resulted in a higher abundance of Strecker aldehydes in ribose-derived MRPs.

Sulphur-containing volatiles. Sulphur-containing compounds are well-recognised contributors to the characteristic meaty aroma of meat products (Liu *et al.*, 2015; Bassam *et al.*, 2022). Due to their low odour threshold, they play a significant role in the overall aroma profile. Dimethyl disulphide, known for its sulphurous aroma and abundance in chicken and beef, has been linked to positive quality perception in grilled beef steak (Gardner & Legako, 2018). Similarly, dimethyl trisulphide, characterised by an onion-like odour, is commonly found in pork and beef (Raza *et al.*, 2020b).

The study identified two specific sulphur-containing compounds (dimethyl disulphide and dimethyl trisulphide) and one thiophene in the MRPs, but not in NYE or NYH. Interestingly, thiazole was found exclusively in fructose-derived MRPs. At the same time, the sulphur-containing compounds were detected only in pentose-derived MRPs. This suggests a potential link between the type of sugar used and these aroma-contributing volatile compounds formed during the Maillard reaction.

Several studies have reported the presence of sulphur-containing compounds in yeast extracts, with dimethyl disulphide and dimethyl trisulphide being the most commonly identified volatiles (Wang *et al.*, 2019; Alim *et al.*, 2020; Raza *et al.*, 2020a, 2020b). Wang *et al.* (2019) characterised dimethyl disulphide as a key aroma compound in yeast extracts derived from the enzymatic hydrolysis of *S. cerevisiae*, attributing its significance to a meaty and burnt aroma profile. Similar observations were also made by Lin *et al.* (2014), who identified both dimethyl disulphide and dimethyl trisulphide as prominent aroma-active volatiles in yeast extract samples. These findings, coupled with the low detection thresholds

1 of the sulphur-containing compounds, highlight their
2 potential influence on the overall aroma profile of the
3 MRPs produced in this study.

4
5 *Oxygen-containing volatiles.* A diverse range of oxygen-
6 containing volatiles in the MRPs, including seven
7 acids, five aldehydes, two ketones, one alcohol, and
8 one lactone, was identified in this study. However, due
9 to their generally high odour thresholds, these volatiles
10 may not significantly impact the overall aroma profile
11 unless present in very high concentrations (Su
12 *et al.*, 2011). Interestingly, aldehydes, alcohols, and
13 methyl ketones, which are considered as lipid-derived
14 volatiles (Karangwa *et al.*, 2015), were identified in all
15 samples, including NYE and NYH. This suggests a
16 potential contribution from the breakdown of starting
17 materials during the Maillard reaction.

18 Three acids (i.e., 2-methylpropanoic acid, 3-
19 methylbutanoic acid, and hexanoic acid) were present
20 among the identified oxygen-containing volatiles. Notably,
21 acetic acid was only detected in pentose-derived
22 MRPs. This specific acid indicates Maillard reaction progress,
23 as it arises from sugar degradation (Li *et al.*, 2021);
24 its presence solely in pentose-derived MRPs suggests a
25 higher reactivity of pentoses compared with hexoses during
26 Maillard reaction. This potentially indicates a more
27 advanced Maillard reaction in pentose-derived products,
28 leading to the generation of acetic acid.

29 Heptanal and benzaldehyde were detected among
30 the aldehydes. This suggests that the aldehydes might
31 have been formed from the breakdown of lipids within
32 the reactant, potentially due to natural oxidation processes.
33 Supporting this notion, He *et al.* (2021)
34 reported heptanal as a common lipid degradation
35 product in various commercial plant protein burger
36 samples. Interestingly, the concentration of most aldehydes
37 (excluding benzaldehyde) in this study decreased
38 by adding reducing sugars and increasing protein-to-sugar
39 ratios. This aligns with observations by Van Ba
40 *et al.* (2013), who suggest that the presence and concentration
41 of reducing sugars can influence the oxidation pathways of fatty acids.
42 Consequently, the types and concentrations of volatile compounds
43 formed during the Maillard reaction might be altered. The exception
44 of benzaldehyde in this trend suggests further investigation
45 into its specific formation pathway.

46
47
48 *Relationship between other volatiles and treatment of NYE.* The relative positions of the samples in the
49 score plot (Fig. 5a) reveal the influence of sugar type
50 on other volatiles, particularly for Strecker aldehydes,
51 sulphur-containing compounds, and oxygen-containing
52 volatiles. The close proximity of NYE and NYH
53 suggests a limited impact of enzymatic hydrolysis on
54
55
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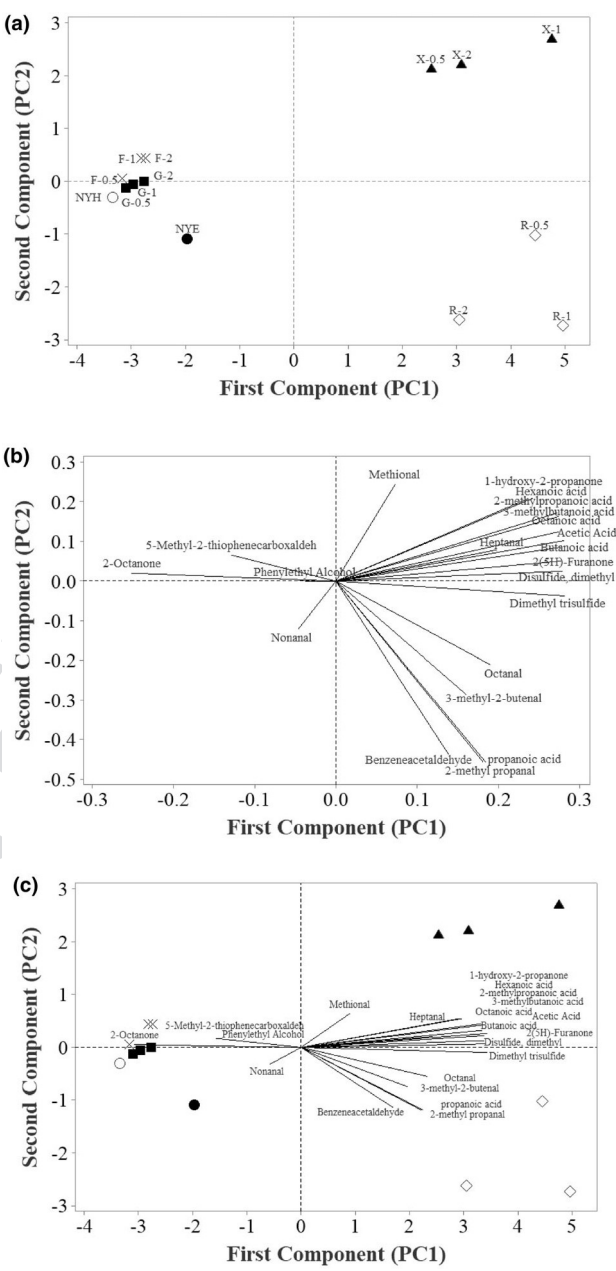


Figure 5 (a) score plot, (b) loading plot and (c) biplot for other volatiles of nutritional yeast extract (NYE), nutritional yeast hydrolysate (NYH) and Maillard reaction products (MRPs) derived from glucose (G-0.5, G-1, G-2), fructose (F-0.5, F-1, F-2), xylose (X-0.5, X-1, X-2) and ribose (R-0.5, R-1, R-2) at different protein-to-sugar ratios, generated from the principal component analysis (PCA). The following symbols represent NYE (●) and NYH (○), followed by the treatment with different reducing sugars: G (■), F (×), X (▲) and R (◇).

Poor quality

volatile formation. However, a greater distinction was observed between pentose-derived and hexose-derived MRPs, indicating a more significant effect of sugar type. Pentose-derived MRPs occupied a distinct region compared with NYE and NYH, suggesting a more substantial difference in their volatile profiles. Interestingly, hexose-derived MRPs clustered closer to NYE and NYH, indicating a similar volatile profile across these samples. Within the pentose group (Fig. 5a), xylose-derived MRPs exhibited strong positive associations with both PC1 and PC2, indicating a more diverse volatile profile enriched with compounds like Strecker aldehydes and acids (Fig. 5c), compared with ribose-derived MRPs, which primarily positively associated with PC1 and likely possess a less diverse profile dominated by other volatile classes (e.g., aldehydes based on Fig. 5c).

The loading plot (Fig. 5b) further clarifies the association of specific volatiles with the observed patterns. Most acids showed strong positive associations with both PC1 and PC2, and they are also closely linked to xylose-derived MRPs in the biplot (Fig. 5c). Additionally, dimethyl disulphide and methional were positioned near xylose-derived MRPs, indicating their prevalence in these samples. Conversely, ribose-derived MRPs are positioned closer and in the same quadrant to Strecker aldehydes (benzeneacetaldehyde and 2-methyl propanal) and dimethyl trisulphide in the biplot, suggesting a higher abundance of these specific volatiles compared with xylose-derived ones. Finally, the positioning of hexose-derived MRPs near certain ketones and alcohols in the biplot suggests a distinct volatile profile compared with pentose-derived MRPs.

Conclusion

This study provides the initial demonstration that sugar types (pentoses and hexoses) and protein-to-sugar ratios significantly influence the pH, browning intensity, and volatile profile of MRPs derived from NYH. The resulting MRPs possessed various desirable volatile compounds known to contribute to roasted or meaty aromas, including pyrazines, Strecker aldehydes (e.g., methional), and sulphur-containing compounds. PCA shows a link between pentoses and the generation of Strecker aldehydes and sulphur-containing compounds. Xylose, in particular, promotes the generation of methional and dimethyl disulphide. At the same time, ribose favours the formation of 2-methyl propanal and dimethyl trisulphide. However, it is important to note that PCA also associates pentose-derived MRPs with furans, which are known for imparting bitter notes at high concentrations. Conversely, hexoses appear to favour the generation of pyrazines. PCA highlights a positive correlation between fructose and the formation of methylpyrazine and 2,6-dimethylpyrazine. Similarly,

glucose promotes the generation of 2-methyl-5-(2-propenyl)pyrazine. However, some aldehydes associated with off-flavours, potentially arising from lipid oxidation, were also observed in hexose-derived MRPs. In conclusion, these findings suggest that MRPs derived from NYH offer promising potential as plant-based enhancers, contributing to developing meaty flavours in the alternative protein industry. Further research could explore the optimisation of reaction conditions to maximise the generation of desirable aroma compounds while minimising off-flavours.

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Author contributions

Alicia Hui Ping Theng: Methodology; investigation; formal analysis; visualization; writing – original draft; writing – review and editing. **Raffael Osen:** Writing – review and editing. **Jie Hong Chiang:** Conceptualization; methodology; visualization; writing – review and editing; project administration; funding acquisition.

Ethical guidelines

Ethics approval was not required for this research.

Conflict of interest

The authors declare that they have no conflict of interest.

Peer review

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

- Alim, A., Song, H., Liu, Y., Zou, T., Zhang, Y. & Zhang, S. (2018). Flavour-active compounds in thermally treated yeast extracts. *Journal of the Science of Food and Agriculture*, **98**, 3774–3783.

- 1 Alim, A., Song, H. & Zou, T. (2020). Analysis of meaty aroma and
2 umami taste in thermally treated yeast extract by means of
3 sensory-guided screening. *European Food Research and Technology*,
4 **246**, 2119–2133.
- 5 The paper identified specific aroma-active compounds associated
6 with desired flavours in yeast extract. This information can be a
7 valuable reference point for identifying similar or related compounds
8 in the MRPs from this study and understanding their contribution
9 to taste.
- 10 Arsa, S. & Theerakulkait, C. (2018). Preparation, aroma characteris-
11 tics and volatile compounds of flavorings from enzymatic hydro-
12 lyzed rice bran protein concentrate. *Journal of the Science of Food
13 and Agriculture*, **98**, 4479–4487.
- 14 Bassam, S.M., Noleto-Dias, C. & Farag, M.A. (2022). Dissecting
15 grilled red and white meat flavor: its characteristics, production
16 mechanisms, influencing factors and chemical hazards. *Food Chem-
17 istry*, **371**, 131139.
- 18 Begum, N., Raza, A., Song, H., Zhang, Y., Zhang, L. & Liu, P.
19 (2019). Effect of thermal treatment on aroma generation from
20 bovine bone marrow extract during enzymatic hydrolysis. *Journal
21 of Food Processing and Preservation*, **43**, e14105.
- 22 Bekatorou, A., Psarianos, C. & Koutinas, A.A. (2006). Production
23 of food grade yeasts. *Food Technology and Biotechnology*, **44**.
- 24 Benjakul, S., Lertittikul, W. & Bauer, F. (2005). Antioxidant activity
25 of Maillard reaction products from a porcine plasma protein–sugar
26 model system. *Food Chemistry*, **93**, 189–196.
- 27 Brands, C.M. & van Boekel, M.A. (2001). Reactions of monosaccha-
28 rides during heating of sugar–casein systems: building of a reac-
29 tion network model. *Journal of Agricultural and Food Chemistry*,
30 **49**, 4667–4675.
- 31 Celia Resconi, V., del Mar Campo, M., Montossi, F., Ferreira, V.,
32 Sanudo, C. & Escudero, A. (2012). Gas chromatographic-
33 olfactometric aroma profile and quantitative analysis of volatile
34 carbonyls of grilled beef from different finishing feed systems. *Jour-
35 nal of Food Science*, **77**, S240–S246.
- 36 Chen, X.M. & Kitts, D.D. (2011). Correlating changes that occur in
37 chemical properties with the generation of antioxidant capacity in
38 different sugar-amino acid Maillard reaction models. *Journal of
39 Food Science*, **76**, C831–C837.
- 40 Chen, X., Yu, J., Cui, H., Xia, S., Zhang, X. & Yang, B. (2018).
41 Effect of temperature on flavor compounds and sensory character-
42 istics of Maillard reaction products derived from mushroom hydro-
43 lysate. *Molecules*, **23**, 247.
- 44 Chiang, J., Loveday, S., Hardacre, A. & Parker, M. (2019). Effects
45 of enzymatic hydrolysis treatments on the physicochemical prop-
46 erties of beef bone extract using endo- and exoproteases. *International
47 Journal of Food Science & Technology*, **54**, 111–120.
- 48 Chiang, J.H., Yeo, M.T.Y., Ong, D.S.M. & Henry, C.J. (2022).
49 Comparison of the molecular properties and volatile compounds of
50 Maillard reaction products derived from animal- and cereal-based
51 protein hydrolysates. *Food Chemistry*, **383**, 132609.
- 52 Cui, Q., Sun, Y., Zhou, Z., Cheng, J. & Guo, M. (2021). Effects
53 of enzymatic hydrolysis on physicochemical properties and solu-
54 bility and bitterness of milk protein hydrolysates. *Foods*, **10**,
55 2462.
- 56 Davidek, T., Robert, F., Devaud, S., Vera, F.A. & Blank, I. (2006).
Sugar fragmentation in the Maillard reaction cascade: formation
of short-chain carboxylic acids by a new oxidative α -dicarbonyl
cleavage pathway. *Journal of Agricultural and Food Chemistry*, **54**,
6677–6684.
- Eric, K., Raymond, L.V., Huang, M. *et al.* (2013). Sensory attributes
and antioxidant capacity of Maillard reaction products derived
from xylose, cysteine and sunflower protein hydrolysate model sys-
tem. *Food Research International*, **54**, 1437–1447.
- Eskin, N.A.M., Ho, C.-T. & Shahidi, F. (2013). Chapter 6 – Brow-
ning reactions in foods. In: *Biochemistry of Foods (Third Edition)*
(edited by N.A.M. Eskin & F. Shahidi). Pp. 245–289. Academic
Press.
- Frank, D., Ball, A., Hughes, J. *et al.* (2016a). Sensory and flavor
chemistry characteristics of Australian beef: influence of intramus-
cular fat, feed, and breed. *Journal of Agricultural and Food Chemis-
try*, **64**, 4299–4311.
- Frank, D., Watkins, P., Ball, A. *et al.* (2016b). Impact of brassica
and lucerne finishing feeds and intramuscular fat on lamb eating
quality and flavor. A cross-cultural study using Chinese and non-
Chinese Australian consumers. *Journal of Agricultural and Food
Chemistry*, **64**, 6856–6868.
- Gardner, K. & Legako, J.F. (2018). Volatile flavor compounds vary
by beef product type and degree of doneness. *Journal of Animal
Science*, **96**, 4238–4250.
- Guo, J., Zhao, R., Li, J. *et al.* (2019). Furan formation from ingredi-
ent interactions and furan mitigation by sugar alcohols and antiox-
idants of bamboo leaves in milk beverage model systems. *Journal
of the Science of Food and Agriculture*, **99**, 4993–4999.
- He, J., Liu, H., Balamurugan, S. & Shao, S. (2021). Fatty acids and
volatile flavor compounds in commercial plant-based burgers. *Jour-
nal of Food Science*, **86**, 293–305.
- Hu, B., Lu, Q., Jiang, X. *et al.* (2018). Pyrolysis mechanism of glu-
cose and mannose: the formation of 5-hydroxymethyl furfural and
furfural. *Journal of Energy Chemistry*, **27**, 486–501.
- Huang, H., Chen, J., Zheng, M. *et al.* (2023). Precursors and forma-
tion pathways of furfural in sugarcane juice during thermal treat-
ment. *Food Chemistry*, **402**, 134318.
- Jayasena, D.D., Ahn, D.U., Nam, K.C. & Jo, C. (2013). Flavour
chemistry of chicken meat: a review. *Asian-Australasian Journal of
Animal Sciences*, **26**, 732.
- Karangwa, E., Zhang, X., Murekatete, N. *et al.* (2015). Effect of
substrate type on sensory characteristics and antioxidant capacity
of sunflower Maillard reaction products. *European Food Research
and Technology*, **240**, 939–960.
- Karangwa, E., de Dieu Habimana, J., Jingyang, Y. *et al.* (2017).
Sensory characteristics of Maillard reaction products obtained
from sunflower protein hydrolysates and different sugar types.
International Journal of Food Engineering, **13**.
- Ko, H.S., Kim, T.H., Cho, I.H., Yang, J.Y., Kim, Y.S. & Lee, H.J.
(2005). Aroma active compounds of bulgogi. *Journal of Food Sci-
ence*, **70**, c517–c522.
- Kwak, E.-J. & Lim, S.-I. (2004). The effect of sugar, amino acid,
metal ion, and NaCl on model Maillard reaction under pH con-
trol. *Amino Acids*, **27**, 85–90.
- Laemont, J. & Barringer, S. (2023). Effect of pH, reducing sugars,
and protein on roasted sunflower seed aroma volatiles. *Foods*, **12**,
4155.
- Laroque, D., Inisan, C., Berger, C., Voulard, É., Dufossé, L. &
Guérard, F. (2008). Kinetic study on the Maillard reaction. Con-
sideration of sugar reactivity. *Food Chemistry*, **111**, 1032–1042.
- The paper offered insights into the different reactivity patterns of
pentoses and hexoses during the Maillard reaction. This knowledge
can help to anticipate how the chosen sugar types (pentoses vs hex-
oses) might influence the Maillard reaction process and the resulting
products in this study.
- Lertittikul, W., Benjakul, S. & Tanaka, M. (2007). Characteristics
and antioxidative activity of Maillard reaction products from a
porcine plasma protein–glucose model system as influenced by pH.
Food Chemistry, **100**, 669–677.
- Li, L., Wang, L., Liu, J. *et al.* (2020). Effect of reaction conditions
on the volatile pyrazines components of defatted flaxseed meal in
the Maillard reaction system. *Journal of the American Oil Chemists’
Society*, **97**, 1385–1399.
- Li, L., Belloch, C. & Flores, M. (2021). The Maillard reaction as
source of meat flavor compounds in dry cured meat model systems
under mild temperature conditions. *Molecules*, **26**, 223.
- Limacher, A., Kerler, J., Davidek, T., Schmalzried, F. & Blank, I.
(2008). Formation of furan and methylfuran by Maillard-type reac-
tions in model systems and food. *Journal of Agricultural and Food
Chemistry*, **56**, 3639–3647.

- Lin, M., Liu, X., Xu, Q., Song, H., Li, P. & Yao, J. (2014). Aromatic components of yeast extract pastes with a basic and characteristic meaty flavour. *Journal of the Science of Food and Agriculture*, **94**, 882–889.
- Liu, S.-C., Yang, D.-J., Jin, S.-Y., Hsu, C.-H. & Chen, S.-L. (2008). Kinetics of color development, pH decreasing, and anti-oxidative activity reduction of Maillard reaction in galactose/glycine model systems. *Food Chemistry*, **108**, 533–541.
- Liu, Q., Li, J., Kong, B., Li, P. & Xia, X. (2014). Physicochemical and antioxidant properties of Maillard reaction products formed by heating whey protein isolate and reducing sugars. *International Journal of Dairy Technology*, **67**, 220–228.
- Liu, J., Liu, M., He, C., Song, H. & Chen, F. (2015). Effect of thermal treatment on the flavor generation from Maillard reaction of xylose and chicken peptide. *LWT – Food Science and Technology*, **64**, 316–325.
- Ma, J., Sun, Y., Meng, D., Zhou, Z., Zhang, Y. & Yang, R. (2023). Yeast proteins: the novel and sustainable alternative protein in food applications. *Trends in Food Science & Technology*, **135**, 190–201.
- Madriga, M.S., Stephen Elmore, J., Dodson, A.T. & Mottram, D.S. (2009). Volatile flavour profile of goat meat extracted by three widely used techniques. *Food Chemistry*, **115**, 1081–1087.
- Mahadevan, K. & Farmer, L. (2006). Key odor impact compounds in three yeast extract pastes. *Journal of Agricultural and Food Chemistry*, **54**, 7242–7250.
- Meinlschmidt, P., Sussmann, D., Schweiggert-Weisz, U. & Eisner, P. (2016). Enzymatic treatment of soy protein isolates: effects on the potential allergenicity, technofunctionality, and sensory properties. *Food Science & Nutrition*, **4**, 11–23.
- Parker, J.K. (2015). Thermal generation of aroma. In: *Flavour Development, Analysis and Perception in Food and Beverages* (edited by J.K. Parker, J.S. Elmore & L. Methven). Pp. 151–185. Amsterdam: Elsevier.
- Perez Locas, C. & Yaylayan, V.A. (2004). Origin and mechanistic pathways of formation of the parent furan a food toxicant. *Journal of Agricultural and Food Chemistry*, **52**, 6830–6836.
- Pham, A., Schilling, M., Mikel, W., Williams, J., Martin, J. & Coggin, P. (2008). Relationships between sensory descriptors, consumer acceptability and volatile flavor compounds of American dry-cured ham. *Meat Science*, **80**, 728–737.
- Phisit, N. & Jiraporn, B. (2013). Characteristics and antioxidant activity of Maillard reaction products derived from chitosan-sugar solution. *International Food Research Journal*, **20**, 1077.
- Purbaningrum, K., Hidayat, C., Witasari, L.D. & Utami, T. (2023). Flavor precursors and volatile compounds improvement of unfermented cocoa beans by hydrolysis using bromelain. *Foods*, **12**, 820.
- Ramírez, R. & Cava, R. (2007). Volatile profiles of dry-cured meat products from three different Iberian X Duroc genotypes. *Journal of Agricultural and Food Chemistry*, **55**, 1923–1931.
- Raza, A., Song, H., Begum, N. *et al.* (2020a). Direct classification of volatile organic compounds in heat-treated glutathione-enriched yeast extract by headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS). *Food Analytical Methods*, **13**, 2279–2289.
- The paper highlighted how heat treatment can significantly affect the volatile profile of yeast extract, potentially generating desirable aroma compounds. This knowledge is relevant to this study on how reaction conditions influence the formation of volatile compounds in MRPs.
- Raza, A., Song, H., Raza, J., Li, P., Li, K. & Yao, J. (2020b). Formation of beef-like odorants from glutathione-enriched yeast extract via Maillard reaction. *Food & Function*, **11**, 8583–8601.
- Resconi, V.C., Escudero, A. & Campo, M.M. (2013). The development of aromas in ruminant meat. *Molecules*, **18**, 6748–6781.
- Ruan, E.D., Aalhus, J.L., Juárez, M. & Sabik, H. (2015). Analysis of volatile and flavor compounds in grilled lean beef by stir bar sorptive extraction and thermal desorption—gas chromatography mass spectrometry. *Food Analytical Methods*, **8**, 363–370.
- Sasanam, S., Rungsardthong, V., Thumthananuruk, B. *et al.* (2021). Properties and volatile profile of process flavorings prepared from D-xylose with glycine, alanine or valine by direct extrusion method. *Food Bioscience*, **44**, 101371.
- Seok, Y.-J., Her, J.-Y., Kim, Y.-G. *et al.* (2015). Furan in thermally processed foods—a review. *Toxicological Research*, **31**, 241–253.
- Shang, Y.F., Cao, H., Wei, C.K. *et al.* (2020). Effect of sugar types on structural and flavor properties of peony seed derived Maillard reaction products. *Journal of Food Processing and Preservation*, **44**, e14341.
- Shurson, G. (2018). Yeast and yeast derivatives in feed additives and ingredients: sources, characteristics, animal responses, and quantification methods. *Animal Feed Science and Technology*, **235**, 60–76.
- Su, G., Zheng, L., Cui, C., Yang, B., Ren, J. & Zhao, M. (2011). Characterization of antioxidant activity and volatile compounds of Maillard reaction products derived from different peptide fractions of peanut hydrolysate. *Food Research International*, **44**, 3250–3258.
- Sun, Y., Hayakawa, S. & Izumori, K. (2004). Antioxidative activity and gelling rheological properties of dried egg white glycated with a rare keto-hexose through the Maillard reaction. *Journal of Food Science*, **69**, C427–C434.
- Sun, H.M., Wang, J.Z., Zhang, C.H. *et al.* (2014). Changes of flavor compounds of hydrolyzed chicken bone extracts during Maillard reaction. *Journal of Food Science*, **79**, C2415–C2426.
- Sun, L., Wang, D., Huang, Z., Elfalleh, W., Qin, L. & Yu, D. (2023). Structure and flavor characteristics of Maillard reaction products derived from soybean meal hydrolysates-reducing sugars. *LWT*, **185**, 115097.
- The paper highlighted how sugar selection can influence the formation and characteristics of MRPs. This aligns with this study on how sugar type (pentoses and hexoses) might affect the MRPs.
- Van Ba, H., Amna, T. & Hwang, I. (2013). Significant influence of particular unsaturated fatty acids and pH on the volatile compounds in meat-like model systems. *Meat Science*, **94**, 480–488.
- Van Lancker, F., Adams, A., Owczarek-Fendor, A., De Meulenaer, B. & De Kimpe, N. (2011). Mechanistic insights into furan formation in Maillard model systems. *Journal of Agricultural and Food Chemistry*, **59**, 229–235.
- Wang, Z., Xiao, Q., Zhuang, J., Feng, T., Ho, C.-T. & Song, S. (2019). Characterization of aroma-active compounds in four yeast extracts using instrumental and sensory techniques. *Journal of Agricultural and Food Chemistry*, **68**, 267–278.
- Watanabe, A., Kamada, G., Imanari, M., Shiba, N., Yonai, M. & Muramoto, T. (2015). Effect of aging on volatile compounds in cooked beef. *Meat Science*, **107**, 12–19.
- Wu, G. (2020). Important roles of dietary taurine, creatine, carnosine, anserine and 4-hydroxyproline in human nutrition and health. *Amino Acids*, **52**, 329–360.
- Xia, B., Ni, Z.-J., Hu, L.-T. *et al.* (2021). Development of meat flavors in peony seed-derived Maillard reaction products with the addition of chicken fat prepared under different conditions. *Food Chemistry*, **363**, 130276.
- Zhang, Z., Elfalleh, W., He, S. *et al.* (2018). Heating and cysteine effect on physicochemical and flavor properties of soybean peptide Maillard reaction products. *International Journal of Biological Macromolecules*, **120**, 2137–2146.
- Zhang, Y., Wang, Y., Jiang, F. & Jin, H. (2019). Sensory characteristics of Maillard reaction products from chicken protein hydrolysates with different degrees of hydrolysis. *CyTA-Journal of Food*, **17**, 221–227.