Sensory Characteristics of Flavour Improvement in Squid and Cuttlefish Ink Hydrolysates by Maillard Reaction

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By-products from seafood usually smell fishy. In this research, squid ink powder enzyme hydrolysates (SIPEHs) and cuttlefish ink powder enzyme hydrolysates (CIPEHs) were produced via 4 h of hydrolysis with Alcalase® and papain, respectively, at 3% E/S ratios. To generate Maillard reaction products (MRPs), D-xylose and L-cysteine or D-xylose alone were introduced to the hydrolysates. The mixture was then heated at 120 °C for 2 hours. The resulting MRPs were labelled as SIPEHs-mx, SIPEHs-x, CIPEHs-mx, or CIPEHs-x. Results showed that extending the reaction time increased browning products and aroma compounds and reduced pH from 7.4 to 6.54-5.55, respectively. Furthermore, the Maillard reaction products (MRPs) also exhibited a reduction in volatile aldehydes that are typically linked to fish-like aroma. The qualitative descriptive analysis (QDA) test revealed that MRPs had a better umami flavour and a less fishy flavour than hydrolysates. SIPEHs-mx and CIPEHS-mx were preferred with the overall acceptance that satisfied consumer preferences. Therefore, the Maillard reaction was found to improve the overall flavour of both SIPEHs and CIPEHs. The results indicated that the flavour compounds developed in the MRPs during the subsequent heat treatment have the potential to serve as a suitable alternative to hydrolysates, producing flavours that meet consumer preferences and are considered acceptable. Such an approach holds promise for application in product development and improvement studies, offering practical implications for the food industry.

Keywords: sensory; Maillard reaction products; hydrolysate; squid ink; cuttlefish ink

I. INTRODUCTION

Cephalopods, which encompass squids, cuttlefish, and octopi, are valuable global fisheries resources of significant importance. The economic value of cephalopods has been rapidly increasing (Kechaou *et al.*, 2015). The growing exploitation of squids leads to a substantial amount of byproducts, which can account for up to 60% of the total weight of the squid (Ezquerra-Brauer & Aubourg, 2019). Among these byproducts, visceral organs and ink sacs are commonly discarded due to their limited profitability.

However, these underutilised marine byproducts possess significant potential as valuable sources of raw materials for the recovery of existing bioactive peptide constituents or the production of bioactive peptides from protein components (Anal *et al.*, 2013).

Enzymatic hydrolysis is a practical approach for converting underutilised fish biomass into a form with increased marketability and acceptability (Salwanee *et al.*, 2013). However, protein hydrolysates obtained primarily from seafood often come with an undesirable fishy and bitter off-

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flavour (Kouakou *et al.*, 2014; Normah & Noorasma, 2018). Consequently, the recognition of seafood protein hydrolysates has been hindered by these fishy off-flavours, which diminish desirability, impact consumer acceptance, and restrict broader application. Numerous attempts have been made to mitigate or conceal this unwanted aroma. These efforts include encapsulation, Plastein reaction, exopeptidase treatment and Maillard reaction. Despite various methods that have been employed, each approach has its limitations. However, the modification through the Maillard reaction appears to be a promising and effective method (Ayu Shazwani & Rabeta, 2021).

The Maillard reaction is a chemical process that happens independently of enzymes. It involves the interaction between carbonyl groups and amine groups found in food, resulting in alterations to colour, functional properties, nutritional value, and flavour (Zhao *et al.*, 2016). The Maillard reaction has shown the capability to modify and enhance the sensory properties and acceptability of fish byproducts. Multiple studies have indicated that MRPs derived from protein-derived peptides play a significant role in enhancing flavour, colour, and bioactive properties (Fu *et al.*, 2020). In addition, MRPs have been widely used as flavour enhancers (Chen *et al.*, 2019). Previous research has shown that fish waste hydrolysates subjected to Maillard reaction generate new flavours (Kouakou *et al.*, 2014).

Nevertheless, there is less data available regarding the Maillard reaction of squid and cuttlefish ink hydrolysates and its potential to mitigate or conceal off-flavours. Therefore, the physicochemical properties and consumer acceptability of the flavour improvement effects demonstrated SIPEHs and CIPEHs and MRPs produced along with D-xylose and/or L-cysteine were assessed in this study.

II. MATERIALS AND METHODS

A. Materials

Squid (*Loligo duvauceli*) and cuttlefish (*Sepia officinalis*) were obtained from the Taman Tun Saadon Wet Market located near Gelugor at Penang in Malaysia. Both samples were packed in a polystyrene icebox and fully covered with ice cubes. The average size of squid combined body and tentacle lengths up to 30 cm and 140 g in weight, and cuttlefish was

around 38 cm and 250 g. The endopeptidases employed in the study included Alcalase® with a stated activity of Anson units (AU) per gram, as well as papain with a stated activity of Anson units (AU) per gram. These enzymes complied with the food grade enzymes suggested specifications outlined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Food Chemicals Codex (FCC). The enzymes were kept at 4°C until use. L-cysteine and D-xylose were purchased from Merk. All solvents and reagents used for the chemical analysis were analytical grade.

B. Preparation of SIPEHs and CIPEHs

A slight modification was made to the enzymatic hydrolysis procedure, following the method outlined by Kim *et al.* (2016). Squid and cuttlefish ink powder were mixed with a safeguard solution and then underwent hydrolysis using Alcalase® derived from *Bacillus licheniformis*, with an activity of 2.972 U/ml) and papain (from *Carica papaya*, with an activity of 3000 USP-U/mg) at a 3% (w/v) E/S ratio, respectively. The optimal temperature and pH for each enzyme were selected based on the recommendations provided by the manufacturer. Hydrolysis was conducted for an extended period of 4 hours. To deactivate the enzyme(s), the reaction was stopped by heating at 85°C for 20 minutes. The resulting hydrolysates were then cooled and subjected to centrifugation (Kubota, Japan, at 10000 rpm for 20 minutes) to collect the supernatant. The resulting products were denoted as the corresponding SIPEHs and CIPEHs.

C. Preparation of MRPs

The preparation of MRPs was conducted following a modified version of the method described by Eric *et al.* (2013). Initially, SIPEHs were dissolved in distilled water along with D-xylose and L-cysteine to achieve a final concentration of 10% (w/v). This mixture was referred to as SIPEHs-mx. Another mixture, called SIPEHs-x, was prepared by combining SIPEHs and D-xylose in distilled water at a final concentration of 10% (w/v). The initial pH of the mixtures was adjusted to 7.4 using either 2N NaOH or 2N HCl. The samples were then heated at 120 °C with continuous stirring using a magnetic stirrer for 120 minutes in an oil bath located in a fume hood. Samples were collected every 30 minutes during the heating process to evaluate changes in browning

intensity and pH. After the heating step, the mixtures were rapidly cooled using ice water. A similar procedure was followed for the preparation of CIPEHs.

D. Physicochemical Properties of MRPs

1. Measurement of pH

The pH values at various time intervals (0, 30, 60, 90, and 120 minutes) during the heating process were determined using a pH meter (Mettler Toledo Delta 320). The pH meter was calibrated multiple times throughout the day to ensure accurate measurements at different temperatures and time points.

2. Measurement of browning intensity

To ensure accurate measurements, the MRPs were appropriately diluted 100-fold until the absorbance values were below 1.5. The absorbance of the samples was measured using a UV-visible spectrophotometer (UV-160A, Shimadzu, Japan) at two specific wavelengths. The absorbance at 294 nm was determined for early MRPs, while the absorbance at 420 nm was measured for late MRPs (Chang *et al.*, 2020). These wavelengths are indicative of the intermediate products of non-enzymatic browning, which serve as precursors to the Maillard reaction, as well as the brown colour developed during the final stages of the reaction.

3. Measurement of colour

Colour measurements were performed using a Minolta colourimeter, CM-3500D (Osaka, Japan). The CIE standard system was utilised, incorporating the following parameters to describe colour: L* (brightness), a* (greenness to redness), b* (blueness to yellowness), and ΔE (colour difference). The results for the various samples were reported in terms of their respective L*, a*, b*, and ΔE values.

4. Volatile compound composition

The procedure for analysing the volatile compounds was adapted from Normah and Noorasma (2018). For the analysis, approximately 1 g of each sample was placed in a 15 ml headspace vial and allowed to reach equilibrium at 50 °C for 15 minutes in a controlled water bath. The vial was then securely sealed using a silicon septum. An Agilent GC-MS system was used for the analysis. The sample was desorbed in the injection port at 250 °C for 2 minutes using the splitless mode. An HP-5MS analytical capillary column was employed, and helium gas was used as the carrier gas at a constant flow rate of 0.8 ml/min. The oven temperature was programmed as follows: initially held at 40 °C for 2 minutes, followed by a linear increase of 5 °C/min until reaching 150 °C (held for 5 minutes), and then a further increase of 10 °C/min until reaching 220 °C (held for 10 minutes). The MS operating conditions included an ion source temperature of 200 °C, an ionisation voltage of 70 eV, and a mass-to-charge ratio range of m/z 33-500 with a scan rate of 2.76 scans/s. To identify the highest peak in the chromatography, the mass spectra were compared with known compounds from the Wiley 6 library (Hewlett-Packard Co., Palo Alto, CA) and NIST 98 library (Hewlett-Packard Co., Palo Alto, CA) mass spectral database. The results were reported as the percentage (%) of the peak area.

E. Sensory Evaluation

1. Sensory evaluation

The sensory analysis was carried out in a Sensory Laboratory School of Industrial Technology, Universiti Sains Malaysia, Pulau Pinang, Malaysia. The samples were prepared in the preparation area and individually evaluated by the subject in the individual booth. For the training session, sensory analysis was conducted in the discussion room. The study protocol followed JEPeM-USM, and the approval code was USM/JEPeM/20050252. The presence of food allergy or intolerance to the samples was considered an exclusion criterion.

2. Quantitative descriptive analysis (QDA) test

Quantitative descriptive analysis (QDA) was conducted in accordance with Normah and Nurhidayati (2018) with a slight modification (without sweet taste). The test was divided into training sessions and sensory evaluation sessions. After selecting 10 panellists, the panellists were trained to discuss the sensory profile and define descriptive terms for each reference sample until a unanimous agreement on the degree of aromatic flavour was reached and

the lowest possible taste intensities were memorised. During training sessions, panellists were presented with reference solutions containing varying concentrations, including fermented anchovies sauce (budu sauce), monosodium glutamate, sodium chloride and caffeic acid, which represented fishy odour and flavour, umami, salty taste and bitter taste, respectively. All descriptors, definitions and references perceived by panellists are shown in Table 1.

Table 1. Description and references for the finalised descriptors.

Attributes	Description	Reference solution	Concentration (%)
Fishy odour	Aroma perceived by smell.	Fermented anchovies sauce (budu sauce)	1 and 8
Fishy flavour	The aroma detected through taste and smell during the act of swallowing.	Fermented anchovies sauce (budu sauce)	0.35 and 1
Umami taste	Basic taste of monosodium glutamate (MSG) solution perceived by tongue.	Monosodium glutamate (MSG)	0.2 and 3
Salty taste	Basic taste of sodium chloride (NaCl) solution perceived by tongue.	Sodium chloride (NaCl)	0.3 and 0.8
Bitter aftertaste	Basic taste of caffeine solution remains after swallowing.	Caffeic acid	0 and 0.03

The validation test was conducted twice to choose panellists with a good ability to differentiate amongst samples and provide good repeatability results and were in agreement with the other panellists. During the sensory evaluation session, the panellists were provided with reference solutions and reference samples that were previously identified during the training session. These were used for comparison purposes to assess the intensity of each flavour in relation to the test samples (SIPEHs, SIPEHs-x, SIPEHs-mx, CIPEHs, CIPEHs-x, and CIPEHs-mx). Each panellist received 20 ml of the test samples (2.5%, w/v) in sensory cups labelled with three codes. Additionally, reference solutions with two different concentrations for each attribute and reference samples labelled as 'A' and 'B' were provided. The panellists were instructed to use a 15 cm line scale to indicate the intensity of each taste, ranging from low to high intensity. Plain water was made available for rinsing purposes, while coffee beans were provided to cleanse the olfactory palate and reduce the possibility of any residual influence carrying over to subsequent evaluations.

3. Acceptability test

The hedonic scale was designed to measure the acceptability of consumers adopting the modified method (change 5% (w/v) to 2.5% (w/v) of Normah *et al.* (2013). The category scale was a seven-point hedonic scale ranging from like very much, neither like nor disliked to disliked very much (modified from 9 scale to 7 scale). Consumers indicated their degree of liking by choosing the appropriate category. Plain rice porridge was used as a carrier to evaluate the acceptability of hydrolysates. A total of 20 ml of 2.5% (w/v) of each hydrolysate was mixed with 50 ml of rice porridge. A minimum of 30 participants were invited to take part in the evaluation and provide their assessment of five attributes: appearance, odour, flavour, aftertaste, and overall acceptability, by indicating their level of liking for each attribute.

4. Statistical analysis

The experimental data was presented as means ± standard deviation (SD). Statistical analysis was performed using SPSS (Version 22.0, IBM Corporation, USA) with a one-way ANOVA to determine significant differences ($p<0.05$). Furthermore, principal component analysis (PCA) was conducted using the XLStat trial version (Addinsoft, New

York, USA) to highlight the average panel scores for each product and sensory descriptor.

III. RESULTS AND DISCUSSION

A. Physicochemical Properties of MRPs

Table 2. Intermediate products and browning intensity, pH and colour evolution of MRPs during heating.

Samples	Parameters	Heating time (min)				
		0	30	60	90	120
SIPEHs-x	pH	7.40 ± 0.02^e	7.10 ± 0.02^d	6.92 ± 0.05^c	6.81 ± 0.01^b	6.51 ± 0.01^a
	294 nm	0.55 ± 0.05^a	0.72 ± 0.02^b	0.94 ± 0.05^c	1.20 ± 1.18^d	1.24 ± 0.03^d
	420 nm	0.45 ± 0.03^e	0.57 ± 0.02^d	0.74 ± 0.03^c	1.03 ± 1.01^b	0.94 ± 0.02^a
	L*	31.47 ± 3.29^b	28.83 ± 1.81^{ab}	26.15 ± 2.67^{ab}	21.51 ± 4.31^a	21.41 ± 0.77^a
	a*	2.73 ± 0.51^a	3.41 ± 0.88^a	4.19 ± 0.11^a	4.30 ± 1.02^a	4.30 ± 0.31^a
	b*	5.63 ± 1.38^a	6.83 ± 1.64^{ab}	9.53 ± 0.23^{bc}	9.93 ± 1.59^{bc}	10.83 ± 1.53^c
	ΔE	-	3.92 ± 0.12^{ab}	6.01 ± 2.65^{bc}	10.31 ± 1.53^{cd}	11.57 ± 2.72^d
SIPEHs-mx	pH	7.41 ± 0.02^e	6.42 ± 0.03^d	6.27 ± 0.02^c	6.13 ± 0.01^b	5.85 ± 0.04^a
	294 nm	0.49 ± 0.08^a	0.71 ± 0.01^b	0.88 ± 0.09^c	1.06 ± 0.03^d	1.42 ± 0.05^e
	420 nm	0.40 ± 0.06^e	0.55 ± 0.01^d	0.67 ± 0.08^c	0.91 ± 0.03^b	1.18 ± 0.04^a
	L*	49.11 ± 3.71^c	47.19 ± 1.13^c	28.53 ± 1.81^b	21.36 ± 0.54^a	16.15 ± 1.78^a
	a*	4.57 ± 0.32^a	4.70 ± 0.05^{ab}	5.10 ± 0.05^{abc}	5.30 ± 0.02^{bc}	5.45 ± 0.38^c
	b*	8.65 ± 0.99^a	9.95 ± 0.2^{ab}	11.50 ± 0.06^b	11.53 ± 0.09^b	11.74 ± 1.49^b
	ΔE	-	18.89 ± 0.94^a	20.79 ± 2.33^a	29.92 ± 3.53^b	33.17 ± 3.5^b
CIPEHs-x	pH	7.40 ± 0.01^c	7.09 ± 0.01^b	7.04 ± 0.03^b	6.78 ± 0.02^a	6.54 ± 0.03^a
	294 nm	1.10 ± 0.08^b	0.69 ± 0.03^a	1.19 ± 0.03^{bc}	1.27 ± 0.02^{cd}	1.38 ± 0.79^d
	420 nm	0.88 ± 0.77^c	0.53 ± 0.03^b	0.92 ± 0.02^b	0.97 ± 0.03^a	0.97 ± 0.03^a
	L*	53.63 ± 2.54^b	37.96 ± 1.76^a	35.52 ± 2.72^a	$30.52\pm2.72a$	28.80 ± 0.28^a
	a*	3.75 ± 0.4^a	3.32 ± 0.22^a	5.24 ± 1.35^a	4.70 ± 0.37^a	5.88 ± 0.35^a
	b*	8.33 ± 1.17^a	6.38 ± 0.86^a	10.08 ± 0.56^a	10.65 ± 2.86^a	10.77 ± 0.87^a
	ΔE	-	15.97 ± 1.68^a	17.99 ± 3.37^a	23.25 ± 3.73^a	24.98 ± 2.50^a
CIPEHs-mx	pH	7.40 ± 0.02^e	6.03 ± 0.06^d	5.92 ± 0.02^c	5.77 ± 0.01^b	5.55 ± 0.04^a
	294 nm	0.76 ± 0.01^a	0.87 ± 0.02^b	0.97 ± 0.03^c	1.03 ± 0.03^c	1.11 ± 0.02^d
	420 nm	0.57 ± 0.01^e	0.75 ± 0.02^d	0.83 ± 0.02^c	0.86 ± 0.03^b	0.93 ± 0.02^a
	L*	45.70 ± 0.39^b	43.03 ± 0.83^{ab}	40.05 ± 5.01^{ab}	37.59 ± 1.15^a	32.89 ± 1.86^a
	a*	5.05 ± 1.26^a	4.88 ± 0.28^a	5.52 ± 1.41^a	6.09 ± 0.34^a	6.90 ± 0.79^a
	b*	9.89 ± 0.62^a	10.92 ± 1.26^{ab}	12.58 ± 4.18^{ab}	14.92 ± 0.93^{ab}	16.89 ± 2.20^b
	ΔE	-	3.34 ± 0.05^{ab}	5.90 ± 5.41^{ab}	9.60 ± 1.27^b	13.21 ± 2.20^b

Note: "SIPEHs-x: SIPEHs + D-xylose, SIPEHs-mx: SIPEHs + D-xylose + L-cysteine, CIPEHs-x: CIPEHs + D-xylose, CIPEHs-mx: SIPEHs + D-xylose + L-cysteine. Standards are expressed as means ± SD (n=3)". Where a, b, c, d, and e represent a significant difference within row (p<0.05).

1. Changes in intermediate products and browning intensity

As shown in Table 2, the absorbance values at 294 nm and 420 nm demonstrated variations during the heating process. A comparison of absorbance values revealed that the absorbance at 294 nm and 420 nm of SIPEHs-mx was significantly higher ($p > 0.05$) compared to SIPEHs-x. Notably, the CIPEHs-x sample heated at 120 °C without cysteine addition exhibited the highest absorbance value. It is worth mentioning that the lower absorbance values observed for CIPEHs-mx at 294 nm and 420 nm align with previous findings, indicating that the addition of cysteine can inhibit the formation of brown pigments during the Maillard reaction and reduce the intensity of browning (He *et al.*, 2019). Afterwards, the absorbance at 420 nm increased rapidly, revealing the increasing depth of the Maillard reaction. In the late stage of the Maillard reaction, some intermediates probably polymerised and produced brown pigments. The colour changes of products produced from the Maillard reaction can be called browning. The Maillard reaction model system showed a significant increase in absorption after 30 minutes of heating at 294 nm, and the formation of nonenzymatic intermediate compounds was proposed. The development of advanced MRPs, including brown polymers, may simultaneously be shown by using the 420 nm absorbance (Cai *et al.*, 2016).

2. Changes in pH

Table 2 showed a noteworthy ($p < 0.05$) decline in terminal pH values was observed with increasing temperatures for SIPEHs, CIPEHs, and MRPs. Interestingly, the SIPEHs and CIPEHs with cysteine addition (SIPEHs-mx and CIPEH-mx) had a lower pH value than SIPEHs-x and CIPEHs-x. Hence, in this study, the initial pH level of the system was adjusted to 7.4. Consistent with the findings reported by Lan *et al.* (2010), the pH of MRPs exhibited a rapid decrease as the heating time increased. The addition of cysteine to the system resulted in a decrease in pH due to the accelerated formation of acidic compounds, such as formic acid, acetic acid, methylglyoxal, and glyoxal (Eric *et al.*, 2014). The presence of amino groups, sugars, free amino acids (FAA), and peptide breakdown facilitated the generation of acidic compounds during the Maillard reaction (Lan *et al.,* 2010). Since the concentration of open-chain sugars and the activity of amino reactants are influenced by pH, this parameter significantly impacts the rate of the Maillard reaction and the production of MRPs (Lan *et al.*, 2010).

According to Ajandouz *et al.* (2008), the reaction rate during caramelisation is enhanced at the initial pH but exhibits minimal effects within the pH range of 6.7 to 8.0. Therefore, in line with the previous Maillard reaction method (Yu *et al.*, 2018), an initial pH of 7.4 was chosen. The Maillard reaction is a chemical process that occurs between carbonyl groups and reducing sugars in the presence of free amino groups found in proteins, peptides, or amino acids. This reaction can result in an elevation of acidity within the system (Eric *et al.,* 2014).

3. Changes in colour

The changes in colour of the MRPs system were depicted in Table 2 as the heating time progressed. The a* and b* values and the colour difference (ΔE) of SIPEHs-mx, SIPEHs-x, CIPEHs-mx and CIPEHs-x were found to increase significantly with the extension of heating time ($p \leq 0.05$). In contrast, the L* value gradually decreased with the prolongation of heating time, confirming that the MR occurred during treatment. The observed increase in the values of a*, b*, and ΔE could be attributed to the generation of diverse brown compounds during the Maillard reaction (Siewe *et al.*, 2020). Additionally, the significant decrease in the L* value with increasing heating time indicated the accelerated formation of dark-coloured pigments through the Maillard reaction (Cai *et al.*, 2016). The MR and caramelisation are the two primary non-enzymatic reactions contributing to the colour change in foods during heat processing (Siewe *et al.*, 2020).

4. *Volatile compounds composition*

The desirable aroma of seafood products plays a crucial role in enhancing consumer acceptance and is influenced by the specific composition of volatile compounds present. The major volatile compounds detected and identified in SIPEHs, CIPEHs and MRPs are shown in Table 3. Fatty acids, such as hexadecanoic acid, nonanoic acid, 2-propenoic acid and tetradecanoic acid, were identified in SIPEHs, CIPEHs and MRPs. These fatty acids are considered vital compounds and precursors to seafood flavour (Laohakunjit *et al.*, 2014).

Table 3. Major volatile compounds of SIPEHs, CIPEHs and MRPs.

Volatile compound	Peak area (%)					
	SIPEHs	SIPEHs-x	SIPEHs-mx	CIPEHs	CIPEHs-x	CIPEHs-mx
Hexadecanoic acid	3.38	0.97	*n. d.	0.51	n. d	0.32
Nonanoic acid	0.12	n. d.	n. d.	n. d.	n. d.	n. d.
2-Propenoic acid	n. d.	n. d.	0.72	n. d.	0.23	n. d.
Tetradecanoic acid	1.13	n. d.	1.21	n. d.	0.40	n. d.
Octanal	n. d.	n. d.	0.15	4.46	n. d.	0.04
Nonanal	0.07	n. d.	n. d.	n. d.	n. d.	n. d.
Decanal	0.11	n. d.	n. d.	1.27	n. d.	n. d.
Pentanal	n. d.	n. d.	n. d.	2.88	n. d.	n. d.
Piperidine	n. d.	n. d.	0.21	0.27	n. d.	n. d.
Octadecanoic acid	0.44	0.31	n. d.	0.29	n. d	0.37
1,2-Benzenedicarboxylic acid	1.05	0.25	n. d.	0.39	4.70	0.50
Propanoic acid	0.11	n. d.	0.33	n. d.	0.97	n. d.
Benzaldehyde	n. d.	n. d.	0.25	0.41	n. d.	n. d.
2-methyl butanal	n. d.	n. d.	6.08	n. d.	n. d.	n. d.
3-methyl butanal	n. d.	n. d.	2.66	n. d.	5.64	0.32
Furfural	n. d.	n. d.	1.51	n. d.	n. d.	n. d.
Dimethyl disulphide	n. d.	n. d.	1.18	n. d.	n. d.	n. d.

Note: SIPEHs: Squid ink powder enzyme hydrolysates, CIPEHs: Cuttlefish ink powder enzyme hydrolysate, SIPEHs-x: SIPEHs + D-xylose, SIPEHs-mx: SIPEHs + D-xylose + L-cysteine, CIPEHs-x: CIPEHs + D-xylose, CIPEHs-mx: SIPEHs + D-xylose + L-cysteine, n.d: not detected.

The volatile compounds, namely octanal, nonanal, decanal and pentanal, were mostly found in SIPEHs and CIPEHs. These volatile aldehydes generally confer fishy and grassy, nutty and pungent odours (Mohamed *et al.*, 2012; Ma *et al.*, 2020). Fruity notes in the form of propanoic acid, hexanal, butanoic acid, 2-methyl ethyl ester, 2-methyl ethyl ester, and heptanal odours have been associated with specific compounds (Théron *et al.*, 2010). Propanoic acid was found in both SIPEHs, SIPEHs-mx and CIPEHs-x. Octadecanoic acid and 1, 2-benzenedicarboxylic acid are described as the sweet flavour of fermented anchovies. In addition, two types of aldehydes were detected in SIPEHs-mx, CIPEHs-x and CIPEHs-mx (2-methyl butanal and 3-methyl butanal). Benzaldehyde was also found in SIPEHs-mx and CIPEHs. According to Eric *et al.* (2013), this particular trace aldehyde

contributes to the development of distinctive aromas, including fruity and nutty aromas.

According to Laohakunjit *et al.* (2014), benzaldehyde contributes to a pleasant and sweet odour profile found in crabs and shrimp. On the other hand, furfural and 5-methyl-2-furfural are known for their caramel-like, sweet, and fruity odours, as described by Mottram (1994). It is worth noting that furfural was only detected in SIPEHs-mx. Additionally, the presence of dimethyl disulphide in SIPEHs-mx can be attributed to the addition of L-cysteine, which contains sulphur amino acids. Sulphide volatile compounds are characterised by meaty or onion/cabbage-like odours, and they have low odour threshold values (Mottram, 1994). These favourable volatile compounds found in MRPs indicate that the Maillard reaction indeed enhances the flavour of ink hydrolysates.

B. Qualitative Descriptive Analysis (QDA) Test

PCA was used to synthesise the sensory profiles of SIPEHs, CIPEHs and MRPs, with standardisation performed on the mean score of consumers obtained for each descriptor and each sample. SIPEHs, CIPEHs and MRPs created the PCA, accounting for 71.58% of the total variance of fishy odour, fishy flavour, umami, salty and bitter criteria (Figure 1). Based on this finding, CIPEHs were described as being bitter and salty with a fishy odour, whereas SIPEHs were described as having a salty, fishy flavour and fishy odour. The presence of fishy flavour and odour was correlated with the significant volatile compounds, including ketones and aldehydes, associated with the fishy aroma. The group with the addition of D-xylose, CIPEHs-x, was described as having an umami taste, and the attributes of SIPEHs-x were lower than those of other products. However, the volatile compounds that corresponded to fishy aroma decreased and were not detected in these groups.
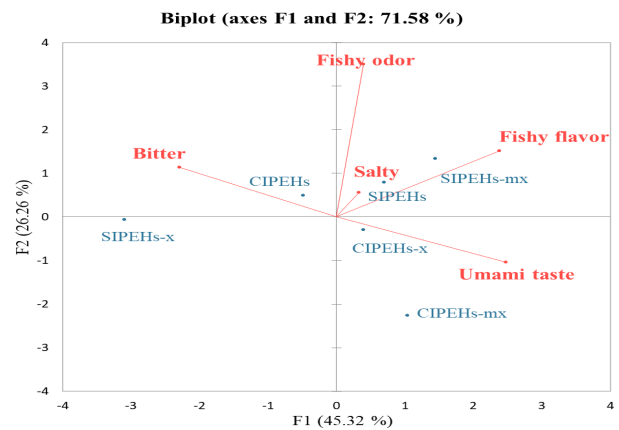

Figure 1. Sensory profiling of QDA using PCA.

Furthermore, the group with the addition of D-xylose and L-cysteine, namely, CIPEHs-mx, was described as having an umami taste, whereas SIPEHs-mx was described as having a salty, fishy flavour and fishy odour. The same was found for CIPEHs-mx. The volatile compounds associated with fishy aroma decreased. The enhancement in flavour can be attributed to the formation of Maillard peptides that possess flavour-enhancing properties. These peptides contribute to the production of volatile compounds, including pyrazines and sulphur-containing compounds (Normah & Noorasma, 2018). The results suggested that the Maillard reaction was capable of reducing the fishy flavour in ink hydrolysates.

C. Degree of Acceptability

The data for the acceptability test performed with 30 consumers were analysed by using PCA and presented in Figure 2. The red lines indicated the distribution of the consumers' preference for a sample. The appearance attribute showed that the consumers preferred SIPEHs-mx, CIPEHs-mx and CIPEHs-x because of their reduced black and brownish colouration after the Maillard reaction. The consumers favoured CIPEHs-mx and SIPEHs-mx for their odour attribute, which might be due to the reduction in fishy aroma via the reaction of xylose and cysteine. The same result was found for flavour.
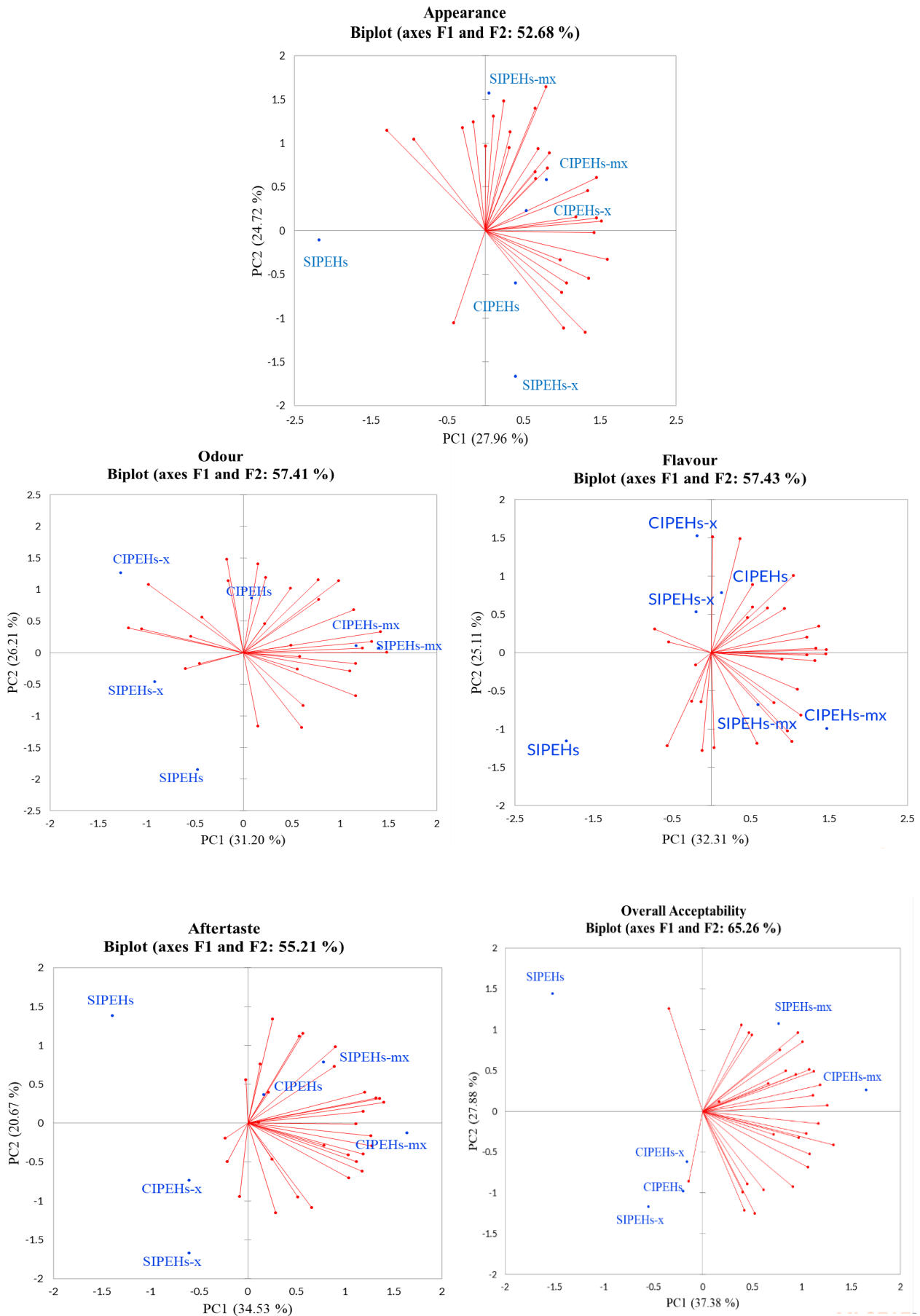
Figure 2. Preference maps were created to visualise the ratings of appearance, odour, flavour, aftertaste, and overall acceptability.

The consumers preferred SIPEHs-mx and CIPEHs-mx because of their weak fishy flavour. Although the hydrolysates contributed to a bitter aftertaste, the consumers favoured CIPEHs-mx because its aftertaste was less bitter than that of others. SIPEHs-mx and CIPEHS-mx were preferred and showed overall acceptability in terms of appearance, odour, flavour and aftertaste. Comparing the sensory profiling of the QDA test and overall acceptability revealed that SIPEHs-mx and CIPEHS-mx were favoured because their properties satisfied consumer preferences (Figure 3).
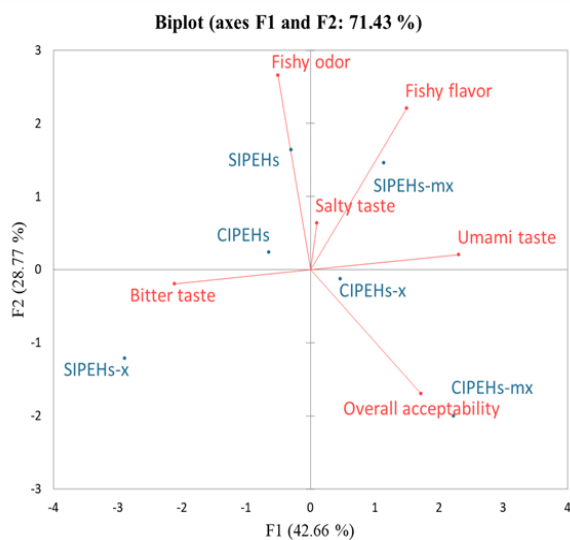
Figure 3. Sensory profiling of QDA test and overall acceptability.

SIPEHs-mx was described as having umami, fishy flavour and salty taste but low fishy odour and bitter taste. Meanwhile, CIPEHs-mx had overall acceptability due to its weak salty, umami, bitter and fishy flavours and fishy odour.

IV. CONCLUSION

In conclusion, the additional advanced MRPs with brown colour but a low pH and volatile compounds could be generated at high heating temperatures and were conducive to reducing bitterness and fishy-like aromas. These findings indicated that the MR with D-xylose and L-cysteine effectively improved the overall flavour of SIPEHs and CIPEHs.

V. ACKNOWLEDGEMENT

VI. REFERENCES

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