COMPONENT ANALYSIS BY SENSOMICS CONCEPT ON FLAVOUR ENHANCEMENT OF SMOKED INGREDIENTS

By

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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University of Reading

2024

Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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February 2024

Abstract

Smoked ingredients are used to improve the organoleptic qualities of culinary products in the food industry. This can be due to the pleasant fragrant aroma derived from the smoke, but we hypothesise that it may also be due to taste enhancement, either directly through the activity of tastant molecules or possibly from odour-induced taste enhancement (OITE). It might be possible to use smoked ingredients to reduce salt or monosodium glutamate (MSG) levels in food products. However, the smoking process, which is required for flavour development, also produces a series of polyaromatic hydrocarbons (PAHs), including the known human carcinogen benzo[a]pyrene. The concentrations of PAHs can now be reduced using Puresmoke technology^{TM} (PST). This technology has been shown to remove aroma compounds from the smoke, but also results in a more balanced aroma. The aim of the thesis is to investigate the contribution smoked water makes to the flavour of a soup matrix, comparing both PST and traditionally smoked water (TR).

In the first instance, it is important to understand what the important aroma compounds in smoke are and the impact of PST on the flavour profile. Smoked water was selected for an in-depth analysis of the aroma compounds using a low (P25) and a high (P50) number of filter plates of PST. The effect of P25 and P50 on 77 volatile compounds using 3 wood types (apple, beech, and oak) was investigated using a sensomics approach. Solid phase microextraction (SPME) and solid phase extraction (SPE) which used diethyl ether as the eluent, were the two most effective extraction techniques for smoked water based on the number of compounds extracted. Seventy-seven aroma-active compounds were detected in P50 and TR apple-wood smoked water. The most abundant compounds were phenol and phenol derivatives, followed by aldehydes, ketones, diketones and guaiacol and guaiacol derivatives, in that order. In general, the main constituents were found in higher concentrations in TR than in PST smoked water.

Aroma extract dilution analysis (AEDA) was employed in both SPME and SPE extracts to determine the most odour-active compounds. A total of 67 aroma-active compounds were detected by gas chromatography-olfactometry (GC-O), the majority of which were phenols and guaiacols. At least 22 compounds with odour activity values (OAVs) more than 1 were identified as potent aroma compounds. To confirm the identity of the odour-active compounds,

the identified 22 potent aromas were combined to generate full and partial recombinates at concentrations corresponding to those in P50 smoked water. The sensory profiling scores (5-point scale) of four descriptors, smoky, woody, ashy, and phenolic, of the recombinates did not closely correspond to the original P50 smoked water, indicating that more refinement of the recombinate was required. The effect of PST on the aroma profile (77 compounds) was analysed in smoked water prepared from three different types of hardwood, each compared to TR smoked water. When the PST was used, the majority of compounds were reduced. The difference between P25 and P50 was significantly less than the difference between TR and P25. The principal component analysis (PCA) plot determined that apple smoked waters were associated with higher concentrations of phenols group, traditionally beech smoked water had high levels of syringols and guaiacols. All three samples of oak-smoked water were similar and had high levels of furans.

Three mechanisms of smoked water on flavour enhancement were investigated using trained sensory panellists. In the absence of MSG, the panel with the aroma excluded through wearing of nose clips, detected an umami taste in the presence of apple-wood smoked water. In the complex mixture of model soup, the smoked water made little difference to the umami taste and when smoked water was added to the mixture of MSG and 5'-ribonucleotides, there was no umami enhancement. However, an umami enhancement was observed in the model soup containing MSG and 5'-ribonucleotides at subthreshold umami levels (below 344 mg/L or 0.038%). Intriguingly, umami was the primary taste that was enhanced when smoked water was combined with 5'-ribonucleotides in salt-reduced soups without using nose clips. This result suggests that odour-induced taste enhancement was the primary mechanism by which smoked water enhanced flavour. In contrast, partial recombinate (17 compounds) did not significantly enhance the tastes of salt-reduced soup compared to salt-reduced soup without recombinate.

Acknowledgement

I would like to express my heartfelt gratitude to my supervisors, Dr Jane K. Parker and Dr Stella Lignou, for their significant assistance, continuous guidance, support, advice, encouragement, and patience throughout my doctoral studies and constant assistance during the preparation of this thesis; this thesis would not have been possible without them. I'd like to thank Dr Lisa Methven in particular for her important comments during registration confirmation, as well as her support and helpful discussion over the 6-month monitoring process over 42 months. My gratitude also goes to Dr Maria Jose Oruna-Concha for her delightful support during registration confirmation and when I was studying at the University of Reading.

My thank should extend to Dr Stephen Elmore and Dr Dimitris Balagiannis for assisting and instructing me while I was having difficulty with the machine, as well as for all of the fun moments we've had together. Thank you to members of my research groups (flavour and sensory) for their assistance with the panellists' sensory support (Suzanne Mouring, Olivia Roman, and my friend Dr Victoria Norton). Dr Jose Piornos and Dr HuiQi Yeo, two former PhD students with the same supervisor as mine, were really helpful. Thank you, too, to my PhD office colleagues. My laboratory work would not have been possible without their assistance. My compliments and gratitude also go to Dr David Baines, who has always been gracious and generous with his knowledge. My gratitude also goes to Huw Griffiths and Dr Elena Cristea of Besmoke Ltd., who gave me samples for my studies and my last experiment session using the company's assessors.

My studies would not have been feasible without the scholarship funding provided by Thailand's Ministry of Science and Technology (former name) to provide higher education and the new valuable experience of studying overseas to government personnel like myself. I would also like to thank the director of the Department of Science Service and the head of the Food Products and Food Contact Materials Division (my workplace) for providing me with this scholarship opportunity. Finally, I'd want to dedicate my work to my wonderful parents in order to express my gratitude for their unconditional love and patience, as well as their unwavering support, both in my home nation of Thailand and during my time studying in the United Kingdom.

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Literature review

Section 1 Smoke and volatiles

Since discovery of fire, the tradition smoking process has been used for food preservation, however smoking is now used mainly for organoleptic reasons to contribute desirable features such as smoke colour, smoke aroma and smoke flavour, which significantly affects the sensory characteristics, overall sensory acceptability and makes the smoked food appealing to consumers (Jaffe et al., 2017; Marušić Radovčić et al., 2016; Wang & Chambers, 2018). The smoking techniques have improved from primary to contemporary technologies. There are different types of smoking method to be classified; it is usually divided into two main categories, direct and indirect (Ledesma et al., 2016), of which traditional smoking and liquid smoking are an example of these two methods, respectively.

Generation of smoke

Pyrolysis is a process in which complex macromolecules of biomass are broken apart by heat in the lack of oxygen into several small molecules. There are two types of pyrolysis based on heating rates: fast and slow pyrolysis. Fast pyrolysis undergoes fast burning and a short time to turn biomass into bio-oil. Slow pyrolysis, on the other hand, mostly turns biomass into biochar. Bio-oil is derived from biomass pyrolysis and is enriched with various compounds, such as alcohols, phenols, aldehydes, and organic acids (Hoang Pham et al., 2021). Through the pyrolysis process, smoke is produced by heating (450-600 °C) wood or similar materials with limited oxygen. Three major wood components, hemicellulose, cellulose, and lignin, are pyrolyzed to form different groups of smoke compounds. According to the mechanism of pyrolysis, various categories of smoke compounds are generated. Hemicellulose and cellulose combustion are a simple process with a narrow range of temperature between 180 °C and 350 °C, producing carboxylic acids and carbonyl compounds, while lignins pyrolysis occurs over a wider range of temperatures between 300 °C and 500 °C, producing phenolic compounds (Hoang Pham et al., 2021; Šimko, 2005). The compounds generated from the pyrolysis of lignin and hemicellulose are shown in **Figure 1.1**.

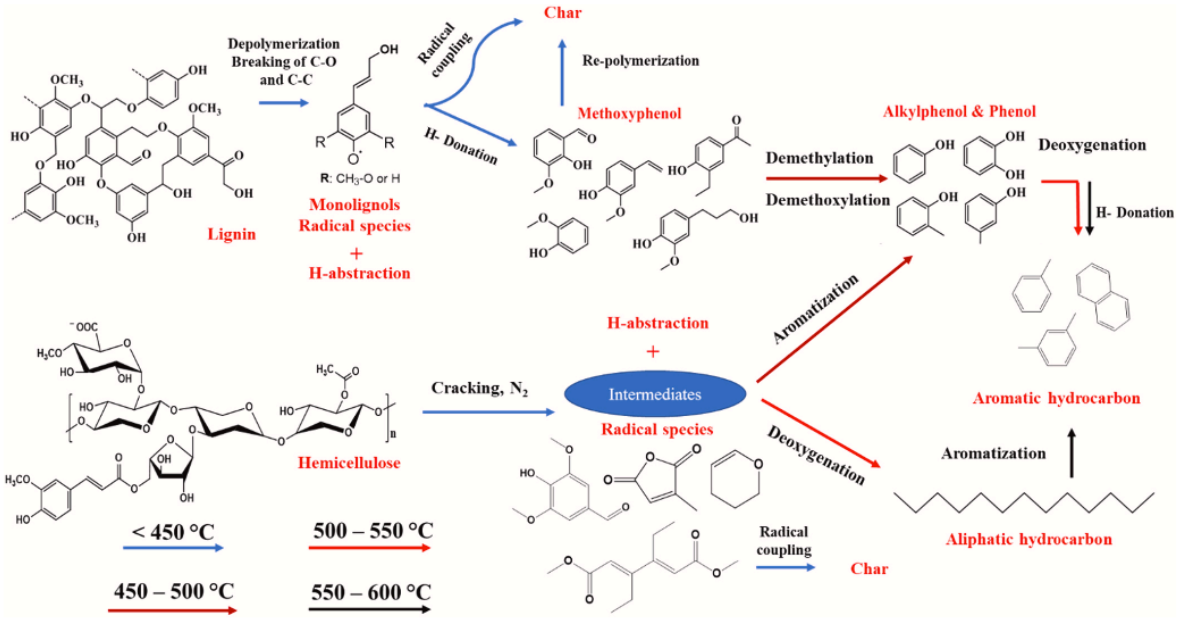


Figure 1.1 Reaction pathways for the pyrolysis of lignin and hemicellulose to form phenolic, alkyl phenol, hydrocarbon, and aromatic hydrocarbon compounds according to Hoang Pham et al. (2021).

The carbonyl compounds impart a sweet aroma and brown colour to smoked products, while the groups of phenols (phenolic compounds) are responsible for a desirable smoky character and function as antimicrobial and antioxidant compounds (Lingbeck et al., 2014; Malarut & Vangnai, 2018). The optimal temperature to produce carbonyls, furans, and phenolic compounds is between 450 °C and 500 °C, but carcinogenic polycyclic aromatic hydrocarbons (PAHs) are also increased from 400 °C to 1000 °C (Lingbeck et al., 2014; Varlet, 2009). The smoke produced by pyrolysis can be used as a direct source of smoke (direct smoking) or collected through a condenser, which liquefies the smoke (vapours), followed by refining and filtering to clarify the solution and remove any remaining dissolved hydrocarbons (Hollenbeck, 1977; Janairo & Amalin, 2018; Lingbeck et al., 2014) to produce liquid smokes (indirect smoking). The production of the required organoleptic volatile compounds free of PAHs contaminants appears to be difficult; therefore, filtration and purification processes are useful for reducing these contaminants (Varlet, 2009).

Hardwood and softwood

There are two types of wood species: hardwood and softwood (Popescu et al., 2009) that are used to produce smoke. In addition to wood, agricultural waste/residues and by-products containing cellulosic products, such as rice husk (Pino, 2014), cacao pod husk (Janairo & Amalin, 2018), energy crops e.g. sugarcane leaves, bamboo (Hoang Pham et al., 2021), are source of biomass used to produce smoke. Hardwoods are derived from angiosperms (flowering plants), while softwoods are derived from gymnosperms (mostly conifers) (Popescu et al., 2011). In general, the structure of hardwoods is more rigid, complex and heterogeneous than that of softwoods (Stelte & Sanadi, 2009). Wood consists of an ordered arrangement of cells whose cell walls are composed of variable amounts of cellulose, hemicelluloses, and lignin, classified as high lignocellulose compounds. Cellulose, hemicellulose, and lignin, which combine to form a composite material consisting of rigid cellulose fibres embedded in a cross-linked matrix of lignin and hemicelluloses that bond the fibres (Le Floch et al., 2015). Cellulose and hemicellulose are hydrophilic and are soluble in water, whereas lignin is hydrophobic and dissolves in organic solvents and alkali solutions (Erfani Jazi et al., 2019).

Cellulose comprises 40–50% of dry wood and has a high molecular weight that contributes to the strength of wood. Cellulose has about 60% crystalline structure of linear glucan polymer chains connected by β-1,4-glycosidic bonds (**Figure 1.2**). Hemicelluloses are heterogeneous polysaccharides made up of various simple sugar units such as pentoses, hexoses, and uronic acids linked by β-1,4 bonds (**Figure 1.3**). Hemicellulose, which binds to cellulose microfibrils to reinforce the cell wall and typically accounts for 25–35% of dry wood mass, is responsible for strengthening the cell wall. Lignin has an amorphous structure which composes of a macromolecule with various linkages between its constituent monomers and branch molecules (**Figure 1.4**). These branches are three basic building blocks of lignin which compose of dimethoxylated (syringyl, S; sinapyl alcohol), monomethoxylated (guaiacyl, G; coniferyl alcohol), and non-methoxylated (p-hydroxyphenyl, H) moieties (Assor et al., 2009; Castro et al., 2020; Le Floch et al., 2015) (**Figure 1.5**). Lignin from hardwood is formed of S, G units, and trace amounts of H units, while softwood lignins have G units and small amount of H units (Assor et al., 2009). The most frequent bond in native lignin is β-O-4 linkage in coupled with other linkages, namely, β-5, 5-5, β- β, 4-O-5, and β-1 linkages (Assor et al., 2009).

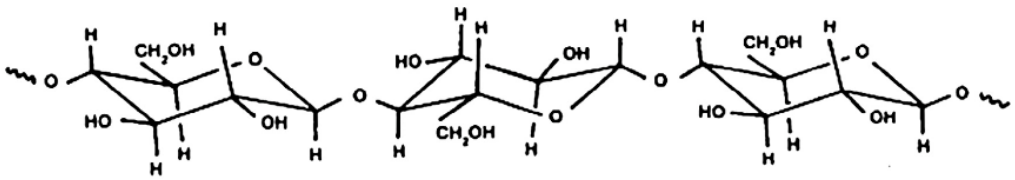


Figure 1.2 Repeating unit of the cellulose (cellobiose), which consists of two β-glucose molecules linked by β-1,4 glycosidic bond (source; Le Floch et al. (2015)).

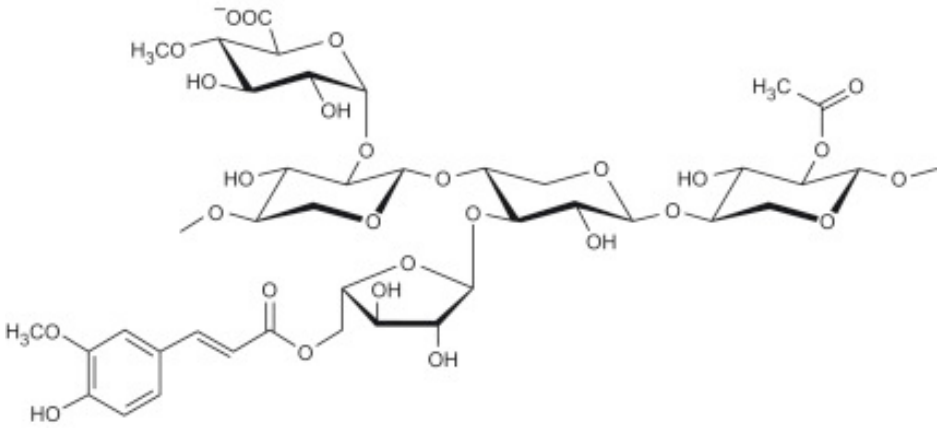


Figure 1.3 Representation of hemicellulose structure (source; Machmudah et al. (2017)).

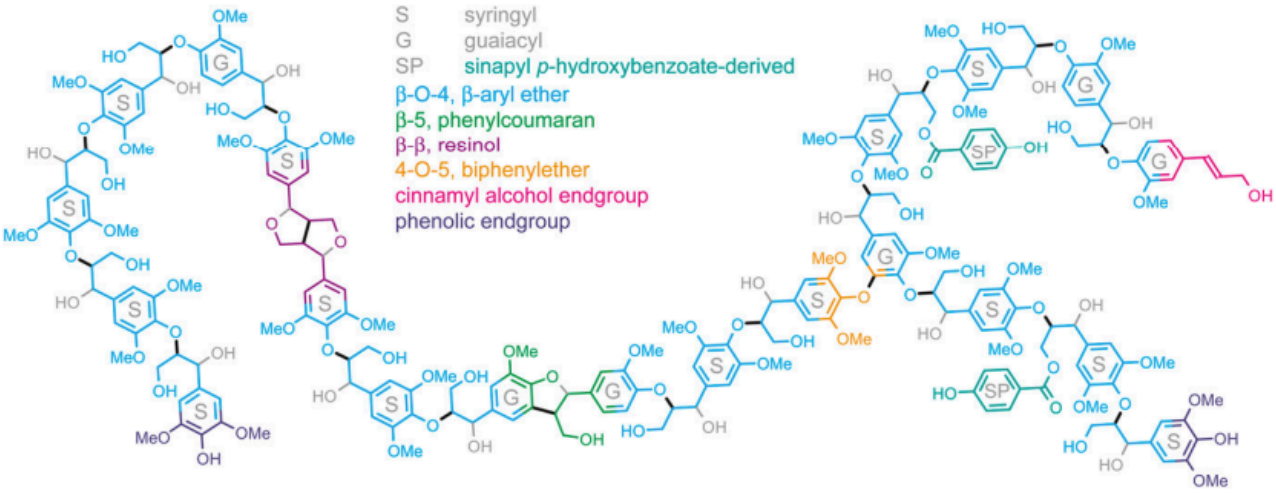


Figure 1.4 Representation of lignin structure (source; Vanholme et al. (2010)).

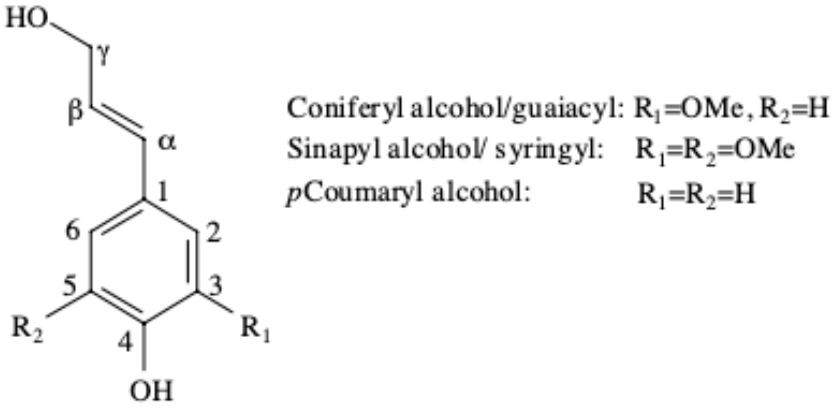


Figure 1.5 The three building blocks of lignin (source; Chakar and Ragauskas (2004)).

Cell walls are made up of different ratios of cellulose, hemicellulose, and lignin. This ratio varies depending on the source of biomass, such as hardwood, softwood, and herbs (Silvy et al., 2018). Hardwoods typically include 15-25% hemicelluloses, 40-50% cellulose, and 15-25% lignin. Softwoods contain a higher lignin content (25-30%) and hemicellulose (24-37%) than hardwoods but have similar cellulose ranges at 40-45% (Popescu et al., 2009; Silvy et al., 2018). The cellulosic contents in some biomass are shown in **Table 1.1**.

Table 1.1 Cellulose, hemicellulose and lignin contents of some biomass.

Biomass samples	**Average content (%)**			**References**
	Cellulose	**Hemicellulose**	**Lignin**	
Hardwoods				
1. Twelve oak wood	22-50	17-30	17-30	Le Floch et al. (2015)
2. Oak wood powder	48-49	29-34	18-22	Popescu et al. (2011)
3. Red Oak	58.6	3.4	24.1	Referred by Lingbeck et al. (2014)
4. White oak	21.4	3.6	39.3	Referred by Lingbeck et al. (2014)
5. Apple	20.7	6.9	37.9	Referred by Lingbeck et al. (2014)
6. Cherry	20.7	3.4	13.8	Referred by Lingbeck et al. (2014)
7. Chestnut	21.4	3.6	32.1	Referred by Lingbeck et al. (2014)
8. Hard maple	17.2	17.2	55.2	Referred by Lingbeck et al. (2014)
9. Hickory	41.4	1.7	24.1	Referred by Lingbeck et al. (2014)

Biomass samples	Average content (%)			References
	Cellulose	Hemicellulose	Lignin	
10. Mesquite	8.0	8.0	44.0	Referred by Lingbeck et al. (2014)
11. Eucalyptus sawdust	35.02	28.34	22.17	Hoang Pham et al. (2021)
12. Eucalyptus woodchip	63.71	15.32	7.91	Malarut and Vangnai (2018)
13. Beech woodchip	54.23	21.01	12.5	Malarut and Vangnai (2018)
14. Beech wood powder	47-48	30-35	18-22	Popescu et al. (2011)
15. Neem woodchip	54.29	19.55	13.21	Malarut and Vangnai (2018)
16. Copper pod woodchip	47.13	25.28	14.37	Malarut and Vangnai (2018)
17. Earleaf acacia woodchip	60.27	11.9	13.71	Malarut and Vangnai (2018)
Softwoods				
1. Fir wood powder	35-37	37-41	24-26	Popescu et al. (2011)
Agriculture wastes				
1. Rice husk	34.32	34.21	28.75	Hoang Pham et al. (2021)
2. Palm kernel shell	7.24	28.45	54.84	Hoang Pham et al. (2021)
3. Corn cob	31.62	45.12	20.29	Hoang Pham et al. (2021)
4. Coconut shell	18.82	38.99	41.04	Hoang Pham et al. (2021)
Energy crop				
1. Sugarcane leaves	28.96	32.77	15.03	Hoang Pham et al. (2021)
2. Napier grass	27.32	35.78	20.93	Hoang Pham et al. (2021)

What are volatiles in smoke?

In general, the compounds of smoke can be roughly categorized into three classes, including acidic, phenolic, and sugar-derived carbonyl compounds, with phenolic substances contributing the most flavour (Hollenbeck, 1977). Liquid smoke is a mixture of many volatiles and certain non-volatiles of different structure, reactivity and sensory activity (Kostyra & Baryłko-Pikielna, 2006). The volatile compounds in smoke ingredients that relate to the desired

flavour and aroma have been found in most recent studies. **Table 1.2** shows some potent odorant volatile compounds and their odour characteristics that are detected in smoke ingredients/products.

It is known that phenolic compounds contribute to the odorant "smoky" structure of wood smoke. Medium-volatility phenolic compounds are regarded as the most important key odorant molecules. The medium-boiling fraction (91 °C - 132 °C) consisting of isoeugenols, syringol, and methylsyringol has a distinctive smoke flavour (Varlet, 2009). The amount of phenolic compounds in final products relies on the type of wood used to generate smoke. The guaiacols, syringols, phenolic derivatives, are specified as the most characteristics of smoked compounds (Jónsdóttir et al., 2008). Hitzel et al. (2013) analysed the content of phenolic substances in Frankfurter sausages and mini-salamis smoked, they discovered that the content of the phenolic compounds varied according to the type of wood in both smoked products.

Table 1.2 Some most potent odorant volatile compounds and odour characteristics in salmon fillets treated by liquid smoke (adapted from Varlet et al. (2007)).

Compounds	**LRI (DB-5)**	**Odour characteristics**
2-Furfural	859	Smoke, green
2-Methyl-2-cyclopenten-1-one	920	Cooked potato, green
2-Acetylfuran	925	Cooked vegetables, potato, usually toasty
5-Methylfurfural	970	Cooked, earthy, green
Phenol	992	Marine, metallic, chemical, mushroom
2,3-Dimethyl-2-cyclopentenone	1052	Spicy, wood fire, roasty
2-Methylphenol	1068	Chemical, spicy, burnt
4-Methylphenol	1093	Animal, spicy, burnt
Guaiacol	1110	Smoked, vanilla, ink
2,6-Dimethylphenol	1130	Chemical, burnt, spicy/woody
3-Ethyl-2-hydroxy-2-cyclopentenone	1140	Solvent, medicinal
2,4- and 2,5-Dimethylphenol	1160-1180	Cucumber, violet, spicy, smoked
4-Methylguaiacol	1192	Candy, spicy, smoked

Compounds	LRI (DB-5)	Odour characteristics
3,5-Dimethoxytoluene	1282	Burnt, green, chemical
4-Ethylguaiacol	1287	Green, smoke, vanilla, clove
4-Vinylguaiacol	1330	Smoke, green, spicy
Syringol	1365	Burnt rubber, spicy
Eugenol	1370	Spicy, smoke, clove
4-Propylguaiacol	1382	Green, spicy, vanilla
1,2,3-Trimethoxy-5-methylbenzene	1400	Cooked, earthy
Isoeugenol	1473	Clove, green, roasty
4-Allylsyringol	1615	Smoke, rotten

Sugar-derived carbonyl molecules have also been implicated in the smoky aroma of wood smoke. These carbonyl-containing chemicals have a sweet or burnt-sweet odour and tend to reduce the strong smoky aroma of phenolic compounds (Montazeri et al., 2013; Varlet, 2009). The phenolic and carbonyl compounds in liquid smoke contribute to the reduction of significant foodborne pathogens due to their antimicrobial (Lingbeck et al., 2014) and antioxidant properties (Kjällstrand & Petersson, 2001). Soares et al. (2016) evaluated the antibacterial capabilities of liquid smoke. The researchers observed that liquid smoke effectively suppressed the growth of prominent pathogens such as *Escherichia coli*, *Salmonella choleraesuis*, *Staphylococcus aureus* and *Listeria monocytogenes* bacteria in bacon products. Additionally, the study unveiled the antioxidant activity of this liquid smoke.

The odour impact molecules in smoke substances are mainly found in different smoked products; for example, phenolic compounds and carbonyl compounds are effective in liquid smoke (Varlet, 2009), while the phenolic compounds are mostly responsible for the smoke odour in the smoked food products (Ai-Nong & Bao-Guo, 2005; Marušić Radovčić et al., 2016; Varlet et al., 2006).

Aroma compounds extraction methods

Aroma compounds typically exhibit a relatively low boiling point and possess restricted solubility in water. Gas chromatography–mass spectrometry (GC–MS) is a sophisticated analytical technique widely employed in identifying aroma compounds due to its exceptional sensitivity. However, in order to obtain a representative extract of the original aroma

compounds and prevent the degradation of the non-polar GC column phase caused by water molecules, it is necessary to use an extract sample that contains a minimal amount of non-volatile components and, ideally, no water. By separating water and non-volatile substances from volatile substances, the ability to identify the aroma's components is greatly enhanced (Elmore, 2015). Several extraction methods have been reported for volatile compound analysis in food matrices, including headspace solid phase micro-extraction (HS-SPME), dynamic headspace extraction (DHE), solid phase extraction (SPE), solvent extraction, solvent-assisted flavour evaporation (SAFE), stir bar sorptive extraction (SBSE), etc. Although numerous methods exist for the analysis of aroma compounds in food, three methods have gained popularity over the past two decades, which were SAFE, SPME, and SPE (Elmore, 2015). The SAFE method extracts aroma compounds from the sample matrix using organic solvents, while the SPME method uses polymer fibre to absorb volatiles in the sample's headspace, and the SPE method is used to clean up the sample and extract the volatiles.

SAFE, involves a mild and exhaustive distillation coupled with acid/neutral/base fractionation, is a method utilised to separate the class of odorants and prevent matrix interference, thereby simplifying the chromatographic outcomes. One method for isolating the volatile is liquid-liquid extraction (LLE), employing multiple solvents without fractionation. This LLE approach is comparatively faster than SAFE; however, it is important to consider that the interference of the matrix may introduce an error in the concentration of the desired odorants. Headspace measurements are traditionally carried out by collecting a sample of the headspace at equilibrium, commonly by the use of Tenax traps or coated fibres (SPME), which extract the analytes from the gas phase. The SPME headspace is solvent-free and does not involve numerous steps of sample preparation like the SAFE technique, which can lead to artefacts and be time-consuming. The SPME headspace technique is widely used for volatile compound extraction and concentrates volatiles from a non-volatile matrix. SPME headspace could be used for routine work because it is a fast and simple technique that can be applied to a variety of samples. However, the SPME headspace does have some drawbacks, including the fact that it only provides information on the chemicals that vaporise in the headspace, one extraction can only be used for one analysis, and the quantitative analysis is challenging. Additionally, even while alternative extraction methods, such as the SPE, LLE, and SBSE techniques, are more suitable for quantifying compound analysis, but they typically contain solvents that can introduce contaminants, and they are also time-consuming methods.

Solid phase extraction (SPE) is a widely used technique that offers fast and specific sample preparation. The adaptability of SPE enables its applications, including purification, trace enrichment, desalting, derivatisation, and fractionation. SPE technique allows for the concentration and purification of analytes from a solution by adsorbing them onto a solid sorbent and purifying the resulting extract following the extraction process. The typical protocol involves applying a solution onto the SPE solid phase, eliminating unwanted components through washing, and subsequently eluting the target analytes into a collecting tube using a different solvent (Żwir-Ferenc & Biziuk, 2006). The extraction techniques used to extract smoke ingredients and smoked food products are shown in **Table 1.3**.

Table 1.3 Volatile extraction techniques for smoke ingredients and smoked food products.

Products	**Extraction methods**	**Extracted compounds**	**References**
Alder smoke	Adsorbent cartridge Tenax TA	Phenols, anhydrosugars, furaldehydes, furans, hydrocarbons	Kjällstrand and Petersson (2001)
An aqueous oak smoke	Solvent (dichloromethane)	Aldehydes, ketones, diketones, furans & pyrans, alcohols & esters & acids, phenols, guaiacols, syringols, lignin dimers, pyrocatechols	Guillén and Manzanos (2002)
Commercial liquid smoke	Solvent (dichloromethane)	Phenolics, aldehydes & ketones, furan & pyrans, organic acids	Montazeri et al. (2013)
Smoke flavouring from rice husk	Solvent (dichloromethane)	Acids, alcohols, carbonyls, esters, furans, phenols	Pino (2014)

Products	Extraction methods	Extracted compounds	References
Commercial smoke flavouring	Solid phase microextraction (SPME)	Furans/pyrans, phenols, guaiacols, syringols, benzenediols, aldehydes & ketones	Giri et al. (2017)
Liquid smoke flavouring	Solvent (dichloromethane)	Carbonyls, phenolics, organic acids	Sokamte tegang et al. (2020)
Wood smoke	Stir bar sorptive extraction (SBSE)	Phenols, ketones, aldehydes, alkanes, acids, furans,esters, ethers, alcohol, heterocycles	Zhang et al. (2020)
Chinese traditional smoke-cured bacon	Nitrogen purge-and-steam distillation	Hydrocarbons, phenols, carbonyls, alcohol, amides, esters, amine, carboxylic acods, heterocyclic	Ai-Nong and Bao-Guo (2005)
Fresh and smoked salmon	Simultaneous distillation extraction (SDE)	Phenolic, furanic, pyrazines & heterocyclic nitrogen, other cyclic & aliphatic	Varlet et al. (2006)
Smoked meat	Solvents followed by solid phase extraction (SPE) for clean-up	Polycyclic aromatic hydrocarbons (PAHs)	Stumpe-Vīksna et al. (2008)
Smoked dry-cured ham	Solid phase microextraction (SPME)	Aldehydes, phenols, alcohols, terpenes, aromatic hydrocarbons,	Marušić Radovčić et al. (2016)

Products	Extraction methods	Extracted compounds	References
		alkanes, ketones, esters, acids	
Smoked bacon	Solid phase microextraction (SPME)	Phenols, furans, aldehydes, ketones, alcohols	Saldaña et al. (2019)

Sensomics approach for identifying key aroma compounds

Sensomics, a multistep analytical procedure involving the human olfactory system, is the standard method for identifying and quantifying key odorants and defining their sensory impact in the overall food aroma profile by aroma recombinates (Nicolotti et al., 2019). The sensomics strategy integrates the use of a machine (GC-MS) and human senses (GC-O) to separate, quantify, and characterise flavour compounds. Experiments involving the reconstitution and omission of aromas can be conducted using both qualitative and quantitative data. This sensomics approach was a unique methodology established by the research group at the School of Life Sciences, Technische Universität München, Germany (TUM, n.d.). The concept sequence procedure and related terminology of sensomics are below (Parker, 2015; TUM, n.d.).

1. Separation of a volatile fraction containing odorants from a non-volatile fraction of food constituents comprising taste-active components, to increase the compound identification ability and select the most suitable extraction method for aroma compounds.

2. Identification of aroma-active compounds using gas chromatography-olfactometry (GC-O) and aroma extract dilution analysis (AEDA). GC-O is crucial for identifying the aroma components. After proper extraction, GC separates the aroma compounds, and trained assessors describe and evaluate the intensity of the aroma that they perceive at the sniffing port of GC-O.

2.1 AEDA uses GC-O to identify all odour-active compounds contained in an aroma extract and then repeats the GC-O on a series of serial dilutions until only the strong aroma compounds are detected. PLEASE add here why AEDA on an SPME extract will give

different results to AEDA on a liquid extract – SPME depends on partition coefficients from water to air to fibre. Heavy molecules have a low partition coefficient and may be underestimated by SPME. With liquid extracts the GCO is not skewed like this.

2.2 Flavour dilution factors (FD factor) is the number of times the original extract can be diluted before the assessor loses the aroma in GC-O sniffing. The odour active compound is given by its dilution factor value. The compounds with the highest FD factors are the compounds of interest. For instance, in a sequence of dilutions where the initial extract is progressively diluted at a 1:1 ratio (Frauendorfer & Schieberle, 2006), the components that no longer retain the aroma after the second dilution possess an FD factor of 4. Serial dilutions can be employed to reduce the number of GC-O assessments needed by utilising a 1:2 dilution ratio, resulting in FD factors of 3, 9, 27, and so on (Parker, 2015).

3. Quantitation of individual odorants by using GC-MS and stable isotope dilution analyses (SIDA) and calculation of odour activity value.

3.1 SIDA is the most widely used technique in sensomics. The extracts are added with known quantities of isotopically labelled standards of all compounds of interest (when possible), which serve as a known reference against which the target compound can be estimated. Since there are only a few isotopically labelled standards, this frequently requires some organic synthesis.

3.2 Odour thresholds are defined as the concentration at which a person first detects a stimulus. This can be a detection threshold where the point at which an individual detects an aroma or a recognition threshold is the point at which an individual recognises an odour.

3.3 Odour-activity value (OAV) is defined as the concentration of the aroma component divided by its odour threshold, therefore an OAV greater than one indicates that the compound is present above its aroma threshold and is likely to contribute to the flavour profile.

4. Validation of analytical and sensory data: a total recombinant of all identified odorants, each in its natural concentration as found in the extract, is compared to the flavour

profile of the authentic sample to confirm that all key odorants have been identified and quantified.

4.1 Recombinates; reconstruct the potent aromas in a base that is neutral but representative to create a recombinate. Typically, a sensory panel evaluates the recombinate and compares it to the aroma of the original extract. If a close match is found, it is likely that all compounds contributing to the aroma have been identified. If not, the search for the missing components continues.

4.2 Omission tests; those compounds that genuinely have an impact on the aroma profile can be determined by systematically removing each compound from the recombinate. In theory, this test shows which volatile components should be targeted for flavour optimisation.

Food products are often aqueous, so the first step of any method is to extract them into an organic solvent. The sensomics approach usually involves the integration of the SAFE technique and GC-O-MS in order to identify volatile chemicals. In addition to the SAFE technique, solvent extraction (SE) and simultaneous distillation extraction (SDE) are other activation techniques also utilised to obtain liquid extracts. SDE is extensively utilised to obtain more representative extract samples (d'Acampora Zellner et al., 2008). Nevertheless, since SDE is not conducted under reduced pressure, higher temperatures are employed. For both SDE and SAFE techniques, substantial quantities of sample are required, and the volatiles extract obtained has to be concentrated prior to GC–O analysis; otherwise, volatiles with high volatility would be lost. Typically, SE yields complex extracts, which can result in numerous co-elution in GC-O and complicate the identification. Additionally, the presence of the solvent peak in GC-O analysis may mean that the early eluting odour active volatiles which coelutes are not reported.

For the purpose of identifying volatile compounds in smoked water samples using the sensomics approach, in this research, some sensomics procedure has been modified. Even though the original sensomics approach for extracting volatile compounds utilised a SAFE technique, the free-solvent SPME extraction technique was employed to extract smoked water samples to preserve and enrich the highly volatile compounds. The SPE technique was also

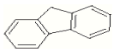
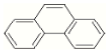
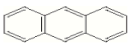
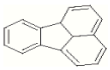
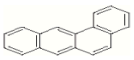
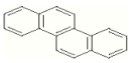
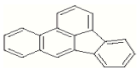
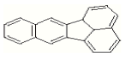
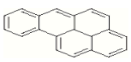
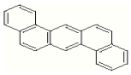
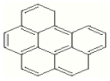
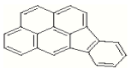
carried out to extract non-polar, aromatic, and low- or medium-volatile compounds from smoked water samples to obtain a wide range of volatile substances.

Polycyclic aromatic hydrocarbons and carcinogenicity

Polycyclic aromatic hydrocarbons (PAHs) contain two or more fused benzene rings. There are no heteroatoms or substituents present on the polycyclic ring. Prior to the 1950s, PAHs were thought to be the primary carcinogen and continue to be one of the most significant classes of carcinogens due to their prevalence in the environment. PAHs with four rings are referred to as light, while those with more than four rings are referred to as heavy. Heavy PAHs are more persistent and toxic than their lighter counterparts. PAHs can be produced in either a natural or an anthropogenic manner and are commonly found in the air, soil, and detritus (Sahoo et al., 2020). PAHs are produced through the pyrolysis of wood during the smoking process, as well as when the fat in the juices of flesh grilled directly over an open flame drips onto the flames and causes them to ignite, then PAHs in these flames adhere to the surface of the meat.

In 2002, the European Commission's Scientific Committee on Food (SCF) proposed 15 categories of PAHs that should be monitored closely in food products to emphasise the highly carcinogenic PAHs. In 2005, the Joint FAO/WHO Expert Group on Food Additives (JECFA) introduced a PAH to EU standards (Sun et al., 2019). These 16 EU PAHs are often known as the "15 + 1 EU priority PAHs" shown in **Table 1.4**. The International Agency for Research on Cancer (IARC) has classified benzo[a]pyrene as a carcinogen (group 1). Group 1 indicates that the chemical is a proven human carcinogen and one of the most hazardous substances found in food. In 2003, a European regulation set the maximum contents of PAH of benzo[a]anthracene and benzo[a]pyrene must not exceed 20 and 10 mg/kg in liquid smoke, respectively. The maximum permissible level of benzo[a]pyrene in smoked fisheries and crustacean products has been established at 5 mg/kg (Varlet, 2009).

Table 1.4 Sixteen PAHs defined by Environmental Protection Agency (modified from Malarut and Vangnai (2018)).

Compounds	Structure	[1/] IARC
Naphthalene		2B
Acenaphthylene		NS
Acenaphthene		3
Fluorene		3
Phenanthrene		3
Anthracene		3
Fluoranthene		3
Pyrene		3
Benz[*a*]anthracene		2B
Chrysene		2B
Benzo[*b*]fluoranthene		2B
Benzo[*k*]fluoranthene		2B
Benzo[*a*]pyrene		1
Dibenz[*a,h*]anthracene		2A
Benzo[*g,h,i*]perylene		3
Indeno[*1,2,3-cd*]pyrene		2B

[1/] IARC = International Agency for Research on Cancer: 1 = Known human carcinogen, 2A = Probably carcinogen, 2B = Possibly carcinogenic, 3 = Not classifiable, NS = Not yet evaluated.

Methods of removal of PAHs

As shown in **Figure 1.6**, there are four basic types of procedures used to eliminate PAHs. These are physical, chemical, biological, and combination treatment approaches.

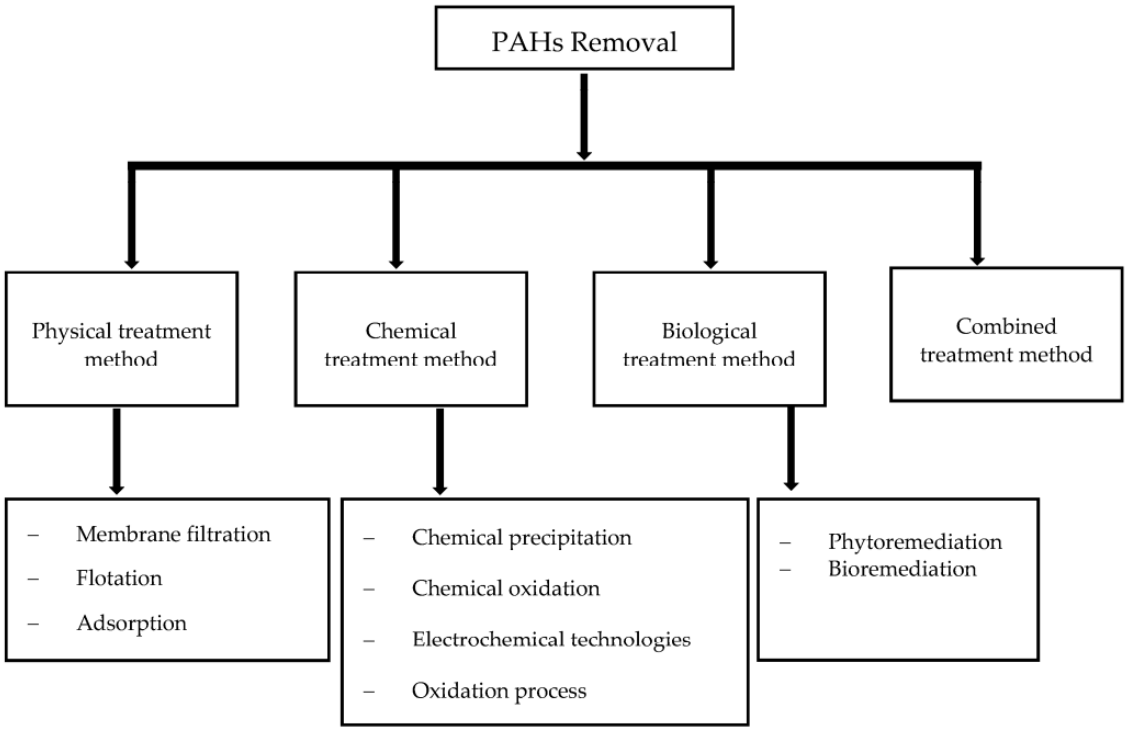


Figure 1.6 Various methods of removal of PAHs from produced water (source; Sher et al. (2023)).

Physical treatment methods can remove coarse particles, organic and inorganic pollutants, and other impurities from wastewater. Physical treatment is frequently utilised as a preliminary step before employing contemporary technology (Sher et al., 2023). According to Hollenbeck (1977), wood smoke consists of two phases, namely a particulate phase and a vapour phase. Due to the fact that the particulate phase contains a large proportion of smoke tars, removing the particulate phase also significantly decreased the level of benzopyrene in the smoke. Rusz (1977) utilised an electrostatic precipitator to eliminate the particulate phase and discovered that electrostatic precipitation also removed benzo[a]pyrene effectively. The original smoke contained an average of 38.5 µg/L of benzo[a]pyrene, but after passing through electrostatic precipitation, the concentration dropped to 0.8 µg/L.

Through chemical reactions, chemical methods are used to treat water, such as chemical precipitation, chemical oxidation, electrochemical technologies, and advanced oxidation processes. In the biological treatment method, PAHs can be biodegraded aerobically and anaerobically. PAHs can be biodegraded by microorganisms such as fungi. Bumpus (1989) incubated PAH-containing anthracene oil for 27 days in nutrient nitrogen-limited cultures of the white rot fungus *Phanerochaete chrysosporium*. Analyses revealed that at least 22 PAHs, including all the most abundant PAH components in anthracene oil, exhibited a reduction ranging from 70% to 100%. Integrated physical, chemical, and biological approaches have demonstrated promising results in effectively degrading, solubilising, and completely eradicating a number of PAHs with high molecular weight from water (Sher et al., 2023).

PST technology

In 2015, Parker et al. (2018) developed PureSmoke™, a new smoke filtration technology that employs molecular size exclusion to prevent harmful smoke components from reaching the food. The active material is a natural zeolite, which is a crystalline alumina-silicate lattice linked by oxygen atoms to form a stable cage-like framework. The interesting characteristic of this material is that the cavities throughout its structure enable it to behave like a molecular sieve, preventing larger molecules and undesirable PAHs while allowing the passage of smaller flavour molecules (see **Figure 1.7**). In accordance with the EU regulations, this technology reduces the number of PAHs, thereby enhancing both consumer safety and taste. A zeolite-based filter eliminated PAHs from a smoke stream, allowing the preparation of smoked ingredients with significantly reduced PAH levels. When controlled smoke was compared to smoke passed through a bed of treated zeolite, the most hazardous PAHs with four or more rings were reduced by up to 95%.

Sensory profiling was performed on two smoked tomato sauce samples, one treated with traditional smoke and the other with smoke treated with PST. The tomato sauce with treated PST smoke was judged to be sweeter and more balanced than the untreated sample due to the loss of some of the harsh, acrid notes of smoke and the oiliness on the palate (Parker et al., 2018). Another study showed no adverse effect on the aroma after the PST was introduced into the smoked ingredient, and they discovered that the flavour was more rounded and balanced when the PST filter was applied (Chua et al., 2019). In sensory profiling and consumer preference studies, smoked tomato flakes (either treated with PST or untreated) were added to

either low-fat or full-fat cream cheese. The sensory analysis revealed a significant decrease in bitterness when the PST was used, as well as a significant decrease in overall smoky aroma and flavour, increasing the perception of cheesy aroma and flavour (Chua et al., 2019).

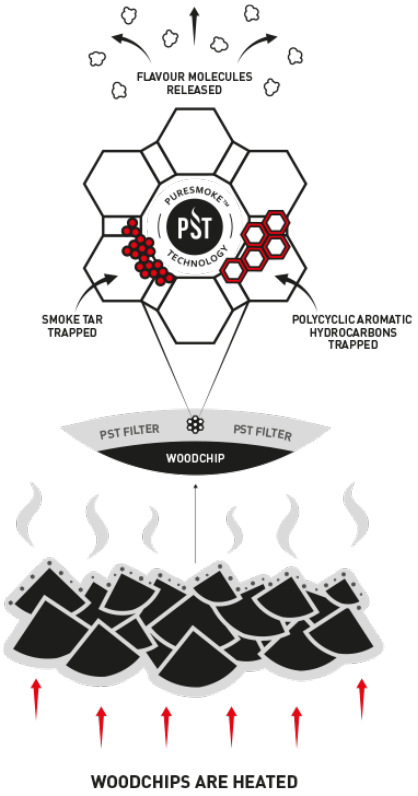


Figure 1.7 The production of smoked ingredient by PureSmoke™ Technology (source; Besmoke (2017)).

The smoked volatiles are regarded as potential contributors that can enhance the aroma and flavour of smoked products. While consuming food, the volatile substances will stimulate the olfactory receptors located in the olfactory epithelium through the retronasal and orthonasal pathways, resulting in the production of an olfactory sensation. The overall food flavour perception is regarded to be an integration of simultaneous sensory which include odour and taste from available chemical stimuli in the mouth and perceptual intra- and cross-modal interactions (Nasri et al., 2013; Small & Prescott, 2005).

Section 2 Cross modal interaction

2.1 Odour-induced taste enhancement

The cross-modal odour-taste interaction implies that taste can boost the intensity of odour meanwhile the odour can enhance the taste perception. The odour-taste interaction may happen when the odour and taste compounds are at levels above or below the threshold and rely on the food matrices (Poinot et al., 2013). Taking into account the effect of aroma on the perception of taste, several studies have reported that the taste perceived, including sweetness, saltiness and bitterness, can be improved by addition of a congruent aroma.

Sweet enhancement

Excess sugar consumption increases the chance of acquiring noncommunicable diseases (such as obesity, cardiometabolic disorders, and dental diseases). Sweetness enhancement by aromas has been proposed as a way to reduce sugar reduction in food products, although enhancement is dependent on the type of aroma and sugar content (Bertelsen et al., 2020). Frank and Byram (1988) studied the odour-induced sweetness of whipped cream products. According to the findings, strawberry odour tended to boost maximum sweetness; however, peanut butter odour did not. This study discovered that odour-enhanced sweetness is odour-dependent. Furthermore, the strawberry odour did not boost the saltiness of sodium chloride, indicating that an odour-enhanced taste is dependent on the tastant. The potential of odour-induced sweet enhancement of strawberry odour was obvious when the panels clamped the nostrils before tasting. The sweetness perception of strawberry-flavoured whipped cream was reduced by 85%.

Sweetness taste enhancement was discovered with the odours of caramel, maracuja, strawberry, and lychee, whereas sweetness taste suppression was observed with angelica oil and damascone. The major discovery was that caramel's sweet-smelling odour enhances the sweetness of sucrose and reduces the sourness of citric acid (Stevenson et al., 1999). Dalton et al. (2000) demonstrated the sensitivity of benzaldehyde perception when paired with congruent and incongruent stimuli (saccharin; sweet and monosodium glutamate; umami, respectively). When benzaldehyde (almond-cherry odour) was combined with saccharin, the participants'

sensitivity to benzaldehyde increased. However, this was not the case when incongruent stimuli (MSG and water) were presented with benzaldehyde. Some perceptual interactions between suprathreshold tastes and odours appear to be dependent on the congruency of specific stimulus combinations (Dalton et al., 2000). For example, the sweetness of sucrose can be improved by strawberry odour but not by peanut butter odour (Frank & Byram, 1988). Several studies have demonstrated that volatile aromas can enhance the perception of taste. For instance, the presence of caramel, vanilla, or fruity notes has been found to increase sweetness (Labbe et al., 2006; Rao et al., 2017; Sakai et al., 2001; Stevenson et al., 1999). Additionally, the aroma of cocoa has been shown to stimulate bitterness (Labbe et al., 2006). The phenomenon of synergy is more commonly observed at lower intensities or concentrations, whereas greater intensities or concentrations are less likely to result in enhancement and may even lead to suppression, as reported by Keast and Breslin (2003).

Salt enhancement

A reduction in sodium quantity is often connected with a decrease in customer acceptance due to the loss of saltiness and flavour of food products (Batenburg & van der Velden, 2011). One feasible approach for reducing salt consumption is to decrease the quantity of salt added to processed foods (Dötsch et al., 2009). Apart from gradually decreasing sodium levels in food products, there are a number of salt reduction strategy approaches presently being effectively used, including; a taste–taste interaction in which the presence of another taste alters the perceived intensity of one taste. Yamaguchi and Ninomiya (2000) investigated the ratio between sodium chloride (NaCl) and monosodium glutamate (MSG) in a clear soup in order to maintain a high palatability score in the event that NaCl levels in the soup needed to be reduced. When used at the optimal level, MSG helped maintain the palatability score of the soup with reduced sodium. However, if the MSG was added higher than the optimum level, the pleasantness was decreased; this indicated that the suitable amount of MSG to add to a salt solution is limited and varied in different food products (Yamaguchi & Takahashi, 1984a). The research has been conducted on the use of soy sauce (rich in glutamate) as a means of replacing and reducing the sodium content of culinary products through salt reduction. McGough et al. (2012) utilised various concentrations of soy sauce to supplant and reduce sodium in emulsified sausages. They found that a treatment containing 50% salt from soy sauce and 50% from flake salt produced the greatest sensory and quality characteristics. Adapted to a 20% NaCl

formulation reduction, the results indicated that a treatment consisting of 50% salt from soy sauce and 30% from flake salt might be suitable for industrial applications.

Another strategy is odour-induced taste enhancement (OITE), in which the presence of an odour enhances the perceived intensity of a taste. The use of tasteless odorants to compensate for the salt reduction through multisensory-integration mechanisms of cross-modally odour-taste interaction has been shown to be a promising method for improving the saltiness (Lawrence et al., 2009; Thomas-Danguin et al., 2019). Recent research indicates that adding a salty-smelling odour, such as that of soy sauce, sardines, bacon, anchovy, peanut, poultry, tuna, cheese, brothy, increases the perception of saltiness (Batenburg & van der Velden, 2011; Djordjevic et al., 2004; Lawrence et al., 2011; Lawrence et al., 2009; Nasri et al., 2011; Onuma et al., 2018; Seo et al., 2013; Syarifuddin et al., 2016). However, despite the fact that the soy sauce odour added to the product was intended to aid in salty enhancement, which can result in an increase in consumer acceptability in salt-reduced food products, it was discovered that the introduction of soy sauce odour strongly influenced the loss of consumer acceptability by significantly increasing off-flavour notes (Lee et al., 2015). Previous evidence suggests that congruent aroma may be necessary for odour induced taste enhancement. Lawrence et al. (2009) demonstrated that participants were able to estimate the saltiness of foods on the basis of their written names. In addition, the authors have shown that a number of specific salt-associated odours (especially anchovy and bacon odours) via the retronasal route could amplify the saltiness of a low-concentrated NaCl solution. The authors also stated, however, that some odours not associated with salt could lead to a reduction in saltiness: tomato and carrot odours; although the intensity of these odours was quite high, the odour-induced saltiness enhancement (OISE) remained very low or negative. The corresponding odour-induced taste enhancement is more significant in the low-concentrated taste solution than in the high-concentrated taste solution (Seo et al., 2013). In another study, Batenburg and van der Velden (2011) studied the effect of single salt-congruent odour components and complex savoury flavourings on saltiness perception. Among the single salt-congruent aromas that could compensate for a 15% reduction in salt, they discovered that sotolon was the best for salt enhancement in salt-reduced bouillons. Meanwhile, when combined with salt-reduced bouillons, the complex savoury flavouring may mask the off-flavour of potassium-based salt substitutes.

Section 3 Umami enhancement

3.1 Umami substances

3.1.1 Monosodium glutamate

Since low-salt diets are generally considered tasteless, it is important to find ways to prepare palatable salt-reduced foods. One well-known method is the addition of monosodium glutamate (MSG) at an optimum level (Manabe et al., 2014). MSG is considered to be a typical umami substance, it also contains one sodium equivalent, thus evoking saltiness (Linscott & Lim, 2016). Glutamates are the salts that can be created from glutamic acid, the most common of which is a sodium salt, monosodium glutamate. All glutamates produce umami taste, but MSG is particularly efficient as it interacts with table salt (sodium chloride). MSG is very stable and does not break down during normal food preparation, but when MSG crystals contact with water, they divide into sodium ions and glutamate ions, taking on the form in which can produce the umami taste (Spence, 2014). MSG is described as a taste stimulant that is brothy, salty, and meaty (Ninomiya, 2002). If MSG is added to suitable foods in moderate concentrations, the palatability and pleasure of the food will increase (Ventanas et al., 2010).

3.1.2 Ribonucleotides

Other important compounds associated with umami taste are 5'-ribonucleotides such as disodium-5'-inosinate (Inosine-5′-monophosphate; IMP), disodium-5'-guanylate (Guanosine-5′-monophosphate; GMP), adenylate or adenosine-5′-monophosphate (AMP), and xanthosinate or xanthosine-5′-monophosphate (XMP) (Spence, 2014; Yamaguchi & Ninomiya, 2000). IMP and GMP have been manufactured industrially since 1960, and are used as additives in many prepared foods, such as pies, chips, noodles, sausages, soups and sauce bases, often in combination with glutamate, to benefit from the synergy between them (Spence, 2014). Ribonucleotides that impart umami are present in many raw ingredients. For instance, inosinate is found in meat, guanylate is found in plants and fungi, and adenylate is found in fish and shellfish, inosinate and guanylate are found in konbu (Spence, 2014). Moreover, the ingredients high in umami of 5′-ribonucleotides (5′-GMP) are yeast extracts and mushrooms such as shiitake, and tomato is rich in 5′-AMP (Dermiki et al., 2013).

3.2 Taste-taste enhancement

The umami taste compounds can be discovered in different levels in many foods. For example, high levels of glutamic acid can be found in kelp, especially Japanese kombu, fermented soy products, cheeses, mature Cheddar, yeast extracts and tomato (Dermiki et al., 2013). Yamaguchi and Takahashi (1984a, 1984b), Mojet et al. (2004) discovered that MSG appear to have a compensatory relationship with NaCl, demonstrating the ability of umami-containing substances to increase a salty taste despite decreases in salt content. The optimal concentration of MSG also enhanced the saltiness of soup samples. More study has shown that umami-tasting substances such as MSG and soy sauce could decrease sodium contents in foods, but the enhanced impacts of MSG and soy sauce on saltiness and palatability appear to depend on food matrices (Kremer et al., 2009).

Yamaguchi (1967) studied the synergistic effect of taste between MSG and IMP by keeping the sum of MSG and IMP concentrations constant and varying the amount of IMP in the mixture from 0% to 100%. The flavour intensity is very mild at the extreme values of MSG or IMP alone. Even when the concentration was significantly raised, the taste intensity of IMP alone barely increased. When MSG and IMP were mixed together, there was a synergistic taste impact, which could imply that IMP plays an important role as a flavour enhancer in the presence of MSG. Another synergistic effect between MSG and 5'-ribonucleotides (IMP and GMP) was explored by Giovanni and Guinard (2001); they discovered that the tertiary mixtures of MSG and both 5'-ribonucleotides had the highest peak intensity of time-intensity profiles. In addition, at lower concentrations, GMP was more effective than IMP.

3.3 Odour-induced umami enhancement (OIUE)

Manabe et al. (2014) investigated the effects of the retronasal odour of dried bonito stock (umami-rich) on the enhancement and improvement of palatability upon reduction of sodium. The results indicated that when the odour of dried bonito stock was added to a 0.68% NaCl solution (15% salt reduction of 0.8% NaCl), the aroma raised umami and enhanced palatability. Frøst et al. (2021) investigated the volatile compounds that may positively contribute to the perception of umami in dashi (aqueous extract of seaweed) stocks made from 16 distinct types of seaweed. The descriptive sensory analysis was performed under two conditions: with and without olfactory input. When the nostril was pinched, there were

significant differences in the perceived umami taste of the 12 dashi samples. The three dashis with lower glutamate levels exhibited a more prominent odour-induced taste enhancement, while those with higher glutamate content showed less effect.

The implementation of odour-induced taste enhancement proved to be an effective approach for reducing certain food ingredients in order to provide health advantages or enhance the overall quality of food products. Sugar, salt and fat reduction methods through the odour-induced taste enhancement pathway have been shown to be promising methods nowadays (Batenburg & van der Velden, 2011; Frank & Byram, 1988; McGough et al., 2012; Onuma et al., 2018; Stevenson et al., 1999; Syarifuddin et al., 2016). Odour-induced taste enhancement not only restores the lost taste from the reduction of certain food ingredients, but it also enhances the umami taste of food products, making them more palatable. However, taste enhancement by odour is considered more efficient with congruent odour-taste than an incongruent odour-taste and more prominent with lower intensity of the tastant compounds (Linscott & Lim, 2016; Nasri et al., 2011; Thomas-Danguin et al., 2019). Odour-taste congruency is the most relevant enhancement that revealed significantly greater activation of brain regions associated with the integration of odour and taste compared to the brain signal of an incongruent combination (Seo et al., 2013).

Research overview

In this research study, we have attempted to understand the reasons behind the unique character and consumer appeal of food products, either those containing smoked ingredients or those that have undergone the smoking process. Numerous studies have investigated the volatile compounds present in liquid smoke and various smoked food products and identified the potent aroma compounds. However, there is currently no evidence to support the assertion that these potent aromas are validated in a recombinant blend solution. The aim of **Chapter 2** is to confirm and validate in a recombinate blend, the identity of the most potent odour active compounds in apple-wood smoked water which is used in subsequent sensory experiments. Moreover, many of the previous studies concentrated on a single processing type of smoked ingredients or a specific variety of wood, so in **Chapter 3** we compared the volatile profiles of smoked water produced using a traditional direct smoking method with a recently developed technology (PST) which reduces the concentration of carcinogenic benzo[a]pyrene in smoked

water. We used three different woods and found similar trends in all three. In **Chapter 4**, we focus on how smoked water and its constituent volatiles may act as taste enhancers and the potential mechanisms of action whereby they impart a savoury taste to smoked food products. We suggested that the group of potent aroma compounds present in smoked water could be congruent with savoury foods and, therefore, can be used to enhance taste.

Research objectives and hypotheses

The overall hypothesis is that the use of smoked water as an ingredient can enhance the taste of salt-reduced soups.

1. **Chapter 2: Characterisation of the key odorants in smoked water by means of the sensomics approach**

   Research question: What are the potent odour active compounds in traditional (TR) and PureSmoke Technology (PST) smoked waters?

   Aim: To identify odour active compounds in smoked water using the sensomics approach and validate the findings in a recombinant blend solution.

2. **Chapter 3: Comparison of volatiles generated in smoked water using PureSmoke Technology (PST) with those generated using a traditional smoking process**

   Hypothesis:

   H_1 - Use of PureSmoke Technology (PST) to produce smoked water (as opposed to use of a traditional direct smoking process) alters the volatile profile of the product.

   H_2 - Use of PureSmoke Technology (PST) reduces some of the harsh notes generated using the traditional process.

   Aim: To evaluate whether or not the volatile profiles change when the smoked water is prepared using PST.

3. Chapter 4: Utilisation of smoked water and smoke recombinates to enhance the taste in salt-reduced soup and investigate the mechanisms

Hypothesis: Addition of smoked water to a low salt soup will enhance the taste of the soup.

H_3 - The components of smoked water are tastants which contribute a salty and/or umami taste to water.

H_4 - The components of smoked water are taste enhancers which enhance the salty and/or umami taste in combination with other tastants such as MSG.

H_5 - The volatile components of smoked water are involved in odour induced taste enhancement. Since smoky notes are congruent with salty and umami foods, we hypothesise that there will be no sweet taste enhancement and that the major enhancements will be in either salt taste, umami taste or both.

Aim: To evaluate the three possible modes of action of smoked water on taste; tastant, or taste enhancer, or odour-induced taste enhancement.

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Chapter 2
Characterisation of the key odorants in smoked water by means of the sensomics approach

2.1 Introduction

The traditional method of food preservation is smoking. Wood smoke also modifies the texture and adds flavour and colour. The three main components in wood are cellulose, hemicellulose and lignin, which undergo thermal decomposition to produce several groups of compounds. In terms of aroma, a large group of phenols is one of the most important groups (Jónsdóttir et al., 2008). Phenolic compounds in smoke produced by burning the lignin component of wood act as natural preservatives and provide their unique flavour characteristics. Smoked flavour has various odour qualities, such as smoky, ashy, woody, musty, etc., rather than just one specific smoky odour (Wang et al., 2018). When smoke compounds were isolated using gas chromatography-olfactometry and gas chromatography-mass spectrometry techniques, several of them were discovered to be a part of the characteristics of smoked flavour. Several phenolic substances, such as 2,6-dimethoxyphenol (syringol), 4-methylguaiacol, 4-ethylguaiacol, 4-vinylguaiacol, guaiacol, 2, 6-dimethylphenol, and 3-ethylphenol, are known to contribute to smoky aroma (Pu et al., 2020; Wang et al., 2018).

Food flavours contain over ten thousand volatile substances; only about 5-10% of these compounds are classified as key aroma compounds (Jelen´ & Gracka, 2017). The odour thresholds of the aroma components span a vast range of values. In order to investigate the impact of the compound on the overall food flavour, the odour activity value (OAV) is used to explain how significant the compound is. The OAV is a ratio of a compound's concentration to its odour threshold and can be used to estimate its impact. The low OAV odorants (less than 1) are commonly considered less important odour compounds. Despite having relatively high odour thresholds, compounds substantially affect a product's overall aroma when present in high concentrations. Yet, even at very low concentrations, substances with extremely low odour thresholds may still function as potent odorants. However, OAVs do not take into account synergies between aroma molecules. The aroma extract dilution analysis (AEDA) is used to analyse most odour-active compounds in food samples using gas chromatography and

olfactometry. The AEDA protocol involves sniffing serial dilutions of aroma extract from low dilution to high dilution to find how many dilution levels each odour compound is still perceived, and is reported as flavour dilution (FD) factor. To ensure the potent odorants are correctly identified in the food samples, recombinate tests are the final step of the sensomics approach. This sensomics approach was selected because it is the most comprehensive method available.

The sensomics approach was used to characterise the volatile compounds in applewood smoked water. Two types of apple-wood smoked water were analysed: traditional (TR) and PureSmoke^{TM} Technology (PST; P50), which utilised natural zeolite to filter contaminants of polycyclic aromatic hydrocarbons (PAHs).

Research question;

What are the potent odour active compounds in traditional (TR) and PureSmoke Technology (PST) smoked waters?

The aim of this chapter was to identify the most odour active compounds in both TR and P50 smoked water samples using applewood in both instances and to determine whether these explain differences in flavour. The identification of odour active compounds in smoked water using the sensomics approach and validate the findings in a recombinate blend solution, including;

(1) to select the most appropriate aroma extraction method (s);

(2) to identify the aroma-active compounds by GC–O, GC-MS using Aroma Extraction Dilution Analysis (AEDA) from different extraction methods;

(3) to quantify (or semi-quantify) the aroma-active compounds and calculate the odour activity values (OAVs); and

(4) to validate and confirm key odorants using model recombinates;

2.2 Materials

2.2.1 Smoked water samples

Apple-wood PureSmokeTechnology (PST) smoked water (P50; batch number; S0201/M/10, manufacturing date; October 2017) which used the natural zeolite for filtering process to trap the polycyclic aromatic hydrocarbons (PAHs) and acrid smoke tar, but enable free flavour compounds to flow (Parker et al., 2018), and apple-wood traditional smoked water (TR; batch number; S0230/M/11, manufacturing date; November 2017) were provided by Besmoke Ltd (Arundel, UK).

2.2.2 Solvents and other chemicals

Dichloromethane (≥99% purity, GC grade), diethyl ether (≥99.5% purity, GC grade) and methyl acetate (anhydrous 99.5% purity) were purchased from Sigma-Aldrich. Methanol (≥99.8% purity, HPLC grade) and sodium chloride (≥99.5% purity, AR) were purchased from Fisher Scientific U.K. Limited (UK). Ethanol (96% purity, FG) was purchased from Kimiauk, Hayman Group Limited (UK), and sodium sulphate (anhydrous, BDH) was purchased from VWR International Limited (England). Distilled water was 18.2 MΩ.

2.2.3 Authentic odorant standard compounds

Thirty-five authentic odorant standard compounds were 1-phenylethanone (acetophenone; 99% purity; Aldrich, Germany), 1-(furan-2-yl)ethanone (2-acetylfuran; ≥99%, FG, Sigma-Aldrich, China), acetic acid (≥99.5%, FG, Sigma-Aldrich, Colombia), 1-(5-methylfuran-2-yl)ethanone (2-acetyl-5-methylfuran; Oxford), 1-(4-hydroxy-3-methoxyphenyl)ethan-1-one (acetovanillone; SAFC^{TM}, Germany), cyclopentanone (99%, Sigma, USA), 1,3-dimethoxy-5-methylbenzene (3,5-dimethoxytoluene; 98+%, Lancaster Synthesis, England), 2,6-dimethoxyphenol (syringol; ≥98%, FG, Sigma-Aldrich, India), 2,6-dimethylphenol (≥99%, FG, Sigma-Aldrich, China), 2,3-dimethylphenol (99.2%, AG, Sigma-Aldrich, Germany), 2,5-dimethylphenol (≥99%, FG, Sigma-Aldrich, China), 2-methoxy-4-prop-2-enylphenol (eugenol; pure, Givaudan, Switzerland), 4-ethylphenol (p-ethylphenol;

≥98%, FG, Merck, China), 4-ethyl-2-methoxyphenol (ethylguaiacol; ≥98%, FG, SAFC, USA), 3-ethyl-2-hydroxycyclopent-2-en-1-one (3-ethyl-2-hydroxy-2-cyclopentenone; >97%, TCI, Japan), 2-ethylphenol (o-ethylphenol; 99%, Sigma-Aldrich, Japan), furan-2-carbaldehyde (furfural; ≥98%, FCC, FG, Sigma-Aldrich, China), furan-3-carbaldehyde (3-furaldehyde; 97+%, Aldrich, USA), 2-methoxyphenol (guaiacol; natural ≥99%, FG, Sigma-Aldrich, China), 2-methoxy-4-[(E)-prop-1-enyl]phenol ((E) isoeugenol; natural 99%, FG, Sigma-Aldrich, China), 2-hydroxy-3-methylcyclopent-2-en-1-one (cyclotene; 98%, Sigma-Aldrich, China), 2-methylfuran (Oxford), 3-hydroxy-2-methylpyran-4-one (maltol; >99%, FG, SAFC Sigma-Aldrich, China), 2-methylbenzaldehyde (Oxford organics, England), 4-methylphenol (p-cresol; 99%, FG, Sigma-Aldrich, USA), methyl benzoate (≥98%, FCC, Sigma-Aldrich, Germany), 2-methylphenol (o-cresol; Acros Organics, Belgium), 2-methylcyclopent-2-en-1-one (2-methyl-2-cyclopentenone; 98%, Sigma-Aldrich, Japan), 2-methoxy-4-methylphenol (methylguaiacol; ≥98%, FG, Sigma-Aldrich, China), phenol (natural 97%, FG, SAFC Sigma-Aldrich, USA), 4-ethenyl-2-methoxyphenol (vinylguaiacol; ≥98%, FG, Sigma-Aldrich, United Kingdom), 5-methyl-2-propan-2-ylphenol (thymol; ≥99%, FCC, Sigma-Aldrich, Germany), 2,4,6-trimethylphenol (97%, Sigma-Aldrich, China), 1,2,3-trimethoxybenzene (98%, Sigma-Aldrich, USA), 4-hydroxy-3-methoxybenzaldehyde (vanillin; ≥97%, FG, Sigma-Aldrich, China).

2.3 Methods

2.3.1 Selection of the most appropriate aroma extraction method for extracting compounds in smoked water

Four main extraction methods, which were solid phase microextraction (SPME), solid phase extraction (SPE), liquid-liquid extraction (LLE), and stir bar sorptive extraction (SBSE) were compared in order to obtain the best extraction efficiency. Smoked water was filtered through the Whatman polypropylene (PP) puradisc syringe filter (pore size 0.45 μm) before extraction.

2.3.1.1 Solid phase microextraction

SPME is the most popular method for extracting volatile compounds. SPME employs a coated fused silica fibre housed in a syringe-like. The sample is placed in a vial and transferred to an incubator to heat the sample to a desired temperature for a specific time to allow the volatile aroma compounds to partition into the headspace After that, the SPME syringe needle punctures the screw cap septum and the fibre is launched to the headspace over the sample vial to allow extraction. After the specified extraction time, the fibre is retracted and then desorbed in the injection port of the GC-MS (Elmore, 2015). In this experiment, a 2.0 mL of filtered smoked water was equilibrated at 60 °C for 15 min before exposure to a 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fibre, Stableflex (Gray) (Supelco®, Merck) for extraction at 60 °C for 30 min.

2.3.1.2 Solid phase extraction

SPE is a sample preparation technique, but due to its versatility, it can also be used for sample isolation, concentration, purification, and cleaning. SPE relies on the partition distribution between a solid phase (sorbent) contained within a column (cartridge) and a sample matrix containing analytes. Collecting analytes from the SPE system is done by eluting the analyte from the sorbent with an appropriate solvent (Ötles & Kartal, 2016). In this experiment, different SPE cartridge types and elution solvents were evaluated for extracting the smoked-related compounds in the smoked water sample.

A: Selection of solid phase cartridge type; three different solid phase cartridges were evaluated; Strata™ -X 33 µm Polymeric Reversed Phase; 100mg/6 mL (Phenomenex) (designed for the extraction of neutral and aromatics compounds), Isolute® NH_2; 100mg/3 mL (Biotage) (designed for the extraction of acids and polar compounds), and Bond Elut ENV; 50mg/1 mL (Agilent Technologies) (designed for the extraction of polar organic compounds), and dichloromethane was used as the eluent. In this study, Strata™ -X 33 µm Polymeric Reversed Phase; 100mg/6 mL (Phenomenex) showed extracted the greatest number of compounds among three cartridge types under the same extraction and elution conditions. The results to justify the suitable cartridge was shown in **Table 2.3**. Next, the selection of eluent type was assessed as below.

B: Selection of the elution solvent; Strata™ -X 33 µm Polymeric Reversed Phase; 100mg/6 mL (Phenomenex) was selected from 2.3.1.2.A, and the eluting solvents were varied. The performance of the eluent dichloromethane, diethyl ether and methyl acetate were assessed. In this study, diethyl ether yielded the greatest number of extracted compounds among the three solvents, shown in **Table 2.3**.

Both SPE extraction methods were started by preconditioning the SPE cartridge with methanol (maximum volume for each cartridge type; 6, 3 and 1 mL for Strata™ -X 33 µm Polymeric Reversed Phase, Isolute® NH_2, and Bond Elut ENV, respectively), followed by distilled water and then smoked water sample, which was attached to a flow control valve of SPE tube vacuum manifold. Filtered smoked water (5 mL) was applied to the top of a conditioned cartridge twice before being rinsed with 5 mL of distilled water. The cartridges were then dried using a vacuum at 70 kPa for 40 min, or until completely dry. Subsequently, the compounds retained on the cartridges were eluted with 5 mL of the selected eluant, and the extracted sample was collected. Finally, water was removed by adding a small amount of anhydrous sodium sulphate and then adjusting the final volume to 10 mL with the eluents before GC-MS analysis.

2.3.1.3 Liquid-liquid extraction

LLE was used to isolate the volatile compounds from the smoked water samples. Dichloromethane and diethyl ether were used as solvents, and the extraction efficiency was compared. Thus, 2 mL of filtered smoked water was diluted 1:1 with distilled water before being transferred to the separating funnel. Next, a volume of 10 mL of the solvent (dichloromethane or diethyl ether) was added, followed by continuous shaking of the mixture for 5 min (releasing the gas at intervals). Then, the funnel was allowed to rest for 10 min at room temperature. Once the phases had separated, the organic phase was collected for GC-MS analysis, (the upper portion for diethyl ether extraction, and the bottom portion for dichloromethane extraction).

2.3.1.4 Stir bar sorptive extraction

SBSE is one method for extracting volatile and semi-volatile compounds from aqueous solution using the coated stir bar (Twister). The Twister comprises a magnetic stir bar coated in glass and an adsorbent layer (polymer). Once the aqueous sample comes into contact with the stir bar, the compounds present in the sample become adsorbed onto the adsorbent layer. Subsequently, these adsorbed compounds are desorbed from the adsorbent layer using either thermal desorption or extracted into an organic solvent. The desorbed compounds are then subjected to analysis using gas chromatography in the subsequent stage.

In this study, one stir bar type (length = 10 mm) coated with 0.5 mm of polydimethylsiloxane (PDMS) (Twister; Gerstel) was used to extract volatile and semi-volatile compounds in smoked water sample. Before extracting the smoked water sample, a stir bar was prepared by undergoing a preconditioned/cleaned-up process. This involved immersing the stir bar in 50 mL of distilled water and subjecting it to agitation for a duration of 1 h. Subsequently, a stir bar was dried using lint-free tissue under ambient conditions for one hour. Afterwards, the stir bar was introduced into a stainless steel automated thermal desorber (ATD) sample tube, wherein glass wool was positioned at both ends of the tube to confine the placement of the stir bar in the centre. The stir bar was then conditioned using an ATD TurboMatrix unit (PerkinElmer) at 240 °C for 16 min in a stream of helium gas. For enrichment purposes, the stir bar was inserted into the smoked water sample and agitated for a specified period of time. Once enrichment was completed, the stir bar was removed from the sample and dried (Gerstel).

To obtain the appropriate extraction efficiency, conventional and sequential SBSE methods were compared. In the conventional SBSE method, a conditioned PDMS coated stir bar was inserted and stirred in 5 mL of smoked water without (extracting non-polar compounds) or with 30% NaCl (extending the polarity range by salting out) for 2 h. In the sequential method, smoked water was extracted in two steps with different conditioned stir bars and with intermediate sample modification by adding 30% NaCl. Smoked water (5 mL) was first extracted at room temperature for 1 h using a preconditioned PDMS coated stir bar. After removing the first stir bar, a second extraction was performed with a new conditioned PDMS coated stir bar after adding of 30% NaCl which was extracted for another hour in agitating condition. When the SBSE-step has been completed, the sorbed stir bar was removed from the

smoked water sample and dried with the lint-free tissue before being desorbed. Analytes from stir bars were desorbed by liquid extraction (back-extraction) using the mixture of dichloromethane:methanol (9:1) in a sonicator for 20 min.

The analysis of an approximate number of aroma components in the smoked water extracts obtained from each extraction method described in section 2.3.1, was predominantly conducted using an Agilent Technologies 7890A gas chromatography with GC sampler 120 series equipped with a capillary Zebron ZB-5MSi GC column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (Phenomenex, USA) (nonpolar column) coupled to an Agilent 5975C inert MSD with Triple-Axis Detector. In the SPME extraction method (section 2.3.1.1), the SPME fibre containing adsorbed aroma compounds was introduced into the injection port of the GC-MS at a temperature of 250 °C using the splitless mode. The desorption of the compound was carried out for 20 min. For the extracts from SPE, LLE and SBSE extraction methods (section 2.3.1.2-2.3.1.4), 2 µL of the extract was direct-injected into the GC-MS injection port at 250 °C in split mode (1:10). The temperature of the oven for SPME injection was programmed from 60 °C to 300 °C at 6 °C/min and then held for 20 min. For direct injection, the oven program started at 40 °C (held for 5 min), then rose to 320 °C at 6 °C/min and held for 20 min. Helium was used as the carrier gas at a flow rate of 1 mL/min.

According to the data presented in **Table 2.3**, the best two extraction methods were chosen based on their ability to yield a greater number of extracted compounds. The first method was SPME (section 2.3.1.1), and the second method was solid-phase extraction (SPE) using diethyl ether as an eluant (section 2.3.1.2. *B*). Then, these two methods were employed for the AEDA technique to identify the potent aroma compounds in smoked water in a further step. For the purpose of analysing the aroma compounds present in the extracts obtained from the two selectively abovementioned methods, an extraction of the smoking water sample was performed again. The tentative identification results of each volatile compounds were given only based on the GC peak chromatogram, utilising the National Institute of Standards and Technology (NIST) 2020 mass spectrum database and ChemStation software, which were shown in **Table 2.5**. To acquire Linear Retention Indices (LRIs) values, a sequence of n-alkanes (C6-C26) was examined under the same conditions for either SPME injection or direct injection. The LRI for each tentative aroma compound was calculated against the retention time of a standard series of n-alkanes.

LRI values were calculated according to *LRI & Odour Database* :

$$LRI = 100\left(\frac{t - t_n}{t_{n+1)} - t_n} + n\right)$$

Where; t = retention time of component

n = carbon number of preceding n-alkane

n + 1 = carbon number of subsequent n-alkane

2.3.2 The identification of aroma-active compounds by Gas chromatography-Mass spectrometry (GC-MS) and GC-Olfactometry (GC-O) using Aroma Extraction Dilution Analysis (AEDA)

The AEDA methodology was employed to identify the potent aroma compounds in smoked water. In order to extract these compounds, SPME and SPE (with diethyl ether) were selected as the two extraction methods since no single extraction could extract all flavour components. For the SPME extraction, 2 mL of smoked water samples were placed into a 20 mL screw-capped round bottom glass vial sealed with a silicone/PTFE-lined screw cap. The vial was placed in a water bath at 60 °C, and after equilibration for 15 min, divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, USA) were exposed to the headspace for 30 min. After extraction, the volatile compounds captured on SPME fibre were manually injected into the injection port (250 °C) of the Agilent Technologies 7890B Gas Chromatography-Flame Ionisation Detector-Olfactometry (GC-FID-O) equipped with a capillary Zebron ZB-5MSi GC column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (Phenomenex, USA). The extracted smoked water samples were injected in splitless and split modes in a three-fold split ratio series corresponding to 1:3, 1:9, 1:27, 1:81, 1:243 and 1:620, respectively. The last split ratio at 1:620 did not follow a three-fold dilution series as the GC-O split ratio limitation, which had the maximum split ratio at 1:620.

On another selected extraction method, SPE (Strata™ -X 33 µm Polymeric Reversed Phase; 100mg/6 mL (Phenomenex)) using diethyl ether as eluted solvent, seven dilutions of extracts were prepared in a three-fold dilution series following the split ratio of SPME method above. 1 µL was manually injected into the injection port of the GC-FID-O at 250 °C in splitless

mode. The temperature of the oven for both splitless and split modes injection was programmed from 60 °C (0 min) to 300 °C at 6 °C/min and held for 20 min. Helium was used as the carrier gas (2 mL/min). The sample was split 1:1 at the end of the column between a FID and an ODO II sniffing port (SGE, Australia). Both extraction method samples above were sniffed by 3 different assessors with one year or more experience in performing GC-O experiments who carried out several training runs on smoke extract prior to starting on the GC-O. In addition, roughly 15 odourant standard compounds, primarily derived from phenols, guaiacols, and syringols groups, were prepared at concentrations between 1-5 mg/L and provided to assessors during olfactory training sessions.

Three trained assessors assessed the samples for 30 min, and this was performed in duplicate. They were asked to describe their perceived odour as the compounds eluted from the column. The flavour dilution (FD) factor for a specific odour region is defined as the highest dilution at which the odour can still be perceived at the sniffing port of GC-FID-O. The FD factor reported for each compound was chosen based on the highest value among the three assessors. Therefore, a sample with a higher FD factor may be more representative of its overall odour (Lok et al., 2022). A homologous sequence of n-alkanes (C6-C26) was analysed under the same conditions. The LRIs of each aroma active compound that the assessors perceived was calculated from the retention time of n-alkanes.

2.3.3 Quantification and semi-quantification of volatile compounds and calculation of the odour activity values (OAVs) of potent aroma compounds

Five-point calibration standards were prepared for 34 authentic odorant standard compounds. These 34 standard compounds were separated into several groups and dissolved in small volumes of methanol or ethanol before being reconstituted with distilled water. A standard solution mixture was generated at concentrations ranging from 0.001-70 mg/L, as shown in **Table 2.1**.

Table 2.1 The concentration range of thirty-four authentic odorant standard compounds preparation.

Authentic odourant chemical standards	**Standard concentrations ranging (mg/L)**
Acetic acid	20,40, 60, 80,100, 120
2-Methyl-2-cyclopenten-1-one	0.01, 0.025, 0.05, 0.075, 0.1
2-Methylfuran	0.125, 0.25, 0.5, 0.75, 1
2-Methylphenol, 4-methylphenol, 2,3-dimethylphenol, 2,6-dimethylphenol, 2,4,6-trimethylphenol, 2-ethylphenol, 4-ethylphenol, guaiacol, 4-methylguaiacol, 4-ethylguaiacol, syringol, 2-furfural	0.001, 0.02, 0.1, 1, 10, 20
Cyclopentanone, 3-furfural, phenol, 2-acetyl-5-methylfuran, maltol, 1,2,3-trimethoxybenzene, 4-vinylguaiacol, eugenol, methyl benzoate, 3,5-dimethoxytoluene, 2-acetylfuran, 2-methylbenzaldehyde, acetophenone, 3-ethyl-2-hydroxy-2-cyclopentanone, isoeugenol, cyclotene, 2,5-dimethylphenol, acetovanillon	0.02, 0.1, 1, 5, 10, 20
Vanillin	1, 5, 10, 20, 30, 40, 50, 70

Two millilitres of each standard point were spiked with 30 µL of internal standard (thymol, 1 mg/L; final concentration of thymol in 2 mL of standard was 0.015 mg/L) and placed into each 20 mL screw-capped vial sealed with a silicone/PTFE-lined screw cap. The standard vials were placed on a sample tray of Agilent Technologies 7890A gas chromatography with GC sampler 120 series coupled to an Agilent 5975C inert MSD with Triple-Axis Detector (GC-MS). The standard vials were then equilibrated at 60 °C for 15 min before exposure to the 50/30 µm DVB/CAR/PDMS SPME fibre at 60 °C for 30 min. The standard compounds captured on SPME fibre were automatically introduced (injection port temperature 250 °C) into GC-MS in the splitless mode, and SPME fibre was desorbed for 20 min. The standard compounds were separated on a capillary Zebron ZB-5MSi GC column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (Phenomenex, USA) (nonpolar column), or a capillary Zebron ZB-WAX GC column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (Phenomenex, USA) (polar

column), and/or DB-WAX UI GC column (60 m, 0.25 mm i.d., 0.50 μm film thickness) (Agilent Technologies, USA) (polar column). The oven temperature gradients were set as follows: 60 °C then rise of 6 °C/min up to 300 °C and held for 20 min for the nonpolar column; 60 °C then rise of 6 °C/min up to 250 °C and held for 20 min for the polar columns. Helium was used as the carrier gas (2 mL/min). Duplicate chromatographic analyses of each standard point were performed. The peak area of each standard compound was integrated using a carefully selected single ion (m/z). The standard calibration curve for each authentic odourant compound was constructed by plotting the response ratio of authentic odourant compound peak areas and thymol peak area (Y-axis) against the ratio of authentic odourant compound concentration and thymol concentration (X-axis).

For quantification and semi-quantification of volatile compounds in smoked water, 2 mL of filtered undiluted and 10-fold diluted smoked waters containing 0.015 mg/L thymol internal standard (final concentration in 2 mL of smoked water) were placed into 20 mL screw-capped vial sealed with a silicone/PTFE-lined screw cap, and then was extracted with SPME and processed under the same GC-MS conditions as applied to authentic odourant standards above. The analysis was performed in triplicate for each smoked water sample. The peak area of each extracted compound was integrated using a selected m/z. Thirty-four compounds were quantified using authentic odorant standard curves, and the remaining were semiquantitative using an estimated response factor. For those where the authentic compound was available, the calibrations were based on the single m/z but for the other compounds detected in the smoked water samples, a factor was calculated to convert the peak area of the selected ion to total peak area. The factor of each compound was calculated from a clean mass spectrum, calculating the fraction of the selected ion relative to the total peak area. For example, there was no authentic standard for 6-methylguaiacol (molecular weight is 138 g/mol, and the main ion used to integrate peak area was 123), so the factor for 6-methylguaiacol was calculated from the formula below;

$$\text{Factor for 6-methylguaiacol} = \frac{\text{Sum of area of all m/z up to ion of Mw + 1 (until ion 139)}}{\text{Area of m/z 123}}$$

A homologous sequence of n-alkanes (C6-C26) was analysed under the same conditions. The LRI of each compound was calculated from the retention time of n-alkanes and

their identity was confirmed by comparison of LRIs of the authentic odorant compounds. Volatile compounds without odourant standards were identified by comparing their LRIs with mass spectra from the NIST 2020 library.

The odour activity values (OAVs) of the potent aroma compounds identified in section 2.3.2 were calculated by dividing the concentrations of aroma compounds by their published odour thresholds in water (Buttery et al., 1971; Czerny et al., 2008; Fiddler et al., 1966; Karagül-Yüceer et al., 2003; Li et al., 2023; Lin et al., 2019; Ong & Acree, 1999; Pu et al., 2020; PubChem; Schaller & Schieberle, 2020; Semmelroch et al., 1995; Strube et al., 2012; Tatsu et al., 2020; Yang et al., 2021; Young et al., 1996; Zhang et al., 2021). The compounds with OAVs greater than 1 were accepted as individual contributors to the smoked water aroma.

2.3.4 Validation and confirmation of key odorants by recombination modelling

The potent aroma compounds of smoked water from section 2.3.2 were prepared for aroma recombinates according to the quantitative data obtained in section 2.3.3 at the approximate actual contents found in P50 smoked water (in order to match the overall intensity of the GC chromatogram peak of P50 smoked water). All 22 authentic potency aroma compounds (food grade and non-food grade) were dissolved in either distilled water or a small amount of ethanol (food grade) as appropriated in a 250-mL volumetric flask and adjusted the volume with distilled water. Along with aroma recombinate containing all the compounds identified and quantitated (both food grade and non-food grade), the other three partial recombinate compounds were prepared, shown in **Table 2.2** as compounds on tick marks symbol.

Sensory profiling of P50 smoked water and 4 recombinates was performed by eight trained sensory panellists from Besmoke company who are experts in profiling smoked ingredients, with a minimum of 2 years of experience. The four recombinates were;

A) the full recombinate using all available aroma compounds, whether food grade or not (22).

B) the partial recombinate using all available food grade aroma compounds (17).

C) the partial recombinate using all available food grade and natural compounds as indicated on the product label (5).

D) a restricted recombinate based on just the most potent and food grade (4).

Four consensus-approved descriptors (smoky, woody, ashy, phenolic) used for routine quality control for smoked ingredient products were scored after sniffing and sipping the samples in a warm water solution (50-60 ºC). For all four recombinates the neat solutions were assessed for aroma. Meanwhile, 1% solutions of the three food-grade recombinate solutions (**Table 2.2**; recombinate B, C and D) were assessed by tasting. The scoring sessions presented the samples in balanced order in a cup labelled with 3-digit codes. The panellists were asked to sniff or sip the samples on different session days and score against each aroma descriptor on the 5-point scale, where 0 means absent, score 1 is very weak, and score 5 is very strong. There were 30 s breaks between recombinates, and only drinking water was used to rinse the palate before tasting the following sample.

Table 2.2 The composition of the full and partial recombinate of P50 smoked water.

Odorant standard compounds	**Content (mg/L)**	**[1/] Recombinate compositions**			
		A	**B**	**C**	**D**
1. Acetic acid	80	✓	✓	✓	-
2. 2-Furfural	35	✓	✓	-	✓
3. 2-Acetylfuran	1	✓	✓	-	-
4. Phenol	3	✓	✓	✓	-
5. 4-Methylphenol	2	✓	✓	-	-
6. Guaiacol	6	✓	✓	✓	✓
7. 2,6-Dimethylphenol	0.1	✓	✓	-	-
8. 4-Ethylphenol	0.3	✓	✓	-	-
9. 4-Methylguaiacol	5	✓	✓	-	✓
10. 4-Ethylguaiacol	1	✓	✓	-	-
11. 4-Vinylguaiacol	0.05	✓	✓	-	-
12. Syringol	30	✓	✓	-	✓
13. Eugenol	0.3	✓	✓	✓	-
14. Vanillin	6	✓	✓	-	-

Odorant standard compounds	Content (mg/L)	[1/] Recombinate compositions			
		A	B	C	D
15. Isoeugenol	0.04	✓	✓	✓	-
16. 2,5-Dimethylphenol	2	✓	✓	-	-
17. Cyclotene	2.5	✓	✓	-	-
18. 2-Methylphenol	2	✓	-	-	-
19. 2,3-Dimethylphenol	0.2	✓	-	-	-
20. 2-Ethylphenol	0.1	✓	-	-	-
21. 2,4,6-Trimethylphenol	0.03	✓	-	-	-
22. Acetophenone	0.05	✓	-	-	-

[1/] Compounds on the tick marks symbol were contained in each recombinate recipes. Compositions of (A) full recombinate (both food grade and non-food grade; 22 compounds), (B) partial recombinate (only food grade; 17 compounds), (C) partial recombinate (food grade natural compounds; 5 compounds), and (D) partial recombinate (the first four highest odour activity value (OAV); 4 compounds) of P50 smoked water.

2.3.5 Data analysis

The mean differences of quantitative and semiquantitative data between P50 and TR apple-wood smoked water samples were carried out with a paired t-test for paired data (IBM SPSS Statistics version 27; New York, USA). The sensory profiling data and panellist performance were analysed using analysis of variance (ANOVA) by SenPAQ (version 6.3, Qi Statistics, UK), which proposes that the variation in panellist by sample interaction and the variation in error adhere to a normal distribution with a constant variance across both panellists and samples (Hasted, 2018). The samples were as a fixed effect and panellists as a random effect. Post hoc multiple pairwise comparisons were conducted using the Tukey-Kramer honestly significant difference (HSD) test to determine which sample means between P50 smoked water and its recombinate samples were significantly different at an alpha level of 0.05.

2.4 Results and Discussion

2.4.1 Selection of the most appropriate aroma extraction method for extracting compounds in smoked water

The suitable separation method was first optimised before identifying and quantifying the odour-active compounds in the smoked water sample. A total of 12 subset methods of the main four extraction methods were compared (see **Table 2.3**), which consisted of solid phase microextraction (1 method), solid phase extraction with varying cartridge types (3 methods) and eluting solvents (3 methods), liquid-liquid extraction with varying solvent types (2 methods) and stir bar sorptive extraction by conventional and sequential methods (3 methods).

Table 2.3 summarised the number of compounds extracted for each of the 12 methods. The two best methods based on providing the highest number of compounds were SPME (method 1; section 2.3.1.1) and SPE (Strata^{TM} -X 33 µm Polymeric Reversed Phase cartridge), which used diethyl ether as eluent (method 6; section 2.3.1.2. *B*). SPME was selected due to its high efficiency in capturing the most volatile compounds, whereas SPE was more effective in extracting the semi-polar aroma compounds. Both methods yielded compounds of approximately the same number accounting for 60 compounds. The SPME method extracted the most phenols, while the SPE with diethyl ether extracted the most guaiacols, syringols, furans, aldehydes, and ketones. On the other hand, the Isolute® NH_2 SPE cartridge (method 3) was identified as less capable of extracting smoked water which only extracted three compounds.

Table 2.3 Approximate number of compounds extracted by 12 methods.

[1/] **Method**	**Compound groups**						**Total compounds**
	Phenols	**Guaiacols**	**Syringols**	**Furans**	**Aldehydes, ketones**	**Others**	
SPME							
1. SPME	20	9	4	8	9	10	60
SPE varied cartridges							

[1/] Method	Compound groups						Total compounds
	Phenols	Guaiacols	Syringols	Furans	Aldehydes, ketones	Others	
2. SPE1-SX	8	6	4	4	2	7	31
3. SPE2-NH_2	0	0	0	1	0	2	3
4. SPE3-ENV	10	6	2	3	1	6	28
SPE varied eluting solvents							
5. SPE1-DCM	10	5	2	4	5	4	30
6. SPE1-DET	18	10	5	10	10	8	61
7. SPE1-MET	12	8	4	3	3	15	45
LLE varied solvents							
8. LLE-DCM	5	6	2	5	4	4	26
9. LLE-DET	4	5	1	4	3	2	19
SBSE varied methods							
10. SBSE-Con	0	3	1	2	0	3	9
11. SBSE-NaCl	4	6	3	1	0	6	20
12. SBSE-Seq	7	5	3	2	1	4	22

[1/] SPME = Solid phase microextraction, SPE = Solid phase extraction (where SX = Strata™-X 33 µm Polymeric Reversed Phase cartridge, NH_2 = Isolute® NH_2 cartridge, ENV = Bond Elut ENV cartridge, DCM = dichloromethane, DET = diethyl ether, MET = methyl acetate), LLE = Liquid-liquid extraction, SBSE = Stir bar sorptive extraction (where Con = Conventional method, NaCl = Conventional method with sodium chloride, Seq = Sequential method).

Table 2.4 Main compounds are found in smoked water extract by at least half of extraction methods.

[1/] Frequency of compounds found in extraction methods	Compounds
11/12	4-Methylguaiacol Syringol
10/12	4-Methylphenol Guaiacol 4-Ethylguaiacol

[1/] Frequency of compounds found in extraction methods	Compounds
9/12	2,3-Dimethyl-2-cyclopenten-1-one
8/12	5-Methylfurfural Phenol 2-Methylphenol Vanillin
7/12	2-Furfural Cyclotene Acetovanillone
6/12	2-Methyl-2-cyclopenten-1-one 2-Acetylfuran Acetosyringone 2-Ethylphenol 2,4-Dimethylphenol 4-Ethylphenol 2,5-Dimethylphenol Syringaldehyde Vanillic acid 4-Allylsyringol

[1/] A total sample of 4 different extraction methods was 12, e.g., 11/12 mean the compound was detected in 11 out of 12 methods.

According to **Table 2.4**, twenty-three tentatively identified compounds were extracted by at least half of the 12 methods. 4-Methylguaiacol and syringol were easily extracted by 11/12 extraction methods. Smoked water was re-analysed in order to identify as many compounds as possible from the two most effective extraction methods, which are shown in **Table 2.5**. Principally, TR smoked water was used to identify the list of compound regions extracted via SPME and SPE methods. Because the TR smoked water provided more concentrated extracts, the TR smoked water chromatograms were used as a reference for identifying the compounds and calculating the LRI. The GC-MS peaks of extracted compounds from each extraction method were only tentatively identified on the basis of a spectral library using ChemStation software (Agilent) by comparing their mass spectra (MS) with the mass spectral library of NIST 2020 on a non-polar ZB-5 column. In this part of the study, the

identification of compounds necessitated two conditions: a matching score of ≥ 700 for mass spectra (match factor or direct match), and an LRI variance of 30 units between the calculated LRI and the values in the database. NIST suggests the following general match factor score ranges: > 900 indicates an excellent match, 800-900 indicates a good match, 700-800 indicates a fair match, and 600 indicates a poor match (JORDI, 2017).

Table 2.5 Comparison of volatile and semi-volatile compounds tentative identified in extracted compounds of smoked water using SPME (section 2.3.1.1) and SPE (Strata™ -X 33 µm Polymeric Reversed Phase cartridge), which used diethyl ether as an eluent (section 2.3.1.2. *B*).

No.	**[2/] Compounds**	**Methods**		**[1/] LRI on ZB-5 (non-polar column)**		
		SPME	**SPE with diethyl ether**	**Exp.**	**Aut. std.**	**NIST**
1	Formic acid	✓	-	<700	515	
2	2-Methylfuran	✓	-	<700		605, 613
3	Acetic acid	✓	-	<700	649	600
4	2-Butenal	✓	-	<700	650	657
5	3-Pentanone	✓	-	<700	690	694
6	Pentanal	✓	-	<700	701	699, 732
7	1-Hydroxy-2-propanone	✓	-	729	655	658, 694
8	Methyl *trans*-2-butenoate	✓	-	757		756
9	Butanoic acid	✓	-	761	833	824, 844
10	Cyclopentanone	✓	-	793	797	
11	3-Furfural	✓	✓	812	814	
12	2-Butenoic acid	✓	-	829		
13	2-Furfural	✓	✓	838	837	829
14	3-Methyl-1-pentanol	-	✓	846		833, 843
15	3-Methylcyclopentanone	✓	-	850	858	
16	Ethylbenzene	-	✓	865	862	
17	2-Pentenoic acid	✓	✓	870		
18	Pentanoic acid	-	✓	876		902, 926
19	3-Methyl-2-butenoic acid	-	✓	881		
20	2-Cyclopentene-1,4-dione	✓	-	891		
21	4-Pentenoic acid	-	✓	896		
22	Cyclohexanone	✓	-	902	896	
23	2-Methyl-2-cyclopenten-1-one	✓	✓	911, 907	912	

No.	[2/] Compounds	Methods		[1/] LRI on ZB-5 (non-polar column)		
		SPME	SPE with diethyl ether	Exp.	Aut. std.	NIST
24	2-Acetylfuran	✓	✓	914, 912	916	893
25	Butyrolactone	✓	-	931	921	
26	3-Methyl-pentanoic acid	✓	✓	929, 937		941
27	2,5-Hexanedione	✓	-	939		920
28	2-Cyclohexen-1-one	✓	✓	941, 936		885, 927
29	2-Ethyl-cyclopentanone	✓	-	945		
30	3,4-Dimethyl-2-cyclopenten-1-one	✓	✓	950, 948		
31	5-Methyl-2(5H)-furanone	✓	✓	954, 950		946
32	1-(Acetyloxy)-2-butanone	✓	✓	960, 964	964	
33	2,3-Dihydro-2-methyl-5-ethylfuran	✓	-	964		
34	5-Methylfurfural	✓	✓	969, 965		946, 978
35	Benzaldehyde	✓	-	971		933, 978
36	Methyl-2-furoate	✓	✓	978, 977		978, 983
37	Phenol	✓	✓	981, 979	983	980
38	Methyl 4-Oxo-pentanoate	-	✓	987		981
39	Benzonitrile	✓	✓	994, 988	994	
40	2-Furanone, 2,5-dihydro-3,5-dimethyl	✓	✓	1010, 1002		993
41	1-(2-Furanyl)-1-propanone	✓	✓	1014, 1011	1016	
42	4-Methyl-4-hepten-3-ol	-	✓	1025		
43	2,3,4-Trimethyl-2-cyclopenten-1-one	✓	-	1031		
44	Cyclotene	✓	✓	1038, 1029	1033	
45	2-Cyclohexene-1,4-dione	-	✓	1032		1024, 1032
46	2-Acetyl-5-methylfuran	✓	✓	1042, 1039	1045	
47	2,3-Dimethyl-2-cyclopenten-1-one	✓	✓	1048, 1043		1043, 1052
48	4-(Pentyloxy)-benzaldehyde	-	✓	1050		
49	2-Methylphenol	✓	✓	1055	1055	
50	2,4-Dimethyl-1,3-cyclopentanedione	✓	✓	1065, 1058		
51	5-Ethylfurfural	✓	-	1062		
52	3,4,5-Trimethyl-2-cyclopenten-1-one	✓	✓	1071, 1067		
53	4-Methylphenol	✓	✓	1075, 1074	1074	1075
54	Acetophenone	✓	-	1078	1077	
55	3-Ethyl-2-cyclopenten-1-one	✓	✓	1080		
56	2-Methylbenzaldehyde	✓	✓	1081	1082	

No.	[2/] Compounds	Methods		[1/] LRI on ZB-5 (non-polar column)		
		SPME	SPE with diethyl ether	Exp.	Aut. std.	NIST
57	Guaiacol	✓	✓	1099, 1094	1098	1089
58	4-Ethyl-2-hydroxy-2-cyclopenten-1-one	✓	✓	1103, 1098		
59	Methyl benzoate	✓	✓	1105, 1100	1104	1103
60	4,4-Dimethyl-2-cyclohexen-1-one	✓	✓	1112, 1107		
61	2,6-Dimethylphenol	✓	✓	1115, 1111	1114	
62	2,3-Dimethyl-4-hydroxy-2-butenoic lactone	-	✓	1116		
63	Maltol	✓	✓	1126, 1118	1122	
64	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	✓	✓	1130, 1123	1127	
65	2-Ethylphenol	✓	✓	1138, 1138	1138	
66	2,5-Dimethylphenol	✓	✓	1151, 1151	1150	
67	Benzoic acid	-	✓	1157	1167	
68	4-Ethylphenol	✓	✓	1168, 1167	1167	1169
69	3,5-Dimethylphenol	✓	✓	1169, 1169		1169, 1196
70	2-Hydroxyphenyl methyl ketone	✓	✓	1179		1167
71	2,3-Dimethylphenol	✓	✓	1182, 1180	1185	
72	1-(3-Methylphenyl)-ethanone	✓	-	1186		1176, 1182
73	2-Methoxy-6-methylphenol	✓	✓	1189, 1186		
74	Resorcinol	-	✓	1195		
75	3,4-Dimethylphenol	✓	-	1197		1190, 1221
76	4-Methylguaiacol	✓	✓	1204, 1199	1202	
77	2,4,6-Trimethylphenol	✓	✓	1213, 1214	1213	
78	2-Propylphenol	✓	✓	1223, 1223		1224, 1244
79	2-Ethyl-4-methylphenol	✓	✓	1230, 1229		
80	2-Ethyl-5-methylphenol	✓	✓	1234, 1241		
81	3,4-Dimethoxytoluene	✓	✓	1241, 1241		1230
82	3-Ethyl-5-methylphenol	✓	✓	1261, 1262		
83	4-(1-Methylethyl) phenol	✓	✓	1266, 1264		1221, 1247
84	3,5-Dimethoxytoluene	✓	-	1274	1275	
85	2,3,6-Trimethylphenol	✓	✓	1278, 1277		
86	4-Ethylguaiacol	✓	✓	1290, 1288	1290	1287
87	2,3-Dihydro-1H-inden-1-one	✓	✓	1303, 1295		1307, 1320
88	5-Acetoxymethyl-2-furaldehyde	✓	✓	1310, 1310		1304
89	2,4,5-Trimethylphenol	✓	✓	1315, 1309		
90	1,2,3-Trimethoxybenzene	✓	✓	1319, 1316	1318	

No.	[2/] Compounds	Methods		[1/] LRI on ZB-5 (non-polar column)		
		SPME	SPE with diethyl ether	Exp.	Aut. std.	NIST
91	4-Ethyl-1,2-dimethoxybenzene	✓	-	1326		
92	4-Vinylguaiacol	✓	-	1329, 1322	1328	1323
93	2,3-Dihydro-1H-inden-5-ol	✓	-	1331		
94	2,3-Dihydroxy-benzoic acid	-	✓	1339		
95	1-Methyl-2,3-dihydro-1H-inden-1-one	✓	-	1340		
96	4-Methoxy-3-(methoxymethyl) phenol	-	✓	1349		
97	Syringol	✓	✓	1360, 1359	1357	
98	Eugenol	✓	✓	1369, 1367	1371	1364
99	Dihydroeugenol	✓	✓	1379, 1377		1352, 1382
100	Unknown	✓	✓	1396, 1392		
101	1,2,3-trimethoxy-5-methylbenzene	✓	✓	1405, 1407		1400, 1435
102	Dodecanal	✓	-	1409	1414	
103	Vanillin	✓	✓	1414, 1411	1415	1410
104	Isoeugenol	✓	✓	1463	1463	
105	3,5-Dimethoxyphenol	-	✓	1480		1472
106	Acetosyringone	✓	-	1485		
107	*trans*-Isoeugenol	✓	-	1491		1447, 1473
108	Acetovanillone	✓	✓	1499, 1500	1499	
109	Butylated hydroxytoluene	-	✓	1525		1497, 1518
110	4-Hydroxy-3-methoxybenzoic acid methyl ester	✓	✓	1531, 1530		1496, 1527
111	5-tert-Butylpyrogallol	✓	✓	1536		
112	1-(4-Hydroxy-3-methoxyphenyl)-2-propanone	✓	✓	1546, 1545		1532, 1541
113	Ethyl ester dodecanoic acid	✓	-	1589		1581, 1597
114	4-Allylsyringol	✓	✓	1613		1602, 1615
115	Syringaldehyde	✓	✓	1677, 1675		1643, 1670
116	Unknown	✓	-	1752		

[1/] Linear retention indices (LRI) on the ZB-5 column from experimental data (Exp.) were calculated from the retention time of the n-alkanes series. LRI of authentic aroma standard (Aut. std.) referred from some authentic aromas in section 2.4.3 (**Table 2.7**) and LRI from an in-house database of the flavour research group at the University of Reading. LRI of NIST was obtained from the NIST Chemistry WebBook.

[2/] All compounds were indicated as tentative compounds by comparing their mass spectra (MS) with the mass spectral library of NIST 2020.

Approximately 116 aroma compounds in smoked water were extracted by these two methods, where the SPME and SPE methods yielded compounds accounting for 100 and 82, respectively. The extracted compounds of smoked water consist of acids, aldehydes, ketones, diketones, furan derivatives, phenols derivatives, guaiacols derivatives, syringols derivatives, and other compounds. The SPME method specifically extracted some acids, aldehydes and ketones compounds with low molecular weight, which contain carbon atoms lower than 6 (**Table 2.5**, e.g., compound numbers 1-10). Although the specified range for the difference between the calculated LRI of the identified compound and the database was set at 30 units, certain acid compounds like formic acid, acetic acid, and butanoic acid (**Table 2.5**; compounds 1, 3, and 9, respectively) exhibited LRI values that did not comply with the established criteria. Furthermore, their LRIs differed by more than 30 units compared to other sources' data.

Despite SPME's effectiveness in extracting highly volatile compounds, which include potent aroma compounds responsible for the flavour of smoked water, it might not be adequate to use it to extract less volatile (and more polar) compounds present in smoked water that may have the potential to be potent aroma compounds. The selected reversed-phase SPE cartridge using diethyl ether as eluent was selective in separating the non-polar compounds, other aromatic compounds or even low/medium volatile compounds, which might also be potent aroma compounds of smoked water. For this reason, in the following step of determining the potency aroma in smoked water by the AEDA, both SPME and SPE methods were performed to extract the greatest number of potent aroma compounds as possible.

2.4.2 The identification of aroma-active compounds by Gas chromatography-Mass spectrometry (GC-MS) and GC-Olfactometry (GC-O) using Aroma Extraction Dilution Analysis (AEDA)

Two extraction methods of SPME and SPE (using diethyl ether as an eluent) were selected from section 2.4.1 to extract the aroma compounds in smoked water samples for identifying the potent aroma compounds by AEDA. For the SPME method, the extracted smoked water samples were sniffed from GC-O in splitless and split modes in a three-fold split ratio series corresponding to 1:3, 1:9, 1:27, 1:81, 1:243 and 1:620, respectively. The split ratio of 1 to 620 was the maximum ratio that this GC-O machine could perform. Therefore, analysed with the same split ratio of SPME, the extracts by SPE extraction were prepared at dilutions 0,

3, 9, 27, 81, 243, and 620 times and then injected into GC-O for sniffing. The AEDA (expressed as flavour dilution (FD) factor (Grosch, 1993)) was used as a criterion for rejecting the least significant compounds. Any compounds that are still detectable by GC-O at high dilution levels (high FD factor number) imply that they are potent and are likely to contribute to the overall flavour of the smoked water. However, SPME GC split mode may not perform well for AEDA because the compounds attached in fibre were diluted instead of all compounds in the real extracts. So, the FD factor of each compound from SPME depends on how well each compound is partitioned onto the fibre at each time of SPME extraction.

For each of the potent aroma compounds that were detected at GC-O through two extraction methods, the LRI value was calculated for each retention time of the perceived odour in comparison to the retention time data of the alkane series (C6-C26) acquired through the injection of alkane standards under the same experimental conditions. To identify the compounds, their LRIs were compared to those of authentic odourant standards (section 2.4.3; **Table 2.7**), aroma standards obtained from an in-house database of the flavour research group at the University of Reading, and LRIs from other databases obtained using a comparable stationary phase of column (non-polar). The criterion for acceptance was a variation of no more than 30 units between the calculated LRI and the LRI of authentic standards or the database. Those potent aroma compounds that lack supporting LRIs in the database will be categorised as tentative potent aroma compounds.

This experiment demonstrated that 97 aroma regions were detected in the SPME extract, compared to 79 in the SPE extract (data not shown). **Table 2.6** shows a total of 67 potent aromas, with FD factor ≥ 3, perceived at the sniffing port of the GC-O by three assessors. Aroma compounds with FD factor < 3 were classed as insignificant aromas, not shown in **Table 2.6**. If the compounds were detected by all three assessors but differed in the FD values, the FD was chosen based on the highest value among the three assessors. About 13 compounds were detected in both methods, 41 were perceived only in SPME extracts, and 13 were detected only in SPE extracts. The top four aroma compounds perceived in both extraction methods with high FD factors (27-243) were 4-ethylguaiacol, 4-ethylphenol, 4-methylphenol and guaiacol. The other two compounds of phenols derivatives with the highest FD factor at 620 in SPME extract were 2,6-dimethylphenol and 3,4-dimethylphenol. Phenols and guaiacols derivatives contributed to the flavour of smoked water as they had high FD factors. The

phenolic compounds found in smoked water come from the thermal degradation of wood through the pyrolysis of lignins between 300-500 °C (Šimko, 2005).

The most potent aroma compounds (FD factor = 243) in the SPE extract were guaiacol (LRI ZB-5 = 1098; cool herb, amine smoke, medicinal aroma) and 3-ethyl-1,2-cyclopentanedione (compound no. 12; LRI ZB-5 = 1099; spicy sweet, tincture aroma) which was detected just after the guaiacol region. Five out of thirteen aroma-active compounds detected by the SPE method alone remained unidentified (**Table 2.6**; compounds 25, 37, 39, 51, and 67) as they were present at concentrations below the instrumental detection limit. Furthermore, some unidentified compound peaks may be obscured by the large peak chromatogram compound adjacent to it. In addition, the final extract compounds by the SPE method may be too diluted because the extract was not concentrated after extraction was complete and then subjected to AEDA. This effect was evident in the FD factor of compounds detected by both the SPE and SPME methods, with the SPE method consistently obtaining lower FD factors than the SPME method. Intriguingly, eugenol and isoeugenol had a high FD factor in the SPE extract but not the SPME extract. This is due to the fact that both eugenol and isoeugenol were nonpolar compounds. These compounds could be retained by the stationary phase of the reversed-phase SPE cartridge and eluted by the organic solvent diethyl ether. From this point of view, SPE (reversed-phase cartridge) with diethyl ether was a more selective method than SPME for extracting eugenol and isoeugenol.

The flavour of smoked water is not confined to a single characteristic but comprises various odour attributes, such as smoky, woody, medicinal, herb, spicy, sweet, burnt, pungent, etc. Phenols, guaiacols and syringols groups are responsible for harsh aromas such as smoky, woody, medicinal, etc., and displayed higher FD factors than furans, aldehydes, ketones, cyclopentenes groups, which showed softer characteristics such as sweet, floral, curry and spice notes. Phenols are typically associated with medicinal, burnt, woody, and green/grass notes. Guaiacol and 4-ethyguaiacol, for example, were classified as smoke, herb, and fragrant notes, whereas eugenol and isoeugenol were described as clove-like spicy notes, which provided the similar result with other studies that investigating/ identifying the aroma compounds of liquid smoke and some smoked food products (Pu et al., 2020; Varlet et al., 2006; Wang et al., 2018).

Table 2.6 Aroma compounds (FD ≥ 3) identified by GC-O in SPME and SPE extracts of TR smoked water arranged from high FD factor to low FD factor.

No.	[1/] Compound	[2/] Odour description given by assessors	Fraction	[3/] LRI in ZB-5 (non-polar)			[4/] FD factor	
				Exp.	Std.	Literature	SPME	SPE
1	* 2,6-Dimethylphenol	Medicinal, smoke, grass, burnt	SPME	1116	1114		620	
2	§ 3,4-Dimethylphenol	Amine smoke, cool herb	SPME	1197		1190-1221	620	
3	* 4-Ethylguaiacol	Fragrant smoke, cool herb, spicy	SPME, SPE	1290	1290		620	81 (2)
4	* 4-Ethylphenol	Medicinal, paints, poo, stink bug	SPME, SPE	1169	1167		620	27 (2)
5	* 4-Methylphenol	Medicinal, paints, poo	SPME, SPE	1072	1074		243	27
6	* Guaiacol	Cool herb, amine smoke, medicinal	SPME, SPE	1098	1098		243	243
7	* 4-Methylguaiacol	Creamy vanilla, sweet, fruity	SPME	1203	1202		243	
8	* 2,5-Dimethylphenol	Green, woody, grassy, soil	SPME, SPE	1146	1150		243	3 (1)
9	§ 3,5-Dimethylphenol	Green, smoke, honey, cool herb	SPME	1173		1169-1196	243	
10	* 2-Ethylphenol	Sweet smoke, fishy	SPME	1139	1138		243	
11	* Methyl benzoate	Medicinal, different smoke	SPME	1102	1104		243 (2)	
12	* 3-Ethyl-1,2-cyclopentanedione	Spicy, sweet	SPE	1099	1101			243 (2)
13	* 2-Methylphenol	Medicinal	SPME	1053	1055		81	
14	! 4-Methoxyphenol	Medicinal, cupboard	SPME	1058			81	
15	* 2,3-Dimethylphenol	Medicinal smoke, green, grass, soil	SPME, SPE	1190	1185		81	27 (2)
16	! Unknown	Medicinal, rubber, burnt vegetables	SPME	1250			81 (2)	
17	§ 3,4-Dimethoxytoluene	Musty, burnt smoke, hay	SPME	1243		1230	81	
18	! Unknown	House smoke, fishy	SPME	1343			81	
19	§ 2-Propylphenol	Soil, green, smoky	SPME	1223		1224-1244	81 (2)	
20	* Syringol	Cool spice, herb, smoke, petrol	SPME, SPE	1354	1357		81 (2)	9 (1)

No.	[1/] Compound	[2/] Odour description given by assessors	Fraction	[3/] LRI in ZB-5 (non-polar)			[4/] FD factor	
				Exp.	Std.	Literature	SPME	SPE
21	* Acetophenone	Paints, poo	SPME	1075	1077		81 (1)	
22	§ Dihydroeugenol	Cool herb	SPME, SPE	1377		1352-1382	81 (1)	27
23	§ 6-Methylguaiacol	Amine smoke	SPME, SPE	1190		1184	81 (2)	3 (2)
24	* Vanillin	Cigarette smoke, smoke	SPME, SPE	1417	1415		81 (1)	9 (1)
25	! Unknown	Medicinal, metallic	SPE	1091				81 (2)
26	* Isoeugenol	Clove, smoky sweet	SPE	1470	1463			81 (1)
27	§ Benzaldehyde	Floral, biscuit, sweet	SPME	990		933-978	27	
28	* 3-Ethyl-2-hydroxy- 2-cyclopenten-1-one	Medicinal, sweet smoke, cupboard	SPME, SPE	1129	1127		27	3 (2)
29	§ 5-Methylfurfural	Green, grassy, earthy	SPME	960		946-978	27	
30	! Unknown	Grass, fragrant, plant	SPME	1162			27	
31	! 1-Ethyl-4-methoxybenzene	Medicinal, soil, rubber burnt, smoke chimney	SPME	1246			27 (2)	
32	§ 1,2-Dimethoxybenzene	Musty, medicinal	SPME	1150		1142-1151	27 (2)	
33	! Unknown	Herb, clove-like, spicy	SPME	1482			27	
34	§ 2,3,5-Trimethylphenol	Rubber, paints	SPME	1265		1275-1296	27	
35	§ 3-Ethylphenol	Sweet/ink	SPE	1176		1153-1195		27 (2)
36	! 2-Ethyl-6-methylphenol	Medicinal, herb, spicy sweet	SPE	1195				27 (2)
37	! Unknown	Herb, fragrant	SPE	1383				27
38	* Cyclopentanone	Green, sweaty	SPE	791	797			27
39	! Unknown	Herbs, fragrant	SPE	1391		1453		27 (1)
40	* 2-Methylbenzaldehyde	Medicinal, herb, sotolon, curry, fenugreek	SPME, SPE	1081	1082		9	27 (2)
41	* 2-Acetylfuran	Sauce, salty,	SPME	917	916		9	
42	§ 2,3,6-Trimethylphenol	Ink, watery stagnant	SPME	1278		1262-1275	9	

No.	[1/] Compound	[2/] Odour description given by assessors	Fraction	[3/] LRI in ZB-5 (non-polar)			[4/] FD factor	
				Exp.	Std.	Literature	SPME	SPE
43	! Unknown	Cheese, sour	SPME	515			9 (2)	
44	! 1-Hydroxy-2-butanone	Green	SPME	775			9 (1)	
45	! 2-Ethyl-4-methylphenol	Green, grass, burnt smoke	SPME	1229			9 (2)	
46	§ Acetovanillone	Clove	SPME	1494	1499	1480-1499	9 (1)	
47	§ 4-Hydroxy-3-methoxybenzoic acid methyl ester	Clove-like, spicy	SPME	1524		1496-1527	9 (1)	
48	* Eugenol	Herb, guaiacol	SPE	1372	1371			9 (2)
49	* β-Damascenone	Jammy	SPE	1402	1390			9 (2)
50	§ p-Cumenol	Sweet, heavy musky fragrant	SPE	1264		1221-1247		9 (2)
51	! Unknown	Smoke, fragrant	SPE	1643				9 (2)
52	§ 3-Methylbenzaldehyde	Green, grass, marzipan	SPME	1095		1059-1086	3	
53	! Unknown	Sweet, clove	SPME	1153			3	
54	! Unknown	Green, smoke	SPME	1112			3 (2)	
55	! 2-Hydroxy-3,4-dimethyl-2-cyclopenten-1-one	Cabinet, musty, mouldy	SPME	1059			3 (1)	
56	§ Butanoic acid	Cheese, sauce, poo	SPME	763		824-844	3 (2)	
57	! Unknown	Cool spice, grass, green	SPME	1017			3 (1)	
58	* Phenol OR 1-Octene-3-ol	Medicinal, mushroom	SPME	979	971/983		3	
59	* 3-Furfural	Sauce	SPME	811	814		3 (1)	
60	§ 3-Methylbutanoic acid	Poo, cheese	SPME	823		833-888	3 (1)	
61	§ 2-Butanone	Vinegar	SPME	589		549-609	3 (1)	
62	! 2-Methylfuran	Sauce	SPME	749		633	3 (1)	
63	* Cyclotene	Curry, sweet, spicy	SPME, SPE	1032	1033		3 (1)	27
64	* 3,5-Dimethoxytoluene	Marzipan-like	SPME	1272	1275		3 (1)	

No.	[1/] Compound	[2/] Odour description given by assessors	Fraction	[3/] LRI in ZB-5 (non-polar)			[4/] FD factor	
				Exp.	Std.	Literature	SPME	SPE
65	[!] Unknown	Fried, fatty	SPME	1222			3 (1)	
66	[*] 4-Vinylguaiacol	Smoke	SPME	1327	1328		3 (1)	
67	[!] Unknown	Cream, green, fruity	SPE	1007				3 (2)

[1/] For (*) compound, authentic standards were used for identification. (§) compound, the identification was from well-matched spectra and literature LRI. (!) compound, only LRI from experimental data was available for the identification of a compound, or if the LRI matched with literature was poor, then it must be considered as a tentative compound.

[2/] Odour descriptors at the sniffing port by all assessors who perceived that odour.

[3/] Linear retention index in ZB-5 column; Exp. was from experiment data; Std. was from authentic aroma standard. Literature linear retention indices with non-polar column (DB-5) obtained from open access databases, including NIST Chemistry WebBook, in-house database of the flavour research group at the University of Reading, www.odour.org.uk (*LRI & Odour Database*), Schranz et al. (2017), and Giri et al. (2017).

[4/] Flavour dilution factor (FD) on capillary column ZB-5 by SPME was solid phase microextraction method, and SPE was solid phase extraction using diethyl ether as eluent. If the compounds were detected by all three assessors but differed in the FD values, the FD was chosen based on the highest value among the three assessors. In case compounds were not detected by all three assessors, the number of those who identified the compound was indicated in parentheses.

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2.4.3 Quantification and semi-quantification of volatile compounds and calculation of the odour activity values (OAVs) of potent aroma compounds

Once the volatile compounds in smoked water have been identified by comparing their calculated LRI with the LRI of authentic aroma compounds, an in-house database, or the open access database, and any unknown compounds have been identified by finding similarities, the next step is to determine the intensity values by quantification. Additionally, the ZB-Wax and DB-Wax UI polar columns were employed to separate the volatile compounds in smoked water. This was done to gather additional data on compound LRIs, which aided in confirming the identification. Using several LRIs from different columns allowed for cross-referencing with existing literature. Seventy-seven out of one hundred volatile compounds (total compounds extracted by SPME in **Table 2.5**) of TR and P50 smoked water samples were quantified/semi-quantified using the SPME extraction method, which was presented in **Table 2.7**. The integrated peak chromatogram of 77 out of 100 compounds was counted from the compound with a peak area of at least 1% of the largest peak area compound (cut-off point selection) of smoked water extracts. If the peak area of any compound is more than 1% of the largest peak area, then the signal will be shown as a peak and can undergo peak area integration. As a result of cut-off point selection, 22 out of 100 compounds were lost due to having a peak area of less than 1% of the highest peak area compound. These 77 compounds included 3 acids, 9 aldehydes, ketones and diketones all based on a cyclopentene or pyran ring, 9 furans, 11 guaiacols, 11 syringols, 21 other phenols, 3 indene derivatives, and 10 other unclassified compounds. All 77 volatile compounds in smoked water samples and 34 authentic aroma compounds were calculated for their LRI against the retention time of the alkane series (C6-C26).

Thirty-four aroma compounds confirmed their identity by comparing their LRIs with the LRIs of 34 authentic aroma compounds. For other compounds with no authentic aroma compounds, their mass spectra were compared with the mass spectra library of NIST 2020, and their LRIs were compared with various sources such as in-house databases, open online databases, etc. The volatile compounds with their LRIs, which were only available for the identification of a compound, must be categorised as tentative compounds. The criterion of acceptance was the matching score (match factor) of ≥ 700 for mass spectra and an LRI variance of 30 units between the calculated LRI and the values in the database. Nevertheless,

there were cases where the differences in LRI between experimental and literature values did not meet the criteria established. This was found with 2-ethylfuran, 3-ethyl-2-cyclopenten-1-one, and 3,5-dimethylphenol (as shown in **Table 2.7**, compounds 10, 26, and 35, respectively). Despite their poor matching scores, the mass spectra of these compounds were found to be more similar to the known spectrum in the library. As a result, they were categorised as tentative compounds. Occasionally, spectra that are compatible may exhibit low matching scores, whereas spectra that are not well-matched may exhibit high scores (JORDI, 2017).

A match factor, also known as a direct match, involves comparing the peaks of the unknown's mass spectrum to those of the peaks in the library's spectra. Hence, this number serves as a measure of the similarity between the spectrum of the unknown and the spectrum of the known in the library (JORDI, 2017). Accurate mass matching is the predominant compound identification technique employed in GC-MS studies. However, relying solely on mass information is insufficient to establish an exact identification with reliability. The combination of retention time matching with accurate mass matching leads to increased certainty in the identification of compounds. The utilisation of broad databases, such as the NIST, for matching purposes, can only be limited to preliminary identification due to the fact that numerous compounds exhibit extremely similar mass spectra and retention times that differ depending on the column and oven temperature (Wehrens et al., 2014).

Thirty-four out of seventy-seven identified compounds were quantified using available authentic odorant standard calibrations, whereas the others were semi-quantified. These 77 volatile compounds were found in both TR and P50 apple-wood smoke but varied in different proportions due to the PST used in the manufacturing (filtering). Comparing the volatile compounds between TR and P50 smoked water, 9 out of 77 compounds were not significantly different between TR and P50 smoked water. There were 1-hydroxy-2-butanone, cyclopentanone, 2-butenoic acid, 2-ethylfuran, 4-cyclopentene-1,3-dione, 2-methyl-2-cyclopenten-1-one, 2-acetylfuran, 4-hydroxy-3,5-dimethoxybenzaldehyde, and 2-hydroxy-4,6-dimethoxyacetophenone. All phenolic and guaiacol compounds were significantly different between the two smoked water samples. However, the amount of almost all compounds in P50 smoked water tended to be lower than in TR, which may indicate that the PST, which was used to filter out carcinogen compounds and tar from smoke also reduced the number of other volatile compounds in smoked water, primarily phenols and guaiacols groups, whereas furans group was not retained by the zeolite filter which may have an effect on the

overall flavour profile of filtered smoked water compared to unfiltered smoked water. Molecular sieving is the primary factor to consider when it comes to the adsorption of chemical types by zeolites. Molecules that have a diameter larger than the size of a zeolite pore are efficiently filtered out. The sieve effect of zeolites can be employed to achieve precise separations of compounds based on their size and shape. Moreover, zeolite highly adsorbed non-polar molecules because of the significant polarising effect of the electrostatic field present within a zeolite cavity, thus, a compound separation can be achieved by zeolites (Abbey, 1996). However, it was not possible to conclude that the PST had an effect on the reduction of compound levels due to the fact that both smoked water samples may not have been produced from the same batch of woodchips and produced at different times (about one month apart). In addition, the smoked water samples were stored for over a year before the GC-MS analysis of aroma compounds for this **Chapter 2** experiment. Consequently, some highly volatile compounds may be lost during storage time. The effect of PST on the aroma compounds contents retention will be conducted in **Chapter 3 (Comparison of volatiles generated in smoked water using PureSmoke Technology (PST) with those generated using a traditional smoking process)**, where TR and PST smoked water samples produced from the same batch of woodchip and same production date.

In this study, 19 of the 77 aroma compounds identified had concentrations greater than 1 mg/L: syringol (125 mg/L), acetic acid (69 mg/L), guaiacol (35 mg/L), 4-methylguaiacol (30 mg/L), 2-furfural (24 mg/L), and vanillin (11 mg/L) were the six compounds with concentrations greater than 10 mg/L. As concentrations are not a direct measure of the potency of aroma compounds, this was standardised by calculating odour activity values (OAVs), which is the ratio between concentrations and odour detection thresholds. The compounds with OAVs greater than 1 were accepted as individual contributors to the smoked water aroma. According to **Table 2.8**, twenty-two potent aroma compounds were chosen and calculated for the OAVs based on the high FD factors and quantitative results. Guaiacol (14,000), 4-methylguaiacol (1,000), syringol (850), 2-furfural (400), 4-methylphenol (300), and 4-ethylguaiacol (225) had the highest OAVs, in descending order. Guaiacol, which matched the FD factor and OAV, was the most potent compound that contributed to the smoke's flavour. Most of the phenolic compounds exhibited higher OAVs. The substances with high concentrations also had high OAV. Nevertheless, the OAV of acetic acid did not align with its concentration. This is an example of a case in which compounds are often detected by GC-O but not odour-active.

In the case of ranking odour-active compounds according to their FD factor, it would be reasonable to assume that compounds with a high FD factor would also have a high OAV. However, there was some opposite result in this experiment for the four compounds of 4-ethylphenol, 2,6-dimethylphenol, 2,5-dimethylphenol and 2-ethylphenol (**Table 2.8**; compound numbers 11-14) that had FD factors (243 and 620) similar to guaiacol, but their OAVs were on the bottom half of OAV ranking list which was not that high compare to the OAV of guaiacol. Interestingly, even though 2-furfural was not recognised at a high FD factor (only perceived in the non-diluted extract) in the AEDA experiment, its OAV was so high, indicating that 2-furfural was a powerful aroma component in smoked water. From this point, it could be suggested that FD factors provide an approximation of the potent aroma components contained in the samples (Piornos et al., 2020). However, despite this, the FD factors could go in the same or opposite direction as the OAVs. Twenty-two substances with OAVs greater than 1 were identified as potent aroma compounds in smoked water (**Table 2.8**). Although, the last three compounds in the OAV ranking (2,3-dimethylphenol, phenol, and acetic acid) had an OAV of less than 1, particularly in P50 smoked water, suggesting that these three compounds are less likely to be significant aroma compounds in smoked water. In the TR smoked water, however, the OAV of 2,3-dimethylphenol and phenol was greater than 1, indicating that these two compounds also exhibited a significant aroma.

As mentioned previously, the P50 smoked water was stored for more than a year prior to the analysis of the aroma compounds by GC-MS and GC-O as we first began to identify its properties by the sensory experiments of **Chapter 4**, so it was possible that the concentration of some compounds could have decreased over time, resulting in an incorrect final value of OAVs. When determining which components were a potent aroma in smoked water based on their OAV values, we instead considered the OAV in TR smoked water because most potent aroma compounds in smoked water had higher concentrations in TR smoked water which resulted in the calculation of the OAV, where higher concentration provided higher in OAV. In addition, even acetic acid had an OAV below 1 in TR and P50 smoked water. However, acetic acid was included in the recombinate since it was the most abundant acid present in smoked water, which may have contributed to the overall flavour of the recombinate model. Hence, these three compounds, 2,3-dimethylphenol, phenol, and acetic acid, were included in the subsequent section of 2.4.4 validation and confirmation of key odorants by recombination modelling.

Table 2.7 Identification and quantitation of aroma-active compounds in the smoked water samples extracted by SPME.

Peak No.	[1/] Compounds identified in smoked water	[2/] LRI				[3/] Mw	[4/] Main ion	[5/] Factor	[6/] R^2	[7/] Concentrations (mg/L)		p-values
		Exp. ZB-5	Std. ZB-5	Exp. ZB-Wax	Std. DB-Wax UI/ NIST					TR	P50	
1	* Acetic acid	701	-	1473	1454	60	43	0.003	0.9130	69.4 ± 10.5	45.2 ± 6.70	*0.028*
2	! 2-Butenal	<700	-	1061	-	70	41	3.98	-	0.049 ± 0.006	0.080 ± 0.004	*0.001*
3	* 2-Methylfuran	<700	-	nd	832-888	82	53	0.008	0.9934	2.26 ± 0.080	3.90 ± 0.291	*0.001*
4	§ Butanoic acid	767	-	1648	1604-1647	88	60	5.10	-	0.009 ± 0.001	0.002 ± 0.003	*0.025*
5	§ 1-Hydroxy-2-butanone	810	-	1409	1391-1395	88	57	2.80	-	0.007 ± 0.003	0.005 ± 0.001	0.377
6	* Cyclopentanone	798	797	1209	1198	84	55	0.010	0.8908	2.98 ± 1.47	2.80 ± 0.466	0.851
7	! 2-Butenoic acid	835	-	nd	1773	86	86	9.86	-	0.006 ± 0.0001	0.004 ± 0.0014	0.106
8	* 3-Furfural	816	814	1451	1434	96	95	0.164	0.9204	0.610 ± 0.064	0.801 ± 0.094	*0.044*
9	* 2-Furfural	838, 842	837	1496	1470	96	96	0.229	0.9898	15.8 ± 1.16	24.2 ± 2.23	*0.004*
10	! 2-Ethylfuran	885	-	1730	944-975	96	81	6.57	-	0.018 ± 0.007	0.018 ± 0.002	0.966
11	! 4-Cyclopentene-1,3-dione	896	-	nd	-	96	96	4.76	-	0.029 ± 0.001	0.032 ± 0.005	0.305
12	* 2-Methyl-2-cyclopenten-1-one	915	912	1401	1386	96	67	0.144	0.9893	1.52 ± 0.470	1.32 ± 0.135	0.512
13	* 2-Acetylfuran	918	916	1537	1515	110	95	0.187	0.9908	2.08 ± 0.208	1.77 ± 0.132	0.092
14	§ 5-Methylfurfural	973	-	1609	1522-1604	110	110	4.91	-	1.10 ± 0.102	1.43 ± 0.090	*0.014*
15	* Phenol	987	983	2043	2019	94	94	0.067	0.9853	5.61 ± 0.851	3.79 ± 0.324	*0.026*
16	! Benzonitrile	998	-	1650	-	103	103	4.49	-	0.177 ± 0.007	0.118 ± 0.007	*0.001*
17	! 1-(2-Furanyl)-1-propanone	1018	-	nd	1558-1571	124	95	11.8	-	0.369 ± 0.044	0.221 ± 0.013	*0.005*

Peak No.	[1/] Compounds identified in smoked water	[2/] LRI				[3/] Mw	[4/] Main ion	[5/] Factor	[6/] R^2	[7/] Concentrations (mg/L)		p-values
		Exp. ZB-5	Std. ZB-5	Exp. ZB-Wax	Std. DB-Wax UI/ NIST					TR	P50	
18	* Cyclotene	1041	1033	1871	1827-1860	112	112	0.005	0.9944	5.36 ± 0.604	2.58 ± 0.141	*0.001*
19	* 2-Acetyl-5-methylfuran	1046	1045	1653	1629	124	109	0.191	0.9945	0.394 ± 0.038	0.214 ± 0.021	*0.002*
20	! 2,3-Dimethyl-2-cyclopenten-1-one	1052	-	nd	1523-1573	110	67	3.92	-	0.258 ± 0.038	0.129 ± 0.006	*0.005*
21	* 2-Methylphenol	1059	1055	2036	2014	108	108	0.086	0.9998	5.22 ± 0.555	2.20 ± 0.095	*0.001*
22	§ 2,3,4-Trimethyl-2-cyclopenten-1-one	1073	-	1531	1539	124	109	5.26	-	0.205 ± 0.033	0.133 ± 0.010	*0.023*
23	* 4-Methylphenol	1079	1074	2120	2097	108	107	0.173	0.9950	3.03 ± 0.261	1.24 ± 0.058	*0.000*
24	* Acetophenone	1080	1077	1694	1670	120	105	0.474	0.9977	0.211 ± 0.008	0.074 ± 0.004	*<0.0001*
25	* 2-Methyl benzaldehyde	1083	1082	nd	1637	120	91	0.998	0.9925	0.029 ± 0.0008	0.005 ± 0.0003	*<0.0001*
26	! 3-Ethyl-2-cyclopenten-1-one	1090	-	1675	1611	110	110	7.63	-	0.066 ± 0.006	0.030 ± 0.002	*0.001*
27	* Guaiacol	1102	1098	1907	1879	124	109	0.052	0.9999	35.0 ± 2.37	17.1 ± 0.746	*0.000*
28	* Methyl benzoate	1107	1104	1660	1638	136	105	0.188	0.9885	0.311 ± 0.011	0.011 ± 0.001	*<0.0001*
29	* 2,6-Dimethylphenol	1117	1114	1944	1923	122	122	0.584	0.9944	0.324 ± 0.021	0.104 ± 0.006	*<0.0001*
30	* Maltol	1127	1122	2022	1954-2004	126	126	0.002	0.9455	6.53 ± 0.559	4.22 ± 0.545	*0.007*
31	* 3-Ethyl-2-hydroxy-2-cyclopenten-1-one	1131	1127	1935	1918	126	126	0.009	0.9991	1.23 ± 0.130	0.42 ± 0.031	*0.000*
32	* 2-Ethylphenol	1141	1138	2102	2082	122	107	0.493	0.9964	0.284 ± 0.023	0.106 ± 0.005	*0.000*
33	* 2,5-Dimethylphenol	1153	1150	2112	2090	122	107	0.074	0.9992	6.39 ± 0.355	1.95 ± 0.080	*<0.0001*
34	* 4-Ethylphenol	1170	1167	2213	2191	122	107	0.500	0.9965	0.828 ± 0.058	0.313 ± 0.012	*0.000*
35	! 3,5-Dimethylphenol	1172	-	2115	2174-2181	122	107	3.84	-	0.207 ± 0.038	0.071 ± 0.009	*0.004*

Peak No.	[1/] Compounds identified in smoked water	[2/] LRI				[3/] Mw	[4/] Main ion	[5/] Factor	[6/] R^2	[7/] Concentrations (mg/L)		p-values
		Exp. ZB-5	Std. ZB-5	Exp. ZB-Wax	Std. DB-Wax UI/ NIST					TR	P50	
36	[!] 1-(2-Hydroxyphenyl)-ethanone	1179	-	nd	-	136	121	7.50	-	0.122 ± 0.001	0.018 ± 0.001	*<0.0001*
37	[*] 2,3-Dimethylphenol	1184	1185	2186	2164	122	107	0.151	0.9998	0.598 ± 0.040	0.185 ± 0.005	*<0.0001*
38	[!] 1-(3-Methylphenyl)-ethanone	1187	-	1804	1786	134	119	4.35	-	0.052 ± 0.0006	0.006 ± 0.0004	*<0.0001*
39	[!] 6-Methylguaiacol	1190	-	1916	-	138	123	3.77	-	0.361 ± 0.007	0.055 ± 0.002	*<0.0001*
40	[!] 2-Ethyl-6-methylphenol	1194	-	nd	-	136	121	2.91	-	0.057 ± 0.002	0.014 ± 0.001	*<0.0001*
41	[!] 3,4-Dimethylphenol	1198	-	nd	-	122	107	6.42	-	0.153 ± 0.001	0.041 ± 0.001	*<0.0001*
42	[*] 4-Methylguaiacol	1205	1202	2003	1977	138	123	0.049	0.9995	29.6 ± 0.858	11.1 ± 0.351	*<0.0001*
43	[*] 2,4,6-Trimethylphenol	1214	1213	2039	2015	136	121	0.879	0.9870	0.112 ± 0.003	0.031 ± 0.001	*<0.0001*
44	[!] 2-Propylphenol	1225	-	nd	-	136	107	3.30	-	0.024 ± 0.0008	0.008 ± 0.0002	*<0.0001*
45	[!] 2-Ethyl-4-methylphenol	1232	-	nd	-	136	121	3.24	-	0.060 ± 0.0018	0.019 ± 0.0003	*<0.0001*
46	[!] 2-Ethyl-5-methylphenol	1236	-	nd	-	136	121	3.78	-	0.048 ± 0.0020	0.012 ± 0.0001	*<0.0001*
47	[§] 3,4-Dimethoxytoluene	1243	-	1837	1798-1806	152	152	8.26	-	0.357 ± 0.006	0.077 ± 0.006	*<0.0001*
48	[!] 1-Ethyl-4-methoxybenzene	1244	-	nd	-	136	121	3.61	-	0.232 ± 0.007	0.065 ± 0.002	*<0.0001*
49	[!] 3-Propylphenol	1262	-	nd	-	136	107	3.27	-	0.077 ± 0.0014	0.019 ± 0.0003	*<0.0001*
50	[!] 3-Ethyl-5-methylphenol	1263	-	nd	-	136	121	4.51	-	0.090 ± 0.003	0.022 ± 0.001	*<0.0001*
51	[!] 2,3,5-Trimethylphenol	1268	-	nd	-	136	121	5.09	-	0.024 ± 0.0006	0.007 ± 0.0002	*<0.0001*
52	[!] 2,3,6-Trimethylphenol	1276	-	nd	2031-2039	136	121	5.78	-	0.122 ± 0.0021	0.030 ± 0.0002	*<0.0001*
53	[*] 3,5-Dimethoxytoluene	1276	1275	1892	1868	152	152	0.218	0.9945	0.104 ± 0.0043	0.009 ± 0.0001	*<0.0001*
54	[!] 3,4,5-Trimethylphenol	1280	-	nd	-	136	136	4.46	-	0.062 ± 0.0030	0.017 ± 0.0004	*<0.0001*
55	[*] 4-Ethylguaiacol	1292	1290	2082	2053	152	137	0.600	0.9958	3.21 ± 0.081	0.858 ± 0.016	*<0.0001*

Peak No.	[1/] Compounds identified in smoked water	[2/] LRI				[3/] Mw	[4/] Main ion	[5/] Factor	[6/] R^2	[7/] Concentrations (mg/L)		p-values
		Exp. ZB-5	Std. ZB-5	Exp. ZB-Wax	Std. DB-Wax UI/ NIST					TR	P50	
56	Thymol (internal standard)	-		-	-			-	-	-	-	-
57	[!] 2,3-Dihydro-1H-inden-1-one	1303	-	nd	-	132	132	5.61	-	0.112 ± 0.002	0.025 ± 0.001	*<0.0001*
58	[!] 5-Acetoxymethyl-2-furaldehyde	1312	-	nd	-	168	126	16.9	-	0.035 ± 0.001	0.021 ± 0.003	*0.001*
59	[!] 2,4,5-Trimethylphenol	1313	-	nd	-	136	121	4.13	-	0.028 ± 0.0011	0.009 ± 0.0001	*<0.0001*
60	[*] 1,2,3-Trimethoxybenzene	1319	1318	2000	1977	168	168	0.212	0.9971	0.104 ± 0.003	0.031 ± 0.001	*<0.0001*
61	[*] 4-Vinylguaiacol	1328	1328	nd	2219	150	150	0.155	0.9657	0.058 ± 0.006	0.017 ± 0.002	*0.000*
62	[!] 1-Methyl-2,3-dihydro-1H-inden-1-one	1340	-	nd	-	146	131	0.549	-	0.004 ± 0.0001	0.001 ± 0.0001	*<0.0001*
63	[*] Syringol	1363	1357	2316	2288	154	154	0.004	0.9940	125 ± 7.18	38.0 ± 6.72	*0.000*
64	[*] Eugenol	1371	1371	2216	2191	164	164	0.169	0.9662	0.829 ± 0.033	0.223 ± 0.007	*<0.0001*
65	[!] 4-Propylguaiacol	1381	-	nd	2103	166	137	2.16	-	0.324 ± 0.021	0.038 ± 0.003	*<0.0001*
66	[!] 5-Methyl-1,2,3-trimethoxybenzene	1408	-	nd	2041	182	182	7.00	-	0.157 ± 0.007	0.034 ± 0.003	*<0.0001*
67	[*] Vanillin	1415	1415	nd	2393	152	151	0.001	0.9709	11.2 ± 3.38	0.577 ± 0.053	*0.005*
68	[!] 2-Propenylbenzene	1415	-	nd	-	118	117	5.18	-	0.024 ± 0.0009	0.003 ± 0.0002	*<0.0001*
69	[!] 7-Methyl-2,3-dihydro-1H-inden-1-one	1430	-	nd	-	146	146	6.46	-	0.021 ± 0.0010	0.003 ± 0.0002	*<0.0001*
70	[!] 4-Methylsyringol	1459	-	nd	-	168	168	4.38	-	0.324 ± 0.026	0.089 ± 0.018	*0.000*
71	[*] Isoeugenol	1466	1463	2379	2192	164	164	0.037	0.9780	0.075 ± 0.007	0.020 ± 0.002	*0.000*
72	[!] Acetosyringone	1485	-	nd	-	196	181	6.11	-	0.038 ± 0.003	0.007 ± 0.001	*<0.0001*
73	[§] *trans*-Isoeugenol	1491	-	2404	2383	236	164	10.7	-	0.012 ± 0.0005	0.003 ± 0.0002	*<0.0001*

Peak No.	[1/] Compounds identified in smoked water	[2/] LRI				[3/] Mw	[4/] Main ion	[5/] Factor	[6/] R^2	[7/] Concentrations (mg/L)		p-values
		Exp. ZB-5	Std. ZB-5	Exp. ZB-Wax	Std. DB-Wax UI/ NIST					TR	P50	
74	* Acetovanillone	1503	1499	nd	2664	166	151	0.048	0.9907	0.094 ± 0.028	0.038 ± 0.005	*0.028*
75	! 4-Ethylsyringol	1538	-	nd	-	182	167	3.25	-	0.242 ± 0.020	0.043 ± 0.009	*<0.0001*
76	! 4-Allylsyringol	1615	-	nd	2563	194	194	6.25	-	0.027 ± 0.003	0.008 ± 0.002	*0.001*
77	! 4-Propylsyringol	1622	-	nd	-	196	167	2.55	-	0.058 ± 0.007	0.009 ± 0.002	*0.000*
78	! Syringaldehyde	1677	-	nd	-	182	182	7.46	-	0.013 ± 0.010	0.006 ± 0.001	0.312

[1/] For (*) compound, authentic standards were used for identification and quantitation. (§) compound, the identification was from well-matched spectra and literature LRI. (!) compound, only LRI from experimental data was available for the identification of a compound, or if the LRI matched with literature was poor, then it must be considered as a tentative compound.

[2/] Linear retention indices (LRI) on ZB-5 column (from experimental data and 34 authentic compounds), ZB-Wax (30 m long) (from experimental data) and DB-Wax UI (60 m long) column (from 34 authentic compounds and NIST Chemistry WebBook). LRI from experimental data calculated from the retention time of n-alkanes C6-C26. nd stands for not detected.

[3/] Mw = molecular weight of compounds.

[4/] Four main ions were used to confirm compounds identity during auto quant, and ion number one is classified as the primary ion used to integrate the peak area of the compounds.

[5/] Response factors were used to calculate the amount of each compound. The response factor is the "m-factor" from the linear equation ($y = mx + c$) for the compound that was quantified using authentic standard compounds. In contrast, the response factor of the compounds that were semiquantitative was a tentative value.

[6/] The coefficient of determination is a goodness-of-fit measure for linear regression models, ranging from 0 to 1.

[7/] Average concentration ± standard deviation (SD) of three replicates. The average value of triplicate is considered significantly different at $p \leq 0.05$.

Table 2.8 Amount, odour threshold, odour activity value (OAV) and flavour dilution factor (FD) of key odorant compounds in smoked water samples.

No.	Odorant	[1/] Odour description given by assessors	[2/] LRI on		[3/] Odour threshold range (µg/L in water)	Concentration (µg/L)		[4/] Flavour dilution factor (FD)		[5/] Odour activity value (OAV)	
			ZB-5	DB-Wax UI		TR	P50	TR	P50	TR	P50
27	Guaiacol	Cool herb, amine smoke, medicinal	1098	1879	0.84-21	35,053	17,107	243	620	14,021	6,842
42	4-Methylguaiacol	Creamy vanilla, sweet, fruity	1202	1977	10-90	29,567	11,075	243	9	1,075	402
63	Syringol	Cool spice, herb, smoke, petrol	1357	2288	29-263	125,177	38,037	81	9	857	260
9	2-Furfural	nd	837	1470	3-3,000	15,771	23,867	nd	nd	394	604
23	4-Methylphenol	Medicinal, paints, poo	1074	2097	1-200	3,028	1,243	243	9	303	124
55	4-Ethylguaiacol	Fragrant smoke, cool herb, spicy	1290	2053	4.4-50	3,607	858	620	27	225	54
67	Vanillin	Cigarette smoke, smoke	1415	2393	25-1,200	11,234	690	81	9	176	11
64	Eugenol	Herb, guaiacol	1371	2191	2.5-150	829	223	9	1	138	37
71	Isoeugenol	Clove, smoky sweet	1463	2192	0.6	75	25	81	3	125	42
21	2-Methylphenol	Medicinal	1055	2014	31-75	5,225	2,204	81	9	120	50
34	4-Ethylphenol	Medicinal, paints, poo, stink bug	1167	2191	13-600	828	313	620	27	26	10
29	2,6-Dimethylphenol	Medicinal, smoke, grass, burnt	1114	1923	14.2	324	104	620	27	23	7
33	2,5-Dimethylphenol	Green, woody, grassy, soil	1150	2090	400	5,560	1,952	243	9	14	5
32	2-Ethylphenol	Sweet smoke, fishy	1138	2082	40	284	106	243	9	7	3
43	2,4,6-Trimethylphenol	Sweet, cool herb	1213	2015	18.59	112	33	1	1	6	2
18	Cyclotene	Curry, sweet, spicy	1033	nd	415	2,227	2,580	27	1	5	6
24	Acetophenone	Paints, poo	1077	1670	65	211	74	81	9	3	1
61	4-Vinylguaiacol	Smoke	1328	2219	3-100	58	17	3	1	3	1

No.	Odorant	[1/] Odour description given by assessors	[2/] LRI on		[3/] Odour threshold range (μg/L in water)	Concentration (μg/L)		[4/] Flavour dilution factor (FD)		[5/] Odour activity value (OAV)	
			ZB-5	DB-Wax UI		TR	P50	TR	P50	TR	P50
13	2-Acetylfuran	Sauce, salty, potato	916	1515	1,000	2,082	1,769	9	9	2	2
37	2,3-Dimethylphenol	Medicinal smoke, green, grass, soil	1185	2164	500	598	185	81	9	1.20	0.37
15	Phenol	Medicinal, mushroom	983	2019	31-10,000	5,611	3,789	3	1	1.12	0.76
1	Acetic acid	nd	na	1454	5,600-320,000	69,369	45,197	nd	nd	0.69	0.45

[1/] Odour descriptors at the sniffing port by all assessors who perceived that odour.

[2/] Linear retention indices (LRI) on capillary columns ZB-5 and DB-Wax UI.

[3/] The range of the odour detection threshold values of the compound in water was according to pieces of literature (Buttery et al., 1971; Czerny et al., 2008; Fiddler et al., 1966; Karagül-Yüceer et al., 2003; Li et al., 2023; Lin et al., 2019; Ong & Acree, 1999; Pu et al., 2020; PubChem; Schaller & Schieberle, 2020; Semmelroch et al., 1995; Strube et al., 2012; Tatsu et al., 2020; Yang et al., 2021; Young et al., 1996; Zhang et al., 2021).

[4/] Flavour dilution factor (FD) determined by AEDA on capillary column ZB-5 by SPME or SPE extraction. If the compounds were detected by all three assessors but differed in the FD values, the FD was chosen based on the highest value among the three assessors.

[5/] Odour activity value (OAV) was calculated by dividing the concentration by the median value of the odour threshold value of the compound in water.

2.4.4 Validation and confirmation of key odorants by recombination modelling

To validate that the identification and quantification of the potent aromas are correct, an aroma model blend (aroma recombinate) experiment was prepared based on the analytical data from GC-O and GC-MS compared with those of the original P50 smoked water. A full recombinate comprising the 22 aroma compounds identified in **Table 2.8** that were commercially available (food grade and non-food grade compounds) and three others partial recombinates (food grade only (17), natural food grade only (5) and the top 4 most odour-active (4)) at their measured concentrations were prepared. A 5-point scale of sensory profiling on four consensus-approved aroma descriptors (smoky, woody, ashy, phenolics) was scored by eight expertly trained panellists for the 4 recombinates and P50 smoked water after sniffing and tasting the samples, which are shown in **Table 2.9**.

The aroma profiles after sniffing were investigated in full recombinate (RC 22; food grade and non-food grade) and partial recombinate formulas (contained only food grade compounds). The three odour attributes, except phenolics, of all non-diluted recombinate models after sniffing were significantly different from P50 smoked water sample but did not differ among the full and partial recombinates. The aroma profiles after sipping were conducted in 1% dilution of all partial recombinates, and P50 smoked water sample. A partial recombinate of 17 compounds (RC 17) had the most aroma attributes similar to P50 smoked water sample. The smoky and woody aroma of RC 17 was significantly less prominent than that of P50 smoked water. It suggests that the loss of smoky and woody aroma intensities may be the result of unidentified aroma compounds that are typically characterised by pleasant descriptors (data from GC-O and GC-MS). However, they remain unidentifiable due to a weak MS signal. Despite having a weak signal and low concentrations, these compounds continue to be highly odour-active (high FD factor), as shown in **Table 2.6** (e.g., compound numbers 16, 25, 30, and 51). Therefore, they could be compounds associated with a smoky and woody aroma, or that altered the overall profile of recombinates.

Table 2.9 The sensory profiling score of aroma attributes after sniffing and tasting the recombinate formulas compared to P50 smoked water.

[2/] Samples	[1/] Attribute scores (1-5)			
	Smoky	**Woody**	**Ashy**	**Phenolics**
Odour				
P50	4.63 a ± 0.7	4.50 a ± 0.8	3.25 a ± 1.5	3.63 ns ± 1.5
RC 22	1.13 b ± 1.1	1.13 b ± 1.4	0.38 b ± 0.5	3.13 ns ± 1.6
RC 17	1.00 b ± 1.1	1.25 b ± 1.6	0.25 b ± 0.5	3.25 ns ± 1.6
RC 5	0.75 b ± 0.7	1.00 b ± 0.8	0.38 b ± 0.5	2.75 ns± 1.4
RC 4	1.00 b ± 1.2	0.75 b ± 0.7	0.50 b ± 0.5	2.25 ns ± 1.2
Significance of difference between samples (p-value)	*< 0.0001*	*< 0.0001*	*< 0.0001*	0.2038
Taste (1%) (food grade compounds)				
P50	4.25 a ± 0.9	4.00 a ± 1.3	3.25 a ± 1.8	2.50 ns ± 1.6
RC 17	2.50 b ± 1.8	1.88 b ± 1.2	1.88 ab ± 1.7	1.88 ns ± 2.1
RC 5	0.75 c ± 0.9	0.88 b ± 1.0	0.50 b ± 0.8	1.00 ns ± 1.8
RC 4	1.50 bc ± 1.3	2.00 b ± 1.5	0.75 b ± 1.2	1.25 ns ± 1.3
Significance of difference between samples (p-value)	*< 0.0001*	*< 0.0001*	*0.0011*	0.1523

[1/] Means of attribute scores in each column not labelled with the same letters were significantly different ($p \leq 0.05$); ns was not significantly different ($p > 0.05$) of means in the same column. Means scores were from 8 trained panels ± standard deviation (SD). Attribute scores; 0 was absent, 1 was very weak, and 5 was very strong.

[2/] P50 was P50 smoked water, RC 22 was full recombinate (both food grade and non-food grade; 22 compounds), RC 17 was a partial recombinate (only food grade; 17 compounds), RC 5 was a partial recombinate (food grade natural compounds; 5 compounds), and RC 4 was a partial recombinate (the first four highest odour activity value (OAV); 4 compounds) of P50 smoked water.

In the sipping session, it was observed that all recombinates were less smoky and woody than the P50 smoked water. However, the phenolic attribute remained consistent across all samples, indicating that the compounds responsible for the phenolic note were at a reasonable level. All recombinates were scored identically apart from RC 5 (acetic acid, guaiacol, phenol, eugenol, and isoeugenol), which was significantly less smoky than RC 17. RC 4 shared similarities with RC 17 regarding methylguaiacol and syringol, which play a significant role in smoky notes. RC 4 and RC 5 had low levels of ashy, suggesting that compounds from RC 17 may contribute to the ashy note. The aroma and flavour enhancement of the recombinate showed more promising when the panellist perceived the sample through a mouth than only sniffing the samples, which resulted from the orthonasal and retronasal effects. From this experiment, even though the recombinates were made for sipping in dilution solution (1%), the result revealed that the panellists scored two aroma attributes (ashy and phenolics) in RC 17, not statistically different from P50 smoked water. In contrast, when the panellists only sniffed, these three aroma attributes, except phenolic, were significantly lower than P50 smoked water, even though the recombinates were not diluted.

Upon validation, it was determined that the identified potent aromas were not accurate in producing the recombinate of P50 smoked water. There were several reasons why the recombinate blend did not closely resemble the original P50 smoked water. First, the recombinate still lacks the compounds only detected in the GC-O sniffing port, as no chromatogram peak of that odorant region was found in the GC-MS. Second, some compounds with a high FD factor that could be referred to as potent aroma compounds remain unidentified in an extract, as this compound is only detectable in SPE extract and not in SPME extract, which was used for the compounds' quantification. Thirdly, the lack of odorant compounds with a high FD factor, such as 3,4-dimethylphenol, 3,5-dimethylphenol, 2-methoxy-4-propylphenol (dihydroeugenol), 2-methoxy-6-methylphenol (6-methylguaiacol), etc., to add to the recombinate. Fourthly, some quantitative data of the potent aromas were incorrect, such as eugenol and isoeugenol contents, as eugenol and isoeugenol are more selective to SPE extraction than SPME extraction, but all quantitative data was conducted using SPME extraction, in which eugenol and isoeugenol were not properly extracted, thus their concentration in recombinate could not be the correct concentration. Fifth, some compounds that cannot be perfectly separated from each other but have LRI values close to each other, such as compound 58 in **Table 2.6**, could be phenol or 1-octene-3-ol, which were potent aroma

compounds in the smoked water sample and we chose phenol to add into the recombinate, which may be incorrect.

Smoked water is a complex blend of over a hundred various substances. Based on the high value of the FD factor, our results identified more than sixty substances that could be the potent aroma components of smoked water. At least 22 compounds with OAVs greater than one was identified as the potent aroma of smoked water. So far, the FD factor does not always correspond with the OAV, and we could not calculate the OAV for all compounds with a high FD factor. Therefore, we attempted to mimic the flavour of smoked water as closely as possible when creating the recombinate blend by using many potent aroma compounds. Nonetheless, the recombinate still does not have a flavour that perfectly matches the original P50 smoked water for the numerous reasons mentioned above.

2.5 Summary

Typically, smoked ingredients are used in commercial products to impart a smoky flavour. The volatile profiles of traditional apple-wood smoked water (TR), and apple-wood smoked water prepared using PureSmoke Technology (PST) (a patented process to remove carcinogens polyaromatic hydrocarbons (PAHs) from the smoke) were analysed to identify the odour-active compounds responsible for the overall smoked water flavour. The two best extraction methods based on providing the highest number of extracted compounds were SPME and SPE, which used diethyl ether as eluent. AEDA was applied in both extraction methods to identify the most odour-active compounds using GC-O in conjunction with GC-MS, which is reported as the flavour dilution (FD) factor. A total of 67 aroma-active compounds were perceived at the sniffing port of the GC-O by two extraction methods, of which most compounds were phenols and guaiacols groups. Acetic acid, syringol, guaiacol, and 4-methylguaiacol were the major constituents and were generally present at greater concentrations in TR than in P50 smoked water. The odour activity values (OAV) identified what compounds could be potent aroma compounds. At least 22 compounds with OAVs ≥ 1 guided as potent aroma compounds in smoked water. Guaiacol was the most potent aroma in smoked water, as it had the highest FD factor and the highest OAV, and it was described as a "cool herb, amine smoke, medicinal". The identified 22 potent aromas were blended to produce full and partial recombinate at the approximately actual contents found in P50 smoked

water to validate whether or not these compounds were potent aromas in smoked water. In addition, a 5-point scale of sensory profiling on four consensus-approved aroma descriptors (smoky, woody, ashy, phenolic) was conducted in full recombinate and partial recombinate compared with the original P50 smoked water. The sensory profiling of the recombinates did not precisely match the actual P50 smoked water. The disparity can be related to either insufficient numbers of potent aroma or a lower concentration of the potent aroma compounds compared to the real quantity present in smoked water.

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Chapter 3
Comparison of volatiles generated in smoked water using PureSmoke Technology (PST) with those generated using a traditional smoking process

3.1 Introduction

Smoked ingredients are becoming increasingly popular as a component of snack seasonings, soups, sauces, rubs and marinades. However, the smoking process which is necessary for flavour development also generates PAHs, of which one, (benzo[a]pyrene) is a known human carcinogen, four are probable carcinogens, two of which are known carcinogens in animals. In 2003, smoke flavourings were assessed in vitro by EFSA, and many were banned from the market due to their high PAH content. This is when Besmoke developed and patented the Puresmoke technology process (PST^{TM}) which produced a natural smoke flavouring with low PAHs (Parker et al., 2018). After in vivo testing, EFSA subsequently allowed more smoke flavours onto the market, but the PST filtered product had superior quality compared to the products produced using the traditional smoking process and is selling well across the globe. The aim of this study was to compare the volatile profiles of PST and traditionally smoked waters.

Smoke production

There are different types of smoking methods classified as either direct or indirect smoking (Ledesma et al., 2016). Smoke is obtained by heating woods or comparable materials to 450-600 °C in a limited supply of oxygen to initiate pyrolysis. The woods usually consist of three main compounds: cellulose, hemicellulose and lignin (Šimko, 2005). Hemicelluloses and lignin are amorphous, whereas cellulose is very crystalline. After water evaporation, pyrolysis of the three primary compounds happens; hemicellulose is combusted first at 180-300 °C, cellulose shows greater thermal resistance and a sharper degradation range (260-350 °C) and lignin decomposition occurs over in the range 300-500 °C and occurs in the last (Lingbeck et al., 2014; Šimko, 2005).

Hard wood and soft wood

Woods are typically divided into two categories: softwood and hardwood, which are based on the natural durability of the material. Hardwood is typically durable, strong, heavy, and has a higher density compared to softwood which is typically non-durable, soft, light and has a lower density. Any type of wood contains cellulose, lignin, and hemicelluloses as its major constituents. Hardwood pyrolysis was easier than softwood because the activation energy of softwood was consistently higher throughout the whole pyrolysis process (Ding et al., 2017). The hardwood type such as oak, beech, hickory, maple, and apple, are the main woods used in the manufacturing of smoke since the chemical composition of softwood tends to produce higher amounts of carcinogenic polycyclic aromatic hydrocarbons (PAHs) (Varlet, 2009).

Polycyclic aromatic hydrocarbons (PAHs)

The group of polycyclic aromatic hydrocarbons (PAHs) is classified as carcinogenic, mutagenic, semi- or non-volatile and non-biodegradable substances (Singh et al., 2016), and have been recognized by the European Scientific Committee on Food (SCF) as genotoxic carcinogens, with specific regard to the highly carcinogenic substance of benzo(a)pyrene (BaP) (Wretling et al., 2010). During traditional smoking, PAHs are generated through incomplete combustion of woods (Ledesma et al., 2016), and increases directly with the decomposition temperature of woods at 400-1000 °C (Varlet, 2009). Even though PAHs are extremely toxic, they have low water solubility, and when dissolved in water or adsorbed on particulate matter, PAHs may undergo ultraviolet light photodecomposition from solar radiation (WHO, 2000), then their concentrations are expected to be rather low in water. This allows the PAHs in smoke to be easily separated using separation and filtration techniques in liquid smoke industry (Lingbeck et al., 2014).

Liquid smoke and smoked water

The smoke produced after pyrolysis is then gathered through the condenser, which liquefies the smoke (vapours), refining and filtering the liquid smoke (Janairo & Amalin, 2018; Lingbeck et al., 2014) to make its final product of liquid smokes. Each type of liquid smoke

has a distinctive aroma and flavour including chemical compositions that depend on the wood types, wood moisture, temperature and time that generated the liquid smoke (Guillén & Ibargoitia, 1996; Janairo & Amalin, 2018; Montazeri et al., 2013). Due to the aerosol smoke being condensed into water and going through a purification process to remove PAHs, the liquid smoke is labelled as smoke flavouring. There have been numerous attempts to lower the PAHs in the smoke aerosol due to the health risk presented by the smoke flavouring process (Parker et al., 2018).

Besmoke Ltd. developed the PureSmoke™ Filtration Technology (PST) to produce smoke ingredients with lower PAH content (Besmoke, 2017). This technology heralds a new generation of natural smoke flavouring systems. Natural zeolite is the raw material used in the filtration process (Parker et al., 2018). The filter traps polycyclic aromatic hydrocarbons (PAHs) but enables free flavour compounds to flow. This technology reduces the number of PAHs, which means greater consumer safety in accordance with EU legislation. By reducing the concentrations of carcinogens, it is proposed that the method leaves only the most desirable volatile smoke, and that the compounds that give the distinctive ashy taste could be retained (Besmoke, 2017) giving a more rounded smoke flavour. After passing through the filter process, the condensed smoke was passed through water to create smoked water.

The hypotheses of this work were that using the PST filter to produce smoked water (as opposed to use of a traditional direct smoking process) alters the volatile profile of the product and reduces some of the harsh notes generated using the traditional process. Two filtration processes (P25 and P50) were compared to a traditional (TR) smoking process. The difference between P25 and P50 was the number of filter plates being used. A higher number of PST (P50), means more filter plates are used and the sample is more filtered. Three different hardwood chips (apple, beech and oak) were produced under all 3 smoking conditions.

3.2 Materials

3.2.1 Smoked water samples

Besmoke Ltd. (Arundel, UK) provided nine smoked water samples made from three types of woodchips (apple, beech, and oak) and three different smoking methods (traditional

and PureSmokeTechnology; PST). Nine smoked water samples consist of Apple-wood traditional (AP-TR) (batch number; CS0047/S/04 & YBES183), Apple-wood PST25 (AP-P25) (batch number; CS0048/S/04 & PUR004), Apple-wood PST50 (AP-P50) (batch number; CS0049/S/04 & PUR0266Q11), Beech-wood traditional (BH-TR) (batch number; CS0032/S/03 & YBES144), Beech-wood PST25 (BH-P25) (batch number; CS0033/S/03 & PUR017), Beech-wood PST50 (BH-P50) (batch number; CS0034/S/03 & PUR), Oak-wood traditional (OK-TR) (batch number; CS0029/S/03 & YBES155), Oak-wood PST25 (OK-P25) (batch number; CS0030/S/03 & PUR007), and Oak-wood PST50 (OK-P50) (batch number; CS0031/S/03 & PUR) smoked water. The three smoked waters were all made with the same batch of woodchip, and all 3 smoking conditions were prepared on the same day for each wood.

PAH analysis carried out by Eurofins showed that the PAH content of the smoked water samples produced by PST were < 0.5 µg/kg (below detection limits), whereas the PAHs content, benzo(a)pyrene (BaP) in particular, was found at 1.4 µg/kg in the untreated sample (TRAD), which was within the limit recommended by EU Commission Regulation No. 1881/2006 (20 µg/kg).

3.2.2 Solvents

Methanol (≥ 99.8% purity, HPLC grade) and ethanol (96% purity, FG) were purchased from Fisher Scientific U.K. Limited (UK) and Kimiauk, Hayman Group Limited (UK) respectively.

3.2.3 Authentic odorant standard compounds

Thirty-five authentic odorant standard compounds were purchased: 1-phenylethanone (acetophenone; 99% purity; Aldrich, Germany), 1-(furan-2-yl)ethanone (2-acetylfuran; ≥99%, FG, Sigma-Aldrich, China), acetic acid (≥99.5%, FG, Sigma-Aldrich, Colombia), 1-(5-methylfuran-2-yl)ethanone (2-acetyl-5-methylfuran; Oxford), 1-(4-hydroxy-3-methoxyphenyl)ethan-1-one (acetovanillone; SAFC^{TM}, Germany), cyclopentanone (99%, Sigma, USA), 1,3-dimethoxy-5-methylbenzene (3,5-dimethoxytoluene; 98+%, Lancaster Synthesis, England), 2,6-dimethoxyphenol (syringol; ≥98%, FG, Sigma-Aldrich, India), 2,6-dimethylphenol (≥99%, FG, Sigma-Aldrich, China), 2,3-dimethylphenol (99.2%, AG, Sigma-

Aldrich, Germany), 2,5-dimethylphenol (≥99%, FG, Sigma-Aldrich, China), 2-methoxy-4-prop-2-enylphenol (eugenol; pure, Givaudan, Switzerland), 4-ethylphenol (p-ethylphenol; ≥98%, FG, Merck, China), 4-ethyl-2-methoxyphenol (ethylguaiacol; ≥98%, FG, SAFC, USA), 3-ethyl-2-hydroxycyclopent-2-en-1-one (3-ethyl-2-hydroxy-2-cyclopentenone; >97%, TCI, Japan), 2-ethylphenol (o-ethylphenol; 99%, Sigma-Aldrich, Japan), furan-2-carbaldehyde (furfural; ≥98%, FCC, FG, Sigma-Aldrich, China), furan-3-carbaldehyde (3-furaldehyde; 97+%, Aldrich, USA), 2-methoxyphenol (guaiacol; natural ≥99%, FG, Sigma-Aldrich, China), 2-methoxy-4-[(E)-prop-1-enyl]phenol ((E) isoeugenol; natural 99%, FG, Sigma-Aldrich, China), 2-hydroxy-3-methylcyclopent-2-en-1-one (cyclotene; 98%, Sigma-Aldrich, China), 2-methylfuran (Oxford), 3-hydroxy-2-methylpyran-4-one (maltol; >99%, FG, SAFC Sigma-Aldrich, China), 2-methylbenzaldehyde (Oxford organics, UK), 4-methylphenol (p-cresol; 99%, FG, Sigma-Aldrich, USA), methyl benzoate (≥98%, FCC, Sigma-Aldrich, Germany), 2-methylphenol (o-cresol; Acros Organics, Belgium), 2-methylcyclopent-2-en-1-one (2-methyl-2-cyclopentenone; 98%, Sigma-Aldrich, Japan), 2-methoxy-4-methylphenol (methylguaiacol; ≥98%, FG, Sigma-Aldrich, China), phenol (natural 97%, FG, SAFC Sigma-Aldrich, USA), 4-ethenyl-2-methoxyphenol (vinylguaiacol; ≥98%, FG, Sigma-Aldrich, United Kingdom), 5-methyl-2-propan-2-ylphenol (thymol; ≥99%, FCC, Sigma-Aldrich, Germany), 2,4,6-trimethylphenol (97%, Sigma-Aldrich, China), 1,2,3-trimethoxybenzene (98%, Sigma-Aldrich, USA), 4-hydroxy-3-methoxybenzaldehyde (vanillin; ≥97%, FG, Sigma-Aldrich, China).

3.3 Methods

3.3.1 Identification and quantification of volatile compounds in smoked water using Gas chromatography-Mass spectrometry (GC-MS)

Each smoked water was filtered through the Whatman polypropylene (PP) puradisc syringe filter (pore size 0.45 μm) before extraction by solid phase microextraction (SPME) and quantification. The identification and quantification of volatile compounds in smoked water samples by GC-MS is described in detail in section 2.3.3.

3.3.2 The organoleptic of smoked water

The sensory characteristics of nine smoked water samples were performed by eight trained sensory panellists from Besmoke company who are smoked ingredients experts with a minimum of 2 years of experience. The sensory characteristics of smoked water samples were evaluated using a qualitative sensory profile. This method involves a trained panel describing the sensory attributes of the product without measuring their intensity levels according to ISO 6658:2017 (ISO, 2017).

The smoked water samples were diluted to a concentration of 0.1% in warm water (50-60 ºC) and presented to the panel in a random order for organoleptic evaluation. Each panel evaluated the sample individually, and the result was recorded on the attribute checklist according to its colour, odour, and taste characteristics. There was a two-minute break between samples, during which just drinking water was used to cleanse the palate before evaluating the next sample.

3.3.3 Data analysis

The quantitative and semiquantitative data for each compound identified in the GC-MS analyses were analysed by both two-way multivariate analysis of variance (MANOVA) (using IBM SPSS Statistics version 27; New York, USA) and principal component analysis (PCA) (using XLSTAT version 2022.4.1; Addinsoft, New York, USA). Two-way MANOVA was performed to check the significance of the main factors (wood type and processing) and interaction effects between the two factors for 77 dependent variables (77 extracted compounds) and the Tukey HSD test was applied to determine which sample means were significantly different ($p \leq 0.05$) between the 9 smoked water samples. The data were reported as mean ± standard deviations, which are shown in **Appendix Table A2**. PCA was applied to visualise the similarities and differences of extracted compounds in all smoked water samples.

3.4 Results and discussion

3.4.1 Identification and quantification of volatile compounds in smoked water using Gas chromatography-Mass spectrometry (GC-MS)

The smoked water samples in this research were a commercial product of unknown composition. The first task was to identify the volatile components in the smoked water samples using GC-MS. Seventy-seven compounds were identified and classified into 8 groups which were summarised in **Table 3.1** These included 3 acids, 9 aldehydes, ketones and diketones all based on a cyclopentene or pyran ring, 9 furans, 11 guaiacols, 11 syringols, 21 other phenols, 3 indene derivatives, and 10 other unclassified compounds. Thirty-four out of seventy-seven identified compounds were quantified using authentic odorant standard calibration, whereas the other compounds were semi-quantified. The volatile constituents discovered in smoked water are presented in **Appendix Table A2**. These 77 identified compounds were found in all smoked water samples made from different woods; this indicated that the smoked waters contained identical volatile compounds but they varied in different proportions depending on the wood type and the main compositions (hemicellulose, cellulose and lignin) of each wood type. These groups of smoke compounds are derived from the pyrolysis of three main components of wood that are hemicellulose, cellulose and lignin. Different groups of compounds are created according to the mechanism of pyrolysis; hemicellulose and cellulose combustion occur between 180 °C and 350 °C, producing carboxylic acids and carbonyl compounds while lignins are burned between 300 °C and 500 °C, producing phenols (Šimko, 2005). A temperature of 450 °C - 500 °C resulted in the best composition to produce carbonyls, furans and phenolic compounds, but the PAHs is also increased from 400 °C to 1000 °C (Lingbeck et al., 2014; Varlet, 2009). According to Varlet (2009), it seems challenging to produce the required organoleptic volatile compounds free from PAHs contaminants; however, filtration and purification procedures can be utilised to eliminate these contaminants.

Table 3.1 Compounds identified in the smoked water samples extracted by SPME.

Peak No.	[1/] Compounds identified in smoked water		[2/] LRI				[3/] Mw	[4/] Compound main ions				[5/] Factor	[6/] R^2
	Codes	Compound names	Exp. ZB-5	Exp. ZB-Wax	Std. DB-5	Std. DB-Wax UI/ NIST		1	2	3	4		
Acids (3)													
1	AA	* Acetic acid	701	1491	-	1454	60	43	45	60	42	0.003	0.9130
4	BA	§ Butanoic acid (butyric acid)	767	1648	-	1604-1647	88	60	73	41	55	5.10	-
7	CA	! 2-Butenoic acid (crotonic acid)	835	nd	-	1773	86	86	69	68	39	9.86	-
Aldehydes, ketones and diketones (9)													
6	CCP	* Cyclopentanone	798	1221	797	1198	84	55	84	41	56	0.010	0.8908
11	4-C-1,3-D	! 4-Cyclopentene-1,3-dione	896	nd	-	-	96	96	68	54	42	4.76	-
12	2-M-2C	* 2-Methyl-2-cyclopenten-1-one	915	1405	912	1386	96	67	96	53	68	0.144	0.9893
18	CCT	* 3-Methyl-1,2-cyclopentanedione (cyclotene)	1041	1871	1033	1827-1860	112	112	69	83	55	0.005	0.9944
20	2,3-D-2C	! 2,3-Dimethyl-2-cyclopenten-1-one	1052	nd	-	1523-1573	110	67	110	95	81	3.92	-
22	2,3,4-T-2C	§ 2,3,4-Trimethyl-2-cyclopenten-1-one	1073	1531	-	1539	124	109	124	81	96	5.26	-
26	3-E-2C	! 3-Ethyl-2-cyclopenten-1-one	1090	1675	-	1611	110	110	81	67	95	7.63	-
30	MT	* 3-Hydroxy-2-methyl-4H-pyran-4-one (maltol)	1127	2022	1122	1954-2004	126	126	71	43	97	0.002	0.9455
31	3-E-2H	* 3-Ethyl-2-hydroxy-2-cyclopenten-1-one	1131	1935	1127	1918	126	126	55	83	69	0.009	0.9991

Peak No.	[1/] Compounds identified in smoked water		[2/] LRI				[3/] Mw	[4/] Compound main ions				[5/] Factor	[6/] R^2
	Codes	Compound names	Exp. ZB-5	Exp. ZB-Wax	Std. DB-5	Std. DB-Wax UI/ NIST		1	2	3	4		
Furans derivatives (9)													
3	2-MF	* 2-Methylfuran	754	1246	-	832-888	82	53	82	81	54	0.008	0.9934
8	3-FD	* 3-Furancarboxaldehyde (3-furfural)	816	1456	814	1434	96	95	96	39	67	0.164	0.9204
9	FF	* 2-Furancarboxaldehyde (2-furfural)	838, 842	1496	837	1470	96	96	95	39	38	0.229	0.9898
10	2-EF	! 2-Ethylfuran	885	1370	-	944-975	96	81	96	53	67	6.57	-
13	2-AF	* 1-(2-Furanyl)-ethanone (2-acetylfuran)	918	1538	916	1515	110	95	110	51	39	0.187	0.9908
14	5-MFF	§ 5-Methyl-2-furancarboxaldehyde (5-methylfurfural)	973	1526	-	1522-1604	110	110	109	53	81	4.91	-
17	1-FPP	! 1-(2-Furanyl)-1-propanone	1018	nd	-	1558-1571	124	95	124	96	67	11.8	-
19	2-A5MF	* 2-Acetyl-5-methylfuran	1046	1653	1045	1629	124	109	124	53	43	0.191	0.9945
58	5-A2FD	! 5-Acetoxymethyl-2-furaldehyde	1312	nd	-	-	168	126	79	43	109	16.9	-
Phenol and derivatives (21)													
15	P	* Phenol	987	2056	983	2019	94	94	66	65	55	0.067	0.9853
21	2-MP	* 2-Methylphenol (*o*-cresol)	1059	2048	1055	2014	108	108	107	77	90	0.086	0.9998
23	4-MP	* 4-Methylphenol (*p*-cresol)	1079	2120	1074	2097	108	107	108	77	79	0.173	0.9950
29	2,6-DMP	* 2,6-Dimethylphenol	1117	1953	1114	1923	122	122	107	121	77	0.584	0.9944
32	2-EP	* 2-Ethylphenol	1141	2102	1138	2082	122	107	122	77	79	0.493	0.9964
33	2,5-DMP	* 2,5-Dimethylphenol	1153	2112	1150	2090	122	107	122	121	77	0.074	0.9992
34	4-EP	* 4-Ethylphenol	1170	2213	1167	2191	122	107	122	77	108	0.500	0.9965
35	3,5-DMP	! 3,5-Dimethylphenol	1172	2115	-	2174-2181	122	107	122	121	77	3.84	-
37	2,3-DMP	* 2,3-Dimethylphenol	1184	2186	1185	2164	122	107	122	77	121	0.151	0.9998

Peak No.	[1/] Compounds identified in smoked water		[2/] LRI				[3/] Mw	[4/] Compound main ions				[5/] Factor	[6/] R^2
	Codes	Compound names	Exp. ZB-5	Exp. ZB-Wax	Std. DB-5	Std. DB-Wax UI/ NIST		1	2	3	4		
40	2-E6MP	! 2-Ethyl-6-methylphenol	1194	nd	-	-	136	121	136	91	77	2.91	-
41	3,4-DMP	! 3,4-Dimethylphenol	1198	nd	-	-	122	107	122	121	77	6.42	-
43	2,4,6-TMP	* 2,4,6-Trimethylphenol	1214	2039	1213	2015	136	121	136	135	91	0.879	0.9870
44	2-PP	! 2-Propylphenol	1225	nd	-	-	136	107	136	77	79	3.30	-
45	2-E-4MP	! 2-Ethyl-4-methylphenol	1232	nd	-	-	136	121	136	91	77	3.24	-
46	2-E-5MP	! 2-Ethyl-5-methylphenol	1236	nd	-	-	136	121	136	91	77	3.78	-
49	3-PP	! 3-Propylphenol	1262	nd	-	-	136	107	108	136	121	3.27	-
50	3-E-5MP	! 3-Ethyl-5-methylphenol	1263	nd	-	-	136	121	136	77	91	4.51	-
51	2,3,5-TMP	! 2,3,5-Trimethylphenol	1268	nd	-	-	136	121	136	135	91	5.09	-
52	2,3,6-TMP	! 2,3,6-Trimethylphenol	1276	nd	-	2031-2039	136	121	136	135	122	5.78	-
54	3,4,5-TMP	! 3,4,5-Trimethylphenol	1280	nd	-	-	136	136	121	135	91	4.46	-
59	2,4,5-TMP	! 2,4,5-Trimethylphenol	1313	nd	-	-	136	121	136	135	91	4.13	-
Guaiacol and derivatives (11)													
27	G	* 2-Methoxy phenol (guaiacol)	1102	1907	1098	1879	124	109	124	81	53	0.052	0.9999
39	6-G	! 2-Methoxy-6-methylphenol (6-methylguaiacol)	1190	1916	-	-	138	123	138	77	95	3.77	-
42	MG	* 2-Methoxy-4-methylphenol (4-methylguaiacol)	1205	2003	1202	1977	138	123	138	95	67	0.049	0.9995
55	EG	* 2-Methoxy-4-ethylphenol (4-ethylguaiacol)	1292	2082	1290	2053	152	137	152	122	138	0.600	0.9958

Peak No.	[1/] Compounds identified in smoked water		[2/] LRI				[3/] Mw	[4/] Compound main ions				[5/] Factor	[6/] R^2
	Codes	Compound names	Exp. ZB-5	Exp. ZB-Wax	Std. DB-5	Std. DB-Wax UI/ NIST		1	2	3	4		
61	VG	* 2-Methoxy-4-vinylphenol (4-vinylguaiacol)	1328	nd	1328	2219	150	150	135	107	151	0.155	0.9657
64	EU	* 2-Methoxy-4-(2-propenyl) phenol (eugenol)	1371	2216	1371	2191	164	149	131	103	149	0.169	0.9662
65	PG	! 2-Methoxy-4-propylphenol (4-propylguaiacol)	1381	nd	-	2103	166	166	122	138	166	2.16	-
67	VN	* 4-Hydroxy-3-methoxybenzaldehyde (vanillin)	1415	2615	1415	2393	152	151	152	109	123	0.001	0.9709
71	IEU	* 4-(1-Propenyl)-2-methoxyphenol (isoeugenol)	1466	2379	1463	2192	164	164	149	103	131	0.037	0.9780
73	tIEU	§ *trans*-4-(1-Propenyl)-2-methoxyphenol (*trans*-isoeugenol)	1491	2404	-	2383	236	164	149	103	121	10.7	-
74	ATV	* 1-(4-Hydroxy-3-methoxyphenyl)-ethanone (acetovanillone)	1503	nd	1499	2664	166	151	166	123	108	0.048	0.9907
Syringol and derivatives (11)													
47	3,4-DMT	§ 3,4-Dimethoxytoluene	1243	1837	-	1798-1806	152	152	137	109	91	8.26	-
53	3,5-DMT	* 3,5-Dimethoxytoluene	1276	1892	1275	1868	152	152	123	109	121	0.218	0.9945
60	1,2,3-TMB	* 1,2,3-Trimethoxybenzene	1319	2000	1318	1977	168	168	153	110	125	0.212	0.9971
63	S	* 2,6-Dimethoxyphenol (syringol)	1363	2316	1357	2288	154	154	139	96	111	0.004	0.9940
66	5-M-1,2,3TMB	! 5-Methyl-1,2,3-trimethoxybenzene	1408	nd	-	2041	182	182	167	139	124	7.00	-

Peak No.	[1/] Compounds identified in smoked water		[2/] LRI				[3/] Mw	[4/] Compound main ions				[5/] Factor	[6/] R^2
	Codes	Compound names	Exp. ZB-5	Exp. ZB-Wax	Std. DB-5	Std. DB-Wax UI/ NIST		1	2	3	4		
70	3,5-DM-4HT	! 3,5-Dimethoxy-4-hydroxytoluene (4-methylsyringol)	1459	nd	-	-	168	168	153	125	107	4.38	-
72	AS	! 1-(4-Hydroxy-3,5-dimethoxyphenyl)-ethanone (acetosyringone)	1485	nd	-	-	196	181	196	182	197	6.11	-
75	ES	! 4-Ethyl-2,6-dimethoxyphenol (4-ethylsyringol)	1538	nd	-	-	182	167	182	168	107	3.25	-
76	ALS	! 4-(2-Propenyl)-2,6-dimethoxyphenol (4-allylsyringol)	1615	nd	-	2563	194	194	119	131	147	6.25	-
77	PS	! 4-Propyl-2,6-dimethoxyphenol (4-propylsyringol)	1622	nd	-	-	196	167	196	168	123	2.55	-
78	SD	! 4-Hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde)	1677	nd	-	-	182	182	181	167	111	7.46	-
Indene derivatives (3)													
57	2,3-D-1H	! 2,3-Dihydro-1H-inden-1-one	1303	nd	-	-	132	132	104	103	78	5.61	-
62	1-M-2,3D	! 1-Methyl-2,3-dihydro-1H-inden-1-one	1340	nd	-	-	146	131	146	103	117	0.549	-
69	7-M-2,3D	! 7-Methyl-2,3-dihydro-1H-inden-1-one	1430	nd	-	-	146	146	117	118	145	6.46	-
Other compounds (10)													
2	2-BT	! 2-Butenal (crotonaldehyde)	0	1070	-	-	70	41	70	39	69	3.98	-
5	1-H-2B	§ 1-Hydroxy-2-butanone	810	1409	-	1391-1395	88	57	29	31	88	2.80	-
16	BZN	! Benzonitrile	998	1650	-	-	103	103	76	50	104	4.49	-

Peak No.	[1/] Compounds identified in smoked water		[2/] LRI				[3/] Mw	[4/] Compound main ions				[5/] Factor	[6/] R^2
	Codes	Compound names	Exp. ZB-5	Exp. ZB-Wax	Std. DB-5	Std. DB-Wax UI/ NIST		1	2	3	4		
24	ATP	* Acetophenone	1080	1694	1077	1670	120	105	77	120	106	0.474	0.9977
25	2-MBZ	* 2-Methylbenzaldehyde	1083	nd	1082	1637	120	91	120	119	65	0.998	0.9925
28	MBZ	* Methyl benzoate	1107	1660	1104	1638	136	105	77	136	106	0.188	0.9885
36	1-2HE	! 1-(2-hydroxyphenyl)-ethanone	1179	nd	-	-	136	121	136	65	93	7.50	-
38	1-3ME	! 1-(3-methylphenyl)-ethanone	1187	1804	-	1786	134	119	91	134	65	4.35	-
48	1-E-4MB	! 1-Ethyl-4-methoxybenzene	1244	nd	-	-	136	121	136	77	122	3.61	-
68	2-PPB	! 2-Propenylbenzene	1415	nd	-	-	118	117	118	91	115	5.18	-

[1/] For (*) compound, authentic standards were used for identification and quantitation. (§) compound, the identification was from well-matched spectra and literature LRI. (!) compound, only LRI from experimental data was available for the identification of a compound, or if the LRI matched with literature was poor, then it must be considered as a tentative compound.

[2/] Linear retention indices (LRI) on ZB-5 column (from experimental data and 34 authentic compounds), ZB-Wax (30 m long) (from experimental data) and DB-Wax UI (60 m long) column (from 34 authentic compounds and NIST Chemistry WebBook). LRI from experimental data calculated from the retention time of n-alkanes C6-C26. nd stands for not detected.

[3/] Mw = molecular weight of compounds.

[4/] Four main ions were used to confirm compounds identity during auto quant, and ion number one is classified as the primary ion used to integrate the peak area of the compounds.

[5/] Response factors were used to calculate the amount of each compound. The response factor is the "m-factor" from the linear equation ($y = mx + c$) for the compound that was quantified using authentic standard compounds.

[6/] The coefficient of determination is a goodness-of-fit measure for linear regression models, ranging from 0 to 1.

3.4.2 The classes of compounds found in different wood of smoked water samples

Appendix Table A2 shows that when considering the effect of the smoking process on the volatile profile, most of the compounds had the highest concentration in the traditional (TR) smoked water, independent of the type of wood. The concentration of each compound tended to decrease when the PST (P25 and P50) was introduced. The concentration of most compounds decreased when the level of filtering increased, which means the concentration of the components of P25 smoked water was higher than the compounds in P50 smoked water (P50 has been used in more filter plates than P25). Two-way MANOVA and Tukey HSD test were performed to check the significance of the main factors (wood types and processing), interaction effects between the two factors, and the compounds mean difference among nine smoked water samples. In addition, the wood types did not significantly affect the change in the amount of acetovanillone (ATV), syringaldehyde (SD), and 3,5-dimethoxy-4-hydroxytoluene (3,5-DM-4HT), whereas 2-furfural (FF), 2-ethylfuran (2-EF), maltol (MT), and 2-butenal (2-BT) contents were not affected by the manufacturing process. 16 out of 77 compounds were unaffected significantly by the interaction between two independent factors (wood * process). The details of the essential group of compounds found in smoked water samples are given below:

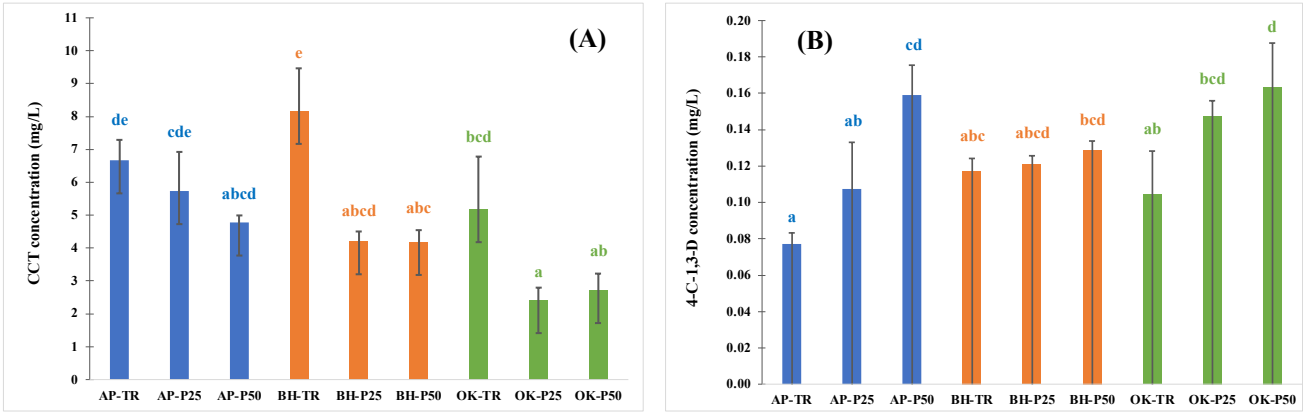

Figure 3.1 (A) cyclotene, (B) 4-cyclopentene-1,3-dione in 9 smoked water samples. The average compound concentrations of three different woodchips (AP = apple, BH = beech, OK = oak) and three different processes (TR = traditional, P25 = PST 25, and P50 = PST 50). Samples not marked with the same letters were significantly different ($p \leq 0.05$).

Aldehydes, ketones and diketones group: there were 9 compounds of this group in smoked water samples (**Appendix Table A2**). From this study, the four compounds with high

concentration were cyclotene, cyclopentanone, maltol and 3-ethyl-2-cyclopenten-1-one. In general, the quantity of all carbonyl compounds dropped as a result of the PST filtering process. Among the carbonyl compounds present in apple-wood smoked water, five out of nine were determined to have the highest concentration compared to the two other wood smoke samples. On the other hand, beech-wood smoked water had the highest amount of cyclotene (**Figure 3.1** (A)) and 3-ethyl-2-hydroxy-2-cyclopenten-1-one. Compounds in the TR sample tended to exhibit considerably greater levels compared to the PST samples ($p \leq 0.05$), with the exception of 4-cyclopentene-1,3-dione (**Figure 3.1** (B)), which had the highest concentration in the PST samples, contradicting all other findings. This compound may have some contamination from other sources rather than a compound genuine to the smoke extracts, which is challenging to elucidate. In addition, all 9 compound contents in P25 were not significantly different from those in P50 samples ($p > 0.05$). Some of the carbonyl components are responsible for the sweet aroma which was created from the pyrolysis of hemicellulose and cellulose of wood (Šimko, 2005).

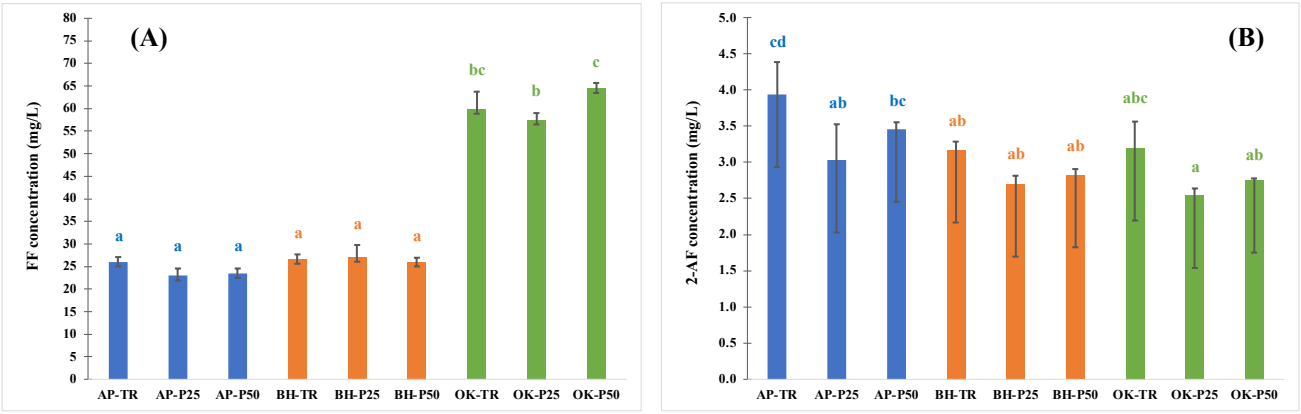

Figure 3.2 Furans derivatives group pattern in (A) 2-furfural, and (B) 2-acetylfuran in 9 smoked water samples. The average compound concentrations of three different woodchips (AP = apple, BH = beech, OK = oak) and three different processes (TR = traditional, P25 = PST 25, and P50 = PST 50). Samples not marked with the same letters were significantly different ($p \leq 0.05$).

Furans group: furans are generated from the pyrolysis combustion of hemicellulose and cellulose. In this study, 9 compounds were found in this group, where the most abundant compounds were 2-furfural, 2-methylfuran, 5-methylfurfural and 2-acetylfuran, respectively. Furfural is the primary product of hemicellulose and cellulose combustion. **Figure 3.2** shows that the PST filtering did not significantly trap these two compounds ($p > 0.05$), except 2-

acetylfuran in apple-wood smoked water. Five out of nine compounds with the highest contents were found in oak-wood smoked water. It is noteworthy that only a small number of these compounds, including 2-acetyl-5-methylfuran, 2-methylfuran, 3-furaldehyde, and 1-(2-furfuryl)-1-propanone, exhibited a significant impact from PST process, specifically in oak-wood smoked water ($p \leq 0.05$).

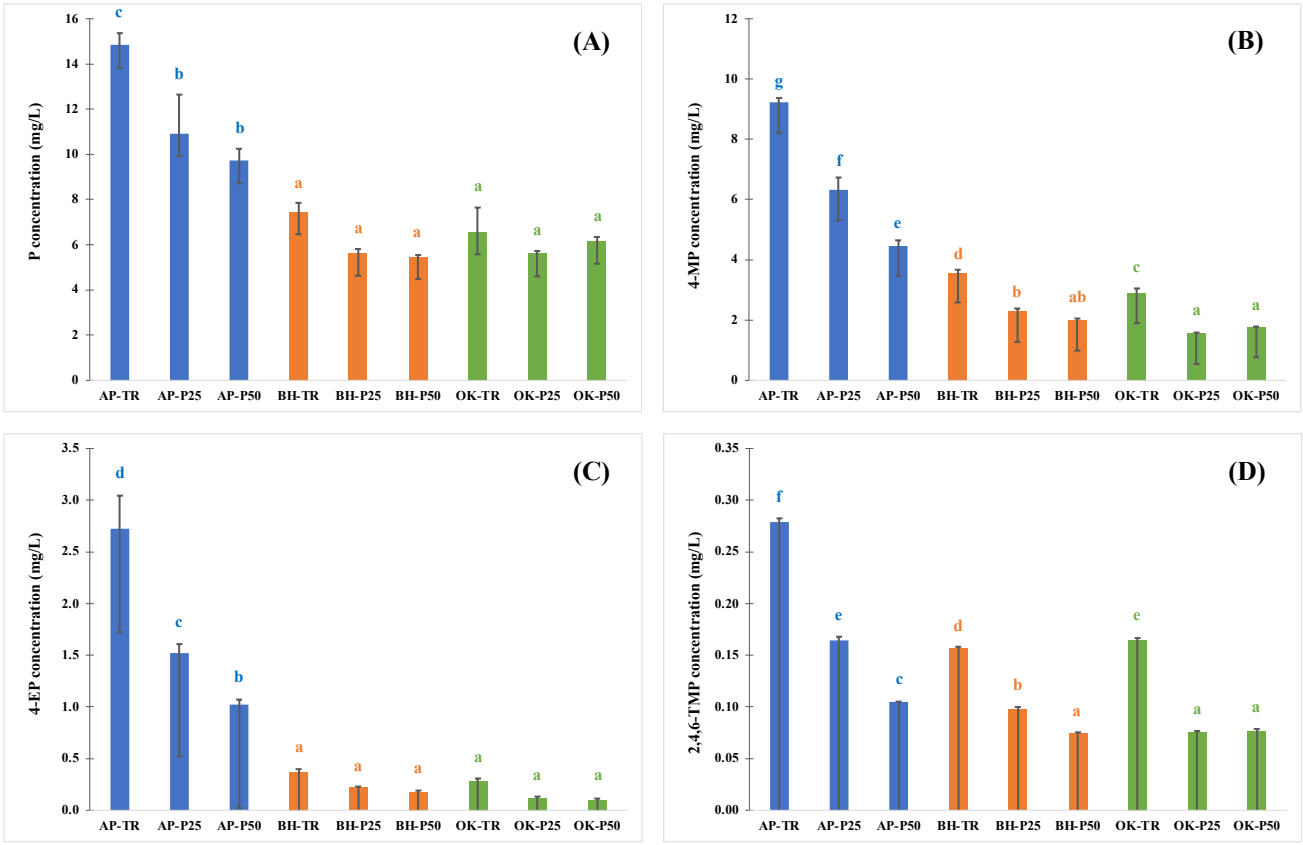

Figure 3.3 Phenol and derivatives group pattern in (A) phenol, (B) methylphenol, (C) ethylphenol, and (D) 2,4,6-Trimethylphenol in 9 smoked water samples. The average compound concentrations of three different woodchips (AP = apple, BH = beech, OK = oak) and three different processes (TR = traditional, P25 = PST 25, and P50 = PST 50) when not marked with the same letters were significantly different ($p \leq 0.05$).

The phenol and derivatives group: is primarily responsible for the phenolic and fragrant notes in smoked water samples, of which 21 compounds were identified. In all the samples analysed (**Appendix Table A2**), 2,5-dimethylphenol had the highest content among other compounds, followed by phenol, 2-methylphenol, 4-methylphenol and 4-ethylphenol. Smoked water produced from apple-wood contains highest concentration of the phenols compared to beech- and oak-wood smoked water, see example compounds of this group in **Figure 3.3**. The

concentration of phenol compounds was greatest in TR smoked water, but it decreased considerably when the PST was introduced, with apple-wood smoked water being the most affected. There were no significant differences seen in the 21 phenol components between P25 and P50 in oak-wood smoked water ($p > 0.05$). However, when excluding oak-wood smoked water, 16 out of 21 components between P25 and P50 showed significant reduction in P50 compared to P25 ($p \leq 0.05$). These phenol compounds come mainly from the thermal decomposition of lignin, providing a desirable smoky flavour (Šimko, 2005) in smoked food products.

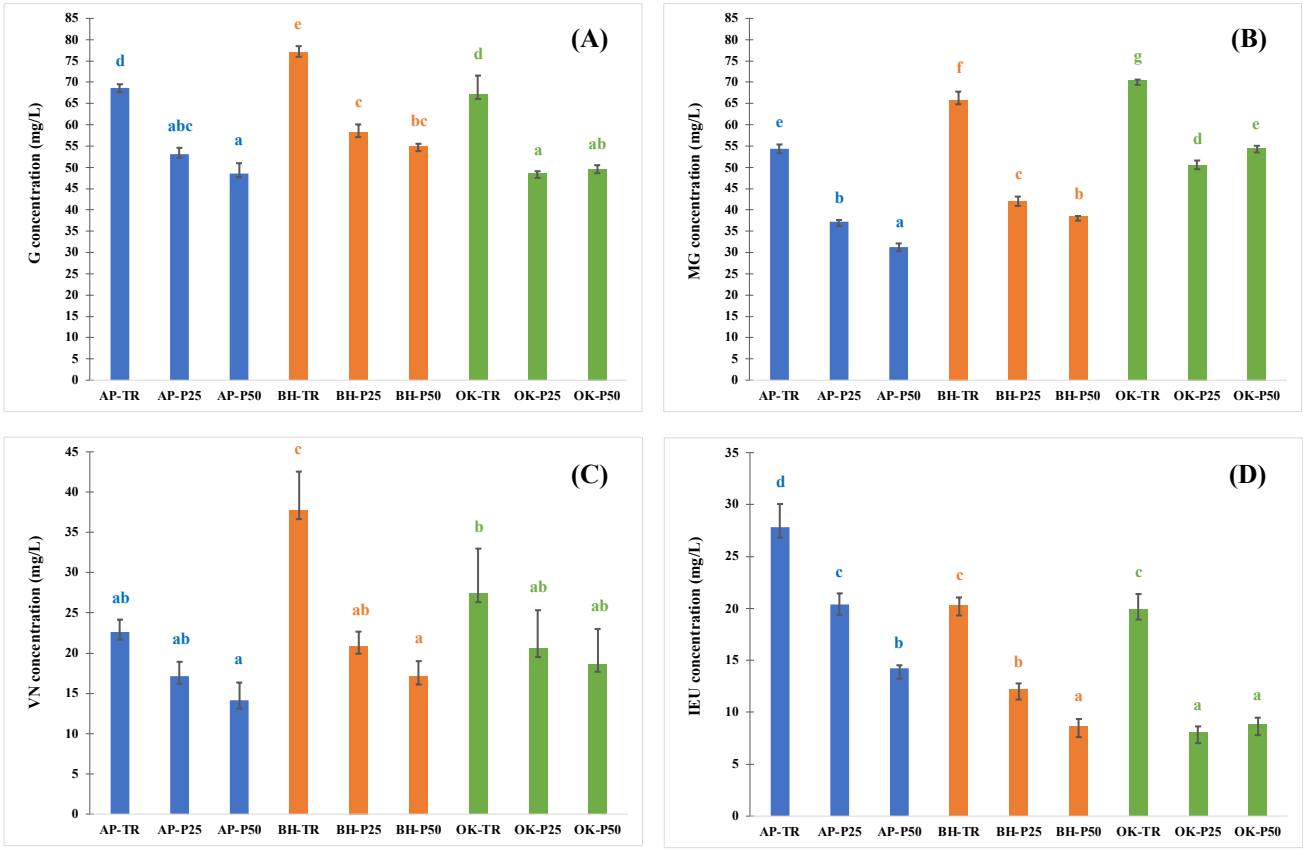

Figure 3.4 Guaiacol and derivatives group pattern in (A) guaiacol, (B) methylguaiacol, (C) vanillin, and (D) isoeugenol in 9 smoked water samples. The average compound concentrations of three different woodchips (AP = apple, BH = beech, OK = oak) and three different processes (TR = traditional, P25 = PST 25, and P50 = PST 50) when not marked with the same letters were significantly different ($p \leq 0.05$).

Guaiacol and derivatives group: is the third most abundant group detected in smoked water samples, accounting for 11 compounds. Guaiacol and derivatives are a product of lignin thermal pyrolysis and are responsible for a smoky fragrant flavour similar to the phenols group

and also provide antioxidant and antimicrobial properties (Lingbeck et al., 2014; Malarut & Vangnai, 2018). In this study, see **Appendix Table A2**, a high intensity compounds of this group that found in smoked water samples were guaiacol, follow by methylguaiacol, isoeugenol, vanillin, and ethylguaiacol, respectively. Four examples of this compound group are shown in **Figure 3.4**; more than half (64%) of this group's compounds were in the highest value in beech-wood smoked water, such as guaiacol and vanillin, while isoeugenol was high in apple-wood smoked water, and methyl guaiacol was rich in oak-wood smoked water. The PST had a significant impact on the quantity of all compounds in this group when compared to the TR smoked water samples ($p \leq 0.05$). The degree of PST filtration also had an effect on the amount of the compounds, with around 64% of compounds between P25 and P50 showing significant differences ($p \leq 0.05$).

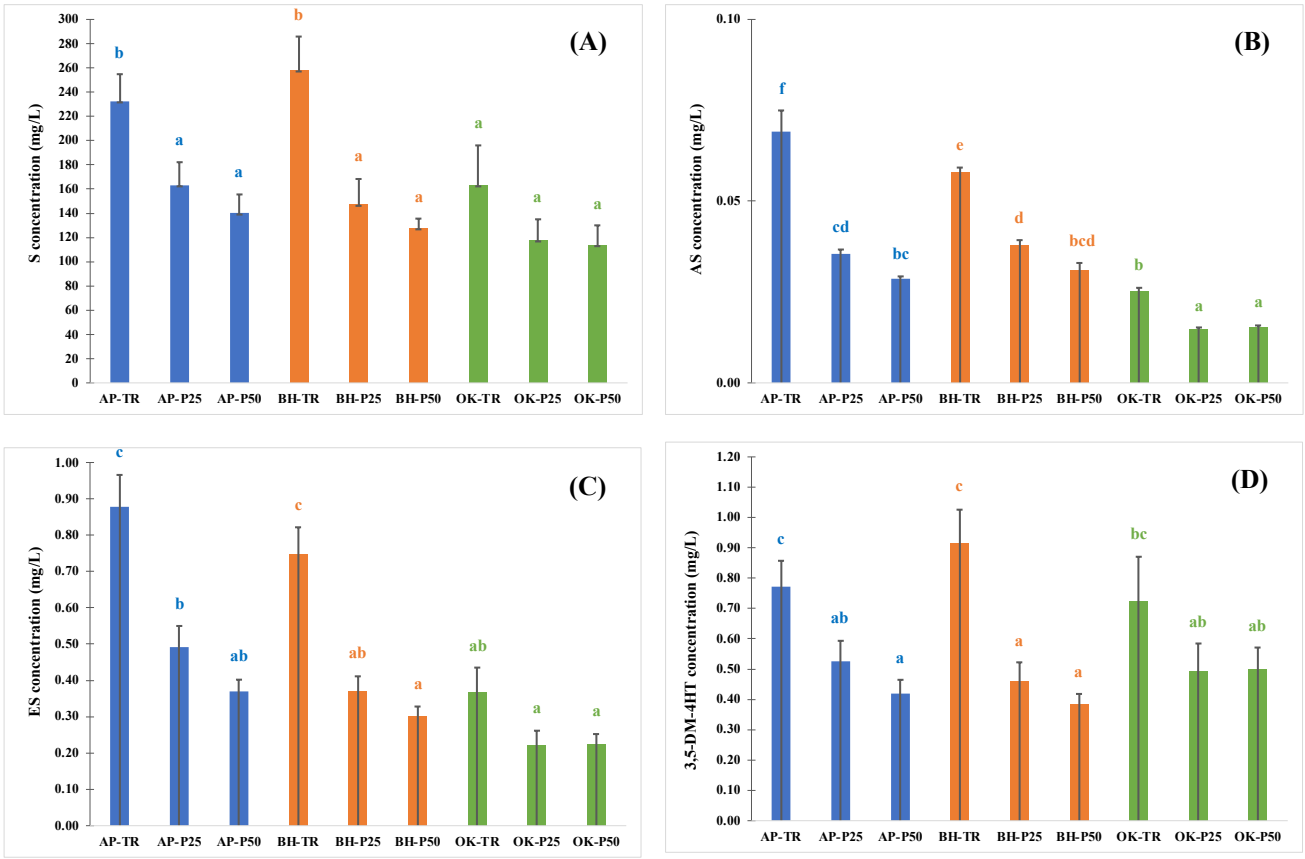

Figure 3.5 Syringol and derivatives group pattern in (A) syringol, (B) acetosyringone, (C) ethylsyringol, and (D) methylsyringol in 9 smoked water samples. The average compound concentrations of three different woodchips (AP = apple, BH = beech, OK = oak) and three different processes (TR = traditional, P25 = PST 25, and P50 = PST 50) when not marked with the same letters were significantly different ($p \leq 0.05$).

Syringol and derivatives group, is generated from the lignin combustion. In this study (**Appendix Table A2**), there were 11 compounds of this group in smoked water samples. The syringol content was very high in all smoked water samples, and the concentration was about 6,000 times higher than the lowest content compound (syringaldehyde). Syringol was the most abundant of the 77 compounds found in smoked water samples. Three compounds were seen highest amounts in beech-wood smoked water, including syringol, and the other eight compounds were highest in apple-wood smoked water. PST process affected the amount of all compounds of this group, as seen by the comparison to the TR smoked water samples ($p \leq 0.05$). This is illustrated in the examples presented in **Figure 3.5**. There was no significant impact of filtration level on the concentrations of any compound between P25 and P50 in oak-wood smoked water ($p > 0.05$). In contrast, the concentrations of two compounds, 3,4-dimethoxytoluene and 3,5-dimethoxytoluene, in apple-wood and beech-wood smoked water varied significantly between P25 and P50 ($p \leq 0.05$).

In addition, smoked water samples contained organic acids. Organic acids are derived from the partial pyrolysis of wood carbohydrates and play a role in the flavour, colour, texture and microbiological stability of foods (Montazeri et al., 2013). The organic acids found in the smoked water were acetic acid, butanoic acid, and crotonic acid, with acetic acid being the most abundant (**Appendix Table A2**). The majority of organic acids have high odour threshold values and contribute little to the overall odour of smoked products (Sokamte tegang et al., 2020).

3.4.3 Effect of smoking processes on retention of the volatile compounds

Table 3.2 showed acids were the most retained after passing through the PST process, followed by furans, ketones and diketones, and guaiacols, which all retained above overall average retention values (66 and 58% for P25 and P50, respectively). Syringol and derivatives, indene derivatives and other phenols were trapped or lost by the PST process more than average. Shown in more detail in **Figure 3.6**, furans (orange bars), and aldehydes, ketones and diketones group (red bars) were less affected by the PST process, particularly furans. Phenols were most affected when PST was introduced. These findings align with those of Parker et al. (2018), who found that all phenols, guaiacols, and syringol in smoke showed a substantial decrease in contents as zeolite weight increased (greater filter), whereas the furans stayed consistent and were not affected by the strength of filter. Zeolites exhibit a sieving role that

separates the compounds according to their size and shape. Compounds larger than zeolites will be filtered out according to the molecular sieving principle. Non-polar molecules exhibit strong adsorption by zeolites due to the electrostatic field in the cavities of zeolites (Abbey, 1996). Phenol and syringol compound groups may have been larger or have non-polar characteristics; in such cases, they would have been captured and passed through the zeolite filter at a reduced rate.

Table 3.2 Percentages of retained compounds in PST smoked water samples compared to TR smoke.

Classes	**Retained compounds compare to TR (%)**	
	P25	**P50**
Acids	78	89
Furans derivatives	79	74
Aldehydes, ketones and diketones	70	66
Other compounds	70	58
Guaiacol and derivatives	68	62
Syringol and derivatives	62	54
Phenol and derivatives	57	46
Indene derivatives	56	48
Overall average retention	***66***	***58***

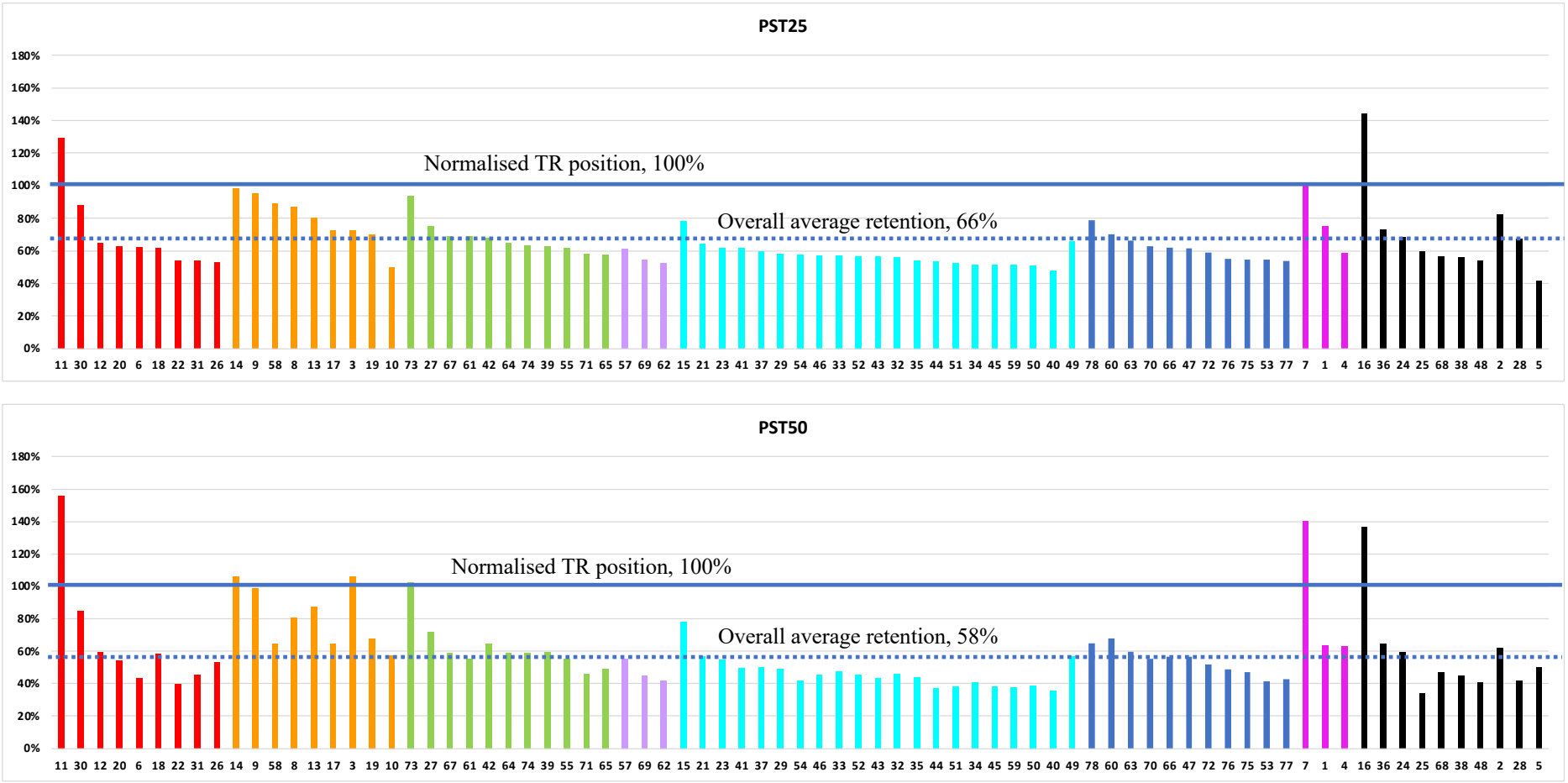

Figure 3.6 Effect of smoking process (PST) on the retention of the compounds classes compared to TR smoked water which was normalised at 100% scale (at blue line position). Where; red bar compounds are aldehydes, ketones and diketones group; orange bar compounds are furans derivatives; green bar compounds are guaiacol and derivatives; purple bar compounds are indene derivatives; bright blue bar compounds are phenol and derivatives; dark blue bar compounds are syringol and derivatives; pink bar compounds are acids; black bar compounds are other compounds. Each compound name of the number given can be found in **Table 3.1**.

3.4.4 The difference aroma among smoked water samples

PCA was carried out on the data (**Figure 3.7**) to visualise graphically the effect of PST and in the three woodchips. Nine smoked water samples and 77 dependent variables (detected compounds) were plotted. The first two principal components accounted for 86% of the variation in the data. The first axis (PC1) represented increased filtering for each of the woods (although OK-50 and OK-25 are reversed) and also showed that in general, AP has more aroma compounds, followed by BH, and OK had the least. The second axis (PC2) mainly discriminated AP-TR and BH-TR, showing AP is particularly associated with the phenols (also seen in the bar charts above, **Figure 3.3**) whilst BH was more associated with guaiacols. The effect of processing for the oak wood was shown to be small compared to other two wood types.

The distribution of the 77 detected compounds were divided into 8 groups which are (1) acids, (2) aldehydes, ketones and diketones, (3) furans derivatives, (4) phenol and derivatives, (5) guaiacol and derivatives, (6) syringol and derivatives, (7) indene derivatives, and (8) other compounds. All phenol and derivatives group compounds (pink colour compounds), all guaiacol and derivatives compounds group (red colour compounds), all syringol and derivatives compounds, all acids compounds, all indene compounds, mostly compounds in aldehydes, ketones and diketones (except 4-C-1,3-D), mostly compounds of other compounds (except BZN), and some compounds in furans derivatives group, were positively correlated with the first axis. Negatively correlated with the second axis was all phenol and derivatives group. AP-TR, AP-25 and BH-TR showed positively correlated with the first axis. AP-TR and AP-P25 were surrounded with all phenol and derivatives compounds, two guaiacol and derivatives (VG and tIEU), and mostly aldehydes, ketones, and diketones group, a few furans (2-EF and 2-AF). BH-TR, positively correlated with all syringol-related compounds, indene compounds, most guaiacols, a few aldehydes, ketones and diketones compounds (including CCT), and a few furans. All oak-wood smoked water (TR, P25, P50), positively correlated with the main compound of furan and pyran derivatives only, particularly FF and 5-MFF, and all three smoked water produced from oak wood were all close together. AP-P50, BH-P25 and BH-P50 were all similar and linked to one compound only.

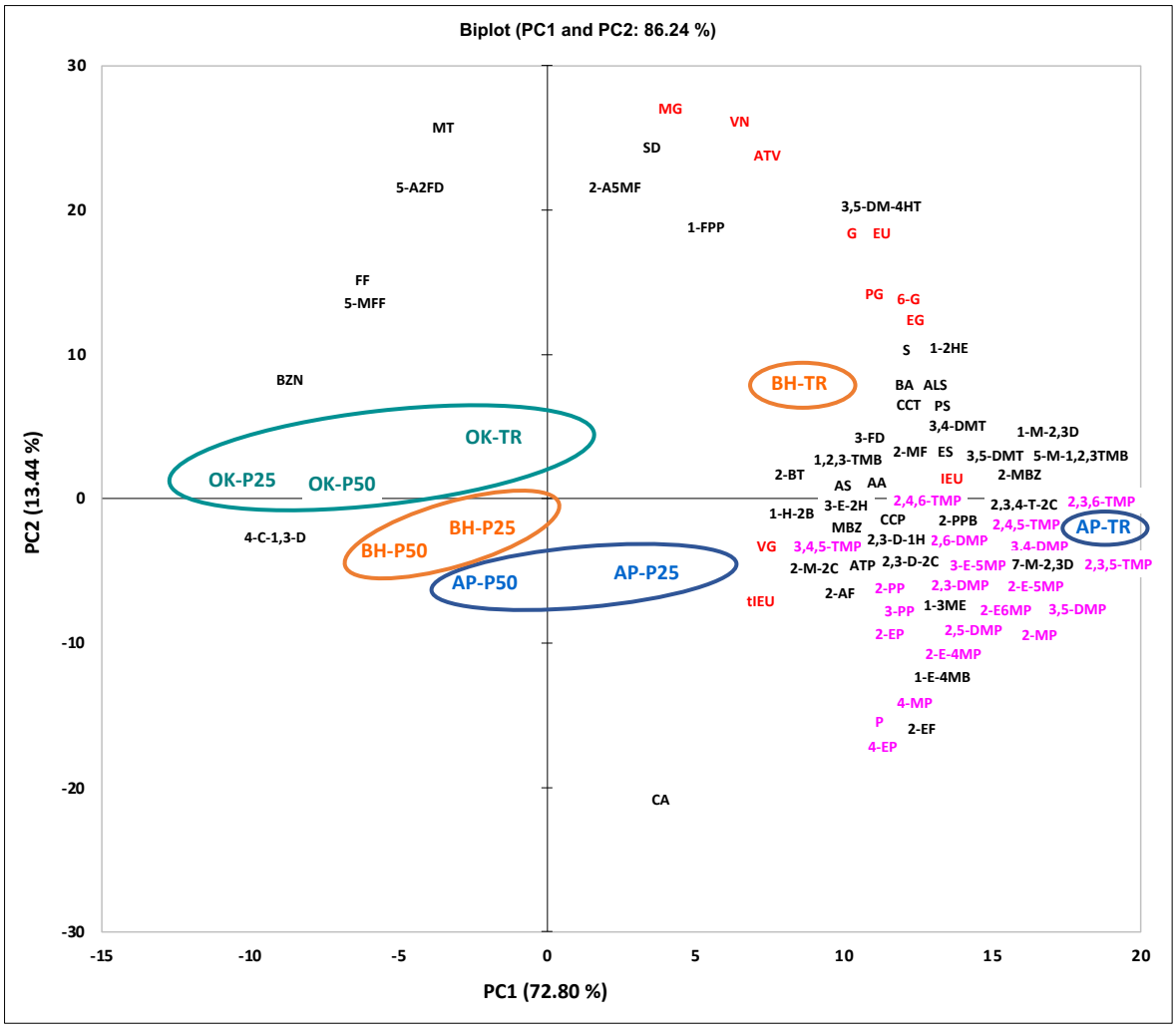

Figure 3.7 Principal component analysis of nine different smoked water samples showing correlation with 77 identified compounds. Where; red compounds are guaiacol and derivatives; pink compounds are phenol and derivatives; black compounds are other compound groups (codes on plot refer to compound codes in **Table 3.1**).

3.4.5 Effect of smoking processes on odour-active volatile compounds retention

In **Chapter 2 (Characterisation of the key odorants in smoked water by means of the sensomics approach)**, at least 22 potent aroma compounds were identified as contributing to the overall flavour of apple-wood smoked water samples. This section investigated whether the PST could affect these 22 potent aroma compounds in freshly produced smoked water. These 22 compounds consisted of 2 furans derivatives (2-furfural; 2-acetyl furan), 9 phenols derivatives (phenol; 2-methylphenol; 4-methylphenol; 2,3-dimethylphenol; 2,6-dimethylphenol; 2,5-dimethylphenol; 2-ethylphenol; 2,4,6-trimethylphenol; 4-ethylphenol), 7 guaiacol derivatives (guaiacol; 4-methylguaiacol; eugenol; vanillin; 4-ethylphenol; 4-

vinylguaiacol; isoeugenol), 1 acids compound (acetic acid), 1 syringols derivatives (syringol), 1 other aromatics (acetophenone), and 1 aldehydes, ketones and diketones group (cyclotene). In **Chapter 2**, the effect of PST, however, could not be concluded because the two apple-wood smoked waters (TR and P50) were too old and were not manufactured using the same batch of woodchips, including the production date period. In this experiment, the TR smoked water was normalised at 100% scale, and the 22 potent aroma compound concentrations from all three wood P50 smoked water were plotted against the TR smoked water. The results are given in **Figure 3.8**. Based on the findings, we might infer that PST had the most impact on phenol derivatives and may retain phenol compounds below 60%. While guaiacols group, acids group, aldehydes, ketones and diketones group had roughly half of the concentration in TR smoked water. The PST process does not appear to impact the furans group.

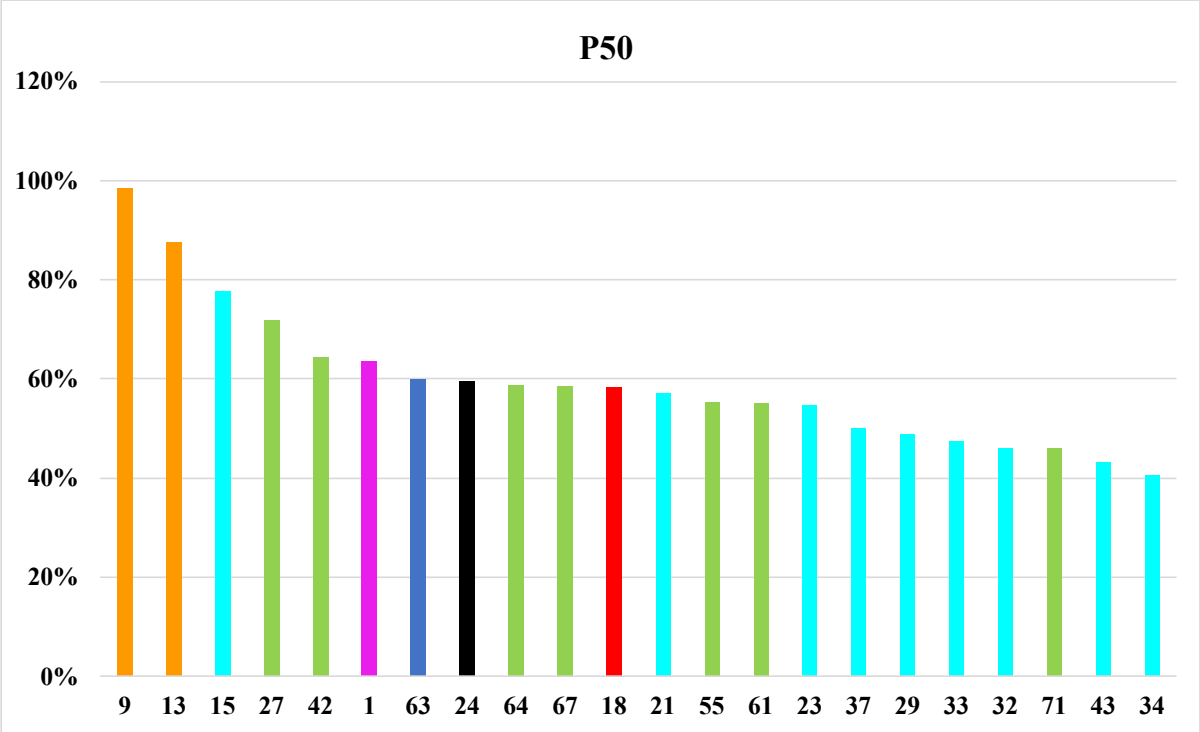

Figure 3.8 Effect of smoking process (PST) on the retention of 22 potent aroma compounds compared to TR smoked water which was normalised at 100% scale. Where; orange bar compounds are furans derivatives; bright blue bar compounds are phenol and derivatives; green bar compounds are guaiacol and derivatives; pink bar compound is acetic acid; dark blue bar compound is syringol; black bar compound is acetophenone; red bar compound is cyclotene. Each compound name of the number given can be found in **Table 3.1**.

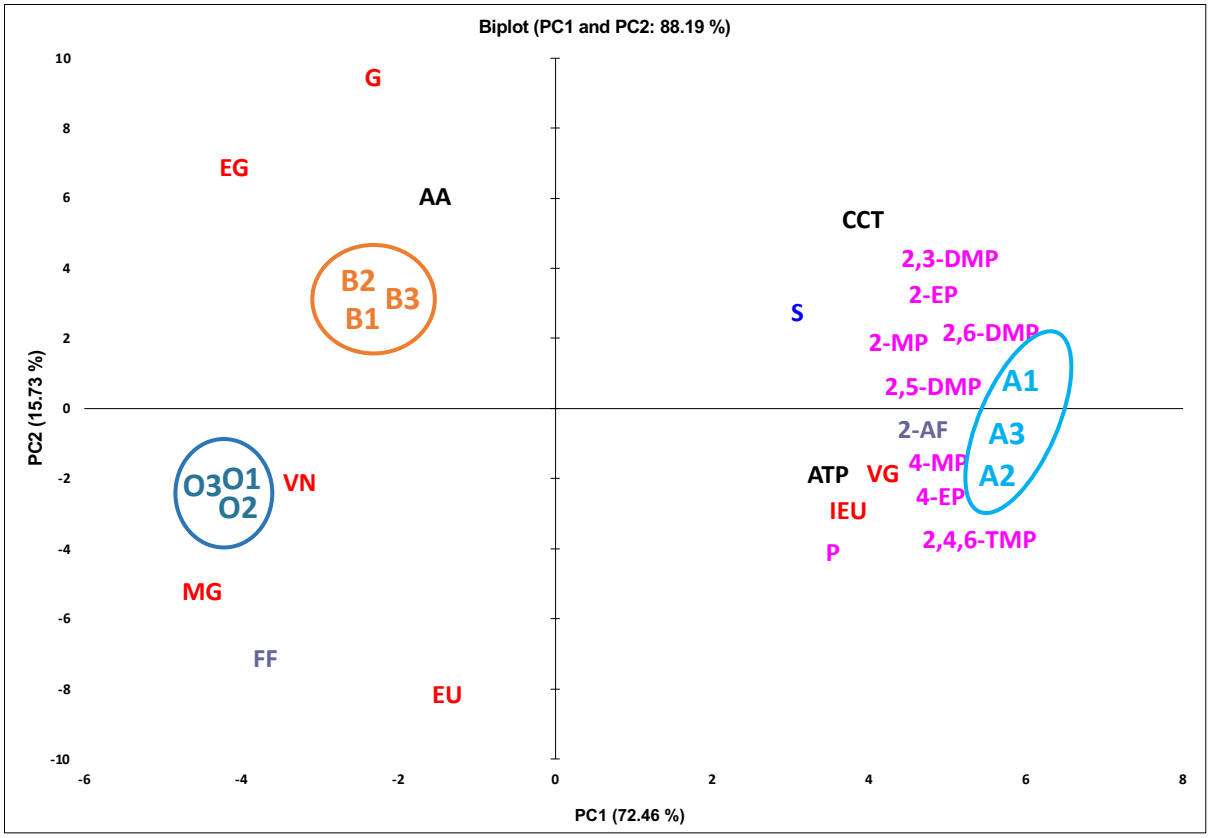

Figure 3.9 Principal component analysis of 22 potent aroma compounds in P50 of three wood-smoked water (A = apple-wood, B = beech-wood, and O = oak-wood smoked water in three replicate values) (codes on plot refer to compound codes in **Table 3.1**).

The distribution of these 22 potent aroma compounds among three types of wood P50 smoked water was plotted in PCA, as shown in **Figure 3.9**. All phenols group (pink colour compounds), some guaiacols, and syringol were related to AP smoked water aroma. This indicated that AP smoked water could be a stronger smoky aroma than BH and OK smoked water. The guaiacol aroma dominates in BH smoked water, while the aroma of OK smoked water will be milder and sweeter than the other wood due to the furans compound.

3.4.6 Effect of different wood

The data of 77 compounds' contents only represent one batch of each wood, so the differences cannot be statistically attributed to the different wood types, as processing day may also have an impact. However, only applewood had two batches and could be plotted for PCA among other wood types. The findings presented in **Figure 3.10** suggest that the degree of variation among the two batches of AP-TR was comparable to that among the various types of

wood. Nevertheless, statistical analysis was not conducted to compare the two batches. This is because all the compounds in the old batch of apple-wood TR smoked water were lower than those in the new batch, except for maltol and syringaldehyde (data not provided). The phenols, guaiacols, and syringols compounds got a reduction of approximately 2-3 times, whereas the furans group got a reduction of approximately 1-2 times in the old batch of applewood. Isoeugenol and 4-vinylguaiacol exhibited the highest reduction, with reductions of roughly 370 and 100 times, respectively. Overall, the old batch of applewood (**Figure 3.10** A4-A6) had far fewer volatiles and this could be attributed to the loss of volatile over times, a milder or shorter smoking process, a different batch of apple woodchips, etc. From these data, it is difficult to draw hard conclusions in relation to the different types of wood. This was a secondary outcome as the main aim was to compare the effect of PST.

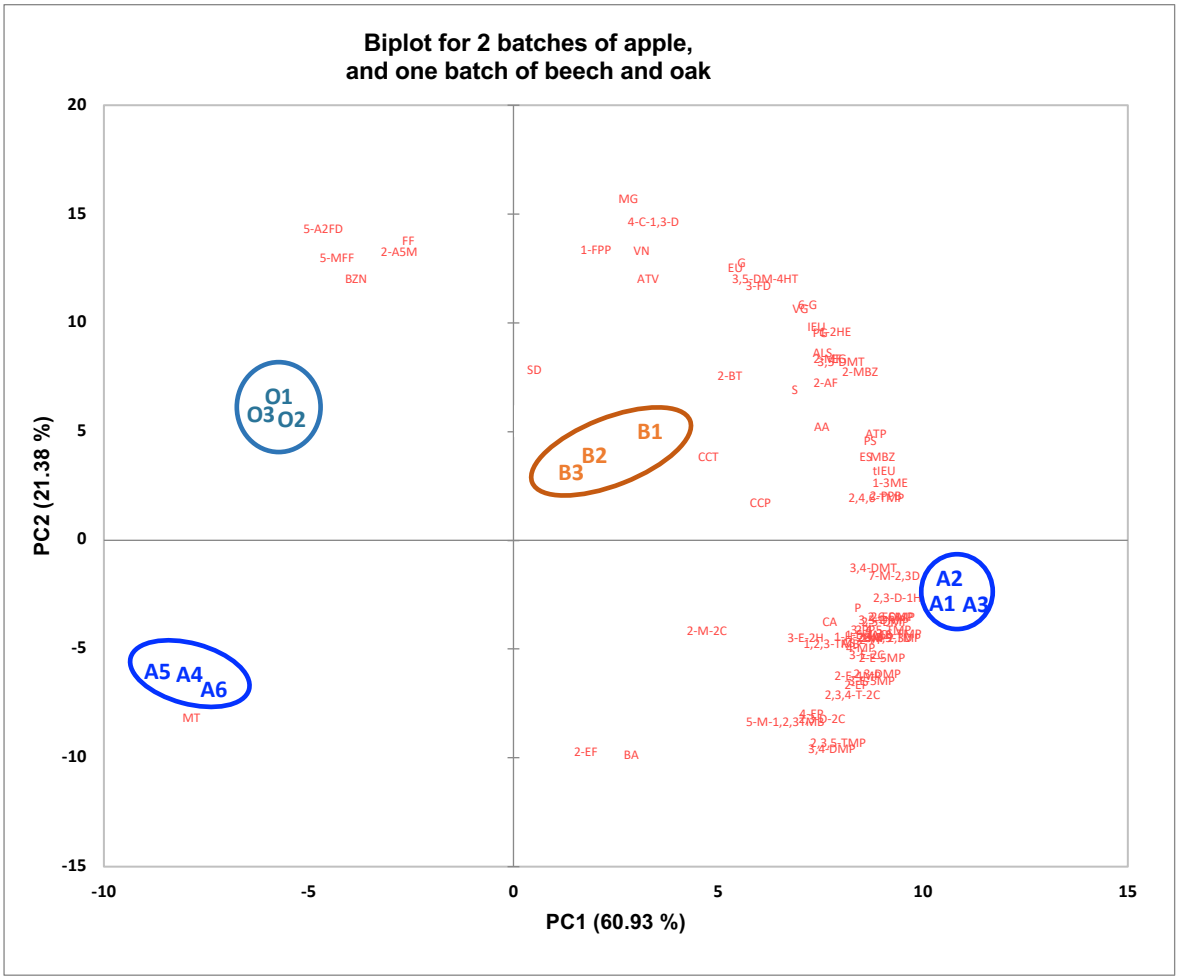

Figure 3.10 Comparison of PCA plots of 77 volatile compounds of 2 batches of apple-wood (A1-A3 were new batch; A4-A6 were old batch), one batch of beech-wood and one batch of oak-wood TR smoked water samples (three replicate).

3.4.7 The organoleptic of smoked water

Table 3.3 summarises a qualitative sensory characteristic of all nine smoked water samples. When the panel sniffed the samples, the "ashy" odour was consistently described in all TR smoked water. When PST was introduced, the ashy odour was changed from "ashy" to "slightly/mildly ashy" in BH smoked water and OK smoked water, whereas in AP, the ashy odour was replaced by a fragrant odour. The ashy note could be related to the phenols compound group, as the PCA plot in **Figure 3.7** showed that AP smoked water was the most related to the phenols group, when PST was used, the concentration of phenolic groups was reduced, which could have a much greater effect in AP smoked water. Because the concentration of the phenols compound has changed, the ashy note has been changed to fragrant. Moreover, the findings from the taste testing consistently showed that the description "astringent and acrid taste" was present in all TR smoked water samples, but was absent in PST smoked water samples.

According to **Figure 3.7**, the PCA plot showed AP-TR and AP-P25 smoked water had positively correlated with all compounds of phenol and derivatives group and mostly aldehydes, ketones, and diketones group. Phenol and derivatives are accountable for a desirable smoky flavour and act as antimicrobial and antioxidant compounds (Lingbeck et al., 2014; Malarut & Vangnai, 2018), whereas carbonyl compounds (aldehydes, ketones, furan and pyran derivatives) provide odour and flavours with sweet, caramel and magi (instant broth), which mellow the smoky harshness from phenolics compounds in liquid smoke (Kostyra & Baryłko-Pikielna, 2006). In terms of smoke flavour from phenolics compounds, it is not a single characteristic but has been described in different categories such as smoky, woody, musty, dusty, ashy, acrid, pungent and others (Wang & Chambers, 2018). Among phenols and derivatives compounds found in smoked water, 2,5-dimethylphenol (2,5-DMP) is the dominant one, and could play an important role in smoked water in corresponding with other phenols derivatives such as 4-methylphenol (4-MP) and 2-methylphenol (2-MP). Wang and Chambers (2018) described 2,5-DMP as smoky, woody, musty/dusty, pungent, cedar, burnt, acrid, petroleum-like in similar character with 4-MP and 2-MP, including the "ashy". However, some research detailed 4-MP as "animal, spicy, burnt" and 2-MP as "chemical, spicy, burnt" (Varlet et al., 2007).

Table 3.3 The qualitative sensory characteristics of nine smoked water (data from Besmoke Ltd.).

Samples	**Description**		
	Appearance	**Odour**	**Taste**
Apple smoked water (AP-TR)	Light yellow or golden-yellow clear liquid. Darkening and evolving towards red tones during storage.	Sweet, fruity, smoky. Intense apple notes. Slightly **ashy**. Green notes may be present.	Acidic, smoky, ashy slightly **astringent, and acrid** taste. Fruity notes.
PST25 Apple smoked water (AP-P25)	Light yellow or golden-yellow clear liquid. Darkening and evolving towards red tones during storage.	Fragrant, sweet, fruity, smoky.	Mildly acidic, smoky, fruity; slightly ashy, some green notes may be present.
PST50 Apple smoked water (AP-P50)	Light yellow or golden-yellow clear liquid. Darkening and evolving towards red tones during storage.	Fragrant, sweet, fruity, smoky.	Mildly acidic, smoky, fruity; slightly ashy, some green notes may be present. Mild ashy notes may be present
Beech smoked water (BH-TR)	Light yellow or golden-yellow clear liquid. Darkening and evolving towards red tones during storage.	Intense, smoky, woody, **ashy**, bacon-like.	Acidic, woody, smoky, ashy, **acrid and astringent**, bacon-like notes.
PST25 Beech smoked water (BH-P25)	Light yellow or golden-yellow clear liquid. Darkening and evolving towards red tones during storage.	Woody, smoky, **slightly ashy**, bacon-like.	Mildly acidic, woody, smoky, slightly ashy, bacon-like.
PST50 Beech smoked water (BH-P50)	Light yellow or golden-yellow clear liquid. Darkening and evolving towards red tones during storage.	Woody, smoky, **mildly ashy,** bacon-like.	Mildly acidic, woody, smoky, mildly ashy, bacon-like.
Oak smoked water (OK-TR)	Light yellow or golden-yellow clear liquid. Darkening and evolving towards red tones during storage.	Intensely smoky, woody, **ashy.**	Acidic, woody, smoky, ashy, **acrid and astringent,** bacon-like notes.

Samples	Description		
	Appearance	**Odour**	**Taste**
PST25 Oak smoked water (OK-P25)	Light yellow or golden-yellow clear liquid. Darkening and evolving towards red tones during storage	Rich, woody, smoky, bacon-like, **slightly ashy**.	Mildly acidic, smoky, woody, bacon-like, slightly ashy.
PST50 Oak smoked water (OK-P50)	Light yellow or golden-yellow clear liquid. Darkening and evolving towards red tones during storage	Rich, woody, smoky, bacon-like, **mildly ashy**.	Mildly acidic, smoky, woody, bacon-like, slightly ashy.

In the case of BH-TR in the PCA plot (**Figure 3.7**), which this smoked water mainly positively correlated with all syringol and derivatives compounds group and mostly guaiacol and derivatives compounds group. Syringol (S) was the most abundant compound found in smoked water samples, followed by guaiacol (G) among these two compounds groups. Syringol and guaiacol contents in smoked water samples were related for each other as both were derived from the pyrolysis combustion of lignin. The concentration of syringol was about 3 times higher than guaiacol which had the same trend of the liquid smoke that produced from hardwood (Guillen et al., 1995). These two compounds group responsible for the smoked flavour in smoked product. Regarding to the odour attributes definition; syringol has the characteristics of smoky, woody, pungent, musty/ dusty (Wang & Chambers, 2018), burn rubber, spicy (Varlet et al., 2007). Guaiacol and derivatives such as 4-methylguaiacol that contained in high amount in smoked water were described in similar characteristic such as smoked and spicy (Varlet et al., 2007). Apart from syringols and guaiacols groups, some compound in aldehyde, ketones and diketones, particularly cyclotene (CCT) was also positive related with BH-TR and it was describe maple syrup (Sokamte tegang et al., 2020).

For the OK smoked water (TR, P25, P50), as it showed positively correlated with the main compound of furan and pyran derivatives only (**Figure 3.7**), particularly 2-furfural (FF) and 5-methylfurfural (5-MFF) and all three smoked water produced from oak-wood were all close together. Furan and pyran compounds have important role in the overall aroma and flavour of smoke products and also help to mellow the harshness aroma from phenolics

compounds. FF was described as “smoke, green” and 5-MFF was described as “cooked, earthy, green” (Varlet et al., 2007).

3.5 Summary

In summary, the results obtained from this study reveal the volatile profiles change when the smoked water is prepared using PST. All nine smoked water samples that produced from three different kinds of wood (apple, beech and oak) and three different manufacturing types (TR, P25 and P50) contain certain types of identical volatile compounds, but the amount was varying regarding to the proportion between the main compositions of wood type and manufacturing effect, which were accounting for 77 identified compounds. Thirty-four out of seventy-seven compounds were quantified using authentic odorant standard compounds, and the remaining compounds were semi-quantified. The 77 identified compounds were divided into 8 groups according to the chemical classes. Phenol and derivatives compounds group were the most abundant compounds found in all smoked water samples, followed by aldehydes, ketones, diketones group (carbonyl-contained group) and guaiacol and derivatives group, respectively. Mostly compounds found in smoked water samples were reduced when the PST was applied. The magnitude of difference between P25 and P50 was much smaller than between TR and P25. In addition, some compound such as 2-furfural was not affected by the manufacturing process. The principal component analysis (PCA) was used to visualise graphically the differences in volatile concentrations in all nine smoked water samples, which classified the smoked water samples into a few groups. There were 4 groups discriminated, AP-TR and AP-P25 were the first group that related with phenol and derivatives, BH-TR was individual sample that related to syringol and derivatives, and guaiacol and derivatives group. All three OK smoked water samples were the most similar and linked with furans derivatives such as 2-furfural and 5-methyl furfural. This suggested that PST was affected to the content of compounds mostly in BH smoked water, whereas not much effect in OK smoked water. Because the PST process reduced the phenol compounds, resulting in the loss of harsher smoke-related aroma, PST smoked water could be rounder/milder and have more aroma balance than TR smoked water.

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3.7 Appendix

Table A1 The linear equation of 34 authentic standard compounds.

No.	Authentic standard compound	Linear equation	R^2
1	Acetic acid	Y = 0.0028X	0.9130
3	2-Methylfuran	Y = 0.0084X	0.9934
6	Cyclopentanone	Y = 0.0100X	0.8908
8	3-Furfural	Y = 0.1636X	0.9204
9	2-Furfural	Y = 0.2294X	0.9898
12	2-Methyl-2-cyclopenten-1-one	Y= 0.1441X	0.9893
13	2-Acetylfuran	Y = 0.1870X	0.9908
15	Phenol	Y = 0.0673X	0.9853
18	Cyclotene	Y = 0.0052X	0.9944
19	2-Acetyl-5-methylfuran	Y = 0.1911X	0.9945
21	2-Methylphenol	Y = 0.0859X	0.9998
23	4-Methylphenol	Y = 0.1734X	0.9950
24	Acetophenone	Y = 0.4741X	0.9977
25	2-Methyl benzaldehyde	Y = 0.9984X	0.9925
27	Guaiacol	Y = 0.0521X	0.9999
28	Methyl benzoate	Y = 0.1877X	0.9885
29	2,6-Dimethylphenol	Y = 0.5841X	0.9944
30	Maltol	Y = 0.0015X	0.9455
31	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	Y = 0.0088X	0.9991
32	2-Ethylphenol	Y = 0.4931X	0.9964
33	2,5-Dimethylphenol	Y = 0.0741X	0.9992
34	4-Ethylphenol	Y = 0.4499X	0.9965
37	2,3-Dimethylphenol	Y = 0.1506X	0.9998
42	4-Methylguaiacol	Y = 0.0494X	0.9995
43	2,4,6-Trimethylphenol	Y = 0.8789X	0.9870
53	3,5-Dimethoxytoluene	Y = 0.2177X	0.9945
55	4-Ethylguaiacol	Y = 0.5995X	0.9958
60	1,2,3-Trimethoxybenzene	Y = 0.2116X	0.9971
61	4-Vinylguaiacol	Y = 0.1554X	0.9657
63	Syringol	Y = 0.0036X	0.994
64	Eugenol	Y = 0.1690X	0.9662
67	Vanillin	Y = 0.0013X	0.9709
71	Isoeugenol	Y = 0.0366X	0.978
74	Acetovanillone	Y = 0.0481X	0.9907

Table A2 Volatile compounds contents identified by GC-MS by SPME in smoked water samples that produced from different kinds of woodchips and manufacturing.

Peak No.	[1/] Compounds	[2/] Concentrations (mg/L)											
		Apple wood			Beech wood			Oak wood			P-value		
		TR	P25	P50	TR	P25	P50	TR	P25	P50	Wood (W)	Process (P)	W * P
Acids (3)													
1	* Acetic acid	217 b ± 18.2	151 ab ± 36.0	92.2 a ± 10.8	153 ab ± 34.9	121 a ± 30.0	116 a ± 37.6	135 ab ± 61.6	104 a ± 23.5	96.9 a ± 2.88	0.048	0.001	0.174
4	§ Butanoic acid (butyric acid)	0.009 b ± 0.001	0.005 ab ± 0.002	0.004 ab ± 0.002	0.009 b ± 0.001	0.005 ab ± 0.001	0.005 ab ± 0.002	0.004 ab ± 0.002	0.003 a ± 0.003	0.004 ab ± 0.002	0.021	0.003	0.261
7	! 2-Butenoic acid (crotonic acid)	0.009 bc ± 0.001	0.011 c ± 0.001	0.011 c ± 0.001	0.007 ab ± 0.001	0.005 a ± 0.001	0.007 abc ± 0.001	0.006 a ± 0.001	0.007 ab ± 0.002	0.009 abc ± 0.001	<0.001	0.042	0.159
Aldehydes, ketones and diketones (9)													
6	* Cyclopentanone	5.91 c ± 2.54	4.94 bc ± 1.77	2.33 ab ± 0.204	4.24 abc ± 0.431	2.92 abc ± 0.288	2.30 ab ± 0.316	3.68 abc ± 0.680	1.26 a ± 0.526	1.37 a ± 0.120	0.001	<0.001	0.284
11	! 4-Cyclopentene-1,3-dione	0.077 a ± 0.006	0.108 ab ± 0.026	0.159 cd ± 0.017	0.117 abc ± 0.007	0.121 abcd ± 0.005	0.129 bcd ± 0.005	0.104 ab ± 0.024	0.148 bcd ± 0.008	0.164 d ± 0.024	0.016	<0.001	0.012
12	* 2-Methyl-2-cyclopenten-1-one	1.92 b ± 0.748	1.48 b ± 0.306	1.36 ab ± 0.122	1.65 b ± 0.093	1.26 ab ± 0.068	1.09 ab ± 0.140	1.32 ab ± 0.279	0.546 a ± 0.019	0.562 a ± 0.020	<0.001	<0.001	0.827
18	* 3-Methyl-1,2-cyclopentanedione (cyclotene)	6.66 de ± 0.634	5.74 cde ± 1.18	4.78 abcd ± 0.209	8.17 e ± 1.29	4.20 abcd ± 0.300	4.18 abc ± 0.360	5.18 bcd ± 1.61	2.42 a ± 0.377	2.72 ab ± 0.500	<0.001	<0.001	0.066
20	! 2,3-Dimethyl-2-cyclopenten-1-one	0.343 e ± 0.026	0.252 cd ± 0.032	0.198 bc ± 0.006	0.275 d ± 0.004	0.186 b ± 0.014	0.155 b ± 0.016	0.191 b ± 0.033	0.095 a ± 0.005	0.090 a ± 0.002	<0.001	<0.001	0.242
22	§ 2,3,4-Trimethyl-2-cyclopenten-1-one	0.317 e ± 0.032	0.188 c ± 0.022	0.132 b ± 0.004	0.236 d ± 0.005	0.148 bc ± 0.013	0.110 b ± 0.003	0.146 bc ± 0.019	0.059 a ± 0.001	0.052 a ± 0.001	<0.001	<0.001	0.001

Peak No.	[1/] Compounds	[2/] Concentrations (mg/L)											
		Apple wood			Beech wood			Oak wood			P-value		
		TR	P25	P50	TR	P25	P50	TR	P25	P50	Wood (W)	Process (P)	W * P
26	[!] 3-Ethyl-2-cyclopenten-1-one	0.109 e ± 0.006	0.065 c ± 0.010	0.058 bc ± 0.004	0.086 d ± 0.004	0.051 bc ± 0.006	0.043 b ± 0.002	0.051 bc ± 0.008	0.025 a ± 0.002	0.026 a ± 0.001	<0.001	<0.001	0.014
30	[*] 3-Hydroxy-2-methyl-4H-pyran-4-one (maltol)	2.40 ab ± 0.691	1.80 a ± 0.212	1.72 a ± 0.183	4.03 c ± 0.295	3.10 abc ± 0.614	2.73 abc ± 0.800	3.58 bc ± 0.272	4.04 c ± 0.065	4.11 c ± 0.754	<0.001	0.140	0.055
31	[*] 3-Ethyl-2-hydroxy-2-cyclopenten-1-one	2.09 e ± 0.268	1.35 d ± 0.100	1.02 cd ± 0.091	2.16 e ± 0.355	0.966 bcd ± 0.162	0.850 bc ± 0.054	0.500 ab ± 0.110	0.261 a ± 0.055	0.245 a ± 0.027	<0.001	<0.001	<0.001
Furans derivatives (9)													
3	[*] 2-Methylfuran	26.4 ef ± 1.98	23.7 de ± 1.79	13.3 bc ± 0.620	28.9 f ± 4.21	20.3 d ± 1.66	8.41 ab ± 0.622	14.2 c ± 0.516	7.99 a ± 0.102	7.06 a ± 0.500	<0.001	<0.001	<0.001
8	[*] 3-Furancarboxaldehyde (3-furaldehyde)	1.05 cd ± 0.052	1.02 cd ± 0.101	0.930 bc ± 0.043	1.14 d ± 0.064	1.02 cd ± 0.081	0.91 bc ± 0.026	0.993 cd ± 0.118	0.739 ab ± 0.015	0.717 a ± 0.011	<0.001	<0.001	0.098
9	[*] 2-Furancarboxaldehyde (2-furfural)	26.0 a ± 1.07	23.0 a ± 1.63	23.5 a ± 1.14	26.6 a ± 1.11	27.0 a ± 2.70	26.0 a ± 1.04	59.8 bc ± 3.93	57.4 b ± 1.63	64.4 c ± 1.30	<0.001	0.077	0.009
10	[!] 2-Ethylfuran	0.018 a ± 0.012	0.015 a ± 0.007	0.014 a ± 0.010	0.009 a ± 0.001	0.007 a ± 0.001	0.006 a ± 0.001	0.009 a ± 0.001	0.003 a ± 0.001	0.005 a ± 0.001	0.004	0.290	0.983
13	[*] 1-(2-Furanyl)-ethanone (2-acetylfuran)	3.94 cd ± 0.447	3.03 ab ± 0.494	3.45 bc ± 0.101	3.17 ab ± 0.113	2.69 ab ± 0.122	2.82 ab ± 0.082	3.19 abc ± 0.370	2.54 a ± 0.100	2.75 ab ± 0.023	<0.001	<0.001	0.711
14	[§] 5-Methyl-2-furancarboxaldehyde (5-methylfurfural)	1.55 a ± 0.151	1.40 a ± 0.130	1.50 a ± 0.072	1.67 a ± 0.123	1.80 a ± 0.070	1.66 a ± 0.025	4.97 b ± 0.530	4.79 b ± 0.100	6.08 c ± 0.161	<0.001	<0.001	<0.001
17	[!] 1-(2-Furanyl)-1-propanone	0.643 d ± 0.015	0.469 abc ± 0.027	0.394 a ± 0.019	0.564 cd ± 0.008	0.455 ab ± 0.025	0.395 a ± 0.016	0.846 e ± 0.087	0.521 bc ± 0.011	0.532 bc ± 0.013	<0.001	<0.001	<0.001
19	[*] 2-Acetyl-5-methylfuran	0.509 c ± 0.022	0.342 a ± 0.070	0.348 a ± 0.027	0.493 bc ± 0.046	0.383 ab ± 0.016	0.353 a ± 0.020	0.784 d ± 0.079	0.515 c ± 0.006	0.505 c ± 0.005	<0.001	<0.001	0.023

Peak No.	[1/] Compounds	[2/] Concentrations (mg/L)											
		Apple wood			Beech wood			Oak wood			P-value		
		TR	P25	P50	TR	P25	P50	TR	P25	P50	Wood (W)	Process (P)	W * P
58	[!] 5-Acetoxymethyl-2-furaldehyde	0.043 ab ± 0.005	0.035 a ± 0.004	0.032 a ± 0.004	0.088 b ± 0.009	0.067 ab ± 0.011	0.043 ab ± 0.003	0.187 c ± 0.041	0.166 c ± 0.021	0.142 c ± 0.021	<0.001	0.003	0.462
Phenol and derivatives (21)													
15	[*] Phenol	14.8 c ± 0.542	10.9 b ± 1.71	9.72 b ± 0.516	7.45 a ± 0.389	5.62 a ± 0.196	5.48 a ± 0.060	6.57 a ± 1.06	5.61 a ± 0.116	6.17 a ± 0.178	<0.001	<0.001	<0.001
21	[*] 2-Methylphenol (*o*-cresol)	10.8 g ± 0.221	7.49 f ± 0.510	5.37 d ± 0.202	6.27 e ± 0.143	4.25 c ± 0.139	3.74 bc ± 0.075	5.12 d ± 0.383	2.88 a ± 0.057	3.19 ab ± 0.054	<0.001	<0.001	<0.001
23	[*] 4-Methylphenol (*p*-cresol)	9.23 g ± 0.155	6.32 f ± 0.420	4.47 e ± 0.168	3.58 d ± 0.086	2.28 b ± 0.095	1.98 ab ± 0.062	2.90 c ± 0.160	1.54 a ± 0.032	1.76 a ± 0.021	<0.001	<0.001	<0.001
29	[*] 2,6-Dimethylphenol	0.67 f ± 0.005	0.410 e ± 0.030	0.303 cd ± 0.006	0.420 e ± 0.005	0.273 c ± 0.003	0.210 b ± 0.012	0.332 d ± 0.017	0.162 a ± 0.003	0.172 a ± 0.004	<0.001	<0.001	<0.001
32	[*] 2-Ethylphenol	0.635 g ± 0.013	0.374 f ± 0.018	0.263 d ± 0.007	0.315 e ± 0.008	0.199 c ± 0.008	0.155 b ± 0.002	0.202 c ± 0.016	0.093 a ± 0.003	0.088 a ± 0.003	<0.001	<0.001	<0.001
33	[*] 2,5-Dimethylphenol	16.9 g ± 0.250	9.90 f ± 0.452	6.72 d ± 0.224	8.69 e ± 0.191	5.45 c ± 0.110	4.29 b ± 0.066	6.76 d ± 0.146	3.37 a ± 0.118	3.58 a ± 0.017	<0.001	<0.001	<0.001
34	[*] 4-Ethylphenol	2.72 d ± 0.321	1.52 c ± 0.089	1.02 b ± 0.050	0.366 a ± 0.035	0.217 a ± 0.014	0.175 a ± 0.013	0.286 a ± 0.021	0.113 a ± 0.021	0.096 a ± 0.018	<0.001	<0.001	<0.001
35	[!] 3,5-Dimethylphenol	0.600 e ± 0.011	0.324 d ± 0.042	0.218 c ± 0.022	0.293 d ± 0.023	0.195 bc ± 0.012	0.146 ab ± 0.012	0.228 c ± 0.005	0.102 a ± 0.003	0.104 a ± 0.004	<0.001	<0.001	<0.001
37	[*] 2,3-Dimethylphenol	1.16 e ± 0.026	0.669 d ± 0.030	0.477 c ± 0.010	0.708 d ± 0.022	0.463 c ± 0.016	0.369 b ± 0.010	0.462 d ± 0.017	0.258 a ± 0.002	0.265 a ± 0.002	<0.001	<0.001	<0.001
40	[!] 2-Ethyl-6-methylphenol	0.176 e ± 0.001	0.093 d ± 0.006	0.060 c ± 0.001	0.096 d ± 0.002	0.059 c ± 0.001	0.041 b ± 0.001	0.057 c ± 0.002	0.022 a ± 0.001	0.019 a ± 0.001	<0.001	<0.001	<0.001

Peak No.	[1/] Compounds	[2/] Concentrations (mg/L)											
		Apple wood			Beech wood			Oak wood			P-value		
		TR	P25	P50	TR	P25	P50	TR	P25	P50	Wood (W)	Process (P)	W * P
41	[!] 3,4-Dimethylphenol	0.227 f ± 0.006	0.136 e ± 0.003	0.095 cd ± 0.004	0.142 e ± 0.002	0.090 c ± 0.002	0.073 b ± 0.002	0.101 d ± 0.003	0.062 a ± 0.005	0.061 a ± 0.002	<0.001	<0.001	<0.001
43	[*] 2,4,6-Trimethylphenol	0.279 f ± 0.004	0.164 e ± 0.003	0.105 c ± 0.001	0.156 d ± 0.002	0.097 b ± 0.002	0.075 a ± 0.001	0.165 e ± 0.002	0.075 a ± 0.002	0.076 a ± 0.002	<0.001	<0.001	<0.001
44	[!] 2-Propylphenol	0.066 e ± 0.002	0.032 d ± 0.002	0.023 c ± 0.001	0.032 d ± 0.002	0.020 c ± 0.001	0.014 b ± 0.001	0.022 c ± 0.001	0.011 a ± 0.001	0.009 a ± 0.001	<0.001	<0.001	<0.001
45	[!] 2-Ethyl-4-methylphenol	0.154 g ± 0.005	0.084 f ± 0.001	0.055 e ± 0.002	0.060 e ± 0.002	0.035 c ± 0.001	0.026 b ± 0.001	0.048 d ± 0.002	0.020 ab ± 0.001	0.017 a ± 0.001	<0.001	<0.001	<0.001
46	[!] 2-Ethyl-5-methylphenol	0.112 e ± 0.006	0.058 d ± 0.001	0.038 c ± 0.001	0.063 d ± 0.002	0.040 c ± 0.001	0.029 b ± 0.001	0.037 c ± 0.001	0.018 a ± 0.001	0.016 a ± 0.001	<0.001	<0.001	<0.001
49	[!] 3-Propylphenol	0.188 f ± 0.004	0.106 e ± 0.002	0.080 c ± 0.003	0.092 d ± 0.002	0.066 b ± 0.002	0.055 a ± 0.001	0.082 c ± 0.002	0.054 a ± 0.001	0.050 a ± 0.001	<0.001	<0.001	<0.001
50	[!] 3-Ethyl-5-methylphenol	0.176 g ± 0.004	0.092 e ± 0.004	0.060 c ± 0.001	0.101 f ± 0.001	0.058 c ± 0.003	0.042 b ± 0.001	0.070 d ± 0.002	0.032 a ± 0.002	0.027 a ± 0.001	<0.001	<0.001	<0.001
51	[!] 2,3,5-Trimethylphenol	0.039 f ± 0.001	0.023 e ± 0.001	0.013 cd ± 0.001	0.022 e ± 0.001	0.012 c ± 0.001	0.009 b ± 0.001	0.015 d ± 0.001	0.007 a ± 0.001	0.006 a ± 0.001	<0.001	<0.001	<0.001
52	[!] 2,3,6-Trimethylphenol	0.235 f ± 0.010	0.125 d ± 0.001	0.088 c ± 0.002	0.154 e ± 0.002	0.098 c ± 0.002	0.072 b ± 0.001	0.115 d ± 0.001	0.061 ab ± 0.002	0.059 a ± 0.002	<0.001	<0.001	<0.001
54	[!] 3,4,5-Trimethylphenol	0.138 f ± 0.009	0.072 d ± 0.003	0.050 c ± 0.002	0.083 e ± 0.006	0.054 c ± 0.003	0.037 b ± 0.001	0.055 c ± 0.002	0.027 ab ± 0.001	0.024 a ± 0.001	<0.001	<0.001	<0.001
59	[!] 2,4,5-Trimethylphenol	0.070 e ± 0.003	0.034 d ± 0.001	0.023 b ± 0.001	0.036 d ± 0.002	0.022 b ± 0.002	0.014 a ± 0.001	0.029 c ± 0.001	0.013 a ± 0.001	0.012 a ± 0.001	<0.001	<0.001	<0.001

Peak No.	[1/] Compounds	[2/] Concentrations (mg/L)											
		Apple wood			Beech wood			Oak wood			P-value		
		TR	P25	P50	TR	P25	P50	TR	P25	P50	Wood (W)	Process (P)	W * P
Guaiacol and derivatives (11)													
27	* 2-Methoxyphenol (guaiacol)	68.7 d ± 0.785	53.2 abc ± 1.49	48.6 a ± 2.30	76.9 e ± 1.62	58.1 c ± 1.98	54.8 bc ± 0.759	67.1 d ± 4.41	48.5 a ± 0.538	49.7 ab ± 0.846	<0.001	<0.001	0.100
39	! 6-Methyl-2-methoxyphenol (6-methylguaiacol)	0.831 f ± 0.008	0.544 d ± 0.020	0.456 ab ± 0.002	0.804 f ± 0.024	0.505 c ± 0.010	0.436 ab ± 0.004	0.705 e ± 0.012	0.434 a ± 0.005	0.470 b ± 0.006	<0.001	<0.001	<0.001
42	* 4-Methyl-2-methoxyphenol (4-methylguaiacol)	54.3 e ± 1.15	37.2 b ± 0.371	31.3 a ± 0.842	65.8 f ± 2.03	42.0 c ± 1.14	38.4 b ± 0.102	70.4 g ± 0.212	50.6 d ± 0.975	54.4 e ± 0.638	<0.001	<0.001	<0.001
55	* 4-Ethyl-2-methoxyphenol (4-ethylguaiacol)	8.07 g ± 0.03	4.69 d ± 0.04	3.64 a ± 0.04	7.71 f ± 0.16	4.74 d ± 0.05	4.12 c ± 0.05	5.76 e ± 0.10	3.76 ab ± 0.07	3.91 bc ± 0.02	<0.001	<0.001	<0.001
61	* 4-Vinyl-2-Methoxyphenol (4-vinylguaiacol)	5.75 d ± 0.455	5.23 d ± 0.199	4.07 c ± 0.093	4.38 c ± 0.128	2.94 b ± 0.150	2.18 a ± 0.134	4.49 c ± 0.220	2.16 a ± 0.081	2.01 a ± 0.111	<0.001	<0.001	<0.001
64	* 4-(2-Propenyl)-2-methoxyphenol (eugenol)	2.29 d ± 0.082	1.78 c ± 0.070	1.42 a ± 0.034	2.94 e ± 0.035	1.62 b ± 0.052	1.37 a ± 0.057	2.20 d ± 0.044	1.37 a ± 0.021	1.488 ab ± 0.055	<0.001	<0.001	<0.001
65	! 4-Propyl-2-methoxyphenol (4-propylguaiacol)	0.770 e ± 0.040	0.430 b ± 0.008	0.316 a ± 0.010	0.769 e ± 0.018	0.483 c ± 0.008	0.391 b ± 0.015	0.600 d ± 0.014	0.334 a ± 0.013	0.333 a ± 0.015	<0.001	<0.001	<0.001
67	* 4-Hydroxy-3-methoxybenzaldehyde (vanillin)	22.6 ab ± 1.51	17.2 ab ± 1.80	14.1 a ± 2.24	37.6 c ± 4.90	20.9 ab ± 1.78	17.1 a ± 1.95	27.4 b ± 5.63	20.5 ab ± 4.82	18.7 ab ± 4.30	0.002	<0.001	0.031
71	* 4-(1-Propenyl)-2-methoxyphenol (isoeugenol)	27.8 d ± 2.25	20.4 c ± 1.05	14.2 b ± 0.329	20.3 c ± 0.789	12.2 b ± 0.592	8.61 a ± 0.709	19.9 c ± 1.51	8.02 a ± 0.631	8.79 a ± 0.658	<0.001	<0.001	0.001
73	§ *trans*-4-(1-Propenyl)-2-methoxyphenol (*trans*-isoeugenol)	0.043 d ± 0.004	0.028 bc ± 0.001	0.024 ab ± 0.001	0.029 c ± 0.001	0.032 c ± 0.002	0.029 c ± 0.002	0.021 a ± 0.001	0.022 a ± 0.002	0.032 c ± 0.002	<0.001	0.001	<0.001
74	* 1-(4-Hydroxy-3-methoxyphenyl)-ethanone (acetovanillone)	0.213 a ± 0.027	0.166 a ± 0.010	0.151 a ± 0.038	0.376 b ± 0.113	0.157 a ± 0.012	0.137 a ± 0.028	0.247 ab ± 0.076	0.178 a ± 0.054	0.172 a ± 0.052	0.221	<0.001	0.042

Peak No.	[1/] Compounds	[2/] Concentrations (mg/L)											
		Apple wood			Beech wood			Oak wood			P-value		
		TR	P25	P50	TR	P25	P50	TR	P25	P50	Wood (W)	Process (P)	W * P
Syringol and derivatives (11)													
47	[§] 3,4-Dimethoxytoluene	0.667 e ± 0.011	0.377 cd ± 0.050	0.319 b ± 0.006	0.625 e ± 0.010	0.403 d ± 0.004	0.346 bc ± 0.001	0.327 b ± 0.006	0.212 a ± 0.003	0.217 a ± 0.002	<0.001	<0.001	<0.001
53	[*] 3,5-Dimethoxytoluene	0.820 d ± 0.034	0.465 c ± 0.107	0.347 b ± 0.004	0.506 c ± 0.021	0.326 b ± 0.020	0.204 a ± 0.003	0.568 c ± 0.032	0.251 ab ± 0.002	0.240 ab ± 0.005	<0.001	<0.001	0.002
60	[*] 1,2,3-Trimethoxybenzene	0.146 f ± 0.006	0.088 cd ± 0.003	0.078 bc ± 0.002	0.144 f ± 0.008	0.109 e ± 0.001	0.092 d ± 0.003	0.071 b ± 0.002	0.054 a ± 0.003	0.056 a ± 0.002	<0.001	<0.001	<0.001
63	[*] 2,6-Dimethoxyphenol (syringol)	232 b ± 22.7	163 a ± 19.3	140 a ± 15.3	258 b ± 28.1	147 a ± 21.2	127 a ± 8.30	163 a ± 33.0	118 a ± 17.0	114 a ± 15.9	<0.001	<0.001	0.039
66	[!] 5-Methyl-1,2,3-trimethoxybenzene	0.187 e ± 0.012	0.111 d ± 0.004	0.088 bc ± 0.001	0.184 e ± 0.002	0.114 d ± 0.004	0.101 cd ± 0.004	0.086 b ± 0.002	0.060 a ± 0.001	0.061 a ± 0.002	<0.001	<0.001	<0.001
70	[!] 3,5-Dimethoxy-4-hydroxytoluene (4-methylsyringol)	0.772 c ± 0.086	0.527 ab ± 0.066	0.420 a ± 0.046	0.914 c ± 0.112	0.461 a ± 0.061	0.383 a ± 0.037	0.723 bc ± 0.146	0.495 ab ± 0.090	0.498 ab ± 0.073	0.929	<0.001	0.052
72	[!] 1-(4-Hydroxy-3,5-dimethoxyphenyl)-ethanone (acetosyringone)	0.069 f ± 0.006	0.035 cd ± 0.001	0.029 bc ± 0.001	0.058 e ± 0.002	0.038 d ± 0.002	0.031 bcd ± 0.002	0.025 b ± 0.001	0.015 a ± 0.001	0.015 a ± 0.001	<0.001	<0.001	<0.001
75	[!] 4-Ethyl-2,6-dimethoxyphenol (4-ethylsyringol)	0.877 c ± 0.088	0.492 b ± 0.05	0.369 ab ± 0.032	0.749 c ± 0.072	0.371 ab ± 0.040	0.302 a ± 0.025	0.367 ab ± 0.069	0.221 a ± 0.039	0.223 a ± 0.039	<0.001	<0.001	<0.001
76	[!] 4-(2-Propenyl)-2,6-dimethoxyphenol (4-allylsyringol)	0.173 c ± 0.022	0.118 b ± 0.015	0.088 ab ± 0.008	0.180 c ± 0.020	0.077 ab ± 0.010	0.055 a ± 0.006	0.104 b ± 0.023	0.055 a ± 0.011	0.056 a ± 0.008	<0.001	<0.001	0.004
77	[!] 4-Propyl-2,6-dimethoxyphenol (4-propylsyringol)	0.275 e ± 0.026	0.128 c ± 0.014	0.095 abc ± 0.005	0.221 d ± 0.012	0.119 bc ± 0.010	0.088 ab ± 0.006	0.119 bc ± 0.018	0.067 a ± 0.010	0.066 a ± 0.007	<0.001	<0.001	<0.001

Peak No.	[1/] Compounds	[2/] Concentrations (mg/L)											
		Apple wood			Beech wood			Oak wood			P-value		
		TR	P25	P50	TR	P25	P50	TR	P25	P50	Wood (W)	Process (P)	W * P
78	[!] 4-Hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde)	0.009 a ± 0.001	0.010 a ± 0.001	0.008 a ± 0.003	0.043 b ± 0.028	0.011 a ± 0.006	0.010 a ± 0.006	0.017 ab ± 0.005	0.017 ab ± 0.009	0.014 ab ± 0.008	0.076	0.049	0.039
Indene derivatives (3)													
57	[!] 2,3-Dihydro-1H-inden-1-one	0.213 f ± 0.014	0.144 e ± 0.005	0.113 d ± 0.002	0.155 e ± 0.004	0.096 cd ± 0.005	0.083 bc ± 0.006	0.113 d ± 0.005	0.064 a ± 0.002	0.066 ab ± 0.003	<0.001	<0.001	<0.001
62	[!] 1-Methyl-2,3-dihydro-1H-inden-1-one	0.007 d ± 0.001	0.004 b ± 0.001	0.003 ab ± 0.001	0.006 c ± 0.001	0.003 ab ± 0.001	0.002 a ± 0.001	0.004 b ± 0.001	0.002 a ± 0.001	0.002 a ± 0.001	<0.001	<0.001	<0.001
69	[!] 7-Methyl-2,3-dihydro-1H-inden-1-one	0.059 f ± 0.006	0.034 de ± 0.002	0.024 bc ± 0.001	0.035 e ± 0.001	0.019 ab ± 0.002	0.015 a ± 0.002	0.027 cd ± 0.002	0.014 a ± 0.002	0.014 a ± 0.002	<0.001	<0.001	<0.001
Other compounds (10)													
2	[!] 2-Butenal (crotonaldehyde)	0.185 ab ± 0.119	0.221 ab ± 0.130	0.153 ab ± 0.028	0.273 b ± 0.018	0.175 ab ± 0.047	0.124 ab ± 0.034	0.118 ab ± 0.049	0.070 a ± 0.013	0.068 a ± 0.004	0.004	0.067	0.364
5	[§] 1-Hydroxy-2-butanone	0.017 b ± 0.003	0.000 a ± 0.000	0.007 ab ± 0.006	0.012 ab ± 0.002	0.011 ab ± 0.008	0.012 ab ± 0.008	0.004 a ± 0.003	0.001 a ± 0.002	0.001 a ± 0.002	<0.001	0.009	0.028
16	[!] Benzonitrile	0.224 ab ± 0.006	0.250 b ± 0.012	0.308 c ± 0.007	0.192 a ± 0.010	0.386 d ± 0.022	0.341 cd ± 0.008	0.530 f ± 0.034	0.627 g ± 0.012	0.478 e ± 0.011	<0.001	<0.001	<0.001
24	[*] Acetophenone	0.798 d ± 0.028	0.538 c ± 0.021	0.434 b ± 0.010	0.551 c ± 0.011	0.392 b ± 0.023	0.304 a ± 0.010	0.436 b ± 0.008	0.288 a ± 0.005	0.297 a ± 0.003	<0.001	<0.001	<0.001
25	[*] 2-Methyl benzaldehyde	0.223 f ± 0.010	0.170 d ± 0.006	0.100 b ± 0.001	0.191 e ± 0.011	0.118 bc ± 0.009	0.054 c ± 0.001	0.126 b ± 0.004	0.050 a ± 0.002	0.044 a ± 0.001	<0.001	<0.001	<0.001
28	[*] Methyl benzoate	1.99 g ± 0.040	0.947 de ± 0.029	0.720 c ± 0.015	1.18 f ± 0.056	1.06 ef ± 0.088	0.612 bc ± 0.033	0.865 d ± 0.022	0.548 b ± 0.003	0.327 a ± 0.002	<0.001	<0.001	<0.001

Peak No.	[1/] Compounds	[2/] Concentrations (mg/L)											
		Apple wood			Beech wood			Oak wood			P-value		
		TR	P25	P50	TR	P25	P50	TR	P25	P50	Wood (W)	Process (P)	W * P
36	[!] 1-(2-hydroxyphenyl)-ethanone	0.295 e ± 0.012	0.212 c ± 0.006	0.168 ab ± 0.003	0.263 d ± 0.014	0.186 b ± 0.008	0.147 a ± 0.003	0.235 c ± 0.009	0.169 ab ± 0.004	0.185 b ± 0.005	<0.001	<0.001	<0.001
38	[!] 1-(3-methylphenyl)-ethanone	0.232 f ± 0.001	0.133 e ± 0.002	0.094 d ± 0.002	0.133 e ± 0.006	0.086 c ± 0.002	0.062 b ± 0.002	0.100 d ± 0.002	0.052 a ± 0.001	0.051 a ± 0.002	<0.001	<0.001	<0.001
48	[!] 1-Ethyl-4-methoxybenzene	0.780 f ± 0.021	0.407 e ± 0.007	0.272 cd ± 0.004	0.286 d ± 0.012	0.176 b ± 0.001	0.133 a ± 0.006	0.254 c ± 0.008	0.119 a ± 0.005	0.111 a ± 0.002	<0.001	<0.001	<0.001
68	[!] 2-Propenylbenzene	0.066 f ± 0.007	0.041 de ± 0.002	0.029 bc ± 0.001	0.043 e ± 0.002	0.024 ab ± 0.002	0.019 a ± 0.001	0.034 cd ± 0.002	0.018 a ± 0.002	0.018 a ± 0.002	<0.001	<0.001	<0.001

[1/] For (*) compound, authentic standards were used for identification. (§) compound, the identification was from well-matched spectra and literature LRI. (!) compound, only LRI from experimental data was available for the identification of a compound, or if the LRI matched with literature was poor, then it must be considered as a tentative compound.

[2/] Average concentration ± standard deviation (SD) of three replicates. Means of triplicate in the same row not labelled with the same letters are significantly different ($p \leq 0.05$).

Chapter 4

Utilisation of smoked water and smoke recombinates to enhance the taste in salt-reduced soup and investigate the mechanisms

4.1 Introduction

Flavour is a multimodal perception, ‘a complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting and may be influenced by tactile, thermal, painful and/or kinaesthetic effects’ (the International Organization for Standardization; ISO, 2008), which contributes to the consumers’ liking on food products. Typically, flavour enhancers such as the amino acid L-glutamate are used to enhance the savoury flavour of food products; besides, they can also be used to enhance other fundamental tastes, such as umami or saltiness (Methven, 2012). Monosodium glutamate (MSG) is a taste enhancer which imparts a brothy, salty and meaty taste (Ninomiya, 2002) and when added to suitable foods at low concentrations, the food's palatability and pleasure increases (Manabe et al., 2014; Ventanas et al., 2010). Another important group of compounds associated with umami taste are the 5'-ribonucleotides such as inosine-5'-monophosphate (IMP), guanosine-5'-monophosphate (GMP), adenosine-5'-monophosphate (AMP), and xanthosine-5'-monophosphate (XMP) (Yamaguchi & Ninomiya, 2000). The synergy between MSG and IMP was investigated by Yamaguchi (1967), MSG and GMP by Rifkin and Bartoshuk (1980), and MSG with IMP and/or GMP by Giovanni and Guinard (2001). These researchers discovered that single solution samples of MSG or ribonucleotides had a lower taste response than binary or tertiary mixtures of MSG and ribonucleotides.

Sodium chloride (NaCl) plays a vital role for providing salty taste, increasing palatability, maintaining texture, playing a preservative role, enhancing flavour intensity, as well as masking certain flavours (such as bitter notes) in food products (Batenburg & van der Velden, 2011; Dötsch et al., 2009; Lad et al., 2012; Xu et al., 2019), but excessive consumption of sodium can cause severe health issues related to cardiovascular disease, which have been reported (He et al., 2011; Morrison & Ness, 2011; Zhu et al., 2018). According to World Health Organisation, a reduction in sodium content of foods (World Health Organization; WHO, 2007) is often connected with a decrease in consumer acceptance due to the loss of saltiness

and flavour of food products (Batenburg & van der Velden, 2011). All new products on the market should meet or fall below the existing maximum salt target for the relevant category, and the average value should be used as the maximum (Public Health England, 2017). According to several food industry trials, potassium-based or other sodium replacers are utilised for salt reduction strategies, but their application is limited. Furthermore, the usage of sodium substitutes has been unsuccessful due to negative effects on product quality (e.g., providing metallic taste) (Public Health England, 2020).

Use of tasteless odorants (aroma enhancers) to compensate for salt reduction through multisensory-integration mechanisms of cross-modally odour-taste interaction has been shown to be a very promising method for improving saltiness (Lawrence et al., 2009; Thomas-Danguin et al., 2019). The odour-taste interaction may happen when the odour and taste compounds are at levels above or below the threshold and depends on the food compositions (Poinot et al., 2013). Recently, attention has been paid to the odour-induced saltiness enhancement (OISE) approach, which is based on the principle that some odours can enhance salty taste (Lawrence et al., 2009). The OISE depends on salt intensity (Nasri et al., 2011), and OISE was also found to be effective even in a null and a low salt solution when the panel sniffed the soy sauce aroma during rating saltiness (Djordjevic et al., 2004). Several studies have evaluated the role of aroma in taste perception. For example, in low NaCl solution level, Chokumnoyporn et al. (2015) found soy sauce odour could induce salty taste which at this level was undetectable if there was no soy sauce odour. In addition, they also reported that even in low levels of soy sauce odour, salty taste was enhanced, which means that the odour of soy sauce could induce and enhance the perception of salty taste. OISE was also found to be effective in the low salt content of solid model cheese of different textures; a significant increase in saltiness perceptions induced by Comté cheese and sardine odours was observed only in soft-textured model cheese. These findings have shown that well-selected aromas which are congruent with cheese product could improve the saltiness of low-salt solid food products of different textures (Lawrence et al., 2011). Batenburg and van der Velden (2011) investigated single salt-congruent aroma compounds and complex savoury flavourings on saltiness perception. They found that sotolon was the best for salt enhancement in salt-reduced bouillons among the single salt-congruent aroma that could compensate for a 15% reduction in salt which suggests that congruent aroma may be necessary for odour induced taste enhancement.

Smoked ingredients are renowned for their aromatic character, and they play a part in improving the perception of taste. Smoke is a complicated mixture of several hundred compounds including phenolics, carbonyls, alcohols, organic acids, esters, furans, lactones and other compounds (Theobald et al., 2012) that are responsible for the unique flavour, colour and taste of smoked food products. The volatile compounds in smoke ingredients that relate to the desired flavour and aroma have been investigated in recent studies (Lingbeck et al., 2014; Theobald et al., 2012).

The present study aimed to evaluate the three possible modes of action of smoked water created using PureSmokeTechnology™ (PST) (in order to reduce polycyclic aromatic hydrocarbons; PAHs), which leads to an enhancement of the perception of flavour: the smoke may contain taste-active compounds, or taste-enhancing compounds which might act synergistically with other taste enhancers, or it may contribute cross-modally whereby the odour compounds induce salt or savoury enhancement when it is added as an ingredient in simple and complex mixtures of food products such as a soup.

4.2 Materials

4.2.1 Fresh soup

Own brand chicken & vegetable broth was purchased from ASDA stores Ltd. It contained water, 9% carrot, 8% onions, 7% swede, 6% chicken thigh, 4% savoy cabbage, 3% celery, 2% pearl barley, corn flour, yeast extract, rapeseed oil, chicken extract, chicken fat, salt, garlic purée, herbs, black pepper, extract of rosemary.

4.2.2 Model chicken soup

A standard recipe of instant chicken soup powder comprised twelve ingredients: 42.36% corn flour (Agglom corn flour, Knighton Foods Ltd, Knaresborough, UK), 39.85% creamer (refined palm oil, maltodextrin, milk protein; SATRO FP 80, FrieslandCampina Kievit B.V.Amersfoort, The Netherlands), 5.20% whole milk powder (Buy Whole Foods Online Ltd, Kent, UK; origin: the Netherlands), 3.17% granulated sugar (Tate and Lyle Sugars, London, UK), 1.58% onion powder (Buy Whole Foods Online Ltd, Kent, UK; origin: India), 0.13%

ground sage (British Pepper & Spice, Northampton, UK), 0.32% parsley flakes (British Pepper & Spice, Northampton, UK), 0.11% pepper extract (British Pepper & Spice, Northampton, UK), 0.10% turmeric extract (Naturex Ltd., Swadlincote, UK), 1.06% chicken flavouring 612761E (Givaudan Schweiz AG, Dubendorf, Switzerland), 0.79% chicken flavouring 610490H (Givaudan Schweiz AG, Dubendorf, Switzerland), and 5.34% salt (sodium chloride; NaCl, Co-operative Group Ltd., Manchester, UK) was prepared.

Soup has a recognised place in the culinary traditions of British cuisine, with people consuming it regularly per week. Soup recipes with chicken were frequently published as medicinal prescriptions (Encyclopedia) and were quite popular. This research produced a model chicken soup entirely made of solid compounds instead of authentic chicken soup. Despite the complexity of this model chicken soup owing to its numerous components, it has advantages in that it is easy to formulate and has consistent replicates.

4.2.3 Palate cleanser

Three palate cleansers were used in the sensory experiments. Full fat yogurt (Onken natural set yogurt, Emmi UK Limited, Putney, London, UK) and cucumber slices which were purchased from the local shops. Tap water was filtered through a filter cartridge (Maxtra MicroFlow Technology, Germany).

4.2.4 Smoked water

Apple-wood traditional smoked water (TR; batch number; S0230/M/11, manufacturing date; November 2017) and apple-wood PureSmokeTechnology™ (PST) smoked water (P50; batch number; S0201/M/10, manufacturing date; October 2017) were provided by Besmoke Ltd (Arundel, UK). PureSmokeTechnology™ (PST) uses the natural zeolite to reduce the polycyclic aromatic hydrocarbons (PAHs) and acrid smoke tar, but it enables free flavour compounds to flow which are transferred to the water to produce PST smoked water (Parker et al., 2018).

4.2.5 Chemicals

4.2.5.1 Taste enhancer and tastant compounds

A mixture of 5'-ribonucleotides (1:1 mix of disodium inosine-5'-monophosphate (IMP) and disodium guanosine-5'-monophosphate (GMP)) was purchased from PT Fermentech Indonesia (Lampung, Indonesia), monosodium glutamate (MSG) from Ajinomoto (99+% purity, Paris, France) and sodium chloride (NaCl; Co-operative Group Ltd., Manchester, UK) from the Coop.

4.2.5.2 Odorant standard compounds and solvent

The following 14 odorant standard compounds were purchased from Sigma-Aldrich; 1-(furan-2-yl)ethanone (2-acetylfuran; ≥ 99%, FG, China), acetic acid (≥ 99.5%, FG, Colombia), 2,6-dimethoxyphenol (syringol; ≥ 98%, FG, India), 2,6-dimethylphenol (≥ 99%, FG, China), 2,5-dimethylphenol (≥ 99%, FG, China), furan-2-carbaldehyde (furfural; ≥ 98%, FCC, FG, China), 2-methoxyphenol (guaiacol; natural ≥ 99%, FG, China), 2-methoxy-4-[(E)-prop-1-enyl]phenol (isoeugenol; natural 99%, FG, China), 2-hydroxy-3-methylcyclopent-2-en-1-one (cyclotene; 98%, China), 4-methylphenol (p-cresol; 99%, FG, USA), 2-methoxy-4-methylphenol (methylguaiacol; ≥ 98%, FG, China), phenol (natural 97%, FG, USA), 4-ethenyl-2-methoxyphenol (vinylguaiacol; ≥ 98%, FG, UK), 4-hydroxy-3-methoxybenzaldehyde (vanillin; ≥ 97%, FG, China). Other three odorant standards compounds were 2-methoxy-4-prop-2-enylphenol (eugenol; pure, Givaudan, Switzerland), 4-ethylphenol (p-ethylphenol; ≥ 98%, FG, Merck, China), and 4-ethyl-2-methoxyphenol (ethylguaiacol; ≥ 98%, FG, SAFC, USA). Ethanol (96%, FG) was purchased from Kimiauk, Hayman Group Limited (UK).

4.3 Methods

4.3.1 Preparation of soups samples

Own brand ASDA chicken & vegetable broth (fresh soup) was passed through a kitchen sieve to remove the chunks prior to use.

For the model soup, 26 g of instant chicken soup powder was mixed with boiling water (250 mL) to make the standard model soup (0.5% NaCl). Whereas low salt model soups were prepared by combining 24.61 g of an unsalted soup base with 0.97 g or 0.83 g salt to obtain the recipe 30% (0.35% NaCl) or 40% (0.30% NaCl) reduced salt soups, respectively (see **Table 4.1**).

Table 4.1 Three model soup recipes.

Soup	**Salt content, g (%)**	**[1/] Soup base, g**	**MSG, g (%)**
Standard (control)	1.39 (0.50)	24.61	0.17 (0.062)
30% salt-reduced	0.97 (0.35)	24.61	0.17 (0.062)
40% salt-reduced	0.83 (0.30)	24.61	0.17 (0.062)

[1/] Soup base consists of 11 ingredients which are corn flour, creamer, whole milk powder, sugar, onion powder, ground sage, parsley flakes, pepper extract, turmeric extract, chicken flavouring 612761E, and chicken flavouring 610490H.

4.3.2 Preliminary screening of smoked ingredients in fresh soup

For initial screening, a sieved fresh commercial soup with or without 1% TR smoked water was presented to the panellists, once for each taste. The panellists wore nose clips and were asked to choose the sample with the strongest taste (sweet, sour, salty, umami and bitter) to identify the possible prominent taste of smoked water in soup sample.

4.3.3 Determination of intensities perception of MSG and NaCl

The perception of umami and salty taste intensities was determined for each panellist using a serial dilution of monosodium glutamate and sodium chloride adapted from ISO 3972:2011 (ISO, 2011). MSG (4.65 g) or NaCl (4.65 g) were dissolved in filtered tap water, and the volume was adjusted to 500 mL (concentration = 9300 mg/L) (level 9), where the highest concentrations of sodium chloride and monosodium glutamate from ISO 3972:2011 were 4000 and 2000 mg/L, respectively (ISO, 2011). Another eight levels of MSG and NaCl solutions were made in serial dilutions at a ratio of 60 mL MSG or NaCl : 120 mL water, as displayed in **Figure 4.1** and **Table 4.2**. Panellists were then given 15 mL of each MSG and/or NaCl solution in disposable cups labelled 1-9. They were asked to rate their intensity perception in ascending order on a general labelled magnitude scale (gLMS) of solution.

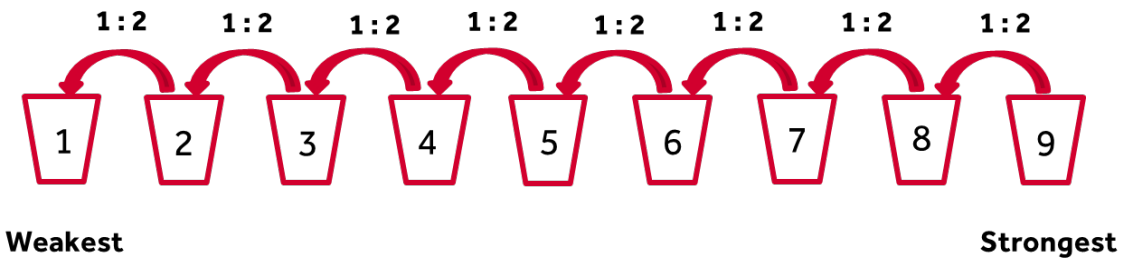

Figure 4.1 Preparation of serial dilution of MSG and NaCl solutions.

Table 4.2 Nine levels of serial dilution of MSG and NaCl concentrations.

Level	MSG and NaCl concentration, mg/L	Concentration, millimolar		Na concentration, mg/L	
		MSG	NaCl	MSG	NaCl
9	9300 (0.93%)	55.0	159	1144	3688
8	3100 (0.31%)	18.3	53.0	381	1229
7	1033 (0.103%)	6.11	17.7	127	410
6	344 (0.034%)	2.04	5.89	42.4	137
5	115 (0.012%)	0.679	1.96	14.1	45.5
4	38.3 (0.0038%)	0.226	0.654	4.71	15.2
3	12.8 (0.0013%)	0.075	0.218	1.57	5.06
2	4.25 (0.00042%)	0.025	0.073	0.520	1.70
1	1.42 (0.00014%)	0.008	0.024	0.170	0.560

The gLMS consisted of a vertical line 230 mm high with labels placed at barely detectable (at the bottom), weak, moderate, strong, very strong, and strongest imaginable (at the top of a line) sensation. On a scale of 0 to 100, the labels were placed at 1.4 barely detectable; 6.0 weak; 17.0 moderate; 34.7 strong; 52.5 very strong; and 100 strongest imaginable sensation (Bartoshuk et al., 2004). The panellists were requested to taste the MSG or NaCl solutions in ascending concentrations from number one to number nine. During tasting, panellists were also asked to indicate the level where they first detected taste, the level where they could first recognise the taste, and the level where the taste became unacceptably strong. After the testing was finished, the concentration at which each panellist detected taste, recognised taste and maximum sensation of taste were recorded.

4.3.4 Determination of the taste-active compounds in smoked water

In the first experiment (**Table 4.3**), two filtered tap water samples and one filtered tap water containing either 1% TR or 1% P50 smoked water were made. Approximately 15 mL of each sample was poured into a cup labelled with a 3-digit code and served to the panellists. Before tasting samples, panellists were asked to wear a nose clip to prevent orthonasal perception. Three-Alternative Forced Choice (3-AFC) test was used. They were instructed to taste all three in the order given and select one sample, that had the stronger taste, was more umami or was saltier, (the question varied for different tests). The test was also conducted in the model soup and fresh soup and with either 1% TR, 1% P50 or 2% P50 which was evaluated using the same methodology, but the panellist was only asked to select one soup sample that had a stronger taste and describe the taste. These tests were performed twice and with a 2-min break.

Table 4.3 Tests conducted to investigate whether the compounds in smoked water are taste-active.

Experiments	Sample base	Smoke	MSG, %	RIBO, %	Nose clip	Question
1.1	Water	1% TR	0	0	Yes	Stronger taste
	Water	1% TR	0	0	Yes	Saltier
	Water	1% TR	0	0	Yes	More umami
	Water	1% P50	0	0	Yes	Stronger taste
	Water	1% P50	0	0	Yes	Saltier
	Water	1% P50	0	0	Yes	More umami
1.2	Model soup	1% TR	0	0	Yes	Stronger taste, what
	Model soup	1% P50	0	0	Yes	Stronger taste, what
	Model soup	2% P50	0	0	Yes	Stronger taste, what
	Fresh soup	1% TR	0	0	Yes	Stronger taste, what
	Fresh soup	1% P50	0	0	Yes	Stronger taste, what

4.3.5 Determination of the taste-enhancing compounds in smoked water

4.3.5.1 Taste enhancement of smoked water with MSG

The 3-AFC were carried out in MSG solutions and model soups to check for any taste-enhancing activity (experiments 2.1 and 2.2) as shown in **Table 4.4**. For MSG solutions, four levels (38, 115, 344 and 620 mg/L) within the range of intensities perception of MSG from section 4.3.3 were chosen. These four selected levels were: (i) a level where only few panellists detected a taste (38 mg/L), (ii) a level where all panellists detected a taste (115 mg/L), (iii) a level where the majority of the panellists recognised umami taste (344 mg/L), and (iv) one which was suprathreshold for all panellists (620 mg/L). For each experiment, two filtered tap water samples and one filtered tap water containing smoked water at 1% sample were prepared. For each sample, 15 ± 2 mL were presented to the panellists in a tasting cup labelled with a random three-digit number in a balanced order, with sample sets randomly assigned. Panellists wore a nose clip to prevent any perception of the aroma compounds in the smoke. They were asked to taste all three samples and identify the sample that had more umami taste. For experiments 2.2, all soup samples were heated up to 75-80 °C before serving. The tests were performed in duplicate, with a 2-min rest between replicates.

Table 4.4 Tests conducted to investigate the taste-enhancing activity of smoked water.

Experiments	Sample base	Smoke	MSG, %	RIBO, %	Nose clip	Question
2.1	Water	1% TR	0.004	0	Yes	More umami
	Water	1% TR	0.012	0	Yes	More umami
	Water	1% TR	0.034	0	Yes	More umami
	Water	1% TR	0.062	0	Yes	More umami
	Water	1% P50	0.004	0	Yes	More umami
	Water	1% P50	0.012	0	Yes	More umami
	Water	1% P50	0.034	0	Yes	More umami
	Water	1% P50	0.062	0	Yes	More umami
2.2	Model soup	1% TR	0	0	Yes	More umami
	Model soup	1% TR	0.004	0	Yes	More umami
	Model soup	1% TR	0.012	0	Yes	More umami
	Model soup	1% TR	0.034	0	Yes	More umami
	Model soup	1% TR	0.062	0	Yes	More umami
	Model soup	1% P50	0	0	Yes	More umami
	Model soup	1% P50	0.004	0	Yes	More umami
	Model soup	1% P50	0.012	0	Yes	More umami
	Model soup	1% P50	0.034	0	Yes	More umami
	Model soup	1% P50	0.062	0	Yes	More umami
2.3	Water	1% TR	0	0.007	Yes	More umami
	Water	1% TR	0.004	0.007	Yes	More umami
	Water	1% TR	0.012	0.007	Yes	More umami
	Water	1% TR	0.034	0.007	Yes	More umami
	Water	1% TR	0.062	0.007	Yes	More umami
	Water	1% P50	0	0.007	Yes	More umami
	Water	1% P50	0.004	0.007	Yes	More umami
	Water	1% P50	0.012	0.007	Yes	More umami
	Water	1% P50	0.034	0.007	Yes	More umami
	Water	1% P50	0.062	0.007	Yes	More umami
2.4	Model soup	1% TR	0	0.007	Yes	More umami
	Model soup	1% TR	0.004	0.007	Yes	More umami
	Model soup	1% TR	0.012	0.007	Yes	More umami
	Model soup	1% TR	0.034	0.007	Yes	More umami
	Model soup	1% TR	0.062	0.007	Yes	More umami

Experiments	Sample base	Smoke	MSG, %	RIBO, %	Nose clip	Question
	Model soup	1% P50	0	0.007	Yes	More umami
	Model soup	1% P50	0.004	0.007	Yes	More umami
	Model soup	1% P50	0.012	0.007	Yes	More umami
	Model soup	1% P50	0.034	0.007	Yes	More umami
	Model soup	1% P50	0.062	0.007	Yes	More umami

4.3.5.2 Taste enhancement of smoked water with MSG and ribonucleotides

The taste-enhancing activity of smoked water was also examined in combination with another taste enhancer, specifically 5'-ribonucleotides, to determine whether or not it is the consequence of a synergistic effect. Four levels of MSG solutions (38, 115, 344 and 620 mg/L) and model soups containing MSG at varying concentrations (38-620 mg/L), were added to 0.007% mixed 5'-ribonucleotides (1:1 mix of IMP and GMP) (RIBO) (**Table 4.4**; experiment 2.3 and 2.4). As previously, the soup samples were heated to 75-80 °C before serving. For each sample, 15 mL of two filtered tap water and one filtered tap water mixture containing either 1% TR or 1% P50 smoked water were provided to the panellists. The panellists wore the nose clip and asked to select the sample with the more umami taste. The test was performed twice, and a 2-min break in between.

4.3.6 Preliminary assessment of smoked water to elicit odour-induced taste enhancement (OITE)

4.3.6.1 Recognised smoky odour threshold

In order to determine an appropriate concentration of smoked water to assess for odour induced taste enhancement, the recognised smoky odour threshold was first identified. The smoky odour recognition was adapted from a rapid detection threshold (RTD) method (Allen et al., 2014) based on a staircase method. Ten concentrations (0.012, 0.021, 0.037, 0.064, 0.111, 0.192, 0.333, 0.577, 0.999 and 1.73%) of P50 smoked water were added into the standard model soup. In each smoke concentration, two control soups and one soup containing different levels of P50 smoked water were made. All soup samples were heated up to 75-80 °C and presented in a cup labelled with 3-digit code. The panellists were asked to select the sample with the strongest smoky odour for each concentration. The soup samples were served to

panellists by starting at a mid-point concentration (0.021%) of smoke. The subsequent samples either decreased in concentration when the correct answer was given or increased in concentration if incorrect. The process was stopped after the third reversal. In the case of the panellists selecting the wrong sample at the highest concentration or selected the right sample at the lowest concentration, these samples were re-presented and the process stopped (Allen et al., 2014). The recognised smoky odour threshold was determined as the geometric mean between the lowest concentration where could be detected and the highest concentration that could not be detected (Kennedy et al., 2010).

4.3.6.2 Preliminary test of OITE of smoked water

In experiment 3 of **Table 4.5**, P50 smoked water at the concentrations between the recognised smoky odour threshold from subsection 4.3.6.1 was used to determine the concentration of smoked water at which the odour compounds induced taste enhancement without detecting smoky odour. Smoked water was introduced to fresh and standard model soups in five concentrations (0.004, 0.012, 0.037, 0.111, and 0.333%). For each smoke concentration, two control soups and one soup contained different levels of smoked water were prepared, heated to 75-80 °C and presented in a cup with 3-digit code. The nose clip was not used during testing since orthonasal assessment was required. The panellists were asked to select the sample with the stronger flavour and describe the flavour attribute perceived. There were no repeats in this tasting.

Table 4.5 The screening test of the odour-induced taste enhancement (OITE) activity of smoked water conducted.

Experiments	Sample base	Smoke	MSG, %	RIBO, %	Nose clip	Question
3.1	Fresh soup	0.004% P50	0	0	No	More flavour, what
	Fresh soup	0.012% P50	0	0	No	More flavour, what
	Fresh soup	0.037% P50	0	0	No	More flavour, what
	Fresh soup	0.111% P50	0	0	No	More flavour, what
	Fresh soup	0.333% P50	0	0	No	More flavour, what
3.2	Model soup	0.004% P50	0	0	No	More flavour, what
	Model soup	0.012% P50	0	0	No	More flavour, what

Experiments	Sample base	Smoke	MSG, %	RIBO, %	Nose clip	Question
	Model soup	0.037% P50	0	0	No	More flavour, what
	Model soup	0.111% P50	0	0	No	More flavour, what
	Model soup	0.333% P50	0	0	No	More flavour, what

4.3.7 Determination of the odour-induced umami enhancement (OIUE) and odour-induced salty enhancement (OISE) of smoked water in salt-reduced model soup

4.3.7.1 Panellist training for reference soup standard anchoring points

The training focused on ensuring each panellist could reliably score saltiness and umami intensity relative to three reference model soups (**Table 4.6**). Each panellist was given three reference soups with increasing salt contents (0.30, 0.35, and 0.50% salt with 0.062% MSG) and asked to taste the soup samples from numbers 1, 2 and 3, respectively. All three reference soups were heated up to 75-80 °C before serving. The panellists were then given 15 mL of each heated reference soup in disposable cups labelled 1-3. They were asked to rate their perception of salty and umami tastes on a structured line scale (0-100).

Table 4.6 Soup reference standards for training on umami and salty scoring.

Standard	[1/] Soup base, g	% Salt-reduced	Salt content, g (%)	MSG, g (%)	Anchoring score (0-100)	
					Salty	Umami
1	24.61	40	0.83 (0.30)	0.17 (0.062)	20	25
2	24.61	30	0.97 (0.35)	0.17 (0.062)	30	40
3	24.61	0	1.39 (0.50)	0.17 (0.062)	50	45

[1/] Soup base consists of 11 ingredients which are corn flour, creamer, whole milk powder, sugar, onion powder, ground sage, parsley flakes, pepper extract, turmeric extract, chicken flavouring 612761E, and chicken flavouring 610490H.

The average panellist ratings for these three references were 20, 30, and 50 for the salty taste and 25, 40, and 45 for the umami taste, respectively (**Table 4.6**). After providing the panellists with the average values of salty and umami tastes for each reference soup level, they were served all these three references once again to taste and asked whether or not they agreed with these average points of each standard to make sure the consensus anchoring points. Thus, these three positions were used as anchors to provide a structured scale for further experiments to rate other model soup samples.

4.3.7.2 Odour-induced taste enhancement activity in P50 smoked water

In experiment 4 (**Table 4.7**), the investigation of the odour-induced taste enhancement of smoked water was carried out using two levels of salt-reduced model soups (30 and 40% reduction). Control and salt-reduced soups were prepared as section 4.3.1 with MSG (0.062%), with/without mixed 5'-ribonucleotides (RIBO) (0.007%), with/without P50 smoked water (0.577%) added according to **Table 4.7**. In experiment 5, salt content was held constant at 0.35% (30% reduction) while MSG content was varied (0.004, 0.012, and 0.034%), with RIBO (0.007%), with/without P50 smoked water (0.577%) (**Table 4.7**). For each experiment, 15 mL of heated soup samples, coded with three-digit random numbers, were served in a balanced order, with sample sets randomly allocated to panellists. The panellist was asked to evaluate the saltiness and umami intensity using structured line scales (0–100), where three levels of salty and umami standards were anchored on the line scale (the anchored point of each standard level is expressed in **Table 4.6**). The three reference soup samples were presented to the panellists at the start of each rating session for re-familiarisation as needed in order that the panellists could score the salty or umami intensity accurately against the standard anchors (reference samples from 4.3.7.1). Each time, the maximum number of soup samples presented to the panellists was 8 including the three references. All panellists scored samples in duplicate and a rest of 2-min was imposed between the duplicate. Compusense Cloud software (Compusense Cloud Software, Ontario, Canada) was used to acquire the sensory data.

Table 4.7 Experimental design for sensory attributes (umami and salty) scoring.

Samples	NaCl reduction (%)	Ingredients (%)						Total Na (mg/L)
		NaCl	[1/] Soup base	MSG	RIBO	Smoke	Water	
Experiment 4 Different levels of salt								
1	40	0.30	8.93	0.062	-	-	90.71	21.3
2	40	0.30	8.93	0.062	-	0.577	90.18	21.3
3	40	0.30	8.93	0.062	0.007	-	90.70	21.3
4	40	0.30	8.93	0.062	0.007	0.577	90.18	21.3
5	30	0.35	8.93	0.062	-	-	90.66	24.7
6	30	0.35	8.93	0.062	-	0.577	90.14	24.7
7	30	0.35	8.93	0.062	0.007	-	90.65	24.7
8	30	0.35	8.93	0.062	0.007	0.577	90.13	24.7
9	0	0.50	8.91	0.062	-	-	90.52	34.9
10	0	0.50	8.91	0.062	0.007	-	90.52	34.9
Experiment 5 Different levels of MSG								
11	30	0.35	8.93	0.004	0.007	-	90.71	24.3
12	30	0.35	8.93	0.004	0.007	0.577	90.18	24.3
13	30	0.35	8.93	0.012	0.007	-	90.70	24.4
14	30	0.35	8.93	0.012	0.007	0.577	90.18	24.4
15	30	0.35	8.93	0.034	0.007	-	90.68	24.5
16	30	0.35	8.93	0.034	0.007	0.577	90.16	24.5

[1/] Soup base consists of 11 ingredients which are corn flour, creamer, whole milk powder, sugar, onion powder, ground sage, parsley flakes, pepper extract, turmeric extract, chicken flavouring 612761E, and chicken flavouring 610490H.

4.3.8 The odour-induced umami enhancement (OIUE) and odour-induced salty enhancement (OISE) of smoked water recombinate in salt-reduced model soup

4.3.8.1 Panellist training for reference soup standard anchoring points

The training focused on ensuring each panellist could reliably score saltiness and umami intensity relative to three salty references (1, 2 and 3) and three umami reference model soups (A, B and C) (**Table 4.8**). Each panellist was given either three salty reference soups or three umami reference soups and asked to taste the soup samples from sample number 1, 2 and 3, for salty references or from sample A, B and C for umami reference standards. All six soup references were heated up to 75-80 °C before being served. The panellists were then given 15 mL of each heated reference soups in disposable cups labelled 1, 2, 3 or A, B, C and wore a nose clip before and during tasting. They were asked to rate their perception of saltiness for salty reference standards and umami taste for umami reference standards on structured line scales (0-100) respectively. The reference scoring training of salty and umami reference standards was performed three times on different days for each reference standard and between the replication.

The average panel ratings for three salty references were 17, 25, and 42 and 16, 27, and 36 for umami reference standards, respectively (**Table 4.8**). After providing the panellists with the average values of salty and umami tastes for each reference soup, they were served all these six references once again to taste and asked whether or not they agreed with these average points of each standard to make sure the consensus anchoring points.

Table 4.8 Soup reference standards for training on umami and salty scoring.

Standard samples	% Salt-reduced	[1/] Soup base, g	Salt content, g (%)	MSG, g (%)	Anchoring score (0-100)
Salt standard					
1	40	24.61	0.83 (0.30)	0.170 (0.062)	17
2	30	24.61	0.97 (0.35)	0.170 (0.062)	25
3	0	24.61	1.39 (0.50)	0.170 (0.062)	42
Umami standard					
A	30	24.61	0.97 (0.35)	0.011 (0.004)	16
B	30	24.61	0.97 (0.35)	0.094 (0.034)	27
C	30	24.61	0.97 (0.35)	0.170 (0.062)	36

[1/] Soup base consists of 11 ingredients which are corn flour, creamer, whole milk powder, sugar, onion powder, ground sage, parsley flakes, pepper extract, turmeric extract, chicken flavouring 612761E, and chicken flavouring 610490H.

4.3.8.2 Odour-induced taste enhancement activity in recombinate of P50 smoked water

At least 22 potent aroma compounds contributed to the overall flavour perception in apple-wood smoked water samples, which were identified as described in **Chapter 2**, **Section 2.3.4. (Validation and confirmation of key odorants by recombination modelling).** To assess the potential of these potent aroma compounds to enhance the taste (specifically, umami and salty) in a salt-reduced model soup, they were combined in recombinate (only 17 food grade compounds) form and then introduced into the salt-reduced soup.

In experiment 6 (**Table 4.9**), the investigation of the odour-induced taste enhancement of recombinate P50 smoked water was carried out using one level of salt-reduced model soups (30% reduction). Control and salt-reduced soups were prepared as per section 4.3.1 with MSG (0.062%), mixed 5'-ribonucleotides (RIBO) (0.007%), and various eight solutions (0.577%) added according to **Table 4.9**. The food grade 17 odour active compounds in P50 smoked water (referred to **Chapter 2, Section 2.3.4**) were produced in the full recombinate, partial recombinate and single recombinate of P50 smoked water (**Table 4.10**).

For each experiment, 15 mL of heated soup samples, coded with three-digit random numbers, were served in a balanced order, with sample sets randomly allocated to panellists. The panellists were asked to evaluate the saltiness and umami intensity using structured line scales (0-100). Two salty references of standard 1 and 3 and two umami references of standard A and C (from 4.3.8.1) were presented to the panellists at the start of each rating session for re-familiarisation of the standard anchors as needed. Each time, the maximum number of soup samples presented to the panellists was 8, including the four references to avoid fatigue. All panellists scored samples in duplicate and a rest of 2-min was imposed between the duplicate. Compusense Cloud software (Compusense Cloud Software, Ontario, Canada) was used to acquire the sensory data.

Table 4.9 Experimental design for sensory attributes (umami and salty) scoring.

[2/] Samples	NaCl reduction (%)	Ingredients (%)						Total Na (mg/L)
		NaCl	[1/] Soup base	MSG	RIBO	[2/] Solutions (0.577 %)	Water	
Experiment 6 Different solutions (PST50, full recombinate, single compound and partial recombinate)								
17	30	0.35	8.93	0.062	0.007	-	90.65	24.7
18	30	0.35	8.93	0.062	0.007	P50	90.13	24.7
19	30	0.35	8.93	0.062	0.007	R17	90.13	24.7
20	30	0.35	8.93	0.062	0.007	G	90.13	24.7
21	30	0.35	8.93	0.062	0.007	S	90.13	24.7
22	30	0.35	8.93	0.062	0.007	M	90.13	24.7
23	30	0.35	8.93	0.062	0.007	G+S+M	90.13	24.7
24	30	0.35	8.93	0.062	0.007	G+S+M+F	90.13	24.7

[1/] Soup base consists of 11 ingredients which are corn flour, creamer, whole milk powder, sugar, onion powder, ground sage, parsley flakes, pepper extract, turmeric extract, chicken flavouring 612761E, and chicken flavouring 610490H.

[2/] Soup varying the solution of (17) no solution, (18) P50 smoked water, (19) full recombinate of P50 smoked water (17 food grade compounds), (20) single compound; guaiacol (G), (21) single compound; syringol (S), (22) single compound; 4-methylguaiacol (M), (23) partial recombinate; (G) + (S) + (M), (24) partial recombinate; (G) + (S) + (M) + 2-furfural (F).

Table 4.10 Solution recipes that added into the 30% salt-reduced model soup.

Odorant standard compounds	[1/] Solution types							
	17	**18**	**19**	**20**	**21**	**22**	**23**	**24**
1. Acetic acid (80 mg/L)			✓					
2. 2-Furfural (35 mg/L)			✓					✓
3. 2-Acetylfuran (1 mg/L)			✓					
4. Phenol (3 mg/L)			✓					
5. 4-Methylphenol (2 mg/L)			✓					
6. Guaiacol (6 mg/L)			✓	✓			✓	✓
7. 2,6-Dimethylphenol (0.1 mg/L)			✓					
8. 4-Ethylphenol (0.3 mg/L)			✓					
9. 4-Methylguaiacol (5 mg/L)			✓			✓	✓	✓
10. 4-Ethylguaiacol (1 mg/L)			✓					
11. 4-Vinylguaiacol (0.05 mg/L)			✓					
12. Syringol (30 mg/L)			✓		✓		✓	✓
13. Eugenol (0.3 mg/L)			✓					
14. Vanillin (6 mg/L)			✓					
15. Isoeugenol (0.04 mg/L)			✓					
16. 2,5-Dimethylphenol (2 mg/L)			✓					
17. Cyclotene (2.5 mg/L)			✓					

[1/] Solution of (17) no solution, (18) P50 smoked water, (19) full recombinate of P50 smoked water (17 food grade compounds), (20) single compound; guaiacol (G), (21) single compound; syringol (S), (22) single compound; 4-methylguaiacol (M), (23) partial recombinate; (G) + (S) + (M), (24) partial recombinate; (G) + (S) + (M) + 2-furfural (F).

4.3.9 Sensory evaluation

Twelve trained in-house sensory panellists (aged 35-65 years, 1 male and 11 females) were used for all sensory evaluations. The panellists used a series of three-alternative forced choice (3-AFC) method with or without nose clip to exclude and include aroma. The sensory evaluations were carried out at the Sensory Science Centre at the Department of Food and Nutritional Sciences (University of Reading) under red light to mask differences in appearance between the samples, in isolated booths and under controlled temperature (21 ± 1 °C).

4.3.10 Data analysis

All data obtained from the discrimination testing by the 3-AFC method were analysed using Thurstonian modelling in XLSTAT (Addinsoft, New York, USA). In all cases, the samples were considered significantly different at the 5% significance level ($p < 0.05$). In general, the number of sensory panellists should use N = 50 or more to get the high power of testing (Lawless & Heymann, 1999). In order to increase the number of responses, two potential approaches are to employ a greater number of panellists or assign more tests (replicates) to a smaller number of well-trained panellists. The data assumes all panellists are independent. However, in this study the number of panellists was set, thus each panellist had to test the samples more than once during a session, although they may not be independent. The potential for non-dependent judgements among replications is therefore examined. To address any potential variability among panellists (gamma - overdispersion) arising from replication in the data, the beta-binomial model was employed in cases of significant overdispersion. However, no significant overdispersion was observed, thus the binomial model was sufficient to be used when all replicates were combined as pooled data.

The 3-AFC method for evaluating the smoky odour detection threshold, the calculation was performed with a geometric mean (GEOMEAN) using Microsoft Excel. In experiment 4, 5 and 6, the data from the salty and umami intensity was analysed by analysis of variance (ANOVA) with the main effects tested against the sample by assessor interaction, sample fitted as a fixed effect and assessor as a random effect using SENPAQ software (version 5.01, Qi Statistics, Kent, UK). Tukey-Kramer honestly significant difference (HSD) test was used to test sample pairs assuming a 5% significance level.

4.4 Results and Discussion

4.4.1 Preliminary screening of smoked ingredients in fresh soup

Screening for different taste modalities in fresh soup showed that 8/11 panellists selected the sample with smoke as the saltiest, 7/11 as the most umami, 5/11 for sweetness, 3/11 for bitterness and 2/11 for sourness. This indicated that salty and umami should be

monitored in discrimination tests to investigate taste-active and taste-enhancing compounds in smoked water.

4.4.2 Determination of intensities perception of MSG and NaCl

Monosodium glutamate has an umami taste and the 5'-ribonucleotides such as disodium-5'-inosinate (Inosine monophosphate; IMP) and disodium-5'-guanylate (Guanosine monophosphate; GMP) also impart an umami taste (Yamaguchi & Ninomiya, 2000). Previous research concluded when the salts of glutamic acid in MSG interact with either IMP or GMP, the taste intensity is remarkably enhanced (Giovanni & Guinard, 2001; Ninomiya, 2002) due to the taste-enhancing synergism.

In order to assess whether the smoke had any taste-enhancing properties in the presence of MSG, an acceptable concentration of MSG had to be determined. The panellists were given nine concentrations of MSG solutions (1.42-9300 mg/L) and were asked to indicate the level where they first detected taste, the level where they could first recognise the taste, and the level where the taste became very strong. The average levels of perceived umami taste intensity are given in **Figure 4.2**. The average levels of umami taste perceived were started between level 4-7 (38.3-1033 mg/L) of MSG solutions. At the MSG level 5 (115 mg/L), the majority of panellists detected a taste. Then at the MSG level 6 (344 mg/L), the majority of the panellists recognised an umami taste. Moreover, at this MSG level 6, only one panellist (H) found that the taste was too strong. Based on these results, it was concluded that the MSG solutions used should be below level 7 (1033 mg/L). In this experiment, the MSG level 4, 5 and 6 (38, 115 and 344 mg/L, respectively) (or 0.004, 0.012 and 0.038% as used in **Table 4.12**) were selected for further study on identification the presence of taste-enhancing compounds in smoked water. These results are consistent with the published average detection threshold for MSG, which is 0.03% (Baines & Brown, 2016).

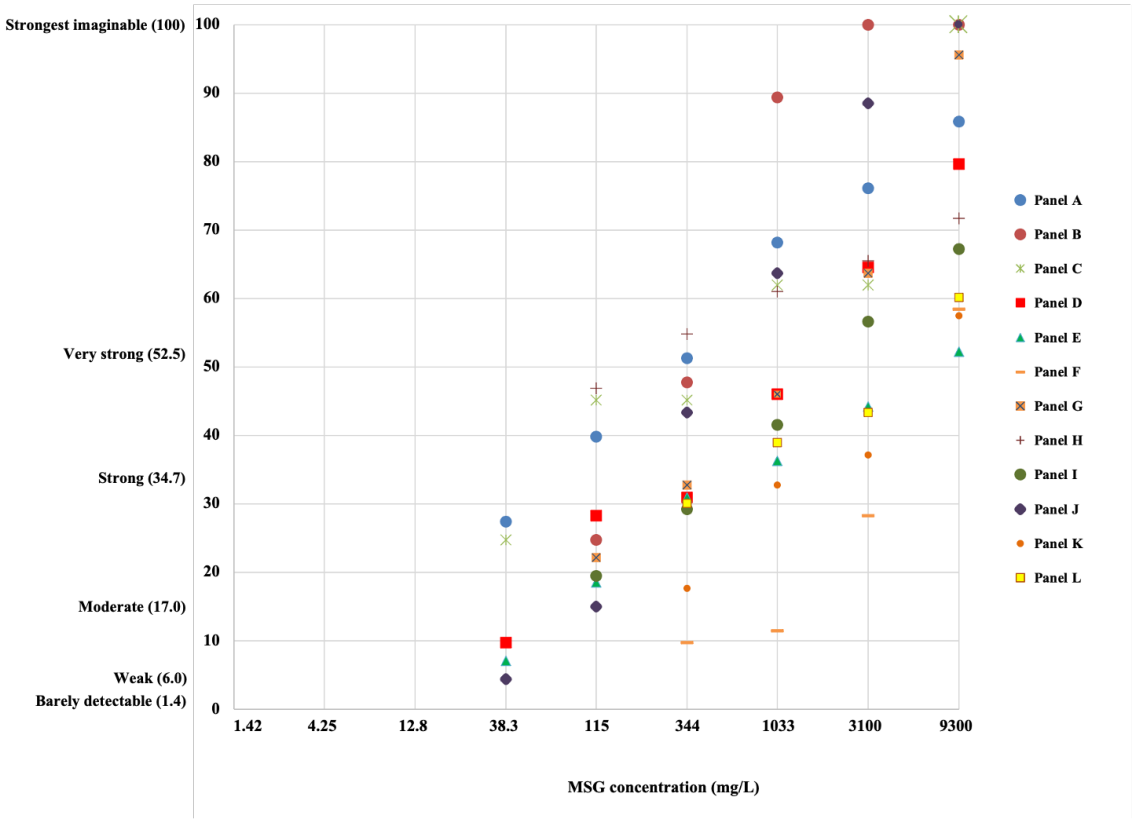

Figure 4.2 The study to determine acceptable concentrations of MSG for the whole panellists (12; A-L) was performed using serial dilutions of MSG (levels 1-9; 1.42, 4.25, 12.76, 38, 115, 344, 1033, 3100, and 9300 mg/L, respectively) and rated their umami perception of intensity on a general labelled magnitude scale (gLMS) (scale 0-100).

For the saltiness recognition threshold determination, 9 levels of NaCl were prepared in the same concentration ranges of MSG (1.4-9300 mg/L or 0.00014-0.93%) solutions. The panellists were given nine concentrations of NaCl solutions and were asked to indicate the level where they first detected taste, the level where they could first recognise the taste, and the level where the taste became very strong. The average levels of perceived salty taste intensity are given in **Figure 4.3**. The average levels of salty taste perceived were started between levels 1-8 (1.42-3100 mg/L) of NaCl solutions. At NaCl level 6 (344 mg/L or 0.034%), 40% of panellists recognised a salty taste. However, most panellists (80%) recognised a salty taste at NaCl level 7 (1033 mg/L or 0.103%). Even though all panellists recognised the salty taste at NaCl level 8 (3100 mg/L or 0.31%), only panellists H and J accepted the saltiness without the feeling of being too strong at this level. From this point of view, this panel group's average recognised salty threshold was between NaCl levels 6-7 (344-1033 mg/L or 0.034-0.103%).

Panellist D was the most sensitive to salty taste as they recognised the salty taste from the lowest concentration of NaCl since. The salty recognition threshold from this study was similar to Hatae et al. (2009), that investigated the detection and recognition thresholds for salt solution in young female students which their average detection threshold was 0.719 mM (0.0042%), and the recognition threshold was 9.68 mM (0.057%). There are many influences for individual differences in the threshold levels of taste perception. The differences in salty threshold among people are affected by age, gender, genetics, etc. Some evidence found that younger people had tastes threshold lower than older people (Kennedy et al., 2010; Mojet et al., 2001).

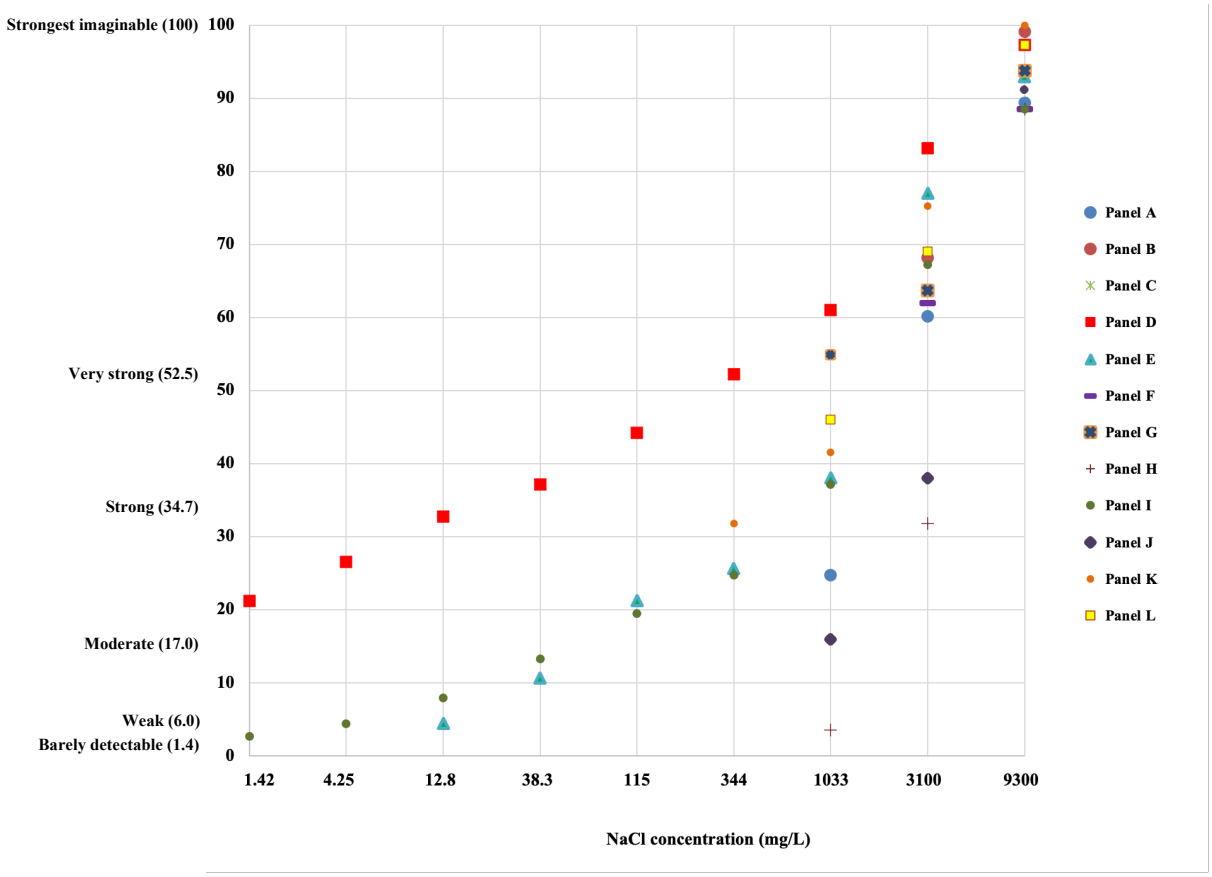

Figure 4.3 The study to determine acceptable concentrations of NaCl for the whole panellists (12; A-L) was performed using serial dilutions of NaCl (levels 1-9; 1.42, 4.25, 12.76, 38, 115, 344, 1033, 3100, and 9300 mg/L, respectively) and rated their salty perception of intensity on a general labelled magnitude scale (gLMS) (scale 0-100).

4.4.3 Determination of the taste-active compounds in smoked water

The taste-active compounds activity in TR and P50 smoked water was investigated in simple (water) and complex mixtures of the model soup and fresh soup with the assumption that the sample containing smoked water would have a stronger taste or flavour than the sample without smoke. The determination used the discrimination test, and the panellists wore the nose clip. Even though there was some limitation concerning whether the nose clips were sufficient to guarantee no odour was perceived during the tasting, some correct answers could come from the fact that the panellists perceived the difference in smoke odour instead of different tastes if the nose clip was not appropriately covered. In order to prevent this error, the sample sets were presented to the panellists after they applied nose clips to their nostrils. They were instructed to wear the nose clips securely throughout the whole tasting of the sample set. With a 2-min break between sample sets, the panellists could remove the nose clip while waiting for the next set. In addition, they were instructed to wear nose clips correctly before beginning testing on each subsequent set.

When two samples are compared in the Thurstonian model, the distance between them is measured by the parameter δ (the degree of difference between products), which is estimated by the statistic d' (Worch & Delcher, 2013). If the relevant distributions are sufficiently far apart that they do not overlap, it means two samples are different and easily identifiable. **Table 4.11** showed when both TR ($p = 0.016$; $d' = 0.971$) and P50 ($p = 0.004$; $d' = 1.191$) smoked water was added to the simple mixture of water, an umami taste was found in the sample that contained smoke. Any taste enhancement in smoked water was not found in the complex mixture of model soup that contained either TR or P50 smoked water. However, in the complex mixture of fresh soup, only P50 enhanced any taste ($p \leq 0.0001$, $d' = 1.342$). Additionally, when a high concentration of P50 smoked water (2%) was added to a model soup, no significant enhancement in umami taste was observed. This finding indicates that even at a high concentration of smoked water, the non-volatile compounds responsible for umami taste are insufficiently concentrated to enhance umami in a complex model soup matrix. These results suggest that there could be evidence of non-volatile compounds in smoked water being taste-active compounds (tastant), especially umami compounds, in small amounts, which the model soup ingredients could suppress. In addition, some components (such as yeast extract) of fresh soup may contain umami compounds that could provide synergy with P50 smoke to enhance the taste. Yeast extracts contain many components such as amino acids, peptides, nucleotides,

and other flavouring compounds that contribute to savoury flavours and umami taste sensations, which are formed during the manufacturing of yeast extract. Yeast extracts are commonly utilised as flavouring agents and taste-active compounds that contribute to the savoury, meaty, kokumi, umami, and salty taste in food. Yeast extracts can elicit an umami taste, similar to monosodium glutamate (MSG), by providing glutamate of less than 1% in food products (Tomé, 2021).

According to Alim et al. (2019), yeast extract was subjected to heat treatment to separate fifteen umami peptides; seven peptides (Pro-Ala-Ala, Gly-Gly-Tyr, Val-Ala-Val, Leu-Val, Leu-Val-Gly, Val-Val, and Pro-Glu-Thr) exhibited different tastes based on concentration; and other eight of these peptides (Lys-Gly, Gln-Leu, Asn-Tyr, His-Val, Glu-Ser, Glu-Leu, Glu-Ala, and Glu-Asn) demonstrated a potent umami taste at high concentration. The threshold range for umami peptides in yeast extracts was determined to be 0.07–0.61 mM (Wang et al., 2023). Additionally, yeast extract was isolated and its salty substances were identified; there were five peptides with a salty taste, which were Asp-Asp, Glu-Asp, Asp-Asp-Asp, Ser-Pro-Glu, and Phe-Ile (Zheng et al., 2021). S. Wang et al. (2019) conducted a comparison between MSG and its substitutes to determine their impact on the sensory attributes of chicken soup. Four substitutes were chosen: two mushroom extracts (CE and MC), tomato extract (TC), and yeast extract (YE). Subsequently, these extracts, together with MSG, were separately distributed at varying concentrations in chicken soup. According to the equivalent umami concentration (EUC) values, yeast extract had the highest value among others and also had the highest levels of inosine 5′-monophosphate (IMP) and guanosine 5′-monophosphate (GMP), measuring 9.49 and 8.48 g/100 g, respectively.

Table 4.11 A 3-AFC test for investigating the taste-active compounds in smoked water by trained panellists with nose clips.

Experiments	Sample base	Smoke	MSG, %	RIBO, %	[1/] p-value	[2/] *d′*
1.1	Water (taste)	1% TR	0	0	0.191	0.397
	Water (salty)	1% TR	0	0	0.263	0.356
	Water (umami)	1% TR	0	0	*0.016*	0.971
	Water (taste)	1% P50	0	0	*0.004*	1.057
	Water (salty)	1% P50	0	0	0.453	0.148
	Water (umami)	1% P50	0	0	*0.004*	1.191
1.2	Model soup (taste)	1% TR	0	0	0.191	0.397
	Model soup (taste)	1% P50	0	0	0.521	0.061
	Model soup (taste)	2% P50	0	0	0.576	n/a
	Fresh soup (taste)	1% TR	0	0	0.079	0.559
	Fresh soup (taste)	1% P50	0	0	*≤0.0001*	1.342

[1/] The p-values were generated using the binomial model and are over two replications with gamma < 0.001.

[2/] *d′* is the distance in perceptual standard deviations between sample pairs used for each sample as determined by binomial calculations; n/a is not applicable.

4.4.4 Determination of the taste-enhancing compounds in smoked water

4.4.4.1 Taste enhancement of smoked water with MSG solution

In order to understand whether taste-enhancing compounds are presented in smoked water, either TR and P50 smoked water was added into both simple mixture models and complex real food products. In the simple model solution, in order to confirm whether the non-volatile compounds in smoked water can interact synergistically with glutamate to increase umami taste, the selected three levels of MSG solutions (38, 115, 344 mg/L or 0.004, 0.012, 0.034%) in the range of umami recognition threshold (from the experiment that determined the recognition levels of umami taste) and one suprathreshold (620 mg/L or 0.062%) were combined with either 1%TR or 1%P50 smoke. These MSG concentrations are consistent with the published average detection threshold for MSG in water which is 300 mg/L (Baines & Brown, 2016; Yamaguchi, 1967).

Even though the addition of MSG will enhance the pleasantness and palatability at the optimum concentration for each food product; however, the pleasantness will be lost beyond the optimum level (Chi & Chen, 1992; Yamaguchi & Takahashi, 1984). In experiment 2.1 (**Table 4.12**), the panellists were asked to wear a nose clip and assess the stronger umami taste of each MSG concentration with TR or P50 smoke using a 3-AFC test. The result showed no synergistic effect between MSG and both smoked water at low concentrations of MSG (subthreshold and threshold levels), except in the highest level of MSG (0.062%), which revealed the synergistic between MSG and TR smoke (p = 0.001, d' = 1.432). This indicated that the synergistic effect between MSG and smoked water in simple mixtures could only occur above umami threshold (MSG level at 0.062%). At below umami threshold levels, the small amount of some taste-active compounds in smoked water could not boost umami perception.

Table 4.12 A 3-AFC test for investigating the taste enhancement compound (effect of MSG on umami taste) in smoked water by trained panellists.

Experiments	Sample base	Smoke	MSG, %	RIBO, %	[1/] p-value	[2/] d'
2.1	Water	1% TR	0.004	0	0.460	0.109
	Water	1% TR	0.012	0	0.848	0.000
	Water	1% TR	0.034	0	0.191	0.397
	Water	1% TR	0.062	0	*0.001*	1.432
	Water	1% P50	0.004	0	0.092	0.559
	Water	1% P50	0.012	0	0.339	0.232
	Water	1% P50	0.034	0	0.521	0.061
	Water	1% P50	0.062	0	0.263	0.356
2.2	Model soup	1% TR	0.004	0	0.263	0.356
	Model soup	1% TR	0.012	0	0.092	0.559
	Model soup	1% TR	0.034	0	0.191	0.397
	Model soup	1% TR	0.062	0	*0.050*	0.763
	Model soup	1% P50	0.004	0	0.339	0.232
	Model soup	1% P50	0.012	0	0.108	0.559
	Model soup	1% P50	0.034	0	0.521	0.061
	Model soup	1% P50	0.062	0	*0.004*	1.116

[1/] The p-values were generated using the binomial model and are over two replications with gamma < 0.001.

[2/] d' is the distance in perceptual standard deviations between sample pairs used for each sample as determined by binomial calculations; n/a is not applicable.

For the complex mixture of real food (**Table 4.12**, experiment 2.2), either 1%TR and 1%P50 smoked water were added to the model soup containing various concentrations of MSG as same as the simple mixtures. The 3-AFC method asked the panellists to identify the sample with the strongest umami taste while wearing a nose clip. None of these differences was statistically significant when TR and P50 smoke was added to the model soup that contained the MSG at the umami recognition threshold levels (MSG concentration at 0.004, 0.012 and 0.034%). Nevertheless, the umami enhancement was revealed significantly only when TR ($p = 0.050$, $d' = 0.763$) and P50 ($p = 0.004$, $d' = 1.116$) smoke were added to the model soup that contained MSG at a suprathreshold level of umami threshold (MSG level at 0.062%). These data suggest that there might be taste-enhancing compounds in smoked water, which interact with the MSG to enhance the umami taste in model soup; however, the MSG content in the soup must be higher than the umami threshold level to suppress the soup ingredient that masked the taste-enhancing compounds in smoked water.

4.4.4.2 Taste enhancement of smoked water with MSG-Mixed 5'-ribonucleotides solution

The taste enhancement of smoked water due to the synergistic was also investigated with another taste enhancer, mixed 5'-ribonucleotides (1:1 mix of IMP and GMP; RIBO), in combination with various MSG concentrations which used methods similar to those in the above experiment. In the simple mixture (**Table 4.13**; experiment 2.3), the results showed that the RIBO did not react synergistically with either TR or P50 smoked water to enhance the umami taste at all MSG concentrations. However, with RIBO present with no MSG, there was a significant umami enhancement when TR and P50 smoke were added ($p = 0.017$ and $p \leq 0.0001$, respectively). These findings indicate that the non-volatile compounds in smoked water can combine with RIBO to enhance the umami taste at the cognitive level. This is evident from the significant difference in taste perception between mixtures with and without smoked water (large d'), despite the fact that 5'-ribonucleotides typically have the characteristic umami taste (Yamaguchi & Ninomiya, 2000). It is likely that for the RIBO solution alone, the umami intensity remained at a low level, which allowed for a significant umami enhancement when it reacted with umami compounds in smoked water. Meanwhile, if considered in the ternary combinations between mixture of umami compounds (MSG and RIBO) and smoked water, for which the umami strength was possibly too high for such effects to be perceived which showed no noticeable enhancement of umami in any MSG concentrations plus with RIBO and smoked

water. The synergism of MSG and 5'-ribonucleotides has been investigated in the binary and tertiary mixtures (Giovanni & Guinard, 2001; Yamaguchi, 1967).

Table 4.13 A 3-AFC test for investigating the taste enhancement compound (effect of MSG and ribonucleotides on umami taste) in smoked water by trained panellists.

Experiments	**Sample base**	**Smoke**	**MSG, %**	**RIBO, %**	[1/] **p-value**	[2/] ***d′***
2.3	Water	1% TR	0	0.007	*0.017*	1.032
	Water	1% TR	0.004	0.007	0.895	n/a
	Water	1% TR	0.012	0.007	0.149	0.559
	Water	1% TR	0.034	0.007	0.310	0.327
	Water	1% TR	0.062	0.007	0.149	0.559
	Water	1% P50	0	0.007	*≤0.0001*	1.710
	Water	1% P50	0.004	0.007	0.453	0.148
	Water	1% P50	0.012	0.007	0.453	0.148
	Water	1% P50	0.034	0.007	0.263	0.356
	Water	1% P50	0.062	0.007	0.834	n/a
2.4	Model soup	1% TR	0	0.007	*0.019*	1.116
	Model soup	1% TR	0.004	0.007	*0.004*	1.432
	Model soup	1% TR	0.012	0.007	*0.019*	1.116
	Model soup	1% TR	0.034	0.007	0.178	0.559
	Model soup	1% TR	0.062	0.007	0.368	0.288
	Model soup	1% P50	0	0.007	0.263	0.356
	Model soup	1% P50	0.004	0.007	*0.004*	1.191
	Model soup	1% P50	0.012	0.007	0.263	0.356
	Model soup	1% P50	0.034	0.007	*0.016*	0.971
	Model soup	1% P50	0.062	0.007	0.108	0.559

[1/] The p-values were generated using the binomial model and are over two replications with gamma < 0.001.

[2/] *d′* is the distance in perceptual standard deviations between sample pairs used for each sample as determined by binomial calculations; n/a is not applicable.

To determine whether the umami enhancement resulted from the synergy between the smoke that possessed any taste-enhancing compounds with other umami compounds (MSG and RIBO), was also conducted in a model soup. In experiment 2.4 (**Table 4.13**), either 1%TR or P50 smoked water was added to a model soup containing various contents of MSG with a fixed amount of RIBO. The results revealed that the umami enhancement of TR smoke was shown in the model soup samples without MSG (only RIBO) and with MSG at the subthreshold levels (0.004 and 0.012%); meanwhile, this umami enhancement of smoke in low levels of MSG was not consistent for P50 smoke. These results indicated that the synergistic effects of umami compounds and smoked water to enhance umami in model soup happened at the umami subthreshold levels. The umami strength was too high to make a difference at the threshold and suprathreshold levels of MSG plus RIBO and smoked water. Any tastants detected in water are masked by the presence of suprathreshold MSG or by the sample complexity. Nasri et al. (2013) reported a similar result, finding that adding salt-associated aroma (sardine odour) to potassium chloride solution alone enhanced the salty intensity. However, when sardine aroma was added to a binary mixture of potassium chloride and sodium chloride, no substantial odour-induced salt enhancement was seen because the salt intensity of the KCl-NaCl mixture was too high and suppressed the odour-induced salt enhancement of sardine aroma.

4.4.5 Preliminary assessment of smoked water to elicit odour-induced taste enhancement (OITE)

4.4.5.1 Recognised smoky odour threshold

The recognised smoky odour threshold was determined before evaluating an appropriate smoked water concentration at which their aroma-induced taste enhancement occurred. The smoky odour recognition threshold is expressed as the geometric mean, as summarised in **Table 4.14**. The recognised threshold of the smoky odour of panellists was between 0.012-0.146%, and the average value among the panellists was 0.204%. However, panellist K could not perceive the smoky odour at the maximum levels of 0.192 and 1.731% P50 smoke, respectively.

Table 4.14 The geometric mean of smoky odour detection threshold by the rapid detection threshold (RTD) method.

Panellists	[1/] Rapid detection threshold (RTD) method		
	Not detected conc.	**Detected conc.**	**Geometric mean**
A	0.111	0.192	0.146
B	0.037	0.064	0.049
C	0.037	0.111	0.064
D	0.037	0.064	0.049
E	-	0.021	≤ 0.021
F	-	0.021	≤ 0.021
G	0.111	0.192	0.146
H	-	0.012	≤ 0.012
I	-	0.012	≤ 0.012
J	0.111	0.192	0.146
K	1.731	-	> 1.731
L	0.021	0.111	0.048
Mean			0.204

[1/] Concentration of P50 smoke were 0.012, 0.021, 0.037, 0.064, 0.111, 0.192, 0.333, 0.577, 0.999 and 1.731%.

The alternative forced choice (AFC) is the most common method used to determine aroma/taste thresholds which require several sample sets to taste but leads to the fatigue of the panellists. A forced choice single ascending series (without repeating concentration steps), is used to determine the threshold to avoid the panellist's cognitive fatigue (Kennedy et al., 2010). Even if this method could be suitable for determining the threshold, some individual thresholds could be inaccurate due to the high false positive of the correct answer given by chance. Therefore, the rapid detection threshold (RTD) method is used to overcome an accurate individual threshold due to correct guessing, as this method selected the right answer from three reversals (Allen et al., 2014). The RTD method also reduces the number of samples sets to taste because it starts from the middle point of the range of sample sets which helps to overcome fatigue.

The efficiency of the RTD method in this experiment showed clearly for panellist K which has the smoky threshold in high concentration. The result suggested that panellist K still

did not perceive the smoky aroma at the highest smoke concentration at 1.731%, which means panellist K is not sensitive to the smoke aroma and could classify panellist K as an outlier value among this panel group. The RTD method is suitable for determining the smoky aroma recognition threshold due to it providing a more reliable value and high sensitivity method. The suprathreshold of smoky odour at 0.577%, however, was used to further evaluate of the odour-induced taste enhancement; in order to confirm most of panellist can perceive the smoky aroma but did not feel it was a very strong odour.

4.4.5.2 Screening test of OITE of smoked water

In order to understand how low levels odour compounds, especially smoky odour, in smoked water induces salt or umami enhancement, the five concentrations (0.004, 0.012, 0.037, 0.111 and 0.333%) between the smoky odour recognised threshold of P50 smoke were added either fresh or model soups (**Table 4.15**). In order to allow panellists to detect the smoky aroma, they were asked to not wear a nose clip for both sessions of the 3-AFC test.

Table 4.15 A 3-AFC test for investigating the odour-induced any taste enhancement (screening) in smoked water by trained panellists.

Experiments	Sample base	Smoke	MSG, %	RIBO, %	[1/] p-value	[2/] *d'*
3.1	Fresh soup	0.004% P50	0	0	0.607	n/a
	Fresh soup	0.012% P50	0	0	0.819	n/a
	Fresh soup	0.037% P50	0	0	0.178	0.559
	Fresh soup	0.111% P50	0	0	*0.0001*	6.923
	Fresh soup	0.333% P50	0	0	*0.0001*	6.923
3.2	Model soup	0.004% P50	0	0	0.213	0.559
	Model soup	0.012% P50	0	0	0.896	n/a
	Model soup	0.037% P50	0	0	*0.020*	1.237
	Model soup	0.111% P50	0	0	*0.001*	2.229
	Model soup	0.333% P50	0	0	*0.003*	1.650

[1/] The p-values were generated using the binomial model and are over two replications with gamma < 0.001.

[2/] *d'* is the distance in perceptual standard deviations between sample pairs used for each sample as determined by binomial calculations; n/a is not applicable.

The first session (**Table 4.15**, experiment 3.1) results with the fresh soup (**Figure 4.4**) revealed that the panellists perceived a significant difference of flavour between fresh soup with or without smoke when the smoked water concentration was over 0.111%. Nine out of twelve panellists recognised taste enhancement when P50 was added. Eight out of twelve panellists perceived salt or umami enhancement. Likewise, five out of twelve panellists recognised taste enhancement at concentrations (0.012-0.111%) of P50, which were lower than the concentrations where they first recognised the smoky flavour (0.111-0.333%).

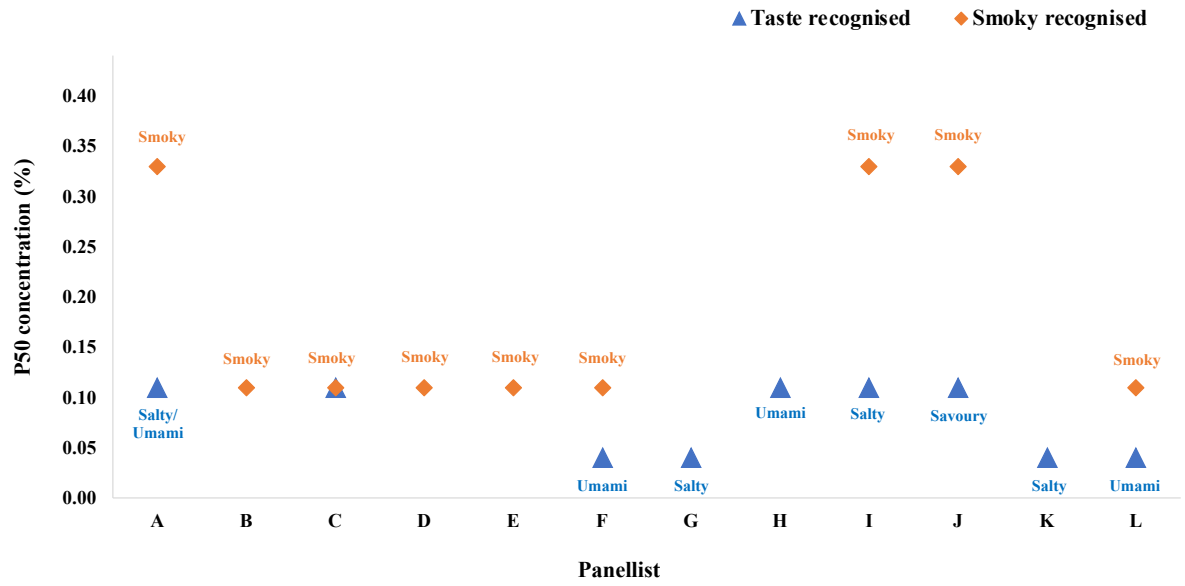

Figure 4.4 P50 concentration at which panellists first perceive a difference in taste and aroma in fresh soup.

The results from the model soup session are shown in **Figure 4.5**. The panellists can recognise the difference in flavour between model soup with or without smoke when smoke concentration was higher than 0.037% onward (**Table 4.15**, experiment 3.2). The results showed five out of nine panellists perceived enhancement in salty or umami taste, but only three out of nine panellists recognised an enhancement in taste at concentrations ranging between 0.004-0.111% of P50, which were lower than those at which the smoky flavour was first recognised (0.037-0.333%).

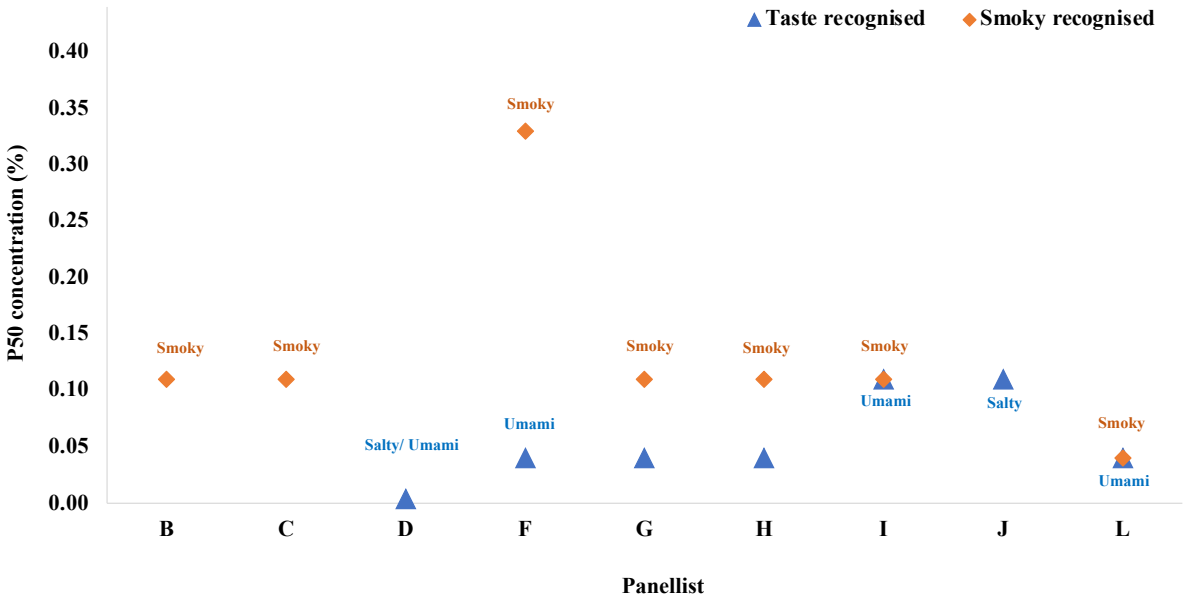

Figure 4.5 P50 concentration at which panellists first perceive a difference in taste and aroma in model soup.

Observing the results from both sessions, it is evident that there is a cross-modal odour-taste interaction generated whereby the odour compounds induced taste enhancement (especially umami and salty tastes), was found when the P50 smoke was added at levels close to threshold in both soups. It may be concluded that the smoky aroma may act cross-modally to enhance taste and flavour, and this smoky aroma probably is a congruent aroma to saltiness or umami taste. A number of studies have shown that the congruent aroma could potentially enhance perceiving of taste such as cocoa aroma induced bitterness and vanilla aroma increases sweetness (Labbe et al., 2006), savoury flavouring improves saltiness (Batenburg & van der Velden, 2011), vanilla aroma enhances sweetness in milk (Q. J. Wang et al., 2019).

4.4.6 Determination of the odour-induced umami enhancement (OIUE) and odour-induced salty enhancement (OISE) of smoked water in salt-reduced model soup

To assess the influence of the cross-modality of the odour-taste interaction on taste enhancement, the study was carried out in a salt-reduced model soup to elucidate the role of smoked water in the perception of salty and umami tastes. For the first set (experiment 4), to evaluate the increase in salty and umami taste induced by odour perception, panellists assessed several model soup samples containing different salt levels with fixed amount of MSG

(0.062%), RIBO (0.007%) and P50 smoked water (0.577%) (Table 4.16) with or without nose clip during the tasting.

Table 4.16 Mean panel scores for sensory attributes (umami and salty) of various samples in model soup.

Samples	[1/] Rating score (0-100 scale)			
	Salty		Umami	
	W/O NC	NC	W/O NC	NC
Experiment 4 Different levels of salt				
(1) 0.30%NaCl+0.062%MSG	26.8 c	25.7 d	40.0 c	35.0 d
(2) 0.30%NaCl+0.062%MSG+Smoke	31.6 c	26.3 cd	47.0 bc	36.7 d
(3) 0.30%NaCl+0.062%MSG+RIBO	27.9 c	31.3 bcd	44.7 bc	45.3 bc
(4) 0.30%NaCl+0.062%MSG+RIBO+Smoke	32.6 c	28.3 bcd	50.1 ab	50.8 ab
(5) 0.35%NaCl+0.062%MSG	30.0 c	27.2 cd	41.5 c	39.3 cd
(6) 0.35%NaCl+0.062%MSG+Smoke	32.2 c	29.0 bcd	42.9 bc	44.3 bc
(7) 0.35%NaCl+0.062%MSG+RIBO	32.4 c	36.5 b	45.0 bc	56.1 a
(8) 0.35%NaCl+0.062%MSG+RIBO+Smoke	39.9 b	34.8 bc	57.0 a	55.3 a
(9) 0.50%NaCl+0.062%MSG	48.0 a	45.7 a	47.4 bc	41.4 cd
(10) 0.50%NaCl+0.062%MSG+RIBO	45.8 ab	50.1 a	50.0 ab	51.2 ab

[1/] Means in each column not labelled with the same letters are significantly different ($p < 0.05$); means are from duplicate samples; data was collected using structured line scales (0-100); W/O NC is without nose clip; NC is with nose clip.

As shown in Table 4.16 (experiment 4), smoked water was able to compensate for the reduction in salt from 0.50% to 0.35% in sample 8 as the saltiness did not differ significantly from the original soup (sample 10). However, this compensation for a reduction in salt was not found when the nose clip was used, confirming the role of the aroma in the perceived salt enhancement. This salt enhancement was not observed when the RIBO were excluded (sample 6), nor was it observed when the salt content of the soup was reduced to 0.30% NaCl (samples 2 and 4). The cross-modal interaction of the smoke aroma also showed a significant enhancement in umami taste in sample 8, but this was not observed in the absence of the RIBO, nor in the 0.30% salt soups. When the aroma was excluded by the use of a nose clip, the umami enhancement was not observed. Looking at the whole set pairwise (with or without smoke), no

significant differences were observed with a nose clip on, and the only significant differences observed were for both salty and umami for the 0.35% salt soup with RIBO. As expected, the umami scores increased when the RIBO were added (sample 1 vs. 3, or 2 vs. 4 etc). This was significant in all cases when the nose clip was worn, but without the nose clip, the trends were there, but they were not always significant.

Table 4.17 Mean panel scores for sensory attributes (umami and salty) at various concentrations of MSG.

Samples	**[1/] Rating score (0-100 scale)**			
	Salty		**Umami**	
	W/O NC	**NC**	**W/O NC**	**NC**
Experiment 5 Different levels of MSG				
(11) 0.35%NaCl+0.004%MSG+RIBO	31.2 a	22.4 b	39.2 b	33.9 b
(12) 0.35%NaCl+0.004%MSG+RIBO+Smoke	34.1 a	27.7 ab	51.1 a	44.0 ab
(13) 0.35%NaCl+0.012%MSG+RIBO	33.8 a	31.3 ab	41.5 ab	45.9 ab
(14) 0.35%NaCl+0.012%MSG+RIBO+Smoke	37.3 a	27.0 ab	50.3 a	41.9 ab
(15) 0.35%NaCl+0.034%MSG+RIBO	37.5 a	34.0 a	45.8 ab	45.7 ab
(16) 0.35%NaCl+0.034%MSG+RIBO+Smoke	34.2 a	28.8 ab	52.5 a	47.8 a

[1/] Means in each column not labelled with the same letters are significantly different ($p < 0.05$); means are from duplicate samples; data was collected using structured line scales (0-100); W/O NC is without nose clip; NC is with nose clip.

In the second experiment session (**Table 4.17;** experiment 5), a 30% salt-reduced model soup (0.35% salt) containing four MSG levels with a fixed amount of RIBO and smoked water were evaluated for saltiness and umami intensity with or without nose clip. The results are shown in **Table 4.17**, whether or not the nose clip was worn, smoked water did not affect the saltiness intensity of any of the samples, even if it contained different MSG contents. For the umami intensity without a nose clip, looking pairwise (with or without smoke), there was no significant increase in umami intensity when smoked water was added, except at the lowest concentration of MSG (0.004%) (sample 11 and 12), and was not influenced by the varying content of MSG. With the nose clip, no umami enhancement was shown when smoked water was added at each concentration of MSG. This shows that the major enhancement of umami is odour-induced at the very low level of MSG. As expected, the umami intensities increased as

the concentration of MSG increased. Observing the results from both sessions, it is evident that there is a cross-modal odour-taste interaction generated whereby the odour compounds induced taste enhancement (especially umami taste), was found when the smoked water was added to salt-reduced model soup. The smoky aroma may act cross-modally to enhance taste and flavour, and this smoky aroma probably is a congruent aroma of umami or saltiness taste.

Previous evidence suggest that congruent aroma may be necessary for odour induced taste enhancement. Lawrence et al. (2009) have shown that a number of specific salt-associated odours, anchovy and bacon odours, via the retronasal route could amplify the saltiness of a low-concentrated NaCl solution. The authors also stated, however, that some odours not associated with salt could lead to a reduction in saltiness, tomato and carrot odours, although the intensity of these odours were quite high, the odour induced salty enhancement (OISE) remained very low or negative. Other studies have shown that the congruent aroma could potentially enhance perceiving of taste such as cocoa aroma induced bitterness and vanilla aroma increases sweetness (Labbe et al., 2006), savoury flavouring, sotolon, improves saltiness (Batenburg & van der Velden, 2011), vanilla aroma enhances sweetness in milk (Q. J. Wang et al., 2019). The cross-modal of odour-taste interaction, this implies that taste can boost the intensity of odour meanwhile the odour can enhance the taste perception. The odour-taste interaction may happen when the odour and taste compounds are at levels above or below the threshold which depends on the food matrixes (Poinot et al., 2013).

4.4.7 The odour-induced umami enhancement (OIUE) and odour-induced salty enhancement (OISE) of smoked water recombinate in salt-reduced model soup

The odour-induced taste enhancement (umami and salty tastes) activity in the P50 smoked water recombinate was evaluated in 30% salt-reduced model soup compared to P50 smoked water (**Table 4.18**). Seventeen food-grade odour-active compounds were prepared in actual contents found in P50 smoked water to make a full P50 smoke recombinate. In addition, the four (sample 24) and three (sample 23) main odour-active compounds were prepared in the form of partial recombinate, and three single compounds were also prepared (samples 20, 21, 22). The panellists were asked to score umami and salty tastes on the structured line scale (0-100) against the three umami (sample A = 16 points, B = 27 points, C = 36 points) and three salty (sample 1 = 17 points, 2 = 25 points, 3 = 42 points) standards that had already anchored

the points on the line scale (data from training). The panellists tasted the samples with and without wearing the nose clip. The results revealed that none of the samples was significantly different in both umami and salty tastes from the soup containing P50 smoked water, whether the nose clip was included or not. Even though all salty and umami scores were not significantly different, the result trends were similar to the result of experiment 4 (**Table 4.16**). When the nose clip was excluded, the panellist perceived a little more umami taste in every soup sample compared to the control one (sample 17), which was found to have the highest score in a soup containing P50 smoked water (sample 18). However, the results were ambiguous for saltiness scores with or without a nose clip and umami scores using a nose clip.

Table 4.18 Mean panel scores for sensory attributes (umami and salty) of the various samples.

[2/] Samples	[1/] Rating score (0-100 scale)			
	Salty		Umami	
	W/O NC	NC	W/O NC	NC
Experiment 6 Different of recombinant compounds				
(17) 0.35%NaCl+0.062%MSG+RIBO	31.2 a	29.9 a	33.9 a	32.0 a
(18) 0.35%NaCl+0.062%MSG+RIBO+PST	29.7 a	28.9 a	36.1 a	30.9 a
(19) 0.35%NaCl+0.062%MSG+RIBO+FRC	30.5 a	27.5 a	34.8 a	30.1 a
(20) 0.35%NaCl+0.062%MSG+RIBO+G	33.4 a	28.6 a	35.4 a	31.8 a
(21) 0.35%NaCl+0.062%MSG+RIBO+S	33.7 a	27.9 a	34.2 a	30.2 a
(22) 0.35%NaCl+0.062%MSG+RIBO+M	31.6 a	27.8 a	34.9 a	27.7 a
(23) 0.35%NaCl+0.062%MSG+RIBO+G+S+M	31.7 a	27.5 a	34.7 a	29.3 a
(24) 0.35%NaCl+0.062%MSG+RIBO+G+S+M+F	32.5 a	28.6 a	35.8 a	29.2 a

[1/] Means in each column not labelled with the same letters are significantly different ($p < 0.05$); means are from duplicate samples; data was collected using structured line scales (0-100); W/O NC is without nose clip; NC is with nose clip.

[2/] Soup varying the solution of (17) no solution, (18) P50 smoked water, (19) full recombinate of P50 smoked water (FRC), (20) single compound; guaiacol (G), (21) single compound; syringol (S), (22) single compound; 4-methylguaiacol (M), (23) partial recombinate; guaiacol+syringol+4-methylguaiacol (G+S+M), (24) partial recombinate; guaiacol+syringol+4-methylguaiacol+2-furfural (G+S+M+F).

Table 4.19 revealed an individual ability to discriminate the difference between soup samples with and without smoked water/recombinants of the panellists. This ability was observed even though there was no significant difference in the average scores for umami and salty tastes across the soup samples, as shown in **Table 4.18.** When a nose clip was excluded, 2 out of 12 panellists could discriminate the umami intensity among samples, and 2/12 panellists discriminated the saltiness. Meanwhile, when including the nose clip, it showed 2/12 panellists could discriminate the umami intensity, and 3/12 panellists discriminated the saltiness among samples. There was a subtle difference in both umami and salty tastes among the soup samples, but only a few assessors were able to detect this difference. The summary of this experiment could support the result of experiment 4 that smoke aroma compounds, particularly the odour-active compounds found in P50 smoked water, act cross-modally with taste enhancers (MSG and RIBO) compounds to enhance the taste, especially umami taste.

Table 4.19 The p-values for panellist's discrimination.

Panellists	**[1/] p-value**			
	Salty		**Umami**	
	W/O NC	**NC**	**W/O NC**	**NC**
P 1	*< 0.0001*	0.4470	*< 0.0001*	0.691
P 2	0.7500	0.6540	*0.0264*	0.3691
P 3	*0.0193*	0.7956	0.8523	0.7130
P 4	0.8591	*< 0.0001*	0.2757	*< 0.0001*
P 5	0.7798	0.3696	0.9076	0.4106
P 6	0.4324	0.1096	0.0881	0.8445
P 7	0.1704	0.3617	0.2127	0.8352
P 8	0.1794	0.4075	0.0551	0.1595
P 9	0.7034	0.3391	0.1925	0.2951
P 10	0.9998	0.1712	0.9998	0.2075
P 11	0.7308	*< 0.0001*	0.2229	*< 0.0001*
P 12	0.5384	*0.0385*	0.2561	0.1048

[1/] p-value is considered significantly different when $p \leq 0.05$; W/O NC is without nose clip; NC is with nose clip.

However, in this experiment section, as mentioned previously in **Chapter 2 (Characterisation of the key odorants in smoked water by means of the sensomics approach)** that 17 potent aroma compounds of P50 smoked water used to produce these recombinates, were identified and analysed in the concentrations using GC-MS and GC-O after storage of P50 smoked water more than a year. Therefore, there was a possibility that the concentration of some compounds could have decreased over time, resulting in an incorrect actual concentration of the potent aroma used to produce these recombinates. Moreover, some compounds in this old P50 smoked water have been reduced over time while used in this experiment section.

4.5 Summary

Three mechanisms of smoked water flavour enhancement were investigated using trained sensory panellists. The results of our study indicate that when smoked water was present, the panel perceived umami in the absence of MSG. In the complex mixture of model soup, the smoke could act synergistically with MSG at a suprathreshold level to enhance umami. When smoke water was added to the mixture of MSG and 5'-ribonucleotides, there was not show any umami enhancement as the result of an excessive umami taste to be perceived as the difference. In contrast, an umami enhancement was observed in the model soup containing MSG-5'-ribonucleotides at subthreshold umami levels. Interestingly, when the panel perceived the smoke-related aroma, umami was the primary taste enhanced when smoked water was combined with 5'-ribonucleotides in salt-reduced soups. This finding implies that the OITE was the primary mechanism by which smoked water enhanced flavours. Thus, the orthonasal odour effect of smoked water may be beneficial in savoury salt-reduced food products, as it may help compensate for the palatability loss associated with salt reduction.

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Chapter 5
Concluding remarks, limitations and future perspective

5.1 Study finding

The purpose of the final chapter of this thesis is to highlight the key findings of the research, introduce the limitations of this study and provide recommendations for future work. To prove the primary hypothesis that the use of smoked water as an ingredient can enhance the taste of salt-reduced soups; so, the aroma profile, quantitative data, key odorants, taste-active compound activity, taste-enhancing compound activity, and odour-induced taste enhancement activity of PST apple-wood smoked water were studied in comparison with TR apple-wood smoked water. In addition, the impact of PST processes on aroma profile, compound distribution, and quantitative was investigated in 3 hardwood smoked water which were apple (AP), beech (BH) and oak (OK). Where the study addressed followed the secondary hypotheses or research question of each experiment chapters (2-4);

Chapter 2 Research question: What are the potent odour active compounds in traditional (TR) and PureSmoke Technology (PST) smoked waters?

- This chapter aimed to identify odour active compounds in smoked water using the sensomics approach and validate the findings in a recombinant blend solution. Two types of smoked water, P50 and TR apple-wood smoked waters, were compared. This was the first time apple wood was analysed for the aroma compounds.

- The first step in the sensomics strategy was to choose the most appropriate extraction method for volatile and semi-volatile compounds. Then, the 12 subset methods made up of the four main extraction techniques—solid phase microextraction (SPME), solid phase extraction (SPE), liquid-liquid extraction (LLE), and stir bar sorptive extraction (SBSE)—were compared. Based on yielding the greatest number of extracted compounds, SPME and SPE, which used diethyl ether as an eluent, were the two best extraction techniques. These two methods retrieved around 116 aroma substances from smoked water, with the SPME and SPE approaches accounting for 100 and 82, respectively.

- To achieve aroma compounds as many as possible (polar, non-polar, etc.,) both SPME and SPE were used in the aroma extraction dilution analysis (AEDA) to identify the potent aroma compounds. The flavour dilution (FD) factor was the primary criterion to consider which compound seemed potent if it had a high value of FD factor. Sixty-seven aroma-active compounds were perceived at the sniffing port of the GC-O by two extraction methods. However, 14 out of 67 aroma-active compounds are still unidentified. The most compounds were phenols and guaiacols groups. Acetic acid, syringol, guaiacol, and 4-methylguaiacol were the major constituents of smoke extracts.

- The odour activity values (OAV) are the main criteria used to identify what compounds could be potent aroma compounds. At least 22 compounds with OAVs ≥ 1 guided as potent aroma compounds in smoke extracts. Guaiacol was the most potent aroma in smoked water, as it had the highest FD factor and the highest OAV, and it was described as a "cool herb, amine smoke, medicinal".

- These 22 compounds were combined to make full and partial recombinate at the approximate actual quantities found in P50 smoked water to validate whether or not these 22 compounds were potent aromas in smoked water. In comparison to the original P50 smoked water, a 5-point sensory profile on smoky, woody, ashy, and phenolic descriptors was performed in full recombinate and partial recombinate. The sensory profile scores of the recombinates did not match the actual P50 smoked water very well because the potent aroma numbers were not yet correct, or the concentration of the potent aroma was still lower than the actual amount in smoked water, indicating that the recombinate needed more refinement.

- In conclusion, P50 apple-wood smoked water had the same aroma profile as TR. Nevertheless, the ratio of compound constituents in P50 apple-wood smoked water was generally lower than in TR apple-wood smoked water.

Chapter 3 Hypothesis 1: Use of PureSmoke Technology (PST) to produce smoked water (as opposed to use of a traditional direct smoking process) alters the volatile profile of the product.

Hypothesis 2: Use of PureSmoke Technology (PST) reduces some of the harsh notes generated using the traditional process.

- This chapter aimed to evaluate whether or not the volatile profiles change when the smoked water is prepared using PST. There were three different kinds of wood (apple, beech and oak) to produced smoked water and three different manufacturing types (TR, P25 and P50).

- All nine smoked water samples had identical volatile compounds, but the amount varied, which accounted for 77 identified compounds. 34 of the 77 compounds were quantified using authentic odorant standards, while the remaining compounds were semi-quantified. The 77 identified compounds were divided into 8 groups according to the chemical classes.

- The most abundant substances discovered in all smoking water samples were phenol and derivatives, followed by aldehydes, ketones, diketones (carbonyl-contained group), and guaiacol and derivatives, in that order. When the PST was applied, the majority of the compounds found in smoked water samples were reduced.

- The principal component analysis (PCA) classified smoked water samples in a few groups. Applewood was related to phenols, beechwood was related to syringols and guaiacols, and oakwood was associated with furans derivatives.

- In conclusion, PST decreased the concentration of smoke components. The degree of the difference between P25 and P50 was significantly less than that between TR and P25. PST smoked water may be rounder/milder and have a better aroma balance than TR smoked water since the PST process lowered the phenol components, resulting in the elimination of harsher smoke-related odour.

Chapter 4 Hypothesis: Addition of smoked water to a low salt soup will enhance the taste of the soup.

- This chapter aimed to evaluate the three possible modes of action of smoked water created using PST (in order to reduce polycyclic aromatic hydrocarbons; PAHs), which leads to an enhancement of the perception of flavour: the smoke may contain taste-active compounds, or taste-enhancing compounds which might act synergistically with other taste enhancers, or it may contribute cross-modally whereby the odour compounds induce salt or savoury enhancement.

- Screening test showed smoked water imparts umami and salty tastes.

- The umami threshold of the trained panel group was between 38-344 mg/L.

- There could be evidence of non-volatile compounds in smoked water being taste-active compounds (tastant), especially umami compounds, in small amounts, which the model soup ingredients could suppress.

- In the simple solution, when smoked water was present, the panel perceived umami in the absence of MSG. In the complex mixture of model soup, the smoke could act synergistically with MSG at a suprathreshold level to enhance umami. When smoke water was added to the mixture of MSG and 5'-ribonucleotides, there was not show any umami. An umami enhancement was observed in the model soup containing MSG-5'-ribonucleotides at subthreshold umami levels.

- Umami was the primary taste enhancement when the panel perceived the smoke-related aroma which combined with 5'-ribonucleotides in salt-reduced soups.

- The odour-induced taste enhancement (umami and salty tastes) activity in the P50 smoked water recombinate (partial recombinate and single compound) was evaluated in salt-reduced model soup. When the nose clip was excluded, the panellist perceived a little more umami taste in every soup sample compared to the control soup that not contained any smoked water or recombinate.

- In conclusion, the OITE was the primary mechanism by which smoked water enhanced flavours.

5.2 Limitations of the study

There are some limitations that need to be considered in the present research:

- *Chapter 2, The identification of aroma-active compounds by Gas chromatography-Mass spectrometry (GC-MS) and GC-Olfactometry (GC-O) using Aroma Extraction Dilution Analysis (AEDA),* to interpret the FD factor and determine the OAV of each potent aroma component. Using standards curves, 34 of 77 compounds were quantified and identified. Many potent aroma compounds with FD factor values greater than 81, such as 3, 4-dimethylphenol, 3,5-dimethylphenol, 4-methoxyphenol, 3,4-dimethoxytoluene, 2-propylphenol, dihydroeugenol, and 6-methylguaiacol, could not be quantified (only semi-quantified) due to a lack of standard compounds. Therefore, precise values could not be provided in order to calculate the OAV of these substances.

- *Chapter 2, Quantification and semi-quantification of volatile compounds and calculation of the odour activity values (OAVs) of potent aroma compounds,* examined the potent aroma of smoked water using a sensomics approach in both TR and P50 apple-wood smoked water to determine if the PST process affects either the number of aroma-active compounds or their concentrations. Even though the detected aroma-active compound classes were identical in both smoked water samples, the concentrations of most P50 smoke compounds were lower than those of TR smoke compounds. We could not be sure that the concentration of the compounds was diminished due to the PST process, since the production dates and woodchip batch samples used to produce these two smoked water samples were not the same. Therefore, the difference in aroma-active compound concentrations may be due to the variability between two batches of woodchip properties, and this was clarified in Chapter 3 by using wood and smoke for the same batch to prepare TR, P25 and P50 smoked water in 3 different woods.

- *Chapter 2, Validation and confirmation of key odorants by recombination modelling,* 67 compounds were identified as the key odorants for smoked water, but only 22 standard compounds (both food grade and non-food grade) with an OAV greater than 1 were prepared as full recombinate, and 17 compounds (only food grade) as partial recombinate. When comparing the recombinate to the P50 smoked water, the recombinate did not have a similar flavour profile to the original P50 smoked water. This could be because some highly potent aromas were omitted from the recombinates. After all, these potent aroma components had no standard compounds to prepare. Also β-damascenone was detected by GC-O in the apple-smoked water but was too low to be quantified by GC-MS. This compound could have provided the typical apple notes which were detected by the panel in apple wood PST but not in the recombinates.

- *Chapter 4, Determination of the odour-induced umami enhancement (OIUE) and odour-induced salty enhancement (OISE) of smoked water in salt-reduced model soup,* and *the odour-induced umami enhancement (OIUE) and odour-induced salty enhancement (OISE) of smoked water recombinate in salt-reduced model soup,* experiments were conducted using only P50 smoked water, which differed from previous experiments (taste-active and taste-enhancement activities experiments), which were always conducted alongside TR smoked water. The purpose of this study is to investigate the flavour enhancement and flavour profile of smoked water, with a focus on PST smoke in comparison to TR smoke in some experiments. As can be seen, the results of the investigation into the taste-active and taste-enhancement activities of P50 and TR were quite similar due to the fact that both samples contained the same compound groups, albeit in different proportions. Therefore, the final two experiments examining the OIUE and OISE in smoked water and recombinate were conducted only with P50 smoked water.

5.3 Recommendations for future work

Regarding the effect of the PST process on potent aroma components in the smoked water flavour profile, some intriguing results were seen throughout this research. However, not all of them were thoroughly investigated due to restrictions on the study's time frame and scope. The recommendations for additional research topics made throughout this thesis are given below.

- The quantification/semi-quantification of SPE (used diethyl ether as eluent) extract should also be investigated. Some compounds in smoked water can be extracted particularly well by SPE methods, but the SPME method used to quantify the aroma compounds in this study did not identify any of these compounds. For instance, 3-ethyl-1,2-cyclopentanedione (LRI ZB-5 = 1101; spicy, sweet aroma) was only detected in SPE extract and had a high FD factor of 243, indicating that it should be a robust aroma compound in smoked water. This compound was not quantified/semi-quantified and was not added to the recombinate because we did not have the compound and did not know its concentration (*Chapter 2*). 3-Ethyl-1,2-cyclopentanedione is also a potent aroma compound found in soy sauce products (Kaneko et al., 2012) and Japanese fermented soybean paste (miso) (Kumazawa et al., 2013). Its aroma has been described as "caramel-like" or "sweet, sugar-like" and has an FD factor of 4.

- Further analysis of volatile compound contents of smoked water made from distinct woodchips in additional batches of each woodchip type (at least twice) to determine the changes in volatile compound contents due to either woodchip type or batch variation (*Chapter 3*).

- An investigation should be conducted to identify all aroma compounds from the GC chromatogram of each type of smoked water derived from various woodchips. In this study, we focused on quantifying/semi-quantifying the same 77 compounds in each sample of smoked water, which began to select compounds from apple-wood smoked water as this was the first time this sort of apple wood would be examined. Each wood type has differences in the contents of the wood's main components (cellulose, hemicellulose, and lignins) or their relative proportions. Then, unique compounds

(minor compounds) could be found in each type of wood. To compare the aroma compounds of various types of wood, it is necessary to monitor both the same compound groups and those that are distinct. The evaluation could be neatly analysed, especially for novel samples/products for which the properties have never been investigated (*Chapter 3*).

- To provide more information, the consumer study might assess the umami and salty enhancement of the recombinate in soup products or applied in another type of food. However, the number of samples should not exceed five in order to avoid fatigue, and the soup should be served and tasted in a warm temperature condition (the soup heating temperature was 75-80 °C) because the taste and texture of the soups will change if the temperature drops (*Chapter 4*).

- Because the recombinate flavour profile did not match the P50 smoked water flavour profile, it implies that the number and content of compounds that contributed to the P50 smoked water flavour is still insufficient. Therefore, further identification and quantification of the compounds that remain unknown in the SPE extract with high FD factor values might be made and added to the recombinates (*Chapters 2 and 4*) and prepare the recombinates both for P50 and TR.

- It would be interesting to study the omission test of the recombinates, taking into account the effects of highly potent, moderately potent, and less potent aroma compound groups and when all potent aroma compound groups are combined (full recombinate). The data could be used to imitate the smoked compounds' constituents from the synthesised standard, thereby saving time and reducing production costs. Even though some synthesised standards may be costly, it is worthwhile to purchase them because only a tiny amount is required to produce an abundance of synthesised smoke ingredients (*Chapters 2 and 4*).

5.3 References

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Appendix I: Achievements

Published conference paper

Panchan, Kanokkan, Lignou, Stella, Griffiths, Huw D., Baines, David A., & Parker, Jane K. (2022, January 28). **Impact of smoked water on umami and salt taste.** Zenodo. https://doi.org/10.5281/zenodo.5913212.

Symposium presentations

Oral: Understanding the use of smoked water as an ingredient to boost the perception of flavour, 7th Nursten Postgraduate Flavour Symposium, University of Reading (2019)

Oral: Investigating smoked ingredients, 8th Nursten Symposium Postgraduate Flavour Symposium, University of Nottingham (2021)

Oral: 16th Weurman Flavour Research Symposium, Dijon (online) (2021)

Oral: Investigating smoked ingredients, 9th Nursten Postgraduate Flavour Symposium, University of Reading (2022)

Poster: 13th Pangborn Sensory Science Symposium, Scotland (2019)

Department of Food and Nutritional Sciences

University of Reading

Understanding the use of smoked water as an ingredient to boost the perception of flavour

Kanokkan Panchan | Stella Lignou | Jane K. Parker

besmoke the art & science of natural smoke

Introduction

Smoked ingredients are often applied to food to deliver a highly desirable and enhanced flavour. Understanding the mechanism of this flavour enhancement is essential for fully exploiting the role of smoke in flavour perception and formulating new products.

Objective

To investigate the three possible modes of action of smoke which lead to an enhancement of the perception of flavour: the smoke may

H1 contain taste-active compounds,

H2 contain taste-enhancing compounds or

H3 contribute cross-modally whereby the odour compounds induce salt or savoury enhancement.

Materials

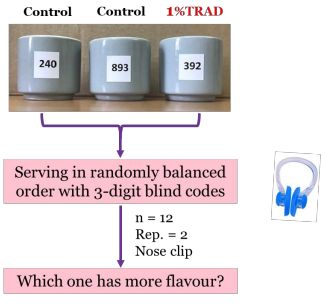
1. **Smoked ingredients**
   1. Unsmoked water (CONTROL)
   2. Smoked water (TRAD)
   3. Smoked water prepared with PureSmoke Technology** (PST)
2. **Simple mixtures**
   1. Tap water
   2. Monosodium glutamate (MSG) solution at 3 different levels
3. **Complex mixtures**
   1. Commercial chicken & vegetable broth (FRESH SOUP)
   2. Instant chicken soup base (MODEL SOUP)

Experiments

A series of preliminary three-Alternative Forced Choice (3-AFC) tests was carried out with the trained sensory panellists, with or without nose clips as appropriate. All sensory sessions were carried out with at least two replicates.

H1) Taste-active compounds

Simple mixture: tap water



✗ H1

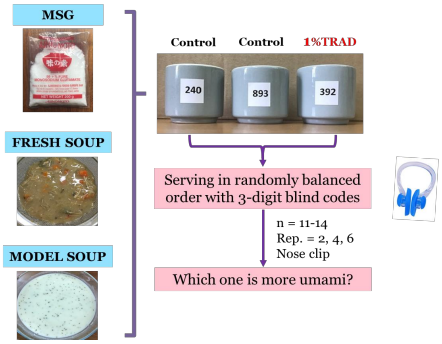
Result: No significant flavour difference (p=0.406) between tap water and tap water containing TRAD smoked.

** PST is a technology developed by Besmoke Ltd (Parker et al. JAFC 2018 p2449) which reduces the content of polyaromatic hydrocarbons in the smoke used to smoke the water, yet retains a good flavour.

H2) Taste-enhancing compounds

(1) Simple mixtures: MSG solutions (38, 115, 344 mg/kg)

(2) Complex mixtures: FRESH SOUP & MODEL SOUP



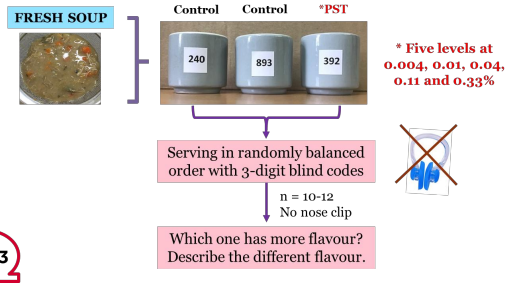
✗ H2

Results: (1) No significant enhancement of umami (p=0.343) when TRAD smoked water was added to solutions of MSG.

(2) No significant enhancement of umami when TRAD smoked was added to either fresh or model soups (p-value=0.111 and 0.542).

H3) Odour compounds induce taste enhancement

Complex mixtures: FRESH SOUP



✓ H3

Results: Significant enhancement in salty or umami taste (p=0.019) when PST was added.

- *5 out of 12* panellists recognised taste enhancement (salty, umami) at concentrations of PST which were lower than the concentrations where they first recognised the smoky flavour.

Conclusion

There is no evidence of smoke being taste-active or taste-enhancing when the aroma is excluded, however, there is some evidence that suggests the smoky aroma may act cross-modally to enhance flavour. Further experiments are in progress.

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