Effect of cold smoking on polycyclic aromatic hydrocarbon content and sensory properties in selected foods

Shaan Kaloti (16940791)

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Abstract

Smoking is a traditional method of food preservation and has improved over time with technology. The biggest problem with smoking is that the combustion of wood results in the formation of polycyclic aromatic hydrocarbons (PAHs). These compounds are carcinogenic and can contaminate food. Thus, the European Union has imposed an upper limit (dependant of food type) of 1 to 50 μg/kg of carcinogenic PAH (sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene). New methods such as cold smoking, friction smoking, liquid smoking, and electrostatic precipitation have been introduced to reduce PAHs in smoked foods. Currently, PAHs are analysed in food using solid-phase extraction, followed by the "QuEChERS" method to clean the extract. PAHs are then quantified using gas chromatography mass spectrometry (GCMS). The purpose of the review section of this study is to give a comprehensive view on the process of food smoking, the benefits, the regulations and ways to quantify PAH in foods

In this study chicken, cheese and crackers were cold smoked with Native New Zealand wood chips (Manuka, Tawa, Rewa Rewa and Pohutukawa). The QuEChERS technique was used to extract PAHs, followed by quantification using gas chromatography in tandem with mass spectroscopy (GC-MS). Cold smoking time (5 to 120 minutes) had the most influence on final PAH concentration (varied from 10.84 ± 1.70 to 112084.74 ± 8784.14 μg/kg). Chicken breast that was cold smoked for 5 minutes using Manuka wood and had the lowest PAH concentration. An untrained sensory panel (n=50) rated chicken breast that had been cold smoked with Manuka, Tawa, Rewa Rewa and Pohutukawa based on overall liking, odour, appearance, texture, smokiness, and flavour. One-way analysis of variance (ANOVA) showed that Manuka cold smoked chicken was most favoured among panellists.

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Name: Shaan Kaloti

Signature

Date: 7/09/22

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Rothman Kam

Signed:

Shaan Kaloti

Signed:

Tony Chen

Signed:

Table of Contents

List of Figures

List of Tables

List of Abbreviations

PAH	Polycyclic aromatic hydrocarbon
EC	European Commission
GC-MS	Gas chromatography mass spectrometry
BaP	Benzo(a)pyrene
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
ANOVA	Analysis of Variance
PCA	Principle Component Analysis

Chapter 1: Introduction

Smoking has been a method of food preservation for centuries. As technology has advanced, so have methods of smoking. Smoking imparts flavour compounds such as phenol derivatives, aldehydes, organic acids, alcohols, ketones, esters and furan derivatives to produce colour and unique smoky flavours in food (Kostyra & Baryłko-Pikielna, 2006).

Industrial smoking can be done one of five ways: hot smoking, cold smoking, friction smoking, electrostatic precipitation or the addition of liquid smoke. Hot smoking, friction smoking and electrostatic smoking are done at temperatures over 90°C, while cold smoking is done at temperatures between 20 to 35 °C (Birkeland, Bencze Rørå, Skåra, & Bjerkeng, 2004; Raffray, Goli, Rivier, Sebastian, & Collignan, 2014). Liquid smoke can be added to precooked foods during marination to impart smoky flavour.

The biggest disadvantage of smoking food is the generation of PAHs. These compounds have multiple six-membered carbon rings adjacent to each other and are formed during incomplete combustion of organic matter. PAHs are toxic and carcinogenic, thus inclusion in food should be limited (Šimko, 2002).

Temperature of wood combustion has a profound influence on PAH concentration in smoke (Šimko, 2002). Traditional hot smoking results in high PAH concentrations, as food is in direct contact with the heat source (Roda et al., 1999). Indirect smoking methods such as cold smoking, friction smoking, liquid smoking, and electrostatic precipitation have been developed to reduce the levels of PAHs in smoked foods.

This study will discuss different smoking techniques, highlight the benefits of smoking and investigate the compounds responsible for smoky flavours. Technologies used for detecting PAHs in foods and legal limits set by governing bodies will also be explored.

The effect of cold smoking on PAH levels in different food types is currently limited, hence this study will broaden knowledge on said topic. The study will also look at the influence of wood type on PAH concentration during cold smoking.

1.1 Research objectives

- To determine if wood-type used to cold smoke chicken, cheese and crackers influences final PAH concentration
- To determine if smoking time influences final PAH concentration in cold smoked chicken, cheese and crackers
- Compare hot smoking and cold smoking in terms of PAH concentration in food
- To determine if surface area of chicken, cheese and crackers influences final PAH concentration
- Use sensory evaluation to ascertain whether panellists prefer chicken breast cold smoked with Manuka, Tawa, Rewa Rewa or Pohutukawa wood chips

Chapter 2: A review on the effect of smoking foods: Benefits and concerns

2.0 Introduction

Humans have used smoking as a method to preserve food for thousands of years. It is one of the oldest food preservation techniques to prolong the shelf life of meat and a way to keep wild animals away from the food due to the presence of fire and smoke (Djinovic, Popovic, & Jira, 2008). As the technology of food smoking is better understood, it has evolved with less emphasis on food preservation but more on improving the organoleptic properties of food. Food smoking technology uses sensory active compounds such as phenol derivatives, aldehydes, organic acids, alcohols, ketones, esters and furan derivatives to induce colouration on food and impart unique smoky flavours in food (Kostyra & Baryłko-Pikielna, 2006). Although smoking isan effective method of preserving food by inactivating pathogenic microorganisms and improving sensory properties, it does have its drawbacks (Šimko, 2005). In the process of generating smoke by combustion or smouldering of woody material, polycyclic aromatic hydrocarbons (PAH) - a class of compounds with multiple six-membered carbon rings adjacent to each other, are also produced from the incomplete combustion of woody materials. PAHs are not desirable, as some of the compounds are found to be toxic and carcinogenic (Šimko, 2002).

Temperature during combustion of wood is an important parameter during smoking because it is directly proportional to the amount of PAH found in the smoke (Šimko, 2002). This is applicable between the temperature range from 400°C to 1000°C. Traditional hot smoking is when the food product is in direct contact with the smoke and will usually result in high PAH concentrations in the food (Roda et al., 1999). Due to this reason, other indirect food smoking methods have been developed like cold smoking, friction smoking, liquid smoking, and electrostatic precipitation to reduce PAH contamination. In some scenarios, it is possible to decrease the concentration of PAHs by further processing of the smoked food via cooking (Simko, Gergely, Karovicova, Drdak, & Knezo, 1993).

In this review, different food smoking techniques will be discussed with emphasis on their advantages over traditional hot smoking. A comprehensive literature survey on the benefits of smoking will be presented by highlighting the volatile compounds responsible for smoky flavour and colourants from the smoke that gives food the brown and red hue. The review will also look at the current technologies in detecting PAH contaminants in food and their legal limits allowed in food according to different food safety governing bodies around the world.

2.1 Methods of smoking foods

2.1.1 Hot smoking by smouldering

Hot smoking refers to processes where heat from the smoke generated by smouldering wood is directly in contact with the food. It is a form of thermal processing and is done at temperatures over 70°C, which will cook food in the process (Raffray et al., 2014) and induce drying (lowering water activity). In an industrial setting, foods are hot smoked in chambers known as smokehouses (*Figure 1*). They have racks where meats can be hung from whilst smoke is pumped into the chamber via a smoke generator where the

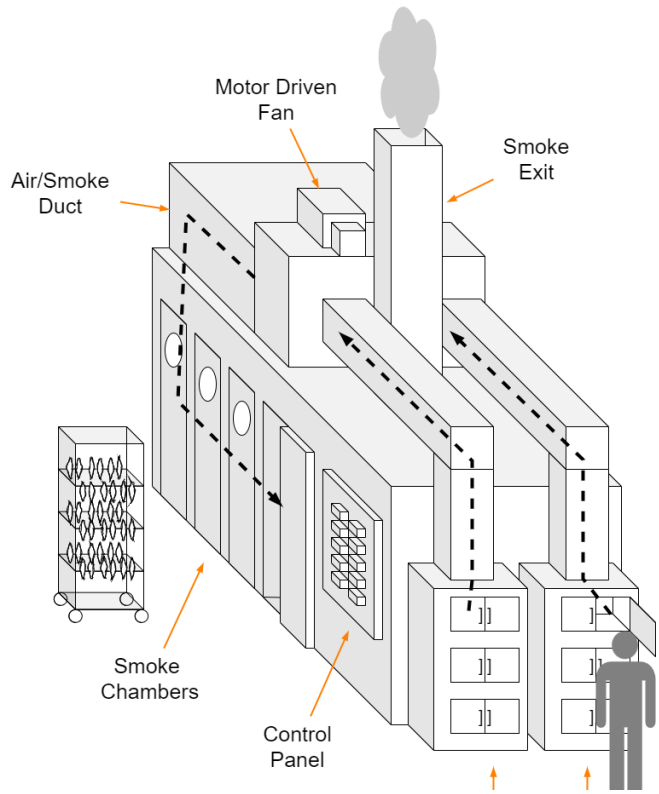

Figure 1. Commercial hot smoking chamber or smoke house.

smouldering occurs. A heating element is at the base of the smoke generator and wood chips or sawdust are added on top to generate smoke (Sikorski & Kołakowski, 2010).

Phenolic compounds are the most dominant volatile flavour compound found in hot smoked foods. During processing, many enzymatic and non-enzymatic reactions happen such as the Maillard reaction, Strecker degradation, oxidation, protein degradation and lipid degradation (Toldrá, 1998; Vestergaard, Schivazappa, & Virgili, 2000). These reactions result in the formation of compounds such as alkenes, aromatic and cyclic hydrocarbons, terpenes, ketones, esters, alcohols, sulfuric and nitrogenous compounds, aldehydes and carboxylic acids. According to Arvanitoyannis and Kotsanopoulos (2012) the rate of volatile compounds generated and depositing on foods during hot smoking depends on many factors. Such factors include source of wood during combustion, flow rate of the smoke, smoking temperature, solubility of the volatile compounds and the composition of the food. For example, increasing the flow rate of smoke will increase the density of the smoke hence encouraging more smoky compounds to deposit on the surface of the food. Temperature of the smoke must be high enough so that the core temperature of the food is at a minimum of 62.8°C for 30 min. This is to ensure that the food is pasteurised in the event of eliminating pathogenic microorganisms (Cavinato, Mayes, Bledsoe, Rasco, & Huang, 2002).

2.1.2 Cold smoking

Cold smoking is when food is indirectly smoked and placed away from the heat source. The smoke from the source is directed to another chamber that is located some distance away. This is to ensure heat energy from the smoke is dissipated before contacting the food, hence the term 'cold smoke'. This process is done between below 35°C, which is significantly cooler than hot smoking so cooking or pasteurisation does not take place under these conditions (Birkeland et al., 2004). Therefore, cold smoking usually involves other steps or methods of food preservation before the actual smoking step. For example, foods are salted, dried, or pre-cooked before cold smoking. These procedures are followed so that the shelf life if cold smoked food is extended as they will not be cooked during the smoking process. Raw fish is commonly cold smoked for sensory purposes only, but the reduction of water activity is necessary to reduce the

risk of food borne pathogens like *Clostridium botulinum* and *Listeria monocytogenes*. From an organoleptic standpoint, the texture and consistency of raw fish is to be maintained by preventing water losses. For this to occur, raw fish is marinated in salted solution with a minimum of 3.5% (weight to solution) to lower water activity without impacting on water loss and the packed under modified atmospheric packaging condition to prolong shelf life. (Messina et al., 2021). Nykänen, Weckman, and Lapveteläinen (2000) reported the use of nisin and sodium lactate extend the shelf life of cold smoked rainbow trout against *L. monocytogenes*. Nisin with concentration of 5000 IU/mL and sodium lactate solution (60%) where injected into the fish fillet so that the final concentration of nisin was 360 IU/g fish flesh and 36 g sodium lactate/kg fish flesh. The combination of these two preservatives was able to extend the shelf life of the fish to 29 days at 3°C and vacuum packed without any effect on the sensory properties of the product. The control with no preservatives, only lasted 16 days before the microbial population reached unacceptable levels.

During cold smoking, typically alcohols are the most dominant volatile flavour compound in cold smoked foods. In the study of Li, Nie, Liu, and Xu (2021), they used gas chromatography–ion mobility spectrometry (GC-IMS) to analyse volatile organic compounds (VOCs) in cold-smoked traditional Chinese bacon. The VOCs that were identified were phenols, alcohols, aldehydes, ketones, and esters. In another study X. H. Huang et al. (2019) they investigated the flavour formation cold smoked Spanish mackerel. It was found that alcohols were the major volatiles with 2-butanol in the highest concentration in their cold smoked mackerel.

2.1.3 Liquid smoking

Liquid smoking was developed as a safer and efficient alternative to the traditional hot and cold smoking techniques (Suñen, Fernandez-Galian, & Aristimuño, 2001). It is produced by condensing smoke from pyrolysed saw dust or wood chips (*Figure* 2). Woody materials are placed in a chamber where it is pyrolysed to generate smoke with intense heat. The smoke is liquefied in the condenser due to cold temperatures and then passes through a series of filtration and purification steps to reach the final product – liquid smoke or smoke condensate. This liquid smoke is safer because toxic compound

and carcinogenic PAHs can be removed during the filtration and purification steps. In addition, the application of liquid smoke can be easily controlled by adjusting the concentration hence making it more versatile and efficient (Lingbeck et al., 2014).

Meats are commonly smoked by marinating the meat product with smoke condensate to infuse the meat with characteristic smoky flavour, sweet aroma and brown colouration. The main smoky flavour/aroma compounds found in liquid smoke are of phenolic type like syringol, guaiacol and pyrocatechol. The sweet aroma from liquid smoke is derived from carbonyl compounds like 5-methyl-2-furancarboxaldehyde and maltol. Finally, the brown colouration associated with liquid smoke are from furfurals likes furan-2-carbaldehyde and furan-3-carbaldehyde (Montazeri, Oliveira, Himelbloom, Leigh, & Crapo, 2013).

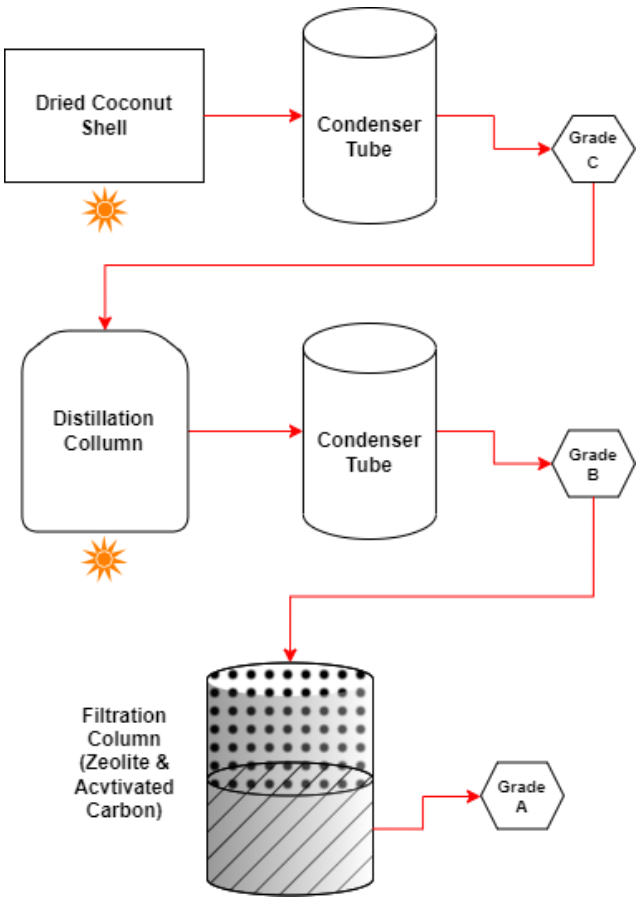

Figure 2. Process flow diagram on the production of liquid smoke for food industries.

2.1.4 Friction smoking

Smoke generation using a friction smoke was introduced in the 1980's to address the short comings of the traditional smouldering of woody material for hot smoking (Seraj, 2018). Controlling the smoking temperature and the smoking environment are the main disadvantages of hot smoking via smouldering because it will lead to product inconsistency in terms of cooking and flavouring. Friction smoking offers better control of the smoke by pressing a log against a rotating friction wheel (*Figure 3*). The temperature of the pyrolysis of wood by friction can reach up to 380°C (Varlet, Serot, Knockaert, et al., 2007) and is controlled by the amount of pneumatic pressure applied (upwards of 3.5 bar) on the wood against the wheel. The amount of smoke generated is controlled by the speed of the rotating wheel (Sérot, Baron, Knockaert, & Vallet, 2004). The additional benefits of friction smoking are lower operation time, wood requirement and lower PAH formation when compared to smouldering (Ledesma, Rendueles, & Díaz, 2016). There are several studies reported using the friction method to smoke salmon (Varlet, Serot, Knockaert, et al., 2007), seafood (Arvanitoyannis & Kotsanopoulos, 2012) and frankfurter sausages (Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2013).

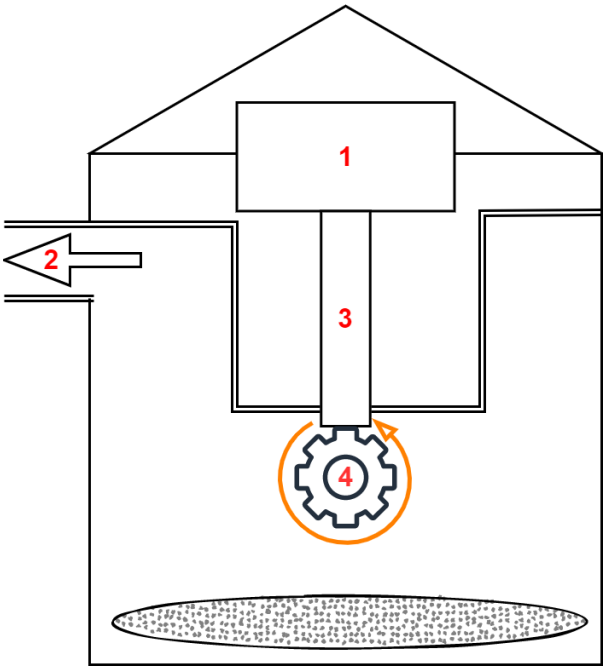

Figure 3. Friction smoker by pressing a piece of wood against a rotating wheel to generate hot smoke. A log of wood being pressed (1 & 3) onto a rotating wheel (4). The smoke that is produced exits chamber (2).

2.1.5 Electrostatic precipitation

The electrostatic smoking method was first applied as a food processing technique in the 1990's. The idea was borrowed from a well establish electrostatic smoke precipitator technology that was used to remove polluting fumes from power-plant smokestacks (Baron, Havet, Solliec, Pierrat, & Touchard, 2008). Although electrostatic smoking does not generate smoke, it is a technique to channel smoke through a tunnel in a controlled manner. This way, the smoke density and flow rates are controlled by the voltage supplied to the anodes. It works by inducing negative charge on the smoke particles when it comes into contact the negative anode (usually a metal mesh), refer to *Figure 4*. In this scenario the smoke is generated by combusting or by friction processing of wood. The positive anode at the top will attract the negatively charged smoke particles and hence creating an upward draft to channel the smoke through the tunnel. In this scenario the food is placed anywhere in the tunnel above the positive electrode (Baron et al., 2008).

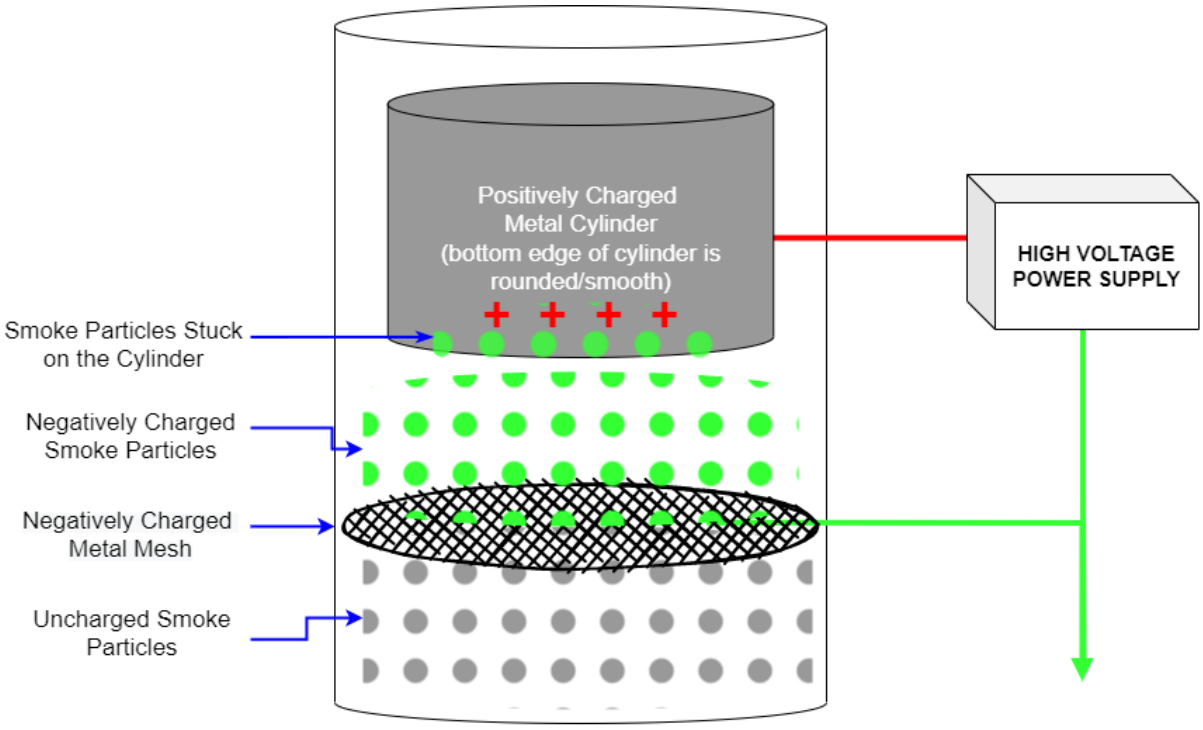

Figure 4. Electrostatic precipitation smoking set up.

2.3 Benefits of smoking food

Traditional hot smoking of food was originally employed as a method of preserving food by inhibiting spoilage due to microbial activity (Adeyeye, 2019). During the hot smoking process, temperature of the smoke can reach between 60°C to 100°C (Stołyhwo & Sikorski, 2005). This can inhibit the growth of microorganisms by cooking, lowering the water activity and introducing bactericidal compounds such as formaldehydes and phenols into the food (Rørvik, 2000). It was reported that hot smoking is effective in inhibiting *Salmonella*, *L.monocytogenes*, *Escherichia coli*, *Aeromonas hydrophila*, *Yersinia enerocolitica* and *Vibrio cholerae* in meat products (Rørvik, 2000; Ruiz-Alonso, Girón-Hernández, López-Vargas, Muñoz-Ramírez, & Simal-Gandara, 2021; Suñen et al., 2001).

As the hot smoking technology has become more widespread, the smoking technique has evolved beyond just food preservation but as food flavour, aroma and colour enhancer (Adeyeye, 2019). Nowadays, foods are smoked for enhancing sensory attributes rather than for preservation. For example, smoked meat products like ham (Jerković, Mastelić, & Tartaglia, 2007), sausages (L. Zhang, Hu, Wang, Kong, & Chen, 2021), bacon (Aaslyng & Koch, 2018), duck (Jo, An, Arshad, & Kwon, 2018) and fish (Kostyra & Baryłko-Pikielna, 2006) are widely consumed and liked because of their smoky flavour. The volatile compounds responsible for the smoky aroma and flavour are diverse and numerous. *Table 1* categorises the smoky volatiles compounds into phenols, alcohols, aldehydes, esters, organic acids and ketones. In most cases, the smoky volatile compounds are derived from the caramelisation of carbohydrates and sugars during the combustion of woody materials . Paravisini et al. (2017) and Pons, Garrault, Jaubert, Morel, and Fenyo (1991) reported that when carbohydrates are exposed to elevated temperature processing, the carbohydrates will undergo caramelisation. During the caramelisation reaction, the derivatives of organics acids, furans and furfural will give the food products pungent, fruity, nutty and almond-like aromas.

Table 1. Volatile compounds responsible for the smoky flavour and aroma in selected foods.

Common volatile compounds found in smoked foods	Aroma description from sensory analysis	Food product	Smoking method	Analytical technique for detecting volatile compounds	Reference
Phenols Methoxyphenols, creosol, Eugenol, Ethyl to trimethyl-phenols	Musty, pungent, smoky, woody, burnt, ashy, cedar, creosote and petroleum-like	Harbin pork sausages	Hot smoked	HS-SPME & GC-MS	L. Zhang et al. (2021)
Alcohols 2-furanmethanol, 1-octanol, 1-decanol 1-undecanol, 12-dodecanol	Fatty, sharp aroma notes	Dalmatian traditional smoked ham	Cold smoked	Flavour compounds were extracted via steam distillation and analysed with GC-MS	Jerković et al. (2007)
Aldehydes Hexanal, 2-furaldehyde, benzaldehyde, 5-methyl furfural, nonanal	Caramel, sweet, butterscotch, brandy, burnt sugar aroma, bitter almond	Liquid smoke extract	Liquid smoked	GC-MS	Montazeri et al. (2013)

Ketones 1-hydroxy-2-propanone, 2-Hydroxy-3-methyl-2-cyclopenten-1-one, 2,3-Dimethyl-2-cyclopenten-1-one, acetophenone	Smoky aroma	Longan (fruit) - *Dimocarpus longan Lour.*	Hot smoked	HS-SPME & GC-MS	Yang and Chiang (2019)
Organic acids Acetic acid 3-methyl-2-furoic acid 3-benzoylacrylic acid	Pungent, sweaty, vinegar odour	Chicken drumstick	Hot smoked	HS-SPME & GC-MS	(L. Zhang et al., 2021)

Surface colour of food is an important attribute that effects consumer acceptance, hence smoking can be of use in this aspect. Smoking introduces brown pigments that can enhance the colour of the food product. For example, Riha and Wendorff (1993) reported from their sensory research that panellists preferred a light golden-brown surface of smoked cheese over light coloured cheeses in the smoked cheese category. Colourants from the non-enzymatic Maillard reaction from the combustion of carbon material has been found to be responsible for the characteristic smoked colour on smoked foods (Ziemba, 1967). Cardinal et al. (2001) reported that cold smoking of Atlantic salmon fillet gave the salmon flesh a red and brown hue when compared to non-smoked salmon fillet. In the event of hot smoking, the intense browning of food is not only contributed by the brown pigments from smoke but also from the reaction of proteins in the food with carbonyls compounds from the smoke (Riha & Wendorff, 1993). A recent study by Chang et al. (2021) showed that the smoke colour is strongly attributed to malondialdehyde and 5-hydroxymethylfurfural. A more detailed analysis of the smoky colouration using Fourier-transform infrared spectra and HPLC–tandem mass spectrometry found that the brown pigmentation is also contributed by melanin-type compounds, polymers of fructose and glucose, and derivatives produced by lipid oxidation (Chang et al., 2021).

2.4 Formation of PAHs during smoking

PAHs are compounds that have two or greater fused aromatic rings. Low molecular weight (LMW) PAHs have 2- 3 rings; whereas high molecular weight (HMW) ones have 4-6 rings (Sampaio et al., 2021). PAHs that have five or more rings are generally carcinogenic and toxic to humans (Nisbet & LaGoy, 1992). Ideally, it is best to limit the formation and inclusion of PAHs in food. The three most important factors that result in PAH formation are temperatures in excess of 300°C, in complete combustion leading to reduced oxygen levels and the presence of organic matter (McGrath, Chan, & Hajaligol, 2003; Zelinkova & Wenzl, 2015). The mechanism of formation is complex and involves many steps (a simplified diagram is shown in *Figure 5*). Short chain organic compounds thermally degrade and condense into larger PAH compounds. Diels-Alder

rearrangements are responsible for the aforementioned reaction (Penning & Huang, 2014).

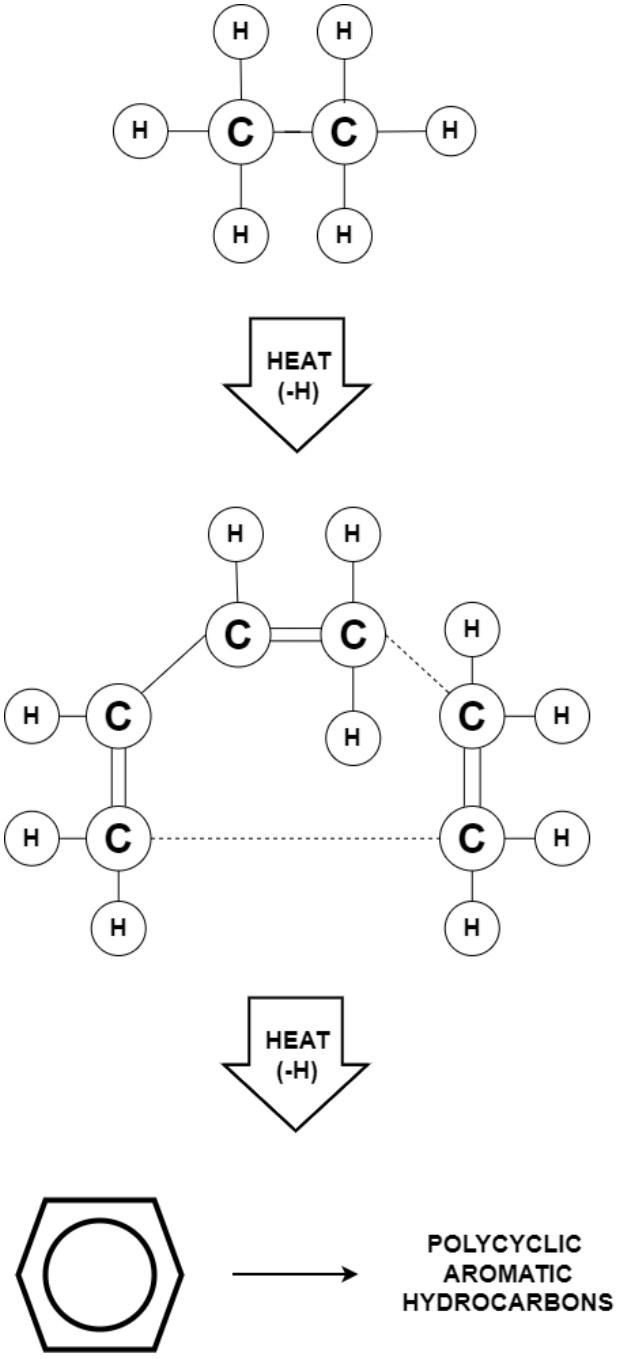

Figure 5. Pyrosynthesis of PAHs starting with ethane.

Benzo(a)pyrene is reported to be one of the most carcinogenic PAHs and is used as an indicator in PAH analysis (Patel, Shaikh, Jain, Desai, & Madamwar, 2020). They tend to accumulate and become bioavailable in human organs due to their lipophilic nature (Abdel-Shafy & Mansour, 2016). Tumours can form in organs such as skin, lungs,

pancreas, colon, bladder and breasts due to long term exposure (Rajpara, Dudhagara, Bhatt, Gosai, & Dave, 2017). Though the mechanism of PAH carcinogenesis is not fully known; it is believed to be induced by PAH metabolites binding to DNA (Stading, Gastelum, Chu, Jiang, & Moorthy, 2021). LMW PAHs are typically weaker carcinogens and require metabolism to become more potent. A possible explanation for carcinogenic properties is the Bay theory. Diol epoxides are intermediate PAH metabolites formed during metabolism of parent PAH by CYP1A1/1B1 cytochromes and epoxide hydrolase enzymes. When epoxides react with DNA, they have a mutagenic effect and form adducts (Stading et al., 2021). In the Bay theory, it is speculated that an epoxide will be extremely mutagenic if it bonds to the bay region of a PAH (mechanism shown in *Figure 6*) (Jerina et al., 1986; Weis, Rummel, Masten, Trosko, & Upham, 1998). The bay region is between the aromatic rings of the PAH molecule. HMW compounds have more bay regions than LMW ones, hence they have a greater tendency to be carcinogenic.

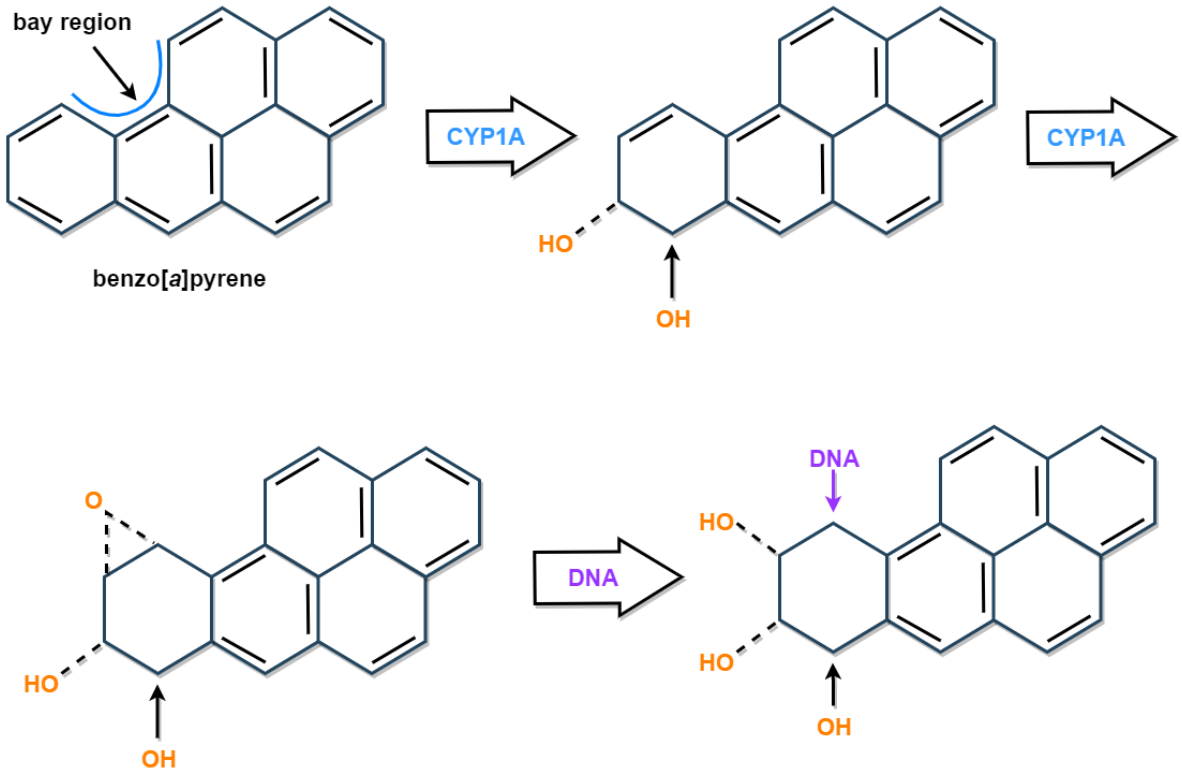

Figure 6. Oxidation of benzo[a]pyrene by CYP1A.

PAHs are volatile compounds that can penetrate food during the process of smoking (Zachara, Gałkowska, & Juszczak, 2017). Total PAH concentration in smoked food depends on factors such as, smoking conditions (temperature, time, humidity and airflow), smoking technique (i.e. hot or cold) and the fuel being used for smoke generation (Ledesma et al., 2016). Smoking can be done using many different fuel sources such as wood, coal and coconut husks. Each fuel will create a unique PAH and flavour profile. For example, Ledesma et al. (2016) reported that spruce wood produced higher PAH levels than apple and alder shells.

Smoking conditions and food composition can also affect PAH concentration. The fat content, distance from the smoking source and cleanliness of equipment will all effect final PAH concentration. Roseiro, Gomes, and Santos (2011) found that when food was further away from the smoke source, the PAH levels decreased. Generally, meats that have less fat content present will see a decrease in PAH levels. Fat is pyrolysed to PAHs or fat drippings will be heated and then pyrolysed (Phillips, 1999). Wretling, Eriksson, Eskhult, and Larsson (2010) found that smoked beef and pork products had higher PAH levels than smoked fish products.

2.5 Techniques for analysing PAHs in foods

Though there is no standardised method for analysing PAHs in foods, there are still many methods that have been developed. These methods have undergone a multitude of improvements to make extraction more efficient. The process of PAH analysis can be summarised in five steps: extraction of PAHs from food sample, purifying the extract, separation of PAHs contained in the extract, detecting and identifying PAHs and quantification of compounds.

2.5.1 Extraction of PAHs from food matrix

PAHs are difficult to analyse as their structures are similar to one another. In fact, Sander and Wise (1997) have indexed approximately 660 different compounds. Due to the lipophilic nature of PAHs, they will heavily accumulate in fatty foods Hence, solvents are used to extract PAHs from a food matrix. Organic solvents such as acetone, hexane,

dichloromethane, methanol and chloroform are used with varying levels of success (Haleyur et al., 2016).

It is important to note that PAHs usually exist in trace amounts; meaning that correct extraction methods and organic solvent need to be used correctly (Onopiuk, Kołodziejczak, Marcinkowska-Lesiak, & Poltorak, 2022). PAH extraction efficiency largely depends on polarity of the solvent being used, preparation of food sample and composition of the food matrix (Ledesma, Rendueles, & Díaz, 2015). When food samples are highly soluble in the solvent being used for extraction, PAH recovery is very high. Complex food matrices can present difficulties during extraction. In meat and fish, PAHs disperse throughout the muscles and can form covalent bonds with nucleic acids (Flesher, Horn, & Lehner, 1998). Therefore, extracting PAH from high protein food matrix has lower PAH recovery rates.

It is common for PAH calibration standards to be added to food samples, as they can improve accuracy of during detection and quantification (matrix calibration) (Michalski & Germuska, 2003). Smoking often leads to PAHs being bound within the food matrix. Whereas PAHs in reference standards are unbound, making them easier to extract.

2.5.2 Purification of extract

Along with PAHs, various hydrophobic and non-polar compounds are also extracted from food samples such as fats and lipids. Extracts are purified in order to remove additional chemicals because they interfere during the detection and quantification stages. Due to the complex and diverse nature of food matrices, a definitive clean up procedure does not exist.

Purification is commonly done using three methods: liquid-liquid extraction (LLE), solid phase extraction (SPE) and chromatography. Luks-Betlej (1997) compared LLE, liquid - liquid chromatography and SPE to determine which one was the most effective clean up method. SPE was found to be the most effective method. Conversely, LLE resulted in too much PAH loss for it to be effective. Anastassiades, Lehotay, Stajnbaher, and Schenck (2003) reported a new dispersive solid-phase extraction (dispersive-SPE), method known as "**Q**uick, **E**asy, **C**heap, **E**ffective, **R**ugged and **S**afe" (QuEChERS). This method involves performing a single-phase extraction using acetonitrile, followed by liquid-

liquid portioning using magnesium sulfate and sodium chloride. Then, dispersive-SPE is done using anhydrous magnesium sulfate, primary secondary amine and acetonitrile. This method has since been validated has been widely used, as it is cheaper and faster than previous clean up methods. Though QuEChERS was originally used in the process of detecting pesticides in fruits and vegetables, it has been modified to aid in the process of PAH detection in smoked meats, fish, cheese, fats and dried herbs (Chiang et al., 2021).

2.5.3 Detecting, identifying and quantification of PAHs

Either gas chromatography (GC) or high-performance liquid chromatography (HPLC) can be used to detect PAHs. GC can be used in tandem with flame ionization detection (GC-FID) or mass spectrometry (GC-MS). HPLC can be used in tandem with ultraviolet (UV) or fluorescence detector and thin-layer chromatography (TLC) with fluorescence detection.

2.5.3.1 GC-MS and GC-FID

Compounds in natural extracts can be separated more efficiently by GC as compared to HPLC because capillary columns have 60,000 plates/30 m (ID 0.25 mm). When food samples are analysed using GC-FID, compounds are identified by their retention times. This means samples often need to be cleaned up to remove non-PAH compounds that have similar retention times as the PAHs. Hence, GC-MS is more commonly used, as compounds can be identified and quantified using retention time and mass fragmentation patterns. The PAH limit of detection (LOD) for GC-MS tend to be lower than GC-FID. Orecchio, Ciotti, and Culotta (2009) reported that the LOD for benzo(a)pyrene whilst analysing coffee brew samples was 0.90 ng/L using a GC-MS. Whereas, Olatunji, Fatoki, Opeolu, and Ximba (2014) reported the LOD while analysing processed meats being 0.1 µg/kg using GC-FID.

2.5.3.2 HPLC

When using HPLC to quantify PAHs, specific columns are used. For example, a Vydac 5 µm C18, 150 x 4.6 mm column can separate 16 PAHs in 35 minutes (Stołyhwo & Sikorski, 2005). HPLC coupled with fluorescence detectors (FLD) is most commonly used because the limit of detection can be controlled by adjusting the wavelength of excitation and emission. FLD can detect smaller concentrations of PAHs than HPLC-UV; however acenaphthylene can't be detected as it doesn't emit fluorescence (Schuster & Schulenberg-Schell, 2000). Dost and İdeli (2012) reported the LOD for benzo(a)pyrene while analysing barbecued meat and edible oils was 0.46 µg/L using HPLC-UV. On the other hand, Kishikawa, Wada, Kuroda, Akiyama, and Nakashima (2003) reported that the LOD for milk samples using HPLC-FLD was 0.0013 µg/kg.

2.6 Regulations on the maximum limit of PAHs permissible in foods

The European Commission (EC) is the governing body of European Union. Commission Regulation no. 1881/2006 of 19 December 2006 outlines the maximum levels for certain contaminants in foodstuffs. As shown in *Table 2*, the maximum limit of benzo(a)pyrene and sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene (total PAH) allowed in various foods is outlined. BaP is only an indicator compound for PAHs, so it is sometimes more useful to analyse total PAH concentration. Thus far, the EC is the only regulatory body that has set limits for PAHs in foods. Smokes meats/fish and their products have BaP limit of 2.0 µg/kg and a total PAH limit of 12 µg/kg.

Table 2. Maximum limit of PAHs allowed in various foods. Adapted from Section 6 of Commission Regulation no. 1881/2006 of 19 December 2006.

Section in 1881/2006	Foodstuffs	Maximum levels (µg/kg wet weight)	
		Benzo(a)pyrene	Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene
6.1.1	Oils and fats (excluding cocoa butter and coconut oil) intended for direct human consumption or use as an ingredient in food	2.0	10.0
6.1.2	Cocoa beans and derived products with the exception of the products referred to in point 6.1.11	5.0 µg/kg fat as from 1.4.2013	30.0 µg/kg fat as from 1.4.2015
6.1.3	Coconut oil intended for direct human consumption or use as an ingredient in food	2.0	20.0
6.1.4	Smoked meat and smoked meat products	2.0 as from 1.9.2014	12.0 as from 1.9.2014
6.1.5	Muscle meat of smoked fish and smoked fishery products , excluding fishery products listed in points 6.1.6 and 6.1.7. The maximum level for smoked crustaceans applies to muscle meat from appendages and abdomen . In case of smoked crabs and crab-like crustaceans (*Brachyura* and *Anomura*) it applies to muscle meat from appendages.	2.0 as from 1.9.2014	12.0 as from 1.9.2014
6.1.6	Smoked sprats and canned smoked sprats (*Sprattus sprattus*); Smoked Baltic herring ≤ 14 cm length and	5.0	30.0

	canned smoked Baltic herring ≤ 14 cm length (*Clupea harengus membras*); Katsuobushi (dried bonito, *Katsuwonus pelamis*); bivalve molluscs (fresh, chilled or frozen) ; heat treated meat and heat treated meat products sold to the final consumer		
6.1.7	Bivalve molluscs (smoked)	6.0	35.0
6.1.9	Dietary foods for special medical purposes intended specifically for infants		
6.1.10	Dietary foods for special medical purposes ᵻ intended specifically for infants	1.0	1.0
6.1.11	Cocoa fibre and products derived from cocoa fibre, intended for use as an ingredient in food	3.0	15.0
6.1.12	Banana chips	2.0	20.0
6.1.13	Food supplements containing botanicals and their preparations Food supplements containing propolis, royal jelly, spirulina or their preparations	10.0	50.0
6.1.14	Dried herbs	10.0	50.0
6.1.15	Dried spices with the exception of cardamon and smoked *Capsicum* spp.	10.0	50.0
6.1.16	Powders of food of plant origin for the preparation of beverages with the exception of the products referred to in 6.1.2 and 6.1.11	10.0	50.0

2.7 Conclusions

Smoking was originally employed to preserve the shelf life of meat products, and it is now mainly used to improve food flavour, colour and smell. However, undesirable carcinogenic PAH compounds are produced during the smoking process, and it is a concern. The formation of PAH during smoking can be explained by incomplete combustion of woody material due to the lack of oxygen and high pyrolytic temperatures. Other methods of smoking such as cold smoking, liquid smoking, friction smoking and electrostatic smoking are developed to reduce the amount of PAH contaminating the food during processing. There are no standard means of extracting and detecting PAH in foods while monitoring these compounds in food. However, solid phase extraction using the QuEChERS method and then quantification of PAH with GC-MS is the most widely reported techniques in literature.

Chapter 3: Effect of cold smoking on polycyclic aromatic hydrocarbon content and sensory properties in selected foods

3.0 Introduction

Smoking is a method of food preservation whereby food is exposed to smoke from combusted material (most often wood). It makes food more desirable because it enhances the appearance (browning) via the Maillard reaction, adds flavour and has an antimicrobial effect (H. Huang, 2016). As such, cheese, fish and cured meats are commonly smoked. Smoked foods have become integral to many countries' cuisines; particularly smoked fish in Scandinavia and smoked ham in USA and Europe (Britannica, 2018). Smoking has long acted as a way of preserving meat because the heat involved in the process removes moisture, which lowers the occurrence of microbial spoilage. This in turn will lower the water activity thus preventing proliferation of bacteria, mold and yeast growth (Toldrá, 2008).

During processing, food can be smoked using one of three ways: hot smoking, cold smoking, or the addition of liquid smoke. Hot smoking involves exposing food to smoke and temperatures over 70°C (Raffray et al., 2014). This process cooks raw meat while imparting smoky flavour compounds such as aldehydes and phenols (Xie, Sun, Zheng, & Wang, 2008). As opposed to hot smoking, cold smoking is done at temperatures between 20 to 35 °C (Birkeland et al., 2004). In this method, food is not cooked, but instead placed in a chamber where smoke surrounds the food so that the smoky flavour is imparted into the food. Liquid smoke can also be added to precooked products as a marinade to produce smoky flavour. These additives are created by condensing wood smoke under conditions with minimal oxygen (Montazeri et al., 2013). Subsequently, liquid smoke is purified and concentrated to an powder or oil product.

In a commercial setting, the chamber used for smoking is referred to as a smokehouse (Britannica, 2018). These chambers have racks and rails for meats to be hung. Smoke is pumped into the chamber via a smoke generator. Wood chips will be heated to created

smoke (The Culinary Institute of America, 2008). A diagram of such setup is shown in *Figure 7*.

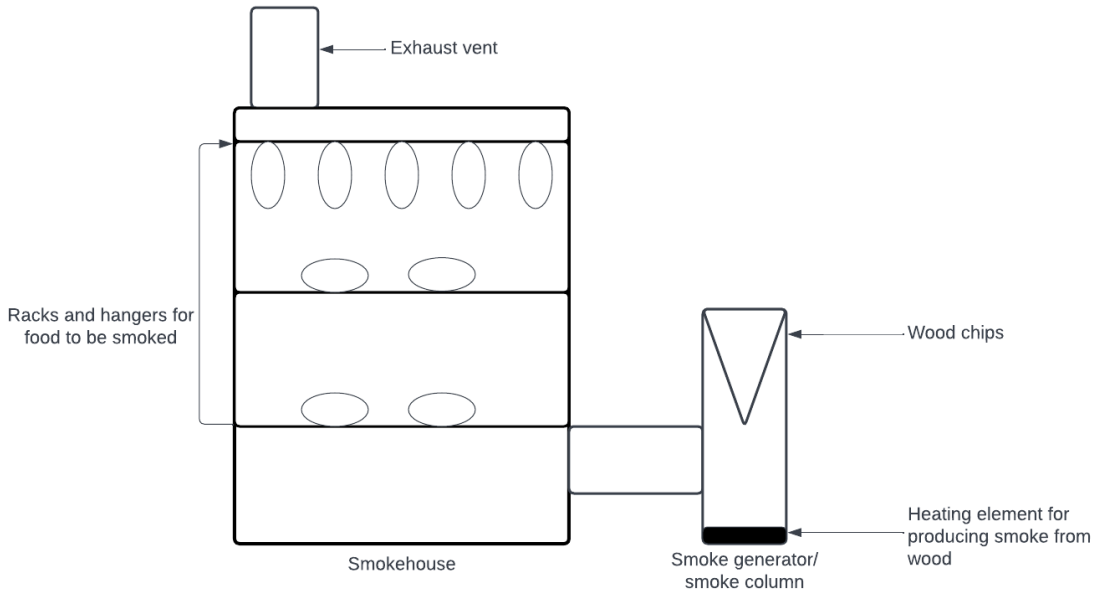

Figure 7. Simplified diagram of a smokehouse cold smoking setup.

Hot smoking is the most popular method, as it is associated with grilling and barbequing. This method presents a problem because it leads to the formation of PAHs during the incomplete combustion of organic matter. PAHs are compounds that have two or more fused aromatic rings (Sampaio et al., 2021). PAH structure with 2-3 rings is considered light, whereas one with 4-6 rings is heavy (Sampaio et al., 2021). PAHs that have five or more rings are typically carcinogenic and genotoxic to humans (Nisbet & LaGoy, 1992). The formation and inclusion of heavy PAHs in foods should be limited. There are three important factors that lead to the formation of PAHs: high temperature, the presence of organic matter and reduced oxygen levels (Zelinkova & Wenzl, 2015).

The mechanism of PAH formation is shown in *Figure 5* . When short chain organic compounds undergo thermal degradation at high temperature, they condense and aggregate into larger PAH compounds. Diels-Alder rearrangements are responsible for the formation of PAHs (Penning & Huang, 2014).

PAHs are volatile compounds that can penetrate food during the process of smoking (Zachara et al., 2017). The concentration of PAHs in the final product depends on factors such as, the fuel being used for smoke generation, smoking technique (i.e. hot or cold smoking) and conditions (temperature, time, humidity and airflow) (Ledesma et al.,

2016). Smoking can be done with a variety of fuel sources such as, coal, wood and coconut husks. These will produce a unique chemical profile and PAH levels. According to Ledesma et al. (2016), spruce wood produced higher PAH levels than apple and alder shells. Attributes of the food being smoked can also influence PAH concentration. The fat content, distance from the smoking source and cleanliness of equipment will all effect final PAH concentration. Roseiro et al. (2011) found that when a food was further away from the smoke source, the PAH levels decreased. Generally, hot smoked meats that have less fat content will see a decrease in PAH levels. This is because fat is pyrolysed to PAHs or fat drippings will be heated and then pyrolysed (Phillips, 1999). According to Wretling et al. (2010), smoked beef and pork products had higher PAH levels than smoked fish products.

The EC once used benzo(a)pyrene used as an indicator for carcinogenic PAH concentration but this, along with limits of PAHs, has now changed. Chrysene, benzo(a)anthracene, benzo(b)fluoranthene and benzo(a)pyrene (referred to collectively as PAH4) are now used as carcinogenic PAH indicators. The allowable limit of PAH4 compounds is a total of 12 μg/kg (Onopiuk et al., 2021).

As reported by Gomaa, Gray, Rabie, Lopez-Bote, and Booren (1993), total PAH concentration in various hot smoked meats can range between 2.6 to 86.6 μg/kg. In contrast, Alomirah et al. (2011) found that non–carcinogenic PAHs made up 60–100% of the total PAH concentration in their hot smoked meat. Though, high levels of carcinogenic PAHs were still found in meat tikka, grilled vegetables, shish tauk and whole grilled chicken. The highest concentration of genotoxic PAHs was found in meat tikka (42.9 μg/kg) because it was grilled near a heat source for a long period of time. Both factors lead to increased levels of PAHs. Additionally, tikka is marinated before it is grilled. Jägerstad and Skog (2005) reported that marinated meats generally have high PAH levels because of addition charring on the surface.

Liquid smoke can also contain a large concentration of PAHs. Yabiku, Martins, and Takahashi (1993) analysed liquid smoke and discovered that benzo(a)pyrene was present in 73% of samples and ranged from 0.1 to 336.6 μg/kg. The PAH concentrations found in the studies are all consistently over the EC limit of 12 μg/kg. This poses a huge problem, as smoked meats are regularly consumed by many people.

Though research about cold smoking is limited, PAH levels do appear to be lower using this method. Varlet, Serot, Monteau, Bizec, and Prost (2007) smoked salmon using a cold smoking, hot smoking and liquid smoke. It was found that PAH levels were 100 times lower than the EC legal limit of benzo(a)pyrene at the time. The friction method of cold smoking had the lowest concentration of PAHs. This is a method where smoke is produced by pressing a log of wood against a friction wheel. Similarly, Duedahl-Olesen, Christensen, Højgård, Granby, and Timm-Heinrich (2010) discovered that in industrially smoked salmon, the average concentration of the sum of genotoxic PAH4 was 9.7 μg/kg in hot smoked and 1.7 μg/kg cold smoked salmon. Cold smoking can be affected by many factors, such as duration, method of smoking and type of food being smoked. The composition of carbohydrate, protein and fat may affect PAH concentration.

Currently, research pertaining to cold smoking is limited and usually only focuses on smoked fish. Hence, there is a need for this knowledge gap to be filled with other food types. The study will also look at the influence of wood type on PAH concentration during cold smoking. Finally, cold smoking has the potential to be a safer smoking method and this matter will be explored.

3.1 Materials and Methodology

3.1.1 Materials

Acenaphthene, EPA 525 PAH Mix A, anhydrous magnesium sulfate ($MgSO_4$), naphthene and anhydrous sodium acetate were sourced from Sigma Aldrich, USA. Acetone, acetonitrile and dichloromethane (DCM) were sourced from Fisher Chemical, USA. Ceramic stones and QuEChERS (quick, easy, cheap, effective, rugged, and safe) clean up columns were purchased from Agilent Technologies, USA. Acetic acid was sourced from Ajax Finechem, Australia. SV Internal Standard Mix (Cat# 31006) was sourced from Restek, Australia. MilliQ water from the Purite Select Neptune Ultimate water purifying unit was supplied by Auckland University of Technology, New Zealand. Arnott's Salada crackers, chicken breast, Carr's Table Water Crackers, Mainland Tasty cheese block, Tegel Manuka Smoked Chicken Breast were purchased from Countdown supermarkets, New Zealand. Manuka, Pohutukawa, Rewa Rewa, and Tawa wood chips were all purchased from Mitre 10 stores, New Zealand.

3.1.2 Cold/hot smoking

3.1.2.1 Food sample preparation

Chicken breast was steamed until the internal temperature reached 75°C. After cooling down to room temperature. It was cut into cubes that measured 3cm x 3cm x 3cm. A Mainland Tasty cheese block was also sliced into cubes that were 3cm x 3cm x 3cm. Both chicken and cheese cubes had a surface area of 54 cm^2. All chicken. cheese and cracker samples had equal surface area.

Additional chicken breasts were cut into three different sizes so that the pieces have surface area measured at 102 cm^2, 78cm^2 and 54 cm^2.

Chicken, cheese and crackers were chosen because they had high protein, fat and carbohydrate content, respectively

3.1.2.2 Cold smoking

250 g of Manuka wood chips were loaded into a UFO Cold Smoke Creator column (Rotorua, New Zealand). The setup is done under a fume hood (setup shown in *Figure 8*). The three food samples; cracker, cheese and chicken samples were placed on the grates of the cold smoking chamber (samples were placed as far possible from smoke source). Smoke was generated by igniting wood chips in the column. An air pump was connected to the cold smoking column with an air flow of 23 L/min Food samples were smoked for 5, 15, 30, 60 and 120 mins using Manuka wood chips. All samples were smoked in triplicate. The temperature in the smoking chamber was 35°C. Subsequently, the samples were stored in zip lock bags at -18°C until they were ready for PAH extraction. The same process was repeated using Tawa, Rewa Rewa and Pohutukawa wood chips but food samples were only smoked for 60 mins. The chamber was cleaned with warm water and soap followed by acetone after each smoking cycle was finished.

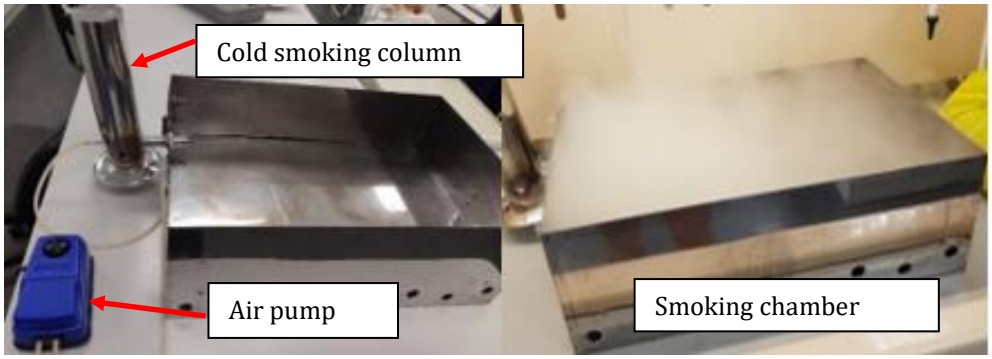

Figure 8. UFO cold smoking setup. Cold smoking column and air pump were attached to the chamber.

3.1.2.3 Hot smoking

Raw chicken breast was sliced into six cubes measuring 3cm x 3cm. 135 g of Manuka wood chips were soaked in water for 30 mins. After that, they were drained and placed into a Samba Smoker box. The box was placed into a Jumbuck 2 Burner Barbeque (as shown in *Figure 9*). After the grates had been put back over the burners, both burners were ignited and put on the highest setting. Once the temperature of the barbeque had reached 275°C and smoke was seen coming out of the box, raw chicken cubes were placed onto the grates. Chicken cubes were smoked for 5 and 15 mins; ensuring the internal temperature had reached 75°C. This was repeat two more times. Subsequently, the samples were stored in zip lock bags -18°C until further analysis.

Figure 9. Hot smoking setup in barbeque.

3.1.3 Moisture content analysis of wood chips

Ten grams of Manuka, Tawa, Rewa Rewa and Pohutukawa wood chips were weighed out and dried for 24 hours at 105°C in a Sanyo Laboratory Convection Oven (Osaka, Japan). All samples were done in triplicate. Moisture content was calculated and presented in *Table 3*.

Table 3. Inherent moisture content of wood chips used for cold and hot smoking.

Wood chip type	Moisture content (%)
Rewa Rewa	7.67
Pohutukawa	7.67
Tawa	13.33
Manuka	6.33

3.1.4 PAH extract and GC-MS analysis

3.1.4.1 Standard curve and matrix-matched calibration

A stock solution of 100 µg/mL EPA 525 PAH Mix A was prepared using DCM (Stock A). The second stock solution of 0.1 g/mL acenaphthene was prepared with DCM (Stock B). The third stock solution of 0.1 g/mL naphthene prepared using DCM (Stock C). 1mL of Stock A, B and C were pipetted into a 100mL volumetric flask and topped up with DCM to make the working solution. The internal standard was prepared by making a 1 µg/mL SV Internal Standard Mix (Cat# 31006) solution using DCM. The SV internal standard mix contained acenaphthene-d10, chrysene-d12, 1,4-dichlorobenzene-d4, naphthalene-d8, perylene-d12 and phenanthrene-d10. Both the working solution and internal standard were stored in the freezer until they were to be used.

To perform matrix-matched calibration, the method outlined in section 3.7 was used. Working solution was added along with DCM and internal standard in the following concentrations from 0 to 1000 µg/kg of sample.

3.1.4.2 PAH extraction

Method adapted from Chiang et al. (2021). Smoked chicken and cheese samples were ground in a mortar and pestle with the addition of liquid nitrogen. The same was done for cracker samples but without liquid nitrogen. 2g of each sample was mixed with 10mL of deionised water and 100µL of PAH internal standard. All samples were done in triplicate. This was then put into a 50mL centrifuge tube along with ceramic stone and vortexed for 1 minute. 10mL of acetone (for chicken and cheese) was then added and

vortexed for 1 minute. For samples with high carbohydrate content like crackers, Chiang et al. (2021) recommended, 10ml of acetonitrile with 1% acetic acid was added to the sample instead. 4g of $MgSO_4$ and 1g of sodium acetate was added to the mixture and vortexed for 1 minute. The mixture was then centrifuged at 4000 rpm for 10 mins at 4°C. After that, the cheese extractions were allowed to sit for 24 hours at 4°C. 8ml of the supernatant was transferred to into a QuEChERS clean-up column (900mg of $MgSO_4$, 300mg of primary secondary amine (PSA) and 300mg of endcapped octadecylsilane silica gel particles) for purification. This was vortexed for 1 minute. and centrifuged at 4000 rpm for 10 mins at 4°C. A 1mL aliquot was pipetted into a 1mL GC-MS vial.

3.1.4.3 GC-MS analysis

The smoked samples and standards were analysed using the Agilent 7890A GC equipped with 5977A mass spectrometer detector (MSD) with an Electron Impact ionisation source. Separation was done using an Agilent DB-5MS-UI column (30m x 0.25mm x 0.25µm). The inlet temperature was 300°C and the samples were injected using splitless mode. Column temperature was initially held at 55°C for 1 minute and increased by 25 °C/min to 320°C and held for 18 minutes. The MSD transfer line was held at 280°C, ion source at 250°C and the quad at 150°C. Data was collected after 29.6 minutes with 3-minute solvent delay. Selective ion monitoring (SIM) mode was used for the data collection. The ion mass program used during quantification is outlined in *Table S1* in Supplementary Information S1. This method was adapted from (Lynam, Smith, & Stevens, 2011). In this study, PAH4 are heavy PAHs that include benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene and benzo(g,h,i)perylene. PAH12 are light PAHs with five or less aromatics rings, making them non-carcinogenic. The compounds included in PAH12 are listed in *Table 4* caption. All GC-MS data were analysed using Microsoft Excel and Agilent MassHunter Quantitative Analysis.

3.1.5 Sensory evaluation

3.1.5.1 Preparation of smoked chicken samples

Raw chicken breast was cut into approximately 3.4cm x 1cm x 2.7cm. These were then placed into a bowl and mixed with 5g of salt per 500 g chicken. After that, they were steamed until they reached an internal temperature of 75°C. This was then repeated five times. Control samples were immediately places into a zip lock bag and stored at -18°C. The chicken was then smoked with 100 g of Manuka, Tawa, Pohutukawa and Rewa Rewa wood chips for five minutes, respectively (using the set up in section 3.2.2).

Each respective chicken sample had a random three-digit code assigned to it for presentation (control = 688, Manuka = 125, Rewa Rewa = 537, Tawa = 914, Pohutukawa = 821). Samples were presented in 105 mL lidded transparent sample cups, which were labelled with a three- digit code and contained 1 piece of chicken each. Four water crackers were packaged in a zip lock bag so they could be presented along with the chicken.

3.1.5.2 Sensory Evaluation

Untrained panellists (51% female, majority aged 25-34, 33% Chinese) were recruited around the Auckland University of Technology (City campus). A total of 50 people participated in the sensory session. Panellists were given five chicken samples, crackers and an instruction sheet that they took home in order to evaluate samples. The instruction sheet had a link and QR code which directed panellists to an online Qualtrics form. They were instructed to consume chicken, answer the questionnaire, drink water, eat a water cracker and then move onto the next sample. The questionnaire asked panellists to assess overall liking, odour, appearance, texture, smokiness on a 3- point just- about- right (JAR) scale (See Supplementary Information S2 for online survey form, consent form, participant information sheet and Ethics approval).

Panellists completed a check all that applies (CATA) analysis for sensory descriptors. They were askedto select properties from the following list: smokey, bitter, sour, earthy, acrid, ashy, dusty, burnt, pungent, petroleum-like, creosote/tar, cedar and metallic.

3.1.5.3 Sensory data analyses

Sensory evaluation was analysed using XLSTAT (Addinsoft, New York). One–way ANOVA was used to determine if there were significant differences in overall liking, odour, appearance, texture, smokiness and flavour of smoked chicken samples. For the attributes that did show differences, Tukey's HSD post hoc test was performed on the data. The null hypothesis for ANOVA was that there is no difference in overall liking, odour, appearance, texture, smokiness and flavour between cold smoked chicken samples.

Principal component analysis (PCA) was used to generate a simplified view of the interaction between all the descriptive analysis attributes. Frequency scores were determined by counting the number of times panellists assigned an attribute to each sample. A Pearson correlation PCA was selected to produce the biplot.

3.2 Results and discussion

3.2.1 Influence of time on PAH concentration

The overall trend appears to be that benzo(a)pyrene (BaP) concentration and cold smoking time are positively correlated (*Figure 10*). From observing *Table 4*, smoked chicken appears to have the lowest PAH4 concentration, followed by cheese and crackers respectively. Cold smoking chicken breast for five minutes produced a PAH4 concentration of 10.84 ± 1.70 µg/kg. This is below the EC limit of 12 µg/kg. Therefore, cold smoking time of 5 minutes is the most ideal for the current smoking setup in this study. Smoked cheese and crackers all have PAH4 concentration above the limit (*Table 4*), which makes them not the ideal food to be cold smoked.

It is important to consider the food matrices of chicken, cheese and crackers and how they interact with smoke. Gomes, Roseiro, Almeida, Elias, and Santos (2011) conducted a study in which they compared PAH concentration of cold smoked sausages with 10% and 40% fat content. BaP concentration in the smoked sausages with 40% and 10% were 0.23 µg/kg and 0.09 µg/kg, respectively. PAHs are lipophilic compounds, so they will readily absorb into fat-rich foods (Wu, Gong, Yan, Sun, & Zhang, 2020). The cheese being cold smoked in this study had a fat content of 37.4%; considerably higher than

chicken breast (3.6% fat). The effect of the differing fat content is made evident when comparing PAH4 concentration of cheese and chicken. PAH4 concentration at 5 minutes of smoke time was 54.79 ± 22.89 µg/kg and 10.84 ± 1.70 µg/kg for cheese and chicken, respectively. Hence, fat content is a factor that needs to be considered when choosing a suitable food to be cold smoked.

The water crackers that were cold smoked contained mostly carbohydrates. Contrary to the findings of this study, smoked breads often contain low PAH concentrations. Fasano, Yebra-Pimentel, Martínez-Carballo, and Simal-Gándara (2016) measured the PAH concentration in traditional Spanish bread. The bread is hot smoked as it is baked in an oven with smoke being generated from oak wood. The total PAH concentration (carcinogenic and non-carcinogenic PAHs) was 3.4 µg/kg, significantly lower than what was found in the crackers. Alomirah et al. (2011) also found that smoked plain pita bread had a low concentration of PAHs; most of which were non-carcinogenic (17.6 µg/kg).

Of the foods that were smoked cold smoked, water crackers consistently had the highest PAH4 and BaP concentration. This could be because crackers are porous in nature, resulting in increased absorption of PAHs from smoke. Ukalska-Jaruga, Debaene, and Smreczak (2020) discovered that soils with highly porous biochar more rapidly absorb PAHs because of their structure. PAHs linger and condense inside porous structures due to vapour pressure, liquid solubility and capillary forces (Pignatello, 1998). Hence, porous foods are not suitable for cold smoking.

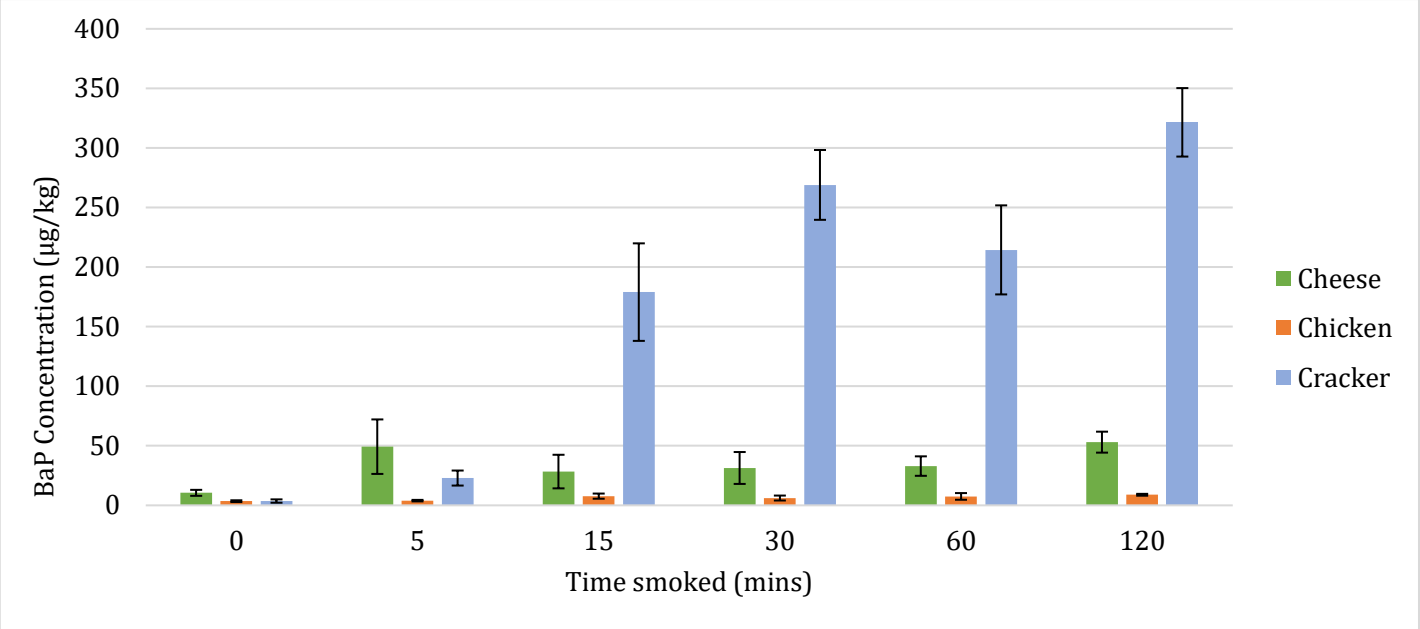

Figure 10. Benzo(a)pyrene (BaP) concentration in Manuka smoked cheese, chicken and crackers over various periods of time (error bars = standard deviation). Experiment was done in triplicates (n=3).

Table 4. PAH4 and non-carcinogenic PAHs in Manuka cold smoked cheese, chicken and crackers over various periods of time. Note that carcinogenic PAH4 include benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene and benzo(g,h,i)perylene. PAH12 includes naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene.

	Time smoked (mins)	**PAH Concentration (µg/kg)**	
		PAH4	**PAH12**
Cheese	0	10.9 ± 31.22	145.92 ± 197.37
	5	54.79 ± 22.89	2241.67 ± 603.0
	15	53.29 ± 21.62	3225.24 ± 445.53
	30	53.17 ± 13.96	4993.52 ± 1687.26
	60	144.65 ± 48.19	7642.58 ± 2460.43
	120	88.86 ± 9.21	6558.77 ± 750.02
Chicken	0	4.96 ± 1.86	76.41 ± 26.50
	5	10.84 ± 1.70	1247.31 ± 29.96
	15	18.06 ± 2.82	2985.33 ± 2052.10
	30	16.82 ± 3.22	1527.49 ± 340.84
	60	18.56 ± 4.03	1353.30 ± 1271.67
	120	20.49 ± 1.92	1992.94 ± 311.18
Crackers	0	15.35 ± 1.99	41.03 ± 14.61
	5	67.67 ± 11.60	9157.84 ± 459.30
	15	360.43 ± 57.54	53296.76 ± 2364.45
	30	507.89 ± 63.99	49884.52 ± 7076.28
	60	246.30 ± 31.18	27773.15 ± 4064.23
	120	612.32 ± 33.05	61254.85 ± 4401.94

3.2.2 Wood chip type on the effect on PAH concentration during cold smoking

BaP concentration in chicken, cheese and crackers varied greatly depending (7.5 µg/kg – 633.9 µg/kg) on which wood chip type was used for cold smoking. Overall, foods smoked with Rewa Rewa had the highest BaP concentration and PAH4s (shown in *Figure 11* and *Table 5,* respectively). Conversely, the concentration in Manuka smoked foods were the lowest amongst all the wood chips. Thus, of all the wood chips used to cold smoke, Manuka would be the most ideal in terms of PAH concentration.

As seen in *Figure 11*, BaP concentration varied among the wood chips. Bruschweiler et al. (2012) conducted a study to determine if various wood dusts contained PAHs. Total PAH concentration varied from 0.24–7.95 ppm; with the highest being found in wood melamine dust. This is likely because different woods are composed of many different compounds. This mainly includes cellulose, lignin, hemicelluloses, polar organic compounds and water soluble compounds (Bruschweiler et al., 2012). PAHs are formed when these compounds are combusted. Lignin is an aromatic precursor to PAHs, so it is likely that lignin content has an effect on BaP concentration in smoked foods (Racovita, Secuianu, Ciuca, & Israel-Roming, 2020). However, no information can be found in literature regarding lignin content in the woodchips used in this study.

Wood moisture content also influences final PAH concentration. As shown in *Table 3*, Manuka wood chips had the lowest moisture content. As a result, food smoked with Manuka had the lowest BaP and PAH4 concentration. Conversely, Tawa the highest moisture content, which resulted in the second highest BaP and PAH4 content. This is because incinerating wood that has high moisture content causes oxygen deficiency in the surrounding environment, resulting in incomplete combustion (H. Zhang et al., 2022). Consequently, this results in greater PAH production. As reported by both Seko et al. (2022) and Shen et al. (2013), moisture content of wood positively correlates to PAH formation.

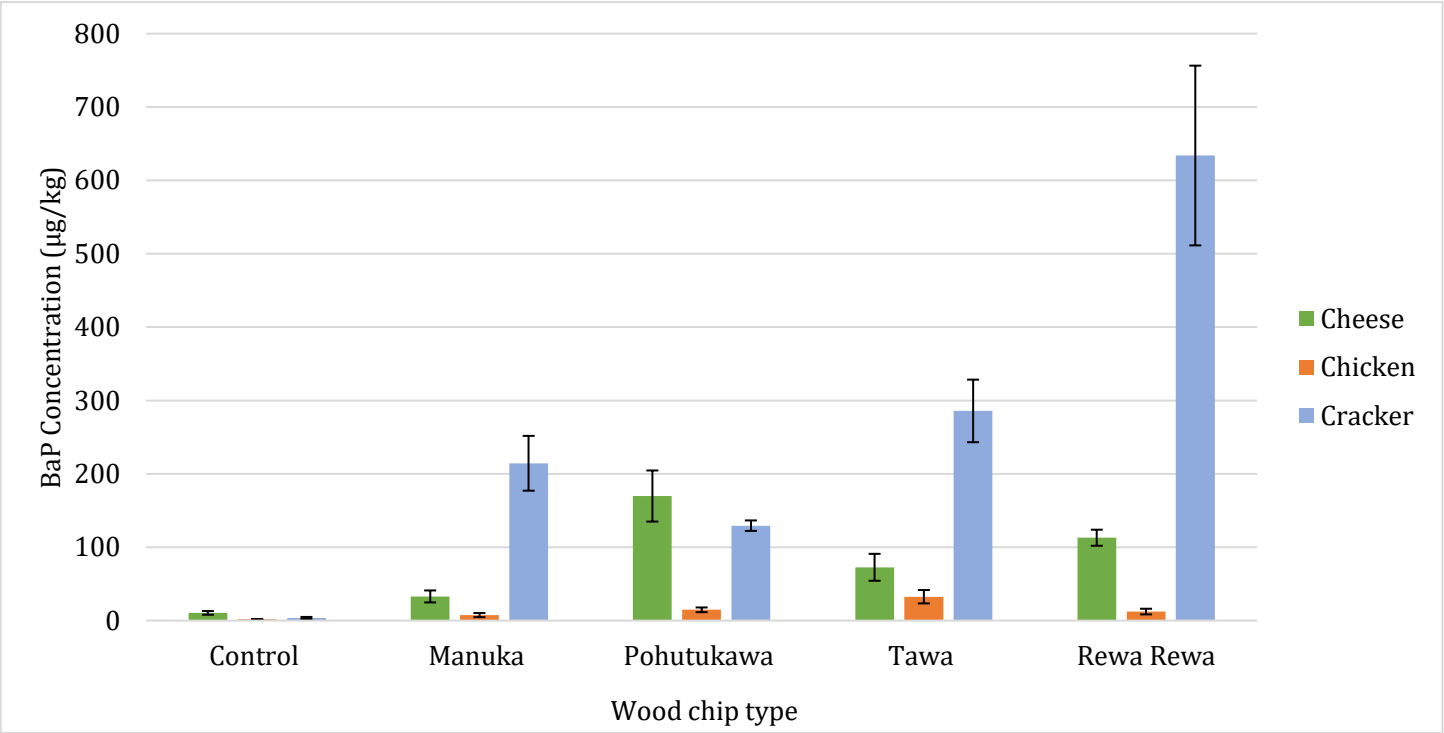

Figure 11. BaP concentration in cheese, chicken and crackers smoked for 60 mins with various wood chips (error bars = standard deviation). Experiment was done in triplicates (n=3).

Table 5. PAH4 and non-carcinogenic PAHs in cheese, chicken and crackers smoked for 60 mins with various wood chips.

	Wood chip type	PAH Concentration (µg/kg)	
		PAH4	PAH12
Cheese	Rewa Rewa	204.66 ± 24.58	13503.16 ± 845.24
	Pohutukawa	362.17 ± 49.23	71322.35 ± 25353.29
	Tawa	170.83 ± 28.81	6728.38 ± 3016.29
	Manuka	144.65 ± 48.19	7642.58 ± 2460.43
Chicken	Rewa Rewa	29.95 ± 5.50	2128.98 ± 711.13
	Pohutukawa	87.72 ± 14.64	4550.47 ± 2093.32
	Tawa	37.67 ± 4.72	4429.77 ± 539.32
	Manuka	18.56 ± 4.03	1353.30 ± 1271.67
Cracker	Rewa Rewa	112084.74 ± 8784.14	1548.19 ± 216.77
	Pohutukawa	24476.71 ± 2061.37	267.65 ± 19.71
	Tawa	41077.57 ± 459.30	522.12 ± 50.99
	Manuka	170.22 ± 82.74	19635.56 ± 1634.37

3.2.3 Hot smoking vs. cold smoking comparison

It has been well established in literature that hot smoking leads to substantial carcinogen formation (Jägerstad & Skog, 2005). As seen in *Table 6,* the PAH4s for chicken that has been smoked for 5 minutes is 105.73 ± 6.11 µg/kg. This greatly exceeds the PAH4 limit of 12 µg/kg that has been set by the EC. In contrast, the same length of smoking time done under cold conditions resulted in PAH4 concentration of 10.84 ± 1.70 µg/kg (shown in *Table 4*). It is also worth noting that the commercially available Tegel Manuka Hot Smoked Chicken Breast had a PAH4 concentration of 73.86 ± 14.34 µg/kg (*Table 6*). Again, this is over the EC limit. Similarly, Duedahl-Olesen et al. (2010) conducted a study where the concentration of 25 selected PAHs (PAH_{25}) were analysed in various Danish smoked fish products. It was reported hot smoked products had higher PAH_{25} concentration compared to cold smoked salmon. The lowest average PAH_{25} concentration was found in cold smoked trout (26 µg/kg) and the highest in hot smoked herring (320 µg/kg). This means that cold smoked food products have lower PAH levels overall when compared to their hot smoked counterpart.

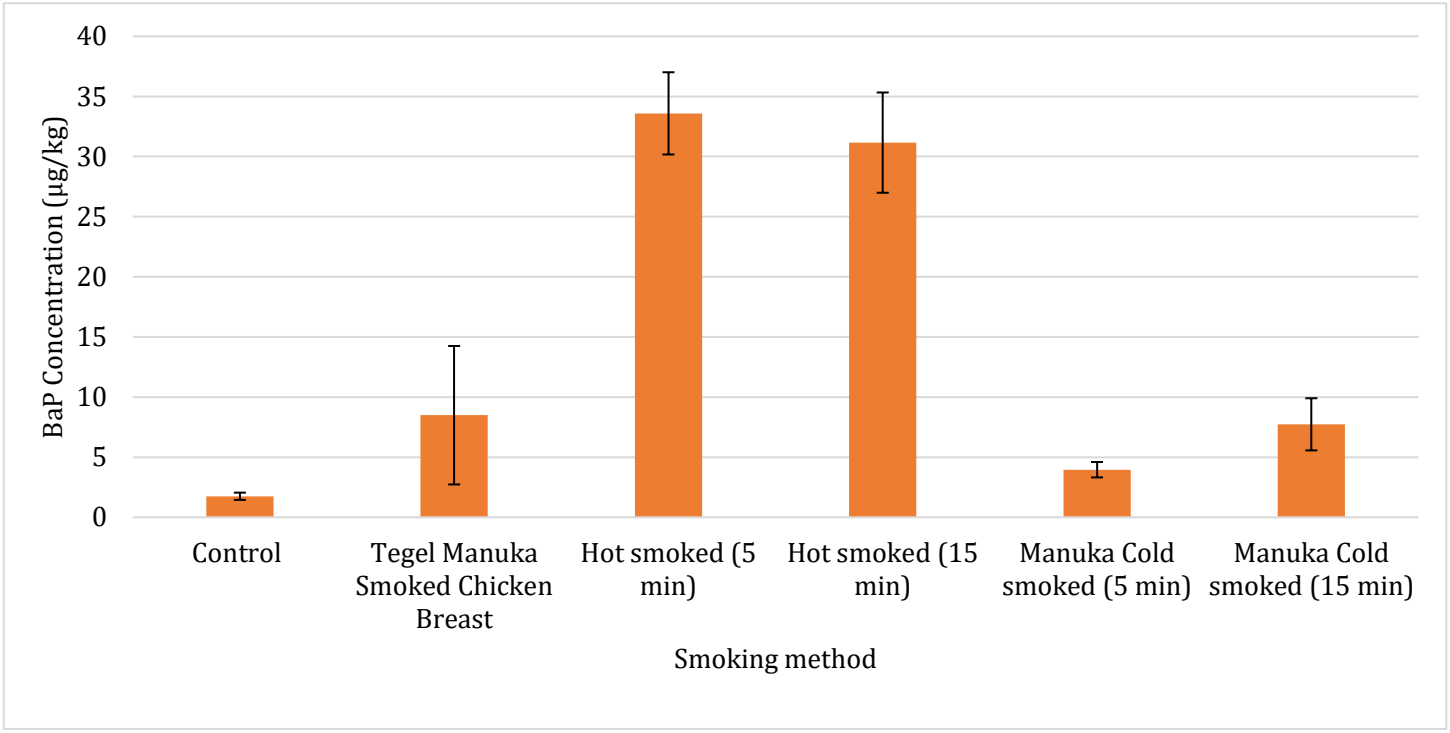

Figure 12. BaP concentration comparison between Manuka hot smoked chicken, cold smoked chicken and Tegel Manuka smoked chicken breast (error bars = standard deviation). Experiment was done in triplicates (n=3).

Table 6. PAH4 and non-carcinogenic PAHs in Manuka hot smoked chicken over various periods of time

Time smoked (mins)	PAH Concentration (µg/kg)	
	PAH4	**PAH12**
0	10.84 ± 1.70	764.41 ± 26.50
5	105.73 ± 6.11	2413.33 ± 90.38
15	98.92 ± 5.22	1701.54 ± 74.02
Tegel Manuka Smoked Chicken Breast	73.86 ± 14.34	513.69 ± 30.86

3.2.4 Effect of total surface area of chicken on PAH concentration during cold smoking

The general trend shown in *Table 7* is that as the surface area of chicken increases, so does BaP concentration. PAH4 concentration was 30.00 ± 6.81 µg/kg, observed in chicken that had a SA/g of 1.68. This would suggest that 1.68 SA/g is the most ideal size for cold smoking.

The weight to surface area ratio has a profound impact on the final PAH concentration in food; as combustion gases adhere onto the external surface and increase BaP content within the food being smoked (Andrée, Jira, Schwind, Wagner, & Schwägele, 2010). Mejborn et al. (2019) compared PAH content of Frankfurter sausages, bacon and pork fillet. The PAH content of sausages has found to be the greatest of all the meats because of high weight to surface area ratio.

Table 7. PAH4 and non-carcinogenic PAHs in Manuka smoked chicken for 60 mins with various surface areas.

Surface area to weight ratio (cm^2/g)	PAH Concentration (µg/kg)		
	PAH4	**PAH12**	**BaP concentration**
1.60	31.74 ± 8.10	4567.63 ± 1756.39	11.38 ± 3.40
1.68	30.00 ± 6.81	2445.13 ± 553.60	9.93 ± 0.96
1.41	79.82 ± 8.21	8518.37 ± 681.11	33.76 ± 3.01

3.2.5 Sensory evaluation

One-way ANOVA was done to determine if differences in perception of sensory attributes existed at the 5% level (See Supplementary Information S3 for ANOVA tables). If differences were present, Tukey's HSD test was done as a post-hoc test to accurately pinpoint differences (See Supplementary Information S4 for Tukey's HSD tables). The null hypothesis for ANOVA was that there is no difference in overall liking, odour, appearance, texture, smokiness, and flavour between cold smoked chicken samples.

As seen in *Figure 13*, significant differences between samples were found for every attribute. In terms of overall liking, both Manuka and Tawa smoked chicken were assigned A. This means that they were the most liked among the panellists. The same applies for odour, smokiness and flavour. Rewa Rewa was most favoured with respect to appearance. Manuka appeared to be the most favoured across all attributes, as was assigned an A for overall liking, odour, texture, smokiness and flavour. Conversely, Pohutukawa was the most disliked, as it was assigned a B for all attributes. Panellists correctly identified the control sample as having the least amount of smokiness, as it was assigned a C. Thus, the null hypothesis can be rejected, as differences were found between all attributes.

It also important to consider how PAH concentrations in each sample effect overall liking and perception of sensory attributes. Pohutukawa smoked chicken breast had the most PAH4, which meant that it was overly smokey and disliked the most by panellists (*Table 7*). On the other hand, Manuka had the lowest PAH4 but was most liked by panellists The data presented does not provide evidence to claim that the PAH are responsible for the negative sensory perception ("overly smokey") and lower consumer acceptance. In this case, a correlation does not prove a causative relationship that the PAH are responsible for this flavour. It is likely that flavour intensity may be correlated or co-varying, but not necessarily responsible

The outcome of this sensory evaluation showed that cold smoking using Manuka wood would be most appealing to consumers. If Manuka cold smoked products were to be commercialised in the New Zealand market, further testing should be considered to determine optimum smoking time.

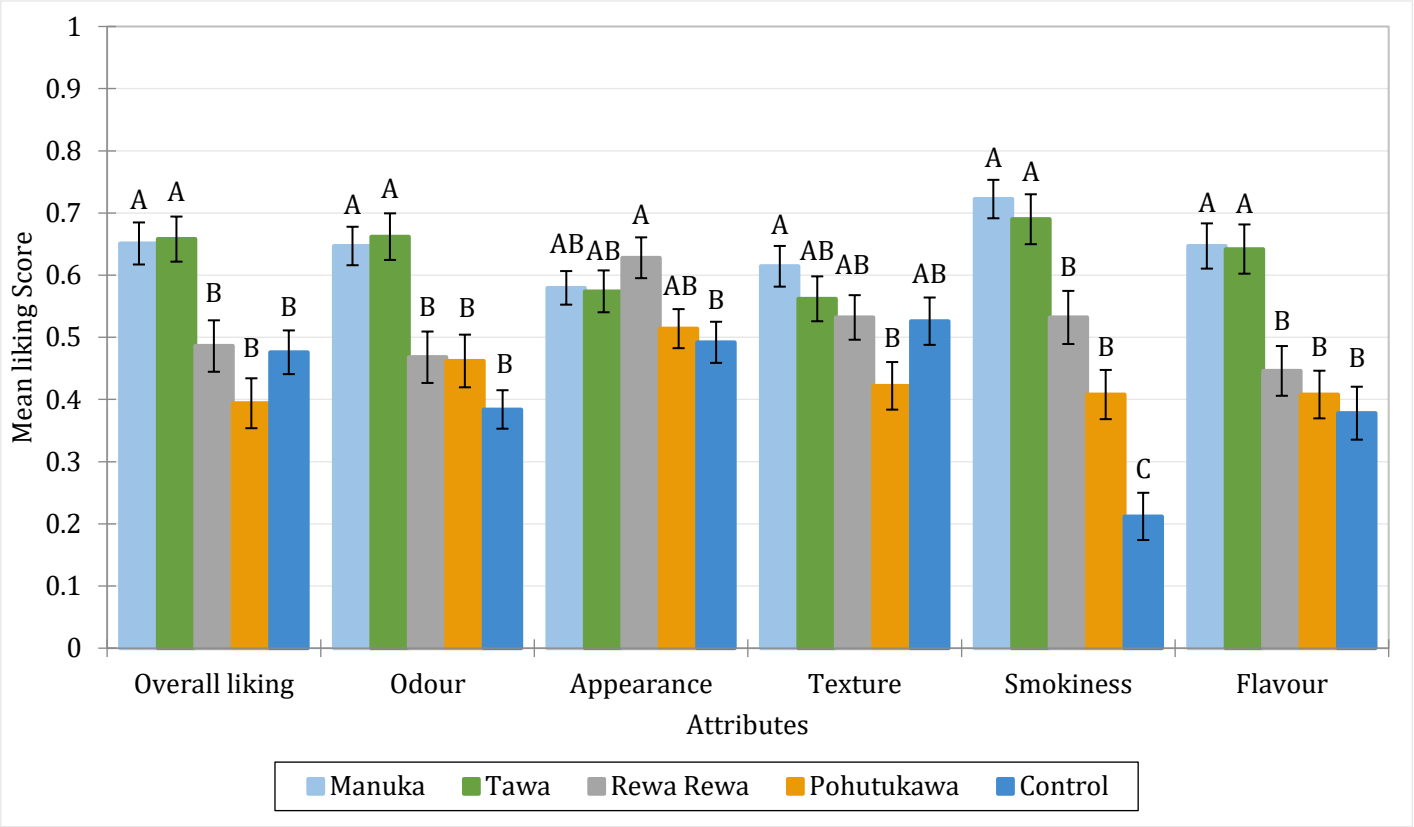

Figure 13. Mean liking score of overall liking, odour, appearance, texture, smokiness and flavour of chicken breast cold smoked with various woods (n=50). Error bars = standard error

As seen in *Table 7*, the attributes that were most chosen via CATA were smokey, earthy and ashy. Smokey was assigned to Tawa the most, followed by Manuka.

PCA analysis was done to visualise the relationship between the various wood used to smoke chicken breast and frequency of attributes that panellists assigned to each sample. Additionally, Pearson correlation matrix can be found in Supplementary Information S5

As shown in *Figure 14*, there is an apparent clustering of Pohutukawa, Manuka and Tawa smoked chicken in the second quadrant. These were all grouped closest to the smokey attribute. Rewa Rewa was also close to smokey but was isolated into the first quadrant. Ashy and petroleum- like were clustered in the first quadrant, meaning that

they were mostly assigned to the Rewa Rewa smoked chicken by panellists. The control sample is isolated in the fourth quadrant, along with the cluster of pungent sour and bitter attributes. Cedar, burnt, dusty, metallic, creosote/tar and acrid are all grouped in the third quadrant with none of the chicken samples being present. This indicates that these attributes were less frequently assigned by the panellists.

Table 8. Frequency of attribute assignment to each sample via CATA

	Sample type					
Attribute	**Control**	**Tawa**	**Manuka**	**Rewa Rewa**	**Pohutukawa**	**Frequency**
Smokey	1	49	47	42	42	**181**
Earthy	8	18	16	10	17	**69**
Ashy	1	11	12	16	15	**55**
Petroleum-Like	1	2	7	17	14	**41**
Burnt	0	7	6	15	6	**34**
Cedar	0	7	10	9	6	**32**
Dusty	2	8	5	5	6	**26**
Pungent	4	3	3	8	3	**21**
Metallic	1	2	4	5	8	**20**
Sour	4	1	5	6	3	**19**
Bitter	4	0	3	5	5	**17**
Creosote/Tar	0	0	0	6	8	**14**
Acrid	2	0	2	4	2	**10**

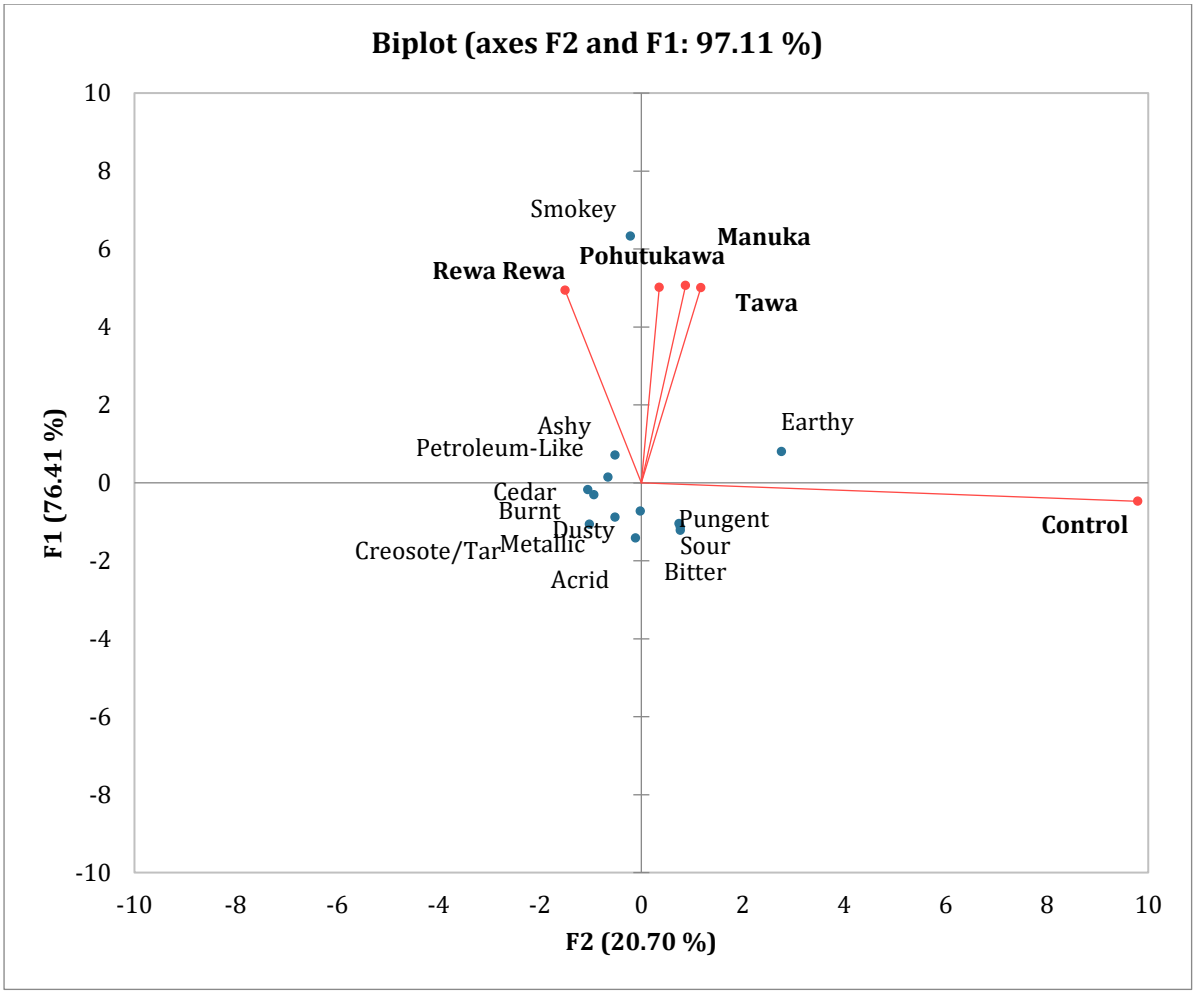

Figure 14. PCA biplot displaying the relationship between wood used to cold smoke chicken and frequency of sensory attributes being assigned to each chicken sample.

3.3 Conclusion

The aim of this study was to determine the effect of cold smoking on the PAH content of chicken, cheese and crackers. This research expanded upon existing literature, as cold smoking using New Zealand native wood chips has never researched. It was found that time and PAH concentration in food were positively correlated. Overall, chicken had the least PAH concentration and five minutes of smoking time was the most ideal for surface area of 54 cm^2. Out of all the woods that were used for cold smoking, Manuka generated the least amount of PAH content in the food. Lignin content of the woods used in this study should be researched further, as it may a profound influence on PAH generation in smoke.

Via sensory evaluation, Manuka cold smoked chicken breast was found to be the most liked among panellists. Manuka cold smoking, especially using chicken breast, has the potential to be commercialised in the New Zealand food market as an alternative to hot smoked foods. Further research should be done to optimise cold smoking methods.

Chapter 4: Thesis conclusions

The use of food smoking technology to improve the organoleptic properties of food is increasingly popular nowadays. However, the dangers of contaminating the food with elevated levels of PAH during smoking of food is of concern. Therefore, food regulators have imposed an upper limit of 1 to 50 µg/kg of carcinogenic PAH – sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene in food depending on food type. Other methods of introducing smoke to food include cold smoking, friction smoking, liquid smoking, and electrostatic smoking are developed to reduce the amount of PAH contaminants during the smoking process. Solid-phase extraction (dispersive-SPE), method using the "QuEChERS" technique is a current way to extract PAHs in food matrices and then quantified using GC-MS. The objective of this review article was to describe a comprehensive view on the process of food smoking, the benefits, the regulations and ways to quantify PAH in foods.

Chicken, cheese and crackers were cold smoked with Native New Zealand wood chips (Manuka, Tawa, Rewa Rewa and Pohutukawa). The PAH concentration of these foods was extracted using QuEChERS technique and analysed using GC–MS. Cold smoking time (5 to 120 minutes) had the largest influence on PAH4 concentration in the foods which varied from 10.84 ± 1.70 to 112084.74 ± 8784.14 µg/kg. The lowest PAH content was in chicken that had been cold smoked with Manuka for five minutes. An untrained sensory panel (n=50) rated chicken breast that had been cold smoked with Manuka, Tawa, Rewa Rewa and Pohutukawa based on overall liking, odour, appearance, texture, smokiness, and flavour. One-way analysis of variance (ANOVA) revealed that Manuka cold smoked chicken breast was the most liked sample among the panel.

Cold smoking could be a safer alternative to hot smoking, as the concentration of PAHs was less. One of the major limitations of this study was the lack of previous research on the topic of cold smoking. Thus, cold smoking should be studied further as it has the potential for greater commercialisation.

Supplementary Information

Supplementary Information S1

Table S1. Ion mass program for GC-MS quantification

Start time (min)	Compound	Ions (m/z)
3	Naphthalene-d8	136
	Naphthalene	128, 127, 129
6	Acenaphthylene	152, 151, 153
	Acenaphthene-d10	164
	Acenaphthene	154, 153, 152
7.2	Fluorene	166, 163, 165
8	Phenanathrene-d10	188
	Phenanathrene	178, 177, 179
	Antrhacene	178, 177, 179
9	Fluoranthene	202, 201, 203
	Pyrene	202, 201, 203
10.4	Chrysene-d12	240
	Benzo[a]anthracene	228, 229, 226
	Chrysene	228, 229, 226
11.6	Benzo[b]fluoranthene	252, 253, 126
	Benzo[k]fluoranthene	252, 253, 126
	Benzo[a]pyrene	252, 253, 126
	Perylene-d12	264
13	Indeno[1,2,3-cd]pyrene	276, 277, 138
	Dibenz[a,h]anthracene	278, 279
	Benzo[g,h,i]perylene	276, 277,138

Supplementary Information S2

Quick Poll

Please make sure you have signed the consent form before beginning this survey

Lay out all samples in front of you and be sure to have a glass of water.

Please enter your reference number (e.g. P1)

Please assess **SAMPLE 914** based on the following criteria:

	Strongly dislike	Neutral	Strongly Like
Overall liking			
Odour			
Appearance			
Texture			
Smokiness			
Flavour			

Select all that you associate with **SAMPLE 914**

- ☐ Smokey
- ☐ Bitter
- ☐ Sour
- ☐ Earthy
- ☐ Acrid
- ☐ Ashy

- [ ] Dusty
- [ ] Burnt
- [ ] Pungent
- [ ] Petroleum-Like
- [ ] Creosote/Tar
- [ ] Cedar
- [ ] Metallic

Please eat a cracker and have a sip of water before moving onto the next sample.

Please assess **SAMPLE 688** based on the following criteria:

	Strongly dislike	Neutral	Strongly Like
Overall liking			
Odour			
Appearance			
Texture			
Smokiness			
Flavour			

Select all that you associate with **SAMPLE 688**

- [ ] Smokey
- [ ] Bitter
- [ ] Sour
- [ ] Earthy
- [ ] Acrid
- [ ] Ashy
- [ ] Dusty
- [ ] Burnt

- [ ] Pungent
- [ ] Petroleum-Like
- [ ] Creosote/Tar
- [ ] Cedar
- [ ] Metallic

Please eat a cracker and have a sip of water before moving onto the next sample.

Please assess **SAMPLE 125** based on the following criteria:

	Strongly dislike	Neutral	Strongly Like
Overall liking			
Odour			
Appearance			
Texture			
Smokiness			
Flavour			

Select all that you associate with **SAMPLE 125**

- [ ] Smokey
- [ ] Bitter
- [ ] Sour
- [ ] Earthy
- [ ] Acrid
- [ ] Ashy
- [ ] Dusty
- [ ] Burnt
- [ ] Pungent
- [ ] Petroleum-Like

- [ ] Creosote/Tar
- [ ] Cedar
- [ ] Metallic

Please eat a cracker and have a sip of water before moving onto the next sample.

Please assess **SAMPLE 537** based on the following criteria:

	Strongly dislike	Neutral	Strongly Like
Overall liking			
Odour			
Appearance			
Texture			
Smokiness			
Flavour			

Select all that you associate with **SAMPLE 537**

- [ ] Smokey
- [ ] Bitter
- [ ] Sour
- [ ] Earthy
- [ ] Acrid
- [ ] Ashy
- [ ] Dusty
- [ ] Burnt
- [ ] Pungent
- [ ] Petroleum-Like
- [ ] Creosote/Tar
- [ ] Cedar

☐ Metallic

Please eat a cracker and have a sip of water before moving onto the next sample.

Please assess **SAMPLE 821** based on the following criteria:

	Strongly dislike	Neutral	Strongly Like
Overall liking			
Odour			
Appearance			
Texture			
Smokiness			
Flavour			

Select all that you associate with **SAMPLE 821**

☐ Smokey
☐ Bitter
☐ Sour
☐ Earthy
☐ Acrid
☐ Ashy
☐ Dusty
☐ Burnt
☐ Pungent
☐ Petroleum-Like
☐ Creosote/Tar
☐ Cedar
☐ Metallic

Please eat a cracker and have a sip of water before moving onto the next sample.

Demo.

Please answer the following questions

Gender

- ○ Male
- ○ Female
- ○ Other

Age

- ○ Under 18
- ○ 18 - 24
- ○ 25 - 34
- ○ 35 - 44
- ○ 45 - 54
- ○ 55 - 64
- ○ 65 - 74
- ○ 75 - 84
- ○ 85 or older

How often do you consume meat?

- ○ Never
- ○ Once a month
- ○ Once a fortnight
- ○ Once a week
- ○ More than once a week

How often do you consume smoked meat?

- ○ Never
- ○ Once a month
- ○ Once a fortnight
- ○ Once a week
- ○ More than once a week

Which ethnic group do you associate with most?

- ○ European
- ○ Maori
- ○ Polynesian
- ○ Chinese
- ○ Indian
- ○ Mixed ethnic background
- ○ [ ] Other (type below)

Powered by Qualtrics

Consent Form

Project title: **Sensory analysis of chicken breast cold smoked with New Zealand sourced woodchips – Manuka, Rewa Rewa, Tawa, Pohutakawa**

Project Supervisor: ***Dr. Rothman Kam***

Researcher: ***Shaan Kaloti***

- ○ I have read and understood the information provided about this research project in the Information Sheet dated 18/05/2020.
- ○ I have had an opportunity to ask questions and to have them answered.
- ○ I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without being disadvantaged in any way.
- ○ I understand that if I withdraw from the study then I will be offered the choice between having any data or tissue that is identifiable as belonging to me removed or allowing it to continue to be used. However, once the findings have been produced, removal of my data may not be possible.
- ○ I agree to take part in this research.
- ○ I agree to report on the taste of consumed chicken samples
- ○ I can
    - Consume chicken
    - Consume wheat products
- ○ I wish to receive a summary of the research findings (please tick one): Yes○ No○

Participant's signature: .....

Participant's name: .....

Participant's Contact Details (if appropriate):

.....

.....

.....

.....

Date:

Approved by the Auckland University of Technology Ethics Committee on 06/07/2022. AUTEC Reference number 22/175

Participant Information Sheet

Date Information Sheet Produced:

18/05/2022

Project Title

Sensory analysis of chicken breast cold smoked with New Zealand sourced woodchips – Manuka, Rewa Rewa, Tawa, Pohutakawa

An Invitation

Dear Sir/Madam,

My name is Shaan Kaloti, I am a Master's student in the Faculty of Health and Environmental Science at the Auckland University of Technology. I would like to invite you to participate in a study that examines our cold smoked chicken and comparing different in sensory attributes.

Participation in this research is completely voluntary and confidential. You are under no obligation to complete the questionnaire nor taste the food provided, and you have the freedom to withdraw at any stage without question.

This Participation Information Sheet will help you decide if you would like to participate in this study. It explains why we are doing this study, how you were chosen for this invitation, how your privacy is protected and what happens after the study is completed. You do not have to decide today whether or not you will participate in this study. Please feel free to discuss your decision with family or friends.

What is the purpose of this research?

The aim of this research is to determine if cold smoking causes less carcinogen formation in foods. Additional factors, such as wood chips used for smoking, time and surface area of the food being smoked have also been considered. There are big gaps in literature regarding cold smoking and this project has the potential to fill them in. This research could also lead to a healthier alternative to hot smoked foods. Testing has been done prior to this sensory to ensure carcinogens in the chicken you are consuming are below the legal limit. The results obtained from this study will identify and determine which wood chips have the most desirable sensory attributes.

How was I identified and why am I being invited to participate in this research?

You are invited to participate in this research because you have responded an invitation from the researcher. We welcome all individuals to participate in this study that meets the following criteria:

- You are able to consume chicken
- You do not have an allergy to wheat
- You are able to attend to set aside 20 minutes to do the evaluation

Your participation in this research is voluntary (it is your choice) and whether or not you choose to participate will neither advantage nor disadvantage you. You are able to withdraw from the study at any time. If you choose to withdraw from the study, then you will be offered the choice between having any data that is identifiable as belonging to you removed and allowing it to continue to be used. However, once the findings have been produced, removal of your data may not be possible.

What are the exclusion criteria?

You are not to participate in this study if:

- You are unable to consume chicken
- Have a wheat allergy
- Are a student of the supervisor

How do I agree to participate in this research?

Your participation in this research is voluntary (it is your choice) and whether or not you choose to participate will neither advantage nor disadvantage you. You are able to withdraw from the study at any time. If you choose to withdraw from the study, then you will be offered the choice between having any data that is identifiable as belonging to you removed or allowing it to continue to be used. However, once the findings have been produced, removal of your data may not be possible.

What will happen in this research?

Should you agree to take part in this project, you will take a pack containing smoked chicken samples and complete a sensory evaluation at home. During the evaluation, you will consume five samples and answer an online survey. You will be assessing rating odour, appearance, texture, smokiness, flavour and overall liking. You will also be asked to select attributes that you associate with each sample from a list.

What are the discomforts and risks?

It is not anticipated that you will experience discomfort or risk in this study. All food products provided in this study will be made from commercially available food ingredients and food grade materials. All food prepared for the research will be prepared, stored and handled according to current New Zealand food hygiene standards. Since chicken is a perishable product, the evaluation will need to be done within three days of receiving the samples. They will also need to be refrigerated before consumption.

What are the benefits?

The potential benefit of this research is that it could lead to innovation in current food processing methods.
Current smoking methods lead to high levels of PAHs in foods, which lead to health problems such as cancer. If cold smoked foods are found to have lower PAH levels and are liked by consumers, it could lead to healthier food for the wider community. There are currently large gaps in literature relating to cold smoking, which need to be explored. By participating in this research, you're helping to widen the knowledge in this are

What compensation is available for injury or negligence?

In the unlikely event of a physical injury as a result of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation, providing the incident details satisfy the requirements of the law and the Corporation's regulations.

How will my privacy be protected?

Your responses are confidential, and your privacy are protected by the use of a unique numeric code that will be assigned. Upon completion of the sensory tests, the data will be combined with all the other participants' data.

What are the costs of participating in this research?

There will be no cost in this study if you wish to take part. We understand that time is an important factor, and we value your participation and responses. This experiment will take 20 minutes to complete.

What opportunity do I have to consider this invitation?

Your participation in this study is completely voluntary. If you require further information or want to ask questions about this research, please contact me via email. If you would like to discuss the requirements, please email me with a suitable time and I will reply back to you in a reasonable time frame.

Will I receive feedback on the results of this research?

A summary of the findings can downloaded here:
https://aut.au1.qualtrics.com/reports/public/YXV0LTYyYzRkYWQ5NjNiNmU3MDAwZjQ5NDg4Yi1VUl8zeDlSSTZzMHZENGJhc3Q=

What do I do if I have concerns about this research?

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor, Dr. Rothman Kam, Rothman.kam@aut.ac.nz, and +64 9 921 9999 ext. 7620.

Concerns regarding the conduct of the research should be notified to the Executive Secretary of AUTEC, *ethics@aut.ac.nz*, (+649) 921 9999 ext 6038.

Whom do I contact for further information about this research?

Please keep this Information Sheet and a copy of the Consent Form for your future reference. You are also able to contact the research team as follows:

Researcher Contact Details:

Shaan Kaloti

shaankaloti@gmail.com

0212066401

Project Supervisor Contact Details:

Rothman Kam

Rothman.kam@aut.ac.nz

+64 9 921 9999 ext. 7620

Approved by the Auckland University of Technology Ethics Committee on 06/07/2022. AUTEC Reference number 22/175

Auckland University of Technology Ethics Committee (AUTEC)

Auckland University of Technology
D-88, Private Bag 92006, Auckland 1142, NZ
T: +64 9 921 9999 ext. 8316
E: ethics@aut.ac.nz
www.aut.ac.nz/researchethics

12 July 2022

Rothman Kam
Faculty of Health and Environmental Sciences

Dear Rothman

Re Ethics Application: **22/175 Sensory analysis of chicken breast cold smoked with New Zealand sourced woodchips – Manuka, Rewa Rewa, Tawa, Pohutakawa**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 12 July 2025.

Standard Conditions of Approval

1. The research is to be undertaken in accordance with the Auckland University of Technology Code of Conduct for Research and as approved by AUTEC in this application.
2. A progress report is due annually on the anniversary of the approval date, using the EA2 form.
3. A final report is due at the expiration of the approval period, or, upon completion of project, using the EA3 form.
4. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form.
5. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
6. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.
7. It is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard and that all the dates on the documents are updated.
8. AUTEC grants ethical approval only. You are responsible for obtaining management approval for access for your research from any institution or organisation at which your research is being conducted and you need to meet all ethical, legal, public health, and locality obligations or requirements for the jurisdictions in which the research is being undertaken.

Please quote the application number and title on all future correspondence related to this project.

For any enquiries please contact ethics@aut.ac.nz. The forms mentioned above are available online through http://www.aut.ac.nz/research/researchethics

(This is a computer-generated letter for which no signature is required)

The AUTEC Secretariat
Auckland University of Technology Ethics Committee

Cc: shaankaloti@gmail.com

Supplementary Information S3

Table S3.1. ANOVA for overall liking

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	2.703	0.676	9.650	**<0.0001**
Error	244	17.084	0.070		
Corrected Total	248	19.787			

Computed against model Y=Mean(Y)

Table S3.2. ANOVA for odour

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	3.022	0.755	11.108	**<0.0001**
Error	244	16.594	0.068		
Corrected Total	248	19.615			

Computed against model Y=Mean(Y)

Table S3.3. ANOVA for appearance

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	0.595	0.149	2.963	**0.020**
Error	244	12.254	0.050		
Corrected Total	248	12.849			

Computed against model Y=Mean(Y)

Table S3.4. ANOVA for texture

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	0.983	0.246	3.747	**0.006**
Error	244	16.009	0.066		
Corrected Total	248	16.992			

Computed against model Y=Mean(Y)

Table S3.5. ANOVA for smokiness

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	8.815	2.204	29.793	**<0.0001**
Error	244	18.049	0.074		
Corrected Total	248	26.864			

Computed against model Y=Mean(Y)

Table S3.6. ANOVA for flavour

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	3.376	0.844	10.879	**<0.0001**
Error	244	18.931	0.078		
Corrected Total	248	22.307			

Computed against model Y=Mean(Y)

Supplementary Information S4

Table S4.1. Tukeys HSD for overall liking

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
Tawa vs Pohutukawa	0.264	4.989	2.748	**<0.0001**	Yes
Tawa vs Control	0.182	3.439	2.748	**0.006**	Yes
Tawa vs Rewa Rewa	0.172	3.250	2.748	**0.011**	Yes
Tawa vs Manuka	0.007	0.131	2.748	1.000	No
Manuka vs Pohutukawa	0.257	4.832	2.748	**<0.0001**	Yes
Manuka vs Control	0.175	3.290	2.748	**0.010**	Yes
Manuka vs Rewa Rewa	0.165	3.102	2.748	**0.018**	Yes
Rewa Rewa vs Pohutukawa	0.092	1.738	2.748	0.412	No
Rewa Rewa vs Control	0.010	0.189	2.748	1.000	No
Control vs Pohutukawa	0.082	1.549	2.748	0.531	No
Tukey's d critical value:			3.887		

Table S4.2. Tukeys HSD for odour

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
Tawa vs Control	0.278	5.330	2.748	**<0.0001**	Yes
Tawa vs Pohutukawa	0.200	3.835	2.748	**0.001**	Yes
Tawa vs Rewa Rewa	0.194	3.720	2.748	**0.002**	Yes
Tawa vs Manuka	0.015	0.287	2.748	0.999	No
Manuka vs Control	0.263	5.016	2.748	**<0.0001**	Yes
Manuka vs Pohutukawa	0.185	3.528	2.748	**0.005**	Yes
Manuka vs Rewa Rewa	0.179	3.413	2.748	**0.007**	Yes
Rewa Rewa vs Control	0.084	1.611	2.748	0.492	No
Rewa Rewa vs Pohutukawa	0.006	0.115	2.748	1.000	No
Pohutukawa vs Control	0.078	1.496	2.748	0.566	No
Tukey's d critical value:			3.887		

Table S4.3. Tukeys HSD for appearance

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
Rewa Rewa vs Control	0.136	3.034	2.748	**0.022**	Yes
Rewa Rewa vs Pohutukawa	0.114	2.544	2.748	0.085	No
Rewa Rewa vs Tawa	0.054	1.205	2.748	0.749	No
Rewa Rewa vs Manuka	0.048	1.075	2.748	0.819	No
Manuka vs Control	0.088	1.944	2.748	0.297	No
Manuka vs Pohutukawa	0.066	1.456	2.748	0.592	No
Manuka vs Tawa	0.006	0.124	2.748	1.000	No
Tawa vs Control	0.082	1.830	2.748	0.359	No
Tawa vs Pohutukawa	0.060	1.339	2.748	0.667	No
Pohutukawa vs Control	0.022	0.491	2.748	0.988	No
Tukey's d critical value:			3.887		

Table S4.4. Tukeys HSD for texture

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
Manuka vs Pohutukawa	0.192	3.734	2.748	**0.002**	Yes
Manuka vs Control	0.088	1.715	2.748	0.427	No
Manuka vs Rewa Rewa	0.082	1.598	2.748	0.500	No
Manuka vs Tawa	0.052	1.015	2.748	0.848	No
Tawa vs Pohutukawa	0.140	2.733	2.748	0.052	No
Tawa vs Control	0.036	0.703	2.748	0.956	No
Tawa vs Rewa Rewa	0.030	0.586	2.748	0.977	No
Rewa Rewa vs Pohutukawa	0.110	2.147	2.748	0.204	No
Rewa Rewa vs Control	0.006	0.117	2.748	1.000	No
Control vs Pohutukawa	0.104	2.030	2.748	0.255	No
Tukey's d critical value:			3.887		

Table S4.5. Tukeys HSD for smokiness

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
Manuka vs Control	0.510	9.337	2.748	**<0.0001**	Yes
Manuka vs Pohutukawa	0.314	5.752	2.748	**<0.0001**	Yes
Manuka vs Rewa Rewa	0.190	3.484	2.748	**0.005**	Yes
Manuka vs Tawa	0.032	0.594	2.748	0.976	No
Tawa vs Control	0.478	8.788	2.748	**<0.0001**	Yes
Tawa vs Pohutukawa	0.282	5.184	2.748	**<0.0001**	Yes
Tawa vs Rewa Rewa	0.158	2.905	2.748	**0.032**	Yes
Rewa Rewa vs Control	0.320	5.883	2.748	**<0.0001**	Yes
Rewa Rewa vs Pohutukawa	0.124	2.280	2.748	0.155	No
Pohutukawa vs Control	0.196	3.603	2.748	**0.003**	Yes
Tukey's d critical value:			3.887		

Table S4.6. Tukeys HSD for flavour

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
Manuka vs Control	0.269	4.803	2.748	**<0.0001**	Yes
Manuka vs Pohutukawa	0.239	4.267	2.748	**0.000**	Yes
Manuka vs Rewa Rewa	0.201	3.589	2.748	**0.004**	Yes
Manuka vs Tawa	0.005	0.088	2.748	1.000	No
Tawa vs Control	0.264	4.739	2.748	**<0.0001**	Yes
Tawa vs Pohutukawa	0.234	4.200	2.748	**0.000**	Yes
Tawa vs Rewa Rewa	0.196	3.518	2.748	**0.005**	Yes
Rewa Rewa vs Control	0.068	1.221	2.748	0.739	No
Rewa Rewa vs Pohutukawa	0.038	0.682	2.748	0.960	No
Pohutukawa vs Control	0.030	0.539	2.748	0.983	No
Tukey's d critical value:			3.887		

Supplementary Information S5

Table S5. PCA Pearson correlation matrix

Variables	Tawa	Control	Manuka	Rewa Rewa	Pohutukawa
Tawa	**1**	0.020	**0.985**	**0.896**	**0.939**
Control	0.020	**1**	-0.007	-0.229	-0.054
Manuka	**0.985**	-0.007	**1**	**0.925**	**0.954**
Rewa Rewa	**0.896**	-0.229	**0.925**	**1**	**0.929**
Pohutukawa	**0.939**	-0.054	**0.954**	**0.929**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

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