University of Nottingham
UK | CHINA | MALAYSIA

Interaction of flavour compounds with the seasoning base

By

Nacchiappan Annamalai

Supervisors: Prof. Ian D Fisk, Dr. Ni Yang

Thesis submitted to the University of Nottingham for the degree of Master of Research

Acknowledgement

I'd like to first thank my supervisors, Professor Ian D Fisk, and Dr. Ni Yang for supporting my growth throughout my MRes and for the advice Ian gave me before the end of this chapter in my life. I would like to thank my parents for encouraging me and supporting me in every which way possible by going above and beyond their capabilities. I would also like to thank all my friends who helped me through this amazing journey of a masters, it is those friends whom we do not give enough credit to, who will stand beside you supporting morally and emotionally. I could not have asked for a better place than the University of Nottingham to do my MRes, and I will forever be grateful to the friends I made and the ones I became close to, and for all the memories which will forever stay in my memory and apparently in google ;).

Table of Contents

1 Abstract 5
2 Introduction 6
2.1.1 Formulation 6
2.2 Aroma compounds 8
2.2.1 Interaction with the base 10
2.2.2 Physical attributes and flavour release 14
2.3 Summary 15
3 Materials and methods 16
3.1 Materials 16
3.2 Evaluation of cheese and onion flavour profile 17
3.2.1 GC-MS SPME 17
3.2.2 Statistics 18
3.2.3 GC-O-MS 18
3.3 Evaluation of seasoning base 19
3.3.1 Scanning Electron Microscopy 19
3.4 Physicochemical data 19
4 Results and discussions 20
4.1 Characterisation of flavour isolates in cheese and onion 20
4.1.1 Comparison of bases 24
4.2 Physical characterisation of seasoning powders 25
4.3 Interaction of aroma with seasoning base 26
4.4 Odour activity of seasoning 29
4.5 Overview 31
4.6 Future work 32
5 Conclusion 33
6 Appendix 34
7 Reference 35

List of figures

Figure 1 ..... Section 4.2

SEM images of seasoning bases

Figure 2 ..... Section 4.3

Concentration of aroma compounds present in seasoning base

Figure 3 ..... Section 4.3

Figure shows the increase in ratio of abundance of butanoic acid ester compounds with different Log K_{ow} Values, in the starch bases when compared to flour bases.

Figure 4 ..... Section 4.5

PCA overview of SPME GC-MS data

Figure 5 ..... Section 6

An example of how the ImageJ was used to measure the particle size

List of tables

Table 1 ..... Section 3

Stepwise description of the processes undertaken in this work.

Table 2 ..... Section 4.1

Physio-chemical properties and functional group description of the flavour compounds in the seasoning

Table 3 ..... Section 4.4

GCO data for the seasonings

1 ABSTRACT

Industrial seasonings are flavourless bases mixed with aroma and taste active compounds which maybe formulated using different techniques for different applications, each with variable loading capacity and delivery efficiency. Depending on the macro molecule composition and the type of the base, the flavour compounds are bound in the seasoning base differently affecting the effective flavour release. The focus of this research was on the topical application of seasonings, specifically cheese and onion seasoning that is applied to crisps. The model cheese and onion flavour developed in this study contained aroma compounds with a wide range of physio-chemical properties. The aim was to investigate the impact on aroma release from five different exemplar bases with varying chemical compositions: corn starch, wheat starch, corn flour, wheat flour, and maltodextrin using GC-Olfactometry, and SPME GC-MS. Seasoning powders were further evaluated using scanning electron microscopy to explain the impact of particle size on the flavour release. Corn flour had the maximum particle size with 650 µm and corn starch had the least (38 µm). maltodextrin (particle size of 95 µm) was the most successful flavour carrier with improved flavour release and a flavour profile containing 17 compounds. Wheat flour was the least improved flavour carrier with the weakest flavour profile. There were some compound specific effects, for example wheat flour retained higher levels of butanoic acid and benzyl alcohol. This study has scope for application across the food industry.

Keywords*: Aroma release, Cheese and onion, Flavour profile, Particle size, Seasoning base*

2 INTRODUCTION

Seasonings are a blend of herbs, spices, and salt that are added to enhance the flavour of foods. Seasonings may also be formulated using specific flavour compounds or blends of compounds (aroma and taste actives) mixed with a flavourless base which is then added to different food preparations including dry foods like crisps and fries. The topical application of powdered seasoning is of particular interest to the food industry as it helps them enhance the flavour of their product.

Flavour is the combination of mainly aroma, taste, and trigeminal senses. The aroma receptors are triggered by multiple volatile compounds and therefore are responsible for the odour that we sense. Similarly for taste, G-Protein Coupled Receptors help in most chemosensory activities and are responsible for the detection of sweet, umami, and bitter tastes (Boughter & Munger, 2013). The other two tastes, saltiness, and sourness are sensed through ion channels that detect ions (Na^+, H^+). Trigeminal stimulation is associated with certain compounds and leads to pain response, it is perceived through the activation of a vanilloid receptor, which is reactive to temperature and capsaicin (Voilley & Etievant, 2006). Enhancing the flavour of a product plays a very important role in terms of customer acceptance enabling manufacturers to diversify their portfolio, flavour preference and perception varies with different culture (A. J. Taylor & Linforth, 2010). Flavour is an important factor in the seasoning industry and characterising the flavour profile is an important tool used by industries, this makes formulation and sensory testing of seasoning important.

SEASONING

2.1.1 Formulation

The formulation of seasonings from across the world stands as a challenge to the industry, as seasonings should be more palatable to the local consumers and satisfy the sensory consistency with the original version of the food (Handbook of Industrial Seasonings, 1994). It should also consider many factors like the quantity of a particular component in the seasoning, the cost of manufacturing, perception of seasonings on different bases or fillers, appearance, and organoleptic properties. The formulation of seasonings has two major steps, choosing the base and incorporating the flavourings with the base.

Seasoning base plays an important role in the formulation as it is the major ingredient in terms of quantity. The base can have different compositions of the three macro nutrients. MD (a product of starch hydrolysis with a lower dextrose equivalent (Y.-J. Wang & Wang, 2000) dextrose equivalent is the quantification of reducing sugars present in a product) is frequently used as a filler or a bulking agent (Underriner & Hume, 1994) during formulation of seasonings. MD is a preferred filler because of its various benefits like extended shelf-life, reduced nutrient loss, and aids free flow of the seasoning particles with no or minimal granulation (Marcus, 2019).

Other factors such as appearance and cost are also important in the seasoning manufacturing industry. When it comes to appearance, colour of the seasoning(s), is crucial for consumer acceptance. Industries use two ranges of colours, natural colour (example, turmeric, paprika), and artificial colour (example, orange colour of cheese from yellow lake pigment that are the salts of aluminum) (Underriner & Hume, 1994). On the other hand, the cost of seasonings depends on the process used to encapsulate the flavour, shelf-life, and the type of base used. Flavour molecules are vulnerable to oxidation and evaporation, and encapsulation of these flavour molecules will provide better protection and better flavour delivery. Encapsulation has two types, single and multiple core encapsulations. The core of an encapsulated product is a flavour molecule, and the coating is a carrier material. For a carrier material to be effective, it has many requirements like good film-forming properties, low viscosity, effective encapsulation, and emulsifying property (X. Wang et al., 2015). Selection of the right material for encapsulation is crucial for the shelf-life of the product with respect to the flavour release of the seasoning.

In the case of industrial seasonings the flavour encapsulation is carried out by the process of plating. Plating is the coating of flavouring on the base, where the flavour compound is entrapped by binding it in the base's matrix (Reineccius, 2005). For example, starch is used to entrap flavour molecules in the plating process. There are various types of starch materials that can be used for encapsulation depending on the property of the starch material and the flavour compound. In the case of cheese and onion seasoning, onion oil and hydrolyzed starch can be used for the encapsulation of cheese aroma using cheese emulsion (Mongenot et al., 2000). Even though plating is the most popular method of producing seasoning due to its cost-effectiveness, it has certain drawbacks like the loss of flavour upon storage due to evaporation and oxidation on exposure to air resulting in a shorter shelf life (Bolton & Reineccius, 1992).

Coacervation and drying are less commonly used methods of encapsulation in industries. Coacervation is the process used to form dry flavourings or seasonings where two oppositely charged hydrocolloids are dissolved in water, one with flavouring and the other without; the mixture is continuously stirred and maintained at a pH while the third hydrocolloid is added resulting in positively charged particles to attract each other which is then diluted with water and allowed to cool and is hardened using glutaraldehyde to form particles that can flow freely (Reineccius, 2005). This process is not widely used because of its numerous disadvantages like the toxicity of glutaraldehyde, high cost, and low efficiency.

Spray drying and tray drying are more commonly used for encapsulation of flavour compounds amongst other drying techniques. Spray drying is a predominantly used technology in the market due to its low operational costs and better product stability (Madene et al., 2006). Aroma compounds with low boiling point can vaporize during the process and there is a possibility of oxidation of flavour compounds on the surface of the particle (Desobry et al., 1997). There are other methods of encapsulation through drying, like tray drying, where the material is spread in a thin layer on the tray at an angle and the air is blown on the material until its dried, this is done as a batch process while the same is done as a continuous process in tunnel drying.

Particle size distribution is a factor that affects flavour binding and flavour release, (Afoakwa et al., 2009) noted that there was a reduced flavour release with increasing particle size distribution and hypothesized that it maybe because of the structural and texture difference that causes higher retention of the flavour compound.

Scanning electron microscopy (SEM) can be used for various measurements like structure, topography, particle size, and chemical composition of particles (Henini, 2000). Ideal samples for SEM should be conductive in nature but, when the sample are food ingredients, it requires some preparation before usage. Environmental scanning electron microscope is suitable for food samples as they usually contain moisture and has minimal sample preparation (Morris & Groves, 2013).

2.2 AROMA COMPOUNDS

Every food has its own aroma profile and similarly, different types of cheese have different aroma compounds. For example, cheddar cheese has acetic acid, butyric acid, ethyl butyrate, acetoin, and methional compounds giving the green, cheese, fruity, sour milk, and baked potato odour respectively (Fox et al., 2016). The Solid Phase Micro Extraction Gas Chromatography Mass Spectrometry (SPME GC-MS) technique can be used for the headspace analysis of aroma

compounds as the fiber used is highly effective in absorbing volatiles in the headspace (Gioacchini et al., 2010).

The aroma of cheese is complex with sweet, buttery, chocolaty, meat-like, fruity, and can be more complex depending on various factors like maturation period, type of cheese, type of milk used and microorganism activity. While the fruity, peachy, and buttery aroma is from ketone-based compounds like 3-hydroxy-2-butanone, 2,3-butanedione, δ-dodecalactone, the lactone-based compounds (γ-decalactone, γ-nonalactone, δ-octalactone) gives a coconutty aroma (Whetstine et al., 2005). Acids like acetic acid or butanoic acid produce a sour or rancid vinegar like odour, acids like isovaleric acid or phenylacetic acid produce a sweet aroma (J. Wang et al., 2021). Aldehyde and sulfide containing compounds help with the complexity of the aroma profile like (E)-2-nonenal with its old books like smell, 2-methylbutanal having a hint of dark chocolate smell, and dimethyl trisulfide producing cabbage like odour (Whetstine et al., 2005). Alcohols give a range of odour in case of cheese like 2-phenethanol with rose aroma, 2-methoxyphenol with burnt odour, and 2-methyl-3-furanthiol with notes of brothy odour (Whetstine et al., 2005) although alcohol usually has a more pleasant aroma. The other types of compounds found in cheese aroma profile were acetals and carboxylic acid groups. Acetals are usually formed by the reaction of alcohol and glycol with aldehyde in the presence of anhydrous acid, and which are considered to add unpleasant odour to the product, it also replaces the more desirable aldehydes (Reineccius, 2005). Whereas short chain carboxylic acid groups have a strong pungent odour, mid chains with carbon number between 4 and 8 have cheesy and buttery notes, and the odour becomes unpleasant beyond chains that have more than 9 carbons (Reineccius, 2005).

There are many types of alcohol, the long chain ones are usually solids with no odour while the short chain and mid chain ones usually have a pleasant aroma (Reineccius, 2005). Aldehydes are very reactive and oxidize easily which is why compounds like benzaldehyde oxidize to form benzoic acid which when added to food gives a bitter note, and higher molecular weight aldehydes usually have a pleasant aroma (Reineccius, 2005). Esters are formed by the reaction of alcohol with acid, these usually have a wide range of odour profile with usually pleasant notes while sulfur containing compounds are particularly important in natural flavours, they have been unidentified until late due to its low sensory threshold (Reineccius, 2005).

Onions usually have a pungent smell, while the aroma of onions vary with factors like Millard's reaction, and the time from when it is cut, the usual compounds found in onions are hydrogen sulfide, methanethiol, propanethiol all having a cabbage like eggy odour, and dipropyl disulfide that has a sulphureous fresh scallions odour (Løkke et al., 2012).

2.2.1 Interaction with the base

Each aroma compound will interact differently with different bases depending on the composition of the base (e.g., carbohydrate, protein or fat), this interaction also varies within a class of base (e.g., chain length of carbohydrate or chemical composition of side groups on a carbohydrate backbone). These interactions can be at a molecular level, or at a higher macroscopic level.

The molecular chemical interactions can be segregated into four types, covalent or irreversible bonding, hydrogen bonding, hydrophobic sp bonding (where s and p stands for the corresponding orbitals), and inclusion complexes (usually found between flavour compounds and a carbohydrate base) (McGorrin & Leland, 1996).

The macroscopic level binding between flavour compounds and the base can be classified into three types. They are, either the adsorption or absorption of the flavour compounds in the solid base, the distribution of flavour compounds between phases, or the availability of the compounds in the headspace (McGorrin & Leland, 1996). The binding of aroma compounds with its base depends on how each aroma compound interact with its base. The interactions of compounds with each base is discussed below.

2.2.1.1 Lipids

Most aroma compounds are hydrophobic, Log K_{OW} or the Octanal-Water coefficient describes the partition of the compound between water and octanal in equilibrium, which increases with the hydrophobicity of the compound, lower is the retention of the polar compounds in the matrix (Naknean & Meenune, 2010). Myristic acid has a high Log K_{OW} value of 6.09 (Janeš et al., 2009) and has a waxy pineapple like aroma, 2,3-butadione has a negative 1.22 Log K_{OW} value and has a buttery note (Table 2). Most aroma compounds are hydrophobic, they tend to have a positive Octanal-Water coefficient.

Lipid's contribution to storing flavour molecules is known and used in various applications (Voilley & Etievant, 2006). Lipids not only carry flavours but also have their own flavour in many cases and for this reason, lipid oxidation is a major problem as this causes certain off-

notes. Furthermore, these oxidised lipids have a low detection threshold in humans so even small quantities can affect the flavour profile significantly (Voilley & Etievant, 2006).

Lipids are considered to be reservoirs for flavour compounds, more so for hydrophobic compounds. And increase in lipid concentration therefore tends to affect the headspace flavour availability of aroma compounds. Hydrophilic compounds will not be affected to a greater extent by this factor (Guichard, 2002). Lipids have been extensively studied and are used as active ingredient carrier in the food industry through microencapsulation (Nahum & Domb, 2021).

2.2.1.2 Protein

Protein can form reversable bonds with aroma compounds through hydrogen bonding (Guichard, 2002) there are a few flavour compounds such as volatile disulfides that can form irreversible bonding with the protein molecules (Adams et al., 2001). Various factors affect protein-flavour interaction like pH (Jouenne & Crouzet, 2000; Mills & Solms, 1984), thermal denaturation (Damodaran & Kinsella, 1980; O'Neill & Kinsella, 1987), addition of salt (Damodaran & Kinsella, 1980), foaming (Phillips et al., 1990), and presence of co-solvents (Damodaran & Kinsella, 1980).

Protein can affect flavour perception as aroma molecules tend to bind with protein (Guichard, 2002). Protein-flavour interaction depends on the type of protein in consideration and the functional group of the flavour compound, certain types of protein like globular protein tends to increase the stability of the system by decreasing the interaction of non-polar compounds with water but this will change with the environmental conditions like temperature or acidity as globular protein will get denatured easily (Voilley & Etievant, 2006).

2.2.1.3 Carbohydrates

Carbohydrates are a significant part of our food and are also used commonly as seasoning bases, and there are four types of carbohydrate molecules: mono, di, oligo, and polysaccharides. Mono and disaccharides like glucose and sucrose are observed to reduce the vapor pressure and hence making the solution more hydrophobic attracting the lyophilic flavour compounds, this is usually observed when simple sugars are added to a matrix, it interacts with water molecules and reduces the water availability for the flavour compounds increasing the flavour concentration in the headspace (Voilley & Etievant, 2006).

Carbohydrates like starch, a polysaccharide, tend to increase the viscosity of the food, the increase in viscosity reduces the diffusivity of flavour molecules and slowly release into the headspace (Naknean & Meenune, 2010).

Starches are also said to have helical structures (amylose in starch has the helical structure) that can bind the flavour molecules (Naknean & Meenune, 2010) and they form inclusion complexes. These complexes are sometimes formed within a few min, while it might take several days for some molecules to form inclusion complexes with starch (Reineccius, 2005). Hydrophobic interaction, London Dispersion forces, and Hydrogen bonds play a role in complex formation. To improve the sensory attributes, ligands like alcohol and esters are trapped in starch matrices where starch forms inclusion complexes with the ligands(Quiroga Ledezma, 2018). The inclusion complexes are important as they affect the flavour perception in food (Conde-Petit et al., 2006), these complexes are formed with ligands that have low solubility with the help of lipids present in the starch base (Tapanapunnitikul et al., 2007).

Starches have different percentages of amylose and amylopectin, the starches with higher concentration of amylose can bind higher quantity of flavour molecules. Therefore explaining why commercially available waxy starches that are entirely amylopectin are less likely to be used as a seasoning base. Factors like texture and the interaction of aroma molecules with amylose (Arvisenet et al., 2002) and the inclusion complex formed by amylose helix using hydrophobic bonding around the aroma compound (Madene et al., 2006) will affect the retention of the aroma compounds and their binding to the starch base.

Retention of aroma compounds by a carbohydrate base depends on various factors like the molecular weight, functional group present, volatility, and polarity of the compound (Goubet et al., 1998). Higher molecular weight flavour compounds have a lower release rate compared to lower molecular weight compounds due to their better capacity to diffuse through the matrix and hence lower molecular weight flavour compounds have a better availability (Naknean & Meenune, 2010). Functional groups also play a role where alcohols, ketones, and aldehydes tend to interact with polysaccharide base better, in that order (Goubet et al., 1998), than other groups.

The method of manufacture of the seasonings play an important role in flavour release, spray drying, and freeze-drying techniques are often used. In these cases, the release of the flavour compounds depend on how quick they can reach the surface due to the carrier’s reduced mobility caused by the encapsulation of the compound and factors such as the pore and the

particle size of the base and the thickness of the layer of base that entraps the flavour molecule (Whorton, 1995). The volatility of the flavour compound has a key role in the polysaccharide flavour interaction. The loss of aroma increases with the increase in volatility of the flavour compound due to higher degree of diffusion from encapsulated molecule (Voilley & Etievant, 2006).

Micro regions formed due to the bonding between polysaccharides during the drying process entraps flavour compounds by reducing its mobility whereas the presence of moisture makes it easier for the hydrophilic flavour compounds to be released(Voilley & Etievant, 2006). Diffusion of aroma compounds affects the flavour availability, and diffusion depends on factors like the size of the flavour compound, the steric hinderance and the molecular weight; Moisture availability increases the mobility of hydrophilic flavour compounds (Goubet et al., 1998).

When there is an increase in the moisture content beyond a certain level, the result is the loss of pores which entraps the aroma molecules and in which case the release depends on the concentration gradient of the penetrant, this phenomenon is known as Fickian diffusion (Goubet et al., 1998). Fickian diffusion is any diffusion that follows Fick's law, which states that flux is proportional to the concentration gradient (Eq. 2.1),

$$F = -D\frac{dC}{dx} \qquad (2.1)$$

Where, 'F' is the flux ($mol.m^{-2}.s^{-1}$), D is the diffusion coefficient ($m^2.s^{-1}$), C is the conc. of the permeating compound ($mol.m^{-3}$), and x is the position coordinate in the direction of the flow (m) (Islam, 2004).

Certain polysaccharides tend to form gels in the presence of water and reduce the free water and thereby trap the flavour molecules resulting in the reduced availability of flavour compounds (Guichard, 1996).

While the chain length of the carbohydrate base affects the flavour interaction, the interaction is proportional to the chain length (Naknean & Meenune, 2010), for flavour molecules of the same carbon number, high polarity of flavour molecules are attracted towards polysaccharides (Bangs & Reineccius, 1982). The length of flavour compounds are also important as the 'Thijssen selective diffusion' theory states that moisture continues to evaporate affecting the volatiles, while longer chain volatiles have a reduced mobility depending on the diffusion (Bhandari, 2013).

The state of the matrix also affects aroma interaction, polysaccharides can be in amorphous or crystalline state, crystalline structures are tightly packed, and in general the absorption of aroma compounds occurs only in amorphous regions (Voilley & Etievant, 2006).

2.2.2 Physical attributes and flavour release

Flavour release during oral processing is dependent on diffusion of flavour compound through the matrix. Mass transfer occurs between the food matrix and air to attain equilibrium and hence nonequilibrium is the driving phenomena of flavour release from the base (A. J. Taylor & Linforth, 2010).

Diffusion increases when the food is chewed in comparison to when the food is sucked on, due to the rapid increase in surface area exposed to saliva during chewing (A. Taylor & Mottram, 1996), this shows that the surface area plays a vital role in flavour release.

There are various other factors that play an important role in the flavour release such as the physiochemical properties of the compound similar to the example in section 2.2.1.1. The relative concentration of a compound between two phases can be described using partition coefficient (equation 2.2) which is given by,

$$\text{Log } K_{ab} = \text{Log}\left(\frac{Conc.in\ a}{Conc.in\ b}\right) \quad (2.2)$$

Where ‘a’ represents solute and ‘b’ represents solvent (Işık et al., 2020).

Henry’s law states that the concentration of a compound in the headspace can be predicted under equilibrium and infinite dilution conditions,

$$H = \frac{Conc.in\ gas}{Conc.in\ solvent} \quad (2.3)$$

Where ‘H’ is henry’s law constant (Gascons Viladomat et al., 2006).

The availability in headspace depends on the hydrophobicity of the compound, the amount of lipids present in the solvent, and the volatility of the compound. The Octanol-water partition coefficient (equation 2.4) can help us understand the availability of a compound in the headspace as it determines the hydrophobicity of a compound,

$$\text{Log } K_{ow} = \text{Log } \left(\frac{Conc.\ of\ the\ compound\ in\ octanol}{Conc.of\ the\ compound\ in\ water}\right) \text{ (Fisk, 2015).} \quad (2.4)$$

2.3 SUMMARY

Whilst the chemistry of aroma compounds and carbohydrates are well understood, little is known about how these interact and impact aroma release in a complex seasoning product. Seasonings were therefore prepared by plating aroma compounds on bases of different physio-chemical properties. Volatile aroma compounds were evaluated by SPME GC-MS and GC-O and seasoning powders were evaluated by SEM, and the physio-chemical data of the aroma compounds predicted using EPI suite. The role of the seasoning (base chemistry and particle size) and type of aroma compound (physio-chemical properties) on flavour release was therefore explained.

3 MATERIALS AND METHODS

3.1 MATERIALS

3-heptanone (98%, Acros organics, Geel, Belgium) in methanol (>99.99%, Sigma-Aldrich, Missouri, United States). Alkane standard (C_7 – C_{30}, Sigma-Aldrich, Missouri, United States). The internal standard (I.S.) was 0.001% (v/v) 3-heptanone in methanol. The Cheese and Onion flavours (date of order: 26th July 2021, brand name: Flavour Factory) and the bases, Corn Flour (CF, date of order: 2nd September 2021, brand name: Balsara's), Corn Starch (CS, date of order: 2nd September 2021, brand name: yourhealthstore), Wheat Flour (WF, date of order: 2nd September 2021, brand name: Tre Grazie), Wheat Starch (WS, date of order: 2nd September 2021, brand name: Flck), and Maltodextrin (MD, date of order: 2nd September 2021, brand name: Bulk) were bought from Amazon online shopping website.

Table 1: Stepwise description of the processes undertaken in this work.

STEP	PROCESS	DESCRIPTION
1	Seasoning preparation	Seasoning base mixed with flavourings at 10% w/w% concentration and was left for 2 days to equilibrate.
2	SPME GC-MS	The prepared seasonings were analysed for headspace flavour concentration using SPME.
3	Statistics	The data from SPME was analysed using TraceFinder software and statistical significance was found using XLStat software.
4	GC-O-MS	The seasoning samples were analysed also using GC-Odour port to collaborate with the SPME data.

5	SEM	The particles were coated for conductance and were observed under SEM to find the relation of particle size with flavour release.
6	Physio-Chemical data	The Physio-Chemical data was obtained for all the flavour compounds so as to find a relationship between the data and flavour release.

3.2 EVALUATION OF CHEESE AND ONION FLAVOUR PROFILE

The cheese and onion (C&O) flavour profile was detected using GC-MS ISQ^{TM} series Single-Quadrupole, Thermo Fisher Scientific (Hemel Kempstead, UK), and extracted by Solid-Phase Microextraction (SPME) – 50/30 µm DVB/CAR/PDMS SPME fiber (Supelco, Sigma Aldrich, UK). Five different seasoning mixtures were prepared using different bases (CF, CS, WF, WS, and MD). The base was coated with the flavouring liquid, mixed thoroughly, and was left to equilibrate for 48 hours before the analysis. The flavourings were added at a concentration of 10% (w/w%).

3.2.1 GC-MS SPME

All the C&O seasoning mixtures were analyzed using the same method. Seasoning (1 gram) was added to a GC headspace vial (20 ml, 22.5 mm×75.5 mm, Sigma-Aldrich, UK) using a spatula. The analysis conditions were standardised to get the maximum number of peaks in the chromatogram, which was fixed as 1 g seasoning added to a headspace vial and 5 ml of water was added, along with 10 µl internal standard (I.S.) (Caporaso et al., 2018) was added to the vial and was sealed using a 20 mm magnetic crimp cap. The sample was incubated at 50 ℃ for 5 min and was extracted at the same temperature for 30 min using SPME.

The SPME fiber was then desorbed at 250 ℃ with splitless injection for 1 min in the GC with a ZB-WAX column (30 m × 0.25 mm inner diameter × 1 µm thickness, Phenomenex Inc., Macclesfield, UK) with helium as carrier gas at a flow rate of 20 ml/min. The fiber was conditioned for 3 min before extraction at 250 ℃. The GC oven was set at an initial temperature

of 40 ℃ (for 2 min) and increased to 250 ℃ at a rate of 6 ℃/min (and held at 250 ºC for 5 min).

The Mass Spectrometer parameters were set as follows; transfer line temperature being 250 ℃, scanning at 35-300 amu, and ion source at 200 ℃. The background subtraction was done using the results from blank vials.

The chromatogram was analyzed using Trace Finder® (2017 version, Thermo Fischer Scientific, US) software and manually cross-verified using XCalibur Qual browser version 2.2 (Thermo Fischer Scientific, US) for accuracy. During cross-verification the peaks were identified by comparing them with library spectra (NIST 2017) and only included if the probability ≥ 70%. After the peak identification, The Good Scents website was used to filter only the flavour compounds. The results were compiled and quantified to get the flavour profile of cheese and onion in each base by comparing the peak area of I.S. with the to the ion peak area.

The cheese and onion flavouring was separately analyzed using SPME and the obtained results were used to eliminate any flavour that may have occurred in the result chromatogram of seasonings due to the flavours in the base.

3.2.2 Statistics

Each sample was run six times to minimize errors in sample preparation and the samples were randomized to remove machine error. The samples were prepared and allowed to equilibrate for a period of two days and the flavouring liquid was separately run using the same method to cross reference and eliminate any flavours from the base.

The concentration of each compound was calculated by comparing the peak area of the compound to the peak area of the internal standard where we already know the concentration.

XLSTAT (Addinsoft, US) software was used to obtain statistical data where Analysis of Variance (ANOVA) was used to compare the significance of the difference in flavour profile after which it was graphically represented using Principal Component Analysis (PCA).

3.2.3 GC-O-MS

The seasoning mixtures were then analysed using GC-O MS, with 1 g of seasoning in the GC headspace vial (20 ml, 22.5 mm×75.5 mm, Sigma-Aldrich, UK), 5 ml of water and 10 µl I.S., and this was sealed using 20 mm magnetic crimp cap.

This was then extracted using a SPME fiber for 30 min at 50 ℃ and was desorbed in the GC ZB-WAX column (30 m × 0.25 mm inner diameter × 1 µm thickness, Phenomenex Inc., Macclesfield, UK) with helium as carrier gas at 250 ℃ with the GC oven was set at an initial temperature of 40 ℃ and increased at a rate of 6 ℃/min to 250 ℃ and was held for 5 min.

The flow rate of 20 ml/min was split in half between the MS and the olfactometry and the analysis was done at the odour port by one panelist and the time, odour, and the duration was recorded using custom built application http://googleplex.biz/gcms-timer/ created at the University of Nottingham International Flavour Research group. This was done to cross verify the results with the results obtained from SPME GC-MS.

3.3 EVALUATION OF SEASONING BASE

3.3.1 Scanning Electron Microscopy

The prepared seasonings were sprinkled on an aluminum base with a layer of carbon tape for conductivity. This was then sprayed with nitrogen gas to remove any loose particles. Since the seasoning is a non-conductive material, it was subsequently coated with gold particles (Rosenberg et al., 1985) using Polaron SC7640 sputter coater (Quorum Technologies, UK) at 16 mA after bringing it to 8×10^{-2} mbar vacuum pressure and flushing with argon gas to remove any loose particles.

The prepared samples were kept under XL30 series SEM and was imaged at 20, 100, and 200 µm to analyze the particles. Multiple images were captured for measuring the particle size.

3.4 PHYSICOCHEMICAL DATA

The physicochemical data (octanol-water partition coefficient Log K_{OW}, Melting point, Boiling point, and Vapor pressure) for each flavour compound found was obtained using the EPI Suite software (US Environmental Protection Agency). The software uses many models to evaluate the physico-chemical properties like MPBPWIN™ for the melting point, boiling point, and the vapour pressure and KOWWIN™ for the octanal-water partition coefficient (Card et al., 2017).

4 RESULTS AND DISCUSSIONS

In this thesis, we discuss the topical application of seasonings, specifically cheese and onion seasoning that is applied to bases used in crisp manufacturing. Firstly, we discuss the flavour profile (flavour isolates) of the cheese and onion seasonings incorporated into the bases CF, WF, CS, WS, and MD along with their physiochemical properties. Secondly, we discuss the physical properties of the bases and relate it to the physiochemical profile of the aroma compounds present in the seasonings, which sets the stage to uncover an efficient base for improved flavour profile and retention.

4.1 CHARACTERISATION OF FLAVOUR ISOLATES IN CHEESE AND ONION

Aroma compounds were identified within the samples using SPME GC-MS. Nineteen aroma compounds were found in the headspace of the seasonings. These compounds were grouped into 8 classes: alcohols, aldehydes, acetals, esters, acids, acetates, ketones, and sulfides (Table 2). The olfactory properties are described using literature references in Table 2.

Table 2 gives us the various flavour compounds present in the seasonings with their physiochemical properties, odour compounds are very important in the flavour quality of cheese, most of the flavour compounds found in cheese occur during the ripening of the cheese. The alcohols (3,5,5-trimethyl-1-Hexanol**,** (Z)-3-Hexen-1-ol, and Benzyl alcohol), acids (Butanoic acid), sulphur containing compounds (dipropyl disulfide), aldehydes (Benzaldehyde), ester (Acetic acid heptyl ester, acetic acid hexyl ester, Butanoic acid 3-methylbutyl ester, butanoic acid butyl ester, butanoic acid ethyl ester, butanoic acid propyl ester, heptanoic acid ethyl ester) acetate (Ethyl Acetate), acetal (1,3-Dioxolane, 2,2,4-trimethyl-), and ketones (2,3-Butanedione, .alpha.-Pinene) present in the cheese and onion seasoning are shown in Table 2 and has various formation mechanisms. The alcohols, aldehydes, and acids are formed during the catabolism of free amino acids in cheese and during lipolysis of triglycerides (Andiç et al., 2015), although we should note that the aldehydes formed from triglycerides are only straight chained but the aldehyde found in the C&O flavour profile was a cyclic aldehyde Aldehydes are scarcely found here because they tend to convert into acids and alcohol almost immediately (Gioacchini et al., 2010). Cyclic aldehyde with an aroma of burnt sugar (Table 2) suggests that this particular compound contributes to the onion flavour profile of the seasoning.

Table 2. Physio-chemical properties and functional group description of the flavour compounds in the seasoning

	Compound name (CAS number)	Log K(ow)	M.Pt. (°C)	B.Pt. (°C)	V.P. (mmHg)	I.D.	Odour	Functional group
1	**1-Hexanol, 3,5,5-trimethyl- (3452-97-9)**	**3.11**	**N/A**	**194**	**0.3**	**MS**	**Oily, herbal** (Lee et al., 2008)	**Alcohol**
2	**3-Hexen-1-ol, (Z)- (928-96-1)**	**1.61**	**N/A**	**156.5**	**N/A**	**MS**	**green, grassy** (Zhu & Xiao, 2019)	**Alcohol**
3	**Benzyl alcohol (100-51-6)**	**1.08**	**-15.2**	**205.3**	**0.094**	**MS**	**sweet, flower** (Kashima & Miyazawa, 2014)	**Alcohol**
4	**Benzaldehyde (100-52-7)**	**1.71**	**-26**	**179**	**1.27**	**MS**	**almond, burnt sugar** (Kashima & Miyazawa, 2014)	**Aldehyde**
5	**Acetic acid, heptyl ester (112-06-1)**	**3.32**	**-50.2**	**193**	**N/A**	**MS**	**green, herbaceous** (*Heptyl Acetate | C9H18O2 - PubChem*, n.d.)	**Ester**
6	**Acetic acid, hexyl ester (142-92-7)**	**2.83**	**-80.9**	**171.5**	**1.32**	**MS**	**Fruity, floral** (Fan & Qian, 2005)	**Ester**
7	**Butanoic acid, 3-methylbutyl ester (106-27-4)**	**3.25**	**-73.2**	**179**	**0.95**	**MS**	**fruity, floral, acid** (Jordán et al., 2001)	**Ester**

8	**Butanoic acid, butyl ester (109-21-7)**	**2.83**	**-91.5**	**166**	**1.81**	**MS**	**Fruity, pineapple** (Moio & Addeo, 1998)	**Ester**
9	**Butanoic acid, ethyl ester (105-54-4)**	**1.85**	**-98**	**121.5**	**12.8**	**MS, GCO**	**sweet, fruity, apple like, banana** (Moio & Addeo, 1998)	**Ester**
10	**Butanoic acid, propyl ester (105-66-8)**	**2.34**	**-95.2**	**143**	**5.95**	**MS**	**sweet, rancid, sharp** (Moio & Addeo, 1998)	**Ester**
11	**Heptanoic acid, ethyl ester (106-30-9)**	**3.32**	**-66.1**	**187**	**0.68**	**MS**	**Fruity, wine like, brandy** (Moio & Addeo, 1998)	**Ester**
12	**Butanoic acid (107-92-6)**	**1.07**	**-5.7**	**163.7**	**1.65**	**MS**	**cheesy, rancid** (Fan & Qian, 2005)	**Organic acid**
13	**Ethyl Acetate (141-78-6)**	**0.86**	**-83.6**	**77.1**	**93.2**	**MS**	**Pineapple** (Fan & Qian, 2005)	**Acetate**
14	**Disulfide, dipropyl (629-19-6)**	**3.84**	**-85.6**	**193.5**	**0.512**	**MS**	**Strong raw onion** (Studsgaard Nielsen & Poll, 2004)	**Sulfur containing**
15	**1,3-Dioxolane, 2,2,4-trimethyl- (1193-11-9)**	**1.69**	**-37.36**	**121.4**	**17.5**	**MS, GCO**	**Mild, musty, sweet** (*2,2,4-Trimethyl-1,3-Dioxolane | C6H12O2 - PubChem*, n.d.)	**Acetal**

16	**1,3-Dioxolane, 4-methyl-2-phenyl- (2568-25-4)**	**1.74**	**21.18**	**240.62**	**0.0448**	**MS**	**Almond-like** (*4-Methyl-2-Phenyl-1,3-Dioxolane | C10H12O2 - PubChem*, n.d.)	**Acetal**
17	**2,3-Butanedione (431-03-8)**	**-1.34**	**- 88.2**		**56.8**	**MS, GCO**	**creamy, buttery** (Roberts & Acree, 1996)	**α-diketone**
18	**α-Pinene (80-56-8)**	**4.27**	**-64**	**155.9**	**4.75**	**MS**	**Piney, woody** (Lehtinen & Veijanen, 2011)	**Cyclic Ketone**
19	**p-Cymene (99-87-6)**	**4**	**-68.9**	**177.1**	**1.46**	**MS**	**Lemon, spicy, grapefruit** (Hu et al., 2011)	**Hydroxyl**

Aroma compounds found in the seasonings using SPME GC-MS and its CAS are listed along with their physicochemical properties (vapour pressure, boiling point, melting point, and octanol-water coefficient) obtained from US EPI Suite, organoleptic properties obtained from literature, and the functional group present in the compounds. MS stands for mass spectroscopy and GCO for gas chromatography Olfactometry, being the ways of identification used in the work. Log $K_{(ow)}$ (Octanal-water coefficient), M.Pt. (Melting point), B.Pt. (Boiling point), V.P. (Vapour pressure), I.D. (Identification method).

The sulphur compound is formed during the amino acid catabolism and (Andiç et al., 2015) while straight chain acid is formed by enzyme lipolytic activity during the ripening stage (Curioni & Bosset, 2002). Aldehyde, ketone, sulphur containing compounds are formed during the maillard reaction in onions (Villière et al., 2015)

Ester compounds are usually found in abundance in cheese, which are known for their fruity odour (Moio & Addeo, 1998). Butanoic acid, acetic acid, and heptyl ester are likely to contribute to the cheesy, buttery, and creamy notes of the cheese and onion flavour profile. Disulfide and dipropyl are likely to contribute to the onion like sulfurous odour which is produced from the sulfoxide, 1-prencso, when the tissue is disrupted (Eady et al., 2008). The amount of each compound bound by a base varies based on many factors like the compound's physio-chemical properties.

4.1.1 Comparison of bases

The concentration of compounds perceived in MD is significantly higher than for the same compounds in other bases. Starches have a wide range of compounds released when compared to flour as shown in Fig. 2, and it was observed that the concentration of the compounds in the headspace of starches were found to be much less than for the same compounds in flour. This difference between starches and flours maybe explained by their difference in lipid content and lipid oxidation as suggested by Pico et al., 2017. The hydrophobicity of the compounds play an important role in the availability in the bases, for example, the most hydrophobic compound (α-pinene) was found only in MD base suggesting that the hydrophobic domains in base may have bound the available α-pinene, the same effect was noticed in MD previously by Siccama et al., 2021 where they studied the effect of MD on 1-octen-3-ol which has a Log K_{ow} value of 2.5. Other factors like porosity and degree of integrity of particle is said to have an influence on the binding of compounds (Rosenberg et al., 1985) which will be discussed below.

4.2 PHYSICAL CHARACTERISATION OF SEASONING POWDERS

The physical attributes like the smoothness and particle cracks on the particle affect the encapsulation of compounds (Rosenberg et al., 1985) which is why the physical characteristics of the seasoning base is considered important. SEM is a microscopic technique used to characterise and analyse particles in the range of micro and nano meters. In this work the SEM was used to photographically capture the particles in the seasoning base to obtain and compare the particle size of the base.

The seasoning bases were observed under SEM after coating with gold to make the non-conducting particles to make it better conducting under vacuum. The images were obtained at various scales from 20 µm till 200 µm to compare. The images at 100 µm gave the best comparison which is shown in fig. 1(a,b,c,d,e). The particle size of CF, CS, WF, WS, and MD were on an average 650 µm, 38 µm, 122 µm, 66 µm, and 95 µm respectively.

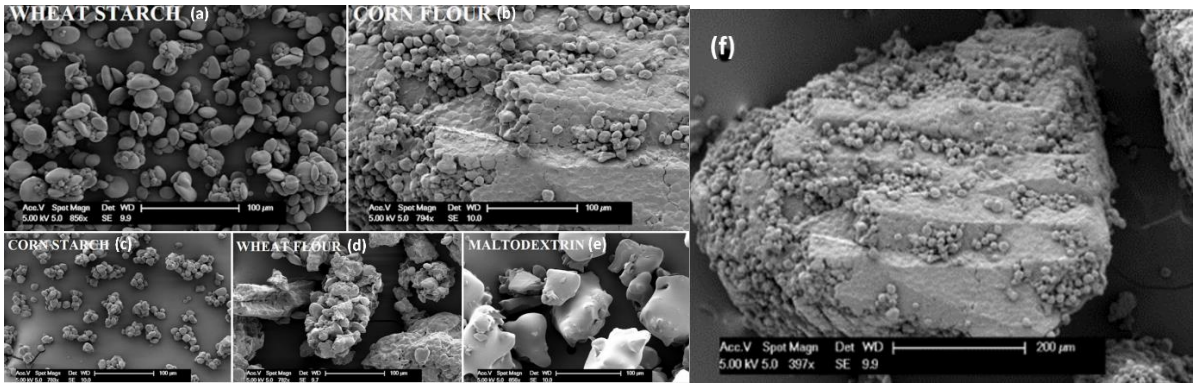


Fig 1. SEM images of seasoning bases, WS (a), CF (b), CS (c), WF (d) and MD (e). Micrograph (f) shows larger field-view of (b).

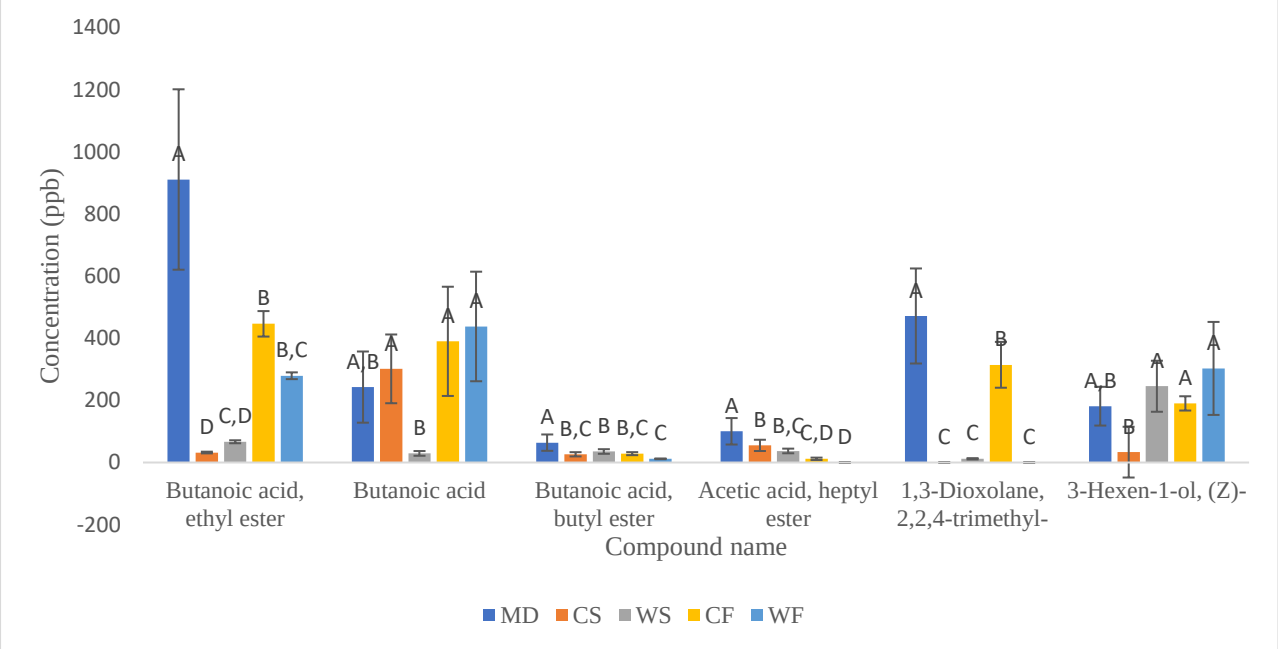
CF particles were the largest out of all with an average particle size of 650 µm and the smallest was CS (38 µm). CF due to its larger particle size was only observed at 200 µm and is shown in Fig 1(f). In the case of WF, the particles seemed to be fused (Fig 1(f)), it is the same in the case of CF (Fig. 1(f)), which may have affected the flavour release and the number of available sites for flavour binding as compared to the other bases which are minimally agglomerated. The results from the SPME analysis discussed in section 4.3 agrees with this as MD has the highest number of flavour compounds in its headspace and the flours with the minimum number of headspace compounds.

4.3 INTERACTION OF AROMA WITH SEASONING BASE

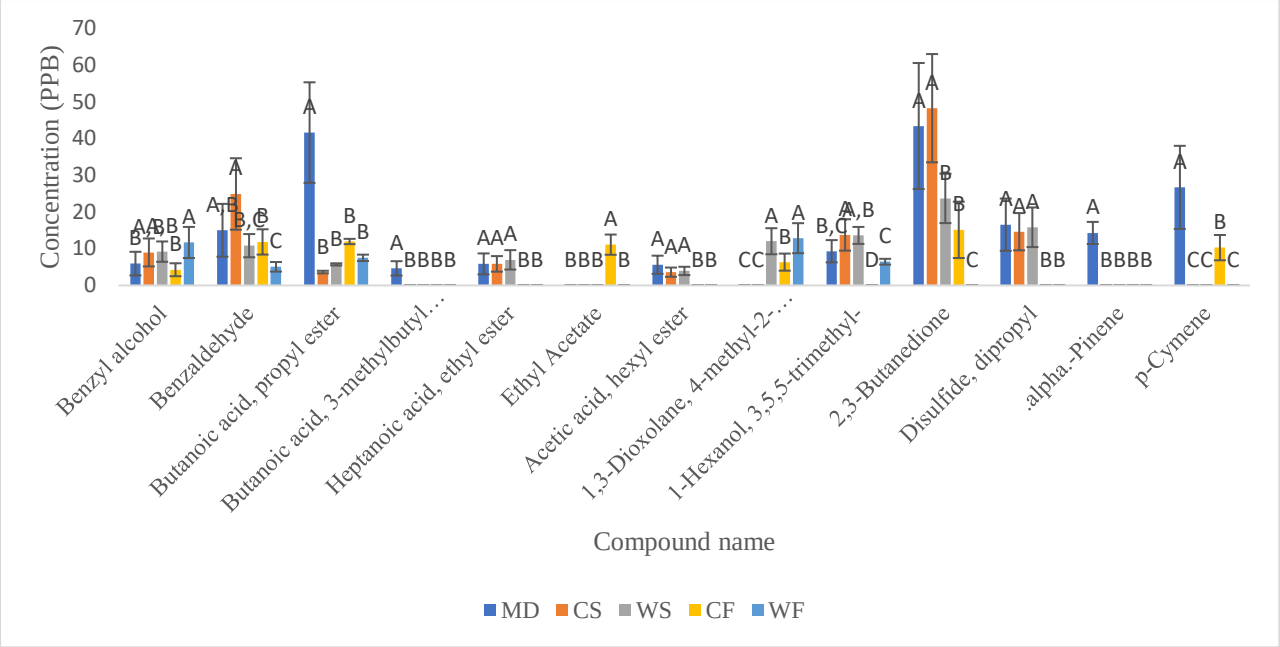
Samples were analysed in six replicates obtaining a total of 19 volatile aroma compounds across all samples where each compound is represented by their concentration in ppb which was obtained from the chromatogram as peak areas.

Ethyl butyrate, Butanoic acid, 3-Hexane-1-ol, and 1,3-Dioxolane, 2,2,4-trimethyl- are all found in high concentrations, all of those corresponds to odours observed in cheese whereas the major compound responsible for onion flavour, Disulfide, dipropyl, is in lower concentration relative to cheese odour (Fig 2). This maybe because sulfur compounds in general have a low odour detection threshold and since the flavouring liquid was commercially manufactured, the manufacturer may have added these compounds in a comparatively lower quantity.

There was a maximum of 17 compounds found in headspace analysis of the seasoning made from MD base, WS had 15 compounds while the CS and CF had an availability of 13 compounds each, WF had the least number (9) of aroma compounds found in the headspace. The concentration of compounds found in the headspace for MD base was higher when compared to the other bases. Both CS and CF has equal number of compounds in its headspace although the compounds present in the headspace were sometimes different.



(a)



(b)

Fig 2. Concentration (ppb) of aroma compounds present in seasoning bases. The codes MD, CS, WS, CF, and WF stand for MD, CS, WS, CF, and WF respectively. Different letters (A,B,C,D) indicate statistical significance ($p<0.05$). Fig 2 (a) represents compounds present in higher concentration while Fig 2 (b) represents compounds present at lower concentrations.

The binding of flavour compounds with same functional group increases with increase in chain length (Kuhn et al., 2006) whereas the effective flavour release of MD also depends on the dextrose equivalent and the surrounding temperature (Buljeta et al., 2021). The presence of a cyclic structure like benzene ring in a compound causes a decrease in binding (Krikunova et al., 2006) as cyclic compounds are thermodynamically more stable than aliphatic compounds. Other changes in structure and molecular weight also affect binding of flavour compound to the base (Krikunova et al., 2006). As seen in the data, in case of benzyl alcohol (aromatic compound) and 3-hexene-1-ol (aliphatic compound), the compound with the benzene ring is significantly less in the headspace of the seasoning than the aliphatic compound (Fig. 2(a), 2(b)). It was observed that higher molecular weight compound (1-hexanol, 3,5,5-trimethyl-) had a similar headspace concentration to that of aromatic ring compound suggesting that molecular weight influences the sorption of chemical compounds.

It is interesting to note that heptanoic acid ethyl ester, acetic acid hexyl ester, 1-hexanol 3,3,5-trimethyl-, dipropyl disulfide are found in CS but not in CF whereas ethyl acetate, 1,3-

Dioxolane 4-methyl-2-phenyl- p-cymene, and 1,3-Dioxolane 2,2,4-trimethyl- were found in CF and not in CS because the compounds found in CS and not in CF have a higher Log K_{ow} value than the compounds found in CF and not in CS which suggests that CS binds hydrophobic compounds better. This is further confirmed when considering the series of butanoic acid esters (ethyl, propyl, and butyl), the higher the Log K_{ow} value, the greater is the concentration of the compound found in starches when compared to flours (Fig. 3).

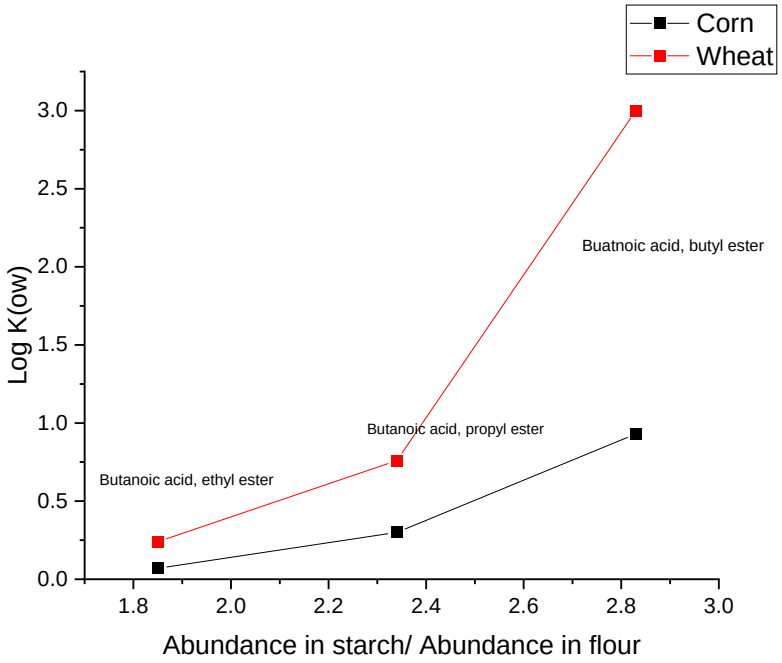


Fig 3. Ratio of abundance of butanoic acid ester compounds in the starch bases when compared to flour bases, for 3 butanoic acid esters with various Log K_{ow}.

The same work should be carried out using waxy starches to understand the effect of amylose or amylopectin in the flavour encapsulation and release, as it contains only trace amounts of amylose which forms nonspecific bonds with the aroma molecules by trapping them in its helical structure around the hydrophobic chain of the flavour molecules (Naknean & Meenune, 2010) while amylopectin is vice-verca.

In all these three compounds discussed in fig. 3, the concentration of the compounds in the headspace of MD based seasoning is significantly higher when compared to the other bases, this can be because of the difference in the macronutrient composition of the bases. While flours have a higher content of proteins and fats than starches and MD, the only significant difference between starches and MD is the degree of hydrolysis. This might also be the reason for MD delivering a higher number of compounds and having a higher concentration of the

compounds in the headspace but, we should also note that in certain cases like Butanoic acid or 3-Hexene 1-ol, (Z)-, WF has higher concentration of the compound.

4.4 ODOUR ACTIVITY OF SEASONING

Table 3. GCO data for the seasonings

Odour Profile	Compound	CAS	MD Retention time (min)	CS Start (min)	WS Start (min)	CF Start (min)	WF Start (min)
onion	Propyl mercaptan	107-03-9	4.738	4.634	4.731	4.893	4.742
sulphur	2-Butanethiol	513-53-1	5.238	5.219		5.296	5.212
Earthy	1,3-Dioxolane, 2,2,4-trimethyl-	1193-11-9	6.94	6.93	6.963	7.005	6.919
Fermented	Ethyl propanoate	105-37-3			7.559	7.585	
cheesy cheddar	2,3-Butanedione	431-03-8	8.083	8.073	8.065		8.072
Fruity	Butanoic acid ethyl ester	105-54-4	9.535	9.465	9.484	9.538	9.463
cheesy	Disulfide, bis(1-methylpropyl)	5943-30-6		21.101	21.171		

Retention time of the odour detected from the GC-O analysis for each seasoning is given in the table. The name and CAS number of the compound corresponding to the odour was obtained using the MS results of the GC-O MS analysis.

Gas-Chromatography-Olfactometry Mass-Spectrometry (GC-O MS) is a method to identify which of the compounds found in the data from SPME are odour active, GC-O also provides a better understanding of the aroma properties and odour threshold.

It is interesting to observe that there are many compounds in the GCO results (Table 3) verified using mass spectrometry data which weren't observed in headspace analysis, this might be because of the change in odour detection threshold between humans and the GC column (Wardencki et al., 2013) which also explains why a few flavour compounds in onion, even though low in concentration, has better odour perception. The odour threshold (OT) of these compounds are very low, the OT of propyl mercaptan is 0.00075 ppm (Wilby, 1969), for 2-Butanethiol is 0.00062 ppm (Wilby, 1969), for ethyl propanoate is 0.01 ppm (Niu et al., 2019)

which means they have strong odour activity and are easily perceived. These values suggest that there are no significant changes in the number of odour compounds from each base although there could have been a change in intensity of the flavour compound which can be measured using GCO although this wasn't possible here because of the lack of replicates or expert sensory panel needed for intensity measurement.

4.5 BASE CHARACTERISTICS AND FLAVOUR RELEASE

The properties of the base like its shape and morphology will affect the flavour release and as we can see in section 4.2, the bases have very different structures. Agglomeration causes flavour loss in carriers (Buffo et al., 2002), it is noted that flour samples being the most agglomerated carriers, correspondingly have a very high flavour loss. WF not only being agglomerated but also has a lot of dents and cracks while CF particles are agglomerated but relatively smooth (Fig. 1). MD, CS and WS samples are smooth and close to a spherical structure. CS and WS seem to be fused with minimal agglomeration. The authors of Rosenberg et al., 1985, states that the binding of compounds is better with a smooth and spherical surface particle, this may explain why MD has the highest number of compounds in the headspace when compared to the other bases.

It must also be noted that while WF, CF, and CS are complex carbohydrates, MD is obtained from starch through the process of hydrolysis, changing the macronutrient composition of the base, which will affect the interaction of compounds with the base as each of protein, starch, and fats react differently with different compounds (Voilley & Etievant, 2006). Please note, that proteins are composed of amino acids that contain positively charged amino groups and negatively charged carboxyl groups. Due to these charged regions, most proteins can function as buffers by binding hydrogen and hydroxyl ions. When water is added to flour bases, the proteins in the flour can alter the pH of the solution and act as buffers. Ideally, the more protein present in the flour, the higher its buffering capacity (Imm et al., 2018). Therefore, increasing the protein content of the flour base can enhance its ability to resist changes in pH when water is added. Saliva acts as a buffer during oral food processing and the buffering effect of saliva influences the sensory perception of certain flavour compounds (Zhang et al., 2022) and hence the buffering effect of protein in flours could be the reason for the lower number of flavour compounds released by flours. Although the change in pH from 2 to 5 did not have any significant effect on the headspace concentration of the flavour compounds (Hansson et al., 2001) suggesting that change in pH at very acidic environments per se does not influence the

flavour release of compounds. Further evaluation might provide detailed information on the effects of proteins and pH on partitioning but is beyond the scope of this work.

4.6 Overview

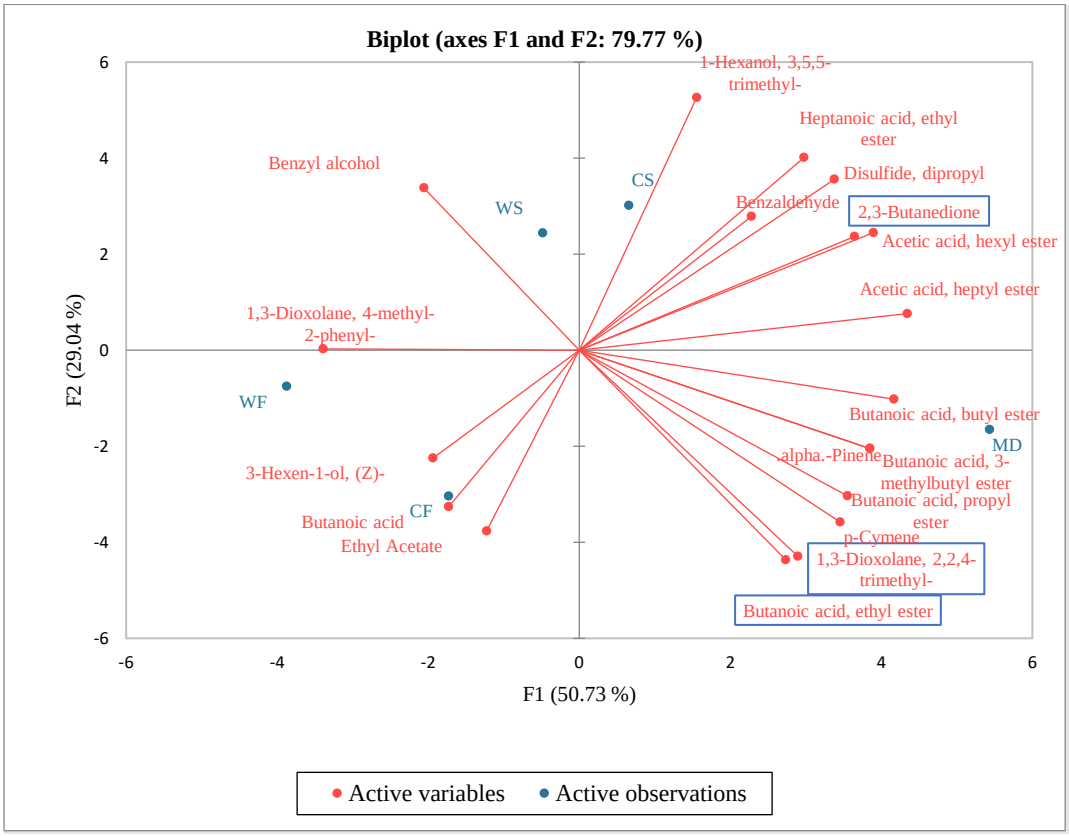


Fig 4. PCA overview of SPME GC-MS data, blue points represent the carrier base, red lines refer to the flavour compounds. WS (WS), CS (CS), WF (WF), CF (CF), and MD (MD).

The principal component analysis (PCA) is a statistical approach that is used here to interpret the distribution of flavour compounds. The overview of the data of each compound and its relative abundance is plotted using PCA in XLStat software. To obtain an overview of the presence of the volatile aroma compounds in the bases, PCA was performed.

The results obtained from the PCA indicate the greater number of compounds available in MD with the number of compounds clustered near MD. It is also evident that WF remains the base with least number of compounds in its headspace as well as that the concentration of compounds present is also low when compared to other bases, and the only prominent

compound in WS is Benzyl alcohol. It is clearly shown that even though CS and flour has equal number of compounds they are opposite to each other in the PCA suggesting that the compounds present in both of them are quite different.

This result is consistent with the SPME GC-MS data presented earlier in the results. Based on the PCA data along with the previous SEM and SPME results, MD should be considered for future works.

4.7 FUTURE WORK

The discussion above proves that the physical properties of the particle play an important role in flavour sorption and thereby making the work with SEM important. The reproducibility of data across a greater number of panelists could not be accomplished which should be done in further studies conducted. More work is needed with different particle size for the same base to observe the changes in flavour release for better understanding. The different dextrose equivalent of MD along with different types of starches (porous, modified starched, and waxy starches) should be also considered. It will also be interesting to consider the presence and the diameter of the helical structure of amylose present in the starch and their influence in affecting the headspace flavour availability.

5 CONCLUSION

This study compared five seasoning bases for effective flavour release, and it was found that MD had a higher flavour release than any of the other bases considered. It was found to be because of its relatively smooth and spherical particles in comparison to the other samples considered with minimum to no agglomeration of particles.

The physiochemical properties of the aroma compounds found in the seasoning through GC-MS headspace analysis were obtained using EPI suite and was compared. The comparison provided the relation between the hydrophobicity of the chemical compound and the carrier, the extent of this variation between starches and flours were established using an example of butanoic acid-based esters.

The key flavour compounds found in the bases from the flavouring liquid were established and the statistical significance was found in the flavour release between the bases for the same compound, and across various compounds. To find the reasoning for the difference in flavour release the physiochemical data and the particle size data from SEM were used, this work provided us with a strong reasoning for the maximum number of flavour compounds and high flavour concentration in the headspace of MD-based seasoning. Further studies considering the different dextrose equivalent MD, similar particle sizes across all the bases to study the effect of particle size on flavour release, different types of starches and flours with varying macro nutrient content and many other factors needs to be performed to establish a better relation between the flavour release and the bases. This study has a huge scope and high industrial application with a lot of further work to be done for better understanding.

6 APPENDIX

ImageJ software was used to measure the particle size (Fig 5), where the scale is set using the reference scale in the image and particles are manually selected and the length is measured using the software and the average is used as the particle size.

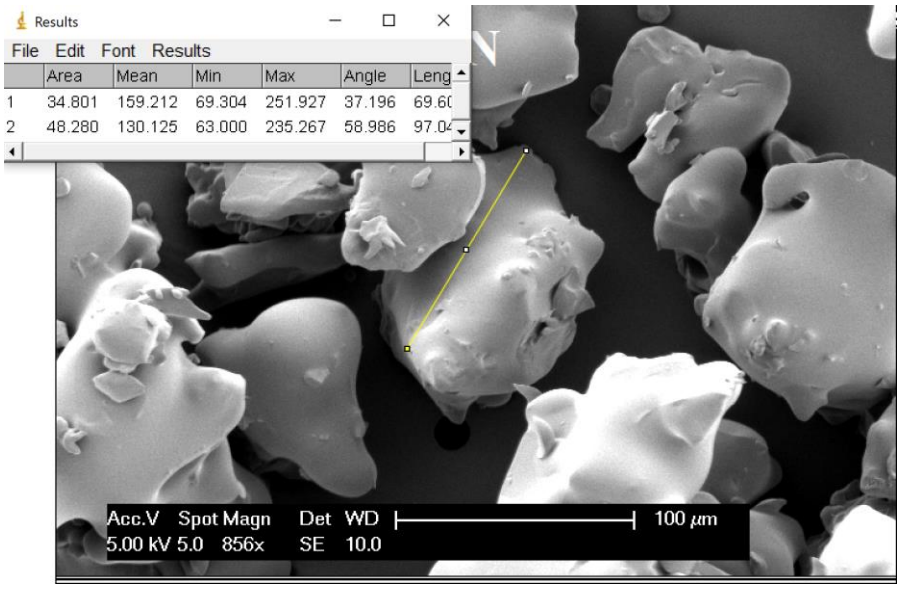


Fig 5. An example of how the ImageJ was used to measure the particle size shown using SEM image of MD.

7 REFERENCE

2,2,4-Trimethyl-1,3-dioxolane | C6H12O2 - PubChem. (n.d.). Retrieved February 17, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/2_2_4-Trimethyl-1_3-dioxolane

4-Methyl-2-phenyl-1,3-dioxolane | C10H12O2 - PubChem. (n.d.). Retrieved February 17, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/4-Methyl-2-phenyl-1_3-dioxolane

Adams, R. L., Mottram, D. S., Parker, J. K., & Brown, H. M. (2001). Flavor−Protein Binding: Disulfide Interchange Reactions between Ovalbumin and Volatile Disulfides. *Journal of Agricultural and Food Chemistry*, *49*(9), 4333–4336. https://doi.org/10.1021/JF0100797

Afoakwa, E. O., Paterson, A., Fowler, M., & Ryan, A. (2009). Matrix effects on flavour volatiles release in dark chocolates varying in particle size distribution and fat content using GC–mass spectrometry and GC–olfactometry. *Food Chemistry*, *113*(1), 208–215. https://doi.org/10.1016/J.FOODCHEM.2008.07.088

Andiç, S., Tunçtürk, Y., & Boran, G. (2015). Changes in Volatile Compounds of Cheese. *Processing and Impact on Active Components in Food*, 231–239. https://doi.org/10.1016/B978-0-12-404699-3.00028-7

Arvisenet, G., Le Bail, P., Voilley, A., & Cayot, N. (2002). Influence of Physicochemical Interactions between Amylose and Aroma Compounds on the Retention of Aroma in Food-like Matrices. *Journal of Agricultural and Food Chemistry*, *50*(24), 7088–7093. https://doi.org/10.1021/JF0203601

Bangs, W. E., & Reineccius, G. A. (1982). Influence of Dryer Infeed Matrices on the Retention of Volatile Flavor Compounds During Spray Drying. *Journal of Food Science*, *47*(1), 254–259. https://doi.org/10.1111/J.1365-2621.1982.TB11072.X

Bhandari, B. (2013). Introduction to food powders. In *Handbook of Food Powders: Processes and Properties* (pp. 1–25). Elsevier Inc. https://doi.org/10.1533/9780857098672.1

Bolton, T. A., & Reineccius, G. A. (1992). The Oxidative Stability and Retention of a Limonene-Based Model Flavor Plated on Amorphous Silica and Other Selected Carriers. *Perfume & Flavourists*, *17*(2).

Boughter, J. D., & Munger, S. D. (2013). Taste Receptors. In *Encyclopedia of Biological Chemistry: Second Edition* (pp. 366–368). Elsevier Inc. https://doi.org/10.1016/B978-0-12-378630-2.00397-2

Buffo, R. A., Probst, K., Zehentbauer, G., Luo, Z., & Reineccius, G. A. (2002). Effects of agglomeration on the properties of spray-dried encapsulated flavours. *Flavour and Fragrance Journal*, *17*(4), 292–299. https://doi.org/10.1002/FFJ.1098

Buljeta, I., Pichler, A., Ivić, I., Šimunović, J., & Kopjar, M. (2021). Encapsulation of Fruit Flavor Compounds through Interaction with Polysaccharides. *Molecules 2021, Vol. 26, Page 4207*, *26*(14), 4207. https://doi.org/10.3390/MOLECULES26144207

Caporaso, N., Whitworth, M. B., Cui, C., & Fisk, I. D. (2018). Variability of single bean coffee volatile compounds of Arabica and robusta roasted coffees analysed by SPME-GC-MS. *Food Research International*, *108*, 628–640. https://doi.org/10.1016/J.FOODRES.2018.03.077

Card, M. L., Gomez-Alvarez, V., Lee, W.-H., Lynch, D. G., Orentas, N. S., Lee, M. T., Wong, E. M., & Boethling, R. S. (2017). *History of EPI Suite™ and future perspectives on chemical property estimation in US Toxic Substances Control Act new chemical risk assessments*. https://doi.org/10.1039/c7em00064b

Conde-Petit, B., Escher, F., & Nuessli, J. (2006). Structural features of starch-flavor complexation in food model systems. *Trends in Food Science & Technology*, *17*(5), 227–235. https://doi.org/10.1016/J.TIFS.2005.11.007

Curioni, P. M. G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. *International Dairy Journal*, *12*(12), 959–984. https://doi.org/10.1016/S0958-6946(02)00124-3

Damodaran, S., & Kinsella, J. E. (1980). Stabilization of Proteins by Solvents. Effects of pH and anions on the positive cooperativity of 2-Nonanone binding to Bovine serum Albumin. *Journal of Biological Chemistry*, *255*(18), 8503–8508. https://doi.org/10.1016/S0021-9258(18)43525-9

Desobry, S. A., Netto, F. M., & Labuza, T. P. (1997). Comparison of spray-drying, drum-drying and freeze-drying for β-carotene encapsulation and preservation. *Journal of Food Science*, *62*(6), 1158–1162. https://doi.org/10.1111/J.1365-2621.1997.TB12235.X

Eady, C. C., Kamoi, T., Kato, M., Porter, N. G., Davis, S., Shaw, M., Kamoi, A., & Imai, S. (2008). *Silencing Onion Lachrymatory Factor Synthase Causes a Significant Change in the Sulfur Secondary Metabolite Profile 1[W][OA]*. https://doi.org/10.1104/pp.108.123273

Fan, W., & Qian, M. C. (2005). Headspace Solid Phase Microextraction and Gas Chromatography−Olfactometry Dilution Analysis of Young and Aged Chinese "Yanghe Daqu" Liquors. *Journal of Agricultural and Food Chemistry*, *53*(20), 7931–7938. https://doi.org/10.1021/JF051011K

Fisk, I. D. (2015). Aroma release. In *Flavour Development, Analysis and Perception in Food and Beverages* (pp. 105–123). Elsevier. https://doi.org/10.1016/b978-1-78242-103-0.00006-0

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2016). Fundamentals of cheese science, second edition. In *Fundamentals of Cheese Science, Second Edition*. Springer US. https://doi.org/10.1007/978-1-4899-7681-9

Gascons Viladomat, F., Souchon, I., Athès, V., & Marin, M. (2006). Membrane air-stripping of aroma compounds. *Journal of Membrane Science*, *277*(1–2), 129–136. https://doi.org/10.1016/J.MEMSCI.2005.10.023

Gioacchini, A. M., De Santi, M., Guescini, M., Brandi, G., & Stocchi, V. (2010). Characterization of the volatile organic compounds of Italian 'Fossa' cheese by solid-

phase microextraction gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry*, *24*(23), 3405–3412. https://doi.org/10.1002/RCM.4782

Goubet, I., Le Quere, J. L., & Voilley, A. J. (1998). Retention of Aroma Compounds by Carbohydrates: Influence of Their Physicochemical Characteristics and of Their Physical State. A Review. *Journal of Agricultural and Food Chemistry*, *46*(5), 1981–1990. https://doi.org/10.1021/JF970709Y

Guichard, E. (1996). Interactions between Pectins and Flavor Compounds in Strawberry Jam. *ACS Symposium Series*, *633*, 118–129. https://doi.org/10.1021/BK-1996-0633.CH011

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International*, *18*(1), 49–70. https://doi.org/10.1081/FRI-120003417

Handbook of Industrial Seasonings. (1994). In E. W. Underriner & I. R. Hume (Eds.), *Handbook of Industrial Seasonings*. Springer US. https://doi.org/10.1007/978-1-4615-2141-9

Hansson, A., Pehrson, K., & Andersson, J. (2001). Effect of changes in pH on the release of flavour compounds from a soft drink-related model system. *Food Chemistry*, *74*(4), 429–435. https://doi.org/10.1016/S0308-8146(01)00158-3

Henini, M. (2000). Scanning electron microscopy: an introduction. *III-Vs Review*, *13*(4), 40–44. https://doi.org/10.1016/S0961-1290(00)80006-X

Heptyl acetate | C9H18O2 - PubChem. (n.d.). Retrieved February 17, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/Heptyl-acetate#section=Physical-Description

Hu, L., Wang, Y., Du, M., & Zhang, J. (2011). Characterization of the volatiles and active components in ethanol extracts of fruits of Litsea cubeba (Lour.) by gas chromatography-mass spectrometry (GC-MS) and gas chromatography- olfactometry (GC-O). *Journal of Medicinal Plants Research*, *5*(14), 3298–3303. https://doi.org/10.5897/JMPR.9000333

Imm, J.-Y., Kim, M., & Oh, S. (2018). Buffering Capacity of Dairy Powders and Their Effect on Yoghurt Quality. *Korean Journal for Food Science of Animal Resources Korean J. Food Sci. An*, *38*(2), 2234–2246. https://doi.org/10.5851/kosfa.2018.38.2.273

Işık, M., Bergazin, T. D., Fox, T., Rizzi, A., Chodera, J. D., David, ·, & Mobley, L. (2020). Assessing the accuracy of octanol-water partition coefficient predictions in the SAMPL6 Part II log P Challenge. *Journal of Computer-Aided Molecular Design*, *34*, 335–370. https://doi.org/10.1007/s10822-020-00295-0

Islam, M. A. (2004). Fickian Diffusion Equation-an Unsolved Problem. *Physica Scripta*, *70*, 119.

Janeš, D., Kantar, D., Kreft, S., & Prosen, H. (2009). Identification of buckwheat (Fagopyrum esculentum Moench) aroma compounds with GC-MS. *Food Chemistry*, *112*, 120–124. https://doi.org/10.1016/j.foodchem.2008.05.048

Jordán, M. J., Tandon, K., Shaw, P. E., & Goodner, K. L. (2001). Aromatic Profile of Aqueous Banana Essence and Banana Fruit by Gas Chromatography−Mass Spectrometry (GC-MS) and Gas Chromatography−Olfactometry (GC-O). *Journal of Agricultural and Food Chemistry*, *49*(10), 4813–4817. https://doi.org/10.1021/JF010471K

Jouenne, E., & Crouzet, J. (2000). Effect of pH on retention of aroma compounds by β-lactoglobulin. *Journal of Agricultural and Food Chemistry*, *48*(4), 1273–1277. https://doi.org/10.1021/jf990215w

Kashima, Y., & Miyazawa, M. (2014). Chemical Composition and Aroma Evaluation of Essential Oils from Evolvulus alsinoides L. *Chemistry & Biodiversity*, *11*(3), 396–407. https://doi.org/10.1002/CBDV.201300234

Krikunova, N. I., Terenina, M. B., Ruchkina, E. L., & Misharina, T. A. (2006). Binding of Aromatic Compounds to Cornstarch Polysaccharides. *Applied Biochemistry and Microbiology*, *42*(3), 379–382. https://doi.org/10.1134/S0003683806030203

Kuhn, J., Considine, T., & Singh, H. (2006). Interactions of Milk Proteins and Volatile Flavor Compounds: Implications in the Development of Protein Foods. *Journal of Food Science*, *71*(5), R72–R82. https://doi.org/10.1111/j.1750-3841.2006.00051.x

Lee, G. H., Shin, Y., & Oh, M. J. (2008). Aroma-Active Components of Lycii fructus (kukija). *Journal of Food Science*, *73*(6), C500–C505. https://doi.org/10.1111/J.1750-3841.2008.00851.X

Lehtinen, J., & Veijanen, A. (2011). Odour monitoring by combined TD-GC-MS-sniff technique and dynamic olfactometry at the wastewater treatment plant of low H2S concentration. *Water, Air, and Soil Pollution*, *218*(1–4), 185–196. https://doi.org/10.1007/S11270-010-0634-3/FIGURES/3

Løkke, M. M., Edelenbos, M., Larsen, E., & Feilberg, A. (2012). Investigation of Volatiles Emitted from Freshly Cut Onions (Allium cepa L.) by Real Time Proton-Transfer Reaction-Mass Spectrometry (PTR-MS). *Sensors 2012, Vol. 12, Pages 16060-16076*, *12*(12), 16060–16076. https://doi.org/10.3390/S121216060

Madene, A., Jacquot, M., Scher, J., & Desobry, S. (2006). Review Flavour encapsulation and controlled release-a review. *International Journal of Food Science & Technology*, *41*(1), 1–21. https://doi.org/10.1111/j.1365-2621.2005.00980.x

Marcus, J. B. (2019). Flavor Enhancement Ingredients. *Aging, Nutrition and Taste*, 173–206. https://doi.org/10.1016/B978-0-12-813527-3.00006-5

McGorrin, R. J., & Leland, J. V. (Eds.). (1996). *Flavor-Food Interactions* (Vol. 633). American Chemical Society. https://doi.org/10.1021/BK-1996-0633

Mills, O., & Solms, J. (1984). Interaction of selected flavour compounds with whey proteins. *AGRIS*, *17*(6).

Moio, L., & Addeo, F. (1998). Grana Padano cheese aroma. *Journal of Dairy Research*, *65*, 317–333. https://doi.org/10.1017/S0022029997002768

Mongenot, N., Charrier, S., & Chalier, P. (2000). Effect of ultrasound emulsification on cheese aroma encapsulation by carbohydrates. *Journal of Agricultural and Food Chemistry*, *48*(3), 861–867. https://doi.org/10.1021/jf990494n

Morris, V. J., & Groves, K. (Eds.). (2013). *Food Microstructures -Microscopy, Measurement and modelling* (1st ed.). Woodhead publishing.

Nahum, V., & Domb, A. J. (2021). Recent Developments in Solid Lipid Microparticles for Food Ingredients Delivery. *Foods*, *10*(2), 1–25. https://doi.org/10.3390/FOODS10020400

Naknean, P., & Meenune, M. (2010). Factors affecting retention and release of flavour compounds in food carbohydrates. *International Food Research Journal*, *17*, 23–34.

Niu, Y., Wang, R., Xiao, Z., Zhu, J., Sun, X., & Wang, P. (2019). *Characterization of ester odorants of apple juice by gas chromatography-olfactometry, quantitative measurements, odour threshold, aroma intensity and electronic nose*. https://doi.org/10.1016/j.foodres.2019.01.064

O'Neill, T. E., & Kinsella, J. E. (1987). Flavor Protein Interactions: Characteristics of 2-Nonanone Binding to Isolated Soy Protein Fractions. *Journal of Food Science*, *52*(1), 98–101. https://doi.org/10.1111/J.1365-2621.1987.TB13980.X

Phillips, L. G., Schulman, W., & Kinsella, J. E. (1990). pH and Heat Treatment Effects on Foaming of Whey Protein isolate. *Journal of Food Science*, *55*(4), 1116–1119. https://doi.org/10.1111/J.1365-2621.1990.TB01612.X

Pico, J., Bernal, J. L., & Gómez, M. (2017). Influence of different flours and starches on gluten-free bread aroma. *Journal of Food Science and Technology*, *54*(6), 1433–1441. https://doi.org/10.1007/S13197-017-2562-3/TABLES/4

Quiroga Ledezma, C. C. (2018). Starch Interactions With Native and Added Food Components. *Starch in Food: Structure, Function and Applications: Second Edition*, 769–801. https://doi.org/10.1016/B978-0-08-100868-3.00020-2

Reineccius, G. (2005). Flavor Chemistry and Technology. *Flavor Chemistry and Technology*. https://doi.org/10.1201/9780203485347

Roberts, D. D., & Acree, T. E. (1996). Effects of Heating and Cream Addition on Fresh Raspberry Aroma Using a Retronasal Aroma Simulator and Gas Chromatography Olfactometry. *Journal of Agricultural and Food Chemistry*, *44*(12), 3919–3925. https://doi.org/10.1021/JF950701T

Rosenberg, M., Kopelman, I. J., & Talmon, Y. (1985). A Scanning Electron Microscopy Study of Microencapsulation. *Journal of Food Science*, *50*(1), 139–144. https://doi.org/10.1111/J.1365-2621.1985.TB13295.X

Siccama, J. W., Pegiou, E., Zhang, L., Mumm, R., Hall, R. D., Boom, R. M., & Schutyser, M. A. I. (2021). Maltodextrin improves physical properties and volatile compound retention of spray-dried asparagus concentrate. *LWT*, *142*, 111058. https://doi.org/10.1016/J.LWT.2021.111058

Studsgaard Nielsen, G., & Poll, L. (2004). *Determination of Odor Active Aroma Compounds in Freshly Cut Leek (Allium ampeloprasum Var. Bulga) and in Long-Term Stored Frozen Unblanched and Blanched Leek Slices by Gas Chromatography Olfactometry Analysis*. https://doi.org/10.1021/jf030682k

Tapanapunnitikul, O., Chaiseri, S., Peterson, D. G., & Thompson, D. B. (2007). Water Solubility of Flavor Compounds Influences Formation of Flavor Inclusion Complexes from Dispersed High-Amylose Maize Starch. *Journal of Agricultural and Food Chemistry*, *56*(1), 220–226. https://doi.org/10.1021/JF071619O

Taylor, A. J., & Linforth, R. S. T. (2010). Food Flavour Technology. In A. J. Taylor & R. S. T. Linforth (Eds.), *Food Flavour Technology: Second Edition*. Wiley. https://doi.org/10.1002/9781444317770

Taylor, A., & Mottram, D. (Eds.). (1996). *Flavour science: recent developments*.

Underriner, E. W., & Hume, I. R. (1994). *Handbook of Industrial Seasonings* (E. W. Underriner & I. R. Hume, Eds.). Springer US. https://doi.org/10.1007/978-1-4615-2141-9

Villière, A., Le Roy, S., Fillonneau, C., Guillet, F., Falquerho, H., Boussely, S., & Prost, C. (2015). Evaluation of aroma profile differences between sué, sautéed, and pan-fried onions using an innovative olfactometric approach. *Flavour 2015 4:1*, *4*(1), 1–19. https://doi.org/10.1186/S13411-015-0034-0

Voilley, A., & Etievant, P. (2006). *Flavour in Food* [Book]. Elsevier Science & Technology. https://doi.org/10.1533/9781845691400

Wang, J., Yang, Z. J., Xu, L. Y., Wang, B., Zhang, J. H., Li, B. Z., Cao, Y. P., & Tan, L. (2021). Key aroma compounds identified in Cheddar cheese with different ripening times by aroma extract dilution analysis, odor activity value, aroma recombination, and omission. *Journal of Dairy Science*, *104*(2), 1576–1590. https://doi.org/10.3168/JDS.2020-18757

Wang, X., Yuan, Y., & Yue, T. (2015). The application of starch-based ingredients in flavor encapsulation. *Starch - Stärke*, *67*(3–4), 225–236. https://doi.org/10.1002/star.201400163

Wang, Y.-J., & Wang, L. (2000). Structures and Properties of Commercial Maltodextrins from Corn, Potato, and Rice Starches. *Starch*, *52*(8–9), 296–304.

Wardencki, W., Chmiel, T., & Dymerski, T. (2013). Gas chromatography-olfactometry (GC-O), electronic noses (e-noses) and electronic tongues (e-tongues) for in vivo food flavour measurement. In *Instrumental Assessment of Food Sensory Quality* (pp. 195–229). Elsevier. https://doi.org/10.1533/9780857098856.2.195

Whetstine, M. E. C., Cadwallader, K. R., & Drake, M. A. (2005). Characterization of Aroma Compounds Responsible for the Rosy/Floral Flavor in Cheddar Cheese. *Journal of Agricultural and Food Chemistry*, *53*(8), 3126–3132. https://doi.org/10.1021/JF048278O

Whorton, C. (1995). Factors influencing volatile release from encapsulation matrices and controlled release of food ingredients. *American Chemical Society*, 135–142.

Wilby, F. V. (1969). Variation in Recognition Odor Threshold of a Panel. *Journal of the Air Pollution Control Association*, *19*(2), 96–100. https://doi.org/10.1080/00022470.1969.10466466

Zhang, D., Jiang, H., Chen, J., & Wang, X. (2022). Buffering capacity of saliva influences the perception of acid-related sensory properties. *Food Quality and Preference*, *97*, 104454. https://doi.org/10.1016/J.FOODQUAL.2021.104454

Zhu, J. C., & Xiao, Z. B. (2019). Characterization of the key aroma compounds in peach by gas chromatography–olfactometry, quantitative measurements and sensory analysis. *European Food Research and Technology*, *245*(1), 129–141. https://doi.org/10.1007/S00217-018-3145-X/FIGURES/1