Application of Black Soldier Fly Larvae to Convert Municipal Organic Waste to Value-Added Chemicals

Doctoral Thesis

by

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ABSTRACT

In the PhD thesis, waste remediation via insect "biocatalysis" was investigated, with particular focus on the production of value-added chemicals from fatty oils, and chitin obtained from black soldier fly larvae. The project aimed to combat two concurrent global issues: increasing amounts of municipal organic waste, and issues surrounding the use of unsustainable natural resources. The viability of the process from waste management to complete synthesis of value-added chemicals using BSFL was investigated and created. In the first stage of the project, the process of extracting oils resulting from feeding larvae on specific organic waste diets was examined and optimised. The fatty acid profile of BSFL oil was identified by chromatographic techniques and purified by distillation. Subsequent to isolation of the oil, the next stage of research involved the synthesis of different valuable chemicals. This ranged from biofuels to surfactants, and was completed using BSFL oil; following various organic chemistry and novel chemical lab-based methodology. In the third stage, chitin was extracted from the insect cuticle, and converted to chitosan and its application as a catalyst support tested in the selective synthesis of jasminaldehyde. Within this, the feasibility of switching the traditional demineralisation acid from a mineral acid to an organic one was explored. A review on previous literature research was completed on processes in the syntheses leading to, and including, the production of value-added chemicals, and it is hoped that the research completed herein will be impactful to society in the future.

Keywords: Waste remediation, BSFL, Fatty acid, Surfactant, Chitin, Sustainability

DECLARATION

I declare that the work in this thesis titled "*Application of Black Soldier Fly Larvae to Convert Municipal Organic Waste to Value-Added Chemicals*" has been carried out by me in the Department of Chemical and Biological Engineering at the University of Sheffield. All the material presented for examination is my own work and has been written by me. The information provided by literature research has been accordingly acknowledged in the text and referenced at the end of this document.

ACKNOWLEDGEMENTS

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CONFERENCES AND RESEARCH AWARDS

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CONTENTS

KEYWORDS/ABBREVIATIONS

In Alphabetical Order

% DA	– % Deacetylation (Chitin)
% DD	– % Deacetylation (Chitosan)
(w/v)	– (weight/volume)
A.V.	– Acid Value
ABS	–Alkylbenzene Sulfonate
BO	– Better Origin Ltd
BOP reagent	– Benzotriazol-l-yloxytris(dimethylamino)phosphonium Hexafluorophosphate
BSF	– Black Soldier Fly
BSFL	– Black Soldier Fly Larvae
Ca	– Calcium
CHD	– Coronary Heart Disease
CMC	– Critical Micellar Concentration
CO	– Carbon monoxide
CO_2	– Carbon Dioxide
DCM	– Dichloromethane
DIPEA	– N, N-Diisopropylethylamine
EA	– Elemental analysis
EDS	– Energy Dispersive X-ray Spectroscopy
EI	– Electron Impact Ionisation
EtOAc	– Ethyl acetate
FAME	– Fatty Methyl Ester
FAO	– Food and Agricultural department of UN
FFAs	– Free Fatty Acids
FFB	– Fresh Fruit Bunches
FTIR	– Fourier Transform Infrared (Spectroscopy)
GC -MS	– Gas Chromatography in tandem with Mass Spectrometry
GPC	– Green Principle of Chemistry
H_2SO_4	– Sulphuric Acid
HCl	– Hydrochloric Acid
He	– Helium
IC	– Ion Chromatography

ICP-MS	– Inductively Coupled Plasma – Mass Spectrometry
IUPAC	– International Union of Pure and Applied Chemistry
K	– Potassium
KOH	– Potassium Hydroxide
LAB	– Linear Alkylbenzene Sulfonate
LCA	– Life Cycle Assessment
LCFA	– Long Chain Fatty Acid
$LiAlH_4$	– Lithium Aluminium Hydride
LiCl	– Lithium Chloride
LiOH	– Lithium Hydroxide
M/Z	– Mass to charge ratio
MCFA	– Medium-chain fatty acid
MeOH	– Methanol
MES	–Methyl Ester Sulfonate
$MgSO_4$	– Magnesium sulfate
MS	– Mass Spectrometry
MSW	– Municipal solid waste
Na	– Sodium
$NaBH_4$	– Sodium Borohydride
NaCl	– Sodium Chloride
NaOH	– Sodium Hydroxide
QAC	– Quaternary Ammonium Compound
S.V.	– Saponification Value
SAS	– Sodium alkyl Sulfate
SDS	– Sodium Dodecyl Sulfate
SEM	– Scanning Electron Microscopy
SMP	– Slip Melting Point
SO_3	– Sulphur Trioxide
Spans	– Sorbitan Ester
STP	– Standard Temperature and Pressure
Surfactant	– **Surf**ace-**Act**ive **A**ge**nt**
TGA	– Thermogravimetric analysis
THF	– Tetrahydrofuran

TLC	– Thin-Layer Chromatography
UN	– United Nations
UV-Vis	– Ultra Violet – Visible Spectroscopy
WVOs	– Waste Vegetable Oils
XRD	– X-ray Diffraction

1 INTRODUCTION

Work completed in this thesis focuses on the valorisation of the remediation of waste into chemicals using Black Soldier Fly Larvae (BSFL). Oil collected from the BSFL is a result of the rapid assimilation of municipal organic waste by the insects in their larval instar and proposes a contemporary alternative to oils conventionally sourced from palm and coconut plantations or the petrochemical industry. Moreover, the chitin located in the insect exoskeleton is an alternative source to the global contemporary shellfish-derived sources and its unique properties can be applied in a multitude of industries.

1.1 WASTE REMEDIATION

United Nation (UN) estimates indicate that 1.3 billion tonnes of food waste are generated globally per annum (Gustavsson, Cederberg and Sonesson, 2011). In the UK alone, 10 million tonnes of food and drink waste is disposed to landfill every year, organic waste constituting for over 60 % of all avoidable municipal solid waste (MSW) produced industrially (House of Commons, Environment, 2017). Along with an ever increasing quality of life, there is a greater population size and therefore the management of food waste is becoming a large burden to society and the environment (Czajczyńska *et al.*, 2017; Paritosh *et al.*, 2017). In particular, the remediation of urban food waste is an ever increasing issue. At present, there exists a large population percentage of young adults under the age of 24 years old, leading to an increase in urban migration (Tan *et al.*, 2018), predominantly seen in Asian countries (Diener, Zurbrügg and Tockner, 2015). Besides food and land shortages, food waste also contributes to global warming via greenhouse emissions; as estimations of around 4.4 billion tonnes of CO_2 are believed to be emitted into the atmosphere every year according to the Food and Agriculture (FAO) UN 2011 assessment (Gustavsson, Cederberg and Sonesson, 2011; Scialabba, 2015). BSFL have the potential to be a very useful insect in waste management, particularly in being a compact urban environment solution.

Waste is traditionally processed using four main methods; sanitary landfill, controlled incineration, compositing and on a minor scale, anaerobic digestion (Kantor, Lipton, Manchester and Oliveira, 1994; Heller and Keoleian, 2003; Melikoglu, Lin and Webb, 2013). BSFL could hypothetically provide a waste-to-energy scheme on a national scale, providing a means of recycling food waste conventionally incinerated or sent to landfill (Agarwal, Singhmar, Kulshrestha and Mittal, 2005). Current methods of municipal waste disposal can

cause severe health and environmental issues (Paritosh *et al.*, 2017), and BSFL waste remediation could provide a safer alternative. Leachate, an unsanitary and possibly harmful liquid that seeps from MSW into the ground, is prominent from improper landfill sites in developing countries, and potentially affects ground water (Beede and Bloom, 1995). Composting is considered a sustainable alternative to landfill when considering isolated food waste, but the process is time-consuming, arduous and requires constant monitoring of parameters such as; temperature, oxygenation, carbon to nitrogen ratio and moisture content (de Bertoldi, Vallini and Pera, 1983). In most cases, meat products and waste are not suitable for this method. Therefore, traditional composting methods are problematic to commercialise on an industrial-sized scale. Alternatively, modern technology, such as previously mentioned anaerobic digestion, can be capital intensive and dependent on government subsidies (Sam, Bi and Farnsworth, 2017).

In addition to municipal food waste, manure management by BSFL proposes an alternate solution to the problem of large accumulations of animal faeces in the modern dairy industry (Li, Zheng, Qiu, *et al.*, 2011) and potentially that of human waste (Banks, Gibson and Cameron, 2014). In wastewater treatment, human waste follows a highly complex series of purification and cleaning steps in order to obtain fresh drinking water (Dhote, Ingole and Chavhan, 2012). In developed countries, manure from the dairy industry is estimated to contribute significant GHG emissions depending on the practice and farm size: 2200 to 12,000 g CO_2-eq for collection, 200 to 2400 g CO_2-eq for transportation, 16,000 to 84,000 g CO2-eq for storage, and 16,400 to 33,500 g CO_2-eq for application in agricultural fertilisers (Aguirre-Villegas and Larson, 2017). In developing countries, it is estimated that up to two thirds of organic waste is still not collected or processed, despite its high energy recovery potential (Diener, Zurbrügg and Tockner, 2009). BSFL waste remediation of human faeces filtered from wastewater treatment could be an alternative urban economic solution.

An emerging practice is insect biomass conversion; whereby municipal organic waste is fed directly to insects. The insects convert biomass thought to be 'low value' into higher value substances such as proteins, fats and chitin stored in the insect mass through a 'biocatalysis' route. These substances can be extracted and refined from the larval body mass, and used as sustainable resources for a range of sectors, predominantly being high quality animal feeds desired by the premium pet food market (Wang and Shelomi, 2017), but lately, in the production of biofuels and bio-solvents (Figure 1).

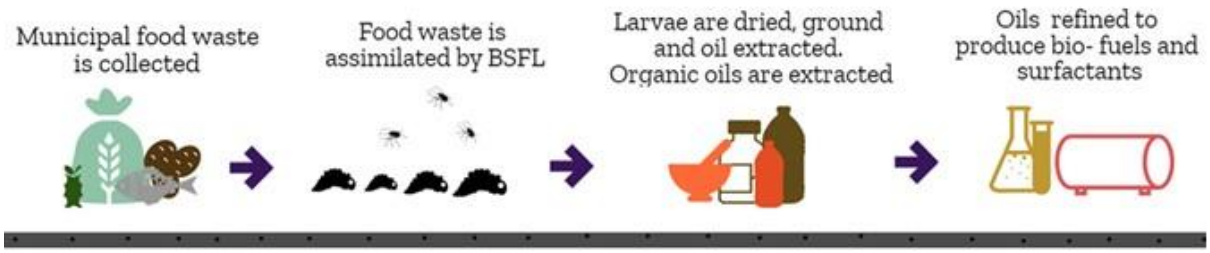


Figure 1: The life cycle of extracting and processing valuable chemicals from BSFL

Several species of insects have been shown to be feasible for this route, with the industrial preference being mealworms, *Tenebrio molitor*, and the projects' focus, black soldier fly. Species can vary in their ability to grow from different types of municipal organic waste and the efficacy in which they assimilate that waste into higher value substances, however the underlying concept remains the same.

1.2 BLACK SOLDIER FLY LARVAE

There is potential offered by Black Soldier Fly Larvae (BSFL) (Figure 2), to provide a truly sustainable feedstock for the synthesis of fuels and valuable chemicals. Black soldier fly (BSF), *Hermetia illucens*, are a Dipteran fly of the Stratiomyidae family and larvae develop through tenaciously eating throughout six instar stages. Larvae rapidly consume organic waste for the purpose of fat accumulation, storing energy to be used in its short adult life where it does not have the opportunity to eat, as adults do not develop mouthparts or digestive organs in metamorphosis (Nguyen, Tomberlin and Vanlaerhoven, 2015). In this way, they bio-catalytically digest and convert biomass into proteins and lipids, the latter representing roughly 30 % of the total larval body mass (Hale, 1973; Li, Zheng, Cai, *et al.*, 2011). Previous studies on BSFL have been based upon the production of biodiesel from the collected and separated lipids, although bio-solvents and other petrochemical products are pertinent for investigation (Li, Zheng, Cai, *et al.*, 2011; Nguyen, Liang, Doan, Su and Yang, 2017).

Figure 2: *Hermetia illucens* larvae prior to drying

Current uses for BSFL include use as a composting means in industry and in domestic households, as well as being utilised as an alternative protein source in insect meal for the Brazilian poultry industry (Allegretti, Talamini, Schmidt, Bogorni and Ortega, 2018). Despite their wasp-like appearance, BSF are harmless insects, their lack of mouthparts preventing them from being a disease vector in adulthood through regurgitation (Gayatri and Madhuri, 2013). Possible ingestion of unsanitary, uncooked BSF is the only way unintentional harm may result to humans or animals (Goddard, 2003). In general, the species are disinterested in human residences and habitation near other common fly species and therefore cannot be considered a pest (Newton *et al.*, 2005). The common house fly, *Musca domestica*, are driven out by the presence of BSF colonies in animal manure, and poultry faeces are reduced by 42-56 % when chickens are fed a BSFL based diet (Furman, Young and Catts, 1959; Axtell and Arends, 1990; Sheppard, Newton, Thompson and Savage, 1994).

The species was first identified in Europe in 1926, but are thought to be native to neotropical regions of the globe (Gladun, 2019). Nowadays, BSF are naturally distributed throughout tropical and sub-tropical regions (Figure 3). International transportation development and commercial breeding of BSF means that presently, distribution extends to Europe, Asia and Australia and Africa, covering 80 % of the globe (Nyakeri, Ogola, Ayieko and Amimo, 2016).

Figure 3: Natural worldwide distribution of BSFL lies between latitudes of 45°S and 40°N (Dortmans, Diener, Verstappen and Zurbrügg, 2017)

In nature, the black soldier flies will lay their eggs in or around moist organic waste, where the larvae will be able to easily assess the food source. Female black soldier flies can lay, or oviposit, up to approximately 500 eggs at one time following copulation, with eggs hatching up to 4 days later (NCIPMI, 1998; Dortmans, Diener, Verstappen and Zurbrügg, 2017). Larvae consume all matter of organic waste, and will begin eating immediately following hatching (Nyakeri, Ogola, Ayieko and Amimo, 2017). The larvae require roughly two weeks to reach maturity, passing through six instars, but in hostile circumstances, the larvae stage can be extended for up to six months (Newton, Sheppard, Watson, Burtle and Dove, 2005). Once maturity is reached, the larvae undergo another metamorphosis - pupation, obtaining a 'pupal cuticle' before hatching as a fully grown adult *Hermetia illucens* (Gullan and Cranston, 2010). In metamorphosis, there is no gastrointestinal development, and adults survive only on water and stored nutrients for approximately one week (D. C. Sheppard, Tomberlin, Joyce, Kiser and Sumner, 2002; Li, Zheng, Qiu, *et al.*, 2011; Dortmans, Diener, Verstappen and Zurbrügg, 2017). Adults copulate, females search for suitable breeding ground, and the cycle begins again.

The earliest recorded attempts at artificial rearing were by Tingle *et al.* (Tingle, Mitchell and Copeland, 1975). Initial conditions were completed at 29.3 °C, and mating and oviposition occurred frequently in an outdoor cage. Cage size was seen to make a key difference, but Tingle *et al*. was unable produce a multi-generational population of BSF. To successfully rear and maintain a colony, there must be a sufficient light source, sustained humidity of between 50-

70 % and a temperature ranging from 25-30 °C (D. Sheppard, Tomberlin, Joyce, Kiser and Sumner, 2002).

The raw material of live BSFL, dried BSFL and pressed BSFL fat was provided by the industrial partner, Better Origin ltd., who fed developing larvae a variety of plant based waste diets. Larvae received were pale in colour, and ranged between 3 – 6 mm in width and 1.5 – 2.2 mm in length (Figure 4). Size was seen to be affected by diet.

Figure 4: *Hermetia illucens* larvae are pale in colour, with a small darker head (left)

1.3 BSFL BIOMASS COMPOSITION

Primary literature-based research found that dried BSFL contains approximately 40 % crude protein and 30 % crude lipid extract, although these figures fluctuate dependent on the chosen diet (Newton *et al.*, 2005; Li, Zheng, Cai, *et al.*, 2011; Li, Zheng, Qiu, *et al.*, 2011). Within the exoskeleton, exists a hard fibrous natural protective material called chitin, which has useful applications as a catalyst support material (Rinaudo, 2006). BSFL compares very favourably when assessing the fat and protein content of other waste consuming insects, Table 1, as well as in its ability to assimilate waste quickly (Diener, Zurbrügg and Tockner, 2009; Kim *et al.*, 2021).

Table 1: Weight percentage composition of different waste consuming insects

	Crude protein (weight %)	Crude fat (weight %)	Ref.
House Fly, *Musca domestica*			(Ravindran and Blair, 1993) (Ogunji, Nimptsch, Wiegand and Schulz, 2007)
Pupa	63	15	
Larva	38	20	
Mealworm, *Tenebrio molitor*	57	29	(Ng, Liew, Ang and Wong, 2001)
Mealworm, *Alphitobius diaperinus*	20	8-9	(Yi *et al.*, 2013)
Superworm, *Zophobas morio*	20-21	15-17	
House cricket, *Acheta domesticus*	21-22	3-4	
Dubia roach, *B. dubia*	18-20	7-8	
Black Soldier Fly, *Hermetia illucens*			
Prepupa	44	33	(St-Hilaire *et al.*, 2007)
Larvae	42-45	31-35	(Hale, 1973)

Of the dried weight, when separated, there are large quantities of protein, fatty acids, vitamins, minerals, fibre and chitin which can be utilised in many applications (Barroso *et al.*, 2017). The protein has potential for animal feed, with estimated values comparable to fish meal (Tomberlin, Sheppard and Joyce, 2002). If used as live feed, the value of the feed is higher with additional essential fatty acids and chitin (Sheppard, Newton, Thompson and Savage, 1994). Literature research into BSFL diet found that the waste feedstock effects the % composition of protein and fat (Table 2), a factor later explored and discussed further in this research.

Table 2: Content of protein and fat of dried BSFL reared on different diets

Diet	% Protein	% Fat	Ref.
Poultry manure	37.9	18.7	(Sheppard, Newton, Thompson and Savage, 1994)
Swine manure	43.2	28.0	(Newton, Sheppard, Watson, Burtle and Dove, 2005)
Restaurant waste (vegan)	43.1	38.6	(Spranghers *et al.*, 2017)
Vegetable waste	39.9	37.1	
Fruit and Vegetable waste	39.0	42.1	(Nyakeri, Ogola, Ayieko and Amimo, 2016)
Food waste	36.0	36.0	(Nyakeri, Ogola, Ayieko and Amimo, 2017)
Banana peels	35.0	38.0	
Brewers waste	42-44.0	26-28.0	

Of the 30 % oil derivative, the largest component commonly found by chromatographic analysis is lauric acid (dodecanoic acid), along with other medium and long-chain free fatty acids (FFAs) (Nguyen, Liang, Doan, Su and Yang, 2017), Figure 5.

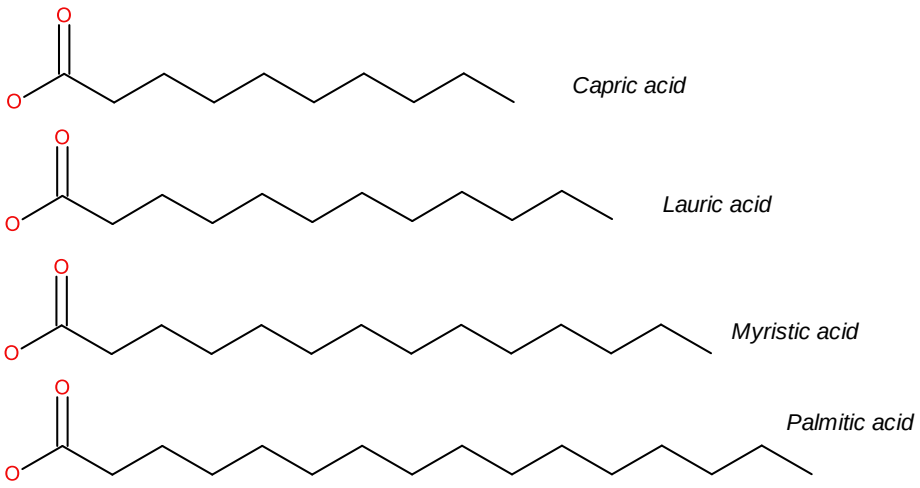


Figure 5: Common FFAs found within BSFL oil

Lauric acid is one of the most widely distributed fatty acids existing in nature, found in edible oils such as coconut and palm kernel oil (Cepeda, Bravo and Calvo, 2009). It is considered a very useful medium-chain fatty acid (MCFA) pertaining to approximately to 45-53 % of the

overall fatty acid composition in coconut oil, similar to that seen in preliminary BSFL fatty acid composition calculations. MCFAs refer to those between C_6-C_{12} and long chain fatty acids (LCFA) are defined those C_{14} and longer (Bach and Babayan, 1982; Dubois, Breton, Linder, Fanni and Parmentier, 2007). Despite a popular bias against saturated fatty acids in the medical industry created by Keys in 1957 (Keys, Anderson and Grande, 1957; Keys *et al.*, 1984), recent evidence suggests that the long-standing belief that saturated fat causes coronary heart disease may in fact be false (Kuo and Huang, 1965; Nevin and Rajamohan, 2004; Dayrit, 2014; Harcombe *et al.*, 2015). Nevertheless, the antibacterial properties of lauric acid and its digested derivative, monolaurin, have not been disputed epidemiologically, and therefore pertinent for further investigation (Lieberman, Enig and Preuss, 2006; Widiyarti, Hanafi and Soewarso, 2009; Choi, Yun, Chu and Chu, 2012; Čičková, Newton, Lacy and Kozánek, 2015; Nitbani, Jumina, Siswanta, Sholikhah and Fitriastuti, 2018).

Along with lauric acid, exists palmitic acid in high concentrations, which is LCFA commonly found in large quantities within palm and corn oils (Dayrit, 2015). Fats and oils are typically classified according to their fatty acid profiles, with coconut being classified as a medium-chain fat and lard a long-chain-saturated fat (Dayrit, 2014) . If triglyceride content is high, the fatty acids are hydrolysed and the corresponding fatty acids separated using fractional distillation.

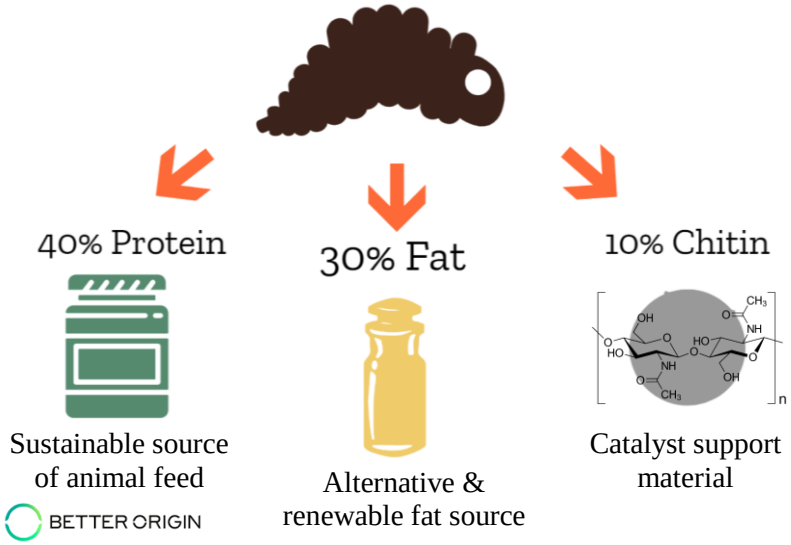


Figure 6: Dried BSFL can be separated into three main components, crude protein, crude fat and chitin

1.4 BSFL PRODUCT IN APPLICATION

The long-term viability of this work has also been carefully considered with applications beyond waste remediation. Insects are rich sources of proteins and fats, and hence have the potential to be a sustainable source of protein in the future for both humans and animals (Van Huis, 2013; Spranghers *et al.*, 2017). The greatest advantage they have over conventional animal meat rearing is their lower impact to the environment on a large scale (Bosch *et al.*, 2019). Insects have a lower feed-to-protein conversion ratio than cattle or swine and in some instances poultry (Oonincx, van Huis and van Loon, 2015). When scaled industrially, farming insects requires less water and space than conventional livestock especially when they are fed organic waste (Allegretti, Talamini, Schmidt, Bogorni and Ortega, 2018). Previous studies have shown insects produce fewer greenhouse gases and lower ammonia emissions in comparison (Ng, Liew, Ang and Wong, 2001; D. Sheppard, Tomberlin, Joyce, Kiser and Sumner, 2002). A life-cycle assessment of the sustainability of insect use for feed and food found that, the use of insect based protein powder or a meat substitute could be approximately 2-5 times more environmentally sustainable than current meat products (Smetana, Palanisamy, Mathys and Heinz, 2016). An additional LCA focused solely on the use of BSFL protein in the animal feed industry (Smetana, Schmitt and Mathys, 2019), and not encompassing any other application of its oil or chitin components as discussed in this work.

In industrial application, the use of larvae would not compete with the food chain and instead be supplementary by reducing the CO_2 emissions arising from it. A convenient and constant source of feed is required but easily resolved by placing insect farms near to waste streams such as breweries or food-processing factories. Or alternatively, near food industry quality control factories, where there are large amounts of food wasted through not being deemed suitable for sale – some of which was used in the investigation on diet in this work. In developing countries, insect rearing can use low-tech and low-capital investment, but if budget is no issue, rearing can be accomplished with highly automated systems. As BSFL thrive at temperatures over 25 °C, cultivation is best suited to countries with a more tropical climate such as India or Pakistan (Gayatri and Madhuri, 2013). In European countries, factories would require an additional heating element in order to successfully breed and rear BSFL.

The process is very scalable and the demonstration of such can be seen in the scaling of BSFL for live feed for chickens and pigs in countries such as brazil (Allegretti, Talamini, Schmidt,

Bogorni and Ortega, 2018). Other business scalability can be seen in Europe, for example mass scale mealworm production for fish food and bird food is already taking place (Ng, Liew, Ang and Wong, 2001). In 2019, approximately 6 kilotons of insect protein meal was produced in Europe used in animal nutrition (Derrien and Boccuni, 2018). There is a tangible possibility that in the near future, insect protein will become increasingly desirable in the animal feed sector and therefore utilisation of the other components is of importance.

This project aims to conform to several 2030 SDG goals set by the UN in 2015, namely; SDG 11 sustainable cities and communities, SDG 12 responsible consumption and production, SDG 12 climate action and SDG 15 life on land (UN General Assembly, 2015). Agreement with SDG 11 would be accomplished through the use of BSFL on a large municipal recycling scale, creating a more sustainable scheme of remediating waste in a developing city. The use of BSFL in the production of sustainable materials from its protein for animal feed, oil for speciality chemicals, and chitin for sustainable materials would work in favour of completing SDG 12. Likewise, the development of products from waste via the biocatalysis action of BSFL, would both mitigate overwhelming CO_2 production from current waste remediation methods, and the emissions associated with the transportation of essential commodities like plant oil from tropical regions to the European countries were they are used. Furthermore, for SDG 15, the switch to BSFL oil for speciality chemicals, would lessen the pressure on plant oils or petrochemical resources and protect further land degradation and biodiversity loss in tropical rainforests.

There are certain matters that must be considered in the development of sustainable products from the utilisation of BSFL. With an ever increasing demand for sustainable retail practices by consumers, such marketing of insects as eco-friendly in itself could be problematic for some activists such as members of the vegan community (Noguerol, Pagán, García-Segovia and Varela, 2021). The use of BSFL products cannot be labelled as vegan as they would be taking advantage of an animal source. However, the topical risks associated with the production of plant oils, such as deforestation, loss of biodiversity and animal habitats (Meijaard *et al.*, 2018) would not pose an issue in BSFL industrial farming. Moreover, although BSFL in itself do not produce lower carbon footprints in comparison to their plant alternatives when considering protein resources (Wang and Shelomi, 2017), there is significant reduction of emissions from their waste remediating capability and rearing BSFL have an edge over plants in terms of the greatly reduced land and water requirements for their growth. In addition, there are land

shortages for the farming of viable crops, and it is questionable that this valuable resource should be used for the cultivation of commercial goods that can finally labelled vegan, rather than for food.

The social impact of creating consumer BSFL products is that the products would need to be labelled sustainable but animal-derived. It is worthy to note that contemporary materials of animal origin in the personal care market such as ambergris and civet musk are already widely accepted by fragrance customers (Mookherjee and Wilson, 1982). There also is a possibility of a religious contention with BSFL derived consumer products, especially in highly religious countries where Hinduism and Sikhism is widespread such as India, where animal-derived medical products are already rejected (Eriksson, Burcharth and Rosenberg, 2013). However, it is also pertinent to note that India is a country where the slaughter house waste management system is very poor (Jayathilakan, Sultana, Radhakrishna and Bawa, 2012), and BSFL would be an excellent alternative remediating methodology, assimilating a wider range of waste material in comparison to most other insect species. It may be advantageous therefore to utilise BSFL in countries where the derived products are considered undesirable, and trade the merchandise elsewhere.

The potential industrial applications of fatty acids are vast, ranging from uses in chemical, fuel, lubricants, pharmaceutical, food, medical and personal care product industries (Thomas, Matthäus and Fiebig, 2015; Japir, Salimon, Derawi, Bahadi and Yusop, 2016; Murali, Srinivas and Ahring, 2017). It is that with this knowledge, that the potential markets for BSFL crude oil and chitin (Figure 6) were considered in this project and the laboratory based means to complete them.

2 THE EXTRACTION AND SEPARATION OF BSFL OIL

In this chapter, the principal aim was to optimise the extraction of lipids from BSFL using conventional and topical methods. Yields obtained as a result of different diets fed to BSFL were compared and evaluated for how diet affects the composition of the oil. An extensive literature review of the subject area found that lipid proportion is generally seen to be around 30 % of the larval weight, depending on diet (Li, Zheng, Qiu, *et al.*, 2011; Cang *et al.*, 2019). The oil consists of various MCFA and LCFAs, along with other free fatty acids (Li, Zheng, Cai, *et al.*, 2011; Li, Zheng, Qiu, *et al.*, 2011; Ramos-Bueno, González-Fernández, Sánchez-Muros-Lozano, García-Barroso and Guil-Guerrero, 2016). Lauric acid, commonly found naturally in coconut and palm oil, was seen to be the highest acid in concentration within the fatty acids (Caligiani *et al.*, 2018). In this work, following oil extraction and characterisation, attempts to separate individual FFAs occurred.

2.1 LITERATURE REVIEW

2.1.1 The Petrochemical Industry and Alternatives

Oil is the most important global commodity currently, but its source is finite (MacKenzie, 1998). For many years, fossil fuel has been considered the cheapest and easiest feedstock for the chemical industry (Akhtar and Amin, 2011). Its chemical versatility led to the massive surge of industrial development of petroleum based products in the twentieth century (Yergen, 1991). However, factors such as the increasing demand by emerging economies and the market demand for more consumer choice, means that the global stock of crude oil is nearly at an end (Sadorsky, 2009). Economically speaking, procuring and suppling energy (in all its forms) is a very important factor in the growth and success of a country. It is pertinent to find chemical alternatives to ensure that economic regression does not occur in spite of a developing countries' demand for energy increasing (Sadorsky, 2009; Kutasovic, 2012).

Currently, certain valuable chemicals are sourced from crude oil and natural gas, which undergo a series of complex separation and purification steps to yield pure and functioning fuels and solvents. Naphtha is the main fraction at 35-40 % obtained from crude oil in atmospheric distillation in oil refineries (Speight, 2008). Naphtha, along with other fractions, is then further processed to produce fuels such as liquefied petroleum gas, petrol, diesel oil, kerosene, and heavy fuel oil (Housmans and Oprins, 2016). The petrochemical industry

produces countless valuable materials on which modern life depends such as solvents, lubricants, medicines, polymers, detergents (Speight, 2006).

Regardless of several economic crises over half a century, the demand and price of oil has been steadily increasing (British Petroleum, 2011). Based upon the Hubbert peak oil theory (Hubbert, 1956), researchers suspect that "peak oil" has or will soon be reached (Figure 7). Hubbert proposed that any limited natural resource, such as oil and gas, follows a bell-shaped curve in its discovery lifetime, with peak oil referring to the time at which the maximum or half the global supply of crude oil will be found and the rate of oil production will be at its highest (Kutasovic, 2012).

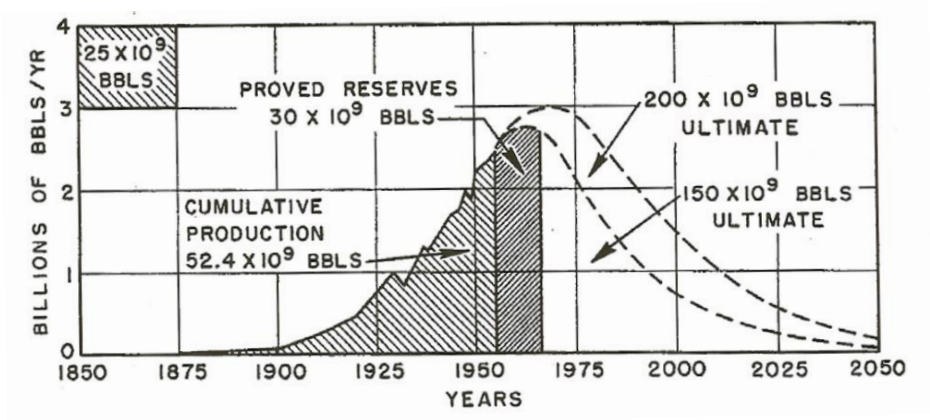


Figure 7: M. King Hubbert's 1956 peak oil graph, based on the assumption there was 150-200 billion barrels of oil (Hubbert, 1956)

Some analysts fear that once peak oil is reached, the shift in the demand of oil will result in widespread problems including economic depression and global warming (Aleklett, Lardelli and Qvennerstedt, 2012), whilst others debate the reliability of Hubbert's theory (Priest, 2014), but not the finite nature of fossil fuel resources. Society and governmental bodies will need to review chemical feedstock alternatives in order to keep up with the market demand for products ordinarily derived from fossil fuels. In addition, as well as economic and environmental drivers for change, there are political drivers to produce alternatives to fossil fuel resources, with public perception of the industry becoming increasingly negative. The interests of politicians lie in the capturing of public attention through policy changes, and therefore shifts towards eco-friendlier ideas have become significant worldwide (Eisenack, Hagen, Mendelevitch and Vogt, 2021; Hawes and Nowlin, 2022).

In addition to affecting economic stability, a dependence on fossil fuel processes affects the environment, with the increasing amount of carbon dioxide (CO_2) in the atmosphere being the

main driver behind global warming (Meher, Vidyasagar and Naik, 2006; Pahla, Mamvura, Ntuli and Muzenda, 2017). Creating a process that relieves or reduces the impact of CO_2 emissions in value-added chemical synthesis would be both beneficial environmentally and economically.

2.1.2 The Plant Oil Industry

The global climate crisis is resulting in changing customer attitudes towards household products conventionally sourced from petrochemical supplies. With customer focus seeking more bio-based environmentally friendly manufactured goods comes an emergence of chemical feedstocks from the plant oil industry, particularly palm and coconut oils (Noguerol, Pagán, García-Segovia and Varela, 2021). In 2005, roughly 115 million metric tons of vegetable oils were produced, of which an estimated 80 % was used as food, 15 % as chemical feedstock and 5 % was consumed animal feed. A substantial share of the 15 % chemical feedstock comprised of 7.5 million metric tons of high-lauric content, of which approximately 50 % was used in toilet soaps, and the other half consumed in the manufacture of detergent grade alcohols used in surfactant production (Zoller, 2009). In the detergent industry, the European market is the largest consumer of bio-surfactants, with consumption of products nearing 178.9 thousand tonnes in 2013, nearly 50 % of the total global consumption (Farias *et al.*, 2021). Current sources for the surfactant industry include petroleum derivatives and tallow, palm and coconut oils (Hayes, 2017).

At present, the European oleochemical industry relies heavily on imported plants oils (Pham, 2016), with 11.41 million metric tonnes of major vegetable oils imported into the EU in 2021 (USDA, 2021). In recent study commissioned by the WWF, Indonesia and Malaysia account for 86% of global palm oil production (WWF and Segi Enam Advisors Pte, 2021), countries which pertain to the most biodiverse tropical forests on earth (Hughes, 2017).

Oils vary in composition (Table 3), with some resources having higher amounts of saturated and others with more unsaturated fatty acids. In addition to food uses, plant oils are currently used as resources for products in the adhesive, surfactant, ink, lubricants, pharmaceutical and plastic sectors, especially those with saturated FFAs. Further need for bio-based products stems from compliance with the Green Principles of Chemistry (GPC) (Horváth and Anastas, 2007; Chen *et al.*, 2020), as using these products would satisfy several principles, including lower

toxicity and being biodegradable in nature. Plant oils are capable of being a direct alternative to petrochemical oils, are cost-competitive and consumer friendly.

Table 3: Typical fatty acid distribution (% wt) of some common fats [1](Zoller, 2009) [2](Hilditch and Williams, 1964) [3](Cang *et al.*, 2019)

	Fat/oil content (~%)	Saturated fatty acids (%)						Unsaturated fatty acids (%)			Ref.
		Caprylic C8	Capric C10	Lauric C12	Myristic C14	Palmitic C16	Stearic C18	Oleic C18:1	Linoelic C18:2	Other	
Palm		-	-	>1	1	47	4	38	9	-	[1]
Sunflower		-	-	-	-	6	2	25	66	-	[2]
Palm Kernel		5	3	50	16	8	2	14	2	-	[1]
Beef Tallow		-	-	-	5	28	21	40	3	3	[2]
Coconut	34	9	6	47	18	9	2	5	2	2	[1]
Olive		-	-	-	0.4	18	-	68	12	1.6	[2]
BSFL	30	-	0.8	32	5.5	22	2.7	20	13	4	[3]

Palm oil and palm kernel oil are edible plant oils primarily extracted from the African oil palm, *Elaeis guineensis* and at times from the American oil palm, *Elaeis oleifera*. Whereas palm kernel oil is derived from the kernel of the oil palm, palm oil is taken from the mesocarp of the fruit of the oil palm (Meijaard *et al.*, 2018), as seen in Figure 8. Although naturally occurring, in recent years' cultivation of palm trees has been taken to a mass industrial scale (Figure 9), resulting in 71 million tonnes of edible oils being produced in 2018 (FAOSTAT database, 2021).

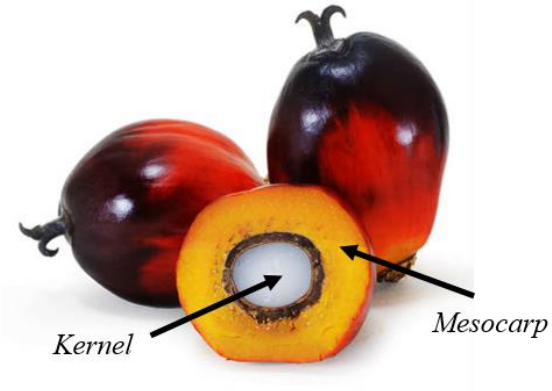


Figure 8: The Palm Fruit is the edible seed of the *E. guineensis*, oils are extracted from both the mesocarp of the fruit and the kernel within (Getty Images)

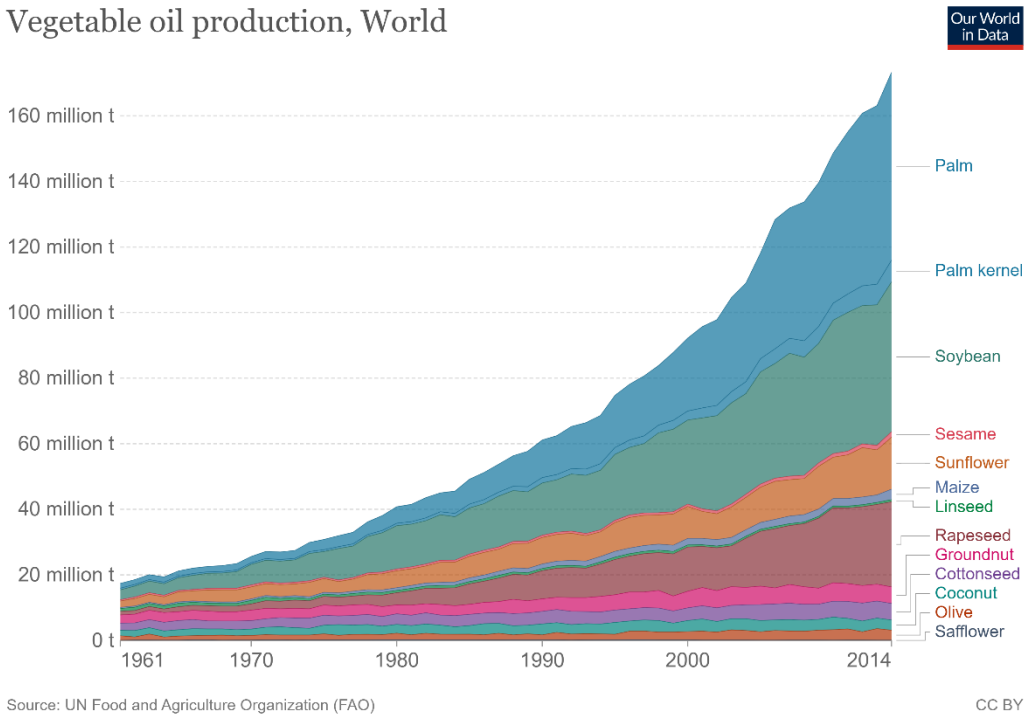


Figure 9: World vegetable oil production variable time span 1961 – 2014 (Taken from Ritchie and Rose, 2021)

It takes 4 to 6 years for *E. guineensis* to reach maturity, by which point the tree has a large crown of between 30 and 45 palms, measuring approximately 5 to 9 metres long (Berger, 2003; Perera, 2003). Generally, the lifespan of the palm tree is defined by its height, which grows by up to 60 cm per year depending on environmental, pest and genetic factors. Fruits can be harvested for up to 30 years, at which point the 15 m height of the tree hinders fruit harvest. (Meijaard *et al.*, 2018). There are three main types of oil palm, which are classified on the thickness of their shell, and bear a variety of oil yields. The Dura produces between 15 and 17 %, the Tenera produces between 21 and 23 % and the Pisifera commonly greater than 23 % oil (Obibuzor, Okogbenin and Abigor, 2012) . The Tenera is a hybrid of Dura and Pisifera and is usually utilised to guarantee a reliable yield because the Pisifera has the ability to spontaneous abort its fruit (Soh, 2012) . Plants are genetically bred focusing on ensuring high yields of fresh fruit bunches (FFB) and a high FFB oil and kernel content. A high level of unsaturation, with high amounts of vitamin E and carotenoids are usually desirable qualities (Bockisch, 1998). Both oils are high in saturated FFAs, with palm kernel oil having a high C_{12} lauric acid content and palm oil having a high concentration of C_{14} palmitic acid, to which it gives its name (Wahid, Abdullah and Henson, 2005; Zoller, 2009).

Coconut oil is extracted from the dried kernel of the *Cocos uncifera* palm nut, denoted the "copra", which has a fat content of between 60-70 % oil. The copra can be sun-dried or dried over an open fire, using the fibres of the nut as fuel (Bockisch, 1998). Like palm, plantations exist in equatorial areas of the globe, with large territories existing in south east Asia and pacific islands, from which they originated (FAO, 2020). The coco oil has a high lauric acid content (up to 52 %), as is often referred to as a lauric oil (Aureles, Odriguez, Ean, Ae and Endoza, 2002; Zoller, 2009), with trace amounts of unsaturated FFAs present. Harvesting occurs 1 month before ripeness, around 11 months succeeding the pollination of the blossom. The palm tree fruits for between 80-120 years (Pham, 2016), and harvesting fruit heavily depends on moisture content as the copra is susceptible to mould infections (Bockisch, 1998). The high medium chain fatty acid content of coco oil makes it extremely desirable in certain applications and nations, such as detergents and lubricants in European and the US (Zoller, 2009).

Although plant oils pose a renewable and sustainable feedstock, there are certain controversial environmental concerns and illegal labour issues associated with the industry. Firstly, increased interest in local development of plantations has resulted in globally around 0.5 % of all deforestation, with up to 50 % deforestation on the island of Borneo between 2005 and 2015 (Meijaard *et al.*, 2018). Where natural tropical rainforests are removed, the change in biodiversity is significant (FAO, 2015). Habitat loss and land conversion often leads to a decline in animal and plant populations, with orangutan citied as the most common species affected, but other ground-dwelling rainforest species are greatly affected (Savilaakso *et al.*, 2014). Secondly, deforestation often takes place in developing countries where modern slavery still persists, for example in the preparation of farmland for oil palm plantations in Indonesia (Jackson, Decker Sparks, Brown and Boyd, 2020; Brown *et al.*, 2021). Furthermore, issues stem from the environmental impacts of large monoculture plantations which deplete soil, causing soil erosion and degradation, and where possibilities of disease spreading throughout the plantation is much more likely (Liu, Kuchma and Krutovsky, 2018). The utilisation of BSFL oil in place of products derived from plant oils would significantly reduce these issues.

2.1.3 Plant oil refining

In industrial production, once the plant oils are extracted raw materials must undergo the twin processes of bleaching and deodorising. In bleaching, often water is removed and a natural clay is added such montmorillonite (Kheok and Lim, 1982). The clay is activated by addition of dilute acid, which releases aluminium atoms leaving a porous structure (Thomas, Matthäus and

Fiebig, 2015). Upon agitation, impurities like dust, odours and colorants are absorbed into the holes, and removed through pressurised filtration (Zoller, 2009). If an edible oil is required, the oils are steamed under vacuum to ensure all substances capable of going rancid are removed in deodorisation (Thomas, Matthäus and Fiebig, 2015; Pham, 2016). Further to refinement and deodorization, triglycerides in plant oils are hydrolysed to FFAs by hydrolysis with water (Figure 10) and separated using fractional distillation (Muckerheide, 1952; Cermak, Evangelista and Kenar, 2012). FFAs are separated into corresponding saturated and unsaturated fractions, ensuring a colourless and often odourless result.

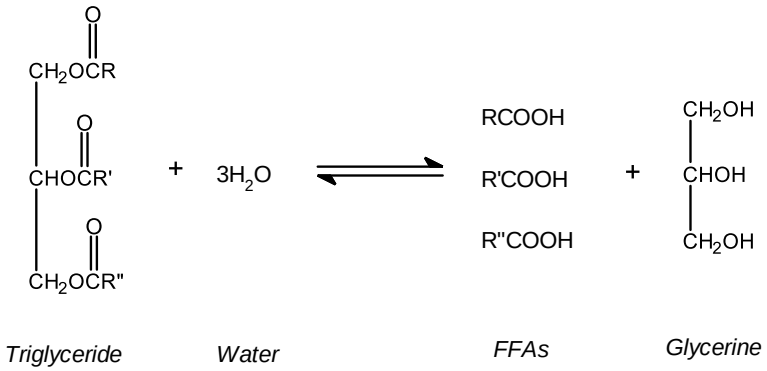


Figure 10: Hydrolysis of plant oils produces FFAs and Glycerine

2.1.4 Fractional Distillation

Distillation is a physical industrialised process used to separate components within a mixture, based upon their constituent boiling points. Early methods of distillation are thought to have originated as early as 800 BC in China, where the concentration of essential oils from their natural sources proliferated (Resetarits and Lockett, 2003). Chemical mixtures are heated until a temperature where the vaporisation of different fractions occurs, and condensed using a cooling method. Distillation is the principal separation technology used in the chemical industry – with fractional distillation being the primary method of crude oil fractionation. The distinction between simple distillation and fractional is in the use of a fractionating column which is used when fractions have boiling points within 25 °C of one another (Figure 11). In addition, fractional distillation is more commonly operated as a continuous steady state process, where simple distillation is batch. An average distillation column at a refinery can be up to fifty metres tall and 4 metres wide, depending on the frequency and type of chemical being separated. (Balaji, Suriya, AnandVikash, Arun and Kumar, 2017)

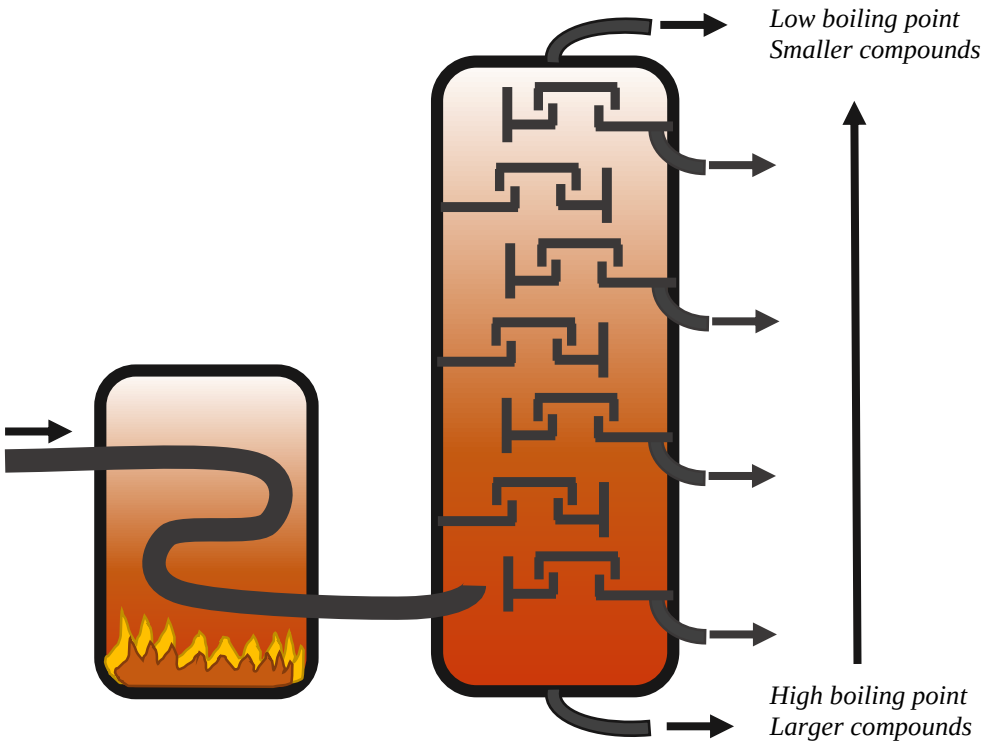


Figure 11: Fractional Distillation relies upon a chemical mixture having different boiling points

Typically, fractional distillation requires a lessening in pressure to complete optimum separation (Muckerheide, 1952). With a decrease in pressure using vacuum pumps, lower boiling points can be achieved and therefore lower operating costs are associated with this method. Additionally, use of pressure correctly improves product stability (Cermak, Evangelista and Kenar, 2012) . The theory behind fractional distillation relies on the difference in vapour pressure between the liquid mixture and fractions that leave it within the column. Higher weight molecular species often have a higher vapour pressure than smaller ones, resulting in fractions hypothetically being separated based upon weight (Resetarits and Lockett, 2003).

2.1.5 Industrial Crude Fat Distillation

Commercial use of fractional distillation is widespread, from the petrochemical industry to the cryogenic air separation plants. For over 100 years, the purification and separation of fatty acids has been practiced, either by batch or continuous processes (Cermak, Evangelista and Kenar, 2012). In one of the first commercial processes, the batch distillation occurred at atmospheric pressure, and fatty acids were charged with a steam sparger and heated up to 315 °C (Muckerheide, 1952). Molar ratio of steam to fatty acid at approximately 5 to 1, and steam and fatty acids were subsequently condensed separately. The presence of steam reduces

anhydride formation (Potts and White, 1953). Due to the high temperatures and great amount of steam vapour needed, this method was often economically poor. In addition, the prolonged heating of fatty acids leads to unwelcome decarboxylation and polymerisation reactions (Cermak, Evangelista and Kenar, 2012). The addition of vacuum equipment brought improvements to batch distillation, requiring lower temperatures and reduced steam intake.

Continuous distillation is often chosen to mitigate issues associated with the batch process. In the continuous process, preheated fatty acids are fed into a series of reaction chambers, heated at the base, and superheated steam is introduced into each chamber by a sparger. Low pressure within the chambers meeting the high temperature of the feedstocks result in almost immediate distillation of fatty acids, which are cooled in the final reaction chamber and collection with aid of a vacuum (Potts and McKee, 1936; Potts and White, 1953). Residence time is approximately 30 minutes. Occasionally in the final condensation step, emulsions are formed with the reaction of fatty acids with trace amounts of magnesium and calcium in the steam. In recovery, this soap is acidified and returned to the feedstock to be processed again (Cermak, Evangelista and Kenar, 2012).

Fractional distillation takes place very similarly to continuous distillation. In continuous fractional distillation, a fractionating column with bubble cap trays is placed instead of a series of reactors (Figure 12). The column separates the fractions depending on boiling point, and side streams remove the fatty acid distillates (Potts and White, 1953).

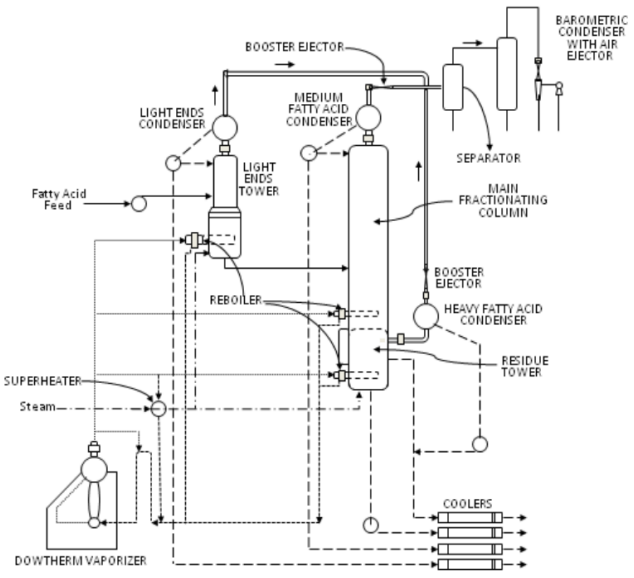


Figure 12: Fractional Distillation operation employed by General Mills to distil fatty acids (1948) (Potts and White, 1953)

2.1.6 Conclusions and BSFL oil objectives

In order to achieve the objectives of oil extraction, characterisation of BSFL oil and purification of extracted oil, the work completed within this chapter followed three distinct work packages. In the first work package, investigation into the optimum methodology for oil extraction was completed, with focus on solvent suitability, as well as ideal duration and temperature for extraction. Within this research, different diets were examined to ascertain whether diet content had an effect on oil composition or yield. In the next work package, the characterisation of the oil would be completed with quantitative analyses of the oil obtained. The final work package would focus on the purification of the oil, with the aim to produce pure fractions of individual FFAs.

2.2 EXPERIMENTAL

2.2.1 Solvents and Materials

Solvents and reagents purchased for use in this work were not further purified. Commercial solvents of purity 99.0 % (Sigma-Aldrich, UK) were used: diethyl ether, acetone, tetrahydrofuran, ethyl acetate, methanol, dichloromethane, hexane and petroleum ether. Commercial reagent pure grade fatty acids (Sigma-Aldrich, UK) were used: capric, lauric, myristic, and palmitic acid. BSFL were acquired from the industrial partner: Better Origin ltd (Cambridge, UK).

2.2.2 BSFL rearing and deactivation

BSFL were reared for a minimum of 30 generations at the Cambridge Better Origin site and fed a variety of diets for a minimum of 4-6 weeks before application in this research. One 1 kg batch of each diet was received within a one-month period. Deactivation of the larvae was completed by heating in an oven at 100 °C for 30 minutes. BSFL material was further dried for 2 days at 60 °C before being mechanically ground using a kitchen blender (Morphy Richards, UK) to obtain a brown powder with an average particle size below 0.2 mm.

2.2.3 Extraction of BSFL oil from BSFL

Several studies were completed in to order to optimise the oil extraction on a lab-based scale. Initially, BSFL oil was extracted using toluene (Fischer Scientific, UK) at varying temperatures and soaking times. It was found that subsequent to extraction, toluene was a difficult solvent to remove, with a boiling point of 115 °C. The impracticality of this property meant that n-hexane and petroleum ether, previously reported by Li *et al.*, were more suitable for this study (Li, Zheng, Cai, *et al.*, 2011). Following evaporation of the solvent, weight of obtained oil was compared to the starting biomass weight, and a percentage yield of oil calculated. Fats and oils obtained in all methods were characterised using FT-IR spectroscopy and GC-MS (2.2.4).

Method 1: Ground BSFL biomass (8 g) was placed filter bag along with 80 mL of chosen solvent to soak and diffuse for a variable reaction time at STP. Following reaction completion, the solvent was collected by vacuum filtration (Vacuubrand GmbH & Co., Germany) and remaining BSFL biomass allowed to dry at room temperature. Coloured BSFL oil was concentrated by rotary evaporation (Büchi, UK ltd., UK) of the solvent, and weighed.

Method 2: Ground BSFL biomass (8 g) were heated under reflux in a round-bottomed flask with 80 mL of solvent at varying temperatures for varying reaction times. BSFL oil was concentrated by rotary evaporation (Büchi, UK ltd., UK) of the solvent, and weighed.

Method 3: Ground BSFL biomass (8 g) were heated under reflux at 80 °C for 1 hour in a soxhlet extractor with 100 mL of solvent, until the solvent was seen to run clear. BSFL oil was concentrated by rotary evaporation (Büchi, UK ltd., UK) of the solvent and weighed.

Method 4: Ground BSFL biomass (8 g) fed a diet of ranges were deactivated by emersion in liquid N_2. The larvae were then ground and allowed to air dry, before being heated under reflux at 80 °C for 1 hour, as described in method 3.

Method 5: Ground BSFL (8 g) were mechanically sieved through sieves of 5 different sizes, and the resulting particle sizes were oil extracted following method 3.

2.2.4 Analysis of BSFL oil

Primary analysis of BSFL oil composition was determined by GC-MS. Analyses were carried out on a Shimadzu 2010 GC combined with a QP2010 SE Mass Selective detector with electron impact ionisation (EI) (Shimadzu Corp., Kyoto, Japan). The GC-MS system was equipped with a purged tee installed after the HP-Innowax (Agilent Technologies, USA) polyethylene glycol phase capillary column (30 m × 0.25 mm I.D.; 0.2 µM). Alternatively, a DB-5MS (Agilent Technologies, USA) nonpolar phenyl arylene polymer capillary column (30 m × 0.25 mm I.D.; 0.2 µM) columns was used. Retention time against relative TIC was collected for analyses using a 0.99 mL/min Helium mobile phase. Automated 0.7 µL injection was performed using AOC 2Oi injector autosampler (Shimadzu Corp., Kyoto, Japan). The oven temperature was programmed at 50 °C and held for 2 minutes and then ramped from 50 °C to 250 °C at 16 °C/min and held at 250 °C for 25 minutes. The MS was operated at scan speeds between 5000 and 20,000 amu covering a range of m/z 30–500. EI mass spectra were recorded at 70 eV. Peaks were identified by analysis of MS fractionated ions, by comparison to a Shimadzu MS computerised database (GCMSsolution software 2012 ©, Shimadzu Corp., Kyoto, Japan).

Characterisation of BSFL oil was undertaken by FT-IR spectroscopy. IR spectra were obtained on a Shimadzu IRAffinity–1S FTIR spectrometer with a Specac Quest ATR attachment

(Smiths Group, London, UK). 16 scans were completed at a resolution of 4 cm^{-1} in the range 4000-600 cm^{-1}.

2.2.5 Isolation of BSFL oil using column chromatography

BSFL oil isolation was undertaken by column chromatography, using a 4.4 cm diameter glass chromatography column (Sigma Aldrich, UK) with 40-60 μm /60 Å silica gel (Thermo Fisher Scientific, US) packed using the slurry method to prevent air bubbles. BSFL oil (2 g) was dissolved in n-hexane and pipetted onto drained silica. To prevent the silica being disturbed, industrial grade sand (Sigma Aldrich, UK) was placed atop. n-Hexane, DCM and diethyl ether were added at a 1:1:1 ratio until the product was seen to elute by Thin-layer chromatography analysis (TLC), and then ratios were adapted to improve separation. TLC was conducted using pre-coated fluorescent indicator (254 nm) silica gel (8.0-12.0μm, 60 Å pore diameter) on aluminium support (Sigma Aldrich, UK).

2.2.6 Preparation of BSFL Biodiesel (FAMEs)

BSFL oil (3 g) and methanol (30 mL) were placed in a round-bottomed flask and heated under reflux at 75 °C at a 1:10 w/v ratio, along with a 1.0 M H_2SO_4 (3 mL) catalyst for 3 hours. Following reaction completion, the biodiesel was separated from the resulting water by a liquid-liquid extraction. The reaction mixture was poured into a separating funnel, and washed twice with diethyl ether (40 mL) and saturated NaCl solution (40 mL) and the aqueous and organic layers allowed to separate. The upper solvent layer containing the biodiesel was removed by rotary evaporation and BSFL fatty methyl esters (FAMEs) analysed using GC-MS (method 2.2.4).

2.2.7 Acid Hydrolysis of BSFL FAMEs

In a 100 mL Erlenmeyer flask of BSFL FAMEs (2 g) were dissolved in methanol (5 mL). Upon addition of NaOH (9 mL), the solution became cloudy. The solution was allowed to age for up to 12 hours, and then the solvent evaporated using rotary evaporation. Ethyl acetate (20 mL) and 1.0 M HCl (8.3 mL) were added, stirred and the solution poured into a separating funnel. The solution was washed twice with ethyl acetate (10 mL), and thrice with saturated NaCl solution (10 mL). The organic layer was collected and solvent removed by rotary evaporation. The BSFL oil product was analysed using GC-MS.

2.2.8 Distillation of BSFL oil

Fractional distillation of BSFL oil was performed using a lab-scale set up, Figure 13. BSFL was placed in pear shaped flask and heated within an oil bath. A small distillation column was attached, with a condensing tube leading to a round-bottom flask to collect different fractions. A high pressure Vacuubrand GmbH & Co. (Wertheim, Germany) diaphragm pump vacuum was attached, along with cooling tubes. The round-bottom flasks were changed at time intervals, or with every change in temperature or pressure. Fractions collected were analysed using GC-MS.

Figure 13: Laboratory apparatus used in the distillation of BSFL oil

2.2.9 Cooling Curve for BSFL Oil

The cooling curve for BSFL oil was completed by following a IUPAC standard method (Paguot, 1979). 3 g of BSFL fat was heated to 70 °C until full melting of the fat was observed. The fat was filtered through a Grade 1 filter paper, and 2.5 g of the fat was placed inside a round-bottomed flask. The flask was immersed into a 17 °C water bath, placed where the meniscus of molten fat was level with the height of the cooling bath. A thermometer was interested inside the flask. When the temperature of the test portion reached 40 °C, a stopwatch was started and the thermometer read every minute until the temperature reached 17 °C.

2.2.10 Melting points of BSFL oil

Melting points were completed by visual inspection of a 2 g vial of fat upon heating upon a heating mantle. Melting point experiments were completed thrice, and the mean calculated.

2.2.11 Determination of Acid Value (A.V.)

Determination of A.V. was completed by titration with potassium hydroxide (KOH). Expected A.V. is dependent on the mass of the test portion (Table 4).

Table 4: Expected A.V. from sample mass (Paguot, 1979)

Expected acid value	Mass of test portion (g)	Weighing accuracy (g)
< 1	20	0.05
1 – 4	10	0.02
4 – 15	2.5	0.01
15 – 75	0.5	0.001
> 75	0.1	0.0002

BSFL oil (2.5 g) was dissolved in ethanol (75 mL) and diethyl ether (75 mL) in a conical flask. Phenolphthalein solution (0.375 mL) was added dropwise, and 0.5 M KOH ethanolic solution was titrated slowly from a 25 mL pipette, with shaking, into the flask mixture. Titration preceded until the end point was reached, a pink colour seen within the flask persisting for at least ten seconds. The volume of KOH was noted and A.V. was calculated using Equation 1.

$$\text{A.V.} = \frac{56.1 \times T \times V}{\text{m}}$$

Equation 1: Determination of A.V.

Where: V is the mL of the KOH solution needed

T is the molarity of the KOH solution

m is the mass, in g, of the test portion

2.2.12 Determination of BSFL oil density

Density determinations for BSFL oils from BSFL fed different diets were completed. The weight of a clean and dry 10 mL measuring cylinder was recorded using and analytical balance to 4 decimal places. BSFL oil (0.2 mL) was heated to 40 °C to obtain a wholly liquid oil and pipetted into the measuring cylinder and the new weight of the cylinder and oil recorded. The procedure was repeated, 0.2 mL BSFL oil added until 2 mL was achieved. BSFL oil weight was plotted against volume, and the density determined from the gradient.

2.2.13 Determination of BSFL oil Viscosity

Viscosity was determined using a 100 mL Ostwald's viscometer. The viscometer was cleaned with acetone prior to use, and DI water used as the standard comparison. A pipette was used to pull BSFL oil up to meniscus point A (Figure 14). The viscometer was placed inside a water bath at the desired temperature. The oil was allowed to flow through the capillary tube until the meniscus reached point B.

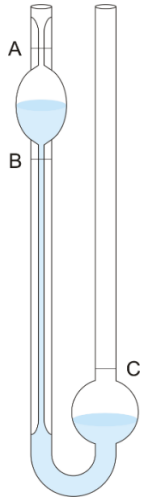


Figure 14 : Ostwald's Viscometer (Generalic, 2018)

The experiment was repeated 3 times at each temperature of 25, 30 and 35 °C and averages calculated. Viscosity was calculated using Equation 2.

$$\frac{\eta_1}{\eta_2} = \frac{t_1 d_1}{t_2 d_2}$$

Equation 2: Determination of viscosity

Where: η_1 is the viscosity of BSFL oil

η_2 is the viscosity of water, 0.00891 poise at 25 °C

t_1 is the flow time of BSFL oil

t_2 is the flow time of water

d_1 is the density of BSFL oil

d_2 is the density of water, 0.997 g/cm^3

Relative viscosity was the calculated using Equation 3.

$$\eta_{relative} = \frac{\eta_{BSFL\ oil}}{\eta_{H_2O}}$$

Equation 3: Determination of relative viscosity

2.2.14 Analysis of BSFL oil using UV-Vis spectrophotometry

UV-Vis spectra were collected using a single beam themoscientific GENESYS 180 UV-Vis spectrophotometer, with a 100 mm pathlength Hellma quartz absorption cuvette. 0.01 g of BSFL oil was dissolved in 1 mL hexane and scans were taken between 190-1100 nm.

2.3 RESULTS AND DISCUSSION

In this chapter, there were three main objectives of focus: optimised extraction of oil from BSFL, comprehensive analysis and characterisation of oils obtained, and the separation and isolation of fatty acid fractions. Further to this, experimental directions that could be taken with saturated long chain fatty acids were considered, focusing on the current and potential applications of BSFL oils.

2.3.1 Optimisation of BSFL oil extraction and separation

Five extraction methods of BSFL oil from dried and ground BSFL biomass were investigated. Achieving the highest volume yield from the starting mass was the primary objective. Initially BSFL fed diets of orange, lime and potato peels were used, but BSFL fed new diets of beetroot, mushroom and a mushroom/potato mixed diet were received at a later date. Gaps seen in the data are result of lack of BSFL fed a specific diet.

Determination of a suitable solvent for extraction was completed by comparison of dielectric constants and visual determination of the solvents ability to dissolve BSFL oil (Table 5). Like most oily substances, smaller chain solvents like methanol are insufficient in breaking up the intermolecular forces existing between MCFAs and LCFAs, especially when existing in a crystalline solid phase. Based upon the calculated Hansen solubility parameters toluene has been found to be the best solvent for the extraction, but is problematic to remove from the oil based upon its high boiling point. Multiple studies on the extraction of BSFL oil on a food-to-waste demonstration (Li, Zheng, Cai, *et al.*, 2011; Nguyen *et al.*, 2018; Kim *et al.*, 2021) have used either hexane or petroleum ether, as they exhibit the good predictive quantitative extraction according to Hansen predictions (Sicaire, Vian, Fine, Joffre and Carré, 2015). In addition, they have favourably low boiling points (Lesten and Kingsley, 2019), ensuring easy recovery and recycling. For these reasons, n-hexane was selected as the solvent in this study.

Table 5: Determination of appropriate extraction solvents for BSFL oil. * Taken at 25 °C, unless otherwise stated

Solvent	C_n	Dielectric constant* $\varepsilon_r(\omega)$	Dissolution	Temperature of Dissolution (°C)
Water	-	78.4	×	-
Methanol	1	32.7	✓	40
Acetonitrile	2		×	-
DCM	2	8.93	✓	18
Acetone	3	20.7	×	-
Ethanol	3	24.55	✓	35
IPA	3	19.92	✓	25
Ethyl Acetate	4	6.02	✓	18
Diethyl ether	4	4.27 at 20 °C	✓	18
Petroleum ether	~5-6	1.9 at 20 °C	✓	18
n-Pentane	5	2.66 at 20 °C	✓	18
n-Hexane	6	1.88	✓	18
1-Hexene	6	2.1 at 21 °C	✓	18
Toluene	7	2.38	✓	18

2.3.1.1 *Method 1*

Extraction of BSFL oil using method 1 (2.2.3) was initially examined using BSFL fed diets of potato, lime and orange, with BSFL fed diets of beetroot and mushroom received at a later date. A principle aim was to determine the efficacy of extracting solvents hexane and petroleum ether, by comparison of final oil yields. It was found that immersing the biomass in a filter bag with exclusively hexane increased the oil extraction on average by 17.2 %, despite petroleum ether being the preferred solvent used in literature (Li, Zheng, Qiu, *et al.*, 2011). It is thought that the aromatics contained within petroleum ether do not extract carboxylic acids well, due to the linear intermolecular forces discussed previously. Using hexane as opposed to petroleum was found to make no difference to the composition of oils extracted by GC-MS analysis. For this reason, hexane alone was used as the preferential solvent for future oil extraction investigations, due to its greater ability to remove oils from BSFL.

Potato-fed BSFL grinds were rinsed with hexane to determine total solvent volume required to fully remove the oils using method 1. The mixture was allowed to soak for one hour, then the biomass rinsed with additional solvent. It was found that 240 mL of hexane was required before the solvent rinses were visually colourless, which amounted to 1.37 g oil on evaporation of

hexane, a 17.1 % yield compared to the starting material. Contextually, this was average yield for potato BSFL but by no means the highest seen from BSFL fed this diet.

Across all diets, there was no distinct correlation found between time spent soaking and oil yield with method 1, seen in Figure 15 and Figure 16. Inconsistencies in yields from the same diet are thought to arise from the lack of homogeneity and small sample size. Larger extraction volumes or a more homogenous mix of BSFL would likely address this issue.

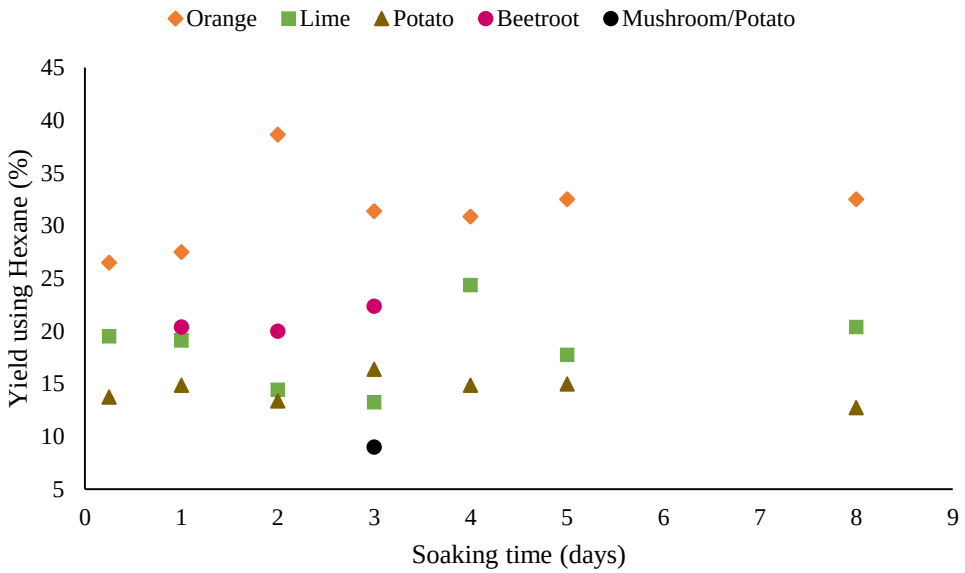


Figure 15: Relation between soaking time with hexane and BSFL oil yield with different diets

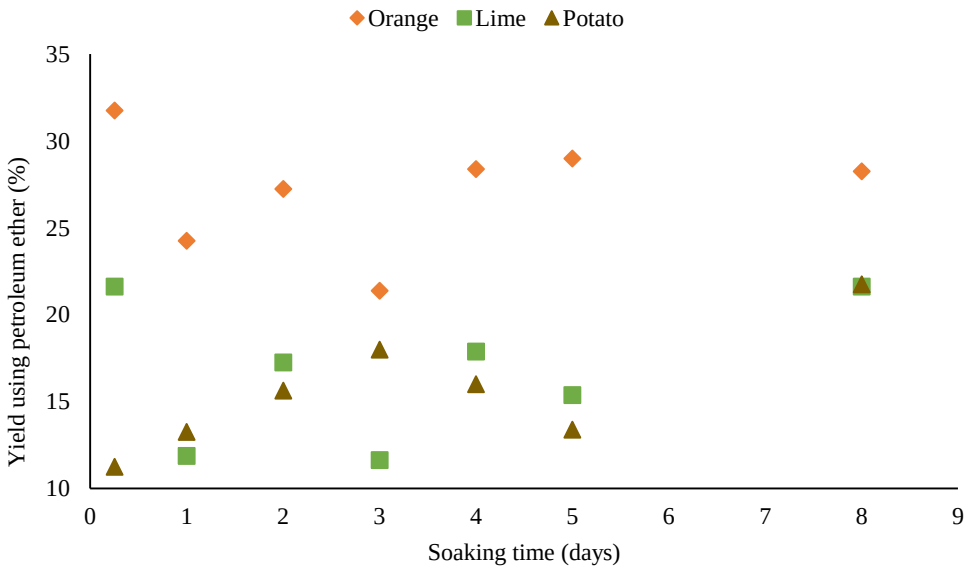


Figure 16: Relation between soaking time with petroleum ether and BSFL oil yield with different diets

The highest yield of BSFL oil were seen with an orange-fed diet (on average around 31 % with hexane and 26 % with petroleum ether), and the lowest seen with potato-fed diets (on average

around 15 % with hexane and 16 % with petroleum ether). Interestingly, oil yields from lime-fed BSFL were similar to that of potato-fed BSFL, not orange as predicted, expected because it is a similar citrus waste feedstock. Beetroot-fed BSFL were found to produce average yields of oils, at an average of 25.8 % when left to soak for 1, 2 and 3 days when completed at a later date. BSFL fed a mushroom/potato diet were found to be low in comparison to those found only potatoes, implying that a mushroom/potato combination is a poor choice of waste feedstock for the objective of maximising oil yield.

The high yields of oil from orange-fed BSFL are thought to be as a result of the high sugar content found in oranges, as seen in Table 6. It was predicted that total carbohydrate content would affect waste to fat conversion, but this trend more closely follows sugar content. Converse to total energy content, which is highest in potatoes at 243 kJ/100g, the highest yields found across all diets were via an orange-fed diet. This is suggestive that the simple sugars are much more accessible carbohydrates to fat conversion, and are key in high production of fat in BSFL. If diets with a lower content sugar carbohydrate content are to be used, it may be advantageous to pre-treat the feedstock to increase the potential availability of energy contained. This is frequently completed in biomass processing to increase the fraction of cellulose available, by procedures of integrated acid or alkaline treatment, milling, extrusion, microwaving, deep eutectic solvents, ionic liquids or steam expansion (Agbor, Cicek, Sparling, Berlin and Levin, 2011; Baruah *et al.*, 2018).

Table 6: Water, lipid, carbohydrate and protein content of food products fed to BSFL (Data sourced from U.S. Department Of Agriculture, accessed December 2021, available at: https://fdc.nal.usda.gov/index.html)

Proximates (g/100g)	**Oranges**	**Limes**	**Potatoes**
H_2O	86.80	88.30	83.30
Lipid	0.12	0.20	0.10
Carbohydrate	11.80	10.50	12.40
Of which sugars	9.35	1.69	0.09
Protein	0.94	0.70	2.57
Total energy (kJ/100g)	197.00	126.00	243.00

The highest yield achieved with method 1 was 39 % with BSFL fed an orange diet and hexane as a solvent after 2 days, which is significantly higher than the 30 % yield reported in literature

(Li, Zheng, Cai, *et al.*, 2011). Diets fed to BSFL were found to slightly affect the composition of the oils extracted.

2.3.1.2 *Method 2*

The extraction yields obtained at a higher temperature with method 2 (2.2.3) were improved in comparison to method 1. BSFL biomass was heated to 40 °C and magnetically stirred under reflux for varying timeframes. Where in method 1 to begin with, but improved with time, around only 10 % of the original biomass weight was found to be oils, using this method, at least 20 % yielded weight was obtained, an improvement towards the 30 % literature value achieved with orange-fed BSFL. This could indicate that extraction is more effective at a higher temperature, or that agitation of the mixture by a magnetic stirrer increases surface area and therefore oil extraction. Because the melting point of the smallest component, capric acid, is 30 °C the ability for the oils to dissolve in a higher temperature solvent may increase the extent of the extraction.

Within this method, oil yield from BSFL fed a diet of orange was seen to decrease with length of heating time, probably due to the lack of homogeneity in particle size seen before (Figure 17). A larger sample size is needed to confirm this hypothesis. For other diets, the heating time did not affect yield, perhaps for the same reason discussed previously. Further investigation of effect of particle size on extraction was completed (2.3.1.5). Overall, the yield produced via either method 1 or 2 is low for diets other than orange peel when compared to that found in literature of around 30 % oils from BSFL fed a variable municipal waste diet (Newton *et al.*, 2005; Li, Zheng, Cai, *et al.*, 2011).

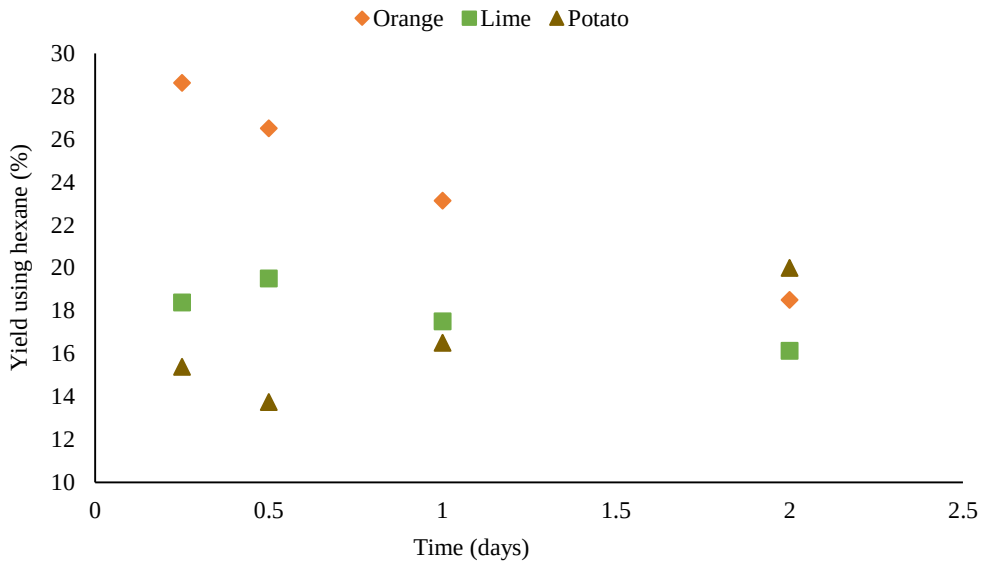


Figure 17: Relationship between heating time (40 °C) with hexane and BSFL oil yield with different diets

2.3.1.3 *Method 3*

Soxhlet extraction was completed on BSFL fed all diets received and found to be the most effective method of oil extraction from BSFL biomass (Figure 18). After 1 hour, no further colouring was seen to the solvent extracting, suggesting that oil extraction was complete. Preliminary yields with this method were seen to be the highest, and therefore are the recommended method for oil extraction from BSFL, and it is clear from results that hexane is the overall superior solvent for extraction.

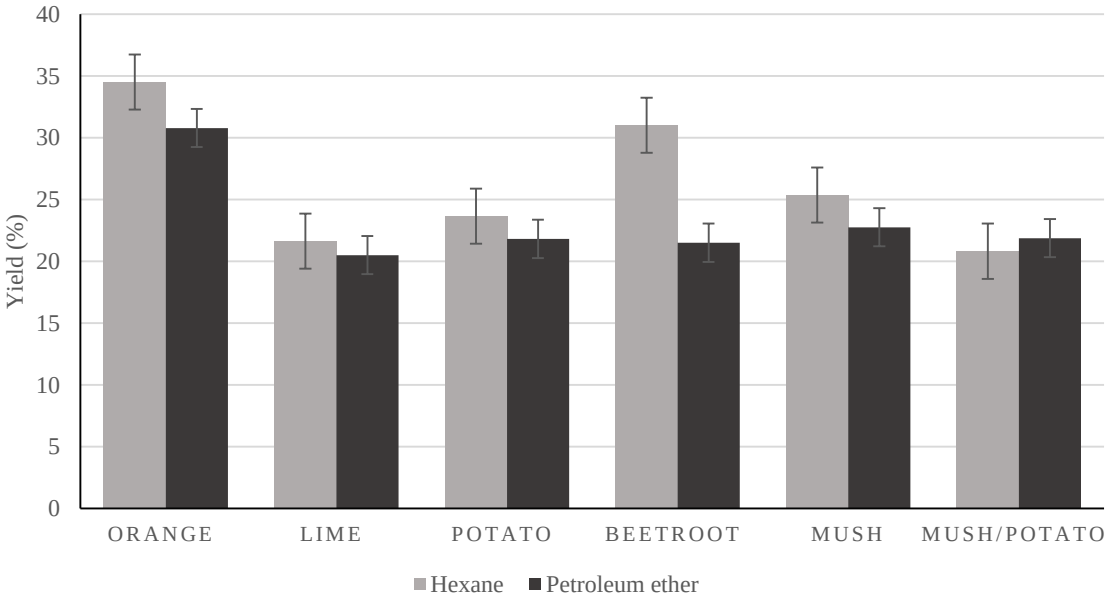


Figure 18: BSFL oil yield generated with hexane and petroleum ether in soxhlet extraction (%)

2.3.1.4 *Method 4*

The prior alternative method of deactivation included heating in 60 °C oven for 2 days. The method of deactivation of the BSFL was investigated by super-critically freezing live orange-fed BSFL before subsequent oil extraction, following the most favourable oil extraction method, method 3. It was found that using this method, oils extracted were richer in colour, an amber colour from orange. However, yields obtained were average at 35.9 %, when compared to heat deactivation. Subsequent literature research found that although heat activation might induce protease activity, resulting in various degraded protein chains, it is unlikely to affect the yield of fat extracted (Manheem, Benjakul, Kijroongrojana, Faithong and Visessanguan, 2013)

2.3.1.5 *Method 5*

It was hypothesised that a smaller particle size would result in greater oil extraction. Therefore, 125 g of mixed diet BSFL was mechanically ground for up to ten minutes, three times and it was found that averagely the majority (43 %) of the particle size existed within 234 – 117 µm (Table 7). Very few particles were found to be under 61 µm. From this, 8 g of BSFL of different particle size were taken and the oil extracted using hexane in a soxhlet extractor. It was found that the smaller the particle size, the greater the extraction from the oil.

Table 7: Oil extraction distribution from different mesh sized BSFL particles (n=3)

Particle size Mesh MG	Microns (µm)	% Weight of starting material	Average Fat Extraction Yield %
Over 35	Over 447	13	20.3
35-60	447 - 234	21	19.5
60-120	234 - 117	43	20.6
120-250	117 - 61	22	36.4
Under 250	Under 61	1	43.6

If larger BSFL particles were attempted to be further mechanically broken down, there was little change in the particle size. This was thought to be due to the large chitin content - around 10 % - present in the exoskeleton of the larvae. The naturally fibrous material is theorised to not easily break up within the blender, but softer internal tissue containing the fat is soft and therefore the smaller particles are more likely to include fat. This explains why smaller particle sized BSFL grinds have higher fat extraction yields.

2.3.2 Analysis of BSFL oil composition

Extracted BSFL oil was seen to vary in colour and consistency. It appeared that the colour reflected the diet fed to the BSFL, and it is hypothesised that the colorants contained within vegetable waste are fat soluble, being taken up by fat cells during BSFL digestion. The solidity of the oil at room temperature was also seen to vary from diet to diet (Table 8), where some oils were solid. Molecular characterisation of oil composition was completed using GC-MS.

Table 8: Visual characterisation of BSFL oil obtained from BSFL fed a variety of diets

BSFL diet	*Colour of Oil*	*State of Oil at 25 °C*
Orange	Orange	Solid
Lime	Yellow / green	Solid with some liquid
Potato	Yellow	Liquid
Beetroot	Dark Yellow	Viscous liquid
Mushroom	Brown	Solid
Mushroom/Potato	Dark brown	Solid

2.3.2.1 *GC-MS analysis of BSFL oil using a HP-Innowax column*

Analysis of BSFL oil found that C_{12} saturated lauric acid was the largest component in existence at around 50 %, along with saturated acids of C_{10} capric, C_{14} myristic, and C_{16} palmitic acid (Figure 19/Table 9), chemical structures of which can be seen in Figure 20. Generally, lauric acid was seen to be at least three times higher in presence than any other FFA by peak area. A calibration curve was determined using lauric acid (r^2 = 0.9994), myristic acid (r^2 = 0.9991), and palmitic acid (r^2 = 0.9994), so that the concentration of those acids could be calculated within the BSFL oil. The concentration of lauric acid ranged between 0.01 - 0.024 mol dm^{-3}, myristic acid between 0.0011 - 0.0025 mol dm^{-3} and palmitic acid 0.0044 - 0.0057 mol dm^{-3} for all diets investigated. GC-MS analysis of lime, mushroom, and beetroot-fed BSFL produced oil with the Agilent HP-Innowax column found the same composition seen in orange-fed BSFL. No triglyceride content was observed using the GC-MS methods.

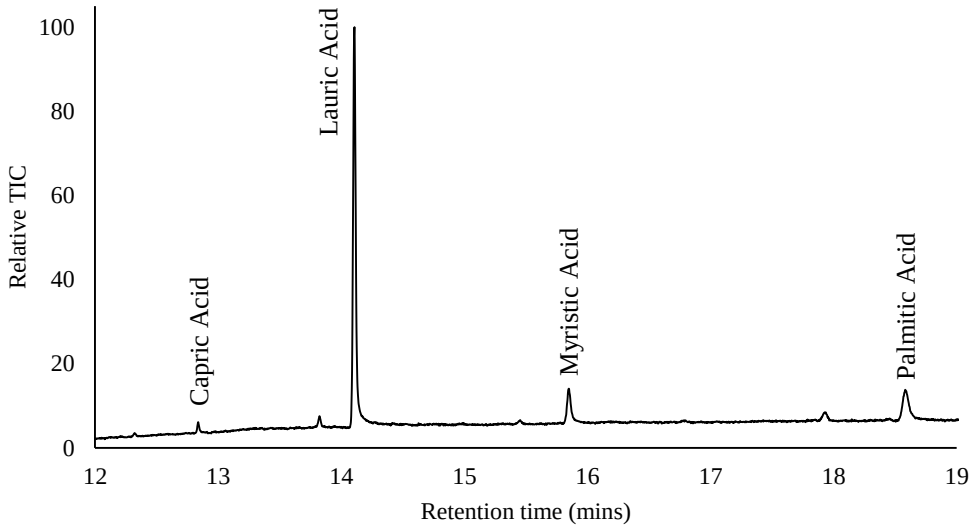


Figure 19: GC-MS chromatogram (Agilent HP-Innowax column) of raw BSFL oil (orange-fed BSFL), extracted via method 2 over the period of 2 hours in hexane

Table 9: Saturated and unsaturated free fatty carboxylic acids found in extracted raw BSFL oil fed a diet of orange (Agilent HP-Innowax column)

C_n	*Common Name*	*IUPAC Name*	M_W	*Retention Time (mins)*	*Conc. (mol dm^{-3})*
10	Capric acid	(n-)Decanoic acid	172	12.8	n/a
12	Lauric acid	Dodecanoic acid	200	14.1	0.024
14	Myristic acid	Tetradecanoic acid	228	15.8	0.0025
16	Palmitic acid	(n-)Hexadecanoic acid	256	18.6	0.0057

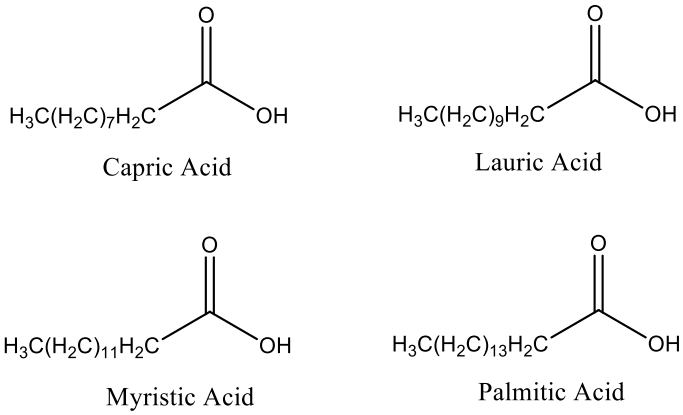


Figure 20: Chemical structures of the predominant species found in BSFL oil

GC-MS analysis of potato-fed BSFL produced oil with the Agilent HP-Innowax column can be seen in Table 10. Interestingly, the presence of myristic acid is more dominant in orange-fed BSFL oil when compared to other acids in the mixture, which could explain why the orange oil is solid at room temperature when potato is not, as the melting point of myristic acid is higher. This evidence is suggestive that the conversion of waste materials to fat by BSFL gives higher lauric acid contents if the sugar composition is higher, allowing for tailoring of the oil in application if a higher lauric acid content is desired. Further studies examining other higher sugar containing feedstocks would be needed in order to confirm this aspect.

Table 10: Saturated and unsaturated free fatty carboxylic acids found in extracted raw BSFL oil fed a potato peel diet

C_n	*Common Name*	*IUPAC Name*	M_W	*Retention Time (mins)*	*Concentraion (mol dm^{-3})*
10	Capric acid	(n-)Decanoic acid	172	12.8	n/a
12	Lauric acid	Dodecanoic acid	200	14.1	0.024
14	Myristic acid	Tetradecanoic acid	228	15.8	0.0011
16	Palmitic acid	(n-)Hexadecanoic acid	256	18.6	0.0044

2.3.2.2 *GC-MS analysis of BSFL oil using a biodiesel column*

In an attempt to determine triglyceride content not seen in previous analysis, a specialised biodiesel column suitable for fatty methyl ester (FAME) analysis was used to examine oil obtained from orange, lime and potato-fed BSFL. A 007-65HT-25-0.1F polyethylene glycol phase capillary column (Quadrex, UK) was used following the previous GC-MS method (2.2.4). The column detected additional unsaturated fatty acids and selected FAMEs (Figure 21/Table 11). A calibration curve for lauric acid was determined (r^2 = 0.9991). The concentration of lauric acid ranged between 0.029 - 0.037 mol dm^{-3} for all analysis of lime, orange and lime oils undertaken. Several methyl esters of fatty acids were seen in analysis, along with several previously unseen unsaturated fatty acids. The presence of methyl esters is thought to be due to esterification with residual ethanol impurities within the GC-MS system. Interestingly, a minor amount of squalene was observed in this method, a chemical commonly obtained from sharks. The application of squalene is notable in modern cosmetic products as a skin softener and more recently, in vaccine formulations (Montana *et al.*, 2010).

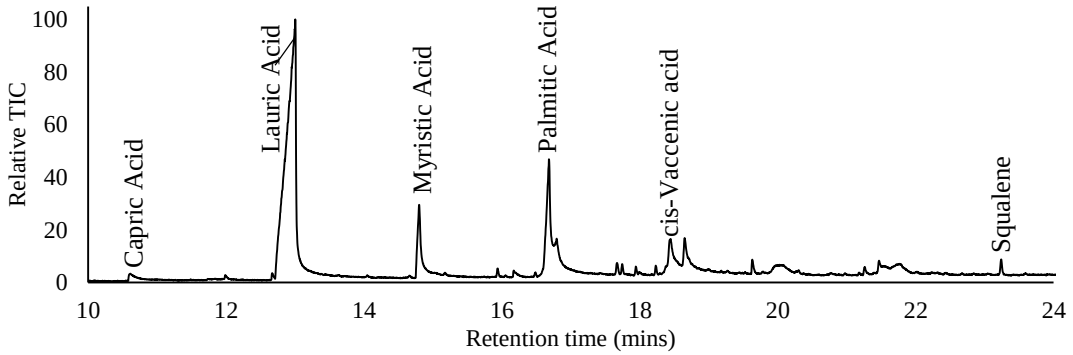


Figure 21: GC-MS chromatogram of raw BSFL oil (orange-fed BSFL), extracted via method 1 over the period of 2 days in hexane using a 007-65HT-25-0.1F GC-MS column (Quadrex, UK)

Table 11: Saturated and unsaturated free fatty carboxylic acids found in extracted raw BSFL oil fed an orange diet

C_n	*Common Name*	*IUPAC Name*	M_W	*Retention Time (mins)*	*Saturation*
10	Capric acid	(n-)Decanoic acid	172	10.6	Saturated
15	Ethyl tridecanoate	Tridecanoic acid ethyl ester	242	12.7	Saturated
12	Lauric acid	Dodecanoic acid	200	13.0	Saturated
14	Myristic acid	Tetradecanoic acid	228	14.8	Saturated
17	Methyl Palmitate	Hexadecanoic acid, methyl ester	270	15.9	Saturated
18	Stearamide	Octadecanamide	284	16.2	Saturated
24	Ethyl docosanoate	Docosanoic acid ethyl ester	369	16.5	Saturated
15	Pentadecylic acid	Pentadecanoic acid	242	16.7	Saturated
20	Arachidic acid	Eicosanoic acid	313	16.8	Saturated
16	Palmitic acid	(n-)Hexadecanoic acid	256	17.6	Saturated
19	Methyl (E)-octadec-10-enoate	10-Octadecenoic acid, methyl ester	254	17.7	Monounsaturated
18	9-Octadecenoic acid, ethyl ester	(E)-9-Octadecenoic acid ethyl ester	311	18.2	Saturated
18	cis-Vaccenic acid	cis-Vaccenic acid	282	18.4	Monounsaturated
18	17-Octadecynoic acid	17-Octadecynoic acid	280	18.6	Monounsaturated
30	Squalene	Squalene	410	23.2	Unsaturated

The validation of MCFA and LCFAs along with other useful compounds such as squalene confirms BSFL as a valuable feedstock. No triglycerides could be identified from the GC-MS analysis completed with this column. M/Z range was increased to 500 m_w but no discernible peaks suggesting the presence of triglycerides could be seen.

Following calibration curves for all main FFAs, concentrations were calculated for oils extracted from differently fed BSFL (Table 12). Analysis found that in general, composition of lauric acid and concentration were highest throughout all oils. High levels are of lauric acid are advantageous for several applications in the personal care and laundry detergent market. Following that, palmitic acid composition and concentration was high. Remaining % composition include the longer C_{18} unsaturated acids seen in GC-MS analysis.

Table 12: Composition and concentration of the dominant FFAs contained within the crude BSFL oil according to diet using a DB-5MS column (Agilent Technologies, US)

	Composition (% by mass)				**Concentration (mol dm^{-3})**			
BSFL diet	*Capric Acid C_{10}*	*Lauric Acid C_{12}*	*Myristic Acid C_{14}*	*Palmitic Acid C_{16}*	*Capric Acid C_{10}*	*Lauric Acid C_{12}*	*Myristic Acid C_{14}*	*Palmitic Acid C_{16}*
Orange	2.7	47.0	19.3	16.3	0.0003	0.010	0.008	0.008
Potato	2.1	44.5	8.6	18.3	0.0002	0.002	0.001	0.004
Beetroot	0.8	48.0	11.2	18	0.0001	0.005	0.003	0.006
Mushroom	0.9	50.6	9.3	15.9	0.0001	0.005	0.002	0.005
Mushroom/Potato	1.4	48.5	9.9	17.1	0.0003	0.004	0.002	0.005

2.3.2.3 *Characterisation of BSFL oil using FT-IR Spectroscopy*

Characterisation of individual MCFA and LCFAs in BSFL oil was not possible using FT-IR spectroscopy, but analysis of pure commercial lauric and palmitic acid (Sigma Aldrich, UK) were completed and compared to a BSFL oil spectrum (Figure 22). Peaks characteristic of carboxylic acids can be seen at the O–H stretch at 2922 cm^{-1}, the intense C=O stretch at 1745 cm^{-1}, the C-O stretch at 1153 cm^{-1} and the O-H bends at 1456 cm^{-1} and 1010 cm^{-1}.

Compared to that of the pure carboxylic acids, the characteristic sharp peak of a carboxylic acid O–H stretch in the 3300-2500 cm^{-1} is alike, but the C=O peak was higher in wavenumber in BSFL oil, where the pure LCFAs have peaks at 1693 cm^{-1}. The lowering of the frequency

of peaks can be attributed to weaker π bonds in the carbonyl functional group, so the higher frequency seen in BSFL oil could be a result of intermolecular bonding between molecules of different sizes, affecting the polarity of the C=O bond.

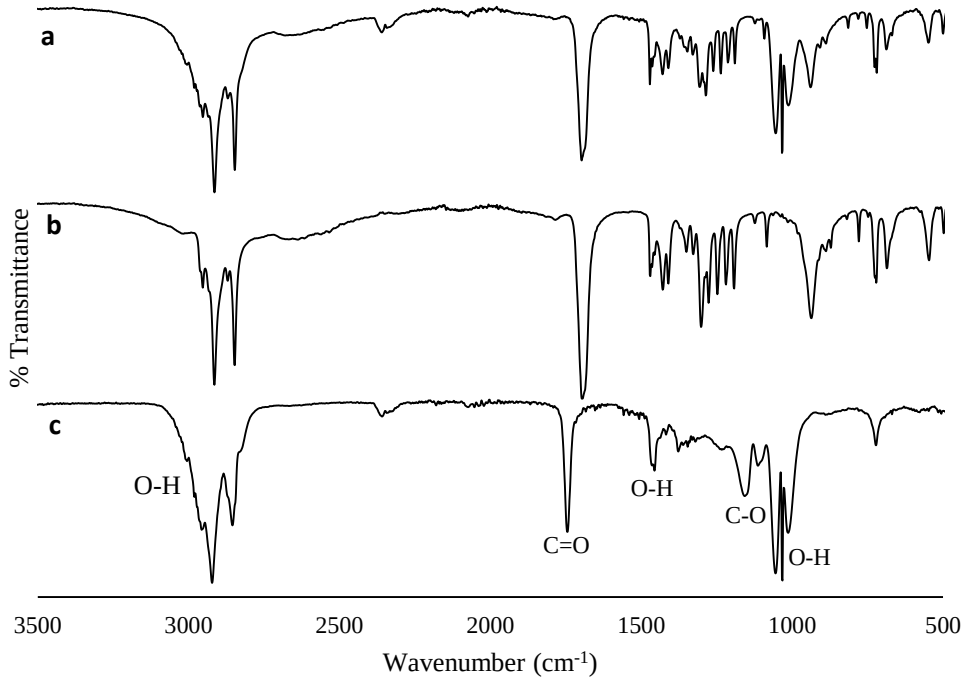


Figure 22: The IR spectrum of BSFL oil compared to that of component FFAs where (a) is palmitic acid, (b) is lauric acid and (c) is BSFL oil

2.3.2.4 *Acid Value*

Quantification of the A.V. of BSFL oils was completed by a titration with KOH, as seen in Table 13. The IUPAC standard procedure (Paquot, 1979) is used to determine FFA content in animal and vegetable oils and fats. Following procedure 2.2.9, expected A.V.s for the quantity used were between 4 – 15. A higher acid value is indicative of a higher amount of FFAs in the oil. A high FFA content is generally indicative that rancidity may occur upon storage.

Table 13: Acid value measurements for BSFL oil extracted from BSFL fed a variety of diets

BSFL diet	***A.V.***
Orange	14.59
Lime	12.34
Potato	11.22
Beetroot	16.04

Beetroot-fed BSFL oil required the largest neutralisation with KOH, suggesting that it contains a higher concentration of FFAs in comparison to oils extracted from BSFL fed other diets. The next highest A.V. was oil extracted from orange-fed BSFL, originally seen to contain the highest lauric acid concentration in GC analysis (Table 12).

2.3.2.5 *Density and Slip Melting Point*

To obtain a quantification of density, BSFL oils were first heated to 40 °C to obtain a homogenous liquid. Following method 2.2.12, density measurements were taken (Table 14).

Table 14: Comparisons for viscosities and SMPs of BSFL oils extracted from different diets

BSFL diet	*Density g/cm^3*	*SMP (°C)*
Orange	0.84	23
Lime	0.82	24
Potato	0.89	23
Beetroot	1.01	19
Mushroom	0.81	21
Mushroom/Potato	0.81	23
Palm oil (unrefined)	0.90	37
Coco oil	0.92	25

Aside from beetroot, BSFL oil from all diets proved to be lighter than the reference of DI water, averaging at around 0.8 g/cm^3. BSFL oil from a beetroot diet was seen to be any extremely vicious in measurements at 1.01 g/cm^3, its density approximately 20 % higher than the rest, but the reason as to why unclear at this stage.

Following method 2.2.10, visual evaluations of slip melting points (SMP) were also completed (Paquot, 1979), Table 14. As BSFL fat is a mixture of many FFAs, there is no sharp melting point, so the SMP provides some information about the melting point (Devi and Khatkar, 2017). Higher melting points would be indicative of a higher concentration of long chain fatty acids. Like density, oil extracted from beetroot fed BSFL had a lower melting point, suggesting that there is a lower amount of LCFAs present with the oil. In comparison to the same determinations with palm and coco oils, the BSFL oil from most diets likens more favourably to coco oil.

2.3.2.6 *Viscosity*

Viscosity measurements were obtained for oils from BSFL fed different diets (Table 15). To obtain a starting homogenous liquid, BSFL oils were heated to 40 °C and then allowed to cool to room temperature, whereby method 2.2.13 was completed.

Table 15: Density measurements for BSFL fat of different diets

BSFL diet	***Viscosity (Poise)***			***Relative Viscosity***		
	25 °C	*30 °C*	*35 °C*	*25 °C*	*30 °C*	*35 °C*
Orange	0.21	0.19	0.17	23.57	21.32	19.08
Lime	0.15	0.104	0.12	16.84	15.71	13.47
Potato	0.30	0.26	0.21	33.67	29.18	23.57
Beetroot	0.45	0.35	0.28	50.50	39.28	31.43
Mushroom	0.17	0.16	0.16	19.08	17.96	17.96
Mushroom/Potato	0.15	0.13	0.11	16.84	14.59	12.53

Viscosity measurements reflected the highly viscous nature of BSFL oil when a direct comparison to water (0.00891 Poise) is made. For the oils tested from different diets, oil extracted from beetroot-fed BSFL was determined to be the highest in viscosity. A viscosity measurement published for BSFL oil fed on broiler and cow manure, is comparatively 0.96 Poise (Cang *et al.*, 2019), generally three times higher than the oils studied in this project. No information on viscosity measurements from vegetable waste fed BSFL is available at this time. However, when compared to canola oil at 30 °C (0.48 Poise) (Diamante and Lan, 2014) for example, BSFL is deemed a less viscous oil in general.

2.3.2.7 *Cooling Curve*

Fats and oils typically have very characteristic cooling curves, describing the phase transitions accompanied by thermal changes, as most contain a complex mixture of compounds. To determine the change of phase matter of different BSFL-fed fat, a IUPAC standard cooling curve was completed (Paquot, 1979). The oil was heated to 40 °C and allowed to cool under STP, until solidification of the fat was observed. BSFL fed a diet of potatoes were seen to solidify sooner, suggested a larger concentration of LCFAs, whereas beetroot and mushroom BSFL took longer to achieve a lower temperature (Figure 23).

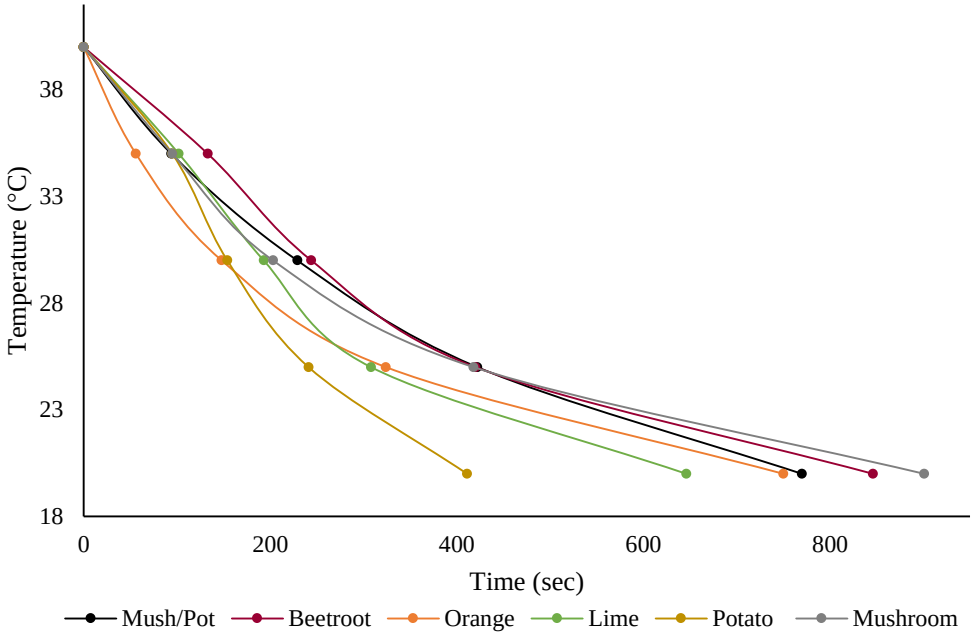


Figure 23: Cooling curves for BSFL fat extracted from BSFL fed different diets

2.3.2.8 *UV-Vis Spectrophotometry of BSFL oil*

UV-Vis spectra was completed to compare the fatty acid profiles (Figure 24) and show the distinction in colour between BSFL fed different diets. UV-Vis data of BSFL was compared to that lauric, capric, myristic and palmitic acid. Peaks for these carboxylic were seen at 224 nm, with similar peaks at 230 nm in the oils. Further peaks at 244 and 246 were seen in the oil, with a broader peak at around 280 nm like that seen in palmitic acid. Whereas the pure acids were translucent, due to the colorants contained within the fat, the absorption of light differed between oils extracted from differently fed BSFL. Mushroom-fed BSFL oil had the most adsorption of light, predictably as it is the oil naturally darkest in colour.

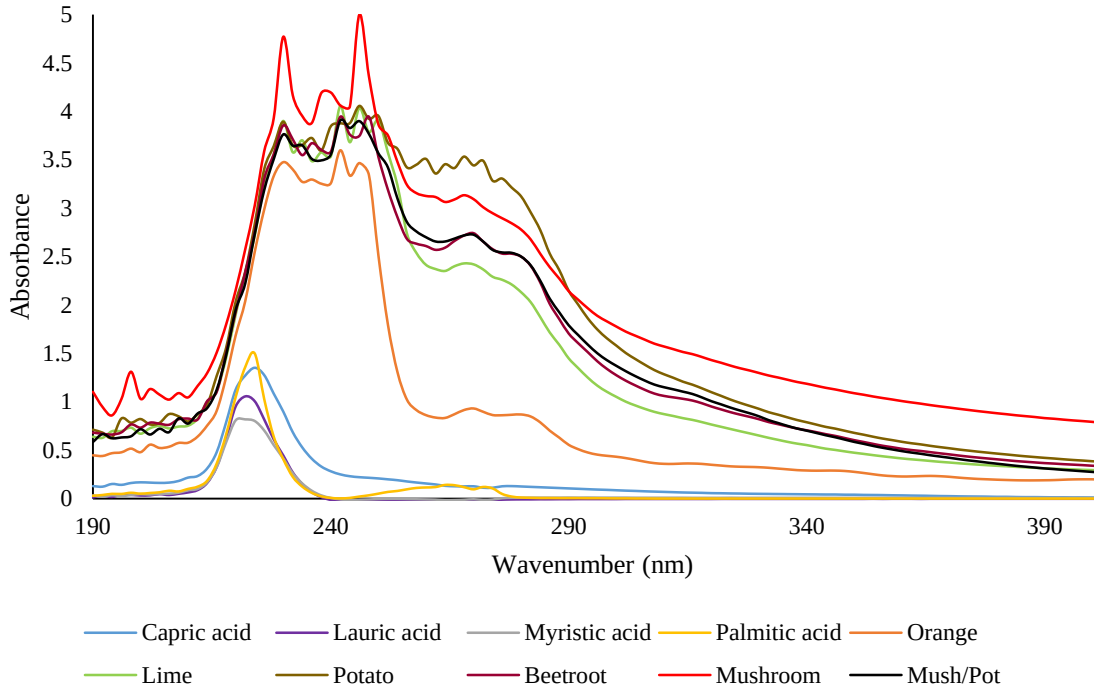


Figure 24:UV-Vis spectra of oils extracted from BSFL fed different diets

2.3.2.9 *ICP-MS analysis of BSFL prior/following fat extraction*

Ground BSFL were analysed using ICP-MS, following nitric acid treatment (Figure 25). Elemental results showed that the highest in concentration were calcium, potassium, magnesium and phosphorous. Concentration of different elements were seen to be higher in larvae that has previously had fat extracted due to reduced proportion matter. The completed analysis confirmed the lack of heavy metals exist in the larvae oil.

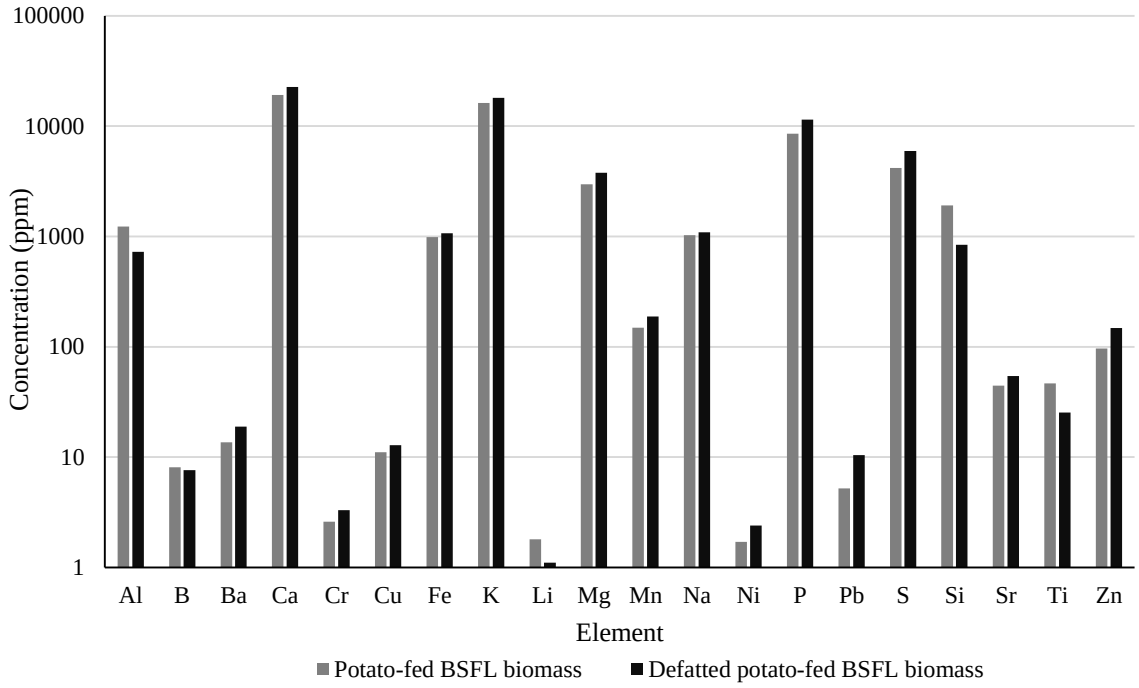


Figure 25: ICP-MS analysis of ground BSFL prior to and following fat extraction

Calcium (Ca) accumulation in the larval bodies is seemingly a result of the larvae having mineralised exoskeletons, where Ca is incorporated into the cuticle along with protein and chitin. In addition to calcium, magnesium is likely to also be contained within chitin complexes in the larval cuticle. High potassium (K) concentrations are likely to be the result of high K concentrations in potatoes, with potatoes with skin on containing 620 mg/g^{-1}. Likewise, high concentrations of phosphorus could be a result of the fertiliser contained within the potato feedstock.

2.3.3 Separation of BSFL oil using column chromatography

In an attempt to obtain pure fractions of FFAs from BSFL oil a column chromatography technique was used. Several separations using column chromatography with a silica gel stationary phase and ethyl acetate/dichloromethane mobile phase were completed. As the solvent mixture was added, fractions were collected every 30 seconds, and elution of products detected using TLC. For the highest yielding column separation of BSFL oil, it was determined by GC-MS analysis that combined fractions of 8-14, 15-20 and 21-25 contained only mixtures of pure FFAs. For fractions 8-14 lauric acid was the only observed FFA, but complete isolation of one FFA in other fractions was unsuccessful, with 15-20 containing lauric acid, myristic acid and palmitic acid, and 21-25 containing the aforementioned along with a high oleic acid

content. Further investigation using a DCM/EtO_2/Petroleum ether solvent mix at a ratio of 1:1:1 found similar results. Elution time was extended to 2.5 minutes and 37 fractions were collected. Of these fractions, 4-16 contained oil when solvent was allowed to evaporate, with varying degrees of colour seen. GC-MS analysis of oil fractions found incomplete separation, with the majority of evaporated fractions containing a mixture of capric, lauric, myristic, palmitic and 9,12-Octadecadienoic acids (*Z*,*Z*).

2.3.4 Separation of BSFL oil using distillation

Distillation was investigated to achieve separated fatty acid fractions from BSFL oil. Initially, BSFL oil was placed inside a round-bottomed flask using the laboratory set-up described in method 2.2.8. Upon heating, a warm buttery smell was observed. At 120 °C, burning of the oil was observed. Increasing the temperature of the oil bath to 220 °C led to no distillation of the oil, even with −0.6 bar applied, the maximum pressure the vacuum pump could obtain. Due to the high boiling points of FFAs, it was decided that using the current set-up distillation would be hard to achieve low enough pressure and therefore BSFL oil was converted to FAMEs using methanol to mitigate the conditions required. Distillation of the ester was found to be possible (Table 16) but when tested, it was found FFAs were not in distinct fractions. Starting with 6.36 g of BSFL FAMEs, fractions were taken every fifteen minutes, with continuous heat and stirring. After distillation completion, 5.25 g of colourless FAMEs were extracted, with 1.11 g of a "tar-like" substance remaining in the flask. GC-MS analysis of the fractions (Figure 27/Table 18) found many unsaturated FAMEs and colorants were removed in comparison to the mixed BSFL starting material (Figure 26/Table 17). Distillation achieved complete removal of containments within the oil, leaving fractions of pure colourless, mixed fatty acids.

Table 16: Distillation of BSFL oil conditions and resulting fraction yield

Fraction	Conditions	Yield (g)
(1)	150 °C / −0.4 bar	1.75
(2)	160 °C / −0.4 bar	1.3
(3)	170 °C / −0.4 bar	1.4
(4)	180 °C / −0.4 bar	0.22
(5)	190 °C / −0.4 bar	0.58

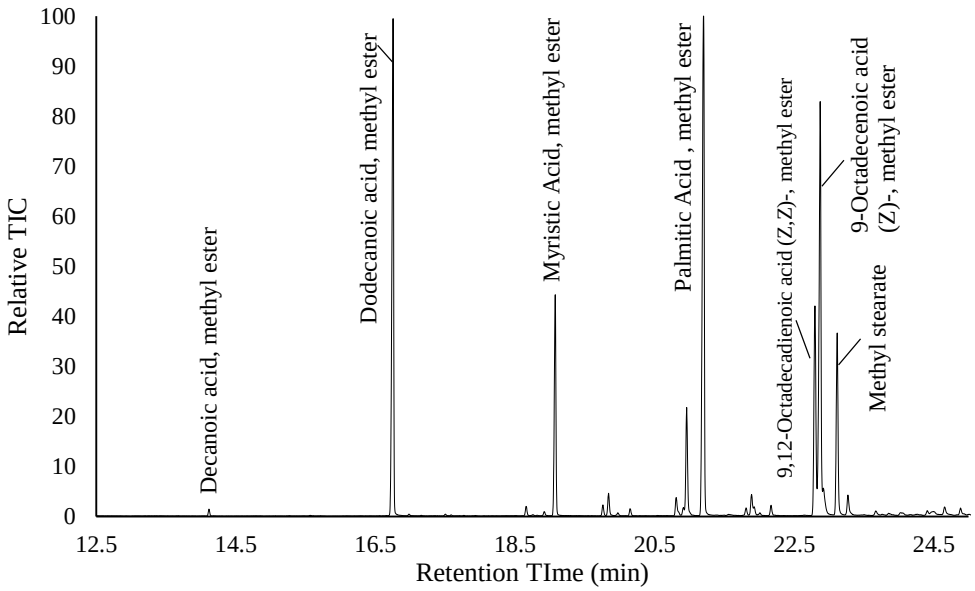


Figure 26: BSFL FAME GC-MS analysis prior to distillation using a DB-5MS column (Agilent Technologies, USA)

Table 17: BSFL FAME GC-MS analysis prior to distillation

Retention Time (min)	Name
14.11	Decanoic acid, methyl ester
16.75	Dodecanoic acid, methyl ester
18.92	Methyl myristoleate
19.08	Myristic acid, methyl ester
19.76	Pentadecanoic acid, methyl ester
19.84	Tridecanoic acid, 12-methyl-, methyl ester
20.15	Pentadecanoic acid, methyl ester
20.94	9-Hexadecenoic acid, methyl ester, (Z)-
21.20	Palmitic acid, methyl ester
22.80	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
22.87	9-Octadecenoic acid (Z)-, methyl ester
23.12	Methyl stearate
23.27	Methyl 10-trans,12-cis-octadecadienoate

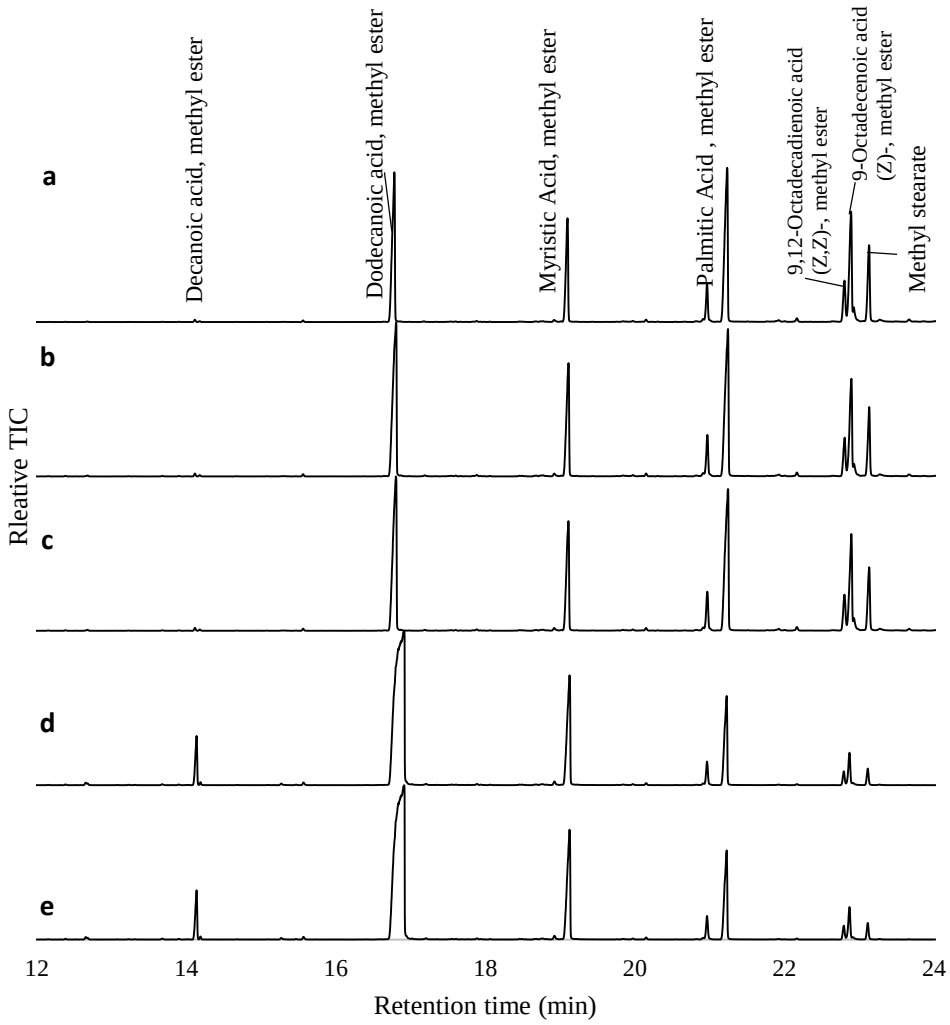


Figure 27: Distillation GC-MS data of five fractions taken at 15 min/10 °C intervals where (a) is FRACT2020003(1), (b) is FRACT2020003(2), (c) is FRACT2020003(3), (d) is FRACT2020003(4), and (e) is FRACT2020003(5) using a DB-5MS column (Agilent Technologies, USA)

Table 18: GC-MS data of five fractions taken at 15 min/10 °C intervals

Retention Time (min)	Name
14.14	Decanoic acid, methyl ester
15.57	Nonanoic acid, 9-oxo-, methyl ester
16.92	Dodecanoic acid, methyl ester
19.13	Methyl tetradecanoate
20.97	9-Hexadecenoic acid, methyl ester, (Z)-
21.23	Hexadecanoic acid, methyl ester
22.80	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
22.87	9-Octadecenoic acid (Z)-, methyl ester
23.12	Methyl stearate

The oil was purified using lab-scale vacuum assisted distillation, as pure fraction of mixed fatty acids were obtained, with no identified contaminants by GC-MS. Fractions were combined, and following method 2.2.7, the esterification was reversed, returning FAMEs to the mixture of most commonly FFAs found in BSFL oil, giving a pure mixture of capric, lauric, myristic, palmitic, 9,12-Octadecadienoic and 9-Octadecenoic acid (Z)- acids (Table 19/Figure 28). The liquid FAMEs returned to solid FFAs, signifying the structural change of the material and its melting point (Figure 29).

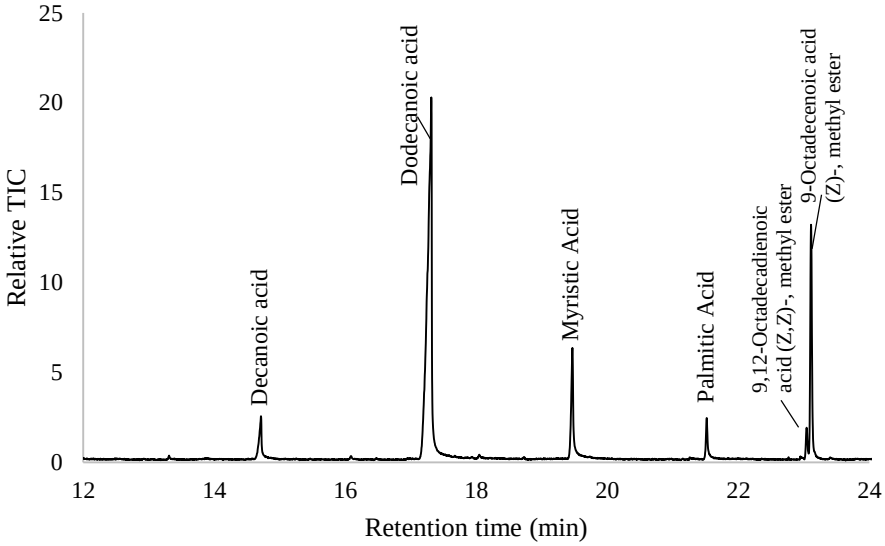


Figure 28: GC-MS chromatogram of reversed distilled BSFL FAMEs using a DB-5MS column (Agilent Technologies, USA)

Table 19: GC-MS analysis of reverse esterification of distilled BSFL FAMEs

Peak#	Ret.Time	Area	Height	Name
1	11.912	477738	130309	Octanoic acid
2	14.713	665440	236198	n-Decanoic acid
3	17.313	9236082	1979326	Dodecanoic acid
4	19.466	1395299	600287	Tetradecanoic acid
5	21.517	476780	225668	Pentadecanoic acid
6	23.043	318018	172637	9,12-Octadecadienoic acid
7	23.11	2290360	1269014	9-Octadecenoic acid (Z)-

Although a thermometer was inserted into the vessel, it is hypothesized that the accuracy of achieving exact boiling points means that complete separation is not possible. Therefore, in

this case, distillation of BSFL oil can be used as a separation method from its impurities and colorants, rather than as a fractionation method.

a ***b***

Figure 29: BSFL oil distillation of resulted in colourless oil following distillation (*a*) of FAMEs and (*b*) FFAs after reverse esterification

2.4 CONCLUSIONS

BSFL oil was successfully extracted using three different methods, where extraction using a soxhlet extractor was seen to be the most efficacious route. Determination of acid value, density, and viscosity was completed for BSFL oil. Refinement of the oil was achieved, with many impurities being removed using a lab-scale distillation. Each product was characterised as far as possible by TLC, GC-MS, UV-Vis and IR spectroscopic analysis.

Extraction and analysis of the BSFL oil were two of the three primary objectives for the research completed in this chapter. The investigation into extraction methods aimed to determine the optimum method for extracting the oil on a lab scale to provide insight on how this might be achieved best on a larger scale. It was found that soxhlet extraction as the best method; exploration suggests it is the superior technique with high yields obtained across all diets, and very sustainable with a recycling solvent mechanism. A commercially scaled version of this process would be energy intensive, but the alternative large scale method of manual pressing of the fat is not sufficient in creating a pure product, and if used would require additional purification steps along the same lines as fat recovery with solvents. As the industrial extraction of coco and palm oils does not involve the recovery of equally high-value proteins - up to 60 % of the larval body - then BSFL could not simply be used a replacement material and new industrial methodology would need to be created. The argument for this infrastructure conception would lie in that BSFL industrial rearing would provide an unconventional but safe, local, waste remediating and sustainable alternative to contemporary medium and long chain oil sources. The current natural resources are often not local to the location of the products for the desired consumer, meaning that there are significant transportation and environmental pollution issues associated with the cultivation of the oil so far from where the final products are required. In addition, the relatively short time needed to generate oil in BSFL, 5-6 weeks, is greatly advantageous when compared to the length of time needed for palm trees to fruit (4-5 years), and the annual yield produced. Along with this, is the substantial loss in biodiversity of such large scale plantations needed for the upkeep in demand for coco and palm based products, an issue that would not occur with locally grown BSFL in northern hemisphere climates. If BSFL escaped they would not be invasive pests, as it is likely the weather of European countries would lead to expiry of the insects outside of the carefully controlled environment required to rear them successfully. On the other hand, the cost of heating the large

scale areas needed to provide a large quantity of oil from BSFL would need to be factored in to overall budgeting.

The analysis of the oil was completed to determine and confirm similarity to coco and palm oils so commercial applications and any potential products from BSFL oil would be better understood and investigated. Full characterisation of the oils obtained from all methods explored found that lauric acid was the chief MCFA seen, conceding with current literature methodology, and very useful in providing a framework as a more sustainable waste remediating alternative to coco oil. As the infrastructure for the applications of the oil are already in existence, namely in this work the global manufacture of surfactants, once fractionally distilled to adequate purity, BSFL oil could readily replace coco and palm oils as a source of medium and long chain FFAs. Density and viscosity measurements were determined according to IUPAC standard methods and were useful in providing context of the oil behaviour with palm and coco. Further analytical determinations in analysis of BSFL oil and its derivatives were also completed, such as: cooling curves of the fat, acid values for all diets tested and UV-Vis spectrometry.

Diet was examined as a means to determine whether higher yields of the desired product, oil, could be increased by feeding the BSFL a singular dietary source during their development. Exploration into the effect of BSFL diet found that an orange-fed BSFL produced higher BSFL oil yields using all extraction methods. This was determined as being a result of the high sugar content in oranges, not fat content, as a 0.1 % lipid content was deemed not sufficient enough to determine any correlation to increasing BSFL fat proportion. Diets high in lipid content should be explored in the future to determine whether carbohydrate and sugar content are solely responsible for accelerated growth, or whether fat content would have a similar relationship. Other diets fed to BSFL were seen to drastically effect the yield of BSFL oil; and most did not conform with the 30 % oil yield frequently quoted in literature when using the optimum soxhlet extraction method (Newton *et al.*, 2005; Li, Zheng, Cai, *et al.*, 2011; Li, Zheng, Qiu, *et al.*, 2011; Manzano-Agugliaro *et al.*, 2012). To further explore the optimisation of oil extraction following solvent selection and extraction method, particle size was examined, where it was hypothesised a smaller particle size would be more likely to achieve higher extracted oils. When ground BSFL was sieved and different particle size grinds were oil extracted, it was found smaller particles were exponentially more successful in extraction with hexane. It is hypothesised that this is not only due to a larger surface area-to-volume ratio, but also because

larger particles contained more chitin, a fibrous robust polysaccharide found within the protective exoskeleton, meaning that they were more difficult to mechanically degrade. For this reason, it might be beneficial on a large scale for BSFL material to be mechanically ground as small as achievable, with the desirable particle size used below 250 µm, to increase fat yield.

Isolation of pure fractions of BSFL FFAs was completed using distillation. Separation of BSFL using silica packed column chromatography was not optimised successfully, with only some isolation of lauric acid achieved. Although several attempts resulted in fractions which contained similar components, no solvent ratio mix was seen to obtain pure fractions of capric, lauric, myristic palmitic acid or minor unsaturated acids by GC-MS analysis. Changes to mobile phase did improve the rate of separation, but did not improve the isolation of FFAs from one another. Subsequently, small scale laboratory vacuum distillations of oils were attempted, but were unsuccessful in also separating the FFAs into fractions. However, using distillation did remove smaller impurities and colorants, resulting in a very "clean" oil by GC-MS analysis. Issues with distillation are concluded to be mainly due to condition limitations, where a high enough vacuum or an appropriate temperature could not be achieved. Undoubtedly, on a large scale, where plant oils are mixed prior to distillation, fatty acids are currently easily fractioned and so it is supposed this would not be an issue industrially.

After achieving clean extracted oil, there are numerous applications available for conversion. A large percentage of palm and coco oils are used in surfactant and personal care products, along with biodiesel in developing countries, and lubricants in the manufacturing industry. As the oil has been proven to be extremely similar in composition to these oils, attempts of following similar pathways on a smaller lab-scale were tried in the following chapter. Along with this, utilising all aspects of BSFL - where protein is already used on mass scale as animal feed – chitin was extracted from the exoskeleton and modified for catalyst application.

In conclusion, the extraction of BSFL oil from BSFL fed a variety of diets was successfully demonstrated and analysed. Significant conclusions on the type of diet fed to BSFL and the most appropriate oil extraction method were made in this study, with a high sugar diet found to have a strong relationship with increased fat yield. Key characteristics such as slip melting point, cooling curves and acid value for oil produced from BSFL were defined. In addition, demonstration of how to achieve the isolated purified FFAs was completed through conversion

to FAMEs, distillation and then reversal to FFAs in a novel lab based route. Diets used in this study proved that the percentage of oil in whole larvae composition adaptable, and if desired, the diet can be tailored to produce more of a certain FFA. Analysis found that the oil, regardless of waste feedstock showed great promise as an alternative sustainable oil to traditional plant oils. This significant conclusion was made on the basis that analysis found the oil highly comparable in composition to coco and palm oil, and with similar properties. BSFL oil adds additional benefits to sustainable sources that plant oils cannot, in that it provides a waste-to-energy scheme, takes up less space than plantations and has a high turnover, unlike plantations were trees can take up to 10 years to fruit. On an industrial scale, the development pathway of the oil to a final commercial product would not be difficult to accomplish, with the means of extraction and distillation already in existence for presently used plant oils. There would be additional infrastructure needed in order to rear BSFL on an industrial scale, but as the protein and chitin parts are also useful components, there is a real possibility that BSFL could provide a sustainable waste-remediating alternative to contemporary oil sources.

3 BSFL Oil Applications

In this chapter, the principal aim was to utilise separated BSFL fatty acids in conversion to value-added products. As the composition has strong likeness to plant-derived oils, with a large proportion containing lauric acid, several potential applications of BSFL were explored using traditional methods, such as biodiesel or alcohols, and further conversion to corresponding surfactants.

Notably, fatty esters derived from naturally occurring fatty acids are prevalent in the bio-solvent market. Methyl laurate is currently derived primarily from plant-based oils (coconuts) and synthesised by esterification (Han *et al.*, 2014). In the industrial process of refining oils and fats (Figure 30), fatty methyl esters are synthesised as well as fatty acids, glycerol and the hydrogenation products of aforementioned products. Products of value include; fatty acid esters which are used are biofuels, alkyl epoxy esters which are plasticizers used in polymers, dicarboxylic acids used in resins, adhesives and surfactants, polyglycerol esters used in food emulsifiers, and fatty alcohol ethoxylates used in non-ionic surfactants (Salimon, Salih and Yousif, 2012). Using fats and oils extracted from BSFL biomass, processes seen in industry were attempted on a laboratory scale.

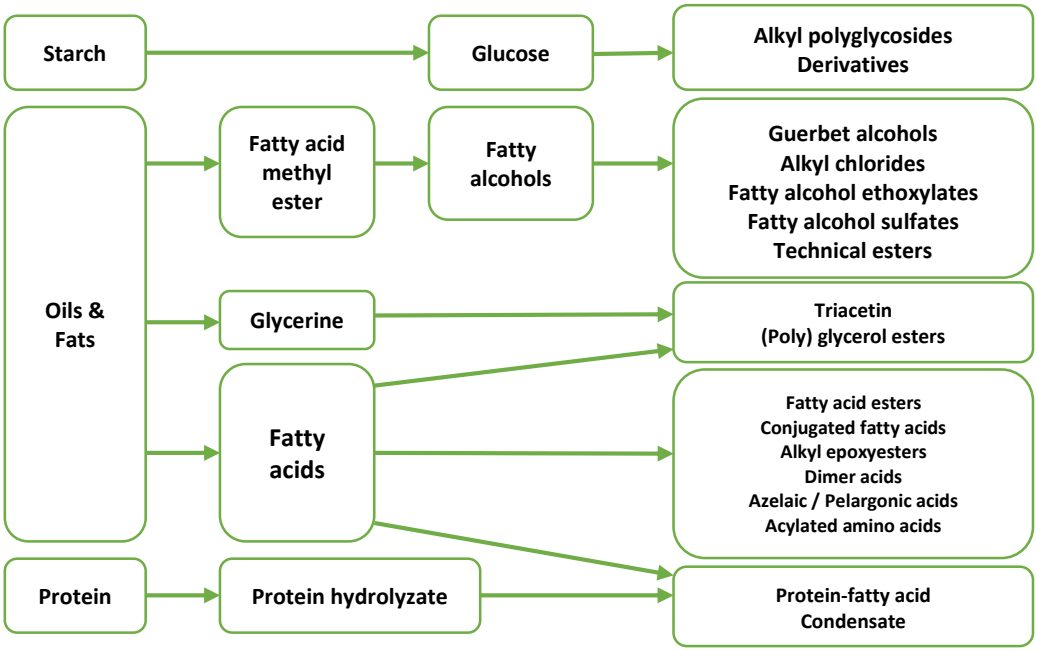


Figure 30: Products deriving from the industrial processing of natural oils and fats (Salimon, Salih and Yousif, 2012)

In May 2019, the concept for BSFL oil to provide a sustainable source for surfactants was submitted into a global collaborative innovation competition "Imagine Chemistry" organised by Nouryon (formerly AkzoNobel Specialty Chemicals). Out of 160 submissions from 30 countries, the research completed in this chapter was chosen as a viable concept, leading to an award of 4 weeks' of collaborative research, from a selection of 14 finalists.

Nouryon representatives emphasised a commitment to achieve sustainable raw material use wherever possible. The primary sources for the Nouryon surfactant product range are based on constituents from tallow, palm and coco oil. During various consultations, the idea of finding an economically stable source for their materials was stressed as of upmost importance, as FFAs from these sources, particularly palm and coco, are often subject to vast fluctuations in price (Figure 31). The partnership sponsor, Better Origin ltd. estimated the price of BSFL oil at around $2000/MT, more expensive on average than the plant alternatives, but only because BSFL oil is considered a by-product of protein production for animal feed, and not focused upon as a profitable commodity of its own accord up until now. If BSFL were reared for the sole goal of oil production with a by-product protein stream, this was estimated to reduce to $1500/MT. With recent prices of palm and coco oil, and historic fluctuations over the last 20 years, this price was not considered an issue by Nouryon as they felt the waste remediation background and carbon emission reduction of the sustainable source was worth the high price point.

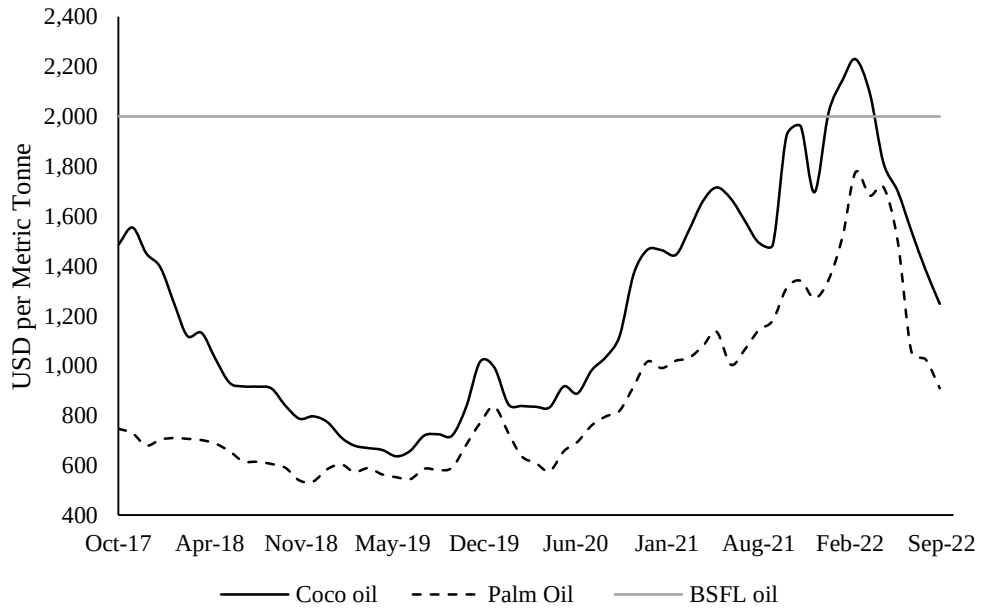


Figure 31: Coconut Oil vs. palm oil price comparison (data extracted from the US Department of Agriculture, assessed online (November 2022) at: https://www.fas.usda.gov/data/oilseeds-world-markets-and-trade

FFAs of particularly high value were described as being of lower carbon number, particularly between the C_{10}-C_{14} size, whereby there is a large existent market for surfactants of this type, with only niche markets for larger carbon chain length. In particular, Nouryon presented great interest in BSFL being a sustainable substitute to oils already used in their commercial synthesis, especially for use in its premium value amphoteric amine oxide surfactants such as lauryldimethylamine oxide. As a premium value application, the cost of this surfactant at €4.5 thousand/MT would justify the high BSFL FFA price point. Nouryon representatives were not greatly interested in the substitution of BSFL oil for in use in lower value surfactants such as sodium dodecyl sulphate at a price of €1.5 thousand/MT. However, additional applications for BSFL oil in markets other than surfactants, such as the lubrication and fuels market were discussed briefly and were shown great interest. To achieve a commercial product from BSFL oil, it was estimated that between €200,000-300,000 of investment would be required, inclusive of research and development, REACH testing and patent and intellectual property consultancy.

As part of the research award grant, it was agreed that use of pilot plant scale distillation equipment was to occur, allowing for the definitive separation of FFAs for purer synthesis. Due to COVID-19 complications, neither these activities nor synthesis of this surfactant group were completed in this research.

3.1 LITERATURE REVIEW

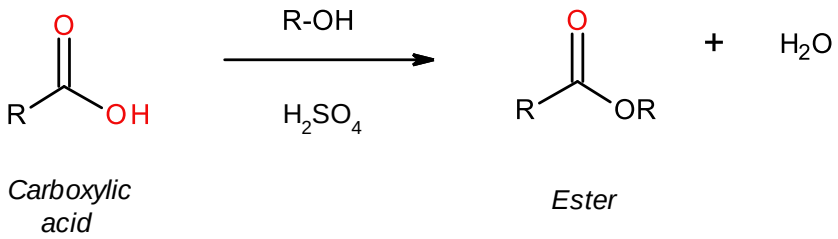
3.1.1 The biofuel and alternative fuel industry

Cellulosic biofuels are liquid fuels made from inedible parts of plants, and they offer an environmentally attractive carbon-neutral reliable alternative to fossil fuels, alleviating issues with the carbon dioxide release associated with refining and consuming crude oil (Huber G. W, 2009; Lee and Lavoie, 2013). Biofuels are traditionally produced using predominantly plant-based sources such as vegetable oils, grains and sugar cane, conventionally referred to as biomass. Biomass encompasses all biological-based matter, including both fauna (zoomass) and flora (phytomass) and is generally considered a completely renewable fuel (Akhtar and Amin, 2011; Ho, Ngo and Guo, 2014; Li, Zhao, Wang, Huber and Zhang, 2015). Biomass resources include; municipal processing waste such as sewage, cooking waste vegetable oil (WVOs) aforementioned 'energy' agricultural crops, aquatic plants such as algae and wood and wood waste such as sawdust (Felizardo *et al.*, 2006; Hung *et al.*, 2010; Jansri, 2015). Examples of zoomass include fish offal, crustacean shells, krill and herein BSFL.

First generation biofuels are obtained from edible biomass, primarily corn, soybeans, and sugarcane, and represent two thirds of bioenergy sources (Gasparatos, Stromberg and Takeuchi, 2013; Ho, Ngo and Guo, 2014). Ethanol fuel is produced through the fermentation of the biomass with a yeast strain (Lee and Lavoie, 2013). First generation biofuel production has numerous socioencomic and environmental impacts (Gasparatos, Stromberg and Takeuchi, 2013). One problem is that often crops are grown on land that could otherwise be used in food production, especially in developing countries such as Brazil, inciting the fuel versus food debate (Huber G. W, 2009; Popp, Lakner, Harangi-Rákos and Fári, 2014). Additionally, redirection of usage of these biomasses from animal food to biofuel would unsustainably raise the price of animal feed. Second generation biofuels propose an alternative, derived from an array of mixed feedstocks, including non-edible biomass such as homogenous wood chip, municipal solid wastes and forest residues (Lavoie, Marie-Rose and Lynch, 2013). Development of fuels from second generation biomass relies upon two distinct pathways "bio" or "thermos", which are currently energy intensive, require pre-treatment steps and therefore uneconomical in their infancy (Lee and Lavoie, 2013). Further development could result in competitive production to first generation. Third generation biofuels are manufactured from algal biomass, and is usually reliant on the lipid content of the biomass (Abbasi, Pishvaee and Mohseni, 2021). Chlorella has a high lipid content of between 60-70 % making it a very

favourable choice (Liang, Sarkany and Cui, 2009). Challenges associated with the production of third generation biofuels revolve around the large volume of water required at lab scale (Chen, Yeh, Aisyah, Lee and Chang, 2011), so large industrial scale production would problematic for northern hemisphere countries where conditions regularly drop below 0 °C in winter. As well as this, removal of the water from the lipid poses issues further in the production line, with additional filtration and centrifugation steps necessary (Lee and Lavoie, 2013). Compared to BSFL, the majority of FFAs in algal oil are unsaturated, ranging from C_{12}-C_{24} (Hippler, Collins and Thompson, 2014), which is often undesirable composition for further applications where saturated are required, such as the surfactant industry (Zoller, 2009).

Biofuels are synthesised using an acid-catalysed esterification, followed by a subsequent base-catalysed trans-esterification, of biomass-derived oils with an alcohol, typically methanol. The biofuels produced are mono-alkyl esters, which have been seen to burn excellently in diesel combustion engines. For example, palm oil based biodiesels have higher flash points that diesel, burning easier within the engine, whilst also reducing CO emissions by 46 % and hydrocarbon emission by 73 % (Abed, Gad, El Morsi, Sayed and Elyazeed, 2019). Esters are ordinarily produced from the heating of carboxylic acids with alcohols in the presence of an acid catalyst, most commonly concentrated sulphuric acid in a Fischer esterification, Scheme 1 (Clayden, 2012). Larger esters resulting from LCFAs tend to form more slowly, so in certain circumstances, it may be essential to increase the temperature, catalyst quantity or reaction duration under reflux. Depending the size of the ester, separation from reagents can be accomplished by fractional distillation or column chromatography.



Scheme 1: The Fischer esterification of carboxylic acids with primary alcohols produces esters

Plant-based oils do represent a potential successful alternative source for current fossil fuel based fuels, but farming of such large land, along with the inorganic fertiliser and water requirements means that this strategy is unsustainable for a global population of 7.6 billion (United Nations, 2017). Furthermore, the farming of crops for the production of first generation

ethanol-based biofuel, or plant oils for biodiesel production could be considered unethical when the UN classifies 1 in 7 of the global population are as 'starving' (United Nations, 2017). There is a potential that the energy crisis could be replaced by a food one instead (Cholakov, Yanev, Markov and Stoyanov, 2013).

Microbial biogas production using presents another alternative, but its optimisation is costly and time-consuming, meaning that it is economically difficult to justify on a large scale (Gebrezgabher, Meuwissen, Prins and Lansink, 2010), although it has proved efficient in off-grid communities (Mao, Feng, Wang and Ren, 2015). Previous studies and research has been based on small-scale micro-grid production using anaerobic digestion by microorganisms as a source of biogas (Khalid, Arshad, Anjum, Mahmood and Dawson, 2011), following the same waste-to-energy methodology as this project.

The project aims to adhere to the 12 principles of green chemistry, a framework for making a greener chemical, process, or product proposed by Paul Anastas and John Warner in 1998 (Anastas and Warner, 1998). This project aims to conform through principles of; the use of renewable biocatalysts – namely BSFL, the use of renewable feedstock, minimization of hazards, and the generation of substances with as little toxicity as possible (Schwartz, Ollilainen, Piironen and Lampi, 2008; Chen *et al.*, 2020) in the creation of sustainable surfactants.

3.1.2 Surfactants

Surfactants, denoted so because of their ability to be surface-active agents, are molecules capable of lowering the interfacial tension within a liquid or solid mixture. Although not a unique property to surfactants, they are amphipathic meaning that self-assembling micellar structures form under specific pH, hydrophobic or hydrophilic conditions (Kronberg, Holmberg and Lindman, 2014; Dave and Joshi, 2017). There are four main surfactant classifications; anionic, cationic, non-ionic and amphoteric. Anionic and cationic are both ionic surfactants, where the head of the surfactant carries a net charge, unlike in non-ionic surfactants where there exists no charge group (Rosen and Kunjappu, 2012). The basic structure of an anionic surfactant can be seen in Figure 32, where the polar "head" group seen on the right has a tendency to dissolve more readily in water environments, and the hydrocarbon "tail" in oilier ones.

Figure 32: Schematic illustration of a surfactant

Micelles are the spontaneously formed colloidal aggregations of surfactant monomers. At a bulk concentration, it becomes energetically favourable to form micelles within the solution, which is dependent on the size, shape and polarity of monomers (Griffiths *et al.*, 2013). In micellization (Figure 33), the hydrophobic tail of the surfactant is removed from contact with water within the environment, thus aiding reduction of free energy of the system (Dave and Joshi, 2017).

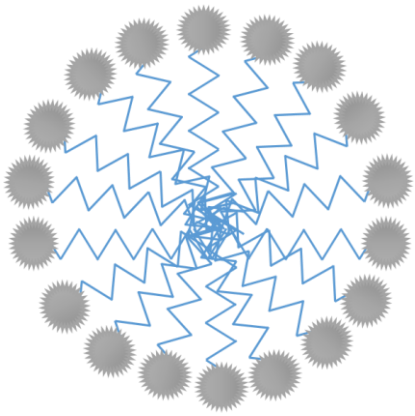


Figure 33: Scheme of a micelle formation by monomers within an aqueous solution

Monomers behave differently in the system to micelles, as the surface tension lowering ability, and therefore characteristics such as wetting and foaming, are dictated by the concentration of free monomers in the system. The concentration at which micelles form is denoted the critical micellar concentration (CMC) (Muller, 1994). Once the CMC is achieved, the surfactant present acts as an emulsifier, removing the insoluble oily target material by encompassing it within the micelle core. Due to the hydrophilic nature of the surfactant head, the micelle is carried away upon rinsing. Efficient surfactants have low CMC values (De, Malik, Ghosh, Saha and Saha, 2015). CMC values are typically quoted as millimolar (mM), or mmol/L.

Surfactants are commonly utilised in many industries as cleaning, wetting, dispersing, emulsifying, foaming and anti-foaming agents. Feedstocks range from those that are petroleum derived, to, more recently, the mass production of bio-based surfactants, due to changes in

consumer preferences and corporate shifts towards sustainability. In 2005, the consumption of surfactants accumulated to almost 2.9 million tons in Europe, Table 20 (Zoller, 2009).

Table 20: The distribution of surfactant classes consumed in Western Europe, 2005 (Zoller, 2009)

Surfactant Type	Percentage distribution (%)
Anionics	40
Non-ionics (ethoxylates)	44
Cationics	9
Amphoterics	2
Other non-ionics	5

There are four main sources for surfactants; petroleum sources, palm oil, coco oil and tallow fat (Zoller, 2009). With palm and coco surfactant sources being similar in composition to the fat separated from BSFL, there are many applications for the free fatty acids, present therein. As the carbon chain length ranges from C_{10-18}, the ability of BSFL to be used in the manufacture of surfactants in the same way as industrial favourites palm, palm kernel and coco are explored in this chapter.

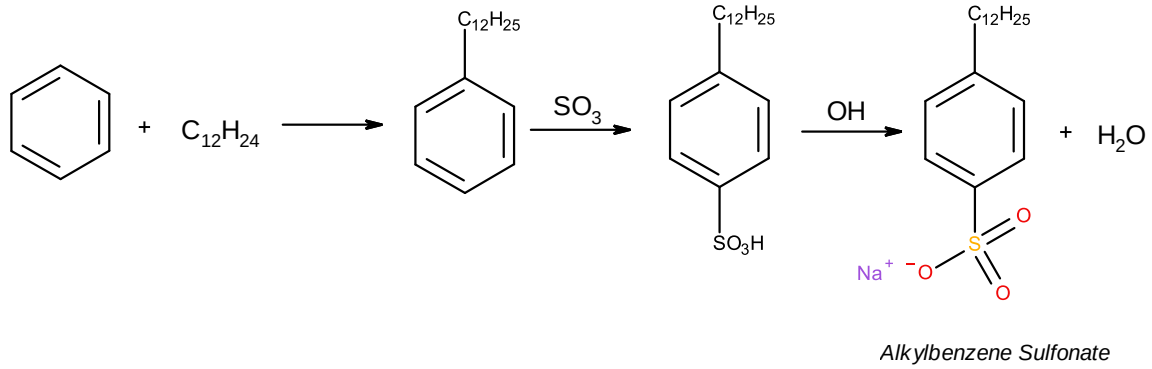
3.1.3 Anionic surfactants

Anionic surfactants have a negatively charged hydrophilic tail group and a positively charged hydrophobic head. They are the most widely used type of surfactants for laundering, dishwashing liquids and shampoos, making up 75 % of the global market (Stache, 1995; Pletnev, 2001). They are particularly good at keeping oily and waxy substances, once dislodged, away from fabrics using micellar aggregation. Examples of anionic surfactants include; alkyl sulfates, akyl ether sulfates, alkyl benzene sulfonates and basic soaps (Bajpai and Tyagi, 2007).

3.1.3.1 *Linear alkylbenzene sulfonates*

Linear alkylbenzene sulfonates (LABs) are formed using aluminium chloride and hydrogen fluoride as a homogenous catalyst or zeolite/acidic heterogeneous catalyst (Kocal, Vora and Imai, 2001). The process is heavily sourced from fossil fuel resources (Zoller, 2009). In the presence of an acid catalyst such as aluminium chloride, benzene, in excess, is mixed with an alkene or chloroalkene along with hydrofluoric acid to yield alkylbenzene. Sulfonation using an air/sulphur trioxide (SO_3) mixture gives rise to the alkylbenzene sulfonate, with resulting

sulfonic acid being neutralised with aqueous NaOH solution (Scheme 2). The alkylbenzene produced will vary in molecular mass depending on the starting materials and catalyst used (Craciun, Reyniers and Marin, 2007).



Scheme 2: Linear alkylbenzene sulfonate production

Legal stipulations restrict the variety of alkenes used, and because branched side chains biodegrade more slowly, branched alkylbenzene sulphonates (ABS) are banned in most developed countries (Hashim, Kulandai and Hassan, 1992). Straight chain alkenes are produced from ethane and a Zieglar-Natta catalyst, a common polymerisation catalyst (Corradini, Guerra and Cavallo, 2004). Alkyl benzene sulfonates are frequently found in powder and liquid laundry detergent formulations (Kocal, Vora and Imai, 2001; Wang, Chuang, Hsu and Tsai, 2017) .

3.1.3.2 *Alkyl sulfates*

Sodium alkyl sulfates (SAS) are linear surfactants extensively used in household liquid detergent products, and personal healthcare products such as toothpaste. They are primarily manufactured by treating long and medium chain alcohols with 2-8 % SO_3 in dry air (Foster, 1997). The immediate is neutralised with NaOH to obtain the SAS (Figure 34).

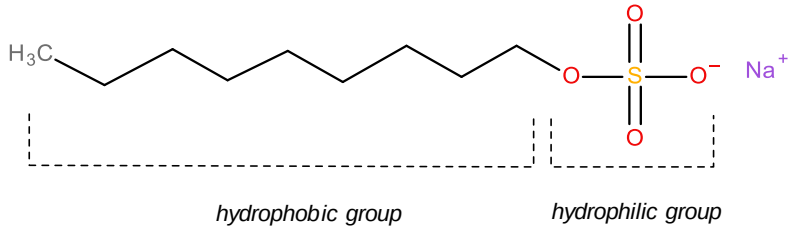


Figure 34: Example of a sodium alkyl sulfate

Reagent long chain alcohols are either produced from the reduction of carboxylic acids from palm kernel or coconut oil sources, or alternatively from the hydroboration–oxidation of long chain alkenes, manufactured from ethene sourced from natural gas or petroleum (Zoller, 2009; Kronberg, Holmberg and Lindman, 2014; De, Malik, Ghosh, Saha and Saha, 2015). Along

with gaseous sulphur trioxide in dry air, there are alternative reagents for sulfonation: sulfamic acid, chlorosulfonic acid, or oleum (Foster, 1997). Dry air in combination with a continuous flow of SO_3 is by far the most common industrial method, Table 21.

Table 21: Industrial reagents used in the sulfonation of alcohols *(Million kg/yr) (Knaggs, 1992)

Plant Type	1980		1985		1990	
	No. of Plants	Capacity*	No. of Plants	Capacity*	No. of Plants	Capacity*
SO_3	44	630	62	855	41	1016
Oleum	51	720	50	605	39	548
Chlorosulfonic Acid	5	35	6	50	6	59
Sulfamic Acid	1	0.45	2	2	1	0.45
Total	101	1385.45	120	1511.35	87	1623.45

Chlorosulfonic acid can be used to sulfonate in either a batch or continuous process. In the batch process, Figure 35, a glass lined, stirred, sealed reactor is fitted with heating and cooling jackets. In operation, an alcohol or ethoxy alcohol feedstock is sent to the reactor and chlorosulfonic acid is gradually added. The reaction is exothermic, and must be kept at below 25 °C to avoid side reactions and colour body formation and to minimize foaming. The rate of chlorosulfonic acid addition is monitored to ensure that this is not exceeded. A glass lined absorber is used with the aid of a vacuum to ensure the removal of HCl gas which is liberated with the breakdown of chlorosulfonic acid. The HCl gas is absorbed within water to make a dilute HCl solution for easy removal. Once sulfonation is complete, immediate neutralization with NaOH is required (Foster, 1997; Dado, Knaggs and Nepras, 2006; Dado and Bernhardt, 2017). Product yield, quality and colour can be high but the throughput efficiency is modest in batch mode (Zoller, 2009) . This process can be easily lab-scaled to provide similar results.

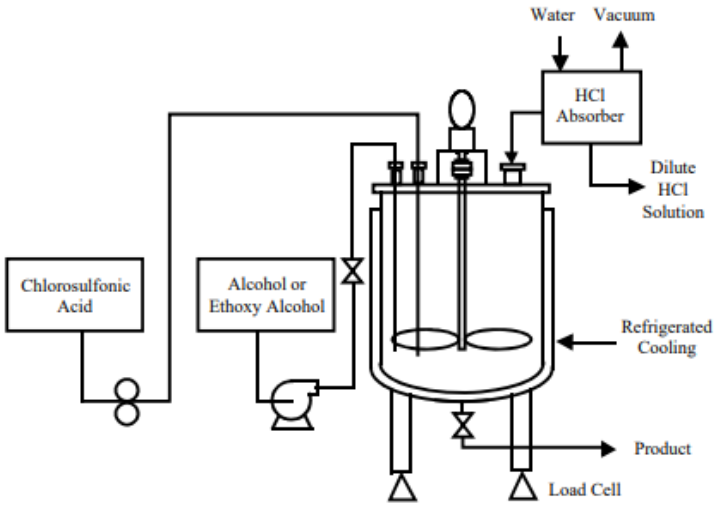


Figure 35: Batch sulfonation of alcohols with chlorosulfonic acid (Foster, 1997)

On an industrial scale, sulfonation with chlorosulfonic acid reagent is mostly continuous, Figure 36. In operation, the fatty alcohol and chlorosulfonic acid are added into a mixing zone, combined and sent to a degasser. Like the batch process, some HCl is removed from the reagents with vacuum assistance. Separated sulfonic acid is sent through a heat exchanger to remove heat and the energy produced is recycled in the mixer to cool the process. A percentage of the reaction mixture is sent to a second degasser to ensure full HCl separation. The HCl is continuously absorbed into water which is continuously neutralized with base. After separation, excess sulfonic acid is sent to a neutralization vessel and mixed with aqueous sodium hydroxide. The process is often economically viable but does create a product with traces concentrations of undesirable chloride ion. (Foster, 1997; Dado, Knaggs and Nepras, 2006; Dado and Bernhardt, 2017)

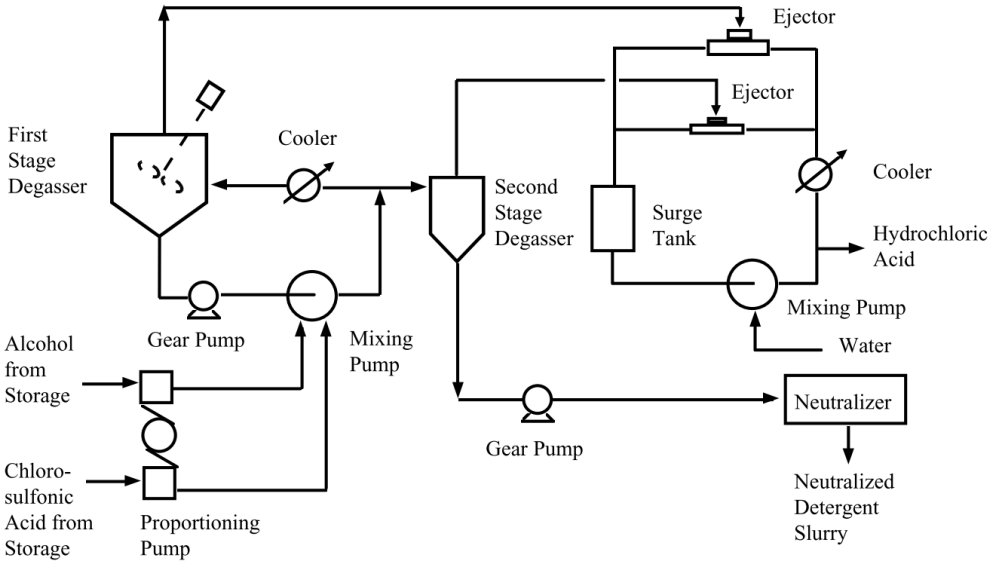
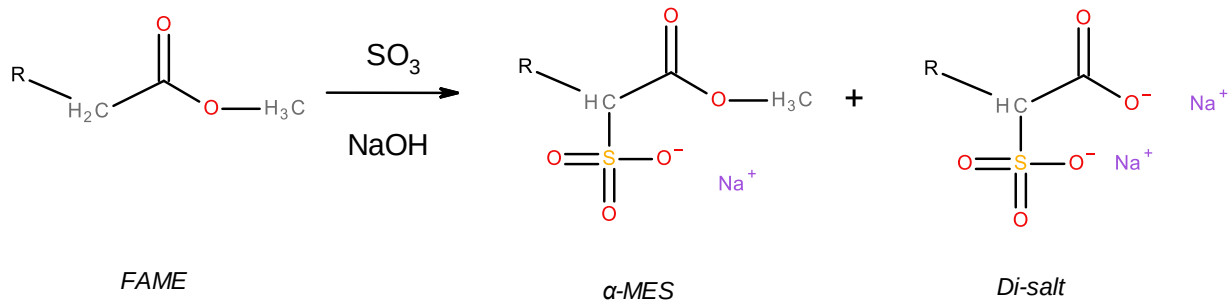


Figure 36: Continuous Alcohol Sulfonation with chlorosulfonic acid (Foster, 1997)

3.1.3.3 *Methyl Ester Sulfonates*

Methyl ester sulfonates (MESs) provide a viable and environmentally friendly alternative to LABs and are therefore growing in popularity, mitigating any issues with variability in crude oil prices (Soy, Kipkemboi and Rop, 2020). Palm, palm olein or palm stearin are all potential feedstocks, as detergent grade MESs often have long $C_{16\text{-}18}$ chain hydrophobic groups (Zoller, 2009; Lim, Baharudin and Ung, 2018). Applications of sulfonates include; emulsifying or demulsifying agents, dispersants, wetting aids, corrosion inhibitors, fluidizers, foamers, and detergents (Zoller, 2009).

In industrial manufacture of MESs, there are two potential routes. In one, fatty acids can be sulfonated using dilute SO_3 with subsequent esterification (Tobori and Kakui, 2019). Alternatively, fatty acid alkyl esters are produced through transesterification of triglycerides or the esterification of free fatty acids with alcohol, typically methanol. The product is then sulfonated using gaseous SO_3 and neutralised with an alkali to obtain the product, α-MES, and its disodium salt by-product, as seen in Scheme 3 (Pletnev, 2001). The solubility of the di-salt is lower than α-MES, resulting in lower solubility when concentrations are high in the final product. As a result, the concentration of di-salt is undesirable above 5 %, otherwise the solubility and surfactant properties of MES are affected, especially within hard water applications (Fujiwara, Miyake and Abe, 1993; Xie, Zeng, Wang and Zhang, 2013).



Scheme 3: Mechanism of fatty acid ester sulfonation

Methyl ester sulfonates are often found in liquid detergent and personal care products because of their milder detergent behaviour, generating less "foam" than their alkyl sulfate counterparts, an advantageous feature in many laundry formulations (Roberts *et al.*, 2008; Zoller, 2009).

3.1.3.4 *Soap*

A soap is the salt of a fatty acid (Figure 37), more specifically referred to as toilet soap when sodium and potassium are the anionic component. They are formed through the reaction of long chain carboxylic acids and a base (Hollstein and Spitz, 1982). The industrial manufacture of basic soaps was introduced to the general public around 1932 (Zoller, 2009). Natural oils were used as feedstocks, vegetable oil or tallow being the main component. Contemporary sources are derived mainly from are palm, palm kernel and coconut oil and occasionally tallow (O'Brien, 2008). If sources are coco or palm kernel based, the carbon chain generally ranges between $C_{12\text{-}14}$, with tallow and palm ranging between $C_{16\text{-}18}$ (Swern, 1964; Schumann and Siekmann, 2000). Longer chain lengths are typically unattractive in laundry detergents due to poor solubility in hard water (Dado, Knaggs and Nepras, 2006).

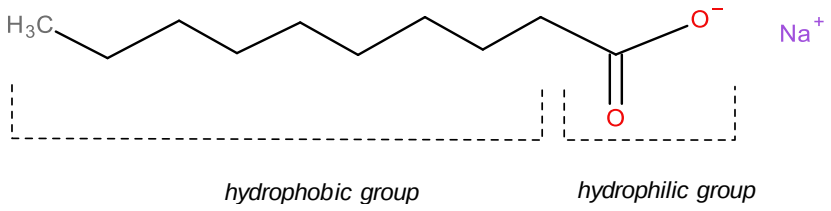


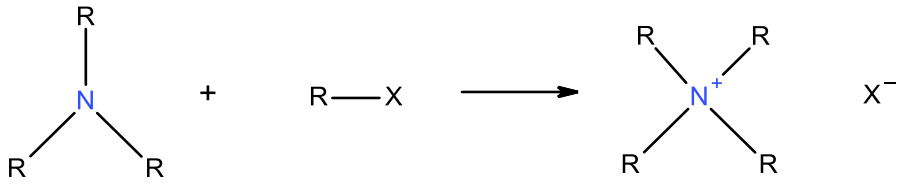
Figure 37: Schematic of a simple toilet soap

Due to the drying nature of simple alkaline soaps on the slightly acidic epidermis of the skin, nowadays, conventional soap bars are a mixture of traditional natural oil derived salts fatty acid and the more complex synthetic surfactants previously mentioned. They are denoted combo "syndet" toilet bars, and have milder properties, with softer detergency, making them more suitable for sensitive skin and advantageous over simple soap (Zoller, 2009). Along with a lower active matter, the bars contain additives to improve performance, for example; colouring to provide a pleasant appearance, perfume to provide a pleasant smell and glycerine as a moisturiser (Hollstein and Spitz, 1982). Formulation of syndet bars can be challenging on an industrial scale.

3.1.4 Cationic surfactants

Cationic surfactants are characterised by the presence of a large positive hydrophilic group, and a corresponding negative counter ion, which dissociate in the presence of an aqueous solution (Kronberg, Holmberg and Lindman, 2014; Dave and Joshi, 2017). R groups are adaptable and can be saturated or unsaturated, cyclic or acyclic, branched or unbranched or aromatic, Scheme 4. Up to 90 % of this class of surfactants is made up of quaternary ammonium compounds (QACs) (Pletnev, 2001), but other examples include alkyl amines, ethoxylated amines and alkyl imidazolines (Zoller, 2009). Cationic surfactants are not particularly good

detergents, having relatively poor dirt and oil removing properties, but they exhibit valuable germicidal and antistatic behaviour (Cross and Singer, 2019). The monomers are easily absorbed onto negatively charged surfaces and provide "softening" and therefore antistatic effects (McDonnell, 2009). For this reason, cationic detergents make excellent conditioners, appearing in fabric softeners, emulsifiers and antistatic agents (Kronberg, Holmberg and Lindman, 2014).



Scheme 4: Preparation of cationic surfactants

3.1.4.1 *Quaternary ammonium compounds*

QACs are colloquially referred to "quats" and are produced by reaction of tertiary amines with an alkyl halide or benzyl chloride in a quaternization reaction (Cross and Singer, 2019). A strong acid is used as a neutralising agent. Due to the presence of ester links, they are more biodegradable than previous quats, with 94 % degradation seen after shaking-flask degradation tests (Qin, Zhang, Kang and Zhao, 2005) and therefore they tend to have lower toxicity (Sioris and Schuller, 2007; Steber, 2007; Mishra and Tyagi, 2011). QACs are permanently charged and are effective independent of solution pH (Figure 38) (Rhein, 2007), which is very beneficial in certain applications.

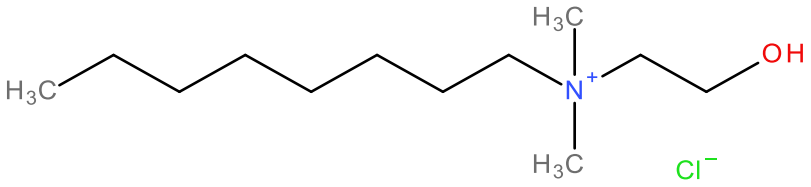


Figure 38: Structure of an alkyl quaternary surfactant

Esterquats are examples of QACs used in fabric softeners in tandem with anionic surfactants (Figure 39). They are excellent at bonding strongly to negatively charged solid surfaces such as fabric, but poor at being effectively removed in the rinsing process (St. Laurent *et al.*, 2007). The esters are synthesised by reaction of fatty acids with a tertiary alkanolamine, followed by reaction with an alkylating agent to the corresponding quaternary in catalyst presence (Cross and Singer, 2019). To ensure a high ester conversion rate, reagents are heated up to

temperatures of 250 °C, and water removal is completed either by vacuum, or by stripping with an inert gas. Reaction times vary from several hours to up to 10 hours, depending on conditions used and catalyst and reagent reactivity. In the quaternisation, reaction temperatures of up to 100 °C are used, with an alkylating agent such as dimethyl sulfate or methyl chloride (Foster, 1997). Due to corrosion concerns from the alkylating agent, this is usually completed in a glass lined or stainless steel reactor. In addition, the reaction takes place with a lower stoichiometric amount of dimethyl sulfate, to ensure there is no trace of the toxic reagent in the end product (Zoller, 2009).

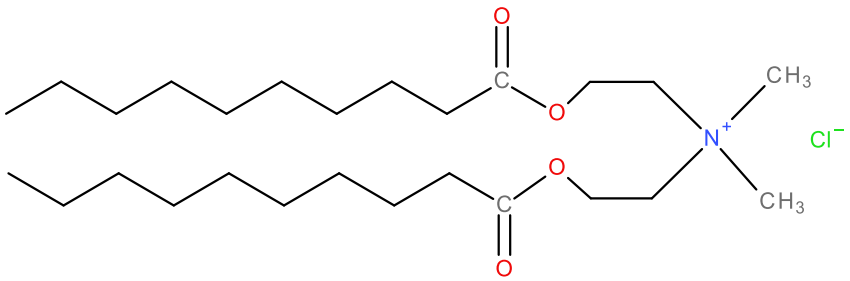
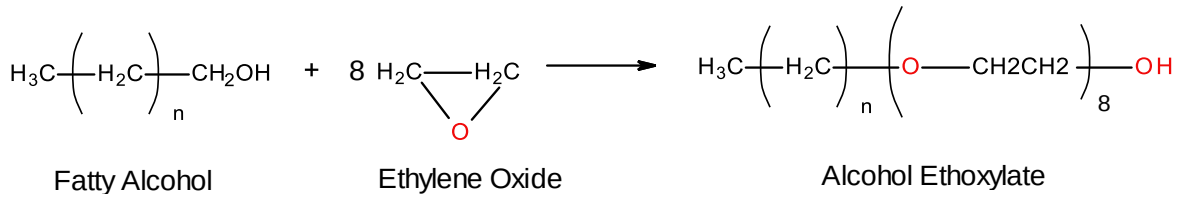


Figure 39: Structure of an esterquat

3.1.5 Non-ionic surfactants

Non-ionic surfactants are amphiphilic detergents with no charged counterparts and when dissolved in a solvent, such as water, non-ionics do not dissociate to form ions (Pletnev, 2001). Despite a lack of an ionic main hydrophilic group, hydrophilic properties exist through the presence of a number of oxygen atoms in one part of the molecule, making the monomer capable of strong hydrogen bonding (Dekker, 1997; Kronberg, Holmberg and Lindman, 2014). In cases where the long hydrophobic ethylene chains exist, this effect is cancelled out (Shinoda, 1963). On the other hand, some non-ionics are able to acquire a charge according to pH level. Examples include long chain carboxylic acids, which act like non-ionic surfactants under neutral pH, and then anionic in basic conditions (Pletnev, 2001). In the majority of cases, the lack of ionization in solutions means that water hardness deactivation is avoided (Bajpai and Tyagi, 2007). Furthermore, non-ionic detergents are low sudsing, so are advantageous in some general purpose applications. Examples of non-ionic detergents include alcohols, ethoxylates, polyethers esters or variable combinations of both (Foster, 1997). Synthesis typically involves the condensation of a long chain alcohol with ethylene oxide, forming an ether, Scheme 5.



Scheme 5: Non-ionic surfactant synthesis of alcohol ethoxylates

When discussing non-ionics, the term HLB is frequently used. HLB refers to the hydrophile–lipophile balance within the monomer. Those with a high HLB (greater than ~12) are predominantly hydrophilic and water-soluble (Gelardi *et al.*, 2016). Detergents with lower HLB's, are hydrophobic and insoluble. The concept is vital in formulation chemistry, as the HLB of the surfactant will need to be considered when blending with a higher HLB surfactant (Attwood and Florence, 2012). The HLB value can be quantified by Griffin's method for non-ionic surfactants, as seen in Equation 4, where M_h represents the molecular mass of the hydrophilic portion of the molecule, and M the molecular mass of the whole molecule (Griffin, 1954).

$$HLB = 20\left(\frac{M_h}{M}\right)$$

Equation 4: Calculation of HLB values of non-ionic surfactants(Griffin, 1954)

3.1.5.1 *Fatty alcohols*

Primary medium and long chain alcohols can be used singularly as surfactants, as they behave with similar properties to non-ionics, negating any micellar formation in water (Attwood and Florence, 2012; Noweck and Grafahrend, 2012). They are used widely as co-surfactants in detergent formulations along with anionic surfactants, contributing in emulsifying and foam stabilising (Pletnev, 2001). Traditional sources of long chain alcohols are natural fats and oils, Figure 40, but synthetic oils have been used too (Noweck and Grafahrend, 2012; Rebello, Asok, Mundayoor and Jisha, 2013).

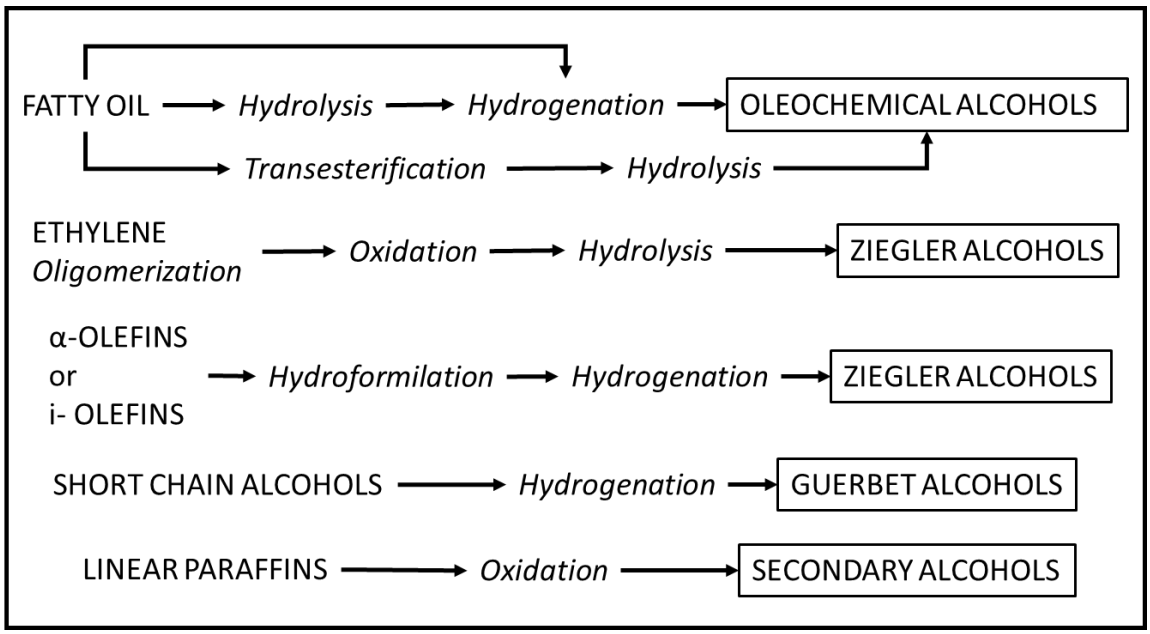


Figure 40: Non-ionic fatty alcohol surfactants have lots of potential sources (Pletnev, 2001)

3.1.5.2 *Sorbitan Esters*

Sorbitan esters, commercially known as "Spans", are mixtures of the partial esters of sorbitol and its mono- and di-anhydrides with a long chain fatty acids (Pletnev, 2001). They generally have lower HLB values and are therefore used as emulsifying oil and water mixtures (Dekker, 1997; Attwood and Florence, 2012), and as food additives in animal feed formulations (Orozco, 2014). Sorbitan monolaurate is a long chain example of a span, formed by the reaction of lauric acid and sorbitol with an acid catalyst (Figure 41).

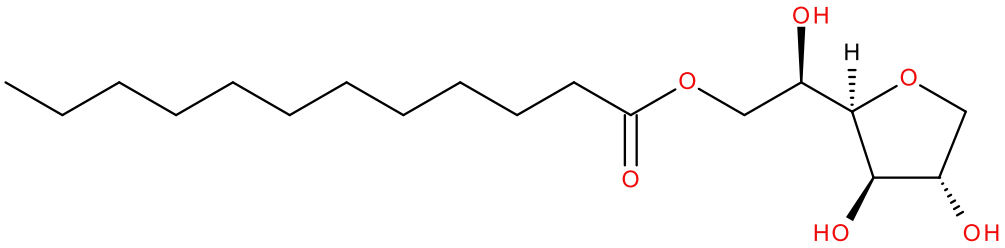


Figure 41: Structure of sorbitan monolaurate

3.1.6 Conclusions and BSFL value-added chemical objectives

The research objective for this chapter study was to examine the use of BSFL oil as FFA source for a particular class of value-added chemicals, surfactants. A large proportion of surfactants use a medium or long chain fatty acid precursor in synthesis, synthetically or naturally derived. With BSFL fat composition comprising mostly of medium and long chain saturated fatty acids,

this means that there are many applications for the extracted oil in the surfactant market, Figure 42.

There exists great interest in providing a more sustainable oil source for surfactants than that of the current global leading choices. At present, there is an issue with maintainable feedstocks for modern surfactants. Current supplies are deemed unsustainable, either sourced from hazardous petrochemical origins or over-farmed coco and palm plantations. Therefore, sustainable alternative oil feedstocks are desired. In this chapter, the objective was to provide a novel and sustainable route to consumer products via an alternative oil source, applying BSFL as an insect "bio-catalyst" to rapidly convert organic waste into an oil with very similar composition to coconut and palm oils. Insects such as BSFL can be grown anywhere in the world, and are easily reared making them suited to locations with limited infrastructure. BSFL were fed organic waste, and the resultant oil produced in chapter 2 demonstrated as a suitable feedstock for surfactant production, evidenced by the thorough composition analysis completed. The idea also wields a significant environmental benefits as food waste is a vastly overlooked driver of climate change, with the waste sector found to be responsible for nearly 8.2 % of total global greenhouse gas emissions produced every year (International Energy Agency 2014).

To address the objective of demonstrating successful sustainable surfactants from BSFL oil, a variety of surfactants from different surfactant classes were produced from isolated BSFL FFAs and characterised by standard laboratory techniques. This encompassed the synthesis of anionic, cationic and nonionic surfactants products from the refined BSFL FFAs, including the most prevalent global surfactant in production, sodium alkyl sulfates. In order to achieve the objective, multiple stage synthesis occurred with several intermediates of BSFL alcohols and biodiesel needed in order to achieve the final surfactant products.

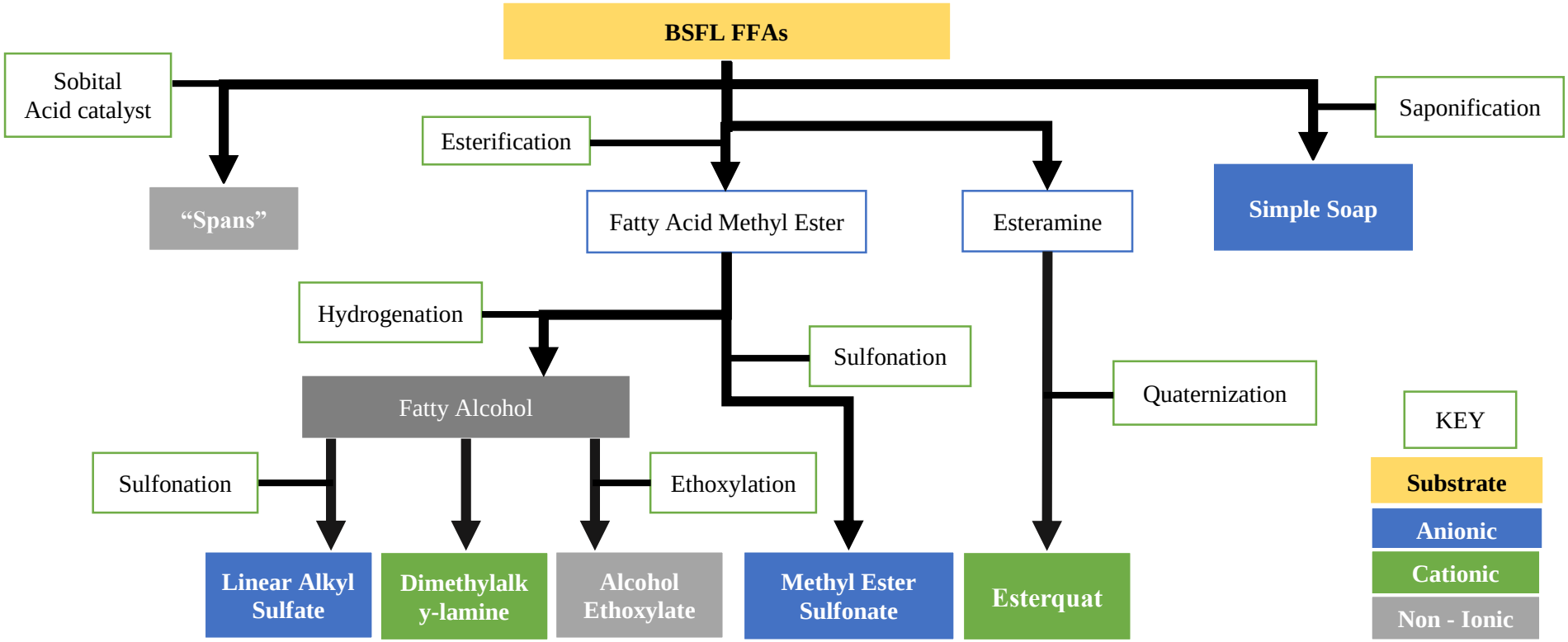


Figure 42: Applications of FFAs found in BSFL oil in the surfactant market

3.2 EXPERIMENTAL

3.2.1 Solvents and Materials

Solvents and reagents purchased for use in this work were not purified further. Commercial materials (ACS reagent grade) (Sigma-Aldrich, UK) were used: NaOH, HCl, H_2SO_4, diethyl ether, lauric acid, NaCl, methanol, THF, $NaBH_4$, $NaHCO_3$, *N*,*N*-Diisopropylethylamine, benzotriazol-l-yloxytris(dimethylamino)phosphonium hexafluorophosphate, benzethonium chloride, Mg_2SO_4, dimethyl sulphide, ethanol, chlorosulphonic acid, triethanolamine, phosphoric acid, *p*-toluenesulfonic acid, sorbitol, KOH, monoethanolamine, CaO, dimidium bromide-disulphine blue indicator, phenolphthalein indicator and chloroform. BSFL were sourced from the industrial partner, Better Origin ltd (Cambridge, UK). Following oil extraction by hexane in chapter 2, the oil was subjected to purification by distillation and use in the following syntheses. Concentrations of acids and alkalis were made-up using deionised water (8.2 Ω) with solid mass analytically weighed, and liquid solutions pipetted and subsequently diluted to required volumes/concentrations using water.

3.2.2 Preparation of methyl laurate intermediate

Lauric acid (10 g) and methanol (80 mL) were placed in a round-bottomed flask and heated under reflux at 75 °C at a 1:8 w/v ratio along with 1.0 M H_2SO_4 (3mL) catalyst. Following reaction completion, the biodiesel was separated from the resulting water by a liquid-liquid extraction. The reaction mixture was poured into a separating funnel, and washed twice with diethyl ether (40 mL) and saturated NaCl solution (40 mL) and the aqueous and organic layers allowed to separate. The upper solvent layer containing the biodiesel was removed by rotary evaporation and methyl laurate analysed using GC-MS (method 2.2.4)

3.2.3 Preparation of BSFL biodiesel intermediate

BSFL oil (3 g) and methanol (30 mL) were placed in a round-bottomed flask and heated under reflux at 75 °C at a 1:10 w/v ratio along with 1.0 M H_2SO_4 (3 mL) catalyst for 3 hours. Following reaction completion, the biodiesel was separated from the resulting water by liquid-liquid extraction, as described above, and the biodiesel obtained by rotary evaporation of the solvent. BSFL FAMEs were analysed using GC-MS (method 2.2.4)

3.2.4 Preparation of BSFL fatty alcohol intermediates

BSFL oil (0.143 g) was added to a beaker containing THF (5 mL), along with *N*, *N*-Diisopropylethylamine (0.21 mL) and benzotriazol-l-yloxytris(dimethylamino)phosphonium hexafluorophosphate (0.487 g) and stirred at room temperature. After 5 minutes $NaBH_4$ (0.038 g) was added and the mixture stirred for an additional 20 minutes. THF was removed by rotary evaporation and the solid dissolved in diethyl ether (50 mL). Using liquid-liquid extraction, the organic solution washed thrice with 5 % HCl (10 mL), saturated $NaHCO_3$ (10 mL) and twice with saturated NaCl solution (10 mL), then dried overnight using Mg_2SO_4. Evaporation of diethyl ether yielded alcohols as a colourless oil.

3.2.5 Preparation of anionic BSFL solid soap product

Various concentrations of NaOH were used to determine the best concentration for the solidness and yield. BSFL oil (10 g), NaOH (20 mL), ethanol (10 mL) were added to a round-bottom flask and heated at 80 °C for 1 hour. Saponification of oil was determined by addition of 3 drops of reaction mixture to 10 mL of cold water. If the formation of fat droplets were observed, NaOH (5 mL) and ethanol (5 mL) were added and the reaction mixture heated together. After 10 minutes, DI water (25 mL) and saturated NaCl solution (50 mL) was added and the mixture cooled in an ice bath. After the cooling time was completed, the liquid portion of the soap reaction mixture, was decanted from the beaker. An additional 50 mL cold, saturated NaCl solution was added and stirred thoroughly in the "salting out" process. The solid soap was collected using a vacuum filtration and washed twice with cold DI water (20 mL).

3.2.6 Preparation of anionic sodium alkyl sulfate product

Chlorosulphonic acid (0.625 g/0.357 mL) was added to lauryl alcohol / BSFL alcohol (1 g) at 0 °C within an ice bath, an extremely low temperature is crucial in order to prevent side reactions. An alkylsulfate intermediate was created, along with gaseous HCl, which was captured by bubbling through 100 mL DI water or 0.1 M NaOH solution. Residual HCl can be removed by air sparging. Following that, reaction with NaOH (0.21 g) yielded SASs.

3.2.7 Preparation of anionic methyl fatty acid ester sulfonate product

BSFL FAMEs were prepared using aforementioned method 3.2.3, using BSFL oil (10 g) and methanol (80 mL) with a 1.0 M H_2SO_4 catalyst (3 mL). Assuming a 70 % yield of FAMEs (7 g), sulphur trioxide pyridine (17.4 g) was added to the FAME. If the mole ratio of SO_3 to FAME is significantly lower than 1:2, full conversion of the methyl ester sulfonate (MES) cannot be achieved. With an excess of sulphur trioxide ~ 20 g (10-20 %) a dark-colour

sulfonation mixture was made, which further to neutralisation with 0.1 M NaOH (50 mL) gives MESs.

3.2.8 Preparation of cationic esterquat product from BSFL oil

Synthesis of the diester from BSFL takes place by the addition of triethanolamine to the FFAs in a 2:1 ratio in the presence of phosphoric acid catalyst (0.5 mL/ 0.94 g). BSFL oil (2 g) was added to triethanolamine (0.8g, 0.71 mL) and heated at 165 °C for 8-10 hours. After the time had elapsed and the mixture cooled, the intermediate diester was dissolved in petroleum ether (10 mL) in a round-bottom flask. DMS (1.1 mL) was added dropwise and heated the mixture heated under reflux at 65-70 °C for 2 hours. Esterquats were transferred to a beaker and the petroleum ether was evaporated slowly at 40 °C until a viscous compound was obtained.

3.2.9 Preparation of non-ionic span product from BSFL oil

Sorbitol (5 g) was stirred with *p*-toluenesulfonic acid (0.5 g) at 140 °C for 1 hour, and then esterified with lauric acid or BSFL oil (5 g) at 1:1 w/w ratio for 100 minutes under reflux at 160 °C. Final sorbitan monolaurate products (1,4 sorbitan - 2 monolaurate/1,4 soribtan - 5-monolaurate/ 1,4 sorbitan -3-monolaurate) were analysed using GC-MS.

3.2.10 Preparation of cationic long chain amide product from BSFL oil

In the first stage of long/medium chain dimethylalkylamine oxide surfactant synthesis, BSFL biodiesel was converted into fatty amides. In a two-neck round-bottom flask BSFL biodiesel (5 g), monoethanolamine or diethanolamine (3 g, 2.82 mL) and CaO commercial catalyst (1 g) was magnetically stirred and heated to 125 °C. A side arm condenser was attached to one neck of the flask and the reaction mixture was heated for approximately 1 hour, until methanol ceased to be removed by condensation. Ethanol (20 mL) was heated to 80 °C and added to the reaction flask, and filtration of the hot mixture completed, followed by cooling to room temperature. Amides were detected and analysed using GC-MS.

3.2.11 Titrimetric analysis of anionic surfactants (Standard method)

A benzethonium chloride 0.004 M (1.79 g in 1 L deionised water) solution was prepared and standardized against sodium lauryl sulfate. 20 mL of mixed dimidium bromide-disulphine blue indicator solution was transferred to a 500 mL volumetric flask, and 50 mL 1 M H_2SO_4 was added, and diluted to volume with water. 1 mEq of anionic surfactant (0.07 g) was weighed, dissolved in around 100 mL water and neutralized to pH 8.3 with 1 M H_2SO_4 and 2 drops of phenolphthalein was added. This was further diluted to 125 mL with DI water. A 20 mL aliquot

was transferred to a 100 mL graduated flask along with 10 mL water, 15 mL DCM, and 10 mL indicator solution. This was titrated with the benzethonium chloride solution, and shaken well after each addition of titrant. The end point was signified by the development of a blue colour in the aqueous phase, and the disappearance of a pink colour from the methylene chloride layer.

3.2.12 Titrimetric analysis of cationic surfactants (Standard method)

Cationic surfactant solution (0.5 g esterquat dissolved in 5 mL DI water), chloroform (15 mL), methylene blue solution (25 mL) were added to a 250 mL Erlenmeyer flask. The mixture was shaken and allowed to stand until the organic and aqueous layers were separate. 0.0004 M solution of sodium lauryl sulfate (prepared by dissolving 1.464 g of SDS in 1 L water was titrated into the mixture until the blue colour seen in the top organic layer migrated down to the colourless aqueous layer. When the same colour intensity was seen in both aqueous and organic layers, titration was complete and the titrate volume was noted.

3.2.13 Determination of the CMC

Surface tension was measured using two methodologies. In the first, the reverse meniscus tension of surfactants was measured by using a Nima Technology Langmuir Film Balance for Brewster Angle Microscope. Whatman grade 1 filter paper was hung upon a hook and slowly lowered into a petri dish containing surfactant diluted in deionised water (Figure 43). The pressure of the filter paper when a reverse meniscus was achieved was noted as the surface tension of the sample. The method was validated by calibration of commercial SDS, where the CMC was confirmed to be 8.2 mM at 25 °C, a well recorded value. For unknown concentrations, surfactants where filtered through a 0.2 µm filter and dissolved into deionised water, and continuously diluted. Surface tension (mN/m) was plotted against concentration (g/mL) and the CMC noted when the regression of the curve was seen to change.

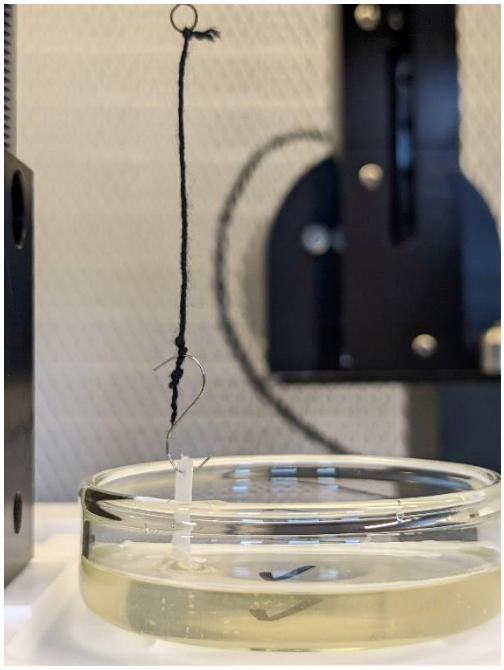


Figure 43: CMCs were recorded through reverse meniscus surface tension measurements

Conductivity was also used to confirm the CMC, measured using a Hanna HI-98311 EC, TDS and Temperature Tester (Hanna instruments, UK). As a consequence of micelle formation, the solution composition is changed after reaching the CMC, resulting in conductivity change as well and a change in linear regression. Conductivity (µS/m) was plotted against concentration (g/mL) and the CMC obtained from the concentration at which two almost straight lines intersect. This concentration value was compared with and confirmed with surface tension measurements. Due to insufficient quantities of certain surfactant samples CMCs could not be determined as a large concentration range is required.

3.2.14 Analysis of Non-ionic surfactants by GC-MS

Analysis of non-ionic surfactant composition was determined by GC-MS. Analyses were carried out on a Shimadzu 2010 GC combined with a QP2010 SE Mass Selective detector with electron impact ionisation (EI) (Shimadzu Corp., Kyoto, Japan). The GC-MS system was equipped with a purged tee installed after the DB-5MS (Agilent Technologies, USA) nonpolar phenyl arylene polymer capillary column (30 m × 0.25 mm I.D.; 0.2 µM) columns was used. Retention time against relative TIC was collected for analyses uing a 0.99 mL/min Helium mobile phase. Automated 0.7 µL injection was performed using AOC 2Oi injector autosampler (Shimadzu Corp., Kyoto, Japan). The oven temperature was programed at 50 °C and held for 2 minutes and then ramped from 50 °C to 250 °C at 16 °C/min and held at 250 °C for 25 minutes. The MS was operated at scan speeds between 5000 and 20,000 amu covering a range

of m/z 30–500. EI mass spectra were recorded at 70 eV. Peaks were identified by analysis of MS fractionated ions, by comparison to a Shimadzu MS computerised database.

3.2.15 Analysis of surfactants by FT-IR spectroscopy

IR spectra were collected using a Shimadzu IRAffinity–1S FTIR spectrometer with a Specac Quest ATR attachment (Smiths Group, London, UK), 16 scans were taken at a resolution of 4 cm^{-1} in the range 4000-600 cm^{-1}.

3.2.16 Analysis of surfactants by UV-Vis spectrophotometry

UV-Vis spectra were collected using a single beam themoscientific GENESYS 180 UV-Vis Spectrophotometer, with a 100 mm pathlength Hellma quartz absorption cuvette. 0.01 g of BSFL oil was dissolved in 1 mL hexane and scans were taken between 190-1100 nm.

3.3 RESULTS AND DISCUSSION

3.3.1 Esterification of Lauric Acid with Methanol

In order to determine the correct experimental conditions for BSFL FAMEs synthesis, an acid-catalysed esterification of pure lauric acid was completed (Section 3.2.1). Optimum reaction time was determined through the periodic withdrawal of 3 mL samples over the course of six hours, which were analysed by GC-MS to determine methyl laurate conversion. The resulting methyl laurate biodiesel was extracted by liquid-liquid extraction and rotary evaporation of solvent. The product was opaque and had a diesel odour. The optimum conditions were found to be a reaction duration of 3 hours, with a 1:8 w/v ratio of 1 mL 1.0 M H_2SO_4 catalyst.

3.3.2 Esterification of BSFL oil with Methanol

Acid-catalysed esterification of BSFL was completed with a sulphuric acid catalyst for 3 hours. It was found that following repeated attempts, a higher amount of sulphuric acid than the tested 1 mL was required to sufficiently catalyse the reaction. It is hypothesised that, with BSFL oil containing many longer chain carboxylic acids, a greater activation energy is required for the esterification when compared to same reaction using pure lauric acid. Mixed esterified FFA products were characterised using GC-MS, where the highest conversion was seen in methyl laurate, predictable due to the high starting concentration of lauric acid in BSFL oil (Figure 44/Table 22). When higher concentrations of sulphuric acid were used, conversion rates overall averaging 80 %, with the presence of lauric acid not being seen at all.

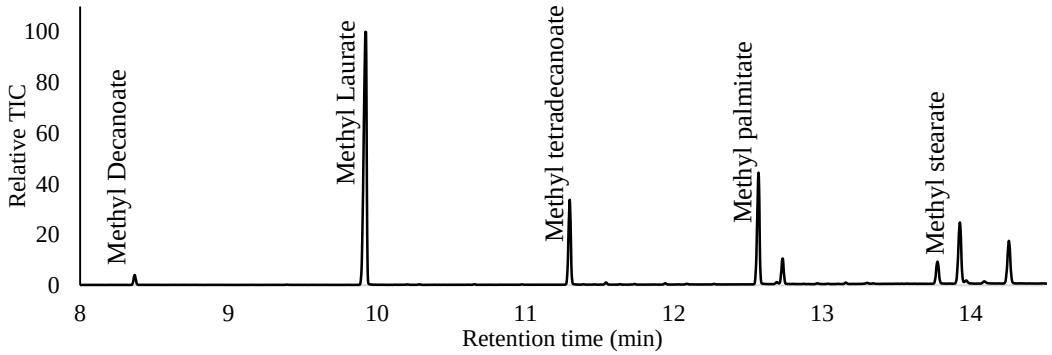


Figure 44: GC-MS chromatogram of the esterification products seen from using a raw BSFL oil reactant

Table 22: GC-MS analysis of products resulting from an optimised BSFL biodiesel production

^{n}C	*Common Name*	*IUPAC Name*	*Chemical Structure*	M_W	*Retention Time (mins)*	*Saturation*
11	Methyl decanoate	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	8.4	Saturated
13	Methyl laurate	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	9.9	Saturated
15	Methyl tetradecanoate	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	11.3	Saturated
17	Methyl palmitate	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	12.5	Saturated
17	Methyl palmitoleate	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268	12.7	Unsaturated
15	Methyl stearate	Methyl stearate	$C_{19}H_{38}O_2$	299	13.8	Saturated
16	Methyl 8-octadecenoate	8-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	13.9	Monounsaturated
18	Linoleic acid, methyl ester	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	14.3	Unsaturated

3.3.3 Hydrogenation of BSFL oil

Hydrogenation of BSFL was completed by an alternative lab-scale hydrogenation method (McGeary, 1998) to the traditional industrial hydrogenation route of H_2 gas and a metal catalyst. On a lab-scale, hydrogenation of chemicals can be achieved through use of sodium borohydride ($NaBH_4$) or lithium aluminium hydride ($LiAlH_4$), but both reagents react violently with water and have low flash points, resulting in spontaneous ignition. Due to the size of the carboxylic acids, the milder $NaBH_4$ reagent is ineffective as a reducing agent. $LiAlH_4$ can produce long chain alcohols from the reduction, but is more readily flammable and therefore impractical for everyday use. A less hazardous alternative was found (method 3.2.4), and proved to be very successful using a base, DIPEA, and electron withdrawing BOP reagent (both commonly used in peptide synthesis) . The mechanism of the reagents together allows for the activation energy of the reaction to be lowered, and hydride to attack the vulnerable C=O bond. A mixture of long chain alcohols, which have potential for use as non-ionic surfactants in blended formulations were formed in the reaction (Figure 45/Table 23). Yield with this method was extremely high, averaging 90 % conversion. Evaporation of the THF solvent and workup with diethyl ether gave long chain alcohols, without need for further purification.

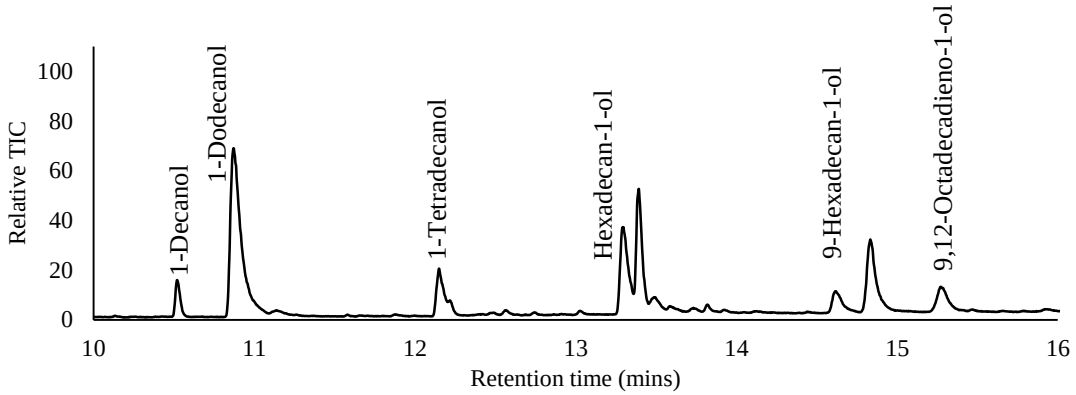


Figure 45: GC-MS chromatogram of the hydrogenation products seen from using a raw BSFL oil reactant

Table 23: GC-MS analysis of products resulting from hydrogenation of BSFL oil

Common Name	***IUPAC Name***	***Product of hydrogenation reaction***	***Retention Time (mins)***
Capric acid	(n-)Decanoic acid	1-Decanol	10.5
Lauric acid	Dodecanoic acid	1-Dodecanol	10.8
Myristic acid	Tetradecanoic acid	1-Tetradecanol	12.1
Palmitic acid	(n-)Hexadecanoic acid	Hexadecan-1-ol	13.2
Palmitelaidic Acid	9-Hexadecanoic acid	9-Hexadecen-1-ol	14.6
9,12-Octadecadienoic acid	9,12-Octadecadienoic acid (Z,Z)	9,12-Octadecadieno-1-ol	14.8

BSFL long chain alcohols were anlaysed using FT-IR spectroscopy and the spectrum compared to that of the orignal BSFL oil (Figure 46). The long chain alcohols in the hydrogenated product were identified through the presence of a broad O-H stretch at 3310 cm^{-1}, in contrast to the sharp carboxylic O-H peak seen for BSFL oil at 2922 cm^{-1}. Aliphatic C-H bonds remain in the 3000-2700 cm^{-1} region, but the strong peak representing a C-O bond representing primary alcohol presence can be seen at 1020 cm^{-1}, instead of at 1153 cm^{-1} as seen in the BSFL oil. A weak peak indicative of the BSFL oil starting material can be identified at 1745 cm^{-1}, suggestive of an incomplete reaction.

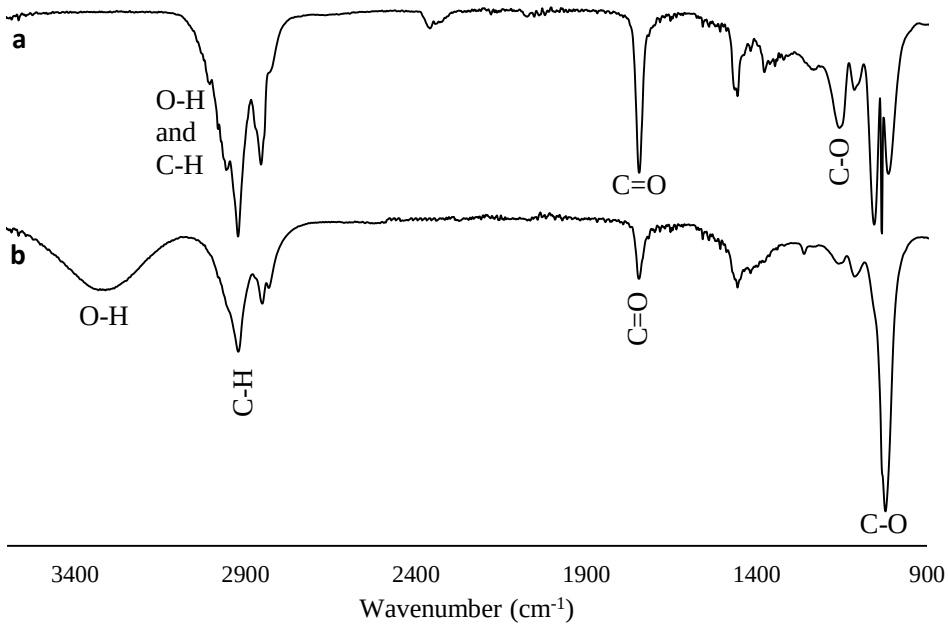


Figure 46: FT-IR Spectrum of BSFL FFAs (a) compared to (b) BSFL alcohols

3.3.4 Saponification of BSFL oil

The synthesis of "toilet" soap was completed by treatment of BSFL oil with varying concentration of NaOH. The ease at which saponification can be accomplished was revealed in the optimisation of the lauric acid esterification, with soap formation seen when NaOH concentration exceeded 0.8 % (w/w). Soap colour was dependent on the colour of the BSFL oil used, and therefore the diet fed to BSFL but generally was seen to be a yellow colour, Figure 47. Soaps were allowed to dry for 2 days at room temperature to remove residual water and hard, malleable soaps were revealed. The BSFL soap was seen to 'bubble' when agitated against skin in water, demonstrating micellar action of molecules surrounding oils and dirt (Morrison and Boyd, 2002).

Figure 47: BSFL basic solid soaps

Using a concentration of 2.0 M NaOH produced the highest performing and least brittle soap from all concentrations tested, although not producing the highest % yield from the concentrations tested, Table 24. Performance was evaluated anecdotally by mixing 0.1 g with water and the flask shaken to see sudsing ability (Figure 48). As well as this, each soap was tested by vigorous application on skin. The higher the NaOH concentration, the more brittle the soap became. Conversely, lower Na concentration made the soap too fatty, making removal from skin to water difficult.

Table 24: BSFL solid soap yields and respective densities

Concentration of NaOH (M)	Dry soap weight (g)	% Yield	Density g/cm^3
1.0	7.23	72.49	1.02
1.25	8.53	77.90	0.80
1.5	8.64	88.60	0.98
2.0	9.63	97.30	0.90
2.5	7.17	85.10	0.68
3.0	8.61	90.40	0.81
5.0	10.36	100	0.82

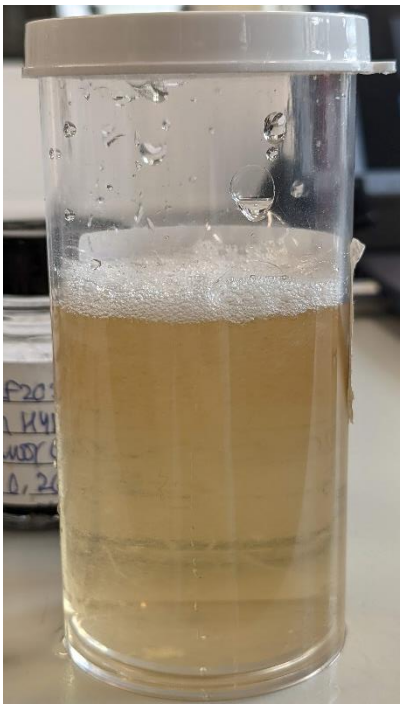


Figure 48: BSFL solid soap performance could be evaluated by mixture with DI water

3.3.5 Synthesis of anionic linear alkyl sulfates

Linear alkyl sulfates are a type of anionic surfactant with a characteristic linear medium or long chain alkyl anion "tail" and a corresponding cationic "head", in this instance, a sulfate counter ion. In industrial production, the surfactant is made by treating FFAs, popularly lauric acid, with gaseous H_2 and sulfonating with gaseous SO_3 to give rise to the SAS product. The synthesis of linear alkyl sulfates products herein was completed by hydrogenation of refined BSFL oil, then sulfonation treatment with chlorosulfonic acid (Figure 49). Industrial methods of sulfonating FFAs include treatment with SO_3 but as the lab lacked the infrastructure for this, chlorosulfonic acid was more appropriate for use in synthesis.

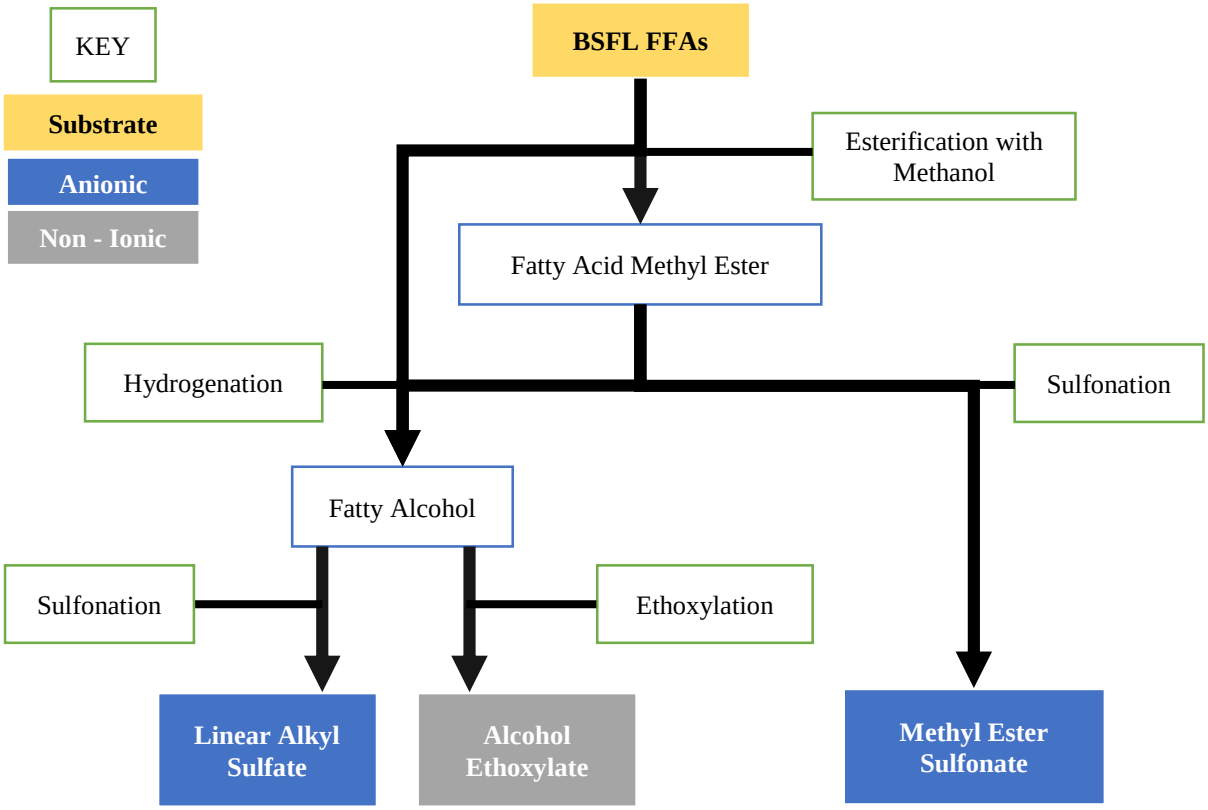


Figure 49: Route taken to achieve LASs and MESs from BSFL oil

The oil was hydrogenated to produce alcohols of the fatty acids following method 3.2.4, and then sulfonated using chlorosulfonic acid, followed by the addition of sodium hydroxide (Figure 49) to yield LASs called sodium alkyl sulfates (SAS). Upon contact with chlorosulfonic acid, the colourless FFAs quickly darkened, resulting in paste like SASs dark in colour, Figure 50. It is likely the dark colour stemmed from excessive exposure to the extremely acidic environment, with chlorosulfonic acid spontaneously splitting with reaction with FFAs to form HCl. HCl was reabsorbed into dilute NaOH, but enhanced removal of HCl with constant

vacuum could have prevented this strong oxidation, but nevertheless, it is believed surfactant performance was unaffected.

Figure 50: BSFL SASs

The experiment was repeated and there was an attempt to characterise and quantify samples using ion chromatography with a variety of columns and eluents. Calibrations for sodium dodecyl sulfate (SDS) - the SAS formed from lauric acid - were attempted but could not be confirmed with other methods such as FT-IR spectroscopy. As other SAS versions of BSFL FFAs - aside from SDS - are expensive, it was the only SAS reference material that could be used.

A mixture of BSFL SASs were detected using an ion chromatography Dionex AS11 Column with a MSM suppressor, pre-column and conductivity detector. Overall, there were peaks seen with multiple different eluent solutions, but often these were not defined or they merged with others, meaning that quantification and identification of SDS was difficult. Eluents were prepared using deionised water (8.2 Ω), and sonicated for 30 minutes prior to flushing the system for ten minutes before use. 0.125 g of an SAS sample was dissolved in 2 mL of deionised water, and the solution filtered twice through a 0.2 µm filter to prevent undissolved solids from entering the column. Samples were manually injected, with a run time of up to 30 minutes performed to ensure comprehensive analysis.

The standard recommended eluent for the AS11 column of 21 mM NaOH proved to be unsuitable, with a single peak at 1.7 minutes produced from most analyte samples. Furthermore, the baseline proved to be poor despite a low back pressure from within the column of 8.2 mPa. In addition, although it was decided that the presence of NaOH within the system could interfere with results, as samples contained strong Na ion content and therefore the cationic eluent was switched to LiOH.

Partially successful peak identification could be seen using a 1.0 Mm LiOH 90:10 MeOH:H_2O eluent (Figure 51). A 1000 µg/L Na^+ reference material (Sigma Aldrich, UK) exhibited a

retention time of 1.4 min. The other 7 peaks using this solvent mixture are suspected to contain SASs from the mixed FFAs, in size order, starting with capric acid (C_{12}) to 9,12-Octadecadienoic acid (Z,Z) (C_{18}), with the largest peak at 8.22 minutes representing SDS. The back pressure of the column increased to averagely 15 mPa using this eluent makeup.

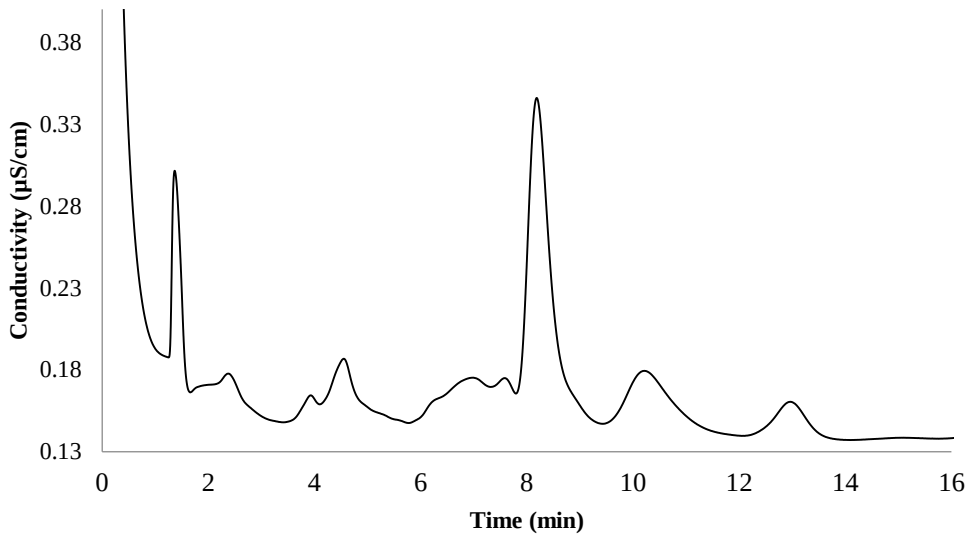


Figure 51: SULF2019002 separated using 1.0 Mm LiOH 90:10 MeOH:H_2O eluent on a Dionex AS11 column, with a sample dilution of 0.125 g to 2 mL

When the sample was diluted by a factor of ten, there was improvement in peak separation and intensity (Figure 52) using the same eluent. It was decided that dilution was important in peak resolution and therefore further samples were prepared as 0.0125 g in 2 mL deionised water.

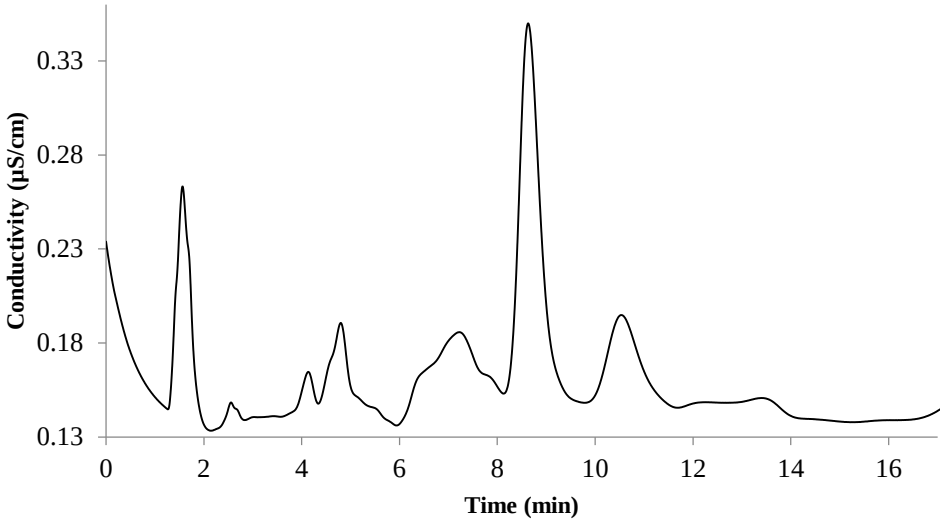


Figure 52: SULF2019002 separated using 1.0 Mm LiOH 90:10 MeOH:H_2O eluent on a Dionex AS11 column, with a sample dilution of 0.0125 g to 2 mL

The MeOH:H_2O eluent ratio was examined to see if resolution between peaks could be improved. Decreasing the methanol content to 1.0 Mm LiOH 75:25 MeOH:H_2O did not improve peak resolution (Figure 53Figure 54), but instead peaks clustered once more. Using this solvent increased back pressure within the instrument to 18 mPa.

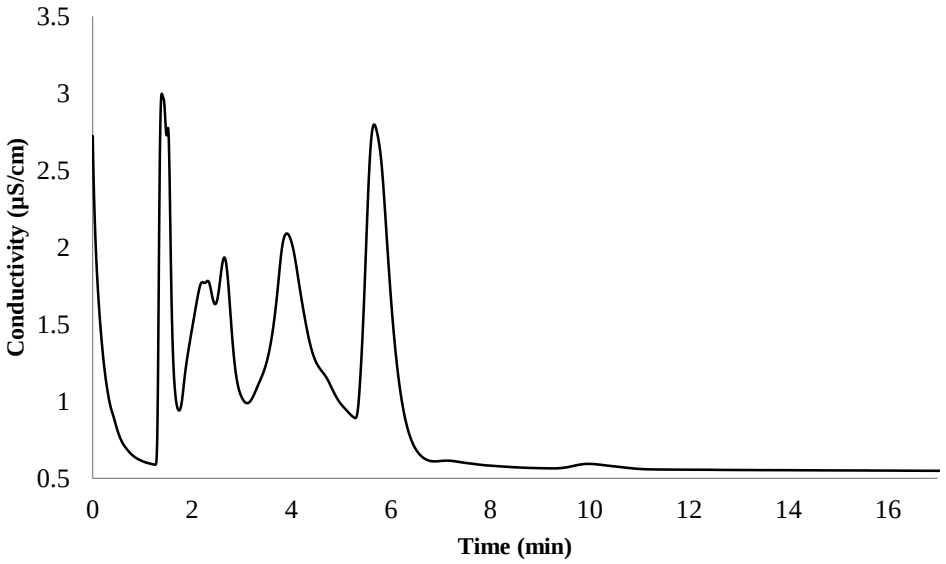


Figure 53: SULF2019002 separated using 1.0 Mm LiOH 75:25 MeOH:H_2O eluent on a Dionex AS11 column, with a sample dilution of 0.0125 g to 2 mL

Further decreasing the methanol portion to 50 % led to excessive pressure rising within the column, exceeding the manufacture recommended 21 mPa. This led to difficult collection of data as mid-run the pump auto shutdown, and it was assumed this excessive back pressure occurred due to complex alcohol-water interactions (Derlacki, Easteal, Edge, Woolf and Roksandic, 1985; Weber, Mitchell, Mcgregor and Gladden, 2009). Compete resolution of peaks could not be accomplished upon additional dilution of the sample, nor by changing the ratio of methanol to water in the eluent. Minimal results collected prior to shutdown resulted in Figure 54, but runs could not be repeated with back pressure conditions.

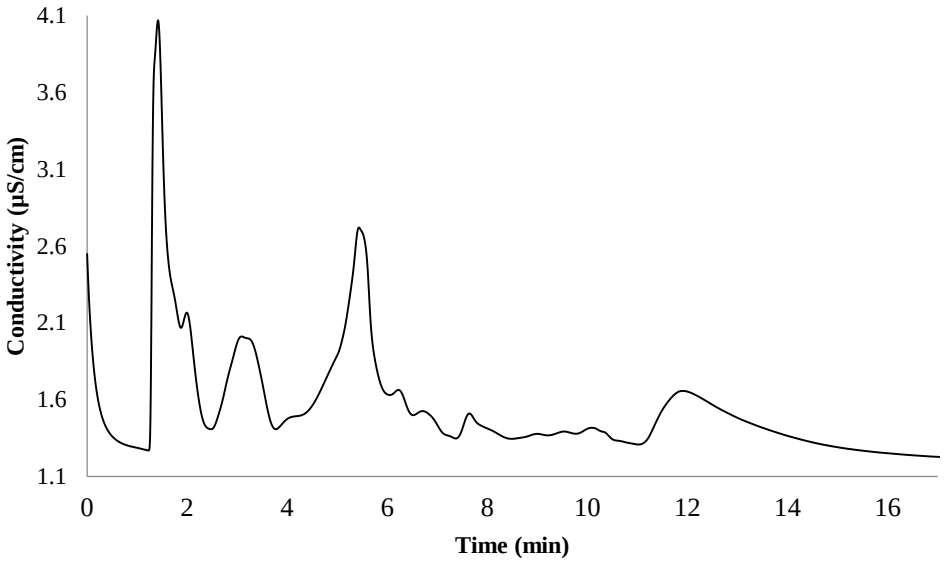


Figure 54: SULF2019002 separated using 1.0 Mm LiOH 50:50 MeOH:H_2O eluent on a Dionex AS11 column, with a sample dilution of 0.125 g to 2 mL

An alternative eluent composition was used to attempt to increase peak resolution and circumvent back pressure issues. In a 20.0 Mm LiOH 30:70 Acetonitrile:H_2O eluent (Figure 55), the mixture had a lower retention time in comparison, but five assumed peaks with higher conductivity value. In this solvent ratio, an increase in LiOH counter ion content seemed to improve separation. However, despite increased peak area, the solvent mixture did not improve peak resolution with any of the 9 samples.

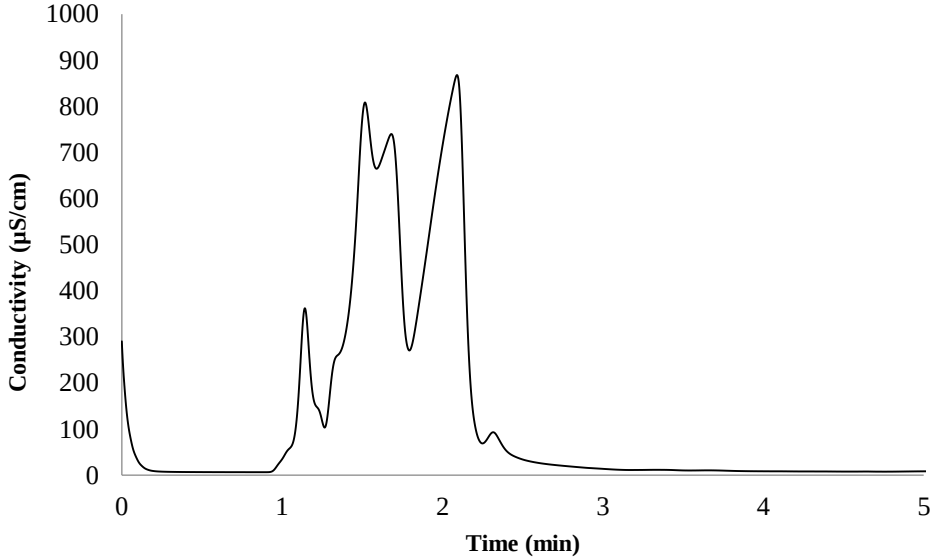


Figure 55: SULF2019011 separated using 20.0 Mm LiOH 30:70 Acetonitrile:H_2O eluent on a Dionex AS11 column, with a sample dilution of 0.0125 g to 2 mL

At every eluent change, an SDS calibration curve was made with concentrations ranging from 0.00001 to 0.5 mol dm^{-3}, using the peak area to plot for concentration (Table 26). Concentration

was difficult to predict, as most chromatograms had overlapping peaks despite many eluent and method modifications. Another IC column was explored but often runs resulted in only 2 peak chromatograms, one representative of a Na peak and the other sulfate anions (Figure 56). A Metrohm A Supp 4 column provided good calibration results, but poor separation with a 1.7 mM $NaHCO_3$, 1.8 mM Na_2CO_3 eluent.

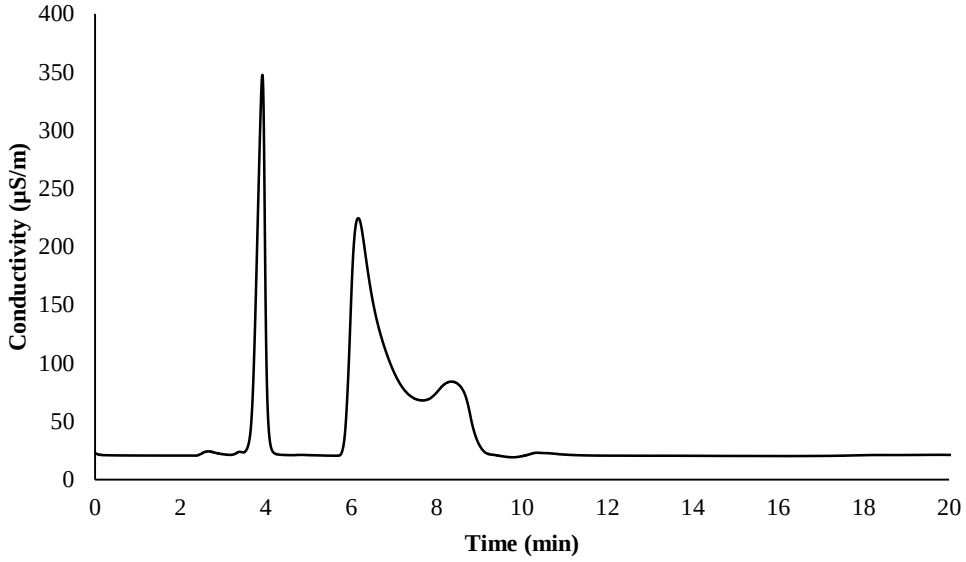


Figure 56: SULF2019004 separated using 1.7 mM $NaHCO_3$, 1.8 mM Na_2CO_3 eluent on a Metrohm A Supp 4, with a sample dilution of 0.0125 g to 2 mL

Samples were also scanned using FT-IR to attempt quantification and validate other calibration methods. A characteristic peak representing skeletal vibration involving the bridge S–O stretch seen between 1120 – 1330 cm^{-1} was chosen (Figure 57), and calibration curve obtained from this peak. This peak was chosen because as it does not appear in reagent BSFL oil. For BSFL SASs additional peaks were observed (Table 25), with 2941, 2922, 2850 and 1465 cm^{-1} peaks corresponding to C-H stretching and bending modes, the 1018 cm^{-1} peak corresponding to C–C band stretching, 864 cm^{-1} peaks corresponding to asymmetric C–H bending of the CH_2 group. In additional, a small peak at 1708 cm^{-1} could be seen in the final BSFL SAS product mixture, which is suggestive that the reaction was incomplete, and residual BSFL FFAs.

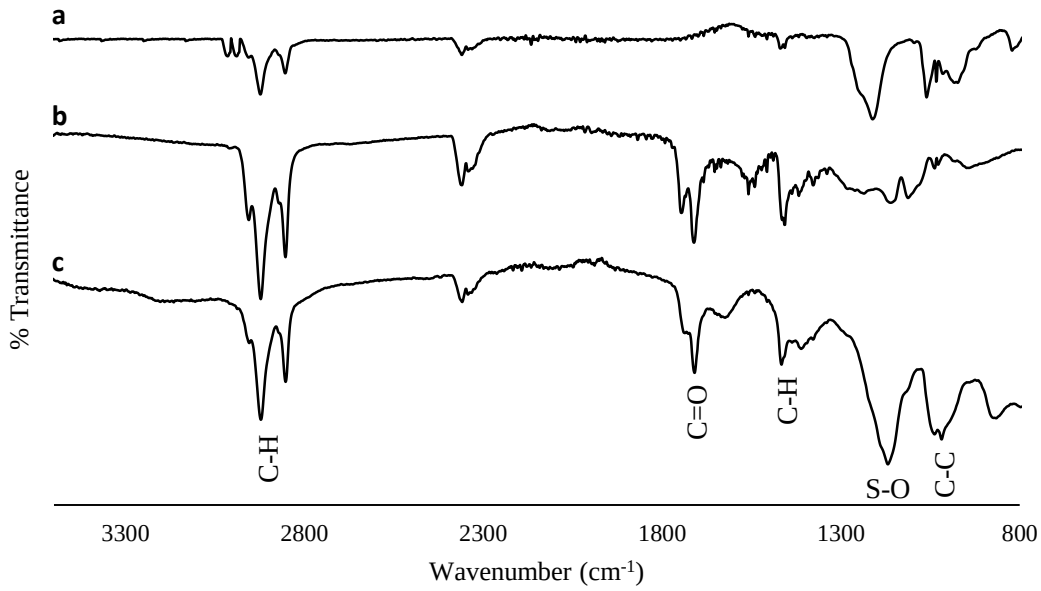


Figure 57: FT-IR spectrum comparing results obtained from a BSFL sulfonated sample, where (a) is commercial SDS (Sigma Aldrich, UK), (b) is BSFL oil and (c) is BSFL SASs

Table 25: FT-IR analysis data table for BSFL SASs

Functional Group	Wavenumber (cm^{-1})	Appearance
Aliphatic C-H (stretch)	2941, 2922, 2850, 1465 and 864	Sharp
C=O (stretch)	1708	Sharp, Medium
S-O	1166	Sharp, strong
C-C	1018	Medium, broad

UV-Vis spectroscopy was used as an alternative concentration determination, because it was thought a peak at 230 nm could be identifiably used (Schmitt, 2001). A calibration curve with the peak area at 230 nm was used to calculate concentrations for sulfonated samples analysed using UV-Vis spectroscopy (Figure 58). The data provided some information about the degree to which conversion was achieved in each sample, where SULF2019010 shows poor conversion to final product.

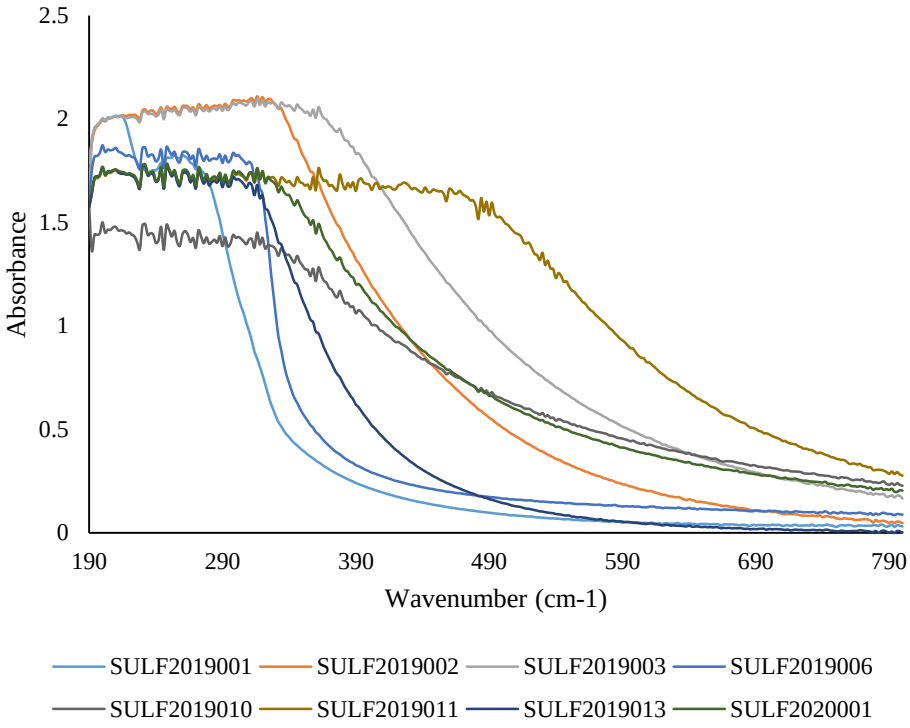


Figure 58: UV-Vis Spectra of BSFL SASs

Table 26: Collection of concentration results from various analytical methods using commercial SDS (Sigma Aldrich, UK) as a calibration reference material

	Conc. (mol dm^{-3})								
	Metrohm A Supp 4 column		Dionex AS11 column	Dionex AS11 column	Dionex AS11 column	Dionex AS11 column	FT-IR	UV-Vis	Standard Anionic Titration
	1.7 mM $NaHCO_3$, 1.8 mM Na_2CO_3 eluent		21.0 mM NaOH eluent	1.0 mM LiOH 30:70 Aceto:H_2O eluent	1.0 mM LiOH 90:10 MeOH:H_2O eluent	20.0 mM LiOH 40:60 Aceto:H_2O eluent			0.004 M Benzethonium chloride solution
	(0.05 g in 2 mL)		(0.025 g in 2 mL)	(0.05 g in 2 mL)	(0.025 g in 2 mL)	(0.025 g in 2 mL)	(0.025 g in 2 mL)	(0.025g in 2 mL)	0.07 g in 125 mL
	(r^2 = 0.9975)		(r^2 = 0.991)	(r^2 = 0.9969)	(r^2 = 0.9997)	(r^2 = 0.9998)	(r^2 = 0.9997)	(r^2 = 0.9974)	-
Sample	Peak at 8 min (SO_4)	Peak at 4 min (Na^+)	Peak at 1.3 min (Na^+)	Peak at 1.3 min (Na^+)	Peak at 1.6 min (Na^+)	Peak at 1.3 min (Na^+)	Peak area in the range of 1120-1300cm^{-1}	Peak area 230 nm	Dimidium Bromide-Disulphine Blue indicator
SULF2019001	0.83	0.40	8.1	0.37	0.19	0.12	0.26	17.21	0.00041
SULF2019002	3.2	0.20	9.4	1.5	0.77	0.14	0.26	19.94	0.00032
SULF2019003	-	9.5	7.8	0.43	0.21	0.013	0.18	19.85	0.00048
SULF2019004	0.19	1.7	-	0.22	0.072	0.036	0.0019	0.2	0.00040
SULF2019006	0.95	0.048	-	0.052	0.026	0.050	0.17	18.17	0.00043
SULF2019010	0.32	10.0	0.80	0.32	0.16	0.030	0.048	14.51	0.00014
SULF2019011	-	10.0	3.2	0.39	0.19	0.040	0.017	17.30	0.00090
SULF2019013	2.0	0.39	23.0	0.084	0.043	0.047	0.034	17.31	0.00016
SULF2020001	0.65	9.4	10.0	0.13	0.066	0.122	0.015	17.36	0.00024
Average	**1.17**	**4.74**	**7.83**	**0.38**	**0.19**	**0.063**	**0.13**	**17.41**	**0.00039**
Std dev.	**1.01**	**4.72**	**6.73**	**0.43**	**0.22**	**0.049**	**0.097**	**1.74**	**0.00021**
Std error.	**0.41**	**1.67**	**2.54**	**0.15**	**0.076**	**0.017**	**0.034**	**0.61**	**0.000076**

Following several attempts to qualitatively identify and qualitatively analyse all SAS samples, Table 26, concentrations of samples were compared to attempt to validate the methodology. Calibration curves were made using SDS, due to the largest component of BSFL oil containing lauric acid, it was assumed that the mixed SAS product would contain roughly 50 % SDS. However, no quantitative method could be validated against another, and concentrations were found to range between 0.0001 to 9.4 mol dm^{-3}, Figure 59. It is hypothesised that due to the nature of having such a mixture of SASs within surfactant samples, it is not possible to validate a single method against another, as each sample was synthesised from a different diet-based oil and therefore has slightly different MCFA and LCFA composition. In addition, most surfactants in IC analysis were calibrated on the basis of a cation Na^+ peak area, which appeared early on at 1.4 minutes, but it is possible higher or lower ratio of cations to anions existed within the surfactant mixture.

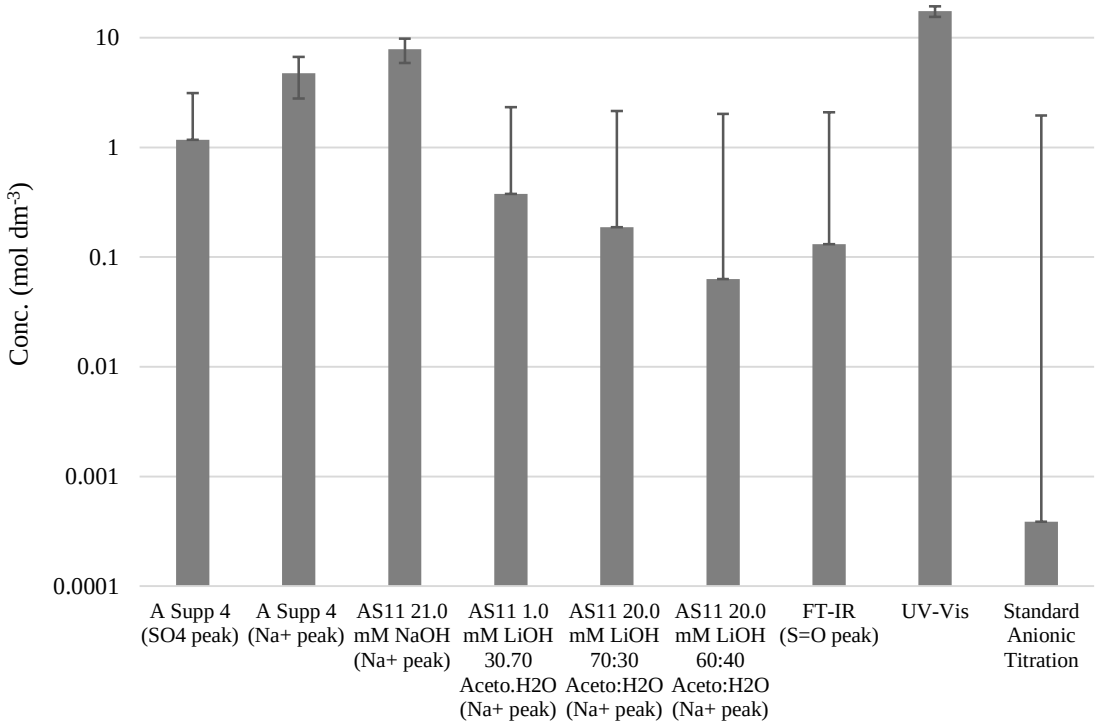


Figure 59: Average concentration results from various analytical methods using 50 %

Next, the CMC of SAS samples were determined according to method 3.2.13, Table 27. The methods used for determining CMC were validated against the CMC of SDS, which is universally documented as 8.2 mM (Moroi, Motomura and Matuura, 1974; Gimel and Brown, 1996). Both conductivity and surface tension were plotted against concentration, and the CMC noted when the regression was seen to change (Figure 60). The regression of

the curves and the CMC intersection point was evaluated visually and supplementary conductivity measurements were completed to confirm the accuracy of the change in regression. Overall, for samples with adequate mass for determination, the average CMC for BSFL SAS samples was found to be 4.66 mM, roughly half that of pure SDS. Concentrations were calculated with the assumption that the majority of BSFL FFA samples contained 50 % lauric acid prior to SAS synthesis, and therefore SAS samples would have a composition of approximately 50 % SDS. However, it is worth noting that CMCs can be considered inaccurate if half of the composition is not SDS and therefore this could count of the discrepancy in CMC values.

Table 27: CMC values of SAS samples

Sample	CMC (mM)
SULF2019002	5.92
SULF2019003	4.94
SULF2019004	3.46
SULF2019006	4.32

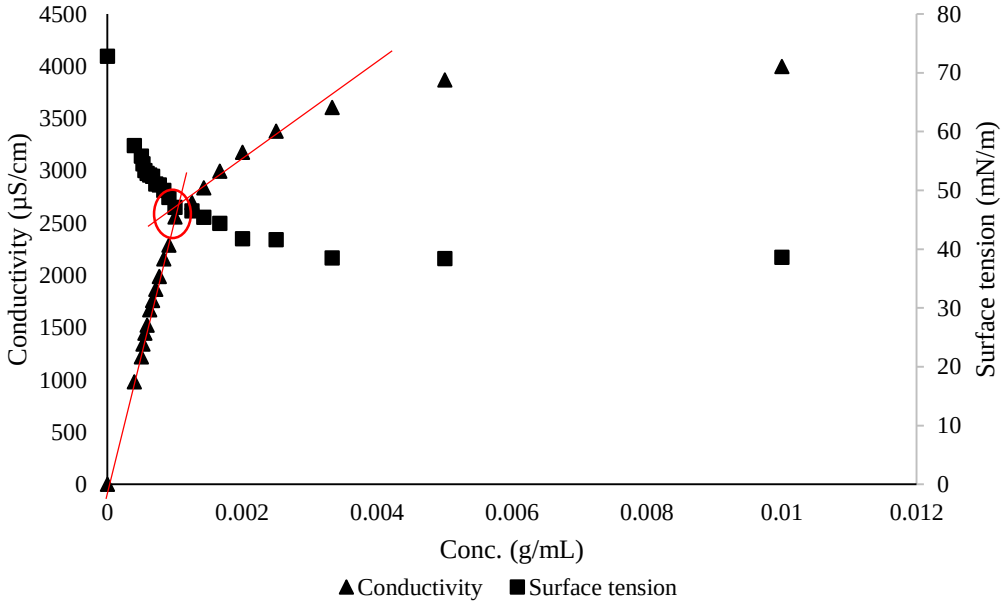


Figure 60: Determination of the CMC for SULF2019002 using surface tension and conductivity with decreasing concentration

3.3.6 Synthesis of anionic methyl ester sulfonates

Methyl ester sulfonate products were obtained through the preparation of fatty acid methyl esters and subsequent sulfonation using chlorosulfonic acid, Figure 49. Like SAS synthesis, MES samples were also dark in colour and were quantified using the standard titrimetric method in section 3.2.11, Table 28.

Table 28: Concentrations of BSFL MES samples obtain and corresponding CMCs

Sample	Concentration (mol dm^{-3})	CMC (mM)
SULF2019007	0.0005	4.07
SUL2019008	0.0003	-
SUL2019009	0.0002	-
SULF2019010	0.0002	4.12
SULF2019012	0.0004	4.15

The critical micelle function was determined by surface tension and conductivity of surfactants with decreasing concentration. For both analyses, the CMC was noted when the trend in the curve change, which for SULF2019007 occurred when the concentration reached 4.07 mM (Figure 61).

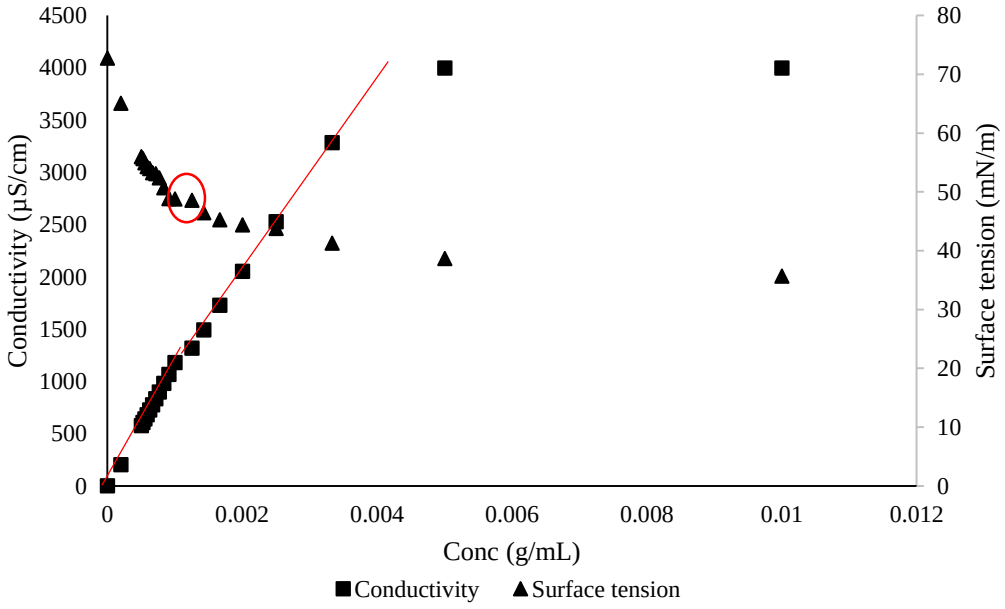


Figure 61: Determination of the CMC for SULF2019007 using surface tension and conductivity with decreasing concentration

FT-IR analysis (Figure 62/Table 29) of the BSFL MESs showed positive identification, assignment of the main functional groups being; 1741 cm^{-1} a C=O asymmetric stretch vibration, 1166 cm^{-1} a SO_3 asymmetric stretch vibration, 1045 cm^{-1} the SO_3 symmetric stretch vibration in $R{-}SO_3$; and 1556 cm^{-1} the strong asymmetric vibration of the O–C–O group. Compared to a previous study on the production of MESs from sesame oil, the FT-IR spectrum shows great likeness with peaks the major bonds being seen, although aliphatic C-H peaks herein display higher intensity (Soy, Kipkemboi and Rop, 2020).

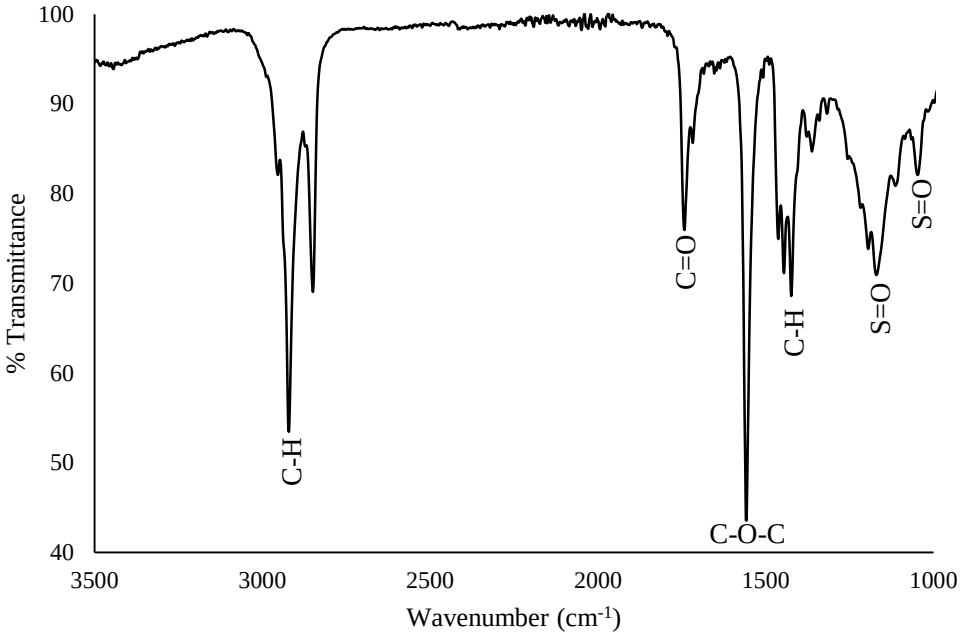


Figure 62: FT-IR chromatogram of mixed MES produced from BSFL oil

Table 29: FT-IR analysis data table for BSFL MESs

Functional Group	Wavenumber (cm^{-1})	Appearance
Aliphatic C-H (stretch)	2922, 2848, 1423	Sharp, strong
Tertiary N	2852	Sharp, strong
C=O (stretch)	1741	Sharp, Medium
Quaternary N^+	1456	Sharp, medium
C-O-C (stretch)	1556	Medium
S=O (stretch)	1166, 1045	Strong, broad

3.3.7 Synthesis of esterquats from BSFL oil

Esterquats are a type of synthetic cationic surfactant with a characteristic quaternary ammnonium compound centre and are generally synthesised by fisher esterification of isolated BSFL FFAs, followed by quarternisation, Figure 63 (Mishra and Tyagi, 2011). Industrial production of esterquats involves subjecting fatty acid glycerol esters are to transesterification with hydroxyl-functionalised tertiary amines in the presence of alkaline conditions (Subirana, Llosas and Oliva, 1999). Application extends to use as antistatic agents in fabric softeners, cosmetic emulsifiers, and antibacterial agents (Mondal and Pratap, 2016).

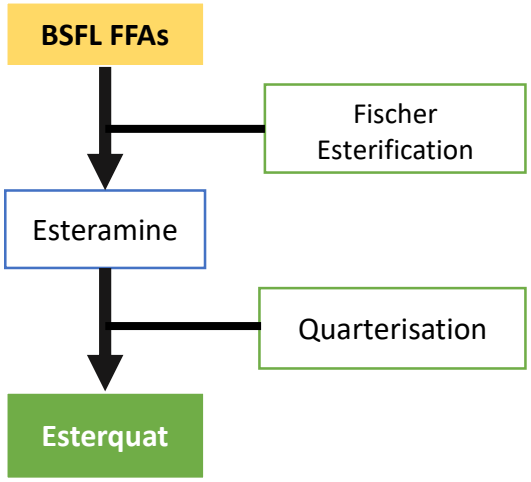
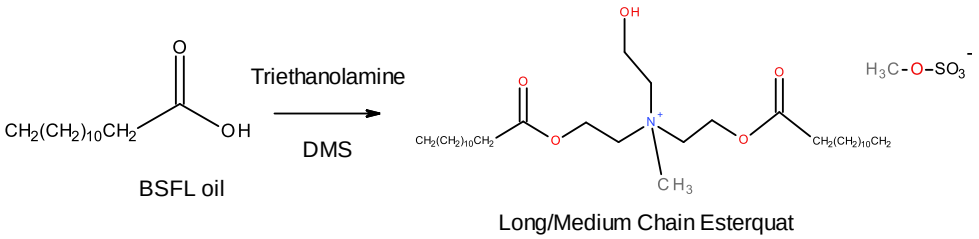


Figure 63: Route taken to achieve cationic esterquats from BSFL oil

Esterquat synthesis was achieved on a laboratory scale by reaction of BSFL oil with triethanolamine, diethanolamine or ethanolamine for 8-10 hours, followed by the addition of DMS (Scheme 6). Palm and coco oils were used as comparative oils in synthesis, to ensure the methodology was comparatively effective in sudsing application.



Scheme 6: Synthesis of esterquats from BSFL oil

The pastes achieved were dark in colour, despite colourless BSFL FFAs and coco oil used, but they produced a good volume of bubbling and were miscible upon mixture with water. Concentration results were achieved by titration against 0.0004 M SDS following method

3.2.12 (Table 30), along with CMC values using surface tension and conductivity measurements. The CMC values are low, which indicates the surfactant is efficient as micellar action occurs with lower amounts of surfactant present. The CMC values were seen to compare favourably to those produced in a study by Mondal *et al*. examining synthesis of esterquats from tallow where CMC were found to be 0.158 and 0.246 mM (Mondal and Pratap, 2016).

Table 30: Concentrations and CMCs of cationic esterquats produced from BSFL, palm and coco oils

Sample	Reaction time (hours)	Yield (g)	Concentration (mol dm^{-3})	CMC (mM)
CATION2020001 (BSFL)	8	5.55	0.00033	-
CATION2020002 (BSFL)	9	6.03	0.00026	2.97
CATION2020003 (Coco)	8	8.03	0.00040	2.09
CATION2020002 (BSFL)	10	6.10	0.00030	2.54
CATION2021003 (Palm)	8	7.65	0.00018	-

The FT-IR data was obtained for synthesised esterquats (Figure 64/Table 31). The formation of esterquats was confirmed by the presence of a O-H stretching vibration at 3292 cm^{-1}, along with peaks of S=O stretching at 1033 cm^{-1} (DMS anion), and C=O at 1735 cm^{-1} (ester group), quaternary N^+ at 1456 cm^{-1}, C-O at 1124 cm^{-1} (aliphatic ether), tertiary N at 2852 cm^{-1} and an aliphatic C-H peak at 2922 cm^{-1}.

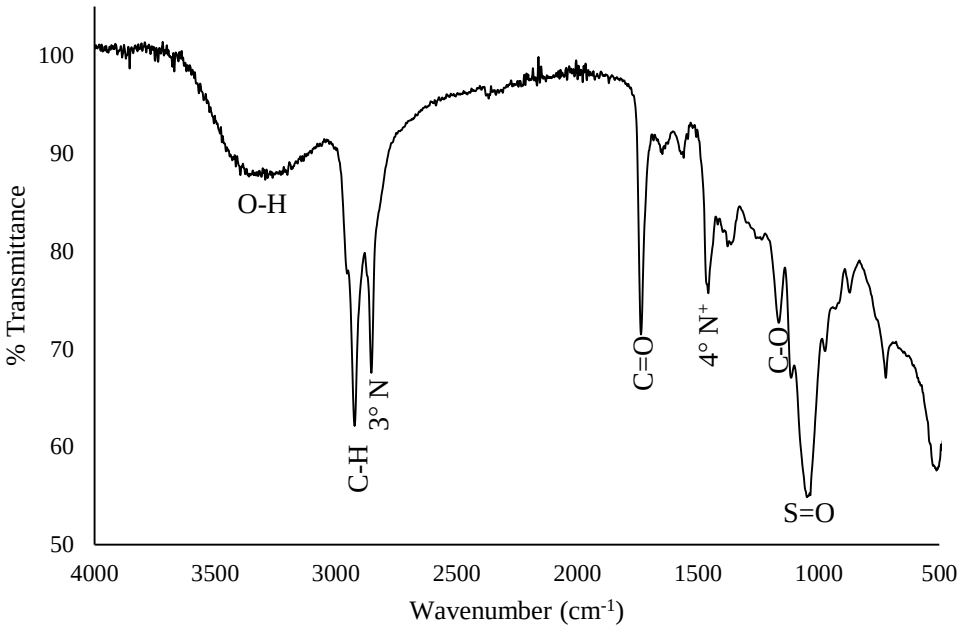


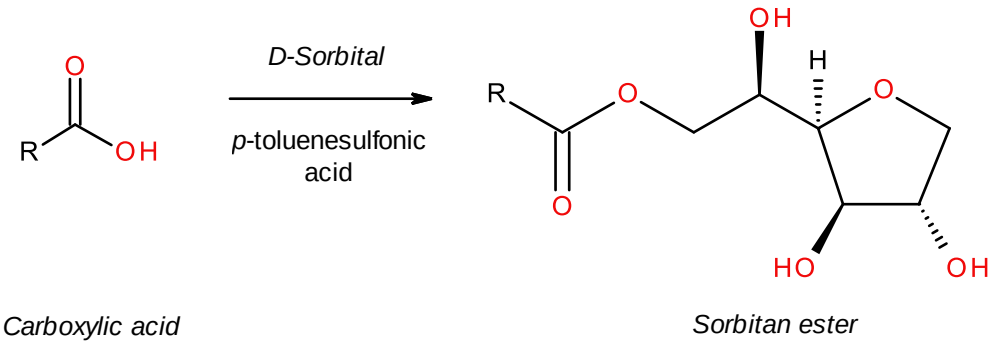
Figure 64: FT-IR spectrum of BSFL esterquats

Table 31: FT-IR analysis data table for BSFL esterquats

Functional Group	Wavenumber (cm^{-1})	Appearance
O-H (stretch)	3292	Broad, strong
Aliphatic C-H (stretch)	2922	Sharp, strong
Tertiary N	2852	Sharp, strong
C=O (stretch)	1735	Sharp, Medium
Quaternary N^+	1456	Sharp, medium
C-O (stretch)	1124	Medium
S=O (stretch)	1033	Strong, broad

3.3.8 Synthesis of spans from BSFL oil

Non-ionic "span" surfactants were produced from BSFL oil using D-sorbital and p-toluenesulfonic acid (Scheme 7). Spans are considered significant due to their performance as being primarily plant-based co-surfactants.



Scheme 7: Synthesis of spans from BSFL FFAs

The presence of sorbitan esters sorbitan monolaurate and sorbitan monopalmitate were confirmed using GC-MS (Figure 65/Table 32). Using peak area, average quantitative conversion of the reagent to product was calculated to be 30.7 %. Low conversion of reagent to product is typical for spans even in industrial production, with commercially available products typically containing ≥ 44 % pure monolaurate, with remaining balance containing reagent lauric and trace amounts of palmitic, myristic and linoleic acids. As spans work best as co-surfactants, this rarely affects their performance, as they continues to display strong emulsifying properties even in low amounts (Attwood and Florence, 2012).

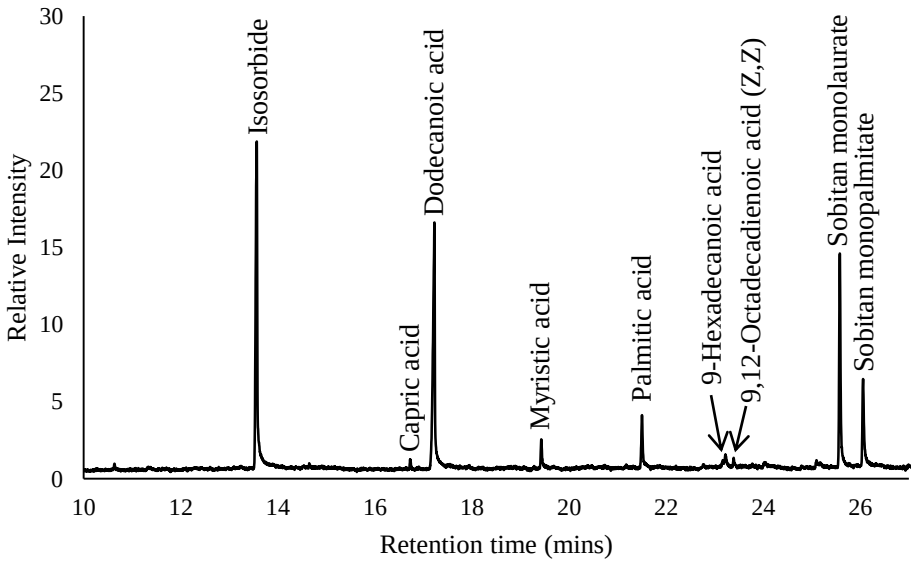


Figure 65: GC-MS chromatogram of non-ionic spans produced from BSFL oil

Table 32: GC-MS results for non-ionic spans produced from BSFL oil

Peak	Retention Time (min)	Name
1	13.5	Isosorbide
2	16.57	Capric acid
3	17.2	Dodecanoic acid
4	19.4	Myristic acid
5	21.5	Palmitic acid
6	23.2	9-Hexadecanoic acid
7	23.4	9,12-Octadecadienoic acid (Z,Z)
8	25.5	Sorbitan monolaurate
9	26.1	Sorbitan monopalmitate

In the reaction, the intermediate isosorbide is first synthesised through the acid-catalysed dehydration of d-sorbitol by *p*-toleuenesulfonic acid. Subsequently, esterification of isosorbide with fatty acids gives rise to isosorbide monoesters or sorbitan esters (Breffa *et al.*, 2013). It likely the reaction conditions were not sufficient for full conversion to products, such as sorbitan monolaurate, to take place, but modification of the parameters might allow for a higher yielding sorbitan ester synthesis, or alternatively using two step synthesis with the isolation of products between steps (Smidrkal, Cervenkova and Filip, 2004) .

Quantification of the surfactants was completed by titration of span samples with 0.1 M KOH-ethanol and phenolphthalein (Table 33), with concentrations based on the majority product being sorbitan monolaurate. The average concentration was found to be 0.0051 mol dm^{-3}. As most non-ionic surfactants are insoluble at room temperature, CMC values were obtained by dissolving samples in DI water and heating to 50 °C, followed by method 3.2.13. The CMCs were found to be low, a beneficial quality to the co-surfactant, meaning that fewer amounts are needed to create micellar action. Compared to a previous study by Smidrkal *et al.* where sorbitan esters were synthesised using a mixture of fatty acids, the CMCs for samples produced herein from BSFL oil were found to be analogous (Smidrkal, Cervenkova and Filip, 2004).

Table 33: Yields and concentrations of non-ionic spans derived from BSFL oil

Sample	Yield (g)	Concentration from titration (mol dm^{-3})	CMC (mM)
NONION2021001	8.58	0.0054	4.62
NONION2021002	8.31	0.0050	5.02
NONION2021003	8.42	0.0048	-

The insoluble nature of the product is further evidence of the presence of spans, which have characteristically low solubility, quantified by a HLB value. For non-ionic surfactants, the HLB refers to the hydrophile–lipophile balance within the monomer and a high HLB (greater than 12) are predominantly hydrophilic and water-soluble (Gelardi *et al.*, 2016). HLB values for products within the BSFL non-ionic surfactant, sorbitan monolaurate and sorbitan monopalmitate are 8.6 and 6.7 respectively, which are extremely low when compared to pure SDS at a HLB of 40 (Griffin, 1954).

FT-IR analysis of the BSFL span product (Figure 67/Table 34) was compared to a commercially available spectrum seen in Figure 66 (Sigma-Aldrich Co. LLC., 2018). The formation of spans was confirmed by the presence of peaks not present in FT-IR spectrums of BSFL isolated FFAs, an O-H stretching vibration at 3388 cm^{-1}, along with peaks of C-O-C stretching at 1078 and 1093 cm^{-1} (heterocyclic and ether). Other peaks were witnessed for C=O at 1728 cm^{-1} (ester group), O-H bend at 1458 cm^{-1}, C-O at 1178 cm^{-1} (alcohol), and a CH aliphatic peak at 2922 cm^{-1}.

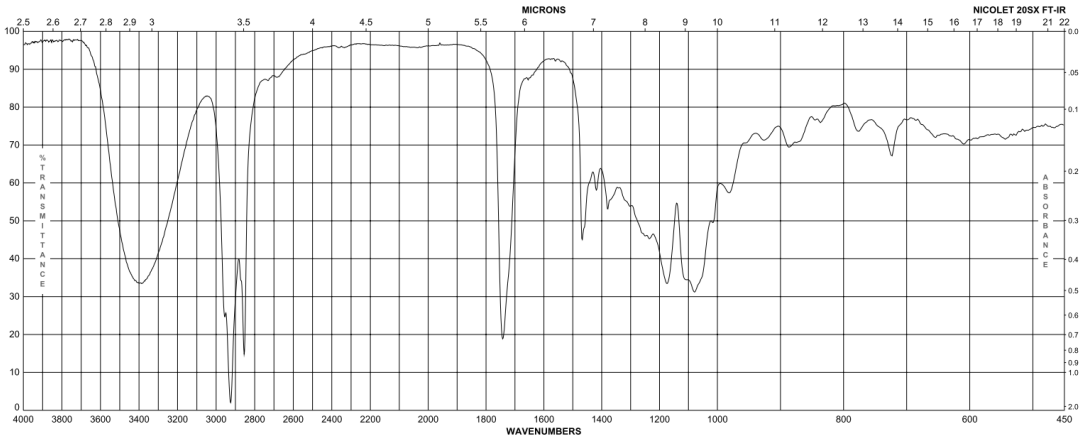


Figure 66: Reference FT-IR spectrum of span 20, accessed 12/12/21 (Sigma-Aldrich Co. LLC., 2018)

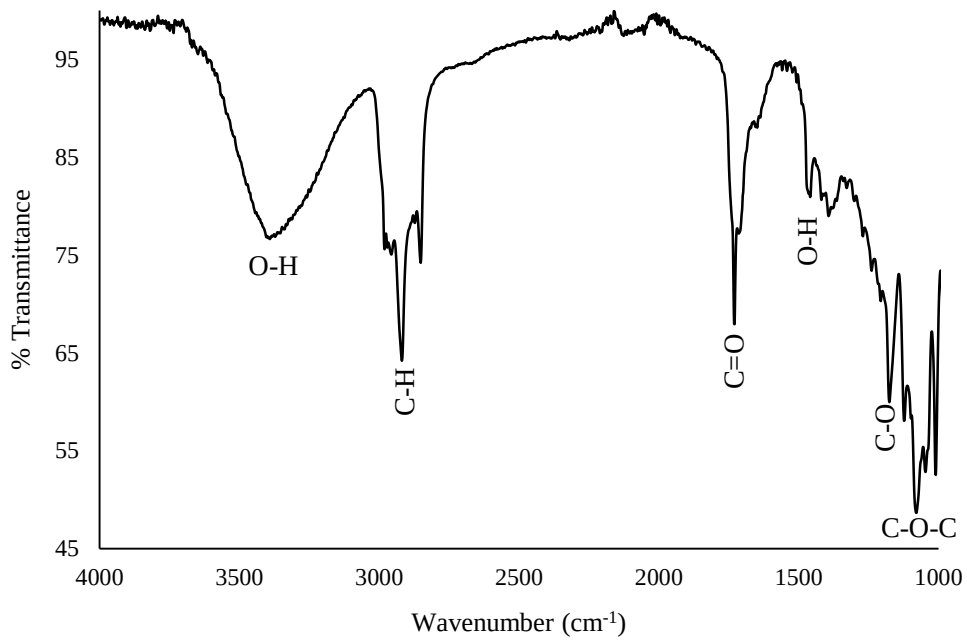


Figure 67: FT-IR spectrum of BSFL non-ionic spans

Table 34: FT-IR analysis data table for BSFL spans

Functional Group	Wavenumber (cm^{-1})	Appearance
O-H (stretch)	3388	Broad, strong
Aliphatic C-H (stretch)	2918	Broad, strong
C=O (stretch)	1728	Sharp, Medium
O-H (bend)	1458	Sharp, medium
C-O (stretch)	1178	Medium
C-O-C (stretch)	1078, 1093	Strong

3.3.9 Synthesis of linear fatty amides from BSFL oil

In the synthesis of amphoteric surfactants dimethylalkylamine oxides, there are two alternative routes and multiple steps required, Figure 68. Amphoteric surfactants behaviour is unique with both anionic and cationic groups, acting dependently on the pH of the solution in which they are dissolved (Bajpai and Tyagi, 2007). In the first synthesis route, FFAs or FAMEs are first converted to amides, followed by dehydration to nitriles, and reduction to amines. The fatty amines may then be further alternated by nucleophilic substitution to secondary fatty amines, or oxidised to obtain the dimethylalkylamine oxide surfactants.

Alternatively, FFAs are hydrogenated to fatty alcohol, followed by nucleophilic substitution The surfactants properties, such as wetting, detergency and foaming, are altered easily by solution pH change (Zoller, 2009).

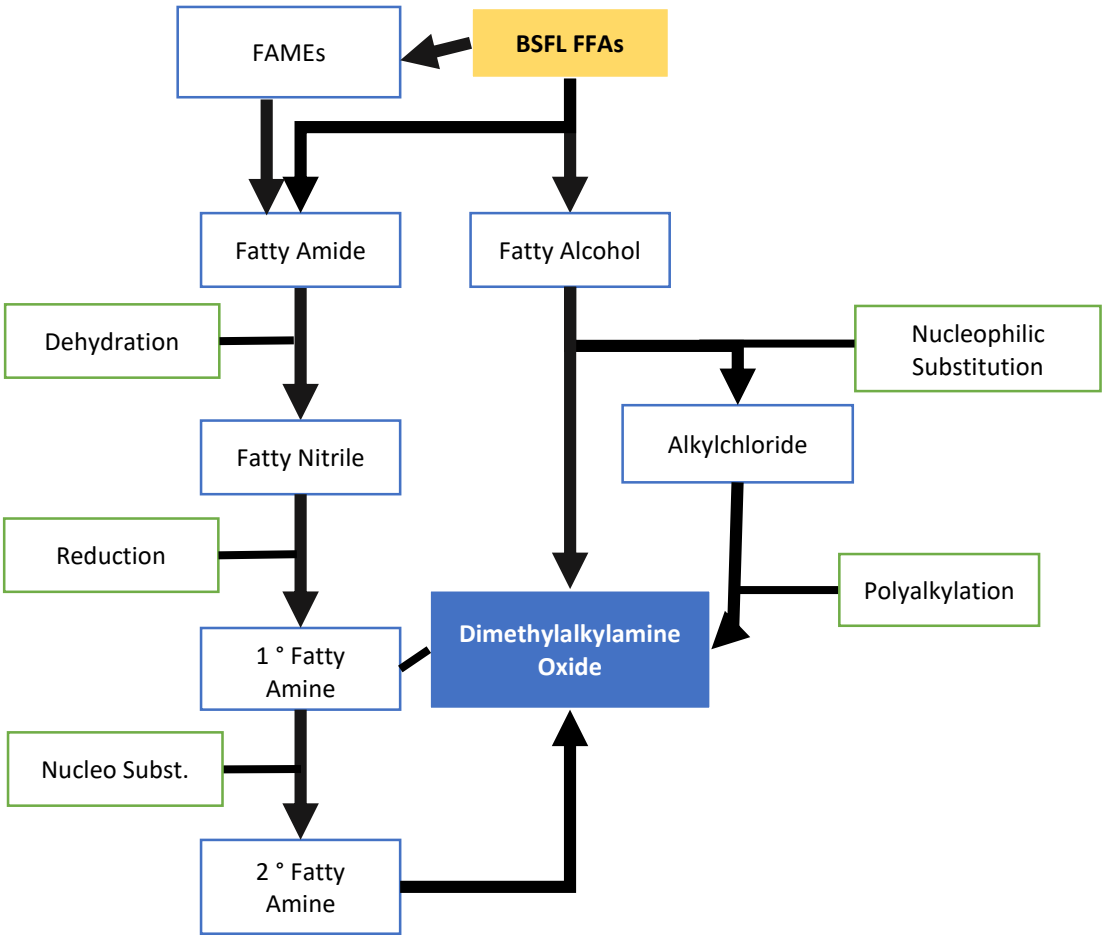
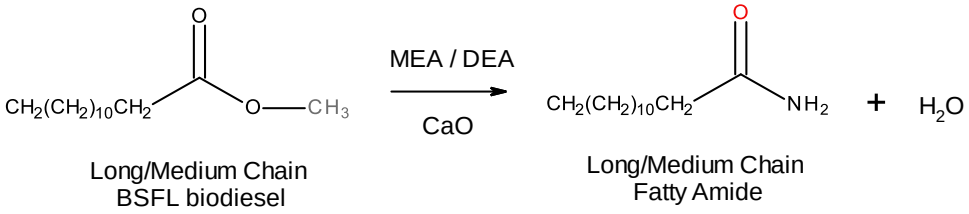


Figure 68: Route taken to achieve anionic surfactant dimethylalkylamine from BSFL oil

In the first stage of dimethylalkylamine oxide from BSFL oil, isolated FFAs were esterified to FAMEs using methanol, and the resulting biodiesel reacted with ethanolamine with calcium oxide catalysis (Scheme 8).



Scheme 8: In the first stage of amine synthesis, a long chain fatty amides are prepared

Subsequent to extraction with hexane, amides were analysed by GC-MS analysis. Analysis of the chromatogram found a mixture of long chain amides, Figure 69 and Table 35, along with dodecanoic acid (lauric acid). The presence of lauric acid is suggestive the conditions of the experiment resulted in reverse esterification of FAMEs, a competing reaction to the production of amides. Despite this, overall conversion to amides by ethanolamine was seen to be more successful than reverse esterificaiton, with peak areas of amides approximately 50 times larger than dodecanoic acid. Interestingly, this methodology resulted in a small amount of N-(2-hydroxyethyl)-decanamide production (Figure 70), undesired in amine oxide synthesis but of great interest in the medical sector as an anti-epileptic drug, desirable due its highly anticonvulsant activity (Guan *et al.*, 2009). As both are established value-added chemicals, further modification of the reaction to tailor the reaction to synthesis or either chemical would be beneficial.

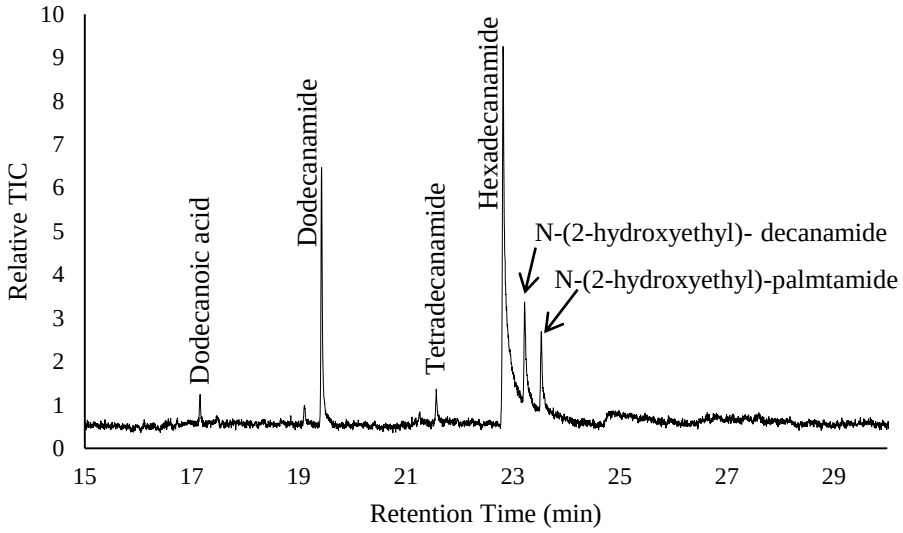


Figure 69: GC-MS chromatogram of the synthesis of BSFL amides from BSFL FAMEs

Table 35: Products resulting from BSFL amide synthesis

Peak	Retention time (min)	Name
1	17.157	Dodecanoic acid
2	19.428	Dodecanamide
3	21.572	Tetradecanamide
4	22.821	Hexadecanamide
5	23.223	N-(2-hydroxyethyl)- decanamide
6	23.54	N-(2-hydroxyethyl)palmitamide

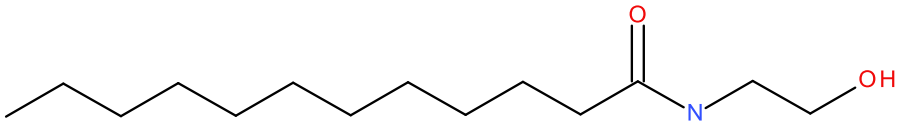


Figure 70: Structure of N-(2-hydroxyethyl)- decanamide

The presence of BSFL amide compounds could be further confirmed by FT-IR analysis, Figure 71. Tabulated results are seen in Table 36. The appearance of a strong broad band at 3311 cm^{-1} was indicative of a N-H amide stretch, occurring both in the long chain fatty amides and in the N-(2-hydroxyethyl)- decanamide derivative. Peaks for amides were also seen, aliphatic C-H stretching vibration at 2922 cm^{-1}, along with a C=O peak at 1024 cm^{-1}, C-O stretch peak at 1122 cm^{-1} and a C-N peak at 1279 cm^{-1}. FFA starting material dodecanoic acid could be seen through the presence of and a O-H bend at 1463 cm^{-1}.

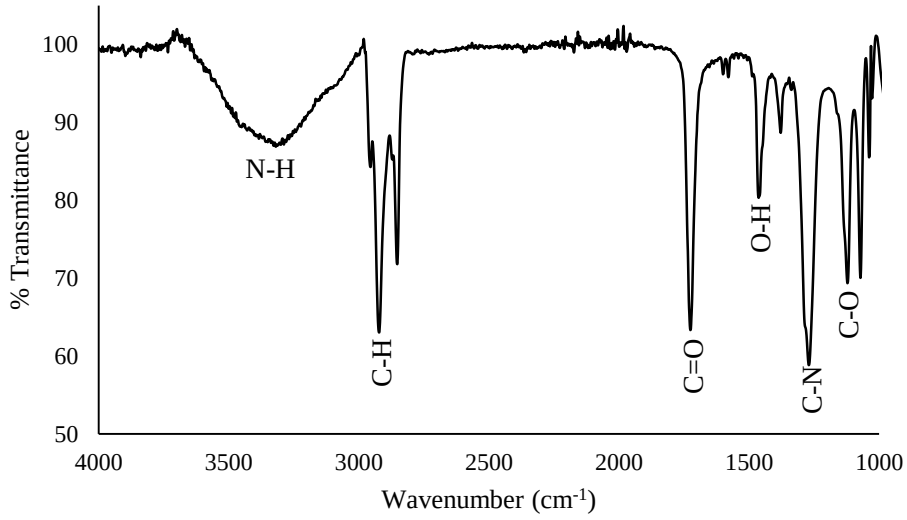


Figure 71: FT-IR spectrum of BSFL amides

Table 36: FT-IR analysis data table for BSFL amides

Functional Group	Wavenumber (cm^{-1})	Appearance
N-H amide (stretch)	3311	Broad, medium
Aliphatic C-H	2922	Sharp, strong
C=O amide (stretch)	1724	Sharp, strong
O-H (bend)	1463	Medium
C-N amide (stretch)	1279	Sharp, medium
C-O (stretch)	1122	Medium

3.4 CONCLUSIONS

The objective of the research contained within this chapter was to produce value-added chemicals through the use of BSFL in waste remediation. Following the extraction and purification of the oil in the previous chapter, the isolated FFAs were subjected to organic lab-scale methodology, alternatives to reactions already occurring on industrial mass scale. As well as providing a method for waste remediation of municipal organic waste in urban environments, the fatty product of BSFLs "bio-catalysis" was demonstrated herein to provide an alternative sustainable and environmentally compliant source of fatty acids for surfactant production to contemporary sources. Current sources of surfactants include petroleum derivatives, tallow, palm and coconut oils, but there is increasing public perception that there are serious environmental issues with the surfactant industry, resulting in a mass loss of biodiversity within tropical rainforests and loss of native habits. In this research, an approach to tackle both of these grand challenges is proposed using BSFL.

Generally, biodiesel obtained from the oils and fats of biomass or zoomass can be used a substitute for or an additive to conventional petroleum-based fuels (Arvanitoyannis and Kassaveti, 2008). Fuel synthesis from BSFL oil was successfully completed in the form of biodiesel with reasonable yields obtained, as an intermediately step to achieve certain surfactant types. Standard esterification reaction methodology was successfully applied to BSFL oil using methanol and acid catalyst, with some alterations to reaction conditions needed; giving a yellow, diesel-smelling fluid that still contained BSFL oil reactant. Although not the objective in this research, it is significant to note that further optimisation is needed improve conversion yield and the properties of BSFL biodiesel must be thoroughly examined before use as a fuel, including engine testing and additionally compared to that seen in WVOs. For that direct comparison to occur, BSFL biodiesel should be compared with biodiesel synthesised from waste vegetable oil, and fresh vegetable oil and tested using standard European methods.

Further investigation into the viability of BSFL to provide biodiesel on a large scale should be completed. The application of BSFL FAMEs in combustion engines should be tested, and the biodiesel fully characterised, especially with the BSFL biodiesel which must meet the approval of European standards (EN 14214) to prevent corrosion in engines if used.

The hydrogenation of BSFL FFAs was completed using sodium borohydride, yielding BSFL primary alcohols. The synthesis of primary alcohols from BSFL oils provides a gateway of many possibilities for the creation of further valuable chemicals, but in itself, acts as a weak anionic surfactant. The preparation of the "hydrogenated" LCFA and MCFA alcohol precursors, with extension beyond the literature methodology, a novel method avoiding the use of hazardous $LiAlH_4$ (McGeary, 1998) was found to be successful with high yields of up to 90 % conversion obtained.

For the primary objective of making value-added chemicals, the synthesis of anionic, cationic and non-ionic surfactants from BSFL oil was successfully demonstrated. The production of solid soap was successfully accomplished using sodium hydroxide. The procedure was simple, and hypothesised to be industrially-scalable and economically feasible following BSFL oil extraction as the process is one of a simple nature and BSFL fats could easily substitute current sources. The smell was seen to be somewhat unpleasant, so in order to be commercially viable, additives such as perfumes or essential oils would need to be added and investigated to ensure that they did not interfere with the saponification mechanism. In addition, the synthesis of BSFL SASs was explored, with successful conversion using lab-scale methodology found using FT-IR and titrimetric analysis. Attempts to quantify SASs were made using a variety of analysis techniques, but method validation by comparison of methods was unsuccessful. It was assumed that the reason for this lay in the nature of the composition of BSFL oil, with the product SASs containing a sulfonated derivatives of mixture of FFAs ranging from C_{10} to C_{18}. Successful quantitation of such a range, and from a biological source would be challenging, without having the commercially pure versions of SASs, which are presently not obtainable easily without great expense and beyond the remit budget of this project. In addition, improved separation and quantification of the BSFL SASs would likely be achievable with more specialised equipment, such as HPLC-ELSD with a Thermo Scientific™ Acclaim™ Surfactant HPLC column, again exceeding the resource budget.

To further complete the goal of synthesising valuable chemicals, the synthesis of anionic BSFL MES surfactants was considered using BSFL FAMEs, and subsequently qualitatively examined using titrimetric and FT-IR analysis. Following this, cationic BSFL esterquats were synthesised, with significant application extending to use as antistatic agents in fabric softeners, cosmetic emulsifiers, and antibacterial agents. Non-ionic spans were produced

using d-sorbitol, qualitative assessed using GC-MS and FT-IR spectroscopy, and quantified by titration. The production of amides was completed using a lab-based reaction of BSFL FAMEs, and the demonstration of such displayed the start of a route to BSFL based amine oxide amphoteric surfactants. In an industrial application, obtaining a measure of the biodegradability of the surfactants produced herein would allow for the "green" label of industrial marketing of surfactants produced from BSFL oil which would be completed through thorough life-cycle assessment analysis.

This innovation provides a novel and sustainable route to consumer products via an alternative oil source. Following lab-scale organic synthesis methodology from BSFL FFAs to advanced surfactants, the basis of BSFL oil adheres to several green chemistry principles, through the use of renewable feedstock and the generation of substances with as little toxicity as possible. The surfactants produced can be denoted sustainable, sourced from the remediation of municipal organic waste and therefore a more environmentally friendly alternative to the current market alternatives of palm, tallow and coco oils. Moreover, the surfactants produced from this investigation are biodegradable, REACH compliant, with minimal effect to the environment.

4 BSFL DERIVED CHITIN AND CHITOSAN

Chitin is a highly basic bio-polysaccharide occurring widely in nature. It has unique properties which make it ideal for use in many chemical applications from catalysis to films. Commercial forms of chitin, or its deacetylated version chitosan, are easily obtained from shellfish exoskeletons, but other sources include fungal mycelia and in this work, black soldier fly.

4.1 LITERATURE REVIEW

Chitin is one of the most abundant naturally occurring amino polysaccharide worldwide (Ravi Kumar, 2000). Chitin is widely distributed in nature, with the main sources being lower eukaryotic organisms such as fungi, molluscs, arthropods and shellfish (Martinez, Pilar Falomir and Gozalbo, 2014). It acts as an important support component, much like its polysaccharide cousin cellulose, sharing common characteristics of low solubility and low chemical reactivity. Unlike cellulose, chitin is not found in plant-based sources, and differs structurally by having an acetamido in place of a hydroxyl group at position C-2 (Figure 72) (Ravi Kumar, 1999). Due to the increased hydrogen bonding this provides, chitin polymer matrices often have increased strength.

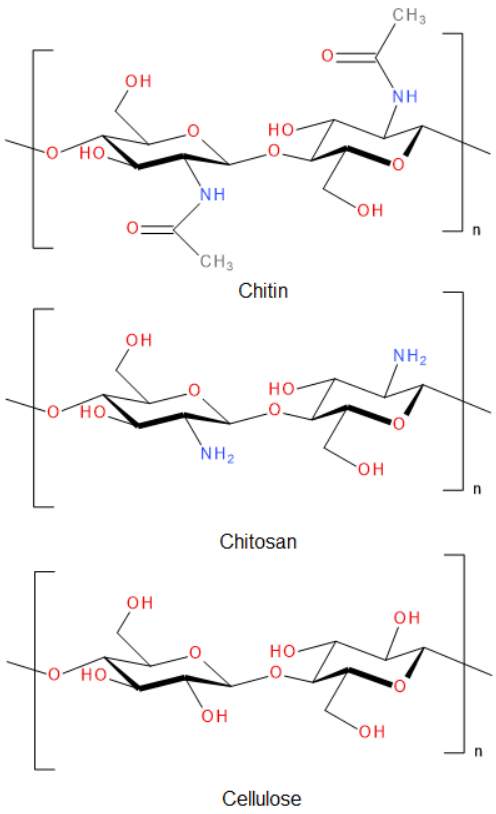


Figure 72: Structures of Chitin, Chitosan and Cellulose

Chitin consists of a 2-acetamido-2-deoxy-b-D-glucose molecules joined through a β (1→4) linkage. Chitosan is the non-toxic *N*-deacetylated version of chitin, with a 'degree of deacetylation' (% DD) being the universal indication to the extent of deacetylation present. The chemical modification usually takes place through strong alkaline treatment. Deacetylation allows the resulting chitosan to increase in solubility, relative to chitin, meaning that there is a wider range of applications available (Kumirska, Kaczy and Bychowska, 2010). As natural polymers, they have excellent properties of adsorption, biodegradability, and biocompatibility (Rinaudo, 2006). As a result, both chitin and chitosan are of commercial interest, with a high percentage of nitrogen present averagely (~6.89 %) compared with cellulose (~1.25 %) (Ravi Kumar, 2000), making both polysaccharides biochemically active. Chitin can form films or chelate metal ions, as well as having structural optical characteristics. Applications being discussed in greater detail in section 4.1.8.

4.1.1 Molecular structure of chitin

Chitin has two naturally occurring allomorphs, α and β forms (Figure 73), a characteristic dependent on the source material. α-chitin form consists of alternating antiparallel arrangements of the polysaccharide chains, whereas β-chitin exists with a conflicting parallel chain arrangement. Allomorphs can be identified using X-ray diffraction, infrared and NMR spectroscopies. Of the two, α-chitin is found in more abundance, occurring in fungus walls (Merzendorfer, 2011), shellfish (Kaya, Baran and Karaarslan, 2015), marine organisms (Atkins, Dlugosz and Foord, 1979) and insect cuticle (Muthukrishnan, Mun, Noh, Geisbrecht and Arakane, 2020). The rarer β-chitin is found less commonly in sources including squid pens, *Aphrodite chaetae* and some protozoa (Rinaudo, 2006). Unlike α-chitin, which systematically reforms in any recrystallization of chitin, β-chitin cannot be formed from solution or by *in vitro* biosynthesis, indicating that the alpha form is more thermodynamically stable (Roy *et al.*, 2017).

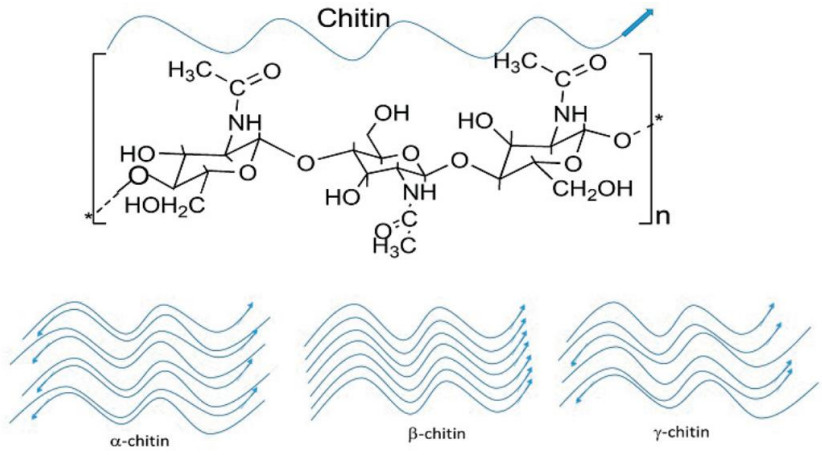


Figure 73: Structure of different chitin conformations (Roy *et al.*, 2017)

The structural integrity of both conformations is maintained through the strong network of hydrogen bonding between chitin chains, by the functional groups of C=O → N-H and C=O → O-H. The improved thermodynamic stability of α-chitin is hypothesised to exist due to additional inter-chain hydrogen bonding between hydroxyl-methyl groups (Kumirska, Kaczy and Bychowska, 2010; Roy *et al.*, 2017). Conversely, β-chitin is weaker, more susceptible to swelling, and is more vulnerable to interruption from water, alcohol and amines. Consequently, β-chitin can be marginally more soluble in organic solvents than its α-chitin counterpart.

4.1.2 Properties of chitin and chitosan

The degree of *N*-acetylation in chitin is important as the number of acetyl groups present has a detrimental effect on chitin solubility and solution properties. Both α and β variations of chitin are highly hydrophobic, insoluble in water and most organic solutions aside from strong halogenic species; lithium chloride, hexafluoroisopropanol, hexafluoroacetone and chloroalcohols (Ravi Kumar, 2000; Rinaudo and Pérez, 2019). Chitosan, once deacetylated, is soluble in most acidic solutions, with the exception of sulphuric acid. If undesired, chitosan must be cross-linked to prevent the polymer from dissolving. Both chitin and chitosan degrade prior to melting, a characteristic typical of polysaccharides with extensive hydrogen bonding throughout. Strong acidic hydrolysis of chitin produces relatively pure amino acid, d-glucosamine (Bertuzzi *et al.*, 2018). In order to obtain the repeating monomeric unit, first cleavage of the glyosidic bonds takes place, followed by severing of the amide bonds, leading to the deacetylation of the glucosamine subunits (Figure 74).

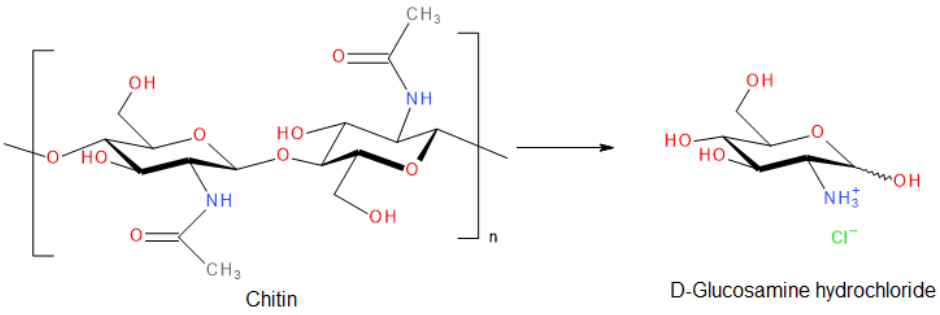


Figure 74: Chitin hydrolysis to obtain D-glucosamine hydrochloride

The functionally of all chitin confirmations is determined by the amino groups within the polysaccharide backbone, denoted as previously mentioned % DD. Nitrogen content varies from 5 to 8 %, depending on the chitin source (Tshinyangu and Hennebert, 1996; Ravi Kumar, 2000). As a result, chitin and chitosan mostly undergo reactions typical of amines, such as Schiff reactions and *N*-acylation. In chitosan, *N*-acylation with acyl halides or acid anhydrides creates amido groups at the nitrogen group. Reactions with acetic anhydride achieves fully acetylated chitin (Tokura, Nishi, Nishimura and Ikeuchi, 1979). Under standard temperature and pressure, chitosan forms aldimines with aldehydes and ketimines with ketones. Chitosan and simple aldehydes produce N-alkyl chitosan upon hydrolysis (Ravi Kumar, 1999). The addition and presence of bulky functional groups to the intra-chain, does affect the prevalence of hydrogen bonding and therefore is significant in desirable film-forming properties (Muzzarelli, 1973).

4.1.3 Sources of chitin

Chitin was first described in 1811 by Henri Braconnot, director of the botanical gardens of the Academy of Sciences in Nancy, France (Morin-Crini, Lichtfouse, Torri and Crini, 2019). The substance, extracted from mushrooms, named "fungine", was found not to dissolve in sulphuric acid and to contain nitrogen. In 1843, the same substance would be discovered in the exoskeleton of butterfly *Bombyx mori* by Jean Louis Lassaigne, similar but markedly different to cellulose which had been identified four years prior (Tucker, 1945). By the 20th Century, large numbers of studies had been completed on the subject of chitin and its derivative chitosan from a wide variety of sources, and research turned to its potential applications.

Nowadays, commercial sources stem from waste shells resulting from large-scale industrial fishing (Mathur and Narang, 1990), discarded by the canning industries in Oregon,

Washington, Virginia and Japan (Ravi Kumar, 2000). The production of chitin from waste is very economically feasible, especially if carotenoids, such as the antioxidant astaxanthin used in vitamin supplements, can be extracted as well. Chitin content ranges between 15-40 %, depending on the species of crustacean (Table 37). However, productivity within the shellfish industry is largely season dependent meaning that there are lags in availability (Pittman and McAlpine, 2003) and more recently, the sustainability of crustacean farming has come under review due to overfishing predictions (Gillett, 2008). Additionally, the global market for chitin and chitosan is expected to grow annually at a rate of 15.4 % (Hahn *et al.*, 2020), due to rising demand for natural fibre biodegradable composites in industrial applications, such as drug delivery systems in the pharmaceutical industry (Tan, Lee and Chen, 2020), highlighting the need to find alternative sources.

The second most prevalent source of chitin after crustaceans is fungi. They present a vegan source material, and as chitin makes up between 1–15 % of the fungal cell wall, are conceivably a good comparable alternative. To date, the scaled-up industrial production of fungal chitin has not been completed.

In essence, there is a potential for insect sources for chitin. Those that include recycling organic waste streams, such as black soldier fly, are of great interest as they present a sustainable alternative to crustacean sources. Insects furthermore have advantageous features of high reproductive rate, easy rearing requirements and not being subject to seasonality (Van Huis, 2013). In the mass rearing of BSF for the purpose of extracting fat for conversion to useful chemicals, chitin is essentially a by-product and therefore its utilisation increases the economic viability of this sustainable approach to waste remediation.

Table 37: Sources of Chitin

Origin	Species	Biomass source	Nitrogen (%)	Chitin in dry biomass (% w/w)	Ref.
Crustaceans *(Arthropoda phylum)*					
Shrimp	*Penaeus monodon*	Shells	5.7	10	(Kaya, Baran and Karaarslan, 2015)
Isopoda	*Oniscus asellus*	Dried Adult	4.7	6-7	(Abdou, Nagy and Elsabee, 2008)
Crabs		Shells	n/a	16.7	
Crayfish	*Procambarus clarkii*	Shells	n/a	20.6	
Insects *(Arthropoda phylum)*					
Honey Bees	*Apis mellifera*	Exoskeleton	5.6	2.5	(Kaya, Baran and Karaarslan, 2015)
Grasshopper	*Schistocerca gregaria*	Exoskeleton	2.9	12.2	
Beetle	*Melolontha melolontha*	Fully dried adult	6.7	13-14	(Kaya *et al.*, 2014)
Black Soldier Fly	*Hermetia illucens*	Whole larvae	n/a	6.4	(Bastiaens, Soetemans, D'Hondt and Elst, 2019)
Fungi					
Button mushroom	*Agaricus bisporus*	Cell wall		43.8	(Wu, Zivanovic, Draughon and Sams, 2004)
Zygomycota	*Mucor rouxii*	Cell wall		50.1	(Wu, Zivanovic, Draughon, Conway and Sams, 2005)
Arachnids					
Spider	*Geolycosa vultuosa*	Whole biomass	6.4	8-8.5	(Kaya, Seyyar, Baran, Erdoğan and Kar, 2014)
	Hogna radiata	Whole biomass	6.4	5.5-7	

4.1.4 Extraction of chitin

For most sources, chitin is found within a complex network of proteins, lipids and minerals comprising the invertebrate cuticle of the insect (Kramer, Hopkins and Schaefer, 1995). Some insect cuticles contain low amounts of mineralisation, while others such as BSFL, contain high amounts of calcium and therefore removal in demineralisation is paramount. The high calcium content however, does make live BSFL animal feed desirable in the agricultural rearing sector (Wang and Shelomi, 2017).

Industrial processing of chitin and chitosan involves three main steps. Initially, chitin is extracted by treatment with an acid to remove minerals present within the shell of most arthropods. In most cases, this is in the form of calcium carbonate. Following this, is the removal of proteins, completed using an alkaline solution. Often there are decolourisation steps to remove colorant if a colourless chitin is desired. Conversion and partial deacetylation of chitin to chitosan takes place through very strong alkaline treatment. Molarity of reagents and duration of extraction is dependent on the structure of the starting material, and therefore the parameters used industrially will not always be applicable on a lab-based scale for BSFL. Both chemical and biological methods can be used, but economically the biological method is less favourable due to lower extraction yields (Finke and Oonincx, 2013).

4.1.4.1 *Demineralisation*

The first stage in chitin recovery, is the chemical treatment of the defatted insect content with HCl to remove minerals. In this step, minerals such as Ca, Mg and K are removed in the form of carbonates or chlorides from the cuticle. Indication of minerals removed can be seen in the production of CO_2 gas, and the change in weight subsequent to drying.

The molarity of acid, ratio of acid to material and time duration of the reaction varies in practice, and varies based upon material (Table 38). In a 2018 study by Caligiani *et al.*, 0.5 or 1 M HCl treatment at STP for 24 hours is deemed a sufficient concentration for the revival of minerals from BSFL (Caligiani *et al.*, 2018), however a study by Waśko *et al.* in 2016 used double the molarity at 80 °C for the same time frame (Waśko *et al.*, 2016). In another study by Kaya *et al.* comparing the extraction of chitin from insects to crustaceans, samples were refluxed in a 4 M HCl solution at 75 °C for 2 hours (Kaya *et al.*, 2014). The conditions employed in these examples are quite harsh when there is evidence to suggest more moderate

conditions are acceptable, as insects generally have low levels of mineral material (< 10 %) (Cauchie, 2002) compared to crustacean shells (20–40 %) (Tolaimate, Desbrieres, Rhazi and Alagui, 2003; Verkerk, Tramper, van Trijp and Martens, 2007). For example, in another study, extracting chitin from *Parapenaeus longirostris* shrimp found that 0.25 M HCl treatment at STP for 120 minutes gave satisfactory extraction (Dahmane, Taourirte, Eladlani and Rhazi, 2014). This provides a strong case for using ambient temperatures in the demineralisation of BSFL. Reaction times and acid molarity must be compared to ensure an adequate investigation into optimised extractions, as a 2020 review by Hahn *et al.* found that the variability in demineralisation conditions for insects ranges from 0.5-4 M HCl for a duration of 0.3 to 24 hours, indicating that the effect of demineralisation time is not well understood (Hahn *et al.*, 2020).

Table 38: Methods for the demineralisation of some insects and crustaceans

Insect Species	Reagent and conc.	Temp (°C)	Duration (hrs)	Ref.
Hermetia illucens **(larvae, pupae, adults)**	0.5 M CH_2O_2	Room temp	1	(Hahn, Roth, Ji, Schmitt and Zibek, 2020)
	0.5 M HCl	Room temp	2	(Khayrova, Lopatin and Varlamov, 2019)
	2 M HCl	Room temp	24	(Caligiani *et al.*, 2018)
	1 M HCl	100	0.5	(Smets *et al.*, 2020)
Tenebrio molitor* / *Allomyrina dichotoma **Beetle (larvae, pupae, adults)**	2 M HCl	Room temp	24	(Shin, Kim and Shin, 2019)
Holotrichia parallela **Beetle *(Adult)***	1 M HCl	30	0.5	(Liu *et al.*, 2012)
Prawn shells	0.5-1 M HCl	Room temp	24	(Mohammed, Williams and Tverezovskaya, 2013)
Squid pens	1 M HCl	Room temp	24	(Kurita *et al.*, 1993)

In this study, the demineralisation performance of a strong mineral acid, HCl, will be compared to an organic acid, lactic acid. Within this, the most appropriate reaction duration

of and demineralisation concentration will be investigated. Compared to HCl, lactic acid has some favourable benefits and green principle compliance, as it is less harmful to the environment, biodegradable, inexpensive and produces a non-toxic waste water stream when used in the demineralisation process (Ameh, Abutu, Isa and Rabiu, 2014).

Lactic acid (2-hydroxypropionic acid or 2-hydroxypropanoic acid) is a resultant by-product from the cheese industry, with the fermentation of carbohydrates in milk by *Lactobacillus* bacteria resulting in lactic acid formation as a by-product (John, G.S., Nampoothiri and Pandey, 2009). It is the most prevalent widely occurring carboxylic acid, with a variety of applications in the food, leather, textile, pharmaceutical among other chemical industries (Wee, Kim and Ryu, 2006). There are two optical isomers: L (+)-lactic acid and D (–)-lactic acid, both obtained naturally though bacterial fermentation with high product isomeric specificity. Lactic acid can also be produced through chemical synthesis, but as this is from a non-renewable petrochemical source, lactonitrile, and it produces a racemic mixture of both D- and L- isomers, it is not favoured industrially (John, Nampoothiri and Pandey, 2007). Notably, in the food industry, the esters of lactic acid by reaction with sodium or calcium are used as dough conditioners and emulsifiers in bakery products (John, Nampoothiri and Pandey, 2006). As calcium is a large component mineral within the exoskeleton of BSFL, this is beneficial to note.

Previous studies on the removal of minerals using lactic acid instead of HCl have shown success in the demineralisation of crustacean shells. In a study by Greene *et al*., the most effective lactic acid concentration was found to lie between 1.14 and 2.28 M, with an increased reaction duration at room temperature seen to be competitive with temperatures of 60 °C for green crab demineralisation (Greene, Robertson, Young and Clyburne, 2016). For this reason, reaction duration will be investigated for BSFL chitin extraction, as it is less energy consumptive than using heat. In the demineralisation of shrimp shells, at a shells to acid ratio of 1:20, 2 hours was a sufficient retention time at room temperature (Mahmoud, Ghaly and Arab, 2007). Furthermore, in the demineralisation of shrimp shells by Ameh *et al*., treatment of 1.0 M lactic acid for 20 minutes was found to be most effective, using residual concentration of calcium afterwards as a measure of reaction completion (Ameh, Abutu, Isa and Rabiu, 2014). Higher concentrations and longer reaction durations will be compared in the demineralisation of BSFL exoskeletons.

4.1.4.2 *Deproteinisation*

Following mineral removal, protein is extracted from the remaining dried insect powder. For BSFL, this means the protein within the cuticle of the exoskeleton. Chemical deproteinisation is commonly completed through alkaline treatment, preferentially NaOH due to its economic viability, along with an increased reaction temperature. Reaction effectiveness is dependent on the process temperature, along with the biomass to reagent ratio (Sagheer, Al-Sughayer, Muslim and Elsabee, 2009). Strong reaction conditions are required to hydrolyse bonds between proteins and chitin, and final chitin yield is characterised by the loss of protein mass in this step. For BSFL, protein contributes approximately 32 % on a dry mass basis (Caligiani *et al.*, 2018), so a loss of around one third of the mass is to be expected.

Previous studies of deproteinisation steps in chitin removal from insects are well established but vary in reaction conditions (Table 39). In the study of chitin extraction for *Musca domestica*, a temperature of 95 °C for 6 hours with a ratio of biomass to 1 M NaOH of 1:10 was used in the deproteinisation step (Ai, Wang, Yang, Zhu and Lei, 2008), with Song *et al.* replicating this method in the extraction from blowfly larvae (Song, Yu, Zhang, Yang and Zhang, 2013). Alternative studies of chitin removal found that using too high of a temperature or concentration resulted in immediate conversion to chitosan, full or partial deacetylation of amino groups, preventing the isolation of chitin (Younes and Rinaudo, 2015).

Table 39: Methods for the deproteinisation of some insects and crustaceans

Insect Species	Reagent and conc.	Temp (°C)	Duration (hr)	Ref.
Hermetia illucens **(larvae, pupae and adults)**	1.9 M NaOH	50	2	(Hahn, Roth, Ji, Schmitt and Zibek, 2020)
	1 M NaOH	80	24	(Khayrova, Lopatin and Varlamov, 2019)
	1 M NaOH	40	1	(Caligiani *et al.*, 2018)
	1 M NaOH	80	24	(Smets *et al.*, 2020)
Tenebrio molitor* / *Allomyrina dichotoma **Beetle (Larave, pupae and adults)**	10 % (w/v) NaOH	80	24	(Shin, Kim and Shin, 2019)
Holotrichia parallela **Beetle *(Adult)***	1 M NaOH	80	24	(Liu *et al.*, 2012)
Prawn shells	1 M NaOH	70	24	(Mohammed, Williams and Tverezovskaya, 2013)
Squid pens	2 M NaOH	Room temp	24	(Kurita *et al.*, 1993)

In a final year project completed by an MEng student working on this topic, it was found that the optimised conditions for the deproteinisation of BSFL were 1 M NaOH 80 °C for 3 hours (Sutcliffe, 2019), and therefore these conditions were applied in the extraction of chitin in this research.

4.1.5 Production of chitosan

The high crystallinity of chitin makes it insoluble in most solvents. For certain applications, modifying chitin to a more useable, soluble form is of importance. Chitosan is the simple *N*-deacetylated derivative of chitin, with deacetylation almost never taking place to completion. Therefore, when chitosan is prepared, % DD is an important evaluation of modification to the acetyl groups. Conversion can be completed using chemical or biological means. Biological deacetylation takes place through enzymatic activity, but as the chitin crystallinity is complex, enzymes are not efficient on a larger scale (Win and Stevens, 2001). Industrially, deacetylation of crustacean shells takes place through chemical deacetylation

due to higher conversion rates, suitability for scaled equipment and lower cost (Hahn, Roth, Ji, Schmitt and Zibek, 2020).

In the chemical conversion, an alkali, preferentially NaOH, deacetylates the amino group, and the product is denoted as chitosan when the % DD reaches 50 %, and it becomes soluble in aqueous acidic solution (Rinaudo, 2006). Change in solubility facilitates application, meaning that chitosan is particularly useful in gels, films and fibre uses. In order for the acetyl group to be removed efficiently, a strong alkali concentration must be used (Pillai, Paul and Sharma, 2011). In the conversion of chitin to chitosan, various concentrations have been investigated. Generally, high concentrations of around 50 % (w/v) have been used in practice, with a stronger concentrations and longer experimental durations often resulting in a higher % DD (Hahn *et al.*, 2020). In an investigation by Mohammed *et al.*, it was concluded that 50 % NaOH at 100 °C for up to 10 hours resulted in a chitosan with 64 % DD over a range of temperatures and durations from prawn shells (Mohammed, Williams and Tverezovskaya, 2013). In addition, a review by Pakizen *et al.* found that a solid to liquid ratio (w/v) (g/mL) of 1:10 produced good conversion from a range of studies (Pakizeh, Moradi and Ghassemi, 2021). For this reason, a concentration of 50 % (w/v), a 1:10 solid to liquid ratio (g/mL) and a temperature of 100 °C for 6 hours will be used for BSFL. Further methods for the deacetylation of some insects and crustaceans can be seen in Table 40.

Table 40: Methods for the deacetylation of some insects and crustaceans

Insect Species	Reagent and conc.	Temp (°C)	Duration (hrs)	Ref.
Hermetia illucens **(larvae, pupae and adults)**	12 M NaOH 10M NaOH	120-140 4	3-6 12	(Hahn, Roth, Ji, Schmitt and Zibek, 2020)
	50 % (w/v) NaOH	100	2	(Khayrova, Lopatin and Varlamov, 2019)
Tenebrio molitor* / *Allomyrina dichotoma **Beetle (Larave, pupae and adults)**	55 % (w/v) NaOH	90	9	(Shin, Kim and Shin, 2019)
Prawn shells	25 % (w/v), 50 % (w/v) NaOH	80, 100	2, 5, 10	(Mohammed, Williams and Tverezovskaya, 2013)
Squid pens	40 % (w/v) NaOH	80	3	(Kurita *et al.*, 1993)

4.1.6 Chitin and chitosan characterisation

In characterisation, the properties of chitin and chitosan are dependent on the source and extraction process. Protein and ash content can be used to assess the quality and purity of extracted material. A variety of methods can be used to assess chitin and chitosan quality, ranging from X-ray diffraction (XRD) for crystalline structure determination, to elemental analysis (EA) for % DD. For BSFL, commercial chitosan derived from crustaceans will be used as a reference material.

4.1.6.1 *XRD*

X-ray diffraction is used to characterise chitin and provide information of the polymorphic form of the crystal structure using the detection of elastically scattered X-rays. Notable peaks for insect-derived highly symmetrical α- polymorph chitin can be seen at 9–11 ° and 19–20 °, along with minor peaks at 12–13 °, ∼21 °, ∼23 ° and ∼26 ° (Liu *et al.*, 2012; Erdogan and Kaya, 2016; Hahn *et al.*, 2020). β-Chitin and γ-chitin polymorphs exhibit different diffraction patterns, having a lower degree of order than α-chitin, differing mainly

at the peak at 12.9 °. Furthermore, the conversion to chitosan from β-chitin produces chitosan with lower crystallinity compared to deacetylation of α-chitin (Kurita *et al.*, 1993). Crystallinity index (CI) is a quantitative indicator of crystallinity and represents a measure of level of order, perfection and average crystallite size in a sample (Reyes-Gasga *et al.*, 2013; Sa *et al.*, 2017). The crystallinity index for chitin and chitosan can be calculated according to Segal *et al.*, 1959, where I_{am} represents the peak intensity at measured at roughly 9 ° and I_{110} the peak at 20 ° (Equation 5).

$$Crystallinity\ index\ (\%) = \frac{I_{110} - I_{am}}{I_{110}} \times 100$$

Equation 5: Crystallinity index for chitin and chitosan calculated using XRD analysis

Crystallinity values range between 40-90 % and differ due to source material, processing conditions, and sample preparation. For example, if the sample experiences vigorous grinding, the crystalline structure is likely to be affected by mechanical force. As a solubility is determined by extent of crystallinity, lower crystallinity in chitosan is beneficial and it facilitates its solubility in acidic solutions, and therefore its utilisation in application.

4.1.6.2 *FT-IR Spectroscopy*

Infrared spectroscopy is one of the most frequently applied characterisation techniques for chitin and chitosan derived from insects (Hahn *et al.*, 2020). Characteristic peaks can be identified (Table 41), due to the functional groups found within the molecules such as the acetamido or amino groups.

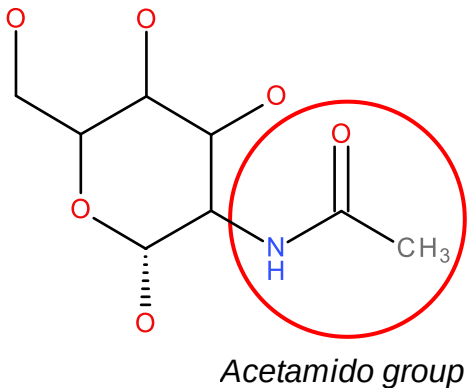


Figure 75: 2D structure of chitin, highlighting the acetamido functional group

Due to the acetamido or amide function group contained within the chitin structure, Figure 75, there are specific amide bonds associated with FT-IR spectroscopy analysis. The amide I band, seen in chitin to occur between 1600 and 1655 cm^{-1}, is primarily related to stretching vibration of C=O and is directly associated with the conformation of the chitin backbone. The Amide II peak originates from a combination of around 18-40 % C-N stretching and 40-60 % N-H bending vibrations. The amide II band position is sensitive to amide conformation. The amide A band (between 3255–3270 cm^{-1}) and amide B (about 3100–3110 cm^{-1}) originate from a Fermi resonance, overtones of amide II and N-H stretching vibrations (Marchessault, Pearson and Liang, 1960; Krimm and Bandekar, 1980; Rinaudo, 2006; Ŝpanêlová, Machoviĉ and Březina, 2006; Sagheer, Al-Sughayer, Muslim and Elsabee, 2009; Kumirska, Kaczy and Bychowska, 2010; Ji *et al.*, 2020).

Table 41: Chitin and chitosan expected FT-IR peaks

Peak	Absorption (cm^{-1})	Appearance
C-N stretching, amide III	1310–1320	Weak, sharp
N-H bending, amide II	1550-1560	Weak, sharp - chitin Strong, sharp -chitosan
NH_2 bending	1590–1600	Weak, sharp
C=O stretching, amide I	1650–1655	Weak, sharp
N-H symmetric stretching (amide B)	3100–3110	Medium
N-H asymmetric stretching (amide A)	3255–3270	Strong, broad – chitosan Weak, broad - chitin
O-H stretching	3430–3450	Strong, broad – chitosan Weak, broad - chitin

Quantitative evaluation of % DD can be completed using this method by calculating and comparing absorption values at different wavelengths, but is challenging as peak position and strength vary depending on subject source and purity. Remaining protein presence can interfere and overlap with chitin and chitosan peak absorptions, and lead to inaccurate quantification. Other impurities like water also limit data validity, as peaks they produce in FT-IR analysis tend to be strong and may mask smaller identification peaks. Nevertheless, FT-IR spectroscopy is a useful tool in identifying the presence of functional groups.

4.1.6.3 *SEM-EDS*

Scanning electron microscopy (SEM) is used in the determination of the chitin and chitosan surface morphology. The surface morphology was seen to be vastly different when comparing different insect sources, ranging from smooth to rough and porous to non-porous, examples of which can be seen in Figure 76.

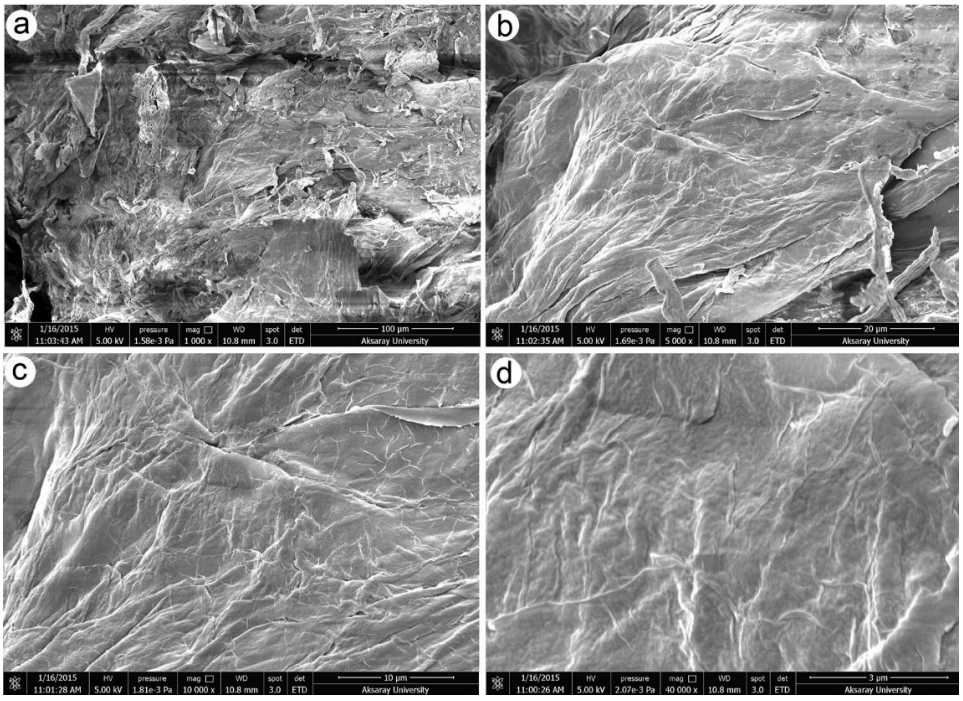


Figure 76: SEM images of chitin isolated from wings of *Argynnis pandora* butterfly with rough (a and b) and smooth surfaces (c and d) of chitin (taken from Kaya *et al.*, 2015**)**

In the analysis of BSFL chitin, energy dispersive X-ray spectroscopy (EDS) was used to determine the presence of remaining minerals after demineralisation, as well as to analyse mineral content within the biomass ash.

4.1.6.4 *Elemental Analysis*

Elemental analysis (EA) provides information on the nitrogen content of chitin and chitosan and therefore an evaluation of the extent of the deacetylation. In the combustion, the carbon, nitrogen, sulphur and oxygen content is determined by the degradation of the sample at 900 °C. In theory, fully acetylated chitin should contain roughly 6.9 % nitrogen content, and deacetylated chitosan around 8.7 %, a higher nitrogen content represents a lower quantity of acetyl groups (de Alvarenga, 2011). Direct comparison of % N values with these values provides good information on the acetylation. The % DA for chitin and the % DD for

chitosan provide a measure of the carbon to nitrogen composition and the evaluation should not exceed 100 %. These are calculated using Equation 6 and Equation 7 respectively:

$$DA(\%) = \left(\frac{\frac{C(\%)}{N(\%)} - 5.14}{1.72} \right) \times 100$$

Equation 6: Degree of deacetylation (% DA) of chitin from EA analysis (Xu, McCarthy, Gross and Kaplan, 1996)

$$DD(\%) = \left(\frac{6.87 - \frac{C(\%)}{N(\%)}}{1.72} \right) \times 100$$

Equation 7: Degree of deacetylation (% DD) of chitosan from EA analysis (Hahn *et al.*, 2020)

It is likely that impure samples could result in % DA higher than 100, as there are no purification steps in the extraction of chitin from BSFL cuticle. For chitosan, a % DD of around 50 % is expected, as previously stated in section 4.1.5.

4.1.6.5 *TGA*

In contrast to EA, where the degradation of a sample is utilised to determine elemental content, thermogravimetric analysis (TGA) is used to evaluate the temperature at which a sample degrades. Sample mass is recorded over time upon increasing temperature. In insect-derived chitin, there are two distinct decomposition steps on a thermogram. The first takes place with the mass evaporation of water at between 50-100 °C, and the second between 300-400 °C thought to be due to dehydration of the polysaccharide backbone, decomposition of the acetyl group and polymerisation of degradation products (Oduor-Odeto, Struszezyk and Peter, 2007). An example of an expected TGA can be seen in Figure 77.

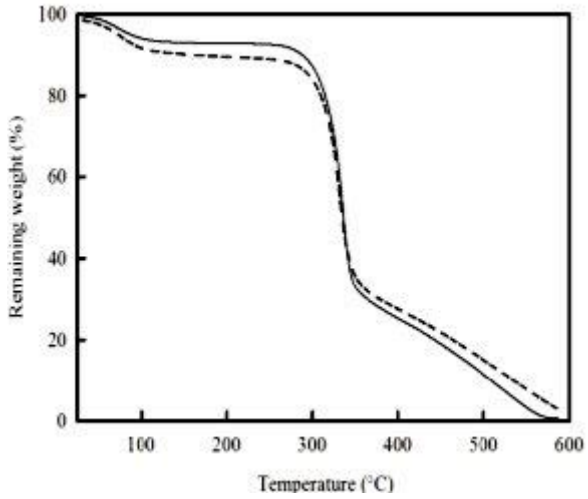


Figure 77: TGA analysis of chitin extracted from fermented shrimp shell waste (- - -) compared with a standard chitin reference material (—) (Bashandy, Raffat, Ibrahim, Tayeb and Gamal, 2017)

4.1.7 Ash content

Ash content was determined by incineration in a furnace at 900 °C. Qualitative ash content took place using FT-IR spectroscopy and quantitative analysis through EA, in order to determine the presence of impurities within the chitin.

4.1.8 Applications of Chitin and Chitosan

Chitin and chitosan are very useful biologically derived support materials, with beneficial economic value. Commercial production of goods from insects is a long-standing, well-established industry, with the production of silk from silkworms dating back to the Neolithic period in China (Vainker, 2004). An increasing insect protein market resulted in 6 kilotons of insect protein meal being produced in Europe in 2019, from a mixture of BSF and mealworms (Derrien and Boccuni, 2018). As BSFL contain up to 35 % chitin (Hahn *et al.*, 2018), there is scope to mass-produce BSFL for its protein, whilst also utilising remaining lipids and isolated chitin from the insect cuticle.

The poor solubility of chitin limits in its utilisation, leading to chitin derivatives, such as chitosan, being much more favourable in application. Despite this, various medical applications using chitin fibres have been reported, with flexible chitin sutures being more resistant to breakdown by bile, pancreatic juices and urine, than comparable man-made sutures (Rathke and Hudson, 1994; Pillai and Sharma, 2010). Additionally, the use of chitosan in tissue engineering for burn victims has been proposed owing to its strong degradation resistance and antibacterial properties, with Liu *et al.* constructing a design from

hyaluronic acid, gelatin and chitosan to give rise to a living cell-matrix-scaffold composite, comparable to an *in vitro* artificial epidermis bilayer (Liu *et al.*, 2004).

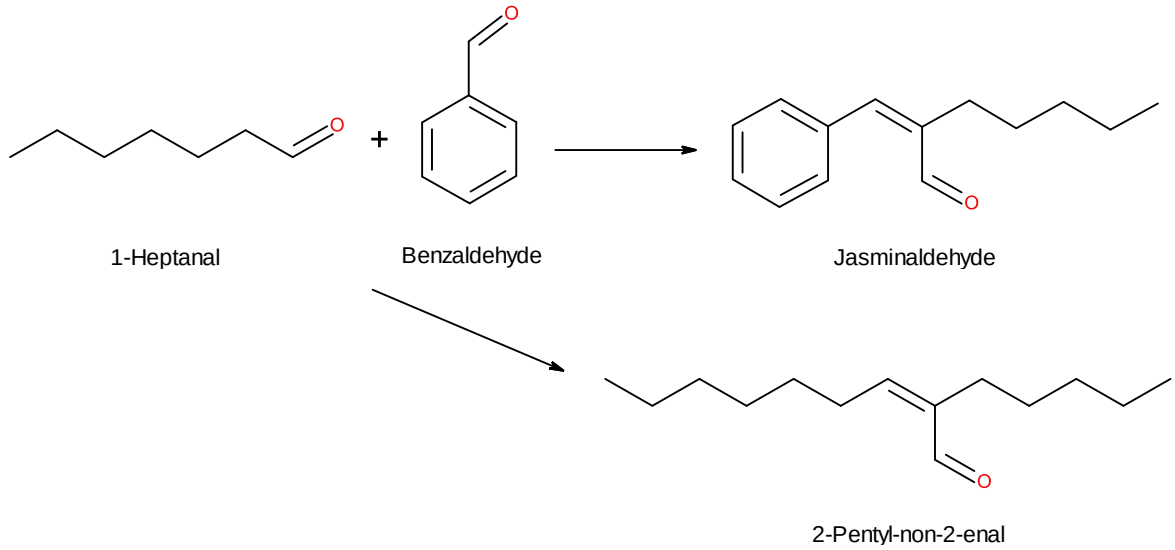
The sorption of dyes by chitosan has been well investigated, as its unique molecular polymer structure lends itself to a high affinity for sorption of many compounds, with reactive functional groups (Ravi Kumar, 2000). As these reactions are typically exothermic, dye sorption rate was high. Moreover, a maleilated chitin derivative has shown promise in waste water treatment as a dye adsorption material (Sridhari and Dutta, 2000). In waste water studies, chitosan was found to be successful in the removal of heavy metal ion containments and selected proteins from water by chelation over a range of pH conditions (Gamage and Shahidi, 2007; Boulaiche, Hamdi and Trari, 2019). A review by Van Tran et al. found that conversion of chitosan to hydrogels improved the removal of contaminants in wastewater treatment through unique encapsulation mechanisms, an area of increasing interest as the hydrogels can be recycled through regeneration, and chitosan is resistant to degradation (Van Tran, Park and Lee, 2018).

There lies a potential to use chitin as a biodegradable and sustainable alternative to some plastics (Van Den Broek, Knoop, Kappen and Boeriu, 2015), having a similar repeating unit polymer structure. In a study by Huang *et al.* a green route from crustacean-derived chitin to transparent films was established using a KOH/urea solution. The resulting tough films were found to have good tensile strength, and good transparency, providing a sustainable alternative to common plastic food packaging (Huang, Zhong, Zhang and Cai, 2017). The limited presence of chitosan was seen to inhibit the growth of *Escherichia coli*, *Fusarium, Alternaria* and *Helminthosporium* bacteria when chitosan was more than 0.025 % present (Hirano and Gebelein, 1995). It is believed that the strong binding ability of chitosan's functional groups, binding at anionic bacterial sites, is responsible for inhibited growth. With proven antimicrobial properties, the use of chitosan as food packaging may reduce prevalence of bacteria in food processing (Shahidi, Arachchi and Jeon, 1999). As chitosan is approved as food safe by the FDA, applications within the food industry as additives, emulsifying agents, preservatives, stabilisers and gelling agents is well known (Ravi Kumar, 2000; Morin-Crini, Lichtfouse, Torri and Crini, 2019).

The optical clarity of chitin from the molecules' "thread-like nature" means they are optically birefringent, and that applications based on its optical properties in different mediums are

being considered (Azofeifa, Arguedas and Vargas, 2012). For example, the development of chitosan-gold sensors based on surface free-electron oscillations has been investigated, showing high specificity for metal ions and selected bacteria (Sugunan, Thanachayanont, Dutta and Hilborn, 2005). An investigation into refraction properties of chitin at several wavelengths within different solvents by Barzic and Albu found that mean refractive index is in the visible range at around 1.592 nm, explaining why some insects have cuticles that appear iridescent or metallic from the multiple reflections of visible light pathways (Barzic and Albu, 2021).

There lies potential to utilise chitosan as a heterogeneous catalyst or a catalyst support, due to the high surface area available as a reaction platform (Abdelkrim, 2015). Industrially, heterogeneous catalysis is often preferential to homogenous due to ease of catalyst removal and recovery, the absence of solvents, wide applicability over a range of reactions and low catalyst loss, all of which lead to lower operating costs (Fadhel, Pollet, Liotta and Eckert, 2010). The use of chitosan as a base hydrogel catalyst in the production of jasminaldehyde (2-benzylideneheptanal) from 1-hepatnal and benzaldehyde has been demonstrated, Scheme 9 (Sudheesh, Sharma and Shukla, 2010). The synthesis of jasminaldehyde is of commercial interest in the cosmetic industry as it is used widely in perfumes, possessing a sweet and pleasant jasmine-like aroma. However, the reaction often results in fast self-condensation of heptanal, producing 2-pentyl-non-2-enal, whose unpleasant aroma interferes with that of the target molecule. Use of crustacean-derived chitosan in the reaction was seen by Sudheesh *et al.* to increase the selectivity of jasminaldehyde synthesis up to 88 % under solvent-free conditions, and up to 99 % when the reaction was optimised. Moreover, the catalyst was found to be easily separated following reaction, and reused up to 6 times.



Scheme 9: Synthesis of Jasminaldehyde

4.1.9 Conclusions and BSFL chitin research objectives

While current applications are limited, owing to unreliable and unsustainable sources for chitin, there exist many applications for chitin and chitosan in a range of industries. As films and fibres, chitin has been seen as beneficial in practice in the biomedical field, in action as biodegradable suture material, and potential as artificial skin. Furthermore, use in the food industry as a plant-based packaging material is notable, synthesised transparent films showing excellent tensile strength, with additional use as an antimicrobial agent very promising. Additionally, strong optical characteristics of the polysaccharide have seen research into sensors as a possible application.

In this research, the principle aim was to assess firstly whether it was possible to use lactic acid in the demineralisation of BSFL exoskeletons and secondly, whether it would act as an appropriate and sustainable alternative to HCl. Therefore, the extraction of chitin from BSFL using a lactic acid demineralisation step would be investigated, as an important area for investigation is the determination of an eco-friendlier extraction from the chitin source. Change of demineralising acid would provide benefits of lactic acid being a renewable source of acid, its biodegradability in water streams, and avoiding the use of mineral acids in the production of a sustainable material, chitin.

Moreover, BSFL-derived chitosan would be used in an experimental application as a catalyst in jasminaldehyde synthesis. Assessment of whether BSFL chitosan can create and perform as a basic hydrogel catalyst was conducted with characterisation of the catalyst prior and

subsequent to jasminaldehyde synthesis. The investigation will determine the ability of the chitosan to form a good catalytic platform, recognise the potential of chitin and chitosan in application, and provide further scope for its utilisation in other heterogeneous applications.

4.2 EXPERIMENTAL

4.2.1 Solvents and Materials

Reagents and solvents used in the reactions were not purified further. Commercial materials (ACS reagent grade) (Sigma-Aldrich, UK) were used: NaOH, HCl, lactic acid, benzaldehyde, and 2-heptanal. BSFL were sourced from the industrial partner, Better Origin ltd (Cambridge, UK). Following oil extraction by hexane in chapter 2, remaining BSFL biomass were subjected to chitin extraction. Concentrations of acids and alkalis were made-up using deionised water (DI) (8.2 Ω) with solid mass analytically weighed, and liquid solutions pipetted and subsequently diluted to required volumes/concentrations using DI water.

4.2.2 Demineralisation of BSFL exoskeleton

In the first stage of chitin extraction, the concentration of HCl and lactic was varied to examine the optimum conditions needed for chitin extraction for both acids (Table 42). Studies for each reaction duration and concentration were repeated three times for reliability. At a solid to liquid ratio of 1:10, a 50 mL of a variable M acid was mixed with 5 g defatted BSFL in a beaker at room temperature and stirred magnetically for a variable time. Following reaction, the demineralised insect material was removed from the acid using vacuum filtration, washed with 20 mL DI water thrice and subsequently dried in an oven overnight at 50 °C to remove remaining water. Demineralised material was weighed following drying and characterised by FT-IR spectroscopy, XRD, TGA, EA and SEM analysis.

Table 42: Parameters used in demineralisation study

Demineralising acid	Concentration (mol dm^{-3})	Time (hrs)
HCl	0.5	1 / 2 / 72
	1.0	1 / 2 / 3 / 4 / 24 / 48
	1.5	24 / 72
	3.0	1 / 2 / 3 / 4 / 24 / 72
Lactic acid	0.5	1 / 2 / 3 / 4 / 24
	1	1 / 2 / 3 / 24
	1.5	1 / 2 / 24

4.2.3 Deproteinisation of BSFL cuticle

At a solid to liquid ratio of 1:10, 1 M NaOH (20 mL) was magnetically stirred under reflux with demineralised BSFL (2 g) at 80 °C for 3 hours. Following reaction, the deproteinised insect material was removed from the acid using vacuum filtration, washed with 20 mL DI water thrice and subsequently dried in an oven overnight at 50 °C to remove residual water. The resulting BSFL chitin was weighed following drying and characterised by FT-IR spectroscopy, EA, XRD and SEM analysis.

4.2.4 Deacetylation of chitin to chitosan

At a solid to liquid ratio of 1:20, of 50 % M NaOH (4 mL) was magnetically stirred under reflux with deproteinised BSFL (0.2 g) at 100 °C for 6 hours. Following deacetylation, the chitosan was separated from the acid using vacuum filtration, washed with 20 mL DI water thrice and subsequently dried in an oven overnight at 50 °C to remove residual water. Chitosan was weighed following drying and characterised by FT-IR spectroscopy, EA, XRD, and SEM analysis.

4.2.5 Synthesis of chitosan base catalyst

BSFL chitosan (0.32 g) was dissolved in 0.1 M HCl (20 mL) at room temperature and stirred until chitosan was seen to dissolve. The solution was then added dropwise into a beaker containing 0.1 M NaOH solution (300 mL). Gelling of the catalyst was observed, and the hydrogel allowed to age without stirring at room temperature for 1 hour, and then recovered by vacuum filtration. During filtration, the chitosan hydrogel was rinsed with DI water until the pH of the filtrate was neutral. Hydrogels were dried in an oven at 80 °C and then ground into a powder by mortar and pestle. Catalyst were characterised using FT-IR spectroscopy, EA, XRD, and SEM-EDS.

4.2.6 Synthesis of jasminaldehyde using chitosan basic catalyst

1-Heptanal (0.9 g), benzaldehyde (4.2 g), and chitosan catalyst (0.1 g), were stirred under reflux in round-bottom flask at 160 °C for 7 hours. The reaction vessel was flushed with nitrogen and a nitrogen balloon attached to create an atmosphere inhibiting the formation of corresponding acids from aldehyde. Following reaction, the product mixture was allowed to cool to room temperature and catalyst recovered through vacuum filtration. Reacted catalyst was weighed and analysed using FT-IR spectroscopy, and SEM-EDS. The analysis of product mixture was carried out by GC-MS.

4.2.7 Alternative chitin extraction

An alternative chitin material was received from Better Origin (BO), which had been produced subsequent to mass fat extraction by pressing and fermentation of the protein within remaining material. The "chitin" had the appearance of exoskeleton shells, skin-like in texture, but it was assumed that shells still contained minerals within the cuticle and residual protein, not removed from pressing, and therefore the BO chitin was subjected to the same treatment denoted in 4.2.2, 4.2.3 and 4.2.4 and analysed prior and after the methodology using FT-IR spectroscopy, XRD, and SEM-EDS.

4.2.8 Determination of ash content

Demineralised BSFL cuticle, BSFL chitin and BSFL chitosan were weighed before incineration in a furnace (Carbolite Furnaces, Sheffield UK) at 800 °C for 6 hours to determine ash content. Samples were subsequently weighed and then analysed for elemental content by EA and SEM-EDS.

4.2.9 Characterisation of Chitin/Chitosan

The XRD measurements on powder samples were carried out (at 40mins = 5–45 ° and RT) using a model Bruker D2 phaser (Bruker Corp., USA) equipped with a secondary Ni-filter. The diffractometer was operated with a 0.6 mm divergence slit at 40 kV and 30 mA and a continuous scan was carried out with a step size of 0.02 °, a step time of 1 s and 15 ° variable rotation. Samples were prepared by mechanical grinding and passing through a sieve of mesh size 120 µm. The crystallinity index for chitin and chitosan was calculated using Equation 5.

FT-IR spectra were recorded using a Shimadzu FT-IR Spectrometer over the frequency range of 4000-500 cm^{-1}, 16 scans were collected at a resolution of 4 cm^{-1}.

Surface morphology characterisation of demineralised BSFL cuticle, chitin and chitosan were comparatively examined using a Joel JSM-6510 Series Scanning Electron Microscope. Chitin, chitosan and catalyst samples are non-conductive and therefore were coated with gold prior to SEM analysis using a SEM sample prep manual Au sputter coater.

Elemental analysis was completed using a Thermo Scientific™ FLASH 2000 CHNS/O analyser, at a temperature of 900 °C. The degree of acetylation, % DA for chitin and the % DD for chitosan, was calculated following Equation 6 and Equation 7 respectively.

Thermogravimetric analysis (TGA) was completed using a Perkin Elmer™ TGA 4000 thermogravimetric analyser, with a temperature change of by heating rate of 10 °C/min from 40 to 600 °C under N_2 purging (10 mL/min).

4.3 RESULTS AND DISCUSSION

In this chapter, three distinct work packages were primarily focused upon: extraction of chitin from BSFL, and the optimum conditions and acid appropriate for the demineralisation step and the thorough characterisation of BSFL chitin and chitosan. Further to this, experimental use of a basic hydrogel catalyst made from BSFL chitosan was used, and its effectiveness demonstrated in practice. Other sustainable applications were considered, focusing on the current and potential applications of crustacean-derived chitin.

4.3.1 Demineralisation of BSFL with HCl

To complete the work package of chitin extraction, initially, demineralisation of chitin cuticle was completed with the industrial preferred acid, HCl. Reaction times varied from 1 hour to 72 hours, with concentrations ranging from 1 M to 3 M at ambient temperatures. The acidic treatment did not result in the removal of colour, with demineralisation products appearing as brown powders. It was generally seen that there was no significant correlation between dry yield (g) following reaction, Figure 78. All reactions had starting weights of 5 g (± 0.1 g), and yields from demineralisation reactions were found to average 3.66 g (± 0.3 g). The percentage weight lost between initial and final weight averaged 26.55 % (± 6.56 %), suggesting that in general, nearly a third of all mineral content from the cuticle was removed using HCl, from all 62 extractions.

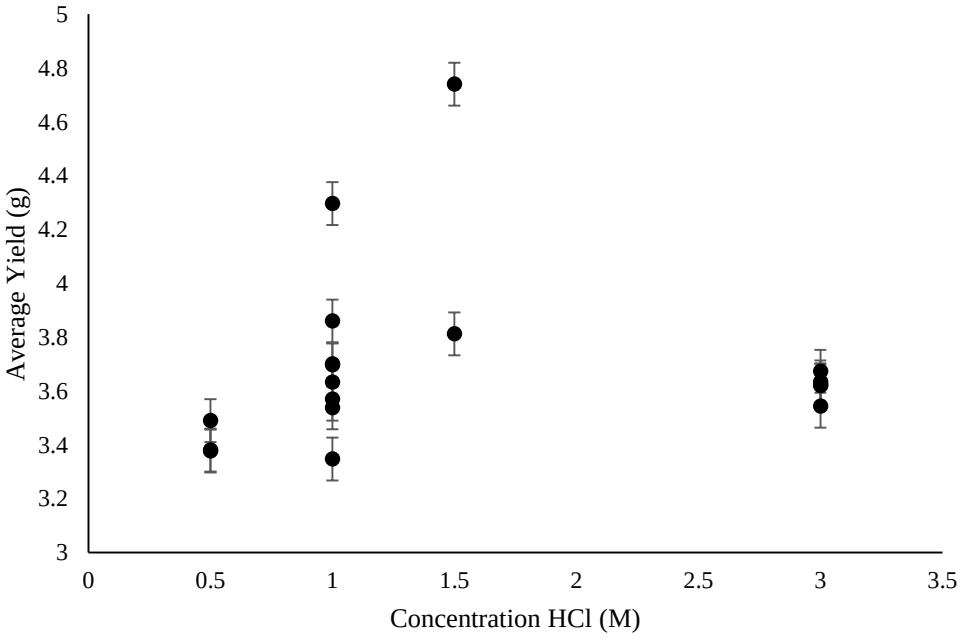


Figure 78: Yields from change in HCl concentration (M) demineralisation study of BSFL

In the studies, yield as a function of time was investigated across all concentrations (Figure 79). There is no strong trend in the demineralisation of BSFL with HCl, but it could be seen that a lengthier reaction duration loosely resulted in lower average yields, across all concentrations. Reactions were completed at room temperature with adequate magnetic stirring, so it is hypothesised that increasing reaction temperature would increase % yield loss. For HCl, the highest yields and least effective reactions were seen by 1.0 M demineralisation treatment for 1 hour and the lowest yields and most effective extraction from 1.0 M treatment for 24 hours.

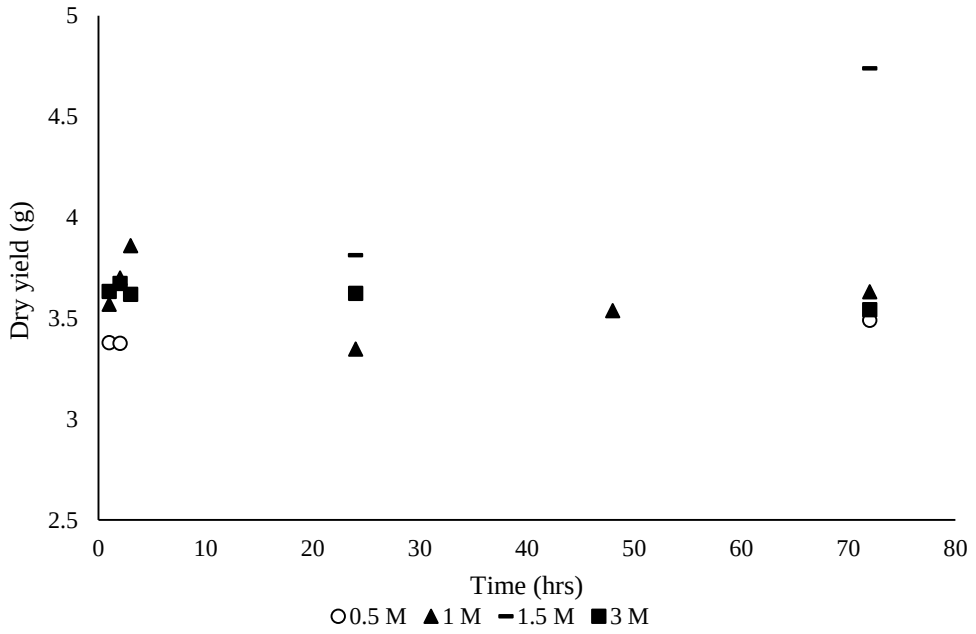


Figure 79: Yields from change in HCl concentration (M) demineralisation study of BSFL over time

XRD diffractograms of isolated samples of BSFL from a HCl demineralisation were collected (Figure 80). Using a 0.6 mm divergence slit for lower noise, reflections creating strong peaks of 9.6 °and 19.6 °, characteristic for α-chitin. A small shoulder peak could be seen at 26 ° was also present, but generally, the sample shows good purity. The crystallinity was improved when compared to a XRD analysis of the starting material BSFL, with that diffractogram showing a completely amorphous solid – no peaks could be seen. The crystallinity index for CHIT2021015 and CHIT2021039 was calculated to be 58.6 % and 61.0 % respectively, which is respectable when compared to pure chitin at 71 % (Ioelovich, 2014).

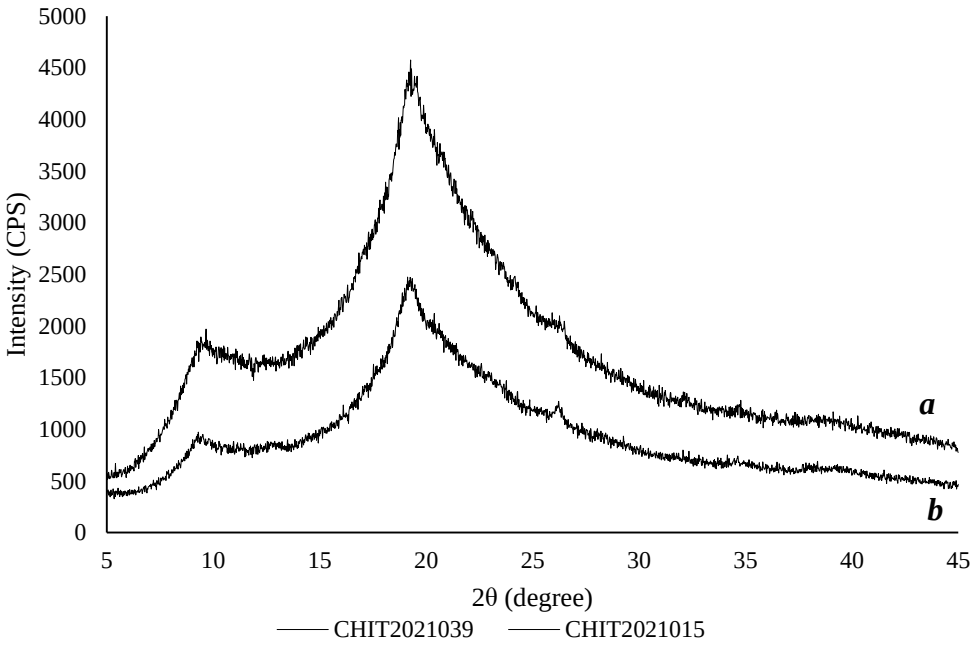


Figure 80: XRD diffractogram of HCl demineralisation of BSFL (*a*) CHIT2021039: 1.0 M for 3 hours, (*b*) CHIT2021015: 1.0 M HCl for 24 hours

FT-IR analysis of the demineralised samples found promising confirmation of the presence of chitin, Figure 81. Characteristic peaks are seen to be emerging in the extraction of chitin: peak observed at 3300 cm^{-1} (N-H and O-H stretching vibration) and sharp peaks at 2915 and 2849 cm^{-1} (intermolecular aliphatic H stretching). There are also two bands at 1659 and 1624 cm^{-1} correspond to the stretching of amide I.

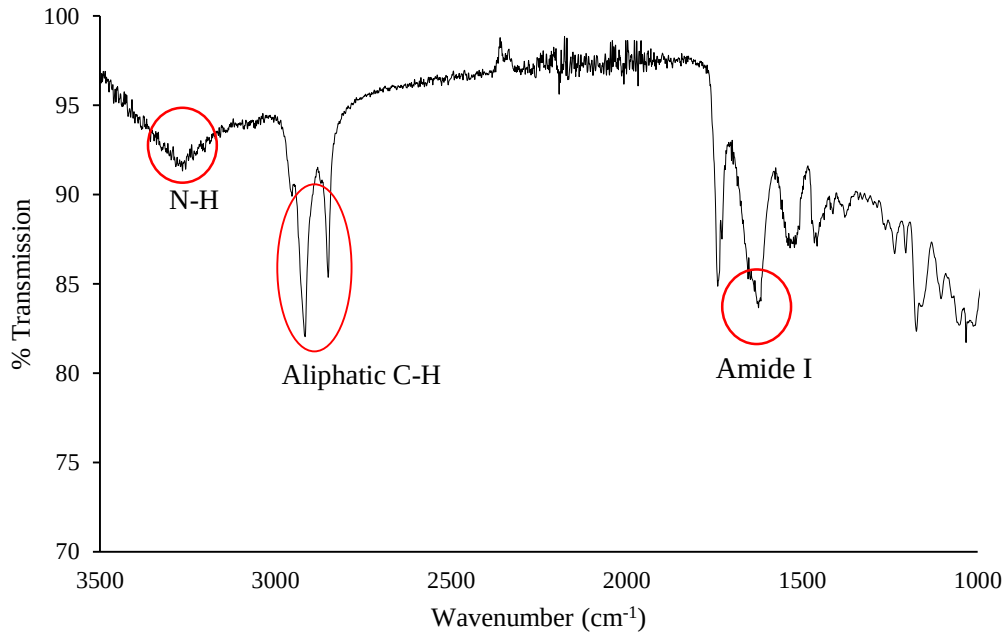


Figure 81: FT-IR spectrum of HCl demineralised BSFL cuticle

Elemental analysis of these example samples found that the elemental composition of the demineralised samples was similar to that of the starting material, Table 43. The remaining material is thought to be mostly oxygen, and any remaining elements from incomplete demineralisation reactions. When compared to commercial values of % N within chitin (6.9 %), the elemental N content in the demineralised samples is higher and therefore would have benefits in some applications.

Table 43: Elemental analysis of BSFL and its HCl demineralised product

Sample	Elemental composition (%)			
	N	C	H	Remainder
BSFL (fat extracted)	7.80	49.05	7.40	35.75
CHIT2021015	7.72	50.16	7.87	34.25
CHIT2021039	7.75	52.96	8.18	31.11

4.3.2 Demineralisation of BSFL with lactic acid

As a comparative method, a more eco-friendly acid, lactic acid, was used in the removal of minerals from the BSFL cuticle. Lactic acid is an organic acid by-product of the dairy industry, and has beneficial properties of bio-degradability and bio-compatibility when compared to HCl (Greene, Robertson, Young and Clyburne, 2016). Reaction times varied from 1 hour to 72 hours, with concentrations ranging from 0.5 M to 1.5 M at ambient temperatures.

It was seen that there was negative correlation between acid molarity and duration of extraction from the dry yield (g) weighed following reaction, as seen in Figure 82. All reactions had starting weights of 5 g (± 0.1 g), and yields from demineralisation reactions were found to average 2.33 g (± 1.01 g) across all concentrations. The percentage weight lost between initial and final weight averaged 52.88 % (± 19.71 %), suggesting that in general, nearly a half of all cuticle is minerals in all cases. This is significantly higher when compared to the extraction of minerals using HCl, suggesting that lactic acid is a more suitable acid for removing minerals. In a previous study using lactic acid demineralisation in green crabs, it was found that 1.14 M was the most effective concentration (Greene, Robertson, Young and Clyburne, 2016), which is consistent with the findings herein as there seemed to be little difference in the % weight loss between 1.0 M and 1.5 M lactic acid. 0.5

M was the least effective acid for short reactions, unless a reaction time of 24 hours was used, which produced yields similar to reactions where higher concentrations were used but shorter reactions. If reaction duration is not an issue in scale up, having 0.5 M concentrations would be safer in mass scale practice.

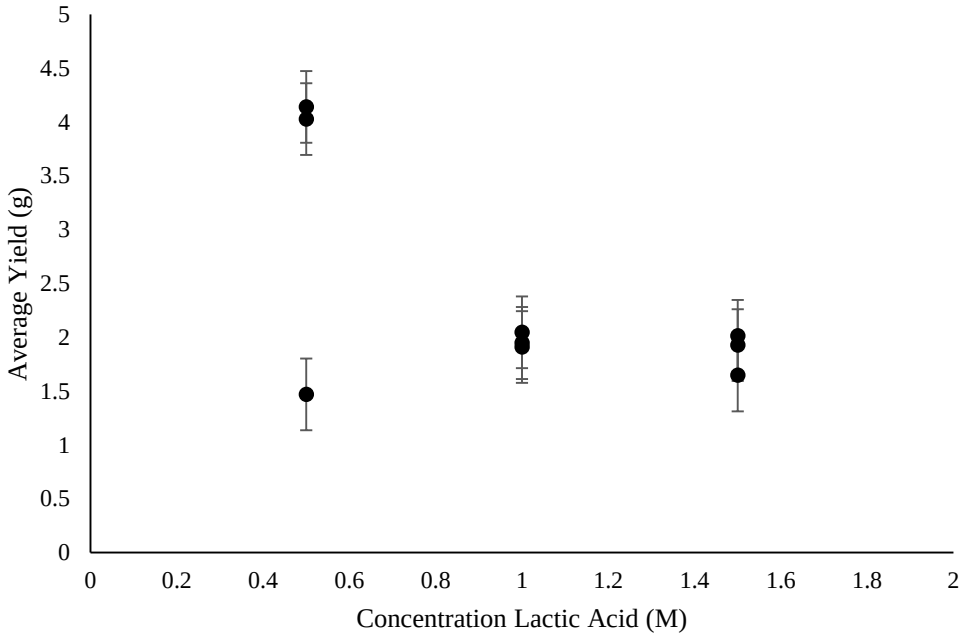


Figure 82: Yields from change in lactic acid concentration (M) demineralisation study of BSFL, where the three points at each concentration are representative of different reaction durations

In the study, yield as a function of time was investigated across all concentrations, Figure 83. There is a trend seen, where longer reaction times resulted in lower yields, across all concentrations. The highest yield was seen by 0.5 M lactic acid treatment for 2 hours, and the lowest from 0.5 M treatment for 24 hours.

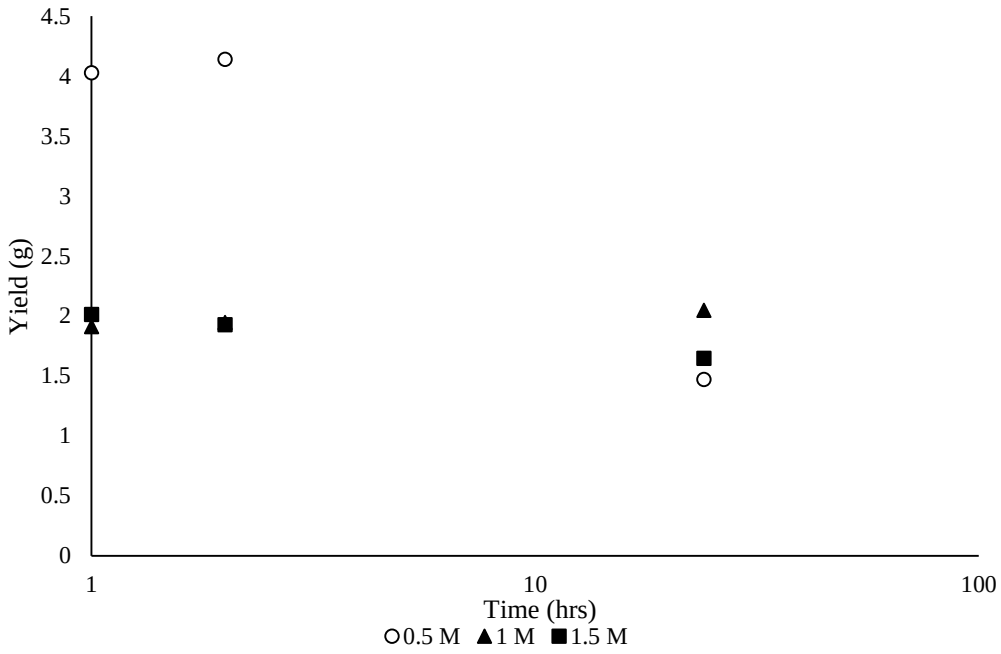


Figure 83: Yields from change in lactic acid concentration (M) demineralisation study of BSFL over time

XRD diffractograms of 2 isolated example samples of BSFL were collected, Figure 84. Using a 0.6 mm divergence slit, reflections creating a shoulder peak of 9.6 ° and a sharper one at 19.4 °, characteristic for α-chitin. A small shoulder peak in CHIT2021067 could be seen at 26 °, but presented as a sharp peak in CHIT2021074. The presence of these peaks compared with the starting material, and with the HCl diffractogram in Figure 84, is suggestive that lactic acid does remove minerals from the cuticle effectively. The crystallinity index for CHIT2021067 and CHIT2021074 was calculated to be 62.6 % and 57.8 % respectively, which is encouraging with comparison to pure chitin at 71 % and the indexes calculated from HCl demineralisation. When comparing the effect of concentration using these examples, the emergence of a sharper peak at 26 ° from CHIT2021074 is positive, as the final product from deproteinisation is effected to contain a stronger peak.

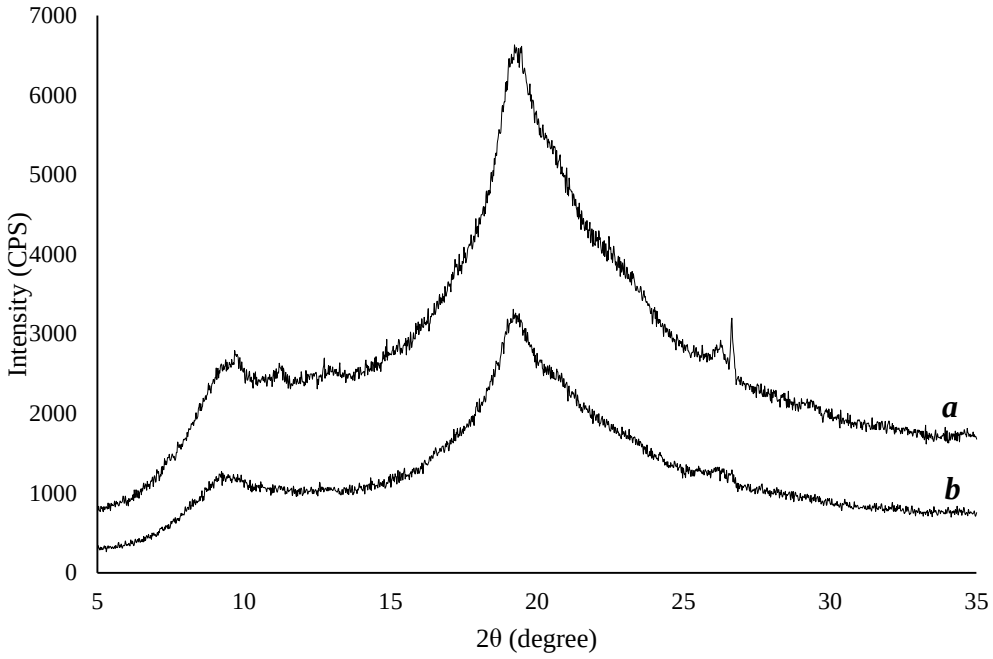


Figure 84: XRD diffractogram of lactic acid demineralisation of BSFL where (*a*) CHIT2021074: 1.5 M for 2 hours (*b*) CHIT2021067: 1.0 M lactic acid for 2 hours

FT-IR analysis of the demineralised samples found promising confirmation of the presence of chitin, Figure 85. Characteristic chitin peaks could be seen in lactic acid extractions similar to HCl extracted samples: 3273 cm^{-1} (N-H and O-H broad stretching vibration), 2920 and 2850cm^{-1} (aliphatic C-H stretching). There are also two bands at 1637 and 1622 cm^{-1} correspond to the stretching of amide I. In addition, peaks at 1537 cm^{-1} and 1369 cm^{-1} could be seen for N-H bending (amide II) and C-N stretching (amide III) respectively.

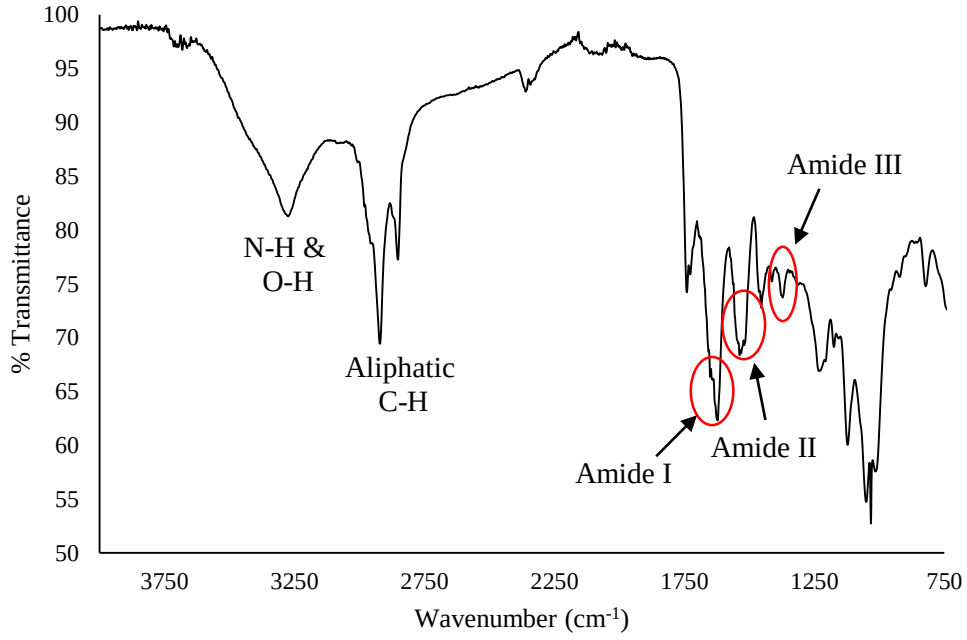


Figure 85: FT-IR spectrum of lactic acid demineralised BSFL cuticle

Elemental analysis of a lactic acid samples found that the elemental composition of the 1.0 M lactic acid treatment (CHIT2021067) was similar to that of the starting material, Table 44. In this instance, it appears that using a stronger lactic acid concentration in CHIT2021074 has resulted in lower nitrogen composition, and when compared to the N % of 6.9 % for commercial chitin (Rinaudo, 2006), it appears the acidic treatment has resulted in a product more similar.

Table 44: Elemental analysis of BSFL and its acid demineralised products where HCl samples analysed (n=10) and LA (n=11)

Sample	Average elemental composition (%)			
	N	C	H	Remainder
BSFL (fat extracted)	7.80	49.05	7.40	35.75
HCl	7.11	46.53	6.28	40.08
LA	7.80	48.82	7.22	36.16

4.3.3 Deproteinisation

Following removal of minerals, protein was removed from the remaining insect material, with the product denoted as BSFL chitin. The parameters in deproteinisation were not subject to change, so that the effect of acid on the demineralisation stage could be appropriately compared. Prior work completed on the subject by a MEng student found that the optimum deproteinisation conditions to be 1 M NaOH 80 °C for 3 hours at a 1:10 solid to liquid ratio (Sutcliffe, 2019), therefore these parameters were used (Figure 86). Contact of demineralized BSFL material with 3.0 M NaOH solution during the deproteinisation stage was not enough to remove the pigments existing in the structure of the insect exoskeleton as evidenced by the dark brown colour of the deproteinised products, congruent with previous findings where a weaker alkaline treatment was used (Ribeiro, Viana, Hattori, Constantino and Perotti, 2018).

Using a starting material weight of around 2.0 g, deproteinisation yields of demineralised BSFL cuticle averaged 0.24 g (± 0.04 g) for samples extracted using HCl and 0.22 g (± 0.06 g) for lactic acid samples. This resulted in a % weight yield loss of 88.23 % (± 2.10 %) and 84.81 % (± 5.26 %) for 62 HCl and 27 lactic acid samples respectively during the strong alkali reaction.

The hypothesis for the deproteinisation process is that the lower the yield, the more effective the removal of proteins, regardless of the demineralisation acid used. When the lowest chitin yielding samples from the demineralisation concentrations are considered, i.e. 0.5 for HCl and 1.0 M for lactic acid, it can be seen from Figure 86 that this made no difference to yields following removal of proteins as these yields were amongst the highest. In general, it was seen that there was little difference in final chitin yields, a discrepancy of around 4 % weight, meaning that lactic acid can be concluded to be a sufficient alternative demineralisation acid when the benefits of using the acid is considered. As the sample selection is vastly bigger for HCl, it cannot be categorically concluded that the discrepancy in % weight loss is not due to a larger sample size. In addition, it is also possible that HCl might be too powerful an acid, leading to hydrolysis of intermolecular bonds instead, which would be expected at very high concentrations.

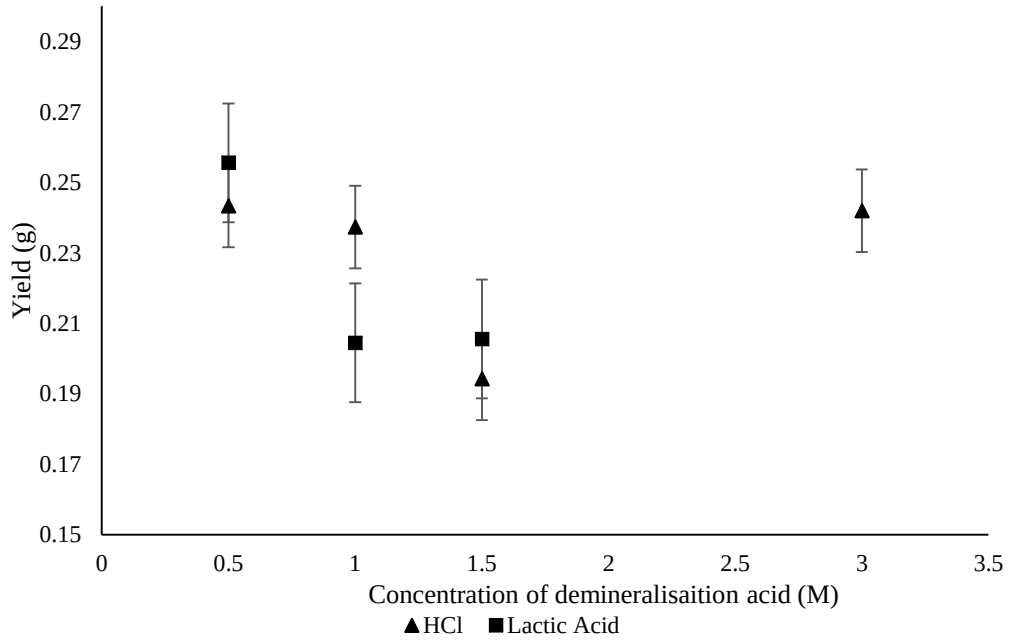


Figure 86: BSFL chitin yields using a deproteinisation method of 1 M NaOH 80 °C for 3 hours

Comparing XRD analysis of chitin samples (Figure 87) resulting from demineralisation of different acids, it appears as if those from lactic acid have a higher crystalline structure, resulting in more reflections. The peak intensities are higher for CHIT2021067, extracted using lactic acid than from CHIT2021015 from HCl demineralisation. As well as the characteristic chitin peaks at 9.6 ° and 19.4 °, strong peaks are observed at 12 ° and 26 °

while no peaks could be seen with samples from HCl extractions. As previously mentioned, it is possible that the strength of the acid has resulted chitin with a reduced crystalline structure. The crystallinity index was calculated using the peaks at 9.6 ° and 19.4 ° and was found to be 61.6 % for HCl and 51.5 % for lactic acid. Although a higher crystallinity index suggests a higher similarity to chitin, a lower one is beneficial in application as lower crystallinity indexes indicate more soluble materials.

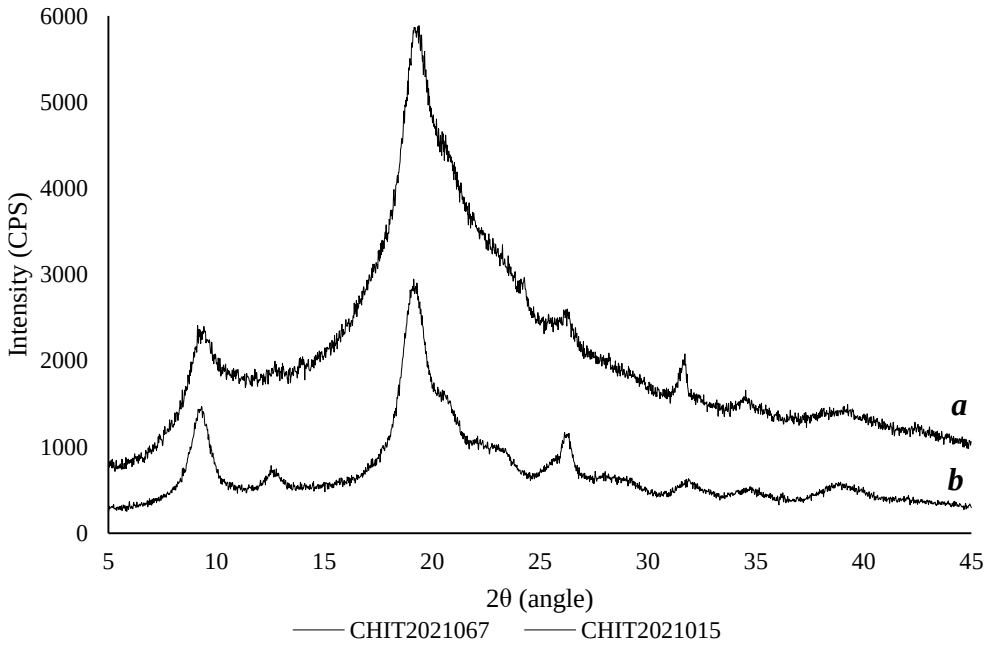


Figure 87: XRD diffractogram of the BSFL chitin where (*a*) CHIT2021067 (1.0 M lactic acid for 2 hours) and (*b*) CHIT2021015 (1.0 M HCl for 24 hours) were recorded

FT-IR analysis of BSFL chitin found (Figure 88/Table 45) peaks at 3400, 3257, 2850, 1926, 1741, 1630, 1620, 1552, 1310, 1155 and 1066 cm^{-1} which is consistent with the structure of α-chitin (Mohammed, Williams and Tverezovskaya, 2013). Peaks typical of *β*-chitin at 972 or 632 cm^{-1} (Acosta, Jiménez, Borau and Heras, 1993) were absent. Identification of a α-chitin allomorph could also be confirmed through the presence of a split amide I band at 1660 cm^{-1} which is attributed to the occurrence of intermolecular hydrogen bond between C-O and H-N, and at 1625 cm^{-1} due to the intramolecular hydrogen bond C-O between H-O-CH_2 (Marchessault, Pearson and Liang, 1960; Rinaudo, 2006; Sagheer, Al-Sughayer, Muslim and Elsabee, 2009; Kumirska, Kaczy and Bychowska, 2010).

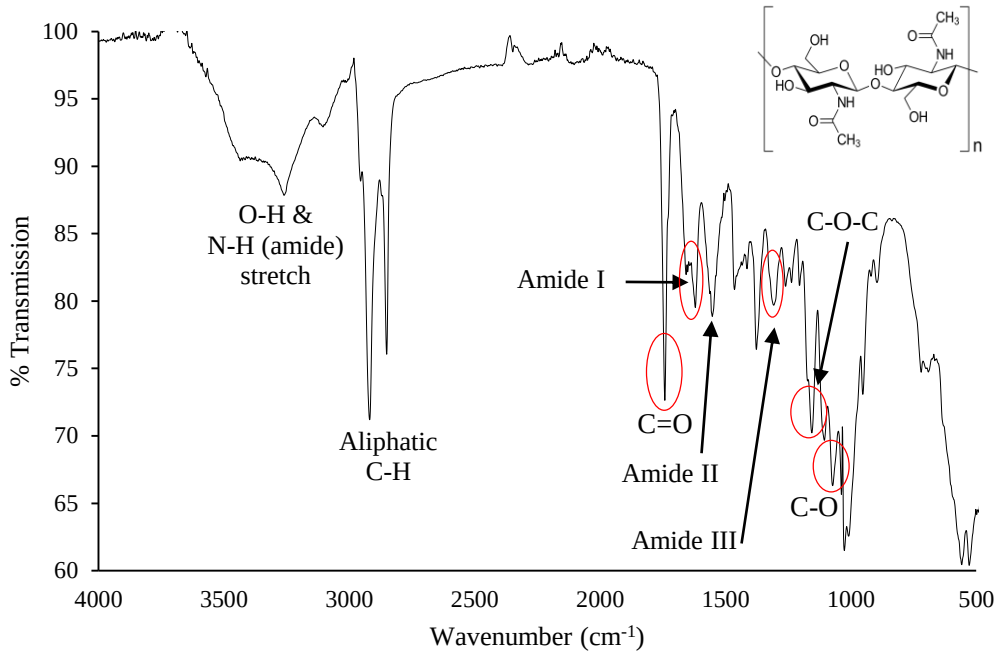


Figure 88: FT-IR spectrum of BSFL chitin

Table 45: FT-IR data table of BSFL chitin peaks

Functional Group	Wavenumber (cm^{-1})	Appearance
-OH	3400	Strong, broad
N-H (stretch)	3257	Strong
Aliphatic CH3	1926 & 2850	Strong, sharp
C=O	1741	Strong, sharp
Amide I - NHOCH3 (bending)	1620 & 1630	Medium
Amide II - NHOCH3 (N-H bending)	1552	Medium
Amide III - NHOCH3 (C-N stretch)	1310	Medium
C-O-C glyosidic link	1155	Medium
C-O	1066	Strong, sharp

SEM analysis of dry deproteinised samples found that in comparison to materials following demineralisation, there was a more homogenous structure (Figure 89), where ***a*** shows 450x magnification of material following demineralisation and ***b*** shows 500x magnification BSFL chitin following deproteinisation. Long "hair-like" strands seen in ***a*** are the setae on the

exoskeleton of the BSFL, which aid in movement, and sensillia, an arthropod sensory organ (Barros, Gutjahr, Ferreira- Keppler and Martins, 2019). Setae and sensillia cannot be seen in chitin samples (***b***), suggesting that these body parts with removed through acidic treatment. ***B*** also is more homogenous and flatter in nature, with the repeating concave "honeycomb" structure seen in reagent ***a*** and literature (Waśko *et al.*, 2016) absent, destroyed through alkaline treatment.

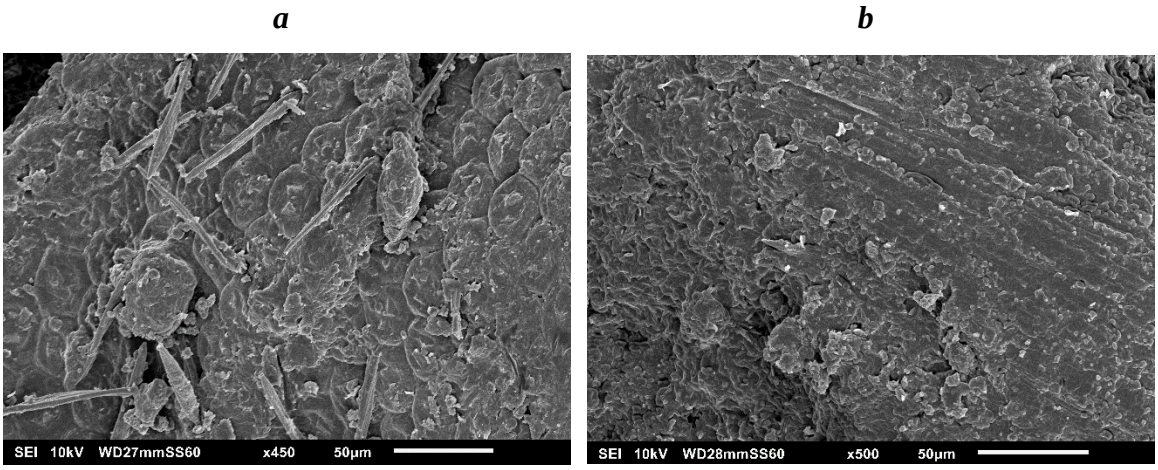


Figure 89: SEM images of *A:* demineralised BSFL cuticle (left) at 450x magnification and *B:* BSFL chitin following deproteinisation (right) at 500x magnification

Elemental analysis of the deproteinised material was completed (Table 46) and it was found that the N content of the chitin was lower than that of the original starting material, and the demineralised cuticle (Table 43/Table 44). For samples extracted form chitin, overall, there was around 4.96 % nitrogen content for HCl samples and 4.49 % for lactic acid samples, which is lower than the % N content of fully acetylated chitin at 6.9 % (Hahn *et al.*, 2020). As a higher nitrogen content is indicative of a lower degree of acetylation, this resulted in calculated % DAs exceeding 100 % for both demineralisation methods using Equation 6. However, this is not unexpected, with previous studies of insect-derived chitin often resulting in lower N values than the expected 6.9 %, and % DA values exceeding 100 %. For example, to compare BSFL chitin, in a study by Erdogen and Kaya, a nitrogen content of 4.6 % and 5.7 % was determined by EA from *D. maroccanus* adults and nymphs, resulting in % DAs of 232 % and 187 % respectively (Erdogan and Kaya, 2016).

Table 46: EA analysis of BSFL chitin samples, with average elemental composition of C, N and H, and average % DA from HCl demineralised (n=10) and lactic acid demineralised chitin (n=11)

Sample	Average elemental composition (%)				Average % DA
	N	C	H	Remainder	
BSFL (fat extracted)	7.80	49.05	7.40	35.75	n/a
Chitin from HCl demineralisation	4.96	36.32	6.84	51.88	126.89
Chitin from LA demineralisation	4.49	37.05	6.26	52.20	180.91

Thermogravimetric analysis was conducted to determine the degradation of the polymorphous crystalline structure of demineralised BSFL cuticle and BSFL chitin over time (Figure 90). There were two main stages of decomposition witnessed from BSFL chitin samples, with the first low mass change noted at between 50 and 150 °C representative of water evaporation. A secondary mass change of was observed between 175 and 400 °C, caused by the decomposition of the saccharide backbone, polymerisation of decomposition products and degradation of the acetyl group (Ogawa, Yui and Miya, 1992). The third stage of mass loss between 400 and 600 °C represents further decomposition of the chitin structure. The mass change calculated was an overall loss of 80.35 % in conditions up to 600 °C, with a loss of 3.79 % in the first step, 65.94 % in the second stage and 10.92 % in the third stage. The TGA analysis shows thermal stability of BSFL chitin, and is in good agreement with previous TGA analysis of chitin (Mohy Eldin, Soliman, Hashem and Tamer, 2012; Bashandy, Raffat, Ibrahim, Tayeb and Gamal, 2017).

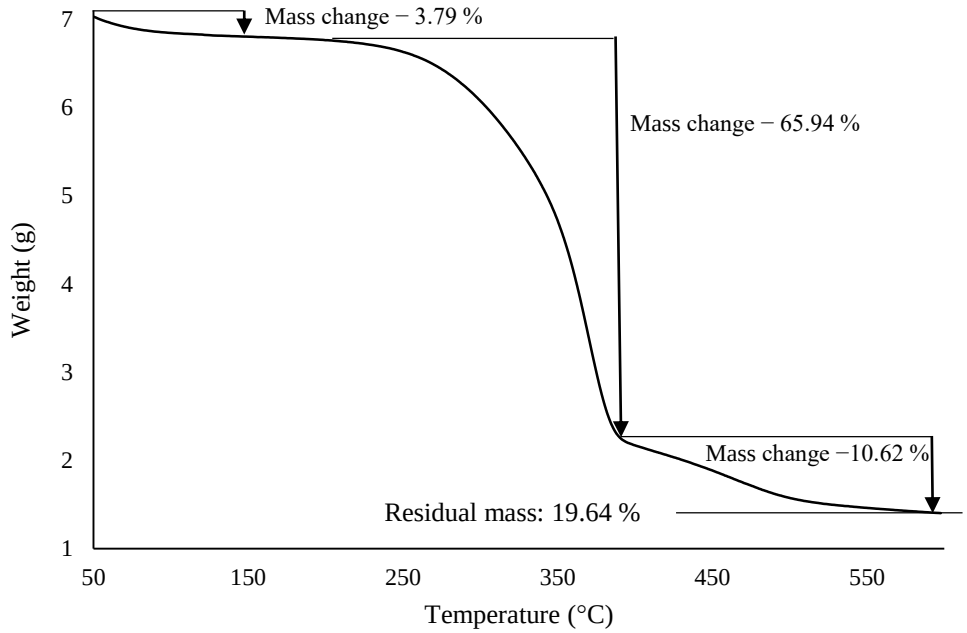


Figure 90: TGA analysis of BSFL chitin by heating rate of 10 °C/min from 40 to 600 °C under N_2 purging (10 mL/min)

4.3.4 Deacetylation of BSFL chitin to chitosan

Chitosan was prepared by harsh alkaline deacetylation of BSFL chitin using 50 % (w/v) NaOH at 100 °C for 6 hours. In this step, partial removal of colour was observed, with BSFL chitosan being a pale grey/brown following overnight drying (Figure 91). Industrially, if a white or colourless chitosan is desired, an additional pigment removal step would be required, as the commercial chitosan used as a reference material (Sigma Aldrich, UK) in this study was a very light grey almost-white substance. Visually, the size of particles appeared smaller than BSFL chitin, with more crystalline-like flakes. The solubility of the product was also good, with noticeable dissolution in weak acidic solution in the subsequently investigated application (section 4.3.6).

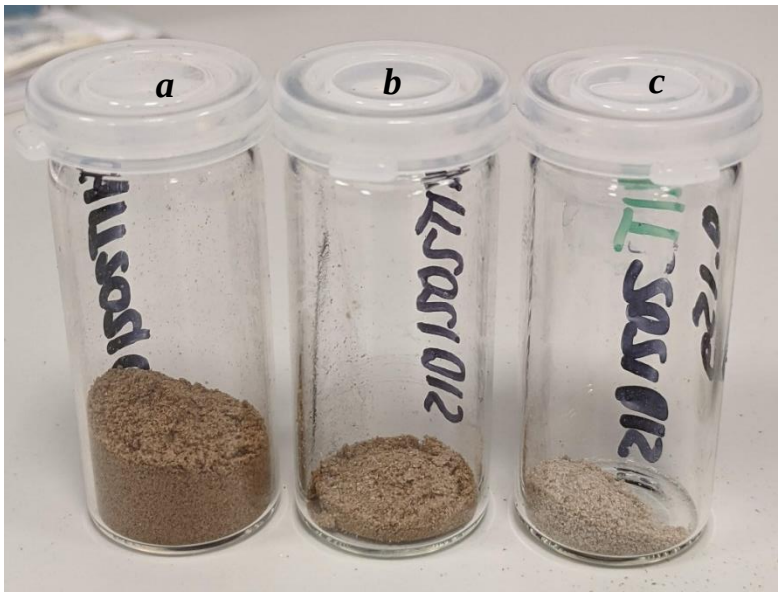


Figure 91: Photographic images of the products resulting from BSFL chitin and chitosan extraction, where pigment loss can be seen at each stage (*a*: BSFL demineralisation product, *b*: BSFL chitin and *c*: BSFL chitosan)

Using an identical deacetylation method, the two demineralisation methods could be directly compared, with a HCl acidic treatment yielding 0.16 g (± 0.04 g) and lactic acid 0.13 g (± 0.03 g) from 0.2 g (± 0.02 g) BSFL chitin starting material. Overall, following the three chitosan retrieval steps of demineralisation, deproteinisation and deacetylation, the overall weight loss from BSFL cuticle to BSFL chitosan was 88.10 % for HCl and 91.02 % and for lactic acid treatments (Table 47), from a starting weight of 5 g defatted BSFL. This yielded a difference of 2.92 %, minimal when the benefits of lactic acid are considered in comparison to HCl, with increased ease of reagent waste disposal, better cohesion with green chemistry principles with bio-degradable and bio-compatible properties.

Table 47: Yields arising from the extraction of BSFL chitosan using two demineralisation methods

Isolation process	**Dry weight of product material (g)**			**Overall % weight loss**
	Demineralisation	Deproteinisation	Deacetylation	
HCl	3.66 g (± 0.3)	0.24 g (± 0.04)	0.16 (± 0.04)	88.10
Lactic acid	2.33 g (± 1.01)	0.22 g (± 0.06)	0.13 (± 0.03)	91.02

Identification of the deacetylated BSFL chitosan was conducted using XRD, by direct comparison with the commercial chitosan (CC) reference material (Figure 92). XRD analysis of chitosan was seen to be in good agreement with previous investigations

(Mogilevskaya, Akopova, Zelenetskii and Ozerin, 2006), seen in Figure 93. Using a 0.6 mm divergence slit, the diffractogram of BSFL chitosan was considerably less noisy than that of the commercial chitosan, with fewer defined reflections, but the chief wide characteristic peaks of 10 ° and 20 ° for chitosan were observed. These peaks are shifted to higher 2θ, differing slightly to the α-chitin starting material, where intensities were seen at 9.6 ° and 19.6 ° (Figure 87). Comparison of XRD diffractograms with analysis by Mogilevskaya *et al*. shows additional intensities corresponding to the presence of chitosan at 24.2 °, 29.3 °, 31.4 ° and 34.5 °, although at lower intensities which is likely a result of sample preparation methods. Using the peaks at around 9 ° and 20 °, the crystallinity index for BSFL chitosan was calculated to be 58.0 %, which is encouraging with comparison to pure commercial chitin calculated at 50.9 %.

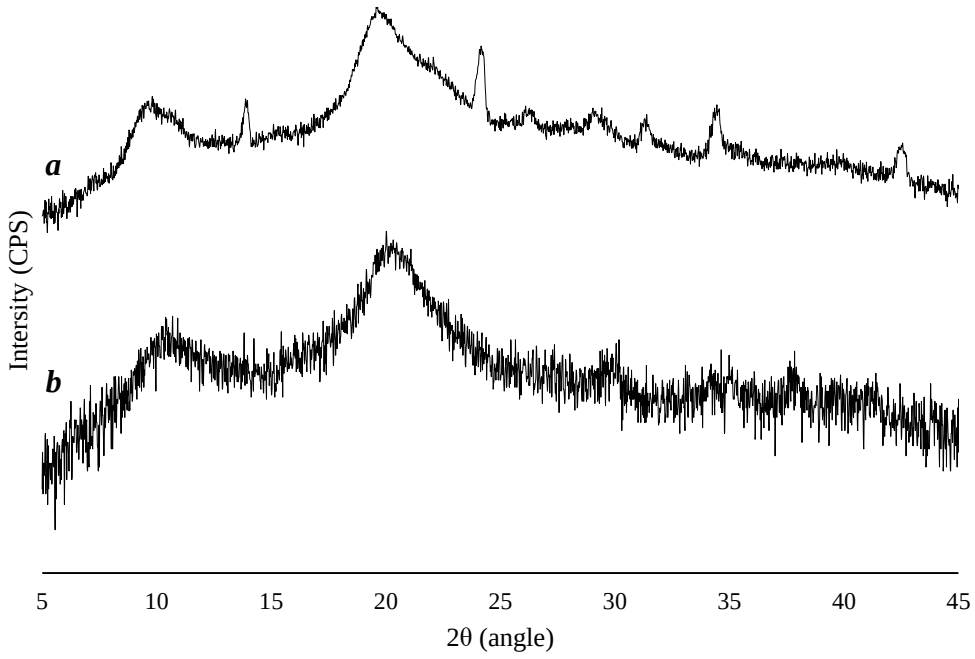


Figure 92: XRD diffractogram of (A) BSFL chitosan compared with (B) commercial chitosan (Sigma Aldrich, UK)

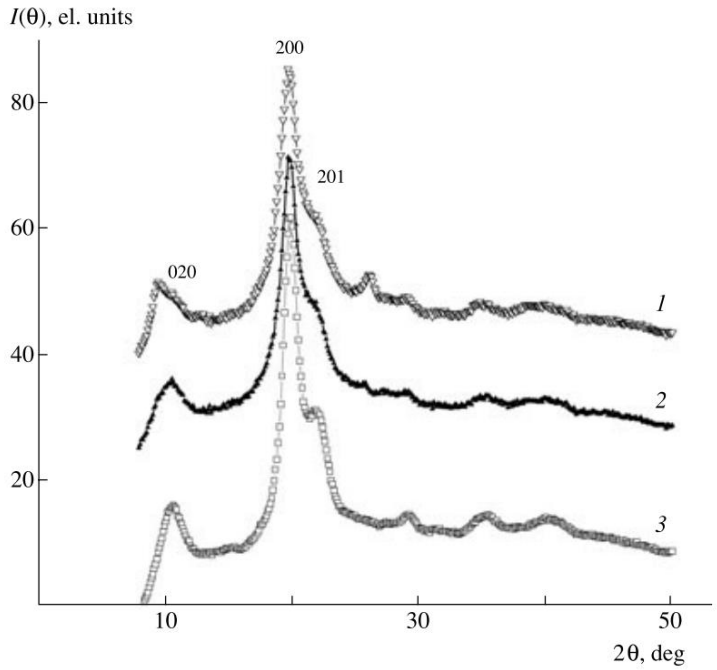


Figure 93: : XRD diffractogram of chitosan from Far East crab shells (Mogilevskaya, Akopova, Zelenetskii and Ozerin, 2006)

FT-IR analysis was completed on BSFL chitosan and compared to that of commercial chitosan to confirm deacetylation (Figure 94/Table 48). Evaluation of both analyses show almost identical IR spectra, which confirms the presence of chitosan and indicates good purity of the end product. Like BSFL chitin, peaks were seen at 3400 cm^{-1} (-NH_2 and -OH groups stretching and intermolecular hydrogen bonding) and 2900 cm^{-1} (aliphatic C-H stretching). After the deacetylation of chitin, the peak at 1560 cm^{-1} (amide II N-H bending) is expected to disappear, accompanied by the appearance of a new sharp peak for NH_2 bending at around 1590 cm^{-1} (Kucherov, Kramareva, Finashina, Koklin and Kustov, 2003). A peak for this occurrence was seen to seen to emerge at 1580 cm^{-1}, where previous chitin FT-IR analysis (Figure 88) exhibited no peak. In addition, the strong carbonyl peak previously seen in chitin FT-IR analysis at 1741 cm^{-1} disappeared, an additional strong indication that removal of the acetyl group has occurred.

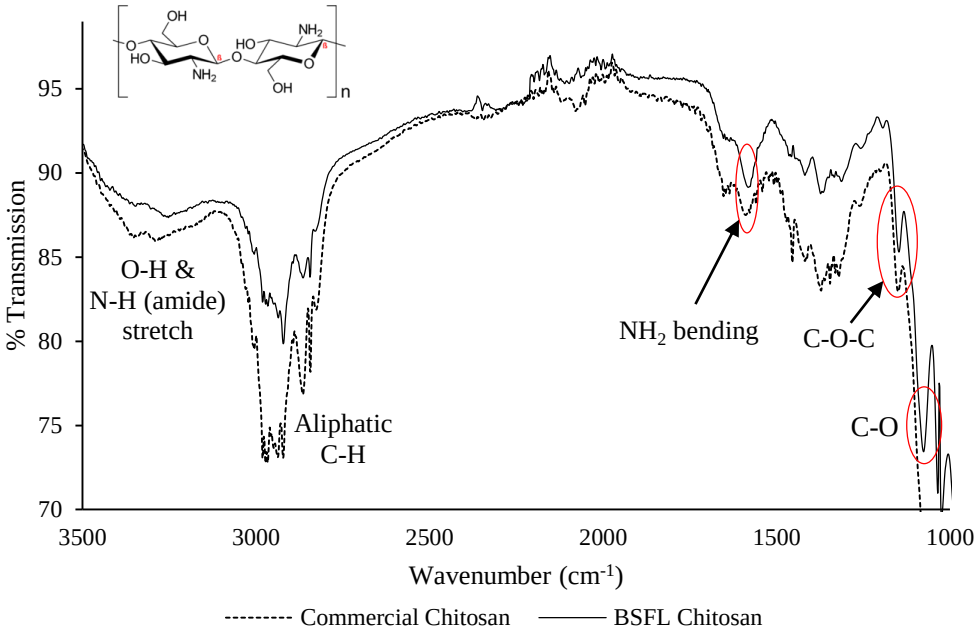


Figure 94: FT-IR spectrum of BSFL chitosan compared with commercial chitosan extracted from shellfish (Sigma Aldrich, UK)

Table 48: FT-IR analysis data table for BSFL chitosan

Functional Group	Wavenumber (cm^{-1})	Appearance
-OH (stretching)	3400	Strong, broad
N-H (stretching)	3257	Strong
Aliphatic CH_3 (stretching)	1926 & 2850	Strong, sharp
Amine NH_2 (bending)	1580	Medium
C-O-C glyosidic link	1150	Medium
C-O	1074	Strong, sharp

The elemental composition of BSFL chitosan products was conducted, once again assessing the difference between the demineralisation processes (Table 49). The N composition was found to be around 0.02 % lower than that of BSFL chitin (Table 46), and lower than the commercial chitosan reference material. However, using Equation 7, % DD was calculated to be near 50 % for both BSFL chitosan, which is promising when materials with a % DD below 50 % can be denoted chitosan, in comparison to commercial shellfish-derived

chitosan which was calculated at 92.22 %. This is suggestive that BSFL chitosan has a good degree of deacetylation.

Table 49: EA analysis of BSFL chitosan samples from different demineralisation treatments following deacetylation

Sample	Average elemental composition (%)				Average % DD
	N	C	H	Remainder	
BSFL (fat extracted)	7.80	49.05	7.40	35.75	-
BSFL chitosan (HCl demineralisation)	4.67	28.01	6.36	61.96	51.87
BSFL chitosan (LA demineralisation)	4.85	28.85	6.12	60.18	54.74
Commercial chitosan	5.4	28.64	6.87	58.89	92.22

The morphology of the chitosan was observed from the SEM micrograph, exhibiting an uneven surface with ridges, interlacing and textured concavities (Figure 95). In comparison to the relatively smooth surface of commercial chitosan ***d***, the BSFL chitosan has ***c*** has a much rougher surface. The particle size appears to be somewhat similar, but much smaller than the products made in the demineralisation and deproteinisation steps (Figure 89), suggesting that the harsh alkaline treatment resulted in chemical degradation of the particles size.

c ***d***

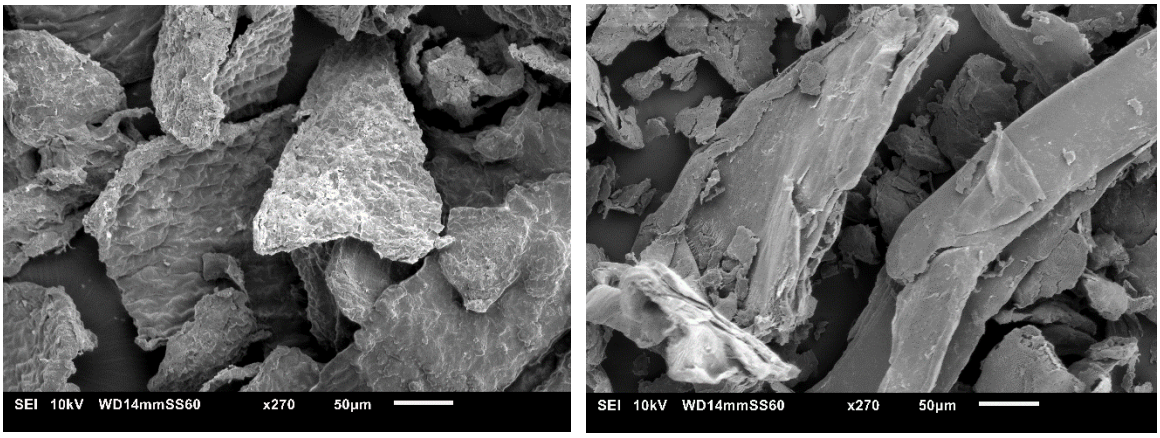


Figure 95: SEM images at 270 x magnification of (*c*) deacetylated BSFL chitosan (left) and (*d*) commercial chitosan extracted from shrimp shells (right) (Sigma Aldrich, UK)

TGA curves were obtained for both BSFL chitosan and commercial chitosan (Figure 96). Both curves show weight loss occurring in two stages. Residual mass of the BSFL was found to be 30.24 %, suggesting good thermal stability of the chitosan. There were two main stages of decomposition witnessed from both samples, with the first low mass change noted at between 50 and 150 °C representative of water evaporation. The secondary mass change of was observed between 200 and 375 °C, caused by the degradation of the chitosan, vaporization and elimination of a volatile product (Ogawa, Yui and Miya, 1992; Bashandy, Raffat, Ibrahim, Tayeb and Gamal, 2017). The third stage of mass loss between 375 and 575 °C represents further decomposition of the chitosan structure. The mass change was calculated an overall loss of 69.76 % in conditions up to 600 °C, and a loss of 8.37 % in the first step, 41.66 % in the second stage and 18.44 % in the third stage. The TGA analysis shows good thermal stability of BSFL chitosan, the shape of the curves produced were in good agreement with previous TGA analysis of chitosan (Sagheer, Al-Sughayer, Muslim and Elsabee, 2009; Phan, Le, Nguyen and Le, 2012; Bashandy, Raffat, Ibrahim, Tayeb and Gamal, 2017).

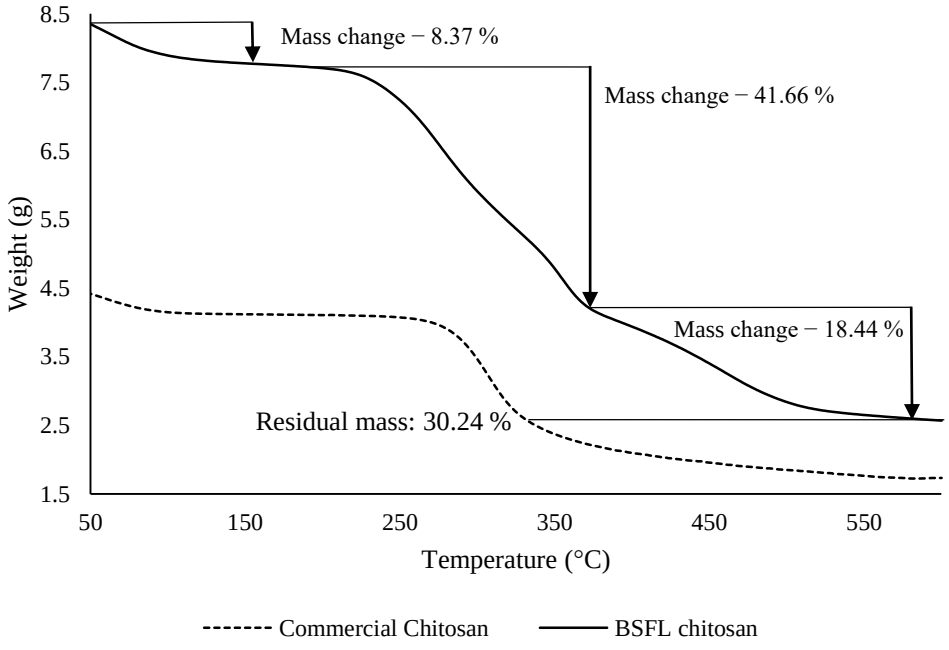


Figure 96: TGA analysis of commercial chitosan (Sigma Aldrich, UK) compared to BSFL chitosan by heating rate of 10 °C/min from 40 to 600 °C under N_2 purging (10 mL/min)

4.3.5 Determination of ash content

Previous elemental analysis found to high amounts of unidentified elemental components with chitin and chitosan (Table 43/Table 44/Table 46/Table 49), so it was suspected that the ash content of BSFL was reasonably high (~50 %). Determination of ash content was completed by incineration of chitin in a furnace at for 6 hours. Resulting products were white powders. Remaining material was weighed (Table 50) and SEM and elemental analysis completed. Based on the % weight remaining after incineration, it was determined that BSFL chitosan contained a higher amount of ash at an average of 49.73 % (± 4.31) in comparison to the commercially shellfish-derived reference material at an average of 5.51 % (± 6.35). The highest ash content was seen in demineralised chitin, with each stage of demineralisation, deproteinisation and deacetylation decreasing the ash amount.

Table 50:Determined ash content of chitin and chitosan samples

Sample	Description	Incineration conditions	% weight determined ash
BURN2021001	BSFL chitosan	600 °C, 6 hours	53.9
BURN2021002	Demineralised chitin	600 °C, 6 hours	92.0
BURN2021003	Commercial chitosan	600 °C, 6 hours	1.02
BURN2021004	BSFL chitin	600 °C, 6 hours	62.1
BURN2021005	BSFL chitosan	600 °C, 6 hours	50.0
BURN2021006	Commercial chitosan	800 °C, 6 hours	10.0
BURN2021007	BSFL chitosan	800 °C, 6 hours	45.3

Elemental analysis of incinerated samples found no nitrogen, and minimal levels of carbon across all samples (Table 51). There were slightly higher levels of H and S seen, but these are still relatable to previous EA analysis of chitin and chitosan. Therefore, it was hypothesised the ash contained primarily residual minerals, from incomplete demineralisation.

Table 51: Elemental analysis of incinerate chitin and chitosan samples

Sample	Elemental composition (%)			
	N	C	H	S
BURN2021001	0	0	0.20	0.03
BURN2021002	0	0	0.5	0.38
BURN2021003	0	0	0.72	0.73
BURN2021004	0	0	0.36	0.13
BURN2021005	0	0.06	0.18	0
BURN2021006	0	0	0.09	0.11
BURN2021007	0	0.06	0.12	0

SEM analysis was conducted to determine mineral content. SEM imaging showed morphology of small particles, very homogenous in size (Figure 97), very unlike the BSFL chitosan with ridges, interlacing and textured concavities (Figure 95). The size of individual grains ranged from less than 10 µm up to 50 µm and was congruent with the morphology of biomass ash (Kalembkiewicz, Galas and Sitarz-Palczak, 2018). Whole area EDS analysis found major elements of C, Ca, O and Na, while minor elements included K and Mg (Table 52). There were significant levels of calcium remaining, along with large levels of oxygen and carbon, in contrast to previous elemental analysis. As BSFL exoskeleton is known to contain high calcium levels, this was not unexpected. It is hypothesised that remaining minerals exist in oxide forms, equating for the high mass % seen across all samples. In addition, there was sodium detected which was suspected to remaining reagent from the deproteinisation and deacetylation stages.

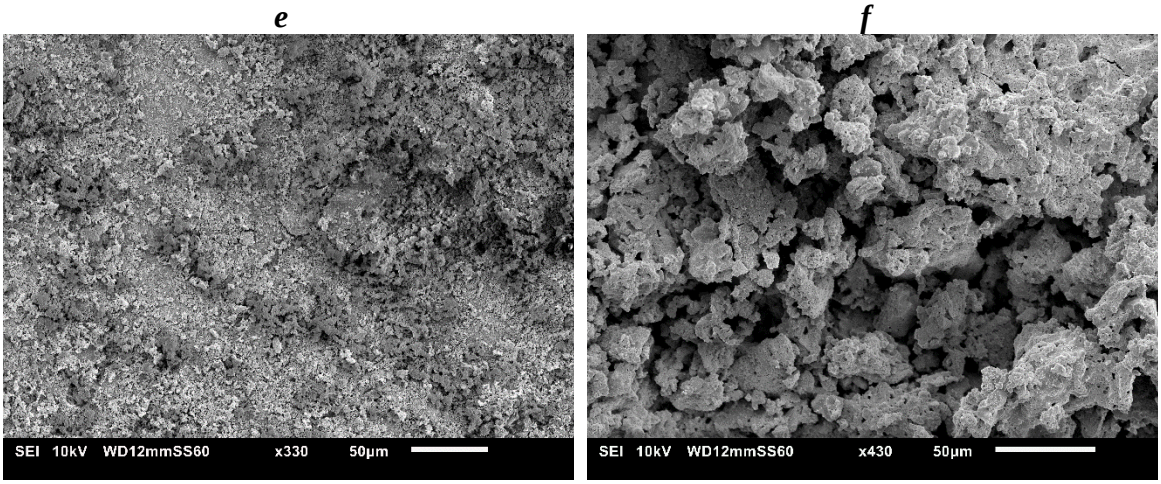


Figure 97: SEM images of *e:* BSFL ash (left) at 330x magnification and *f:* (right) at 430 magnification

Table 52: Elemental composition of incinerated samples performed by SEM-EDS, where remaining % mass is representative of Au

Sample	Mass (%)					
	Ca	C	O	Na	Mg	K
BURN2021001	-	18.31	42.85	21.77	1.74	-
BURN2021002	15.49	24.87	53.77	-	7.51	13.85
BURN2021003	7.40	26.81	19.92	-	8.01	-
BURN2021004	17.85	12.27	44.36	9.01	8.33	-
BURN2021005	-	20.77	28.84	18.40	-	-
BURN2021006	15.20	13.44	30.62	8.34	3.76	-
BURN2021007	-	14.75	49.71	21.49	2.73	-

4.3.6 Use of BSFL chitosan as a catalyst in jasminaldehyde synthesis

BSFL chitosan was tested in application as a catalyst in the selective synthesis of jasminaldehyde. Jasminaldehyde is a desirable product in the cosmetic industry, due to its appealing strong aromatic almond smell, but the reaction often results in an even mixture of jasminaldehyde and the self-condensation product of heptanal, 2-pentyl-non-2-enal. In this project, chitosan from a commercial source, shellfish, was compared in application of BSFL chitosan from both HCl and lactic acid demineralisation extractions. In the production of the basic catalyst, chitosan was dissolved in HCl and added dropwise to NaOH and allowed to age for 1 hour (method 4.2.5). After 1 hour, gelling was observed, and catalysts were removed by vacuum filtration and allowed to dry for 24 hours at 80 °C. The catalysts appeared as crystals of different colours depending on the source material: orange from commercial chitosan, and brown from BSFL chitosan.

Table 53: Synthesised chitosan basic catalyst yield (method 4.2.5), catalyst weight used in reaction (method 4.2.6) and recovered yield from use in jasminaldehyde synthesis

Starting material	Average synthesised catalyst yield (g)	Catalyst reagent weight (g)	Recovered catalyst weight (g)
Commercial chitosan	0.28	0.10	0.046
HCl BSFL chitosan	0.20	0.10	0.031
LA BSFL chitosan	0.19	0.11	0.032

It was found that a higher amount of catalyst was synthesised from the commercial chitosan (Table 53), but this was not unsurprisingly when it was seen previously that impurities exist within the chitosan structure. With a chitosan reagent weight of 3.2 g (± 0.03 g), average catalyst yields were 0.2 g and 0.19 g for HCl demineralised chitosan and lactic acid demineralised chitosan respectively. In the study by Sudheesh *et al.*, catalyst yields were not quoted and therefore it is not known whether these values are competitive (Sudheesh, Sharma and Shukla, 2010). Chitosan catalysts were characterized using a variety of different techniques, including XRD, FT-IR spectroscopy, SEM-EDS, TGA, and EA, prior and subsequent to reaction.

A FT-IR spectrum of the catalyst prior to reaction can be seen in Figure 98. The wide absorption peak at circa. 3300 cm^{-1} corresponds to the vibrational stretching of the hydroxyl groups, with the broadness of the peak indicating a strong amount hydrogen bonding as a result of the subjected basic treatment. In addition, there were strong peaks at 2900 cm^{-1} (aliphatic C-H stretching), 1590 cm^{-1} (NH_2 group bending), 1147 cm^{-1} (C–O–C bond stretching), and 1014 cm^{-1} (C–O bond stretching). The distinction of the catalyst in comparison to BSFL chitosan is determined by the wide peak at ~3300 cm^{-1}, where previous FT-IR analysis (Figure 94) saw a slimmer, less intense peak.

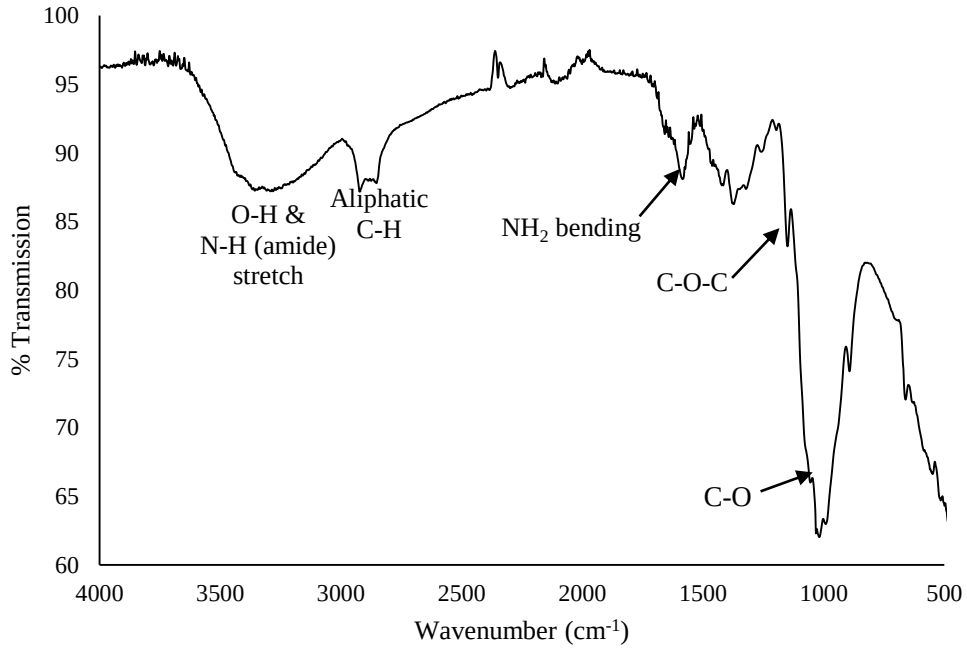


Figure 98: FT-IR spectrum of BSFL basic chitosan catalyst prior to jasminaldehyde synthesis

TGA analysis of BSFL chitosan catalysts was conducted prior to jasminaldehyde synthesis (Figure 99). Thermal stability of the catalysts was seen up to a temperature of 250 °C, a temperature whereby catalyst application would be restricted. The TGA curves showed similar decomposition to that of BSFL chitosan and commercial chitosan (Figure 96), with weight loss at two main stages. Once again, the first weight loss occurred between 80–150 °C with around a 6.81 % weight loss on average across all samples, due to the adsorbed water. The second weight loss was witnessed from 250 and 400 °C with around a 45.90 % weight loss on average across all samples, and is attributed to the decomposition of the polysaccharide chain and degradation of chitosan.

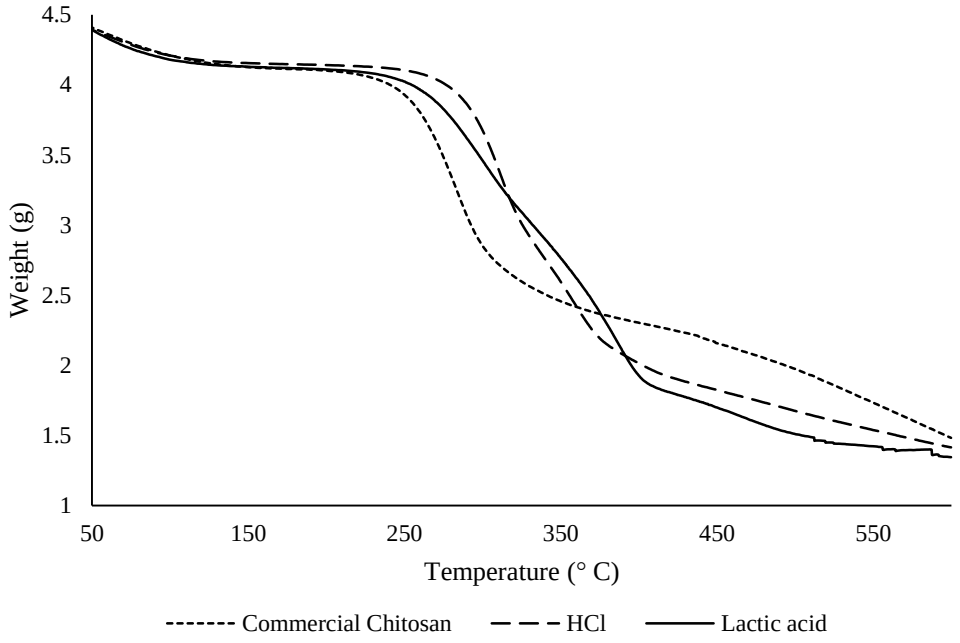


Figure 99: TGA profiles of the chitosan catalysts obtained by heating rate of 10 °C/min from 40 to 600 °C under N_2 purging (10 mL/min), where HCl and lactic acid indicate demineralisation extraction acids used

Catalytic activity of the chitosan catalyst was confirmed by GC-MS analysis of the products resulting from the synthesis of jasminaldehyde by 1-heptanal with benzaldehyde (Figure 100/Table 54). Confirmation of the target material, jasminaldehyde, was seen by a peak at 18.2 minutes using GC-MS analysis, with a peak area significantly higher than that of its competing product 2-pentyl-non-2-enal at 17.2 minutes. Calibration of the peak area would need to be completed to further confirm increased reaction selectivity by chitosan catalyst. The reaction without the catalyst has previously been seen to result in fast self-condensation of heptanal (Sudheesh, Sharma and Shukla, 2010), producing 2-pentyl-non-2-enal in equal

measure. In the present work, the peak area of jasminaldehyde being is 15 times larger than that of 2-pentyl-non-2-enal, suggesting high selectivity of BSFL chitosan as a catalyst.

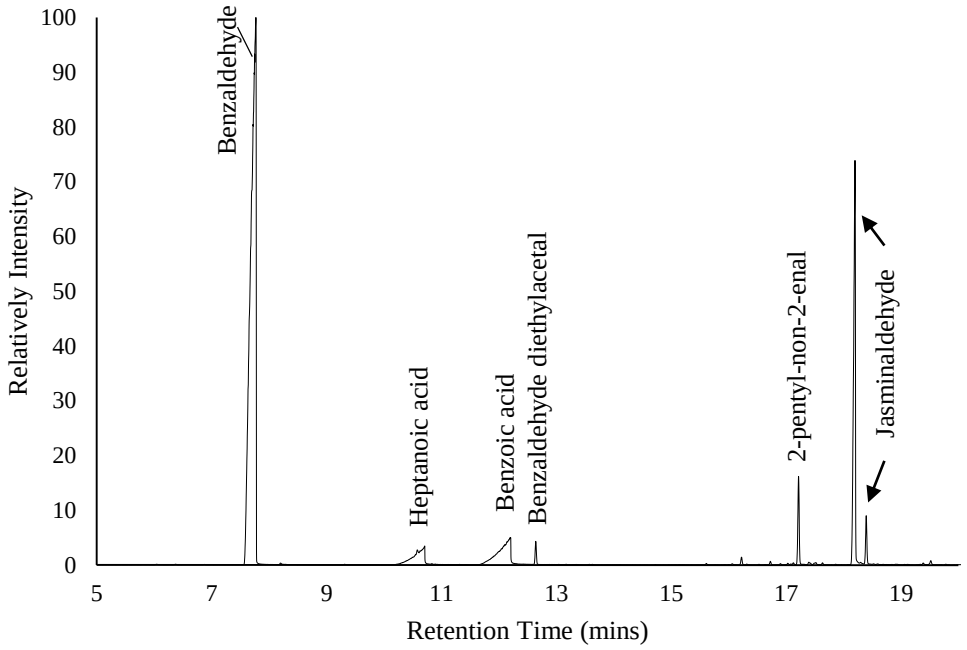


Figure 100: GC-MS chromatogram of products following jasminaldehyde synthesis with a chitosan basic catalyst

Table 54: GC-MS chromatogram data from jasminaldehyde synthesis

Peak	Retention time (min)	Area	Height	Name
1	7.769	1.43 x10^8	21813419	Benzaldehyde
2	10.708	8003859	720570	Heptanoic acid
3	12.204	14075881	1063020	Benzoic acid
4	12.643	1317969	904686	Benzaldehyde diethylacetal
5	17.217	5652680	3512009	2-pentyl-non-2-enal
6	18.2	34692806	16106222	Jasminaldehyde
7	18.396	2949559	1928755	Jasminaldehyde

Following reaction, the catalyst was recovered by vacuum filtration, and recovery weight noted (Table 53). It was discovered that BSFL catalysts lost two thirds of their mass to the reaction. SEM-EDS analysis was completed to compare the appearance and composition of the outer layer of the catalyst, Figure 101. Prior to the reaction, at a 350x magnification, the surface morphology can be described at showing a rounded and layered structure in contrast to previous images where chitosan has seen to be sharp and angular (Figure 97). Following

use in jasminaldehyde synthesis, at a 300x magnification, the catalyst appears brittle, with cracks visible on the surface, and a much smaller particle size, indicating its participation in the reaction.

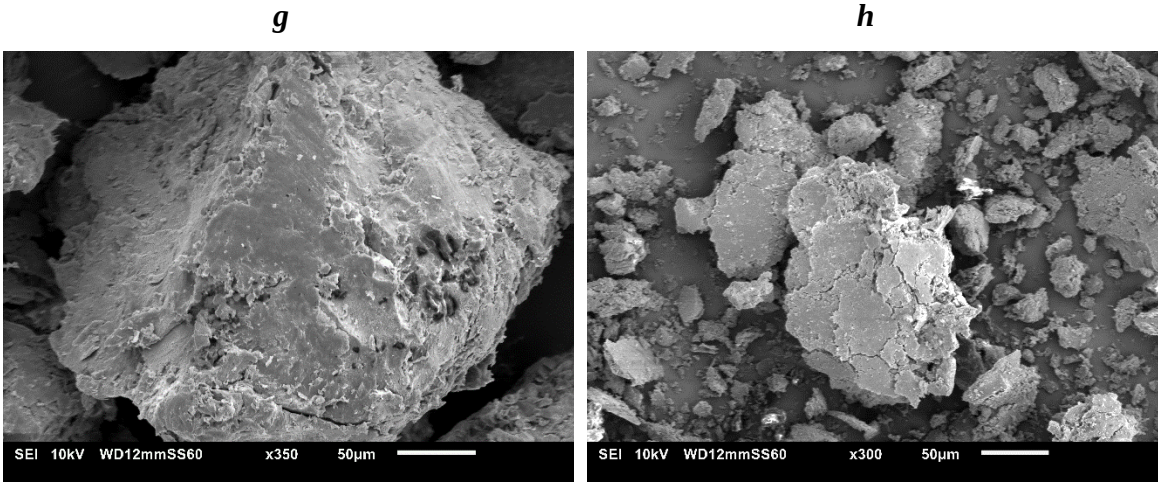


Figure 101: SEM images of catalyst before and after jasminaldehyde synthesis, where *g*: catalyst synthesised from HCl demineralised BSFL chitosan (20 mL 0.1 M HCl , 300 mL NaOH, 1 hour) and *h*: the catalyst subsequent to Jasminaldehyde synthesis (160 °C, N_2, 6 hours)

The FT-IR spectrum for the catalyst after jasminaldehyde is shown in Figure 102. The wide hydroxyl group absorption peak seen at ca. 3300 cm^{-1} in the spectrum prior to catalyst use (Figure 98) is absent, suggestive that hydroxyl groups strongly participated in the reaction. In addition, there were once again strong peaks at 2900 cm^{-1} (aliphatic C-H stretching), 1590 cm^{-1} (NH_2 group bending), 1014 cm^{-1} (C–O–C bond stretching), and 1153 cm^{-1} (C–O bond stretching.

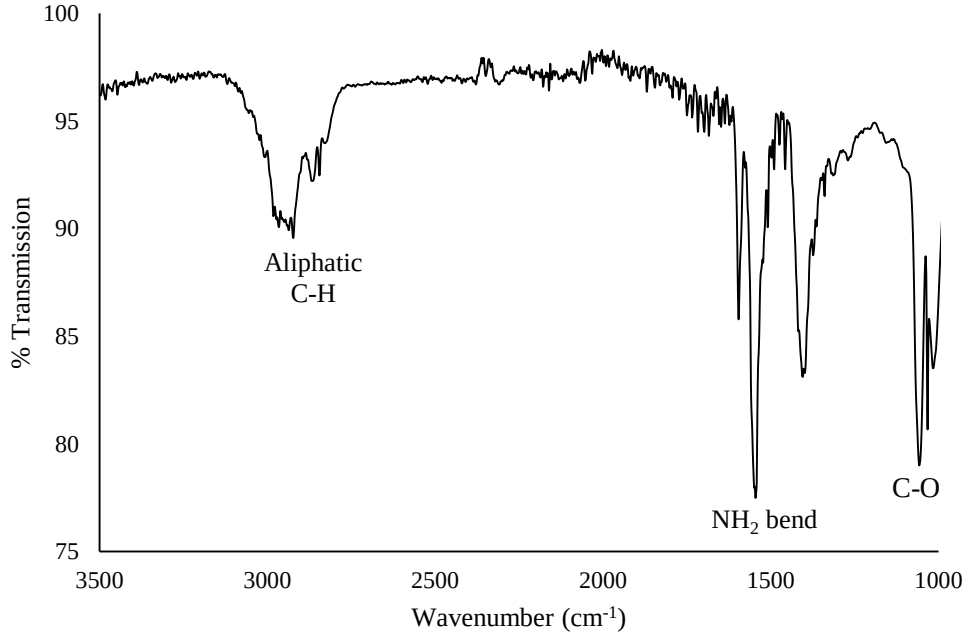


Figure 102: FT-IR spectrum of BSFL basic chitosan catalyst following jasminaldehyde synthesis

4.3.7 Alternative chitin extraction

A chitin containing material was received from Better Origin (BO), as a by-product of a fermentation procedure of whole BSFL. The material was denoted "chitin" due to its hard and fibrous nature, and its likeness to exoskeleton "skins". As previous methodology of chitin extraction extends to biological fermentation means of demineralisation and deproteinisation, it was supposed that there was a possibility fermentation be fruitful in these processes (Tan, Lee and Chen, 2020; Xie *et al.*, 2021). The BO material was investigated to ascertain whether or not it was indeed chitin, or as suspected, needed further refinement to extract the chitin.

Initially, the material denoted "BO chitin" was analysed using XRD, Figure 103. The diffractogram showed the characteristic peaks for chitin at ~9 ° and ~20 °, but these peaks were concealed and not well defined. In addition, there existed a strong intensity at 21 °, which is very atypical for previous BSFL XRD diffractograms. Crystallinity index could not be calculated accurately due to significant merging of peaks on and near to the intensity at 20 °.

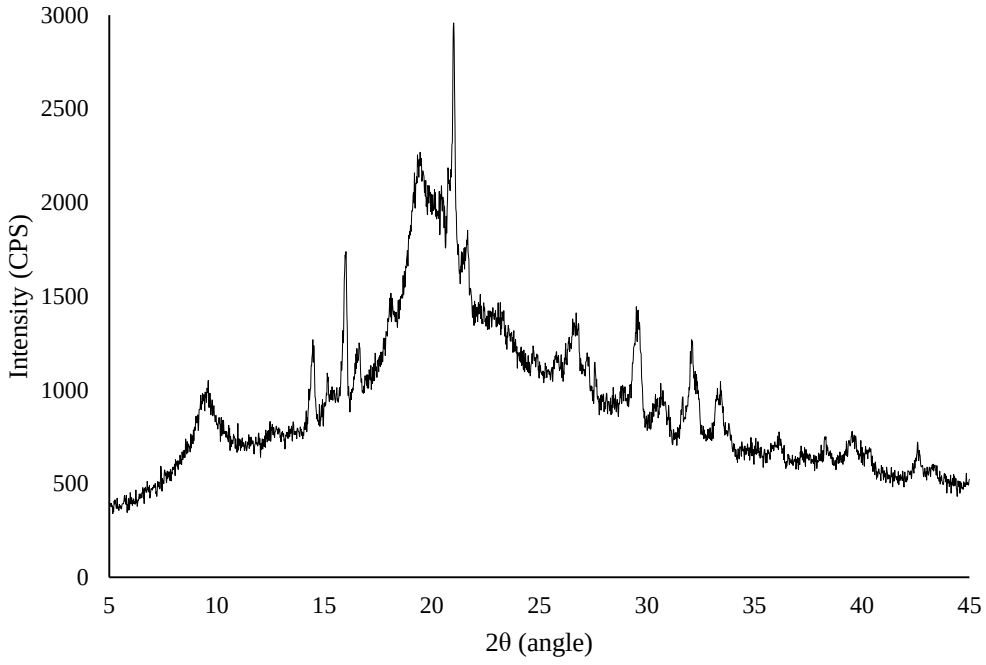


Figure 103: XRD diffractogram of BSFL Better Origin fermented chitin-containing material prior to conventional chitin extraction

To determine the morphology and information about elemental composition, SEM-EDS was conducted on the material. Whole area EDS analysis found major elements of C, Ca, O, Na and Mg, while minor elements included K and Mg (Table 55). There were significant levels of carbon, along with large levels of nitrogen, which is a positive finding and indicative of chitin presence.

Table 55: EDS analysis of BO BSFL fermented material

Sample	Mass (%)					
	Ca	C	O	Mg	K	N
BO fermented material	6.68	50.85	8.51	1.73	4.01	18.22

The surface morphology closely resembled that previously seen by demineralised BSFL, Figure 89(***a***), with setae and sensillia, as well as uneven surface with ridges, interlacing and textured concavities.

i ***j***

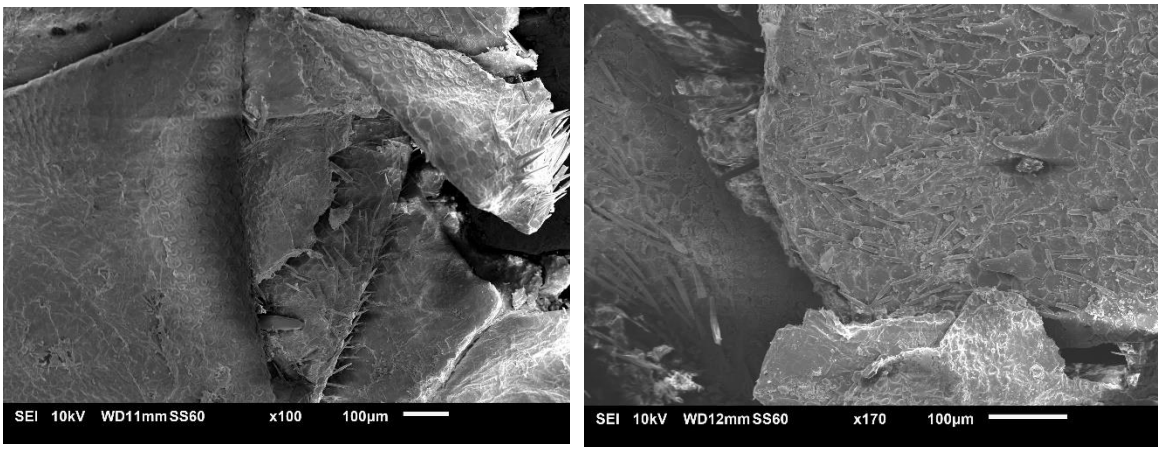


Figure 104: SEM images of *i:* BO fermented BSFL cuticle (left) at 180x magnification and *j:* (right) at 170x magnification

It was therefore decided that the fermented material needed adequate chitin extraction. As demineralisation treatments had found little difference in yield between the two acids, lactic acid treatment was selected for this trial due to its advantageous benefits. BO fermented exoskeleton was dried overnight at 60 °C and then subjected to a demineralisation treatment of 1.5 M lactic acid for 2 hours at a 1:10 solid to liquid, followed by 1 M NaOH 80 °C for 3 hours at a 1:10 solid to liquid deproteinisation treatment (Table 56). The chitin extraction

method resulted in an overall weight loss of 83.47 %, a competitive value with the results from defatted BSFL.

Table 56: Yields arising from the extraction of BSFL chitosan from BO chitin using a LA demineralisation method

Starting weight (g)	Yield after treatment (g)		Overall % weight loss
	Demineralisation	Deproteinisation	
1.20 (± 0.01)	0.56 (± 0.04)	0.20g (± 0.05)	83.47

XRD was used to confirm the extraction of chitin from this alternative source, and analysis was in good agreement with previously extracted chitin results. Strong peaks at 9.6 and 19.6 ° were seen to emerge (Figure 105), with smaller intensities at 13 °, 23 °, 26 ° and 39 °, all characteristic reflections seen by the amorphous chitin structure (Cárdenas, Cabrera, Taboada and Miranda, 2004; Sagheer, Al-Sughayer, Muslim and Elsabee, 2009; Shin, Kim and Shin, 2019). The crystallinity index of the demineralised material was found to be 39.64 %, and the deproteinised material 45.39 %, which can be evaluated as having a lower crystalline perfection than that of the primary method investigated in this project.

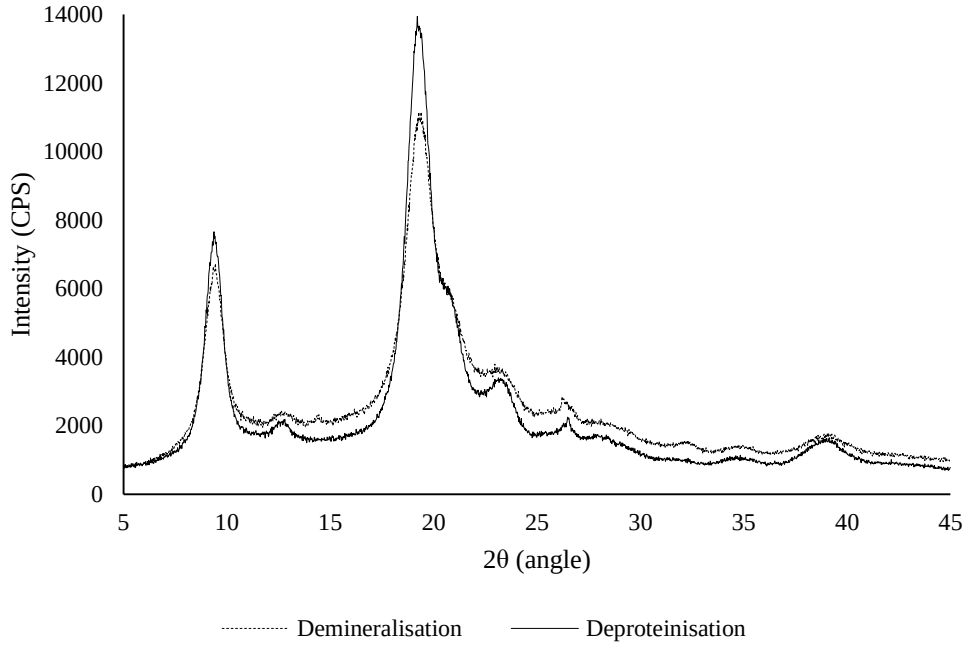


Figure 105: XRD diffractograms of the "BO chitin" product following conventional HCl demineralisation and NaOH deproteinisation treatments

It was concluded that although this method did produce chitin with a good structure and yield, the fermentation process would negate the advantages provided by fat extraction, as fat was spent in the fermentation process. Moreover, the specific fermentation process used by BO did not provide an alternative chitin extraction to the conventional one used in this chapter.

4.4 CONCLUSIONS

Three process steps were investigated for chitosan production from BSFL exoskeletons: demineralization, deproteinisation and deacetylation. The use of an organic acid (lactic acid) as an alternative to a mineral acid (HCl) in the demineralisation of BSFL cuticle was investigated using various acid concentrations and reaction durations. It was found that as the duration of the reaction increased, the degree of demineralisation also increased. Further to this, conventional NaOH alkaline deproteinisation was completed on all materials, and the yield and physiochemical properties of the resulting BSFL chitin compared. Subsequently, the BSFL chitin was converted to chitosan by deacetylation using strong NaOH alkaline treatment, and the yield and physiochemical properties of the resulting BSFL chitosan evaluated.

BSFL exoskeleton was demineralized using two alternative acid treatments and the product of demineralisations characterised using XRD, FT-IR, and EA. The residual yield after acid treatment was used as a measure of the efficiency of demineralisation. Residual yield data was obtained using five acid concentrations (0.5, 1.0, 1.5, and 3.0 M) from treatment with HCl and three acid concentrations (0.5, 1.0 and 1.5 M) using lactic acid, at a solid to liquid ratio of 1:10. For all concentrations, the best conditions determined in the investigation were 1.0 M treatment for 24 hours for HCl treatment and 0.5 M treatment for 24 hours for lactic acid, commonly demonstrating that lengthier reaction duration results in more effective demineralisation. Overall, lactic acid seemed to be more effective in removing mineral content with a percentage weight lost between initial and final weight averaging 52.88 % (± 19.71 %), compared to HCl at 26.55 % (± 6.56 %), across all concentrations and reaction durations. Demineralisation was confirmed by XRD and FT-IR analysis. In characterisation, it was established that the source of demineralisation acid used did not affect the characteristics of the end product and had no significant effect on quality of the chitin extracted. Minimal differences could be seen in yield but these were determined as negligible when the benefits of using lactic acid was considered. Temperature was not investigated in the demineralisation study, but it is hypothesized that the rate of reaction would increase with increased temperature, as found by previous studies (Ameh, Abutu, Isa and Rabiu, 2014; Greene, Robertson, Young and Clyburne, 2016). Nevertheless, knowing that 24 hours promotes efficient demineralisation is noteworthy, as this would result in significant energy savings on an industrial scale.

Characterisation using XRD, FT-IR spectroscopy, TGA and EA indicated that the chitin was isolated from BSFL by the removal of proteins from demineralised cuticle, with high similarity to commercial chitin and previously reported literature chitin studies. The % DA was determined at 126.89 % for samples demineralised using HCl and 180.91 % for samples resulting from lactic acid demineralisation. Although these values exceeded the 100 % mark, this was not unanticipated as previous studies found analogous results from other chitin containing sources. Chitin was seen to display an α crystalline form upon XRD analysis which is typical of insect derived chitin (Hahn *et al.*, 2020). The thermal profile of BSFL chitin indicated characteristic behaviour of the polysaccharide, with three main degradation stages observed. The similarity of the FT-IR spectroscopy analysis of the material to the was promising, with distinctive peaks typical of chitin observed. The durability of BSFL chitin was witnessed, and solubility in solution found to be poor but convenient for processing. The insoluble nature of chitin in general makes application of the material challenging, and so for that reason further treatment of BSFL chitin to chitosan was completed.

Strong alkaline treatment of BSFL chitin brought about the removal of acetyl groups and gave rise to BSFL chitosan. Overall, the following all three treatments, minerals, protein and acetyl groups were removed resulting in 88.10 % yield loss in HCl demineralisation treatments and 91.02 % in lactic acid ones. As predicted previously, this amounts to roughly 10 % of the defatted larvae. Commercial chitosan (Sigma Aldrich, UK) and chitosan from BSFL were analysed for comparison of their nitrogen contents and degree of deacetylation. The deacetylation was observed by elemental analysis and calculation of the degree of deacetylation, whereby it was found that there was an average % DD of 51.87 % and 54.74 % for samples demineralised using HCl and lactic acid respectively, where % DD less than 50 % can be denoted chitosan. This was a very competitive value when compared to the commercial chitosan value calculated at 91.02 % using the same elemental analysis method. Structural differences resulting from deacetylation were detected using XRD, FT-IR, SEM-EDS, EA and TGA. The chitosan was seen to be amorphous by XRD analyses, and soluble in mildly acidic solution. Results collected in this study indicate that BSFL chitin and chitosan have quite high potentials as alternative chitin and chitosan sources.

Subsequent to chitosan production, its potential application as a catalyst was tested by conversion to basic hydrogel catalyst and use in jasminaldehyde synthesis. Following methodology by Sudheesh *et* al., the BSFL chitosan catalyst was found to perform well in

the condensation of 1-heptanal with benzaldehyde to produce jasminaldehyde by GC-MS analysis. The catalyst was found to increase selectivity in the reaction and therefore it was presumed the catalyst provided the basic conditions and surface necessary to prevent the self-condensation of 1-heptanal.

An alternative fermentation based chitin extraction was examined to determine whether the process by Better Origin was fruitful. Significant levels of minerals were seen to exist by SEM-EDS analysis and remaining proteins appeared to affect the structure of the crystal by XRD analysis. It was decided that the material would be subjected to conventional demineralisation and deproteinisation treatments. Following this, the Better Origin material displayed the good characteristic identification peaks seen previously by conventionally extracted BSFL chitin and chitosan, as well as commercial chitosan.

To conclude, within this study, the chemical procedure for the removal of chitin and chitosan from BSFL was examined, and the use of an organic acid was examined as an encouraging alternative to a mineral for the demineralisation. This study showed that the efficacy of lactic acid for the demineralisation of BSFL was comparable to that of HCl. The work described in this study has demonstrated that chitin can be effectively extracted from BSFL following demineralisation using 0.5 M lactic acid for 24 hours and deproteinisation using 3.0 M NaOH for 3 hours. Low molecular mass chitosan samples with % DD of 58.0 % was obtained by treating the BSFL chitin with 50 % NaOH at 100 °C for up to 5 hours. The findings within this study confirmed that BSFL has a potential as an alternative source of chitin and chitosan, from a sustainable waste-remediating source. Further investigation on the chitin extracted from BSFL and chitosan produced herein would help to better discover sustainable products for biotechnological, biomedical, and cosmetic applications. This could include further synthesis of catalysts for its utilisation in other heterogeneous applications, BSFL chitin films, or BSFL chitosan dye absorption materials.

5 CONCLUSIONS AND FUTURE WORK

There are two main challenges that this research sought to address through the utilisation of black soldier fly larvae. These were waste remediation, and the development of more sustainable alternatives to some natural resources. Work completed in this thesis focused on the valorisation of the chemicals products resulting from the remediation of waste by black soldier fly larvae (BSFL). Oil collected from the BSFL was examined as a possible alternative to plant oils already in use as sources for value-added chemicals, and chitin was extracted from the BSFL exoskeleton and examined in its use as a catalyst support in jasminaldehyde synthesis.

In 2011, the UN estimated that 1.3 billion tonnes of food waste were generated globally per annum (Gustavsson, Cederberg and Sonesson, 2011). The problem of municipal waste - otherwise known as household waste - is a challenging issue humankind is facing worldwide, with some countries having a defined structure for its disposal, but others are severely lacking in food waste management (Beede and Bloom, 1995). In particular, disposal of urban waste presents a challenge (Lohri, Diener, Zabaleta, Mertenat and Zurbrügg, 2017). The majority of waste generated is usually incinerated or, in some developing countries dumped in open areas, which may cause severe health and environmental issues (Paritosh *et al.*, 2017). Food waste is a vastly overlooked driver of climate change, with the FAO 2011 assessment estimating the total carbon footprint of food wastage, including land use change, at around 4.4 Gt CO_2 per year. It was concluded through this work that the management of food waste could be achieved through the (bio)chemical conversion using black soldier fly larvae. Alongside the benefits of remediating waste, research completed within this work shows that large scale adoption of BSFL rearing could provide a local, sustainable and carbon reductive method of recycling waste and a novel system of producing valuable materials. The supply chain of products made from BSFL would encompass the municipal food waste industry, and would rely on a constant course of waste. The use of larvae would not compete with the food chain and instead be supplementary by reducing the CO_2 emissions arising from it. A convenient and constant source of feed is required but easily resolved by placing insect farms near to waste streams such as breweries or food-processing factories.

The long-term viability of this work has also been carefully considered with applications beyond solely waste remediation. Declining reserves of fossil resources and environmental concerns by consumers around the use of coconut and palm kernel oil present a need to identify new sources of the raw materials required for the production of fuels and chemicals. Deforestation rates in south east Asia are some of the highest globally, as vast areas of space are needed for palm plantations, and so acres of rainforests are cleared, endangering biodiversity and native habitats (Pacheco, Gnych, Dermawan, Komarudin and Okarda, 2017). There is an increasing public perception that there are serious environmental issues with this industry causing a mass loss of biodiversity within tropical rainforests and loss of native habits (Vijay, Pimm, Jenkins and Smith, 2016). Therefore, palm and coco oils have been established as an unreliable source for the surfactant industry with climate change, neglect and disease contributing to instability in price and availability worldwide (FAO, 2020; USDA, 2021). When scaled industrially, farming insects requires less water and space than conventional palm and coconut oil cultivation. Through large scale waste remediation, BSFL are a proposed tool for reducing the dependence of Europe's oleochemical industry on imported tropical vegetable oils. Oil from BSFL rearing can be deemed a more sustainable version of plant oil, having a beneficial feedstock of organic waste.

Complying with UN SDG 13, there is significant reduction to CO_2 emission if BSFL were to be used as a replacement source of plant oils, not only through the waste remediation of BSFL but also in the transportation of fatty acid oils from south east Asia or Africa to Europe (Barroso *et al.*, 2017; Mahmood, Zurbrügg, Tabinda, Ali and Ashraf, 2021). An additional advantage of mass rearing BSFL compared to contemporary plant oil cultivation exists the possibility of a high turnover of oil comparatively. Where the fastest palm fruits with 4 years (Wahid, Abdullah and Henson, 2005), BSFL are producing oil within only a few months (Manurung, Supriatna, Esyanthi and Putra, 2016), with trees also have a limited oil producing capability of 80 to 100 years, and BSFL with the correct genetic rearing consideration have limitless production. BSFL are highly effective in their consumption of waste, and results from this research show that the biological transformation of this into higher value organic oils was completed, with a variety of chemical applications discussed.

In the production of value-added chemicals, a significant finding of this research is that BSFL was found to produce an oil extremely similar in composition to that of palm and coco oil and therefore could provide a viable alternative source. In the first work package of

research, the extraction of BSFL oil was investigated, and in lab-scale removal, soxhlet extraction at 80 °C was found to give the highest yields when a hexane solvent was used. FFAs in the BSFL oil were subsequently analysed using FT-IR and GC-MS, whereby the latter included quantification of key FFAs commonly found in plant oils, including a significant proportion of lauric acid. Characterisation of the oil included determination of the density, acid value, cooling curves and slip melting point. In the next work package, the composition of BSFL was examined to investigate whether the diet fed to developing larvae effected the final composition of oil. It was discovered that the higher sugar content foods produced higher amounts of oil within final dried larvae, and it is recommended that if a larger oil to protein portion is desired, diet should be tailored accordingly. In a work package investigating oil refinement, a noteworthy result was that lab-scale distillation of the oil yielded colourless, pure mixtures of FFAs by GC-MS analysis, removing the need for palm and petrochemical FFA sources.

The synthesis of value-added chemicals from waste via BSFL assimilation was successfully demonstrated through the synthesis of FAMEs, fatty alcohols, SASs, MESs, esterquats, fatty amides and spans. The surfactants produced were characterised using IC, FT-IR spectroscopy, GC-MS and concentrations quantified by titration. The CMC was determined by reverse meniscus surface tension methodology, along with comparison to conductivity measurements. Demonstration of lab-scale synthesis of these value-added chemicals is highly promising for the hypothesis that BSFL oil could take the place of traditional plant-oils in industrial scale surfactant plants.

In the extraction of chitin from BSFL exoskeletons, the use of HCl and lactic acid in the demineralisation stage was examined, and the results found that the acids where comparable in their removal of minerals. Due to the eco-friendly benefits of using a non-mineral acid such as lactic acid, such as removing a toxic waste water streams, and being less harmful to the environment, it can a recommended in its use of chitin extraction. Subsequent to deproteinisation by 3 M NaOH, the resulting BSFL chitin was characterised using XRD, FT-IR spectroscopy, SEM and TGA analysis. A significant finding of this research was that BSFL chitin was seen to be highly similar to that of traditional shellfish-derived chitin, and would provide an additional bonus of being a sustainable, consistent resource, as well as the benefit of remediating waste. Deacetylation to achieve the chitosan derivative was completed using 50 % (w/w) NaOH treatment, and characterisation accomplished using

XRD, FT-IR spectroscopy, SEM-EDS and TGA analysis. The % DD was calculated to be near to 50 % value indicating chitosan presence, from chitin products of using both demineralisation acids. Synthesis of a basic catalyst from BSFL chitosan occurred and was tested in the selective synthesis of jasminaldehyde from 1-heptanal and benzaldehyde. The BSFL chitosan catalyst was seen to perform well by GC-MS analysis.

The primary next stage of the work would focus on further lab-scale synthesis of alternative surfactants, namely the development of the amphoteric surfactant dimethylalkylamine oxide pathway. Other surfactants of commercial interest would be explored, such as non-ionic fatty alcohol ethoxylates which currently require expensive ethylene oxide and specialised reaction vessels to withstand the resulting strong exothermic reaction. In order to effectively and economically produce surfactants on a grand scale, with an unlimited budget, the large scale distillation of BSFL oil would be completed to separate FFAs into identifiable pure fractions. Following this, the demonstration of large scale SAS production of SDS from fractionated BSFL oil using gaseous SO_3 would display excellent demonstration of direct synthesis of BSFL value-added chemicals.

Further to this, market research of the potential public perception of waste or insect derived cosmetic products is pertinent for examination. Likewise, the development of a life-cycle assessment (LCA) including both the assessment of the environmental impacts associated with all the stages BSFL rearing for the purpose of succeeding commercial manufacture of BSFL oil or chitin based products instead of solely for food, would be extremely beneficial. The assessment of BSFL in particular, where previous research exists on insects in general, would be constructive in comparing the environmental benefits against the use of plant-based oils in the surfactant industry.

In future work, the diet fed to BSFL would be further investigated, to confirm the hypothesis that a high sugar diet results in a high fat to protein composition. In particular, a diet high in sugar, such as tropical fruits mango or lychee, or solely cane sugar, should be fed to the developing BSFL to ensure that the hypothesis denoted in chapter 2 of this work is correct. Other diets such as those with high protein content, raw or discarded meat, or high fat diets such as commercially rejected avocado, nuts or seeds, would be beneficial in understanding how the protein to fat ratio can be tailored. A diet producing a higher yield of lauric acid would be advantageous to the oleochemical industry, where lauric acid is highly desirable.

When ingested, lauric acid is enzymatically transformed to monolaurin, which pertains to its own unique properties. In the medical industry, the application of monolaurin is of interest as a non-traditional antimicrobial agent (Zhang, Wei, Cui, Zhao and Feng, 2009). Previous research has found value in its use as an antibacterial agent against many bacterial strains, including *Staphylococcus aureus* (Widiyarti, Hanafi and Soewarso, 2009), as well as significant demonstration of inhibitory activity against a number of fungi and viruses (Dayrit, 2015). Although digestion of lauric acid containing products yields the monolaurin derivative through enzyme action *in vivo*, the synthesis of monolaurin can be achieved chemically by esterification with glycerol (Nitbani, Jumina, Siswanta, Sholikhah and Fitriastuti, 2018). The synthesis of monolaurin from BSFL oil and its demonstration as a medical antimicrobial agent should be explored in future studies.

There lies further investigation into the development of BSFL chitin and chitosan based films and fibres. Without time constraints, the development of chitosan films would have been investigated as a translucent anti-bacterial alternative to contemporary plastic food packaging. The manufacture of BSFL fibres from chitin, with substantial tensile strength, can be achieved through reaction with trichloroacetic acid and chloral hydrate in a DCM solution (Austin and Brine, 1977). Besides this, further work would include the application of BSFL chitosan hydrogels as dye adsorption materials, where previous studies into shellfish-derived hydrogel beads showed great potential in wastewater treatment (Sridhari and Dutta, 2000; Van Tran, Park and Lee, 2018).

Looking to the future, the successful development of BSFL products would rely heavily on the utilisation of all of its counterparts; oil, chitin and protein. From the work completed and with the consultation from industry partner, Nouryon, it is the recommendation that the counterpart of highest monetary value is the oil, and its application in surfactants as a sustainable alternative to plant oils. It is therefore imperative that the improvement of oil yield is of highest priority, with the investigation into obtaining the highest amount of lauric acids from the FFAs found in BSFL oil, crucial to valorising commercial process. These research findings have shown how BSFL may remove the need for modern plant and petrochemical sources. In the surfactant industry, there is a clear shift towards more sustainable products and therefore a clear market for BSFL products, with Unilever launching its €1 billion Clean Future commitment in 2020, aiming to 100% of the carbon

derived from fossil fuels in formulations with renewable or recycled carbon by 2030 (Unilever Press Release, 2020).

As a by-product of the oil production scale, would be the benefit of high-value protein for animal feed, instead of the alternative currently, where pressed fat is by-product from the current small scale production of BSFL protein in cases where the protein is separated out. As there already exists a viable market for protein (Van Huis, 2013; Maurer *et al.*, 2016; Nyakeri, Ogola, Ayieko and Amimo, 2016), there would be little opposition to the further introduction of BSFL by-product protein as animal feed. For chitin applications, the BSFL chitin product would fit into the biotechnology, food emulsification and packaging markets (Morin-Crini, Lichtfouse, Torri and Crini, 2019; Tan, Lee and Chen, 2020). The biggest appeal for this market would lie in the sustainability and biodegradability of BSFL chitin in comparison to contemporary resources.

In this work, the ability to rear BSFL on a diet of food waste, extraction of organic oils and conversion of these oils to some value-added products has been demonstrated, in the form of surfactants and a chitosan catalyst. In summary, the use of BSFL in waste remediation is notable in this research as an asset that brings about numerous benefits, with the fat and chitin components being explored as useful in a variety of applications.

6 REFERENCES

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