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Identification and quantification of lipids in *T. viridissima*, *C. biguttulus* and *C. brunneus* by GC-MS and off-line SPE GC-MS

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Chemistry

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Summary

The main objective of this study was to elucidate and quantitate the complete fatty acid profiles of the species *Tettigonia viridissima*, *Chorthippus biguttulus* and *Chorthippus brunneus*, all belonging to the order *Orthoptera*. Insects are already a staple food in many parts of Africa, South America and Asia, and have garnered increased attention in the West during the last few decades. The beneficial amino acid and fatty acid profiles of insects could make them a viable alternative to beef, poultry and fish in the West in the coming decades due to the global, exponential population growth. Previous studies on the fatty acids of insects have been mostly focused on species whose habitat is located in more tropical climates. As a result, this study was conducted on three species commonly found in Norway, and whose combined habitats range throughout Scandinavia, continental Europe, temperate Asia and parts of North Africa.

The complete fatty acid profiles of all species were identified and quantitated using gas chromatography coupled to a three-sector mass spectrometer. The analytical method had previously been established, tested and validated in our laboratory several years prior to this study. Fatty acids extracted from the insects, by use of solvents, were derivatized into fatty acid methyl esters prior to analyses. Off-line solid-phase extraction was also implemented for the fatty acids from *T. viridissima* to quantitate the contents of neutral lipids, polar lipids and free fatty acids. The presence of fatty acids such as linoleic acid, α-linolenic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid were subsequently subjected to discussion to evaluate the potential of the species as human food, and impact on human health.

In the comparative study, *T. viridissima* was found to contain 10.4% fatty acids of dry weight, *Chorthippus* contained 6.14%. Both contained comparatively equal amounts of saturated fatty acids (31.1 and 32.7%), while *Chorthippus* was significantly richer in polyunsaturated fatty acids (42.1%) than *T. viridissima* (33.0%). Furthermore, the essential fatty acids linoleic acid and α-linolenic acid were abundant in both, but *Chorthippus* exhibited by far the highest contents of the latter (30.7%). The results suggested that both *T. viridissima* and *Chorthippus* contained nutritionally beneficial FA compositions, however, *Chorthippus* had a more favorable *n*-6/*n*-3 ratio of the two.

Sammendrag

Hovedmålet med denne studien var å identifisere og kvantifisere de komplette fettsyreprofilene til artene *Tettigonia viridissima*, *Chorthippus biguttulus* og *Chorthippus brunneus*, der alle tilhører insektordenen *Orthoptera*. Insekter er allerede et fast innslag i dietten mange steder i Afrika, Sør-Amerika og Asia, og har de siste tiårene opplevd en fornyet interesse i Vesten. De gunstige amino-, og fettsyreprofilene til insekter kan gjøre dem til et aktuelt alternativ til storfe, fjærfe og fisk i Vesten de kommende tiårene grunnet den globale, eksponentielle befolkningsveksten. Tidligere studier som har omhandlet fettsyrer i insekter har hovedsakelig fokusert på arter med utbredelse i mer tropiske strøk. Derfor ble denne studien utført på tre arter som er utbredt i Norge, Skandinavia forøvrig, kontinentale Europa, tempererte Asia, og deler av Nord-Afrika.

De komplette fettsyreprofilene for alle artene ble identifisert og kvantifisert ved bruk av en gasskromatograf koplet til et tre-sektor massespektrometer. Den analytiske metoden tatt i bruk har tidligere blitt etablert, testet og validert i vårt laboratorium flere år før denne studien fant sted. Fettsyrene som ble utvunnet fra insektene, ved bruk av løsningsmidler, ble derivatisert videre til fettsyremetylestere før analysene. Fast-faseekstraksjon ble også inkorporert for fettsyrene fra *T. viridissima*, for å kvantifisere forekomstene av nøytrale lipider, polare lipider og frie fettsyrer. Forekomstene av fettsyrene linolsyre, α-linolensyre, arakidonsyre, icosapentaensyre og docosahexaensyre ble i etterkant benyttet i diskusjonen for å evaluere potensialet til alle artene som menneskelig føde, samt innvirkning på menneskelig helse.

I den sammenliknende studien ble fettsyreinnholdet til *T. viridissima* funnet å være 10,4% av tørrvekten. *Chorthippus* inneholdt derimot 6,14%. Begge inneholdt omtrentlig like mengder mettede fettsyrer (31,1 og 32,7%), men *Chorthippus* hadde betydelig høyere innehold av flerumettede fettsyrer (42,1%) enn *T. viridissima* (33.0%). De essensielle fettsyrene linolsyre og α-linolensyre var tilstedeværende i rike mengder i begge arter, men forekomsten av sistnevnte var betydelig høyere i *Chorthippus* (30,7%). Både *T. viridissima* og *Chorthippus* hadde en gunstig fettsyreprofil fra et ernæringsmessig perspektiv, men *Chorthippus* hadde utelukkende den mest gunstige *n*-6/*n*-3 ratioen av de to.

Abbreviations

AA	Arachidonic acid
ALA	α-linolenic acid
BCFA	Branched fatty acid
DHA	Docosahexaenoic acid
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
FAME	Fatty acid methyl ester
FFA	Free fatty acid
FID	Flame ionization detector
GC	Gas chromatography
LA	Linoleic acid
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometer
MUFA	Monounsaturated fatty acid
NL	Neutral lipid
OA	Oleic acid
PL	Polar lipid
PUFA	Polyunsaturated fatty acid
RIC	Reconstructed ion chromatogram
RRF	Relative response factor
SFA	Saturated fatty acid
SIM	Selected ion monitoring
SPE	Solid-phase extraction
TG	Triglyceride

1. General introduction

Throughout history, insects have been an important part of the human diet, and often as an alternative to meats and fish. The act of entomophagy, consumption of insects, is predominantly practiced in Asia, South-America and Africa (Chakravarthy et al. 2016). Their contents of proteins, fats, vitamins and minerals have in recent times facilitated an increased scientific interest throughout the Western world. The commercialization of insects as human feed in both the developed, and developing world, could potentially result in increased long-term food security for a growing global population, which is currently soaring towards a total of 9 billion by the middle of the century. An increased consumption of insects could also contribute to a more sustainable development, especially when treated as a substitute to red meat. The production of meat from cattle has lately come under especially heavy scrutiny by the public, in part due to the large emissions of the greenhouse gases methane and nitrous oxide, as well as the large quantities of feed required per pound of produced beef. On average, insects are five times more efficient than beef cattle at converting feed into tissue, and twice as efficient as pigs and chickens (Mitsuhashi 2010). When considering the reproduction rates of insects opposed to traditional livestock, these values increase even further. According to Chakravarthy et al. (2016), among the 1700 edible species consumed worldwide, 80% belong to the insect orders *Coleoptera*, *Hymenoptera*, *Lepidoptera* and *Orthoptera*. Locusts, grasshoppers and crickets belong to the latter.

Several studies have been conducted to establish the importance of unsaturated fatty acids in biological functions within the human body, as well as their ability to prevent and treat cardiovascular diseases, coronary heart disease and inflammatory diseases (Connor 2000). An increased intake of *n*-3 PUFAs reportedly also had beneficial effects on patients with certain cancers, as well as a linked association with an overall reduced risk of cancers such as breast cancer (Simopoulos 2008). Additionally, PUFAs such as AA, DHA and EPA act as important regulators of several processes within the brain (Bazinet & Layé 2014). The official stance of FAO (2010) however, was that there was insufficient evidence to establish any relationship between PUFAs and cancer, and further research was recommended.

Also according to FAO (2010), there is convincing evidence that the PUFAs ALA and LA are the two only EFAs, because the human body is incapable of synthesizing either. Through elongation and desaturation, they also act as precursors to the *n*-6 fatty acid AA, and the *n*-3

fatty acids EPA and DHA. Plant materials are the primary source of the EFAs ALA and LA for humans, while EPA and DHA are abundant in oily fish and krill, and cannot be found in the seed oil of plants (Dewick 2009).

Several authors, Simopoulos (2002) included, have established the importance of the *n*-6/*n*-3-ratio in the human diet. Throughout human evolution, the ratio of the two FA groups were close to 1, but Western societies today have an excess consumption of *n*-6 fatty acids, resulting in a *n*-3 deficiency. Simopoulos (2002) postulated that a 4/1 ratio was associated with a 70% decrease in overall mortality, while a ratio of 2-3/1 had anti-inflammatory effects. According to Russo (2009), evidence is in support of the importance of the *n*-6/*n*-3 ratio, first defined by Simopoulos in 1991, thereby making it a useful tool in determining the overall nutritional quality of foodstuffs from purely a FA point of view. FAO (2010) however had no recommendation for the n-6/n-3 ratio, arguing that intakes of *n*-6 and *n*-3 FAs adhering to dietary recommendations established in their report would be sufficient.

To date, many qualitative and quantitative studies have been published on the FA compositions of insects belonging to the order *Orthoptera*. Thompson (1973) reviewed the FA compositions of seven insect orders, including *Orthoptera*, revealing significant inter-order differences in relative percentages of common FAs such as C14:0, C16:0, C18:0, LA and ALA. Grapes et al. (1989) utilized capillary GC-FID, and GC-MS, to analyze the fatty acid contents of the cricket *Acheta domesticus* at various stages of development. Grapes et al. (1989) also utilized solid-phase extraction to fraction the lipids into three different classes. The adhesion secretions of *Schistocera gregaria*, a desert locust, were analyzed by Reitz et al. (2015). Sampling of the lipids was carried out using contact SPME, and the lipids were subsequently analyzed by GC-MS. Paul et al. (2017) compared the FA compositions of three species belonging to *Orthoptera* and the larvae *Tenebrio molitor*. *Chorthippus parallelus* contained an abundance of the EFA ALA, while LA was the most abundant FA in the crickets *A. domesticus* and *C. discolor*. However, no studies have been carried out to acquire and quantitate the complete FA profiles of the three species *Tettigonia viridissima*, *Chorthippus brunneus* and *Chorthippus biguttulus*, all of which are commonly found in Europe and temperate Asia. The latter two also appear in north Africa. Elucidation and quantitation of their complete FA profiles would yield important nutritional information that could potentially mark the three species as viable for human consumption. The contents of EFAs, EPA, DHA, AA, *n*-3 and *n*-6 FAs present would also indicate possible health benefits by consumption of these insects.

2. Aims of the study

The overall aim of this work was to elucidate and quantitate the FA compositions of three different species: the bush cricket *T. viridissima*, and the two grasshoppers *C. brunneus* and *C. biguttulus*, using an in-house designed and validated analytical method for derivatized lipids by GC-MS.

The partial objectives are listed below:

- Obtaining the complete FA profiles of *T. viridissima, C. brunneus* and *C. biguttulus* by using solvents to extract the lipids, derivatization of the extracted lipids into FAMEs, and subsequent analysis by GC-MS.

- Fractioning of the lipids in *T. viridisima* by off-line SPE into three fractions: neutral lipids, free fatty acids, and polar lipids, with subsequent quantitation of each class after analysis by GC-MS.

- Evaluating the three, different species as potential human food based on FA compositions, with an emphasis on PUFAs, MUFAs, and the abundance of the FAs LA, ALA, EPA, DHA and AA, as well as the overall *n*-6/*n*-3 ratio of each species.

3. Theory

3.1 Lipids

Lipids form a diverse class of natural products, which includes fatty acids, triglycerides, phospholipids, waxes, sterols, vitamins who are non-soluble in polar solvents, and polyketides, among others. Although no exact definition of lipids exists (Akoh & Min 2008), they may be defined in several ways. The most basic definition of this heterogenous group of natural products would be their shared characteristic: solubility in nonpolar, organic solvents such as chloroform, hexane, diethyl ether and benzene (Akoh & Min 2008; Gunstone & Norris 2013). This characteristic is due to the presence of hydrocarbon chains of varying lengths. Lipids are also responsible for key biological functions, such as the storage of energy in organisms, most often in the form of triglycerides, commonly referred to as fats and oils (depending on the degree of unsaturation), and biological signaling. They also constitute a significant part of the cell membrane due to the amphiphilic nature of phospholipids, forming continuous bilayers (Dewick 2009; Yeagle 2016).

3.1.1 Fatty acids

Fatty acids are carboxylic acids accompanied by hydrocarbon chains of varying lengths, typically ranging from 4 to 28 carbon atoms. The most common chain lengths, however, range from 10-22 carbon atoms with an even number being the norm, and the majority of natural fatty acids exhibit straight chains whether unsaturated or saturated (Gunstone & Norris 2013). While most fatty acids are insoluble in polar solvents such as water due to the long, aliphatic hydrocarbon chains, some very short fatty acids are readily soluble in water and insoluble in nonpolar solvents (Akoh & Min 2008).

The degree of unsaturation in fatty acids refers to the presence of double bonds within the hydrocarbon chain. Monounsaturated fatty acids (MUFAs) refer to the FAs containing only a single double bond. Polyunsaturated fatty acids (PUFAs) however, contain two or several more double bonds within the hydrocarbon chain. Saturated fatty acids (SFAs) on the other hand are characterized by their absence of any double bonds, containing only single bonds. The presence of double bonds drastically affect their state at room temperature. Triglycerides containing PUFAs generally appear as liquids of varying viscosities, while triglycerides containing SFAs appear as solids. This is due to the inability of TGs containing PUFAs to

align in a crystalline way, owing to the less straight chains formed by the presence of double bonds (Hart et al. 2011). The configuration of the double bonds in unsaturated FAs are most commonly *cis*, rather than *trans*.

3.1.2 Nomenclature of fatty acids

The established IUPAC nomenclature for FAs was published in 1979, and includes information on the number of carbon atoms present in the alkyl chain, as well as the position and configuration of the double bonds relative to the carboxylic acid terminus. The shorthand designation also includes information of the hydrocarbon chain length, the total number of double bonds, as well as the position of the double bond closest to the methyl terminus of the alkyl chain, most commonly by use of the symbols "n" or "ω" (Devle 2013). The trivial names, however, originated before the chemical structures of some common, naturally occurring FAs were elucidated, and are often based on the Latin names of the plants or plant seeds they were first isolated from (Gunstone & Norris 2013). The trivial names have become so established, that they are often used interchangeably with the official IUPAC systematic names and shorthand designations in the literature. An overview of the nomenclature of FAs commonly found in insects are displayed in **table 1**.

Table 1: The systematic name based on IUPAC nomenclature, trivial name, and shorthand designation of some common FAs found in insects belonging to the order *Orthoptera*

IUPAC nomenclature	Trivial nomenclature	Shorthand designation
Tetradecanoic acid	Myristic acid	C14:0
Hexadecanoic acid	Palmitic acid	C16:0
Octadecanoic acid	Stearic acid	C18:0
cis-9-Octadecenoic acid	Oleic acid	C18:1n-9c
cis-9,12-Octadecadienoic acid	Linoleic acid	C18:2n-6c
cis-9,12,15-Octadecatrienoic acid	α-Linolenic acid	C18:3n-3c

Adapted from Devle (2013)

3.1.3 Acylglycerides

FAs are seldom found in nature in their original state purely as carboxylic acids with alkyl chains. However, they appear more commonly as triglycerides (TGs), and are referred to as fats and oils, depending on their state at room temperature. The structure of a TG is comprised of a glycerol unit with three FAs through ester linkages. The TG is termed as 'simple' if the three FA units are identical, and 'mixed' if the FA units differ from each other (Dewick 2009). The latter is the most abundant of the two. TGs are biologically important, because they act as storage lipids, accumulating energy in the tissue, which can be metabolized by the organism in times of need (Devle 2013). The predominant biosynthesis of TGs is achieved through continuous esterification of glycerol-3-phosphate by FA-coenzyme A residues (Dewick 2009). Additionally, diglycerides and monoglycerides are also part of this group, instead consisting of two FAs or one FA through ester linkages, respectively.

3.1.4 Phospholipids

As previously stated in section 3.1, lipids also exhibit biological importance as parts of the cell membrane, forming a continuous and spherical bilayer with amphiphilic properties due to the hydrophilic head and hydrophobe tails (Cevc 1993; Dewick 2009). Phospholipid is the general term employed for this group of lipids. The biosynthesis of phospholipids is achieved in a similar fashion to triglycerides, with glycerol-3-phosphate being twice esterified by FA-CoA residues, and an additional esterification of phosphate with an alcohol, such as choline (Dewick 2009). They may also contain a sphingosyl backbone, instead of glycerol (Devle 2013).

The main groups of phosphoglycerides present in animals are phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol (Tocher et al. 2008).

3.1.5 Free fatty acids

In contrast to TGs and phospholipids, free fatty acids are characterized by a lack of the glycerol or sphingosyl backbone through ester linkages. FFAs circulate in the blood through plasma, available for metabolism by the organism (Boden & Shulman 2002). Thus, they act as an energy source, but they also play an important role in signaling processes. Most notable of which is the secretion of insulin (Itoh et al. 2003).

3.1.6 Fatty acids and human health

Among the FAs, *n*-3 PUFAs have been the most extensively studied, and subsequently established as key to overall human health. They play an integral role in the function and development of the brain in infants and adults, and the *n*-3 FA DHA is present in major quantities in both the brain, and the nervous system (Horrocks & Yeo 1999; Ruxton et al. 2004). Deficiencies of DHA and *n*-3 PUFAs have been associated with several disorders and diseases, including cystic fibrosis, attention deficit hyperactivity disorder, unipolar depression, cardiovascular disease and autoimmune disease, among others (Horrocks & Yeo 1999; Siddiqui et al. 2004). Furthermore, both EPA and DHA have been linked to proper retinal and immune function, as well as hypotriglyceridemic and anti-inflammatory effects (Siriwardhana et al. 2012; Swanson et al. 2012). The latter is achieved by the inhibiting effect of *n*-3 FAs on the production of the proinflammatory prostaglandin E_2, a derivative of the *n*-6 FA AA produced through biosynthesis in organisms (Siriwardhana et al. 2012).

ALA and LA were coined as EFAs in section 1, meaning they cannot be readily biosynthesized by human beings, and are required to be included in the diet. ALA and LA are an *n*-3 and *n*-6 FA, respectively. Both ALA and LA are precursors to AA, which in turn is the precursor to both EPA and DHA. Therefore, the removal of dietary ALA has been linked to an overall deficiency of *n*-3 FAs, including DHA (Barceló-Coblijn & Murphy 2009). A diet rich in ALA, and with low contents of LA, have been linked to comparable levels of EPA in the tissue as diets supplemented by fish oil (Mantzioris et al. 1994). While LA is classified as an EFA, the eicosanoid derivatives from this FA have direct or indirect links to inflammation and metabolic diseases (Choque et al. 2014).

OA, a MUFA, reportedly has properties aiding in wound healing, as well as suggested beneficial effects on autoimmune and inflammatory diseases (Sales-Campos et al. 2013). It also exhibits properties of reversing the inhibitory effect of cytokines on insulin production, thus potentially resulting in beneficial effects in patients currently suffering from diabetes II by increasing levels of OA in the diet (Vassiliou et al. 2009).

In contrast to MUFAs and PUFAs, a high dietary intake of SFAs is associated with adverse health effects. Substitution of SFAs in the diet with PUFAs and MUFAs has been linked to an overall decrease in the risk of cardiovascular diseases (Siri-Tarino et al. 2015). The SFAs C12:0, C14:0 and C16:0 have been reported to have negative effects on human health (Devle 2013).

3.2 Insects as a source of nutrition

Insects have historically constituted an important part of the human diet as a delicacy, staple food or as an emergency resource in times of famine, and is regularly consumed on a daily basis throughout the world today (Bodenheimer 1951; Shockley & Dossey 2014). The consumption of insects is predominantly practiced in Asia, South America and Africa, but has in recent years experienced increased attention in Western countries as a potential substitute to animal proteins from traditional livestock, in large part due to the lower emissions of methane and N_2O associated with production of insects as food (Oonincx et al. 2010). A total of 2163 species of insects have been reported in the literature to be currently utilized globally for human consumption (Shockley & Dossey 2014).

Insects contain significant amounts of protein, and many species are reported to contain levels of over 60% of dry weight (DeFoliart 1992; Verkerk et al. 2007). The proteins found in insects tend to lack the sulphur containing amino acids methionine and cysteine, but are richer in lysine and threonine. Insects also display a beneficial nutritional profile in terms of the composition of essential amino acids, with contents ranging from 46-96% (Verkerk et al. 2007). However, the presence of chitin, which is the dominant constituent of the exoskeleton of insects, causes whole insects to be a lower quality source of proteins than traditional livestock (DeFoliart 1992). This is attributed to the lowered ability of humans to digest chitin.

The contents of FAs in insects relative to dry weight varies among species. The insect orders *Isoptera* and *Lepidoptera* rank amongst the highest in terms of total FA content (DeFoliart 1992). The FA compositions of the different insect orders are similar, but with significant quantitative differences of the most abundant FAs: C14:0, C16:0, C18:0, OA, LA and ALA (Stanley-Samuelson et al. 1988).

Table 2: The total lipid content, and the major FA constituent, in three *Orthopterans* and the larvae of *Tenebrio molitor**

Species:	% lipids of dry weight	Major FA
Acheta domesticus	15	LA
Chorthippus parallelus	10	ALA
Conocephalus discolor	13	LA
Tenebrio molitor larvae	32	OA

* Adapted from (Paul et al. 2017)

Table 2 displays the total FA content relative to dry weight of four different species, three of which belong to the order *Orthoptera*. *A. domesticus* and *C. discolor* are crickets, while *C. parallelus* is a grasshopper. *T. molitor*, however, belongs to the order *Coleoptera* and is a beetle. The EFA LA is the major constituent of the FAs in the two crickets, while the other EFA, ALA, is the most abundant FA in the meadow grasshopper *C. parallelus*. The nutritional compositions of several insect orders are compiled and displayed in **table 3**, including relative contents of proteins and fats to dry matter. Crystalline chitin, forming the fibrous phase of the cuticle, is the major source of fiber in insects (Vincent 2002).

Table 3: Contents of protein, fat and fiber in some of the insect orders. Displayed as percentages of dry matter*

Insect order	Protein [%]	Fat [%]	Fiber [%]
Blattodea (cockroaches)	57.30	29.90	5.31
Coleoptera (grubs, beetles)	40.69	33.40	10.74
Hemiptera (true bugs)	48.33	30.26	12.40
Hymenoptera (ants, bees)	46.47	25.09	5.71
Isoptera (termites)	35.34	32.74	5.06
Orthoptera (crickets, grasshoppers, locusts)	61.32	13.41	9.55

*Adapted from Rumpold and Schlüter (2013)

3.3 *Orthoptera*

The insect order *Orthoptera* includes grasshoppers, bush crickets, crickets, locusts, katydids, among others. It is further divided into two suborders: the long-horned *Ensifera*, and short-horned *Caelifera* (Field 2001). Bush crickets and crickets belong to the former, while grasshoppers and locusts belong to the latter. Approximately 27000 species belonging to *Orthoptera* have been documented. One of the key characteristics of many insects in this order, is the ability to produce sound by either rubbing together their wings or legs, referred to as stridulation. Characteristic songs have functioned as an important aid in differentiating between species (Perdeck 1958). Despite the presence of wings, powerful metathoracic legs make jumping and walking the preferred alternatives of locomotion for many species (Burns 1973). The species of the order *Orthoptera* have been estimated to account for 13% of the insects consumed worldwide (Van Huis et al. 2013).

3.3.1 *Tettigonia viridissima*

T. viridissima is a bush-cricket, belonging to the family *Tettigoniidae* (Arak et al. 1990). Their characteristic, long antennae are a distinguishing feature of species belonging to the suborder *Ensifera*, and may reach sizes several times that of the body length. The males and females are distinguished by the presence, or absence, of an ovipositor. The ovipositor is located directly behind the abdomen, specifically used for laying eggs, and exclusively found in females belonging to the species. Specimens typically appear as green. However, the organs responsible for stridulation in males appear brown. Although males and females exhibit differences in length, the size of the species typically ranges from 2.8 to 4.2 cm. Furthermore, *T. viridissima* is carnivorous, with a diet composed of smaller insects. Their habitat stretches from Europe to Mongolia in temperate Asia. The species is heavily represented in literature due to studies conducted on their adhesion pads, song and wing movements (Brackenbury 1990; Goodwyn et al. 2006; Gorb et al. 2000; Römer & Krusch 2000).

3.3.2 *Chorthippus biguttulus* and *Chorthippus brunneus*

While formerly established as a single species, *Stauroderus variabilis*, *C. biguttulus* and *C. brunneus* are presently identified as two separate species, in large part due to their different songs (Perdeck 1958; Ragge & Reynolds 1988). Both species belong to the family *Acrididae*, which in turn belongs to the suborder *Ensifera*, meaning that short antennae are a mutual characteristic in both *C. biguttulus* and *C. brunneus*. Both are very common species of field grasshoppers, and their combined range and habitat covers a majority of Europe, temperate Asia and parts of north Africa (Bellmann 1988). Their outward appearance is predominantly brown, and the size of adult males and females ranges from 1.5 to 2 cm. In contrast to *T. viridissima*, both species are herbivorous and feed on a diet exclusively composed of grasses. The species are considered to be in the early phase of species divergence, hence the focus of most published literature on their characteristic songs (Butlin et al. 1985; Safi et al. 2006; Von Helversen 1993).

4. Methodology

4.1 Lipid analysis

Quantitative analysis of lipids from biological matrices has changed over the decades. Several techniques utilizing thin layer chromatography (TLC) have previously been employed. The spots on the TLC plates were quantitatively analyzed by measuring a number of parameters such as spot size, reflectance and absorbance (Privett et al. 1965). More novel techniques have emerged during the last few decades for the analysis of lipids from a variety of biological matrices, including matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Schiller et al. 1999), and high-performance liquid chromatography employing a light scattering detector (HPLC-LSD) (Norlén et al. 1998). GC-FID and GC-MS, however, remain the more commonly employed analytical methods for lipid analysis. Both utilize a GC for separation, and require lipids to be derivatized into more volatile methyl esters prior to analysis.

4.1.1 Extraction of lipids

Extraction of lipids from biological matrices by use of solvents may not be discussed without addressing two of the most referenced studies in recent history: those of Bligh and Dyer (1959) and Folch et al. (1957). The latter introduced a simple method for isolating the total lipid content, by exposing animal tissue to a 2:1 chloroform and methanol (v/v) mixture, as well as water containing a mineral salt for liquid-liquid extraction. The combination of a polar and non-polar solvent is necessary to extract neutral lipids as well as polar lipids from the sample tissue (Devle 2013). Furthermore, the 2:1 chloroform and methanol extraction mixture is applicable to animal tissues with relatively low contents of lipids (Folch et al. 1957). In contrast, the method developed by Bligh and Dyer (1959) employed a 1:2 chloroform and methanol (v/v) mixture for rapid lipid extraction, and was initially developed for tissues such as fish muscle, which contains an abundance of water (~80%). However, the authors listed permissible adaptations of the method to different sample materials.

The method of Folch et al. (1957) has been utilized by a number of studies to elucidate the FA compositions of insects. Although erroneously referenced to as the Bligh and Dyer (1959) method, Yang et al. (2006) employed a 2:1 chloroform and methanol mixture to extract the total content of lipids from the species *Gryllotalpa africana*, *Acheta confirmata*, *Chondracris*

roseapbrunner, *Lethocerus indicus*, *Cybister limbatus* and *Hydrous cavistanum*. Paul et al. (2017) also performed a solvent extraction of lipids from the insects *A. domesticus*, *C. parallelus*, *C. discolor* and *T. molitor*, using a 2:1 chloroform and methanol mixture.

However, other methods are most certainly utilized. Solid-phase microextraction (SPME) has been used in a number of studies to sample lipids from insects, and is carried out by direct contact of the SPME fiber with the surface of the insect (Gołębiowski et al. 2011; Reitz et al. 2015). The nature of the sampling method limits the usability of SPME to adhesion secretions from insect feet, as well as the lipid fractions present in the outer layer of the exoskeleton. Thus, the use of solvent extraction is the more appropriate method for determining the total lipid content of insects.

Furthermore, it is imperative that the amount of sample exposed to solvent extraction is representative of the species as a whole, in order to obtain representative results and maintain acceptable precision across analyses. No guidelines or recommendations have been established for the initial amount of sample material to be used, and thus it is left to the judgement of each respective author. The problem is largely circumvented by the thorough homogenization of all the sample material, often by the traditional method of submerging the sample tissue in liquid nitrogen, with a subsequent homogenization by using mortar and pestle. The method is referred to as cryopulverization, and serves a dual purpose. The presence of water in the sample tissue will make it brittle upon contact with liquid nitrogen, thus resulting in a comparatively easier pulverization procedure. Additionally, cells become disrupted, releasing lipids contained within and the lipid constituents of the membranes (Burden 2008). The only limitations attributed to this method is the potential loss of small sample amounts, and a limited capacity to process larger numbers of samples (Burden 2008). The tough exoskeletons of insects, composed of crystalline chitin, renders cryopulverization a particularly useful method for making all the lipids present available for extraction by solvents.

4.1.2 Transesterification procedure

As previously stated in section 4, FAs are required to be derivatized into FA methyl esters (FAMEs) prior to analysis by GC-MS, due to their initial, limited volatility (Devle 2013). The common approach is a nucleophilic addition in the presence of an acid or alkaline catalyst, resulting in the elimination of the alcohol group in FAs (Hart et al. 2011). Using an alkaline catalyst, sodium methoxide, is the most widespread method for acylglycerides, resulting in a rapid transesterification where the glycerol unit is replaced through methanolysis (Christie 2011). The TGs are completely transesterified in a matter of minutes at room temperature (Eder 1995). The mild conditions of this method prevent any undesirable reactions, such as isomerization of double bonds in MUFAs and PUFAs (Christie 2011). Additionally, the reagent is also applicable to phosphoglycerides, due to the presence of glycerol.

Morrison and Smith (1964) developed a simple method for the transesterification of numerous classes of lipids by the use of an acid catalyst, boron-trifluoride. This method results in very few undesirable reactions, and may be used for PLs and FFAs, resulting in quantitative yields (Morrison & Smith 1964). Additional heating is required for the complete reaction to take place.

4.1.3 Solid-phase extraction

Solid-phase extraction (SPE) may be performed on-line or off-line, and is generally considered to be amongst the most popular sample preparation methods employed in analytical chemistry (Fritz et al. 1995; Hennion 1999; Mitra 2004). With on-line SPE, the sample preparation method is directly connected to the chromatographic system used for analysis, and requires no further treatment of the samples (Hennion 1999). The use of off-line SPE, however, entails further handling of samples prior to analysis.

SPE utilizes the principles of retention and elution, based on the affinity of the analyte to either the stationary phase or the mobile phase (Simpson 2000). The stationary phase in SPE is a solid material, acting as a sorbent, and *n*-alkylsilica has traditionally been employed as the universal SPE sorbent, available in disposable cartridges (Hennion 1999). However, the analyte of interest dictates the choice of the sorbent material.

With SPE, the sample matrix containing the analytes is transferred to the column with the sorbent, oftentimes subsequently to a washing/pre-conditioning of the sorbent material by an

appropriate solvent (heptane or hexane) to equilibrate (Grapes et al. 1989). The analytes are retained in the solid phase through either adsorption, or penetration of the outer layer of the solid surface (Simpson 2000). An analyte is eluted from the solid phase by the introduction of a suitable solvent, of which the analyte has a greater affinity to than the sorbent material. Thus, possibly interfering compounds are left in the column (Mitra 2004). A graphical representation of this process is presented in **figure 1**. This relatively simple method may also be used to separate different classes of lipids into multiple fractions, by using several different solvents as mobile phases.

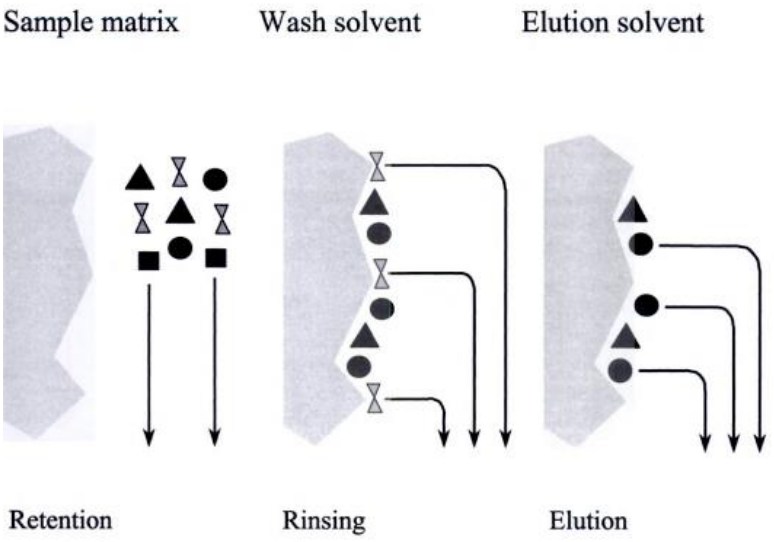

Figure 1: The basic principles of SPE, highlighting the retention of analyte molecules in the sorbent, and elution by the use of a solvent as a mobile phase. An additional rinsing phase is displayed in the middle. From Simpson (2000).

Grapes et al. (1989) successfully employed off-line SPE as a method to fractionate the lipids in the cricket *A. domesticus* into NLs, PLs and FFAs. In their study, Bond-Elut NH_2 columns were conditioned using hexane, prior to the transfer of the sample solutions. NLs were eluted by a 2:1 chloroform and propanol solution, PLs by methanol, and FFAs by a 98:2 diethyl ether and acetic acid (v/v) solution.

4.1.4 Gas chromatography – mass spectrometry

The identification, and quantitation, of analytes represents the final step after any given sample preparation in analytical chemistry. A complex sample mixture containing several different analytes requires the ability of an instrument to separate these compounds to such a degree that all constituents in the sample matrix may be identified. In the world of lipid research, gas chromatography (GC) has become amongst the most utilized methods to ensure the separation of fatty acids in complex mixtures, and for the subsequent quantitative analysis (Dodds et al. 2005; Quehenberger et al. 2011). FID and MS are the most commonly employed detectors, and both are directly coupled with the GC.

The basic principles of GC are similar to the basic principles of SPE. FAMEs are vaporized upon injection, and carried through a column using an inert gas as a mobile phase (most commonly helium). Interactions between the compounds and the column, the stationary phase, directly affects the time of elution of each specific compound, thus resulting in separation. Fused-silica capillary columns have become the most commonly utilized stationary phases for GC, owing to their improved high resolution capacity over packed columns (Eder 1995). However, the use of silica-fused capillary columns facilitates a need for detectors with a higher response, and sensitivity (Eder 1995). The coupling of a MS detector with a GC offers the best solution to this problem, because MS detectors are significantly more sensitive than their FID counterparts (Devle 2013).

MS detectors also offer several other benefits compared to the use of FID. Chief among which is the ability to obtain spectrometric data, including molecular mass and structural information of the FAMEs (Dodds et al. 2005). In contrast, FID relies solely on the comparison of retention times between an analyte, and its respective reference standard (Devle 2013).

5. Key results and discussion

The overall aims of this study are highlighted in section 2. The complete FA profiles of *T. viridissima*, *C. biguttulus* and *C. brunneus* were to be elucidated and quantitated, with an additional fractioning of the lipids in *T. viridissima* into three fractions. These aims were the basis for **paper 1**. To identify and quantitate FAs present in potentially very low concentrations, a highly selective and sensitive analytical instrument had to be employed. A GC coupled with a MS detector was chosen for this purpose, thus allowing for the detection of FAs present in the samples in low concentrations, whom had no representative reference standards. The reference standards used for the identification process are listed in **appendix II**. The MS employed had an EBE geometry (electrostatic-magnetic-electrostatic sectors).

The method validation for the GC-MS as an analytical method for the identification and quantitation of FAMEs, was carried out by Devle et al. (2009) several years prior to this study. LOD, LOQ, linearity, sensitivity, selectivity, accuracy and repeatability were among the analytical parameters subjected to testing in their study, using three acquisition modes: full scan, RIC and SIM. A mix containing 38 FAMEs were utilized for the method validation, as well as derivatized FAs from milk samples. Satisfying results were reported for all analytical parameters, and values for both LOD and LOQ were in the ng/mL range across all three acquisition modes (Devle 2013).

Quantitation of each respective FA in *T. viridissima* and *Chorthippus* samples required the use of RRF-values, and equation 1 displayed below.

(Eq. 1) $$Mass_{FAME} = \left(\frac{Area_{FAME} * C_{mole\ IS}}{Area_{IS} * RRF}\right) * Molecular\ weight$$

The RRF-values used for the quantitation of FAMEs were obtained through the previous work of Devle et al. (2009)**,** in which four concentrations of 150, 300, 600 and 1200 µg/mL of Restek Food Industry FAME Mix were made by diluting with hexane. Duplicates of each concentration were subjected to analysis by GC-MS, as well as two injection replicates of each concentration (Devle et al. 2009). The RRF-value of each FAME may be found in **appendix III**. FAMEs not represented in the Food Industry FAME Mix had to be assigned reasonable RRF-values. Examples include MUFAs such as C18:1*n*-7c and C16:1*n*-9t, which were assigned the same RRF-values as C18:1*n*-9c and C16:1*n*-7c, respectively. All BCFAs

were assigned values corresponding to their longer-chained counterparts, e.g. 10-methyldodecanoic acid was assigned the same RRF-value as C13:0. Furthermore, C26:0 was assigned the same value as C24:0, and C16:2*n*-6t the same as C16:1*n*-7c. C19:1*n*-9c was assigned a value of 1, corresponding to the value of the C19:0 internal standard. While the assignment of RRF-values to selected FAMEs contributes to an increased degree of inaccuracy in regard to the quantitated concentrations, FAMEs with previously uncalculated RRF-values constitute but a minor fraction of the total lipid content of each respective species.

A total of five different internal standards (IS) were utilized for the quantitation of FAMEs. The concentrations and volumes of the internal standards are displayed in **appendix I**. For the quantitation of the complete FA profile of both species, C19:0 TG and C11:0 TG internal standards were used. These two internal standards were also utilized for the NL fraction. C19:0 PL IS was utilized for all FAMEs in the PL fraction. C19:0 FFA and C11:0 FFA internal standards were utilized for the FFA fraction. C11:0 internal standards were used for the quantitation of short-, and medium-chained FAMEs (C10:0-C16:0). C19:0 internal standards were used for all longer-chained FAMEs, C15:0, MUFAs and PUFAs. C19:0 PL IS, however, was used for all FAMEs in the PL fraction.

The method of using a GC coupled with a sector MS detector resulted in the satisfactory separation, and subsequent quantitation, of 37 FAs in *T. viridissima* and 33 FAs in *Chorthippus*. The selectivity and sensitivity of the method, coupled with the use of full scan acquisition, aided in the identification and quantitation of several FAs present in low concentrations. The use of full scan acquisition resulted in a plot of the total ion current (TIC). The plot yields a conventional chromatogram diagram, where each peak is plotted as the relative intensity of acquired mass signals against time (Hübschmann 2015). The spectral information of some of the smaller peaks, FAMEs present in low concentrations, could thus be subjected to library searches in NIST 08 to confirm their identities. Although SIM and RIC offer better specificity and sensitivity by scanning for pre-determined ions (Devle et al. 2009; Jorge et al. 2007), full scan acquisition was considered the more suitable alternative for the routine analysis of FAs in *T. viridissima* and *Chorthippus*. Additionally, the main advantage of the full scan acquisition, as opposed to SIM, is the ability to identify FAMEs through the spectral information and library searches. Two TIC plots are presented in **figure 2** and **figure 3**. Both serve as examples for plots yielded after analysis of replicates to acquire the complete FA profiles of *T. viridissima* and *Chorthippus*.

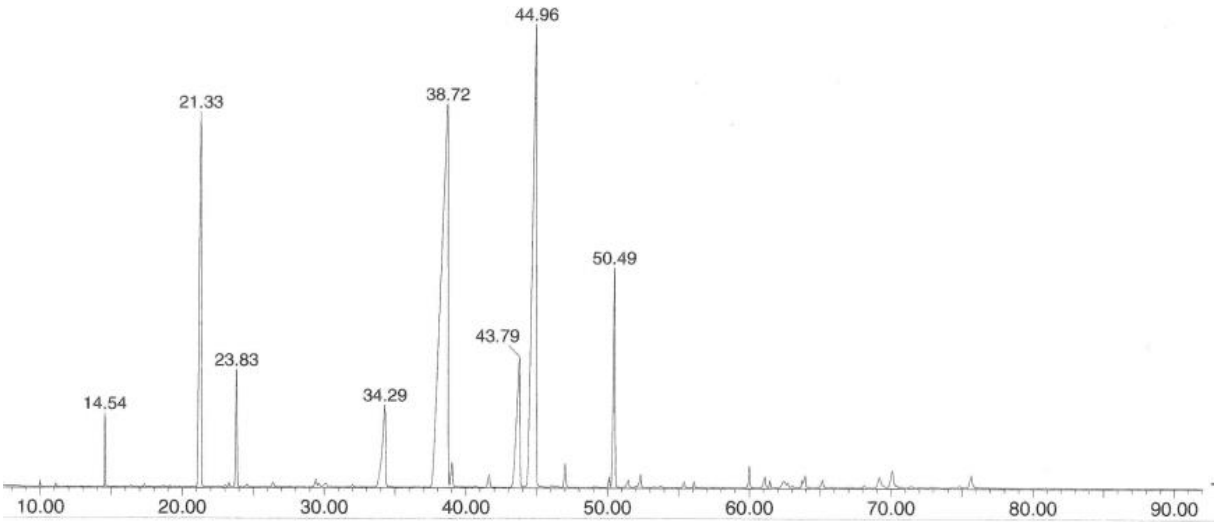

Figure 2: The TIC plot from a *T. viridissima* replicate for the elucidation of the complete FA profile. The peaks of the solvent, heptane, have been removed. The relative intensities of the peaks (y-axis) are plotted against time (x-axis). The numbers above the peaks denote the time of elution from the GC column.

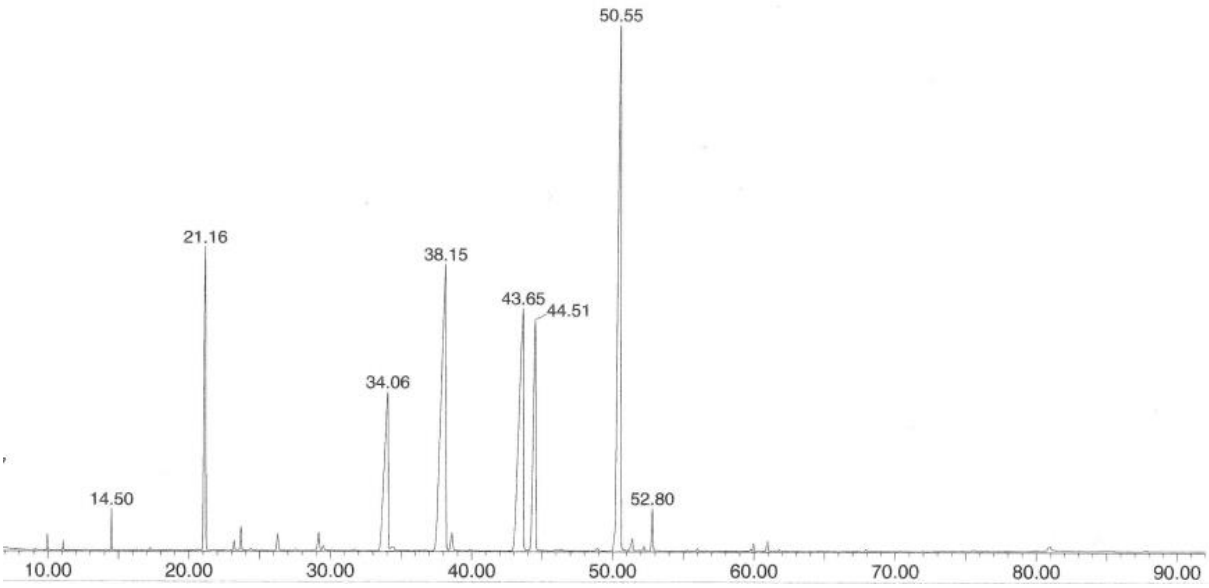

Figure 3: The TIC plot from a *Chorthippus* replicate for the elucidation of the complete FA profile. The peaks of the solvent, heptane, have been removed. The relative intensities of the peaks (y-axis) are plotted against time (x-axis). The numbers above the peaks denote the time of elution from the GC column.

Although the analytical method chosen yielded a satisfactory separation of the derivatized FA components in the samples of both species, possible coelution of components might have occurred. The presence of broad peaks can be observed in both **figure 2** and **figure 3**. The issue is attributed to the initial amount of sample material prior to solvent extraction, as well as analyses of undiluted replicates. Some alkanes are likely to have coeluted with FAs present in significant concentrations. Furthermore, the SFA C20:0 in *Chorthippus* replicates is likely to have eluded detection due to coelution with C18:3*n*-3c. Analysis of diluted replicates, or implementing RIC acquisition mode, could possibly determine its presence. However, analyzing undiluted replicates was deemed necessary to acquire the complete FA compositions of both species.

A total of 19 FAs was identified in the NL fraction, 16 FAs in the FFA fraction, and 6 FAs in the PL fraction for *T. viridissima*. Representative TIC plots for the three fractions are shown in **figure 4**, **figure 5** and **figure 6**, respectively. The lower amount of initial sample size prior to solvent extraction, as well as fractioning of the lipids by off-line SPE, resulted in narrower peaks with a decreased risk of coelution. Several more FAs could potentially have been identified in all three fractions by increasing the initial amount of sample material, albeit in very low concentrations, and potentially below LOQ.

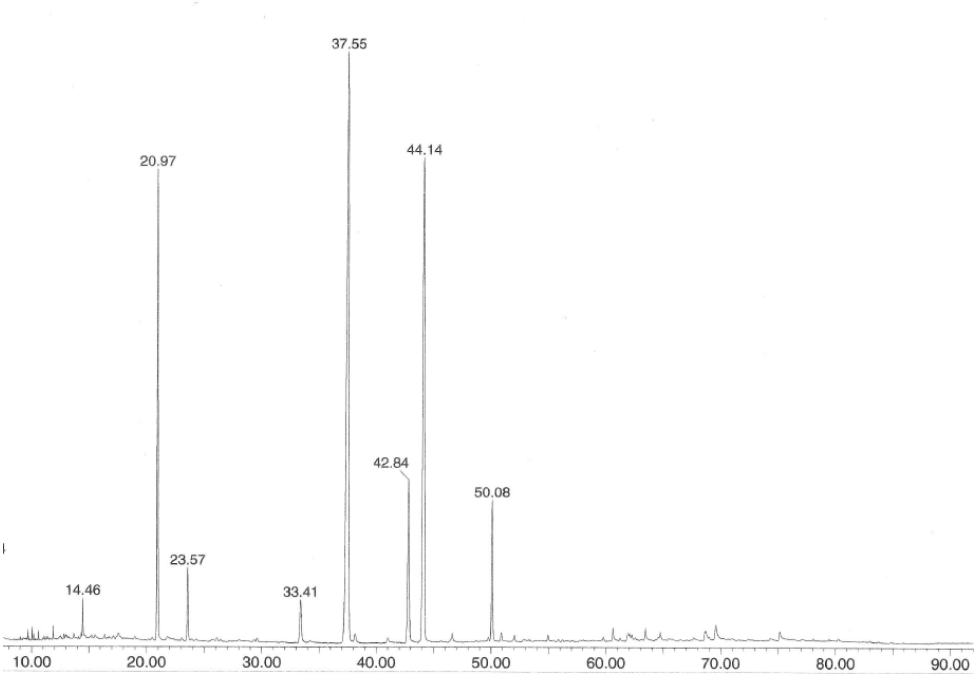

Figure 4: TIC plot for a NL fraction replicate. The peaks of the heptane solvent have been removed. The relative intensities of the peaks (y-axis) are plotted against time (x-axis). Numbers above peaks denote time of elution.

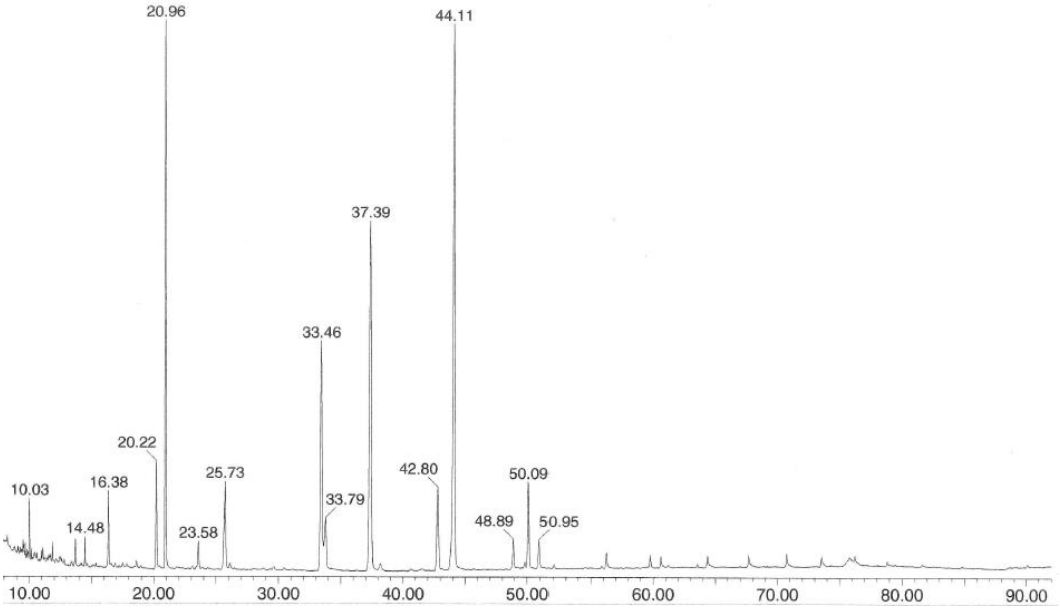

Figure 5: TIC plot for a FFA fraction replicate. The peaks of the heptane solvent have been removed. The relative intensities of the peaks (y-axis) are plotted against time (x-axis). Numbers above peaks denote time of elution.

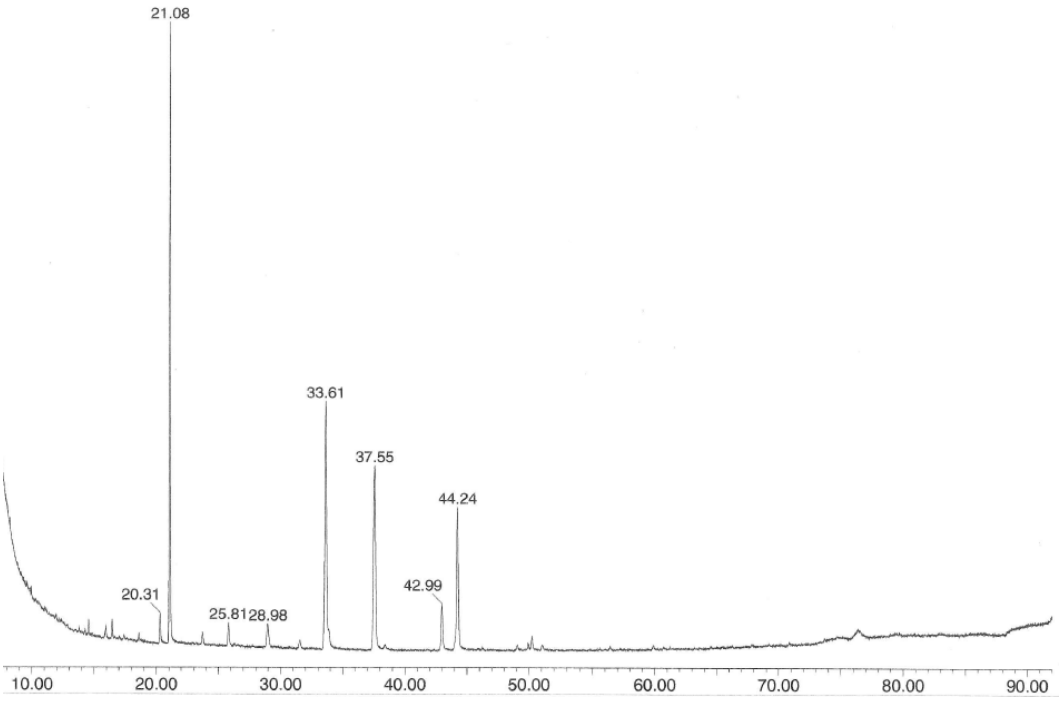

Figure 6: TIC plot for a PL fraction replicate. The peaks of the heptane solvent have been removed. The relative intensities of the peaks (y-axis) are plotted against time (x-axis). Numbers above peaks denote time of elution.

As previously explored in section 3.2, proteins and lipids are the major constituents of insects along with fiber (chitin). Thus, the amino acid profile of the proteins, and the FA constituents of the lipid profile are the most likely to affect human health through the consumption of insects. Additionally, the extraction and utilization of proteins and FAs from insects may become commonplace in Western societies in the future (Van Huis 2013). Therefore, this study sought to determine and quantitate the FA compositions of the carnivorous bush cricket *T. viridissima*, and the herbivorous grasshoppers *C. biguttulus* and *C. brunneus*. SFAs, MUFAs, PUFAs, *n*-3 FAs, *n*-6 FAs, EFAs and the *n*-6/*n*-3 ratio were the focus of the study to evaluate the potential health benefits from consuming these insects from purely a FA composition point of view. The results are discussed at length and are the focus of **paper 1**. The average concentration of each FA, along with standard deviation, for each sample preparation may be found in **appendix IV** and **appendix V**. Retention times and areas are also included in these appendices, along with values for matchfactor, reverse matchfactor and probabilities acquired through library searches in NIST 08 based on spectral information. **Table 4**, displayed below, highlights the quantitative differences found in SFAs, MUFAs, PUFAs, *n*-6 and *n*-3 FAs for *T. viridissima* and *Chorthippus*.

Table 4: Concentrations of selected FA classes relative to 1 g of sample dry weight, for *T. viridissima* and *Chorthippus*.

	Average ± S.D (mg/g d.w.)	
FA class	*T. viridissima*	*Chorthippus*
SFAs	32.33 ± 2.90	20.13 ± 1.68
MUFAs	37.32 ± 0.46	15.44 ± 0.40
PUFAs	34.33 ± 0.41	25.88 ± 0.37
n-6 FAs	28.36 ± 0.39	6.86 ± 0.08
n-3 FAs	5.96 ± 0.14	19.02 ± 0.34
Total lipid content	104.0 ± 3.0	61.45 ± 1.76

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids

T. viridissima was found to exhibit a total FA content of 10.4% of dry weight. The corresponding value for *Chorthippus* was 6.14%. OA was found to be by far the most abundant FA in the bush cricket *T. viridissima* (32.8%), while the EFA ALA was the most abundant in *Chorthippus* (30.7%). The high abundance of ALA in *Chorthippus* was concluded to be reflective of the herbivorous diet of the two species *C. biguttulus* and *C. brunneus*, adhering to the similar conclusion of a contemporary study performed by Paul et al. (2017) on *C. parallelus*. The FAs C14:0, C16:0 and C18:0 were the largest contributors to the total SFA content of both *T. viridissima* and *Chorthippus*. The other EFA, LA, accounted for 26.6 and 11.1% in *T. viridissima* and *Chorthippus*, respectively. DHA was not detected in either species, and only small contents of the FAs EPA and AA were detected, and quantitated, in *T. viridissima*.

Section 3.2 highlighted the fact that the FAs C14:0, C16:0, C18:0, C18:1*n*-9c, C18:2*n*-6c and C18:3*n*-3c accounted for the majority of lipids across all insect orders (Stanley-Samuelson et al. 1988). These six FAs accounted for 95.6 and 96.1% of the lipids present in *T. viridissima* and *Chorthippus*, thus adhering to the claim.

Chorthippus displayed the more favorable *n*-6/*n*-3 ratio of the two (0.36). While no ratio is universally agreed upon by professionals (FAO 2010), dietary ratios below 5/1 seem to offer some health benefits (Simopoulos 2002; Yang et al. 2016). The *n*-6/*n*-3 ratio for *T. viridissima* was 4.7. It is important to note that health benefits associated with increased *n*-3 intakes remain a controversial subject, and may not actually contribute to a lower overall mortality, as claimed by Simopoulos (2002) (Rizos et al. 2012). However, both *T. viridissima* and *Chorthippus* contained high proportions of MUFAs and PUFAs relative to total lipid content, and significant amounts of the EFAs ALA and LA, as well as OA. The potential health benefits associated with increased intake of these FAs were examined in section 3.1.6.

Furthermore, the total FA contents for both species adhered to values reported for similar species in the literature (Paul et al. 2017; Yang et al. 2006), thus confirming the plausibility of the results gathered in this study. The precision was also deemed acceptable, based on the standard deviations, and consistent with uncertainties reported in the similar studies of Yang et al. (2006) and Paul et al. (2017).

Table 5: Concentrations of selected FA classes relative to 1 g of sample dry weight, for neutral lipids, free fatty acids and polar lipids in *T. viridissima*.

	Average ± S.D (mg/g d.w.)		
FA class	NLs	FFAs	PLs
SFAs	24.33 ± 2.58	10.74 ± 0.80	6.61 ± 0.35
MUFAs	24.77 ± 0.62	8.17 ± 0.55	2.53 ± 0.12
PUFAs	16.77 ± 0.31	12.55 ± 0.69	2.07 ± 0.24
n-6 FAs	13.80 ± 0.28	11.15 ± 0.69	1.92 ± 0.24
n-3 FAs	2.96 ± 0.12	1.39 ± 0.09	0.14 ± 0.01
Total FA content	65.87 ± 2.67	31.47 ± 1.19	11.21 ± 0.48

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids

Additionally, the lipids from *T. viridissima* were successfully fractioned, using off-line SPE, into neutral lipids, free fatty acids and polar lipids. The total FA contents of each fraction are displayed in **table 5**. Furthermore, the results following each sample preparation are listed in **appendix VI**, **appendix VII** and **appendix VIII**. The method yielded a total FA content of 10.8% of dry weight for *T. viridissima*. This value is consistent with the value reported for *T. viridissima* following the method for elucidation of the complete FA profile. As expected, the storage lipids comprising the NL fraction contributed the highest FA concentration. Phospholipids, belonging to the PL fraction, are key constituents of the cell membranes and were expected to yield the lowest concentrations. The precision was also found to be satisfactory, thus demonstrating the potential usefulness of off-line SPE in future studies of insect FAs. At the time of this study, the employment of SPE to fractionate insect lipids appears limited in the literature, with the notable exception of Grapes et al. (1989).

6. Conclusions and further work

The in-house developed and validated method of employing GC-MS for the analysis of derivatized FAs was found to yield satisfactory results for lipids extracted from insects. A total of 37 FAs was identified for *T. viridissima*, and 33 FAs were identified for *Chorthippus*. Five internal standards, and previously determined RRF-values, allowed for the quantitation of all FAs present in both species. *T. viridissima* was found to exhibit a total FA content of 10.4% of dry weight, while the corresponding value for *Chorthippus* was found to be 6.14%. Additionally, the total FA content of *T. viridissima* was found to be 10.8% following the fractioning of the lipids into three fractions by SPE. Both species were rich in MUFAs and PUFAs, as well as the two EFAs LA and ALA. *Chorthippus* was especially rich in the latter, possibly as a result of the herbivorous diet, and thus also displayed the most favorable *n*-6/*n*-3 ratio of the two species. Both *T. viridissima* and *Chorthippus* contained roughly equal amounts of SFAs relative to the total FA content, 31.1 and 32.7%, respectively. *Chorthippus*, however, was far richer in PUFAs (42.1%) than *T. viridissima* (33.0%). MUFAs constituted 35.9% of the FAs in *T. viridissima*, and 25.1% of the FAs in *Chorthippus*. C14:0, C16:0, C18:0, C18:1*n*-9c, C18:2*n*-6c and C18:3*n*-3c were by far the most abundant FAs, accounting for over 90% of the total FA contents in both species. Inter-species differences related to MUFA and PUFA contents were thus heavily related to the overall quantitative presence of C18:1*n*-9c, C18:2*n*-6c and C18:3*n*-3c. The intake of *n*-3 FAs, and the possible health benefits, remain a subject of great debate and form the basis of many clinical trials. Nevertheless, the contents of *n*-3 FAs and the *n*-6/*n*-3 ratio were utilized for the discussion of the two species as human food. From purely a FA composition point of view, both species displayed nutritionally beneficial profiles. The significantly higher contents of the *n*-3 FA ALA in *Chorthippus* suggested it displayed the most beneficial FA composition.

However, further studies are needed to conclusively mark both species as safe for human consumption. Employing ICP-MS for the detection of heavy metals potentially present would be greatly beneficial to partly achieve this goal. Rearing *Chorthippus* in strictly controlled environments, and with differing feeding regiments, could also yield interesting results in future lipid studies as to how reflective the FA composition is of the feed.

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Paper I

Identification and quantification of lipids in *T. viridissima, C. biguttulus* and *C. brunneus* by GC-MS

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Abstract:

The complete fatty acid (FA) profiles of the species *Tettigonia viridissima*, *Chorthippus biguttulus* and *Chorthippus brunneus* were determined and quantitated. Extracted lipids were derivatized into FA methyl esters prior to analysis by GC-MS. A total of 37 different FAs was identified in *T. viridissima*, yielding a total FA content of 10.4%/g dry weight. The contents of saturated FAs, monounsaturated FAs, and polyunsaturated FAs were 31.1, 35.9 and 33.0%, respectively. Lipids from *T. viridissima* were also fractioned into neutral lipids, free fatty acids and polar lipids by off-line SPE. For *C. brunneus* and *C. biguttulus*, 33 FAs were identified, yielding a total FA content of 6.14%/g dry weight. SFAs, MUFAs and PUFAs respectively constituted 32.7, 25.1 and 42.1% of the total FA content. The contents of MUFAs, PUFAs, *n*-3 FAs, *n*-6 FAs of each species, and the *n*-6/*n*-3 ratio, were subsequently discussed to evaluate the potential of the three species for human consumption.

Keywords: Fatty acid methyl esters, GC-MS, lipids, *Orthoptera*, nutrition

Abbreviations: FA, fatty acid; **IS**, internal standard; **NL**, neutral lipid; **PL**, polar lipid; **FFA**, free fatty acid; **FAME**, fatty acid methyl ester

1. Introduction

As the world's population surges towards a total of 9 billion people by the middle of the 21st century, the increased global demand for food will inevitably follow. A higher consumption of beef, fish and poultry will be facilitated by the higher purchasing power of the emerging middle class in developing countries, resulting in an increased pressure on the food supply system (Godfray et al. 2010). And even today, roughly 800 million people still experience hunger around the globe, either chronically or transitionally (Borlaug 2007). In order to face the daunting task of feeding a growing population, and those currently lacking in basic nutrition, novel and more efficient foods will have to be studied and consequently utilized for human consumption on an industrial scale. In both the developed and developing world. Historically, insects have played an important part in human nutrition outside of Europe in areas such Asia, Africa and South-America, functioning as a nutritionally viable alternative to meats and fish due to the high contents of proteins and fats (DeFoliart 1992). Insects also provide important micronutrients such as calcium, iron and zinc (Van Huis et al. 2013). Approximately 13% of the insects consumed globally belong to the order *Orthoptera*, which includes grasshoppers, crickets and locusts (Van Huis et al. 2013).

Among the several groups of natural products are lipids, which can be broadly defined as a heterogenous group of substances that are insoluble in water and contain alkyl chains within their molecular structures. Thus, making them readily soluble in organic solvents such as chloroform, diethyl ether and heptane (Akoh & Min 2008). Major constituents of the lipid group include fatty acids (FAs) and their derivatives, such as prostaglandins, thromboxanes and leukotrienes (Dewick 2009). Free fatty acids (FFAs), triacylglycerides and phospholipids are of interest in the work of identifying and quantitating the total amount of lipids in biological matrices. Triacylglycerides, storage lipids, contain a glycerol 'backbone' unit with three FAs through ester linkages, and are most commonly referred to as fats and oils.

Phospholipids are constituents of the cell membranes in animals and plants, forming bilayers, and generally consist of two FA chains and a phosphate group joined by a glycerol unit (Cevc 1993). FFAs, also known as non-esterified fatty acids due to the absence of a glycerol 'backbone', are FAs found in the plasma available for metabolism by the organism (Gordon 1960).

Essential fatty acids, EFAs, are defined as FAs that are essential to growth and development, and crucial in preventing diseases such as: diabetes, coronary artery disease, arthritis, and several inflammatory and autoimmune disorders (Simopoulos 1999). However, these EFAs must be supplied through the diet, as they are not readily synthesized by the human body. Linoleic acid (LA) and α-linolenic acid (ALA), an *n*-6 and *n*-3 FA, respectively, have been identified as the two EFAs required to be included in the diet (FAO 2010). They are precursors to the *n*-6 FA arachidonic acid (AA), and the *n*-3 FAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), all of which are produced through biosynthesis by elongase and desaturase enzymes (Dewick 2009). DHA and EPA have been associated with proper retinal and immune function, and are present in significant quantities in both the brain and the nervous system (Ruxton et al. 2004; Siriwardhana et al. 2012; Swanson et al. 2012). Deficiencies of EPA and DHA have been linked to several diseases and disorders (Horrocks & Yeo 1999; Siddiqui et al. 2004). Furthermore, low levels of dietary ALA has been associated with overall deficiencies of both DHA and EPA (Barceló-Coblijn & Murphy 2009).

The intake ratio of *n*-6 to *n*-3 FAs through diet has also been reported to be of significance in overall health (Liu et al. 2013; Riediger et al. 2008; Russo 2009; Yang et al. 2016). A ratio of ~1 has been highlighted as an optimal balance when compared to the diet throughout the evolution of human beings. Western societies in particular have a high intake of *n*-6 FAs compared to *n*-3 FAs, with ratios ranging from 15/1 to 17/1, according to Simopoulos (2002),

93 resulting in promotion of cardiovascular diseases, as well as inflammatory and autoimmune
94 diseases.

95 Buszewska-Forajta et al. (2014) focused on the lipid fraction constituents from *Chorthippus*
96 *parallelus* abdominal secretion using GC-MS/MS, scanning for bioactive compounds
97 responsible for accelerating wound healing. Vötsch et al. (2002) made a first attempt to
98 identify the chemical composition of the smooth pads of *Locusta migratoria*, responsible for
99 adhesion to surfaces. Additionally, there has been several studies dedicated to the cuticular
100 lipids of species belonging to the order *Orthoptera*, including the work of Jackson (1981) and
101 Gibbs and Mousseau (1994). Yang et al. (2006) carried out analyses of the total lipid content
102 and PUFA composition of six species of insects, including the three species of cricket
103 *Gryllotalpa africana, Acheta confirmata* and *Chondracris roseapbrunner* by GC-FID. Paul et
104 al. (2017) obtained the FA profiles of three *Orthopterans*, also by GC-FID.

105 The objectives of this study were to identify and quantitate all the FAs present in the
106 herbivorous grasshopper species *Chorthippus brunneus* and *Chorthippus biguttulus*, as well
107 as the carnivorous bush cricket *Tettigonia viridissima*. All three species belong to the
108 *Orthoptera* order, and are commonly found in Scandinavia, continental Europe and temperate
109 Asia. Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated
110 fatty acids (PUFAs), *n*-3 and *n*-6 FAs were the focus of the study, as well as determining the
111 *n*-3/*n*-6 ratio, to evaluate the potential health benefits by incorporating these insects into the
112 diet.

113 To the knowledge of the authors, the work presented in this article is the first study conducted
114 to elucidate and quantitate the complete FA profiles of the species *T. viridissima, C.*
115 *biguttulus* and *C. brunneus*.

116

2. Materials and methods

2.1 Chemicals and standards

The chloroform used for lipid extraction from the sample tissues, and internal standard (IS) stock solutions, was supplied by VWR Chemicals and of Chromanorm quality (France). Methanol, used in conjunction with chloroform for the extraction procedure and to make the sodium methoxide solution, was supplied by Sigma Aldrich and of Chromasolv quality (France). The 10% (~1,3 M) boron-trifluoride-methanol solution used for transesterification of the lipids to FAMEs was supplied by Sigma-Aldrich (Switzerland). Heptane ≥99% n-heptane basis (GC) was supplied by Sigma-Aldrich (Israel). Acetic acid and diethyl ether was used in combination as a mobile phase for the elution of FFAs by off-line solid-phase extraction (SPE). The acetic acid 96% puriss. p.a was supplied by Riedel-de Haën (Germany) and the diethyl ether puriss. p.a. ≥99,8% was supplied by Sigma Aldrich (Poland).

A total of five internal standards were chosen for quantitation of the FAMEs. They were all supplied by Larodan AB (Malmö, Sweden): undecanoic acid (C11:0 FFA), triundecanoin (C11:0 TG), nonadecanoic acid (C19:0 FFA), trinonadecanoin (C19:0 TG), 1,2-Dinonadecanoyl-sn-Glycero-3-phosphatidylcholine (C19:0 PL). The IS stock solutions were all prepared by dissolving 10 mg of standard with 10 mL of chloroform to a final concentration of 1 mg/mL, with the exception of C11:0 TG, which was prepared by dissolving 1 mg of standard with 10 mL of chloroform to a final concentration of 100 µg/mL. Furthermore, the C19:0 PL standard was dissolved in a 90:10 (v/v) mixture of chloroform and methanol, respectively, to maximize solubility. All IS stock solutions were transferred to GC sample vials, sealed, and stored in darkness at -20 °C until use.

To identify the fatty acid methyl esters (FAMEs) resulting from the derivatization of FAs from *T. viridissima, C. brunneus* and *C. biguttulus*, a FAME mix containing 37 different

141 FAMEs was chosen. The Food Industry FAME Mix was supplied by Restek (Bellefonte, PA,
142 USA) and had a total concentration of 30 mg/mL.

143

144 **2.2 Samples and sample preparation**

145 Wild individuals belonging to the species *T. viridissima*, *C. biguttulus* and *C. brunneus* were
146 all collected from the Asker area, Norway, during the period of June 2016 until October
147 2016, by using traps. Individuals were macroscopically identified shortly after collection.
148 Males and females are both represented within each species. No cleaning or treatment was
149 carried out on any of the individuals after collection from the traps and subsequent storage at
150 -20 °C.

151 To prepare the insects for lipid extraction and analyses, the specimens were homogenized
152 using liquid nitrogen and cryopulverization. The homogenized sample material was then
153 subjected to freeze-drying for 72 hours to remove all traces of water. Subsequently, the
154 homogenized and dried samples were then kept in the dark at -20 °C. Specimens of both *C.*
155 *biguttulus* and *C. brunneus* were homogenized together, resulting in a sample mixture
156 containing both species. *C. biguttulus* and *C. brunneus* are typical sibling species, and are
157 usually distinguished by their characteristic songs, but difficult to distinguish
158 morphologically (Perdeck 1958; Ragge & Reynolds 1988). Grouping the species together
159 were thus done out of necessity. Henceforth, for the sake of simplicity, *C. biguttulus* and *C.*
160 *brunneus* are referred to by their shared genus name: *Chorthippus*.

161

162

163

2.3 Lipid extraction procedure and transesterification for complete FA profile

For *T. viridissima*, the following amounts of sample material was weighed out for three consecutive sample preparations: 503 mg, 509 mg and 508 mg, respectively. For *Chorthippus*, 252 mg and 251 mg were weighed out for two simultaneous sample preparations.

30 mL of a 2:1 chloroform and methanol (v/v) solution (Folch et al. 1957), henceforth referred to as Folch's extraction mixture, was transferred to a 100 mL Pyrex reagent bottle with a screw cap, along with 3.7 mL of C19:0 TG IS and 600 µL of C11:0 TG IS solutions, the latter two by utilizing Hamilton syringes. The homogenized *T. viridissima* sample was then added to the bottle, with subsequent shaking on 450 rpm for 60 minutes in a horizontal position. The contents of the Pyrex bottle were then transferred to a separatory funnel through a porcelain Büchner filter funnel to retain larger pieces of sample material, and 6 mL 0.1 M NaCl in milli-Q water was added. 10 mL of Folch's extraction mixture was used to wash the Pyrex bottle. The lower phase was decanted off in a glass beaker after shaking the separatory funnel vigorously until separation of the two phases. Two additional liquid-liquid extractions were carried out with 4 mL chloroform and collected in the same glass beaker.

The organic phase was then distributed equally to nine 6 mL Duran® GL 14 culture tubes, and the glass beaker washed with chloroform before transfer to the glass tubes. Removal of the solvent was carried out by inserting the glass tubes in heating blocks at 40 °C in a pure nitrogen atmosphere until a dry residue remained. 1 mL of heptane was then added to each tube.

A sodium methoxide solution was prepared by dissolving metallic sodium, supplied by Merck (Darmstadt, Germany), in methanol to a concentration of 5 mg/mL. 1 mL of the sodium methoxide stock solution was added to each of the glass tubes, with subsequent

188 horizontal shaking on a shaker table for 20 minutes at 450 rpm. 1 mL of 10% boron-
189 trifluoride-methanol solution was then added to each glass tube with an additional 20 minutes
190 of shaking at 450 rpm. The glass tubes were subsequently heated in a water bath at 80 °C for
191 20 minutes. The tubes were cooled to room temperature before the upper heptane phase of
192 each tube was removed and collected in a single glass vial, which in turn was thoroughly
193 homogenized before transfer to GC sample vials, resulting in three undiluted parallels for
194 analysis by GC-MS. The GC-MS analytical method selected had already been developed and
195 evaluated by Devle et al. (2009) in our laboratory several years prior to this study, and has
196 been incorporated into several projects since as a routine analysis.

197 *Chorthippus* sample preparation was identical to the aforementioned method, with one minor
198 modification: 20 mL instead of 30 Folch's extraction mixture was used for the initial lipid
199 extraction, due to the lower amount of sample material used.

200

201 **2.4 Lipid extraction for off-line SPE and transesterification**

202 10 mL of Folch's extraction mixture was added to a 100 mL Pyrex reagent bottle with a cap,
203 along with five internal standards: 600 µL of C19:0 TG, 300 µL of C11:0 TG, 200 µL of
204 C19:0 FFA, 100 µL of C11:0 FFA and 100 µL of C19:0 PL, all transferred from their stock
205 solutions using Hamilton syringes. Lastly, 100 mg of *T. viridissima* was added to the Pyrex
206 bottle, before being subjected to horizontal shaking at 450 rpm for 60 minutes on a shaking
207 table. The contents were poured through a funnel into a 100 mL separatory funnel, along with
208 3 mL of 0.1 M NaCl in milli-Q water. The Pyrex bottle was washed with 5 mL of Folch's
209 extraction mixture. The bottom phase in the separatory funnel was decanted into a glass
210 beaker after rigorous shaking, followed by two additional liquid-liquid extractions using 2
211 mL chloroform. The contents of the glass beaker were distributed equally to twelve 6 mL

Duran® GL 14 culture tubes, along with the chloroform used to wash the beaker. The solvent was removed by inserting the twelve glass tubes in heating blocks at 40 °C in a pure nitrogen atmosphere until dry residues remained, which were then dissolved by adding 1 mL of chloroform per tube. A vortex mixer was used to thoroughly homogenize the contents of each tube before transfer to twelve GC sample vials.

Two blank samples were also made for off-line SPE by spiking 5 mL of chloroform with 100 µL of C11:0 FFA IS, and 200 µL of C19:0 FFA IS, and transferring 1 mL each to two GC sample vials. Six of the twelve sample vials were subjected to off-line SPE along with the two blanks.

The off-line SPE was carried out using a GX-274 ASPEC™ (Gilson, Middleton, WI, USA) and the accompanying software TRILUTION® LH Software version 3.0 (Gilson, Middleton, WI, USA). Bond Elut NH_2 500 mg, 3 mL columns (Agilent Technologies, USA) were used as stationary phases, and conditioned using 7.5 mL heptane. The samples were then transferred to the columns, and neutral lipids (NL) were eluted into glass vials using 5 mL chloroform, FFAs were eluted using 5 mL 98:2 diethyl ether:acetic acid (v/v) and polar lipids (PL) were eluted using 5 mL of methanol. The contents of the glass vials were then transferred to 6 mL Duran® GL 14 culture tubes. FFAs eluted from the blank samples were also collected and transferred to glass tubes.

Preliminary testing of the off-line SPE method revealed a contribution of the FFAs C14:0, C16:0 and C18:0 from the columns, which had to be accounted for by analysing blank samples, and subsequently subtracting their mean areas from their respective counterparts in the FFA samples. However, no such contribution was detected for the NL and PL fractions.

The solvents in all tubes containing the three fractions, and blanks, were then evaporated to dryness in a nitrogen atmosphere at 40°C in heating blocks. 500 µL of heptane was then

added to each tube to dissolve the dry residues. The transesterification procedure was identical to the one mentioned in section 2.3. The NL fraction was added only sodium methoxide, followed by 20 minutes of shaking at room temperature. The FFA fraction and FFA blank samples were added 10% boron-trifluoride-methanol, shaken for 20 minutes and heated in a water bath at 80 °C for 5 minutes. The PL fraction followed the procedure of the latter, but was heated for 20 minutes. The upper heptane phases of all fractions were then collected, homogenized on a vortex mixer and redistributed to GC sample vials for analyses.

2.5 GC-MS analysis of FAMEs

An Autospec Ultima GC-MS (Micromass Ltd, Manchester, England) was used to identify the FAMEs in the samples. The MS was a three-sector instrument with EBE geometry. Electron ionization, EI, was used as the ionization method, accelerating the electrons to 70 eV before impact with the analyte molecules, and 40-600 *m/z* was chosen as the mass range. Additionally, the mass spectrometer was tuned to a resolution of 1000. The temperature of both the ion source and transfer line was kept at 250 °C. Full-scan acquisition mode was utilized.

Furthermore, the gas chromatograph used in combination with the MS was an Agilent HP6890 (Agilent Technology, Wilmington, DE, USA). Separation was carried out on a 60 m Restek column (Rtx®-2330) with 0.25 mm I.D. and a 0.2 µm film thickness of fused silica biscyanopropyl cyanopropylphenyl polysiloxane stationary phase (Restek Corporation, Bellefonte, PA, USA). To inject the sample, a CTC PAL Auto sampler was used (CTC Analytics AG, Zwinger, Switzerland), injecting 1 µL at a split ratio of 1:10 into an injection chamber set to 250 °C, and using helium as a carrier gas (99,9999%, Yara, Rjukan, Norway) at a constant pressure of 95 kPa. The total run time was set to 92 minutes, with the initial GC

oven temperature set to 65 °C for 3 minutes, before increasing, at a rate of 40 °C/min, to 150 °C and held for 13 minutes. Then it was held at 151 °C for 20 minutes after increasing the temperature by 2 °C/min. The temperature was then increased to 230 °C, at a rate of 2 °C/min and held for a total of 10 minutes. Finally, at a rate of 50 °C/min, the temperature was held at 240 °C for 3.7 minutes.

Undiluted triplicates were subjected to analysis by GC-MS after each sample preparation for the identification and quantitation of the complete FA profiles, with a single injection of each replicate. Two injections of heptane were carried out in-between each injection of a sample replicate. For the samples prepared using off-line SPE, undiluted triplicates were made for each of the following fractions: neutral lipids, free fatty acids and polar lipids. Duplicates were made for the free fatty acid blank samples. A single injection was carried out for each sample replicate, and two injections of heptane in-between each sample replicate.

The software used for the GC-MS analysis was Masslynx 4.0 (Waters, Milford, MA, USA), and NIST 08 Mass Spectral Library (Gaithersburg, MD, USA) was used to aid in the identification of FAMEs, along with the retention times of the standards present in Restek's Food Industry FAME Mix. The results were converted to µg/g sample dry weight in section 3.

3. Results and discussion

3.1 Complete FA profile of *T. viridissima*

A total of 37 different FAs was identified for *T. viridissima*, with C17:1*n*-7c being the only FA not to be detected in all replicates. The average concentrations of the FAs were converted to µg/g of dry weight, and are displayed in table 1. Amongst the identified FAs, 21 of the 37 FAs were represented in the Restek Food Industry FAME Mix, and could thus be identified through both retention times and MS library searches. The remaining 16 FAs relied solely on MS library searches, based on returned values of matchfactor, reverse matchfactor and probability, but were identified in all replicates. The alkyl chain length varied from 12 to 26 carbon atoms, and SFAs, MUFAs and PUFAs were all represented, including *n*-3 and *n*-6 FAs.

Several branched fatty acids, BCFAs, were also detected in low amounts. Both *iso*-, and *anteiso*-methyl branched FAs were present: 10-methyldodecanoic acid (C12:0, 10-methyl), 14-methylpentadecanoic acid (C15:0, 14-methyl), 14-methyl-hexadecanoic acid (C16:0, 14-methyl), 16-methylheptadecanoic acid (C17:0, 16-methyl) and 17-methyloctadecanoic acid (C18:0, 17-methyl).

The average total FA content for the carnivorous bush cricket *T. viridissima* was found to be 10.4% ± 0.3 per gram of sample dry weight. This value is consistent with the value reported by Yang et al. (2006), whom reported a lipid content of 10.2g/100 g for the ground cricket *Acheta confirmata*. Although a different species, comparison is advantageous in confirming the plausibility of the data gathered in this study. SFAs constituted 31.1% of the total FA content, and displayed the largest variation in data amongst the FA classes. MUFAs made up 35.9%, and PUFAs 33.0% of the total FA content. Furthermore, *n*-3 FAs and *n*-6 FAs constituted 5.73% and 27.2%, respectively. The values for MUFAs and PUFAs also aligned

with the values reported by Yang et al. (2006). In their study, *A. confirmata* contained 33.5% MUFAs and 33.8% PUFAs.

Most notably, octadecanoic acid (C18:0), hexadecanoic acid (C16:0), tetradecanoic acid (14:0) and their monounsaturated and polyunsaturated counterparts, including C18:1*n*-9c, C18:2*n*-6c, C18:3*n*-3c, were by far the most abundant FAs in *T. viridissima* in both quantity and diversity. There was also significant diversity among the eicosanoids detected. The same trend was observed in the studies of lipids in the cricket *Acheta domesticus* by Hutchins and Martin (1968), and Grapes et al. (1989). This trend was also found by Yang et al. (2006) in *A. confirmata*. C18:0, C16:0 and C14:0 respectively accounted for 3.44%, 25.4% and 1.49% of the total FA content in *T. viridissima*.

C18:1*n*-9 was the most abundant MUFA, and FA, yielding 32.8% of the FA total. The two EFAs previously mentioned in section 1, C18:2*n*-6c, LA, and C18:3*n*-3c, ALA, each contributed 26.6% and 5.60% of the total. Paul et al. (2017) also reported LA as being among the major lipid constituents in the crickets *Conocephalus discolour* and *A. domesticus*. The FAs EPA, C20:5*n*-3c, and DHA, C22:6*n*-3c, are abundant in oily fish and marine organisms and have been extensively linked to several important functions in the human body, including prevention of cardiovascular diseases and inflammations (Swanson et al. 2012). While DHA was undetected in any of the samples, small amounts of EPA were identified and quantitated, accounting for 0.12% of the FAs. The precursor to both EPA and DHA, arachidonic acid (C20:4*n*-6c), only accounted for less than 1%.

Section 1 has already established the importance of the *n*-6/*n*-3-ratio, and a 70% decrease in overall mortality has been linked to a dietary ratio of 4/1 (Simopoulos 2002). However, this claim remains a subject of debate (Rizos et al. 2012). The calculated ratio for *T. viridissima* was 4.7, which is above the recommended value. However, even an overall 5/1 ratio in the

diet may provide beneficial effects for those affected by asthma, according to Simopoulos (2002). Additionally, a 5/1 ratio, or lower, has also been linked to decreased levels of serum cholesterol and proinflammatory cytokines (Yang et al. 2016). Furthermore, the significant abundance of the EFA C18:2*n*-6c, as well as the high contents of MUFAs and PUFAs, would suggest that *T. viridissima* displays a nutritionally beneficial FA composition, that could potentially positively impact human health if incorporated into an already balanced diet. C18:1*n*-9c, OA, was overall the most abundant FA, and is reported to have beneficial effects in patients suffering from diabetes II, as well as an ability to reverse the effects of inflammatory cytokines (Vassiliou et al. 2009). The presence of the other EFA, C18:3*n*-3c, further substantiates the claim that the FA composition of *T. viridissima* may positively affect human health.

3.2 Complete FA profile of *Chorthippus*, and comparisons

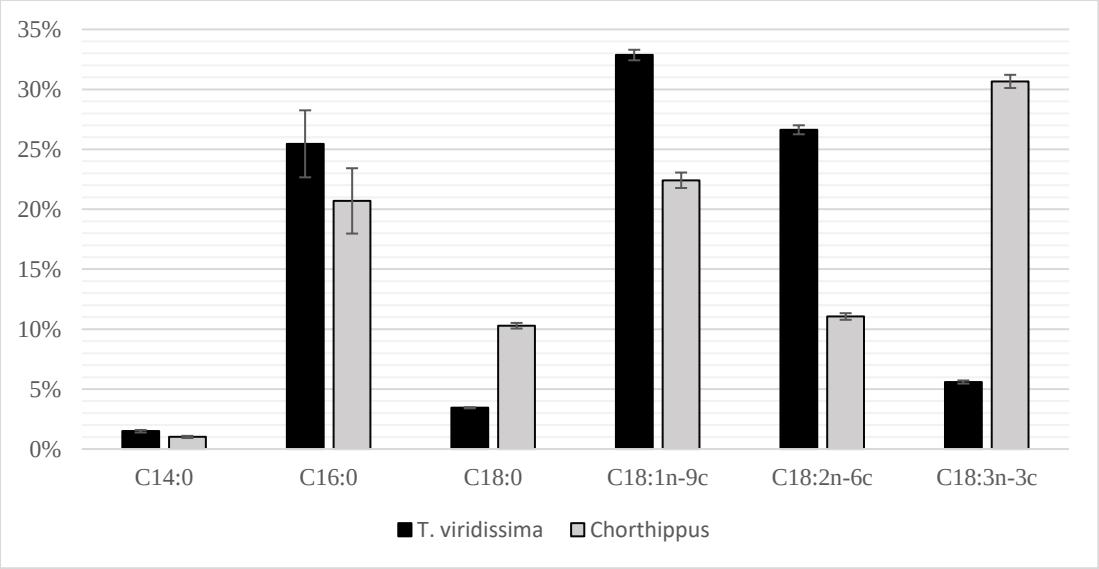

Figure 1: A graphical representation of the average percentages of each of the most abundant FAs found in both *T. viridissima* and *Chorthippus*, relative to total concentration of FAs per gram of sample dry weight. For *T. viridissima*, n = 9. For *Chorthippus*, n = 6.

A total of 33 FAs was identified for *C. brunneus* and *C. biguttulus*. 21 of these were identified using the retention times of reference standards, as well as searches in the MS library. The remaining 12 relied upon the MS library alone for identification, but were present in all replicates. The results for each FA were converted to µg/g dry weight, and are presented in table 1. As with the FAs present in *T. viridissima*, the chain length varied from 10 to 26 carbon atoms, and SFAs, MUFAs and PUFAs were all identified amongst the FAs, including *n*-6 and *n*-3 FAs. In contrast to *T. viridissima*, the presence of BCFAs was scarce, with the *anteiso*-BCFA 10-methyldodecanoic acid being the only representative. The SFAs C10:0, C21:0 and C22:0 were not detected in the *T. viridissima* replicates, but were present in *Chorthippus* in average concentrations of 13.5, 15.1 and 26.2 µg/g d.w., respectively. The SFA C20:0 was not detected in the *Chorthippus* samples, most likely due to coelution with

C18:3*n*-3c, which could be resolved by diluting samples before subsequent analysis by GC-MS. AA, C20:4*n*-6c, and EPA, C20:5*n*-3c, were also not identified in the *Chorthippus* samples. If present, doubling the initial amount of sample, akin to *T. viridissima*, prior to lipid extraction could possibly reveal their presence. As with *T. viridissima*, DHA was not detected in any of the replicates.

The average total FA content of *Chorthippus* per gram of sample dry weight was 6.14% ± 0.17, a lower value than what was discovered for *T. viridissima*. Paul et al. (2017) reported a total lipid content of 10% of dry matter for the species *Chorthippus parallelus*. There were significant, quantitative differences in the FA classes of *Chorthippus* and *T. viridissima*, displayed in table 2. SFAs in *Chorthippus* constituted 32.7% of the total amount of FAs present in the samples, a similar value to the SFA content of *T. viridissima*. The MUFA content in *Chorthippus* was lower, accounting for 25.1% of the FAs, and PUFAs constituted a total of 42.1%. As previously mentioned in section 3.1, the same values for *T. viridissima* were 35.9 and 33.0%, respectively. These differences are largely explained by the variations of the following three FAs: C18:1*n*-9c, C18:2*n*-6c and C18:3*n*-3c, as shown in figure 1. For both species, treating *C. biguttulus* and *C. brunneus* as a single species in this study, the three FAs accounted for the majority of the total FA content. While *Chorthippus* contained comparatively lower amounts of C18:1*n*-9c and C18:2*n*-6c, C18:3*n*-3c accounted for 30.7% of the total FA content. C18:1*n*-9c and C18:2*n*-6c however, respectively contributed 22.4 and 11.1%. The higher concentration of C18:3*n*-3c, ALA, in *Chorthippus* is likely due to the herbivorous diet, as opposed to the carnivorous diet of *T.viridissima.* The results of Paul et al. (2017) also proved C18:3*n*-3c to be present in major quantities in *C. parallelus*, and the authors concluded that the diet was responsible for the abundance of ALA in *C. parallelus*.

The SFAs C18:0, C16:0 and C14:0 were the major contributors to the total SFA content of *Chorthippus*, respectfully accounting for 10.3, 20.7 and 1.01% of the total FA amount. The

same trend was observed in the case of *T. viridissima*. A graphical representation of these FAs, in both *T. viridissima* and *Chorthippus*, is displayed in figure 1.

The *n*-6/*n*-3 ratio of *Chorthippus* was 0.36, a more favourable ratio from a nutritional point of view than the ratio calculated for *T. viridissima*, and below the ratio of 1 championed by Simopoulos (2002). The concentration of the essential *n*-3 FA C18:3*n*-3c was also 5.4 times higher in *Chorthippus*. The other EFA, C18:2*n*-6c was, however, 2.4 times higher in *T. viridissima*. The nutritionally beneficial FA composition of *T. viridissima* was examined and established in section 3.1, emphasizing the high contents of the EFA C18:2*n*-6c, as well as the beneficial effects of its most abundant FA: C18:1*n*-9c. However, the higher PUFA content, the significant higher quantities of the EFA C18:3*n*-3c, and the more favourable *n*-6/*n*-3 ratio would suggest that *Chorthippus* exhibits the more beneficial FA composition of the two species in a comparison as a potential human food.

3.3 Fractioning of lipids in *T. viridissima*

Fractioning of the lipids in *T. viridissima* into three classes, by use of off-line SPE, resulted in an average total FA content of 10.8% ± 0.3 per gram of sample dry weight. This value is consistent with the total FA content reported for *T. viridissima* in section 3.1. However, it is the belief of the authors that the method would have benefited from a larger, initial amount of sample material, which in turn could have resulted in the identification of several more FAs across all three fractions.

The total concentrations of the lipid classes are displayed in table 3. Neutral lipids, including the storage lipids triacylglycerides, were by far the most abundant in *T. viridissima*, yielding a total concentration of 65.87 ± 2.67 mg/g of sample dry weight. Polar lipids consistently yielded the lowest concentrations, with the exception of C18:0, and accounted for a total of 11.21 ± 0.48 mg/g d.w. The overall lower content of polar lipids is attributed to the primary role of phospholipids as constituents of the cell membrane. The FFAs, however, constituted a total of 31.47 ± 1.19 mg/g d.w. Furthermore, the FAs C16:0 and C18:0 constituted the majority of the total SFA content within each respective fraction. The MUFA C18:1*n*-9c, and the PUFAs C18:2*n*-6c and C18:3*n*-3c were all present in major quantities within each fraction. The precision was also deemed satisfactory, thus demonstrating that SPE could become a useful method in future lipid studies of other insects.

4. Conclusions

The work presented through this study highlighted the quantitative diversity of FAs for different species belonging to the order *Orthoptera*. Significant differences in the contents of MUFAs and PUFAs in the carnivorous bush cricket *T. viridissima* and herbivorous grasshoppers *C. biguttulus* and *C. brunneus* were observed, as well as differences in the total FA contents. The FA contents were 10.4 and 6.14% of dry weight, respectively. *Chorthippus* was richer in PUFAs (42.1%) than *T. viridissima* (33.0%), and contained higher amounts of the EFA C18:3*n*-3c (33.7%). In contrast, *T. viridissima* was richer in the EFA C18:2*n*-6c (26.6%), and C18:1*n*-9c (32.9%). Fractioning of the lipids in *T. viridissima* into neutral lipids, free fatty acids and polar lipids resulted in a total FA content of 10.8%. The average concentrations of the three fractions were 65.87, 31.47 and 11.21 mg/g of dry matter, respectively. The abundance of FAs potentially beneficial to human health, high contents of MUFAs and PUFAs relative to SFAs, and favourable *n*-6/*n*-3 ratios suggested all three species displayed favourable nutritional profiles from a FA composition point of view, although further studies are needed to conclusively mark all three species as safe for human consumption.

The authors have declared no conflict of interest.

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Table 1: Complete FA profiles of both *T. viridissima* and *Chorthippus* in order of elution. The average concentrations for *T. viridissima* are the result of three sample preparations, each containing three parallels. The average concentrations for *Chorthippus* are the result of two sample preparations, each containing three parallels. All values are presented as µg/g of sample dry weight.

	Average ± S.D (µg/g d.w.)	
FA	*T. viridissima*	*Chorthippus*
C10:0	n.d. [b)]	13.52 ± 0.56
C12:0	72.80 ± 4.62	113.3 ± 5.6
C12:0 (10-methyl) [a)]	10.81 ± 1.25	7.15 ± 1.63
C14:0	1547 ± 110	622.2 ± 54.2
C14:1n-3c [a)]	6.23 ± 0.44	2.78 ± 0.48
C14:1 other [a)]	3.94 ± 0.48	n.d. [b)]
C14:1n-5c	15.94 ± 0.63	4.57 ± 0.48
C15:0	31.24 ± 1.69	19.28 ± 0.89
C15:0 (14-methyl) [a)]	55.23 ± 4.82	n.d. [b)]
C16:0	26475 ± 2903	12723 ± 1673
C16:1n-9t [a)]	7.17 ± 0.73	6.46 ± 0.36
C16:1 other [a)]	68.54 ± 3.02	103.4 ± 5.6
C16:1n-7c	1854 ± 61	274.3 ± 16.9
C16:1n-5c [a)]	n.d. [b)]	28.23 ± 1.43
C16:0 (14-methyl) [a)]	59.86 ± 2.43	n.d. [b)]
C17:0	109.2 ± 3.4	248.5 ± 14.3
C16:2n-6t [a)]	8.88 ± 1.33	15.90 ± 2.42
C17:1n-7c	71.57 ± 1.98	66.19 ± 2.86
C17:0 (16-methyl) [a)]	149.5 ± 2.5	n.d. [b)]
C18:0	3576 ± 52	6321 ± 143
C18:1n-9c	34178 ± 458	13776 ± 397
C18:1n-7c [a)]	505.2 ± 5.9	359.8 ± 14.3
C18:1n-8t [a)]	n.d. [b)]	20.08 ± 0.88

C18:0 (17-methyl) [a)]	33.15 ± 2.80	n.d. [b)]
C18:2n-6c	27693 ± 390	6798 ± 168
C19:1 other [a)]	n.d. [b)]	15.50 ± 0.89
C19:1n-9c [a)]	73.27 ± 5.96	26.63 ± 1.68
C18:3n-3c [a)]	14.21 ± 2.00	55.06 ± 4.14
C20:0	172.9 ± 25.7	n.d. [b)]
C18:3n-3c	5818 ± 135	18845 ± 339
C20:1n-11c [a)]	26.70 ± 3.89	73.15 ± 1.77
C20:1n-9c [a)]	357.2 ± 11.8	654.1 ± 28.1
C21:0	n.d. [b)]	15.09 ± 1.12
C20:2n-6c	124.9 ± 3.4	44.13 ± 2.02
C22:0	n.d. [b)]	26.18 ± 0.82
C20:3 other [a)]	10.85 ± 0.92	n.d. [b)]
C20:3n-6c	19.07 ± 11.66	n.d. [b)]
C20:3n-3c	n.d. [b)]	124.8 ± 4.0
C20:4n-6c	519.7 ± 17.3	n.d. [b)]
C22:1n-9c	141.1 ± 6.3	27.65 ± 1.60
C20:5n-3c	126.4 ± 34.9	n.d. [b)]
C24:0	26.95 ± 3.21	6.06 ± 0.46
C24:1n-9c	13.14 ± 1.84	n.d. [b)]
C26:0 [a)]	13.60 ± 3.21	12.52 ± 0.56

[a)] The FA is not confirmed by a standard from Restek's Food Industry FAME Mix. [b)] n.d. – not detected.

Table 2: A comparison of the major FA classes found in both *T. viridissima* and *Chorthippus*. All values are presented as mg/g of sample dry weight.

	Average ± S.D [mg/g d.w.]	
FA class:	*T. viridissima*	*Chorthippus*
SFAs	32.33 ± 2.90	20.13 ± 1.68
MUFAs	37.32 ± 0.46	15.44 ± 0.40
PUFAs	34.33 ± 0.41	25.88 ± 0.37
n-3 FAs	5.96 ± 0.14	19.02 ± 0.34
n-6 FAs	28.36 ± 0.39	6.86 ± 0.08

SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids

Table 3: The average concentration, and standard deviation, of each FA across the three lipid fractions: neutral lipids, free fatty acids and polar lipids. The results are based on three sample preparations, each including three parallels of each fraction, for *T. viridissima*. All values are presented as µg/g of dry matter.

	Average ± S.D [µg/g d.w.]		
FA:	NLs	FFAs	PLs
C12:0	87.96 ± 12.79	n.d. [b]	n.d. [b]
C14:0	1311 ± 122	324.9 ± 52.1	85.86 ± 23.25
C16:0	21466 ± 2576	8252 ± 765	3829 ± 325
C16:1 [a] other	52.66 ± 6.12	n.d. [b]	n.d. [b]
C16:1n-7c	1112 ± 50	334.1 ± 73.4	n.d.[b]
C17:0	76.86 ± 10.82	77.35 ± 14.08	n.d. [b]
C17:1n-7c	46.82 ± 10.00	46.40 ± 11.74	n.d.[b]
C17:0 (16-methyl) [a]	96.88 ± 14.77	n.d. [b]	n.d. [b]
C18:0	1212 ± 59	1954 ± 214	2694 ± 145
C18:1n-9c	23018 ± 623	7613 ± 545	2535 ± 214
C18:1n-7c [a]	248.3 ± 9.5	142.8 ± 25.2	n.d. [b]
C18:2n-6c	13652 ± 285	10953 ± 691	1927 ± 247
C19:1n-9c [a]	44.34 ± 4.23	n.d. [b]	n.d. [b]
C20:0	80.96 ± 5.77	88.49 ± 76.13	n.d. [b]
C18:3n-3c	2961 ± 125	1352 ± 93	145.8 ± 15.9
C20:1n-9c	171.3 ± 12.2	53.34 ± 9.18	n.d. [b]
C20:2n-6c	46.99 ± 6.08	33.81 ± 7.89	n.d. [b]
C20:4n-6c	108.6 ± 9.7	168.9 ± 17.5	n.d. [b]
C22:1n-9c	74.36 ± 13.65	31.31 ± 8.13	n.d. [b]
C20:5n-3c	n.d. [b]	45.57 ± 5.23	n.d. [b]
Total [mg/g]:	65.87 ± 2.67	31.47 ± 1.19	11.21 ± 0.48

[a] The FA is not confirmed by a standard from Restek's Food Industry FAME Mix. [b] n.d. – not detected.

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Appendices

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Appendix I: Internal standards

Table A.1: The internal standards utilized for the quantitation of the complete FA profiles of *T. viridissima* and *Chorthippus*, as well as the neutral lipid fraction of *T. viridissima*

Internal standard	Molecular weight [g/mole]	Concentration [mg/mL]	IS used [mL]	Amount IS [mg]	Moles IS	Moles fatty acids
C11:0 TG	596.9	0.1	0.6	0.06	$1.005*10^{-7}$	$3.015*10^{-7}$
C19:0 TG	933.6	1	3.7	3.7	$3.963*10^{-6}$	$1.189*10^{-5}$

Table A.2: The internal standards utilized for the quantitation of the free fatty acid fraction of *T. viridissima*

Internal standard	Molecular weight [g/mole]	Concentration [mg/mL]	IS used [mL]	Amount IS [mg]	Moles IS	Moles fatty acids
C11:0 FFA	186.3	1	0.1	0.1	$5.367*10^{-7}$	$5.367*10^{-7}$
C19:0 FFA	298.52	1	0.2	0.2	$6.70*10^{-7}$	$6.70*10^{-7}$

Table A.3: The internal standard utilized for the quantitation of the polar lipid fraction of *T. viridissima*

Internal standard	Molecular weight [g/mole]	Concentration [mg/mL]	IS used [mL]	Amount IS [mg]	Moles IS	Moles fatty acids
C19:0 PL	818.2	1	0.1	0.1	$1.222*10^{-7}$	$2.444*10^{-7}$

Appendix II: Reference standards

Table A.4: The FAME components of the Restek Food Industry FAME Mix, used as reference standards for FAMEs from *T. viridissima* and *Chorthippus*. Listed in order of elution, along with weight% of each respective FAME in the FAME mix*

FAME	Systematic name	Weight%
C4:0	Butanoic acid methyl ester	4.0
C6:0	Hexanoic acid methyl ester	4.0
C8:0	Octanoic acid methyl ester	4.0
C10:0	Decanoic acid methyl ester	4.0
C11:0	Undecanoic acid methyl ester	2.0
C12:0	Dodecanoic acid methyl ester	4.0
C13:0	Tridecanoic acid methyl ester	2.0
C14:0	Tetradecanoic acid methyl ester	4.0
C14:1n-5c	cis-9-Tetradecenoic acid methyl ester	2.0
C15:0	Pentadecanoic acid methyl ester	2.0
C15:1n-5c	cis-10-Pentadecenoic acid methyl ester	2.0
C16:0	Hexadecenoic acid methyl ester	6.0
C16:1n-7c	cis-9-Hexadecenoic acid methyl ester	2.0
C17:0	Heptadecanoic acid methyl ester	2.0
C17:1n-7c	cis-10-Heptadecenoic acid methyl ester	2.0
C18:0	Octadecanoic acid methyl ester	4.0
C18:1n-9c	cis-9-Octadecenoic acid methyl ester	4.0
C18:1n-9tr	trans-9-Octadecenoic acid methyl ester	2.0
C18:2n-6c	all-cis-9,12-Octadecadienoic acid methyl ester	2.0
C18:2n-6tr	all-trans-9,12-Octadecadienoic acid methyl ester	2.0
C18:3n-6c	all-cis-6,9,12-Octadecatrienoic acid methyl ester	2.0
C18:3n-3c	all-cis-9,12,15-Octadecatrienoic acid methyl ester	2.0
C20:0	Eicosanoic acid methyl ester	4.0
C20:1n-9c	cis-11-Eicosenoic acid methyl ester	2.0
C20:2n-6c	all-cis-11,14-Eicosadienoic acid methyl ester	2.0
C20:3n-6c	all-cis-8,11,14-Eicosatrienoic acid methyl ester	2.0
C21:0	Heneicosanoic acid methyl ester	2.0
C20:4n-6c	all-cis-5,8,11,14-Eicosatetraenoic acid methyl ester	2.0
C20:3n-3c	all-cis-11,14,17-Eicosatrienoic acid methyl ester	2.0
C20:5n-3c	all-cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	2.0
C22:0	Docosanoic acid methyl ester	4.0
C22:1n-9c	cis-13-Docosenoic acid methyl ester	2.0

C22:2n-6c	all-cis-13,16-Docosadienoic acid methyl ester	2.0
C23:0	Tricosanoic acid methyl ester	2.0
C24:0	Tetracosanoic acid methyl ester	4.0
C22:6n-3c	all-cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	2.0
C24:1n-9c	cis-15-Tetracosenoic acid methyl ester	2.0

* From Devle et al. (2009)

Appendix III: RRF-values

Table A.5: The RRF-values of FAMEs present in *T. viridissima* and *Chorthippus*, and the molecular weight of their respective FA counterparts, in order of elution.

FAME	RRF-value	FA Molecular weight [g/mole]
C10:0	0.95	172.27
C12:0	1.05	200.33
C12:0 (10-methyl) [a)]	1.23	214.35
C14:0	1.12	228.38
C14:1n-3c [a)]	1.24	226.38
C14:1 other [a)]	1.24	226.38
C14:1n-5c	1.24	226.38
C15:0	1.22	242.41
C15:0 (14-methyl) [a)]	1.22	256.43
C16:0	1.22	256.43
C16:1n-9t [a)]	1.18	254.43
C16:1 other [a)]	1.18	254.43
C16:1n-7c	1.18	254.43
C16:1n-5c [a)]	1.18	254.43
C16:0 (14-methyl) [a)]	1.22	270.46
C17:0	1.22	270.46
C16:2n-6t [a)]	1.18	252.43
C17:1n-7c	1.22	268.46

C17:0 (16-methyl) [a)]	1.19	284.48
C18:0	1.19	284.48
C18:1n-9c	1.16	282.48
C18:1n-7c [a)]	1.16	282.48
C18:1n-8t [a)]	1.16	282.48
C18:0 (17-methyl) [a)]	1.00	298.52
C18:2n-6c	1.01	280.48
C19:1 other [a)]	1.00	296.52
C19:1n-9c [a)]	1.00	296.52
C18:3n-3c [a)]	0.98	278.48
C20:0	1.17	312.54
C18:3n-3c	0.98	278.48
C20:1n-11c [a)]	1.13	310.54
C20:1n-9c [a)]	1.13	310.54
C21:0	1.00	326.57
C20:2n-6c	1.06	308.54
C22:0	1.18	340.59
C20:3 other [a)]	1.18	306.53
C20:3n-6c	1.18	306.53
C20:3n-3c	0.96	306.53
C20:4n-6c	0.96	304.52
C22:1n-9c	1.10	338.59
C20:5n-3c	0.96	302.52
C24:0	1.19	368.65
C24:1n-9c	1.01	366.65
C26:0 [a)]	1.19	396.71

[a)] No previously calculated RRF-value

Appendix IV: Complete FA profile *T. viridissima*

Table A.6: Summary table of the first sample preparation for *T. viridissima*, using 0.509 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C12:0	538	11.00	913	936	69.90	39.69	0.69
C12:0 (10-methyl) [A)]	89	12.45	742	760	9.71	6.08	0.60
C14:0	10903	14.43	946	948	73.70	863.1	47.5
C14:1n-3c [A)]	147	15.3	804	817	8.73	3.19	0.16
C14:1 other [A)]	87	15.84	837	843	21.40	1.90	0.19
C14:1n-5c	385	16.24	847	863	23.60	8.34	0.32
C15:0	691	17.17	913	915	64.70	16.28	0.48
C15:0 (14-methyl) [A)]	373	18.98	853	882	46.60	30.54	2.83
C16:0	170728	21.18	951	951	82.90	13957	1194
C16:1n-9t [A)]	153	22.54	805	821	36.30	3.91	0.31
C16:1 other [A)]	1328	23.10	906	908	23.90	33.96	1.12
C16:1n-7c	36925	23.63	959	960	45.90	943.9	25.4
C16:0 (14-methyl) [A)]	1148	24.37	856	856	58.10	30.16	1.19
C17:0	2152	26.18	900	906	60.40	56.59	1.28
C16:2n-6t [A)]	199	27.41	853	857	37.00	5.05	0.63
C17:0 (16-methyl) [A)]	2636	29.89	860	912	54.90	74.73	0.29
C18:0	62660	34.07	956	960	78.00	1776	19
C18:1n-9c	606149	38.46	955	955	10.50	17503	82
C18:1n-7c [A)]	8974	38.74	950	950	9.36	259.1	2.0
C18:0 (17-methyl) [A)]	492	40.38	811	829	34.30	17.43	0.16
C18:2n-6c	424659	44.76	960	960	37.30	13983	59
C19:1n-9c [A)]	1027	45.82	883	885	21.20	36.12	0.32
C18:3n-3c [A)]	205	48.81	838	893	26.60	6.91	0.80
C20:0	2881	49.92	935	937	73.60	91.12	13.65
C18:3n-3c	85896	50.29	960	960	71.50	2894	65
C20:1n-11c [A)]	439	51.80	830	847	14.30	14.31	0.37
C20:1n-9c	5709	52.13	929	930	18.90	185.9	2.2
C20:2n-6c	1856	55.92	937	938	43.20	64.04	0.93
C20:3 other [A)]	185	57.53	832	840	26.10	5.72	0.29
C20:3n-6c	486	58.20	763	768	67.50	14.84	5.27
C20:4n-6c	6759	59.81	936	940	64.40	254.1	5.4
C22:1n-9c	1964	61.29	918	919	40.20	71.63	2.90
C20:5n-3c	1656	63.56	868	881	20.80	61.54	17.19
C24:0	490	67.06	832	863	78.80	18.00	0.87
C24:1n-9c	187	68.38	752	763	16.70	8.07	0.60
C26:0 [A)]	222	73.19	727	753	34.30	8.78	0.82

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix

Table A.7: Summary table of the second sample preparation for *T. viridissima*, using 0.503 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C12:0	467	11.09	901	908	62.20	33.36	1.03
C12:0 (10-methyl) [A)]	70	12.55	731	755	11.90	4.59	0.25
C14:0	9119	14.54	950	950	73.70	697.1	23.1
C14:1n-3c [A)]	120	15.43	788	798	15.60	3.07	0.03
C14:1 other [A)]	82	15.97	781	791	19.10	2.09	0.14
C14:1n-5c	316	16.37	843	857	21.10	8.06	0.16
C15:0	562	17.31	902	906	58.10	15.58	0.32
C15:0 (14-methyl) [A)]	308	19.13	840	902	55.70	24.34	1.65
C16:0	148083	21.33	952	952	83.40	11670	398
C16:1n-9t [A)]	121	22.73	802	822	33.20	3.66	0.18
C16:1 other [A)]	1106	23.31	898	900	22.10	33.27	0.64
C16:1n-7c	31062	23.83	960	960	46.70	934.2	10.5
C16:0 (14-methyl) [A)]	941	24.58	857	860	63.60	29.10	0.26
C17:0	1743	26.40	898	900	47.20	53.89	1.35
C16:2n-6t [A)]	140	29.63	812	820	16.90	4.19	0.33
C17:1n-7c [A)]	1197	27.66	904	907	29.10	36.76	0.90
C17:0 (16-methyl) [A)]	2179	30.11	860	915	55.50	72.66	0.84
C18:0	53803	34.30	950	955	77.60	1794	15
C18:1n-9c	512304	38.72	954	954	10.90	17403	188
C18:1n-7c [A)]	7507	39.02	949	949	9.88	254.9	1.9
C18:0 (17-methyl) [A)]	402	40.68	792	819	50.00	16.74	0.86
C18:2n-6c	370117	44.96	960	973	37.60	14335	169
C19:1n-9c [A)]	830	46.06	861	867	16.10	34.35	0.29
C18:3n-3c [A)]	188	49.05	818	883	24.10	7.46	0.21
C20:0	2207	50.11	926	927	60.90	82.26	0.92
C18:3n-3c	74655	50.50	958	958	71.30	2959	28
C20:1n-11c [A)]	321	52.00	813	833	12.90	12.31	0.07
C20:1n-9c	4742	52.32	935	937	22.80	181.7	4.1
C20:2n-6c	1473	56.10	921	921	43.50	59.81	0.90
C20:3 other [A)]	154	57.61	798	808	13.50	5.57	0.29
C20:3n-6c	286	58.40	743	749	35.40	10.33	3.36
C20:4n-6c	5721	59.99	943	945	67.50	253.1	4.2
C22:1n-9c	1529	61.45	913	914	34.50	65.66	0.53
C20:5n-3c	1706	63.72	864	882	21.30	74.92	5.58
C24:0	253	67.20	781	799	62.80	10.90	1.35
C24:1n-9c	108	68.51	728	738	6.79	5.48	0.45
C26:0 [A)]	107	73.32	659	710	9.60	4.98	1.00

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix

Table A.8: Summary table of the third sample preparation for *T. viridissima*, using 0.508 g of sample (d.w.), n=3. Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C12:0	263	11.11	911	914	70.80	38.22	2.63
C12:0 (10-methyl) [A)]	44	12.57	718	730	3.17	5.85	0.09
C14:0	5096	14.58	949	951	73.50	793.6	20.8
C14:1n-3c [A)]	74	15.46	767	773	5.69	3.43	0.21
C14:1 other [A)]	41	16.02	776	820	5.55	1.93	0.09
C14:1n-5c	178	16.42	821	830	21.70	8.24	0.06
C15:0	315	17.37	893	897	60.70	15.85	0.74
C15:0 (14-methyl) [A)]	191	19.18	869	888	49.00	30.74	1.06
C16:0	91000	21.42	954	954	80.30	14645	785
C16:1n-9t [A)]	62	22.8	812	822	26.50	3.39	0.23
C16:1 other [A)]	692	23.38	891	916	15.40	37.78	1.04
C16:1n-7c	17321	23.91	951	952	40.30	946.3	16.1
C16:0 (14-methyl) [A)]	568	24.65	853	854	57.40	31.94	0.71
C17:0	1028	26.50	891	895	61.60	57.70	1.77
C16:2n-6t [A)]	80	27.76	808	812	13.50	4.33	0.15
C17:0 (16-methyl) [A)]	1324	30.29	842	871	54.90	80.28	1.70
C18:0	30808	34.51	947	959	76.50	1867	11
C18:1n-9c	276317	38.96	951	952	9.69	17058	128
C18:1n-7c [A)]	4136	39.22	936	936	7.92	255.4	1.6
C18:0 (17-methyl) [A)]	225	40.85	787	808	27.60	16.97	1.80
C18:2n-6c	195787	45.12	959	959	36.00	13784	85
C19:1n-9c [A)]	548	46.19	888	889	22.40	41.36	3.46
C18:3n-3c [A)]	101	49.12	864	883	33.10	7.31	0.67
C20:0	1327	50.22	925	949	72.40	89.94	1.61
C18:3n-3c	41605	50.60	952	953	69.50	2997	11
C20:1n-11c [A)]	198	52.08	800	809	13.40	13.93	2.76
C20:1n-9c	2529	52.42	929	929	17.40	176.3	4.1
C20:2n-6c	896	56.18	902	903	38.30	66.13	1.35
C20:3 other [A)]	81	57.68	811	818	19.80	5.36	0.23
C20:3n-6c	77	58.46	736	740	5.23	5.04	1.27
C20:4n-6c	3531	60.06	929	942	70.00	283.7	6.4
C22:1n-9c	1002	61.53	899	899	33.70	78.15	1.82
C20:5n-3c	721	63.79	866	872	19.90	57.62	1.71
C24:0	155	67.26	833	851	84.30	12.18	0.89
C24:1n-9c	73	68.58	722	722	3.56	6.69	0.61
C26:0 [A)]	85	73.38	692	716	25.20	7.25	1.71

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix

Appendix V: Complete FA profile *Chorthippus*

Table A.9: Summary table of the first sample preparation for *Chorthippus*, using 0.252 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C10:0	123	9.12	771	853	22.40	3.39	0.09
C12:0	1030	11.09	918	925	61.60	29.14	1.17
C12:0 (10-methyl) [A)]	70	12.52	740	801	11.30	1.83	0.41
C14:0	5299	14.50	943	947	71.10	160.6	4.1
C14:1n-3c [A)]	77	15.39	743	767	4.73	0.76	0.16
C14:1n-5c	121	16.34	780	797	4.41	1.20	0.07
C15:0	465	17.25	878	890	42.20	5.00	0.16
C16:0	103871	21.15	952	952	83.00	3253	180
C16:1n-9t [A)]	140	22.62	785	811	17.80	1.64	0.06
C16:1 other [A)]	2352	23.20	916	920	29.70	27.42	1.37
C16:1n-7c	6041	23.68	937	939	34.60	70.44	3.33
C16:1n-5c [A)]	617	24.38	860	931	20.00	7.20	0.21
C17:0	5370	26.28	929	932	64.40	64.42	2.51
C16:2n-6t [A)]	338	27.53	863	884	29.80	3.92	0.18
C17:1n-7c	1454	29.50	893	897	25.80	17.32	0.63
C18:0	126651	34.06	955	960	77.80	1639	33
C18:1n-9c	271796	38.15	957	958	11.00	3582	78
C18:1n-7c [A)]	6952	38.59	941	942	9.40	91.63	2.96
C18:1n-8t [A)]	385	39.93	803	819	7.73	5.08	0.20
C18:2n-6c	116300	44.51	958	971	37.00	1748	30
C19:1 other [A)]	239	45.95	795	806	8.07	3.85	0.11
C19:1n-9c [A)]	425	46.15	834	845	14.20	6.82	0.31
C18:3n-3c [A)]	957	48.90	840	847	27.50	14.74	0.86
C18:3n-3c	316603	50.55	956	956	71.20	4872	78
C20:1n-11c [A)]	1315	52.21	901	926	18.90	19.56	0.45
C20:1n-9c	11118	52.80	949	950	28.80	165.3	5.40
C21:0	231	55.32	792	826	31.80	4.08	0.26
C20:2n-6c	737	55.99	870	878	22.30	11.63	0.53
C22:0	446	59.78	854	894	68.50	6.98	0.26
C20:3n-3c	1861	59.97	878	929	40.00	32.17	1.47
C22:1n-9c	417	61.80	861	869	23.00	6.95	0.39
C24:0	92	67.12	727	749	32.20	1.55	0.14
C26:0 [A)]	184	73.24	709	744	33.10	3.34	0.14

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix

Table A.10: Summary table of the second sample preparation for *Chorthippus*, using 0.251 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. Matchfactor	Probability [%]	A. amount [µg]	S.D
C10:0	132	9.16	701	782	18.80	3.56	0.10
C12:0	1004	11.11	903	914	57.70	28.59	1.45
C12:0 (10-methyl) [A)]	71	12.55	681	752	5.22	1.82	0.11
C14:0	5018	14.53	945	949	72.80	153.4	13.1
C14:1n-3c [A)]	71	15.41	724	752	11.10	0.70	0.07
C14:1n-5c	120	16.36	737	770	8.54	1.17	0.11
C15:0	450	17.29	865	882	49.80	4.78	0.20
C16:0	99830	21.19	947	947	76.80	3158	378
C16:1n-9t [A)]	140	22.67	787	815	29.50	1.61	0.07
C16:1 other [A)]	2211	23.24	918	922	30.90	25.52	1.28
C16:1n-7c	5907	23.72	935	937	36.80	68.18	3.67
C16:1n-5c [A)]	604	24.42	857	929	19.30	6.97	0.30
C17:0	5138	26.33	924	928	63.50	60.98	3.13
C16:2n-6t [A)]	358	27.58	837	876	29.20	4.10	0.64
C17:1n-7c	1397	29.56	880	886	23.70	16.47	0.43
C18:0	120216	34.08	955	960	77.10	1539	22
C18:1n-9c	257178	38.18	956	956	10.20	3354	61
C18:1n-7c [A)]	6862	38.65	931	934	7.88	89.49	1.97
C18:1n-8t [A)]	392	40.00	804	827	9.08	5.12	0.15
C18:2n-6c	112455	44.54	960	961	36.80	1672	28
C19:1 other [A)]	249	45.99	781	799	6.61	3.95	0.25
C19:1n-9c [A)]	415	46.20	825	840	15.30	6.67	0.30
C18:3n-3c [A)]	900	48.95	845	857	28.90	13.70	0.30
C18:3n-3c	303266	50.57	957	957	69.60	4616	34
C20:1n-11c [A)]	1177	52.26	891	918	18.80	17.33	0.22
C20:1n-9c	11155	52.84	951	952	28.10	164.1	5.4
C21:0	229	55.36	760	905	36.80	4.02	0.20
C20:2n-6c	684	56.04	870	883	19.70	10.67	0.12
C22:0	406	59.81	847	867	69.30	6.27	0.07
C20:3n-3c	1801	60.01	882	928	39.00	30.81	0.25
C22:1n-9c	425	61.85	857	871	25.20	7.01	0.05
C24:0	93	67.16	690	729	31.10	1.55	0.06
C26:0 [A)]	165	73.27	664	716	12.90	2.96	0.13

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix

Appendix VI: Neutral lipid fraction

Table A.11: Summary table of the first sample preparation, using off-line SPE, for the neutral lipid fraction in *T. viridissima*, using 0.100 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A.area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C12:0	150	11.10	687	785	3.43	11.90	0.90
C14:0	1852	14.47	884	921	59.00	156.3	11.3
C16:0	26887	20.97	937	947	75.40	2342	199
C16:1 other [A)]	262	23.08	742	791	4.89	6.75	0.35
C16:1n-7c	4852	23.58	914	923	28.40	124.9	2.5
C17:0	415	26.11	659	726	1.86	11.00	0.93
C17:1n-7c	226	29.35	728	784	4.45	5.94	0.66
C17:0 (16-methyl) [A)]	356	29.63	725	803	11.20	10.16	1.07
C18:0	4616	33.43	833	920	25.80	131.8	3.5
C18:1n-9c	87292	37.57	951	952	9.38	2538	49
C18:1n-7c [A)]	907	38.19	810	844	3.72	26.39	0.39
C18:2n-6c	46961	44.16	950	972	26.20	1512	7
C19:1n-9c [A)]	140	45.66	745	796	9.04	4.97	0.27
C20:0	268	49.79	664	764	3.09	8.57	0.19
C18:3n-3c	9523	50.09	931	937	55.00	323.2	1.1
C20:1n-9c	542	52.06	812	861	14.10	17.79	0.54
C20:2n-6c	157	55.87	733	795	4.18	5.48	0.25
C20:4n-6c	357	59.79	788	855	39.90	13.52	0.72
C22:1n-9c	212	61.25	731	803	9.76	7.79	1.32

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix

Table A.12: Summary table of the second sample preparation, using off-line SPE, for the neutral lipid fraction in *T. viridissima*, using 0.100 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A.amount [µg]	S.D
C12:0	87	11.13	688	764	4.06	7.41	0.47
C14:0	1388	14.51	915	936	66.30	124.2	3.9
C16:0	25010	21.03	949	956	78.70	2295	158
C16:1 other [A)]	170	23.17	768	814	9.07	4.47	0.43
C16:1n-7c	4405	23.63	923	927	28.70	114.2	4.3
C17:0	247	26.19	705	750	6.37	6.50	0.53
C17:1n-7c	151	29.42	742	791	5.57	4.05	0.39
C17:0 (16-methyl) [A)]	319	29.69	760	815	22.60	9.32	0.94
C18:0	4382	33.51	920	943	68.50	125.9	4.6
C18:1n-9c	82915	37.69	921	927	3.58	2414	34
C18:1n-7c [A)]	918	38.32	848	868	6.05	26.86	0.86
C18:2n-6c	43420	44.25	959	960	36.50	1398	17
C19:1n-9c [A)]	108	45.72	715	762	6.16	3.89	0.32
C20:0	264	49.85	729	866	23.60	8.49	0.28
C18:3n-3c	9182	50.16	939	941	62.10	313.8	12.3
C20:1n-9c	523	52.11	830	872	18.10	17.41	1.06
C20:2n-6c	146	55.94	739	786	4.00	5.06	0.15
C20:4n-6c	264	59.84	800	866	42.50	10.12	0.47
C22:1n-9c	201	61.30	747	803	8.79	7.41	0.30

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix

Table A.12: Summary table of the third sample preparation, using off-line SPE, for the neutral lipid fraction in *T. viridissima*, using 0.100 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A.area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C12:0	37	11.13	655	764	4.46	7.06	0.77
C14:0	553	14.50	877	904	61.40	112.8	2.3
C16:0	8571	20.97	940	940	74.70	1802	34
C16:1 other [A)]	71	23.11	730	793	8.35	4.56	0.24
C16:1n-7c	1471	23.60	910	919	26.60	94.60	0.36
C17:0	84	26.10	621	681	0.88	5.55	0.12
C17:1n-7c	61	29.35	696	756	3.11	4.04	0.63
C17:0 (16-methyl) [A)]	134	29.60	670	744	3.44	9.57	0.38
C18:0	1485	33.41	811	849	34.30	105.8	1.4
C18:1n-9c	26877	37.50	925	928	3.50	1951	16
C18:1n-7c [A)]	292	38.17	796	836	5.11	21.22	0.13
C18:2n-6c	14724	44.13	950	969	30.40	1184	20
C19:1n-9c [A)]	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
C20:0	90	49.76	630	792	4.59	7.22	0.46
C18:3n-3c	2966	50.10	885	894	40.50	251.3	2.1
C20:1n-9c	197	52.04	741	804	7.33	16.19	0.23
C20:2n-6c	41	55.89	656	712	1.94	3.55	0.53
C20:4n-6c	94	59.79	760	821	29.60	8.95	0.43
C22:1n-9c	77	61.24	691	730	6.96	7.09	0.07

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix. n.d. = not detected

Appendix VII: Free fatty acid fraction

Table A.13: Summary table of the first sample preparation, using off-line SPE, for the free fatty acid fraction in *T. viridissima*, using 0.100 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C14:0	329	14.48	860	890	60.50	40.09	3.86
C16:0	7762	20.96	941	941	75.80	972	68
C16:1n-7c	917	23.58	839	869	14.60	37.96	2.42
C17:0	310	26.10	646	695	0.88	13.21	0.68
C17:0 (16-methyl) [A)]	133	29.62	641	731	2.64	6.11	0.37
C18:0	5092	33.46	929	954	69.50	233.7	10.6
C18:1n-9c	18242	37.39	914	924	2.91	853.4	11.2
C18:1n-7c [A)]	343	38.18	770	805	3.22	16.05	0.56
C18:2n-6c	24486	44.11	953	969	32.60	1306	12
C20:0	201	49.82	651	755	3.79	10.34	0.55
C18:3n-3c	2815	50.09	907	914	45.30	153.7	3.3
C20:1n-9c	127	52.07	755	790	5.80	6.71	0.30
C20:2n-6c	75	55.88	715	760	4.64	4.24	0.67
C20:4n-6c	337	59.78	834	879	36.30	20.58	0.99
C22:1n-9c	57	61.25	713	766	9.24	3.42	0.67
C20:5n-3c	83	63.57	-	-	-	5.05	0.41

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix

Table A.14: Summary table of the second sample preparation, using off-line SPE, for the free fatty acid fraction in *T. viridissima*, using 0.100 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C14:0	307	14.53	833	879	56.40	30.56	2.66
C16:0	7859	21.02	944	953	77.90	802.2	10.1
C16:1n-7c	915	23.65	846	890	17.60	33.70	6.90
C17:0	116	26.20	668	731	8.69	4.45	0.81
C17:0 (16-methyl) [A)]	84	29.69	602	685	3.73	3.46	0.95
C18:0	4621	33.54	918	952	53.40	190.3	18.0
C18:1n-9c	18024	37.49	929	931	4.41	756	52
C18:1n-7c [A)]	303	38.29	761	808	4.22	12.74	1.35
C18:2n-6c	21882	44.19	959	960	35.50	1046	67
C20:0	213	49.82	607	667	0.71	9.57	7.57
C18:3n-3c	2599	50.16	869	887	37.30	127.2	8.6
C20:1n-9c	87	52.12	684	714	4.99	4.15	0.23
C20:2n-6c	50	55.92	624	752	0.79	2.51	0.41
C20:4n-6c	242	59.84	825	870	37.40	13.26	1.33
C22:1n-9c	53	61.30	668	716	5.12	2.83	0.45
C20:5n-3c	65	63.61	702	749	5.39	3.54	0.23

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix

Table A.15: Summary table of the third sample preparation, using off-line SPE, for the free fatty acid fraction in *T. viridissima*, using 0.100 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability	A. amount [µg]	S.D
C14:0	98	14.51	759	802	34.80	26.83	2.26
C16:0	2501	20.98	926	943	69.80	701.2	32.2
C16:1n-7c	285	23.61	817	850	13.40	28.59	0.58
C17:0	53	26.15	576	632	1.36	5.53	0.92
C17:0 (16-methyl) [A)]	39	29.64	-	-	-	4.34	0.58
C18:0	1459	33.47	887	937	45.70	162.1	4.5
C18:1n-9c	5960	37.40	913	917	3.77	674.5	8.0
C18:1n-7c [A)]	124	38.21	706	732	6.78	14.04	2.04
C18:2n-6c	7229	44.13	945	971	22.40	932.9	8.9
C20:0	53	49.80	606	760	0.59	6.62	0.64
C18:3n-3c	944	50.14	855	867	32.90	124.7	0.9
C20:1n-9c	40	52.08	696	732	6.66	5.14	0.83
C20:2n-6c	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
C20:4n-6c	114	59.82	808	845	34.20	16.84	0.56
C22:1n-9c	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
C20:5n-3c	34	63.60	710	756	10.80	5.07	0.22

[A)] Not represented by a reference standard in the Restek Food Industry FAME Mix. n.d. = not detected

Appendix VIII: Polar lipid fraction

Table A.15: Summary table of the first sample preparation, using off-line SPE, for the polar lipid fraction in *T. viridissima*, using 0.100 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C14:0	56	14.58	664	721	10.50	8.60	1.02
C16:0	2878	21.08	928	953	71.20	456.1	18.6
C18:0	2244	33.61	828	891	25.70	360.9	3.5
C18:1n-9c	1713	37.55	838	893	3.91	280.9	8.2
C18:2n-6c	1131	44.24	893	935	18.40	211.5	6.6
C18:3n-3c	77	50.21	718	835	7.40	14.77	0.41

Table A.16: Summary table of the second sample preparation, using off-line SPE, for the polar lipid fraction in *T. viridissima*, using 0.100 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C14:0	73	14.60	622	686	12.90	9.62	1.03
C16:0	2444	21.09	923	946	72.00	335.6	14.4
C18:0	1474	33.61	849	921	42.80	205.7	8.2
C18:1n-9c	1905	37.55	870	885	7.23	270.9	17.9
C18:2n-6c	1162	44.24	890	936	16.40	188.5	12.9
C18:3n-3c	84	50.24	686	824	10.30	13.9	0.4

Table A.17: Summary table of the third sample preparation, using off-line SPE, for the polar lipid fraction in *T. viridissima*, using 0.100 g of sample (d.w.). Values for matchfactor, reverse matchfactor and probability were acquired through NIST 08 library searches based on spectral information. Also included are the average areas and retention times. Average amount denotes concentration relative to initial sample size. N=3

FAME	A. area	Retention [min]	Matchfactor	R. matchfactor	Probability [%]	A. amount [µg]	S.D
C14:0	26	14.60	589	601	1.23	7.53	1.81
C16:0	1207	21.07	889	944	66.10	357.1	22.5
C18:0	804	33.56	740	807	12.50	241.6	11.4
C18:1n-9c	682	37.48	816	826	4.17	208.9	8.5
C18:2n-6c	510	44.20	803	878	3.98	178.1	20.0
C18:3n-3c	42	50.20	-	-	-	14.98	1.46

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