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Influence of different pre-treatments and drying methods on the dry fractionation behavior of mealworm larvae (*Tenebrio molitor*)

submitted by

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Statutory declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

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List of abbreviations

Acronyms	
[%db]	% dry base
AH / Alkali	Alkaline hydrolysis
Blanched / B	Blanched larvae
C	Chitin
DM	Dry mass
FAO	Food and Agriculture Organization of the United Nations
Fresh / F	Living unprocessed larvae
NfE	Nitrogen free extract
P	Protein
TA	Texture analysis
TML	*Tenebrio molitor* larvae
SD	Standard deviation
TDF	Total dietary fiber

List of sample codes

Abbreviation	
DwB	Oven drying without blanching
DwF	Oven drying without freezing
DaD	Oven drying and defatting
FBD	Fluidized bed drying
FD	Freeze-drying
OD80	Oven drying at 80 °C
OD60	Oven drying at 60 °C
VD	Vacuum drying

1. Zusammenfassung

Insekten werden durch die stetig wachsende Weltbevölkerung als ernstzunehmende Alternative in der zukünftigen Lebensmittel- und Futtermittelversorgung diskutiert. Die Anreicherung des hochwertigen Proteins ist dabei die größte Herausforderung. Eine vielversprechende Technologie ist die trockene Fraktionierung, die, im Gegensatz zu der nassen Extraktion, weniger Energie und Ressourcen benötigt. Ziel dieser Studie war es, den Einfluss von verschiedenen Trocknungsmethoden und Vorbehandlungen auf das trockene Fraktionierungsverhalten von Mehlwurmlarven zu untersuchen. Dazu wurden die Larven unter standardisierten Bedingungen getrocknet (Ofentrocknung, Gefriertrocknung und Wirbelschichttrockung) und teilweise entfettet mittels überkritischen CO_2 und nach Vermahlung via Sieb-Analyse (0 – 1400 µm) fraktioniert. Diverse Analysen wurden verwendet, um die Veränderungen während dieser Prozesse zu bewerten. Die Ergebnisse zeigen, dass eine erhöhte Trocknungstemperatur eine dunklere Farbe (Bräunungsreaktionen) und ein Schrumpfen der Larve (Gewebezerfall) zur Folge hatte. Hingegen wurde die Farbe der gefriergetrockneten und entfetteten Proben, durch die Extraktion von Melanoidin, aufgehellt. Eine Größenveränderung konnte nicht festgestellt werden. Je höher die Temperatur und Trocknungszeit, umso härter und kompakter wurde die Struktur der Larven bei der Heißlufttrocknung. Im Gegensatz dazu wiesen die gefriergetrockneten und entfetteten Proben eine porös-brüchige Textur auf. Dies hatte einen signifikanten Einfluss auf das Mahl- und Trennverhalten. 87 – 89 % (m/m) der heißluftgetrockneten und vermahlenen Larven hatten eine Partikelgröße über 500 µm; Gefriertrocknung 62 % (m/m), Entfettung 58 % (m/m). Dazu konnte eine Proteinanreicherung in kleineren Fraktionen (<500 µm) bei dem teilweise entfettetem Material (+5 % zur größten Fraktion) und der Wirbelschichttrocknung (+3 %) nachgewiesen werden. Chitin reicherte sich in größeren Fraktionen (>500 µm) an; besonders bei der Gefriertrocknung (+12,5 % zur kleinsten Fraktion), der Entfettung (+4,7 %) und der Wirbelschichttrocknung (+4,2 %). Entfettung und geringe Hitze führten zu einer gleichmäßigen Partikelgrößenverteilung und einer guten Trennbarkeit von Protein und Chitin. Die Heißlufttrocknung verursachte eine Partikelkonzentration im Bereich 500 – 1000 µm. Protein und Chitin konnten kaum getrennt werden, da dessen Bindung durch Hitzeeinwirkung zusätzlich verstärkt wurde. Diese Daten zeigen eindeutig, dass Trocknungsmethoden und Vorbehandlungen einen Einfluss auf das Fraktionierungsverhalten von Mehlwurmlarven haben. Die trockene Fraktionierung kann als eine zukunftsfähige Alternative zur konventionellen Proteingewinnung angesehen werden.

2. Summary

Edible insects have emerged as a sustainable source for food and feed for the rapidly growing global population. Enriching the high valuable components such as protein to obtain intermediates, which can be used as nutritious substitutes, is one of the key challenges. An alternative to the commonly used wet protein extraction is the simple, energy- and resource-friendly dry fractionation. Main goal of this thesis was to determine whether different pre-treatments and drying methods influence the dry fractionation behavior of mealworm larvae. For this purpose, larvae were oven dried at different temperatures, freeze dried, fluidized bed dried and partially defatted, followed by roller-milling and sieve-fractionating under standardized conditions. Physio-chemical methods were used to analyze and evaluate the changes that occurred during the processing. Sieve analysis was used to determine the fractionation behavior (0 – 1400 µm). The results show that elevated drying temperatures led to pronounced darkening (browning) and shrinkage (tissue collapse). Freeze drying and partially defatting caused brightening (melanoidin extraction, no browning) and conservation of length and diameter. Extended drying times and high temperatures led to a hard and compact structure, whereas low temperatures and defatting led to a brittle and porous structure. This affected the fractionation and milling behavior significantly. 87 – 89 % (m/m) of the oven dried material had a particle size over 500 µm, freeze-dried 62 % (m/m) and defatted only 58 % (m/m). Additionally, an accumulation of protein in smaller fractions (<500 µm) was observed for the defatted (+5 % compared to biggest fraction) and fluidized bed dried (+3 %) samples. Chitin was enriched in larger particle sizes by freeze-drying (+12.5 % compared to smallest fraction), defatting (+4.7 %) and fluidized bed drying (+4.2 %). Low thermal stress and partially defatting caused an even distribution of particle sizes and showed a good separability of protein and chitin. Oven drying led to a concentration in the particle size ranging from 500 – 1000 µm. Protein and chitin could not be separated due to temperature induced strengthening of the bond between those components. These results show that different drying methods and pre-treatments influence the fractionation behavior. Dry fractionation uses less water and energy to create intermediates, while obtaining the native functionality of the protein. This data can be used to establish and optimize processes to extract insect protein.

3. Introduction and objective of this thesis

At a growth of six million people per month in the near future over nine billion people will inhabit the earth in 2050 (Bongaarts 2009). Factors like the climate change, energy wastage, plant diseases or inequality of food distribution will impair the supply of resources and will inevitably lead to a fundamental re-thinking of our production methods (Gahukar 2011). In 2014 the average European consumed 95 g protein with 65 % covered by meat (developing countries 56 g protein with 25 % meat). Fast growing areas like China will increase their meat consumption by 9 % by 2030 (Mlcek et al. 2014). Alternative sources and technologies must be established to satisfy the demand for easy to produce, always available and sustainable high-quality protein. Regarding these challenges, the use of edible insects for food and feed applications has emerged as a potential alternative to the consumption of conventional animal or plant derived raw materials.

The entomophagy -the consumption of insects- has supplied humans with nutrients for thousands of years and is nowadays pushed forward by the rediscovery of the manyfold benefits of insects. A faster growth rate and the immense biodiversity makes them far superior to conventional warm blooded livestock. Less food energy and nutrients are needed to generate more protein per kilogram biomass, with the possibility of using organic waste as feed (Premalatha et al. 2011, Ramos-Elorduy et al. 2002). Although these promising characteristics makes them more than suitable for sustainable future use, the western society may refuse to implement whole insects in their daily diet, in contrast to the long history of entomophagy in Asia, the Sub-Sahara area or Middle America (Ramos-Elorduy 2009). This acceptance can be increased by implementing processed insects as a substitute for conventional plant- or animal-derived bulk materials in common food (Tan et al. 2015).

The technology necessary to produce protein flours, concentrates or isolates is therefore one of the most important field of interest. Many factors and processes influence the enrichment of insect protein, including the selection of species, variability of drying and comminution, or the extracting method. This thesis will evaluate the influence of different pre-treatments (blanching, freezing and defatting) and drying methods (oven drying, freeze drying and fluidized bed drying) on the dry fractionation behavior of mealworm larvae (*Tenebrio molitor*, TML).

Furthermore, dry fractionation seems to be a promising alternative to the commonly used wet fractionation. Although aqueous extraction produces high protein yields, a manipulation of the techno-functional properties by high salt concentrations and alkaline pH values is indicated (Bußler et al. 2016). The potential of dry fractionation processes is shown by studies on plant protein. Far less water and energy is used while enriching protein with native functionality (Schutyser and van der Goot 2011). In this context, the high protein quality and its natural properties like water binding capacity could be maintained (Yi et al. 2013).

The purpose of this study is to investigate how the physical and chemical properties of the mealworm larvae are altered by these processing steps / pre-treatments and how these changes influence the composition of the size fractions after sieve classification. With the information gathered from this study, drying and processing methods can be optimized. Improving existing methods for processing edible insects can help to further develop a fundamental knowledge about this future-oriented resource.

4. Theoretical background

To fully understand the background and reasons behind this idea, the following subchapters will provide an overview of relevant topics relating to edible insects. The worldwide entomophagy, composition of selected species, important components and processes will be further specified.

4.1. Edible insects

"There are, however, some flying insects that walk on all fours that you may eat: those that have jointed legs for hopping on the ground. Of these you may eat any kind of locust, katydid, cricket or grasshopper" (Leviticus 11:21-22).

Since the early stages of human evolution, the entomophagy has been part of our diet. Like our primate relatives, hunter and gatherer utilized not only plants and animals but also insects as an always available source of energy (Morris 2008). It is estimated that more than two billion people still have insects included in their traditional diet (van Huis and Food and Agriculture Organization of the United 2013). Contrary to regions where insects are still consumed, Europe and most western countries have turned away from entomophagy which led to a loss of know-how and lower acceptance of this practice (Hartmann et al. 2015, Mlcek et al. 2014). A survey on European non-vegetarians showed that only 12.8 % men and 6.3 % women are willing to implement insects in their daily diet. Acceptance decreases with advancing age but does not depend on level of education (Tan et al. 2015). Nowadays, more and more interest is shown in entomophagy. Globalization, cross-cultural connections and a rising awareness for the use of the global resources led to a reevaluation of insects as food. The Food and Agriculture Organization (FAO 2013) reported that over 1900 different species are eaten worldwide: beetles (31 %), caterpillars (18 %), bees, wasps and ants (14 %), grasshoppers, locusts and crickets (13 %), cicadas, leafhoppers, planthoppers, scale insects and true bugs (10 %), termites (3 %), dragonflies (3 %), flies (2 %) and others (5 %). The following Figure 1 shows the worldwide distribution of recorded species of edible insects. Reliable values on the consumption of insects are hard to obtain because the majority is collected in the wild. Although no direct correlation can be made, this map shows which countries have a variety of edible insects in their diet. Central and South America, Sub-Saharan Africa or South-East Asia have a long tradition of entomophagy, whereas Europe, North America or Russia have no significant use of insects as food.

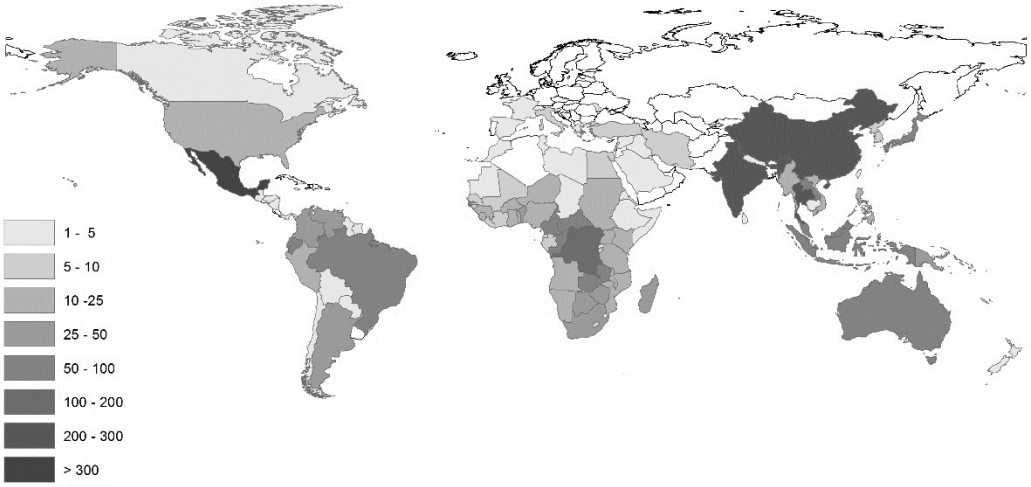

Figure 1: Recorded number of edible insect species, by countrySource: Centre for Geo Information, Wageningen University, based on data compiled by Jongema, 2012 (Halloran et al. 2014).

4.1.1. Composition of edible insects

The wide variety of species makes insects the most diverse living beings on earth. Differences can be found between the classes as well as within the individual families. In addition, the stage of development (egg, larvae, pupae, adult) plays an important role (Mlcek et al. 2014). This is represented by the ratio of protein, fat, fiber (chitin), NfE (Nitrogen free extract) and ash. The average composition of selected edible insect orders is listed in Table 1.

Adult insects, especially crickets, grasshoppers, locusts and cockroaches consists mostly of protein, whereas insects in the larval stage (silkworms, larvae, grubs) have the highest amounts of lipids. Most fiber (chitin) is found in flies and bugs. Since this rigid structural polymer is constantly degraded and newly formed, some stages of development like larvae, eggs and pupae have a varying chitin content, which is mostly lower than in their adult state (Merzendorfer and Zimoch 2003).

Table 1: Composition (protein, fat, fiber, NfE and ash) of selected insect orders based on dry matter [%db] (Rumpold and Schlüter 2013, Melo-Ruiz 2011).

Insect	Nutrient [%db]				
	Protein	**Lipids**	**Fiber**	**NfE**	**Ash**
Blattodea (cockroaches)	57.3	29.9	5.31	4.53	2.94
Coleoptera (beetles, grubs)	40.69	33.4	10.74	13.2	5.07
Diptera (flies)	49.48	22.75	13.56	6.01	10.31
Hemiptera (true bugs)	48.33	30.26	12.4	6.08	5.03
Hymenoptera (ants, bees)	46.47	25.09	5.71	20.25	3.51
Lepidoptera (butterflies, moths)	45.38	27.66	6.6	18.76	4.51
Odonata (dragonflies, damselflies)	55.23	19.83	11.79	4.63	8.53
Orthoptera (crickets, grasshoppers, locusts)	61.32	13.41	9.55	12.98	3.85
Maguey grub (silkworm)	30.88	58.55	0.12	8.16	2.29
Ahuahutle (mosquito egg)	53.6	4.33	3	18.07	21

4.1.2. Tenebrio molitor

With 661 documented species, the *Coleoptera* is the most consumed family of insects worldwide. *Tenebrio molitor*, the yellow mealworm, is one of the most common insects used for human consumption and is also mass-produced as feed for pets and animal livestock (Dossey, Tatum and McGill 2016). The already established production, the high availability, the simple farming and the already existing research makes mealworm larvae the most suitable raw material for this study. Mealworms are part of the darkling beetle family. Like all insects, their body contains three sections: head, thorax and abdomen. Within their life span they go through a metamorphosis, which can be classified in four different phases: 1) egg, 2) larvae, 3) pupae and 4) adult (Schaffer 1999). As seen in Table 2, the composition of the insect varies in each development stage. A high protein and chitin content, as well as a low lipid content is characteristic for the adult stage. The pupae contain the less amounts of protein but similar lipid and chitin values as the larval stage. Low chitin and acceptable protein contents makes the larvae the best choice for processing. The reasons why protein and chitin are the most influencing factors in the production of mealworm flour are elaborated in the following subchapters.

Table 2: Approximate composition [%] of three development stages of Tenebrio molitor (Nowak et al. 2016).

	Water [%]	**Protein [%]**	**Lipids [%]**	**Fiber/Chitin [%]**	**Ash [%]**
Adult	62.1	24.1	6.1	6.8	1.4
Larva	62	17.9	13.1	2.4	1.5
Pupa	61	12	12.9	2	1.3

4.1.3. Chitin

“Chitin is the most abundant natural amino polysaccharide and is estimated to be produced annually almost as much as cellulose” (Ravi Kumar 2000). This biopolymer is the main structural element in exoskeletons of insects, crustaceans, shrimp, crabs and fungi. The chemical structure of one section is displayed in Figure 2. It rarely exists in a purified form and mostly forms a complex with other components like protein (Finke 2007). Even though no health threats are known, the poor digestibility makes it not suitable for human consumption. It can be applied as a fiber, film, sponge or powder for various applications (food, cosmetics, biomedical and pharmaceutical applications). As shown in Table 1, the chitin content in insects varies from 0.12 %db to 13.56 %db (3 – 10 % (Cauchie 2002)).

This biodegradable and renewable material with antibacterial, fungistatic and thickening properties can be used as an resource-friendly alternative for commonly used ingredients (Rinaudo 2006). Studies on poultry showed, that a combination of whey and 2 or 0,5 % chitin improved the feed conservation rate (FCR) an enhanced the intestinal microflora. Chitin improves the utilization of milk lactose and could therefore be used for people with such digestion problems (Ravi Kumar 2000). The binding of specific fatty acids, bile acids or phospholipids is another interesting ability which makes it potentially suitable for the prevention of coeliac disease (Muzzarelli 1996). An extraction of this versatile polymer is not only beneficial for the protein yield but could also enhance the value of the different fractions due to its promising abilities.

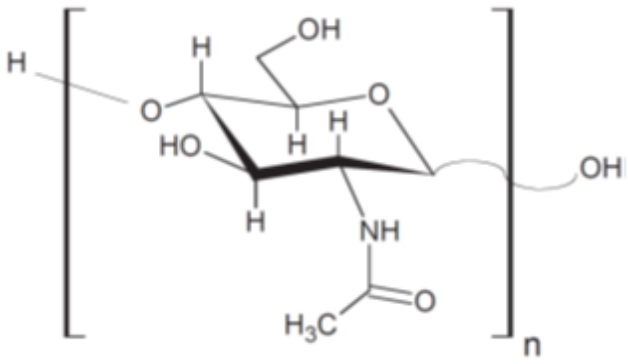

Figure 2: Chemical structure of chitin (Merzendorfer and Zimoch 2003)

4.1.4. Protein

Protein extracted from insects can be used in various ways to substitute animal- or plant-derived protein. A comparison to conventional warm blooded livestock shows that the crude protein in mealworm larvae (51.5 %db) is significantly lower than the lean proportion of poultry (93 %db), beef (86 %db) or pork (73 %db) (Zhao et al. 2016, Branscheid 1998). Though the digestibility varies by species of insect it is similar to animal protein (insects: 77 % – 98 %, egg: 95 %, beef: 98 %) and higher than most plant-derived protein (Mlcek et al. 2014, Ramos-Elorduy et al. 1997). This emphasizes the suitability for the human consumption. In addition, the *Tenebrio molitor* larvae have a good profile of essential amino acids. Except methionine, the quantity of essential amino acids meets the dietary guidelines for humans and are comparable to those of soy or fish (Azagoh et al. 2016).

The biggest challenge for obtaining protein from insects is the purification as well as the separation from chitin and fat. A commonly used method for the isolation is the wet extraction. Alkaline pH values and high salt concentration are applied to alter the solubility and increase the protein recovery (Bußler et al. 2016). This has a negative influence on the functionality respectively the usability in food applications. However, a dry fractionation does not apply any chemical stress to the protein and obtains the natural status, which makes it a promising alternative to the established technology.

4.1.5. Additional fields of interest

Since the work on edible insects is still very fundamental, a lot of research in this field is still to be done. Especially topics related to food safety are important for the introduction into the European food chain. Toxicity, pathogens, contaminants, pesticides or anatomic differences could potentially be a threat for consumers (van Huis and Food and Agriculture Organization of the United 2013). It is assumed that these risks are similar to those of conventionally used livestock. An example for this assertion are studies on the microbiological aspects of insects. Although fresh insects can be highly contaminated, the type of bacteria is similar to food harvested from soil. A preservation step like heat inactivation, fermentation or acidification is necessary but can successfully reduce the contamination risk (Klunder et al. 2012, Stoops et al. 2016).

Only few studies have been published on the potential allergenicity of insect proteins. The similarities to shellfish, which are considered an allergen, underlines the importance of this topic. Researches on leaf beetles or biologically derived carmine showed that some patients reacted sensitively to exposure. Silkworm pupa are known to be allergenic. Over 1000 Chinese people per year experience anaphylactic reactions (Belluco et al. 2013). Each insect species differs in composition and allergenic potential. A broad investigation on the exact triggers is necessary to ensure food safety.

Another interesting field is the production on an industrial scale. Gathering edible insects in the wild is still the most commonly used method worldwide. Projects in Thailand showed that larger scale farming methods can successfully produce higher amounts of edible insects in a sustainable way (Hanboonsong et al. 2013). Although insects are produced as feed for domestic and aquatic animals, the capacities in Europe are not sufficient to supply the demand for protein. For example, the biggest Chinese insect processor exports 200 t of dried mealworms annually. However, producing food-grade quality on this level is still not accomplished but is promoted by initiatives such as *PROtoINSECT* of the European Union. Tests on cultivation and breeding showed promising results for a sustainable production (Smith and Barnes 2015).

4.1.6. Legal regulations

Countries all over the world have a different approach to define which species is suitable or not suitable for human consumption. Southeast Asia has a long tradition of entomophagy and most countries of this area generally have no strict regulations for insects as food. Thailand as the biggest breeder for crickets worldwide is currently working on guidelines and good agricultural practices for breeding insects. South Korea and China have recently implemented some species in their food law. Contrary to that, insects are not and have not been a part of the daily diet in western countries. The USA and Canada do not consider insects as novel food as long as they have a history of safe consumption. Additional to that the production must follow the same strict rules that are standard for food which include the bred for human consumption, bacteriological tests and good manufacturing practice. Australia decided, that some species (mealworms, crickets and moths) are considered traditional food and thus are not novel food (van Huis and Food and Agriculture Organization of the United 2013).

The European Union has a different approach to introduce products into the market. If certain foods or processing methods were not consumed or used to a significant degree by humans prior to March 1997 it is considered a novel food. Although the standards for food law should be the same in all countries which are member of the EU, some countries do not consider insects as novel food. Belgium, Great Britain, the Netherlands and Denmark regulate their marketing and consumption more liberal than others. In contrast Italy and Germany consider all insects as novel food (Lähteenmäki-Uutela and Grmelová 2016).

These different interpretations along with time-consuming and expensive approval process for novel food led to a revision of the regulation. Insects are now implemented into the new version (EU) No 2015/2283 (European Parliament 2015): "However, on the basis of scientific and technological developments that have occurred since 1997, it is appropriate to review, clarify and update the categories of food which constitute novel foods. Those categories should cover whole insects and their parts." Although this statement is open for interpretation insects can now be considered as food that is also traditional in western countries and thus be easier approved in the food market.

4.2. Fractionation

Isolating and extracting components from raw material to obtain enriched fractions of various nutrients can be performed by a fractionation. This process can be performed with two different approaches: wet and dry. Aqueous fractionation uses solvents to extract the desired value proportion, whereas dry fractionation only uses physical separation methods (e.g. air/sieve classification).

4.2.1. Wet and dry fractionation

The following Figure 3 displays an exemplary process scheme for the production of legume protein concentrates (Schutyser et al. 2015). As seen on the left side of the flow diagram, the milled legumes are suspended in water and starch is separated by a hydroclone. This step is similarly conducted for insects with a removal of chitin. After adjusting to an alkaline pH value the protein is precipitated, neutralized and dried. On the other hand, the dry process is only composed of a milling and classification step. This shows the advantages and disadvantages of both procedures.

Wet fractionation can efficiently extract and separate various components and is commonly used to produce highly concentrated protein isolates (Schutyser and van der Goot 2011). A high demand for water and energy results in ecological problems when applied in larger scale production. Additionally, solvents, added salts and the pH value influence the protein functionality altering gelation or emulsifying activity, as shown for pigeonpea and cowpea protein isolates (Mwasaru et al. 2000). However, dry fractionation only uses physical differences between the particles to enrich certain components in size fractions. Without chemical stress and low physical stress, the native status of the protein can be maintained. The simplicity of the process and a low consumption of energy and resources makes the dry fractionation a promising alternative for processing insects. Although lower yields are obtained, protein concentrates with functional properties similar to the native condition can be produced, as shown for dry fractionated yellow field peas (Pelgrom et al. 2013, Pelgrom 2015).

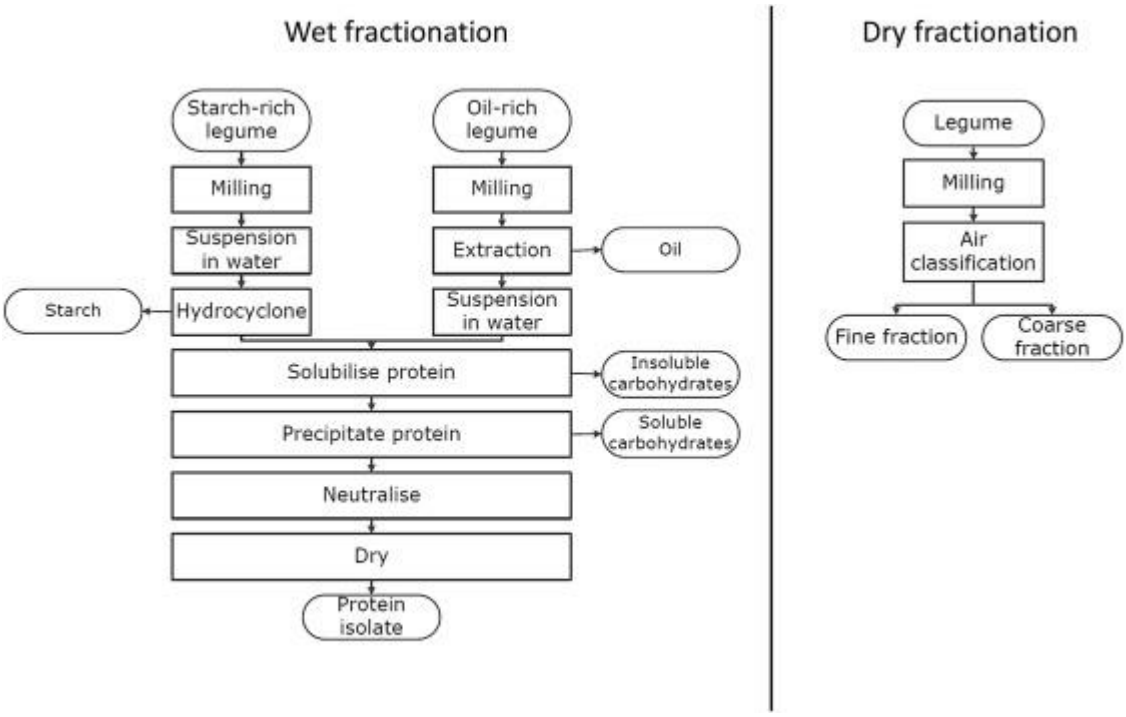

Figure 3: Schematic illustration of wet (left) and dry (right) fractionation process (Schutyser et al. 2015)

4.2.2. Application and advantages of the dry fractionation

Dry fractionation is a commonly used technology used to separate components based on their differences in density or size. Studies on wheat showed that dry processed have better defined properties and can produce fraction with enhanced nutritional interest (Hemery et al. 2007). The fractionation behavior of legumes such as cowpea flours showed that there is a significant difference in the composition of different size fractions. This can be used to obtain flours with different chemical and physical properties (Kerr et al. 2000). Similar results were found in barley meals. Variations in the pre-treatment of barley influenced the composition of the fractions.

It is suggested that the combination of methods that are used for processing have to be adjusted for the enrichment of specific valuable components (Liu, Barrows and Obert 2009). Research in other fields, like the palm oil production, showed that the dry method is often advantageous to the established detergent fractionation (Dijkstra 2012). These examples emphasize the aim of this study.

5. Material and methods

The practical work was performed in the laboratories and pilot plants of the *Institute of Food Science and Technology of the University of Natural Resources and Life Sciences (BOKU) Vienna*. The following chapters describe all processes and analytical methods used within the experimental part of the present thesis. The general work flow is presented in Figure 4. The illustration depicts three different levels of the experimental procedure. 1) Processing level, 2) Material level and 3) Analytical level.

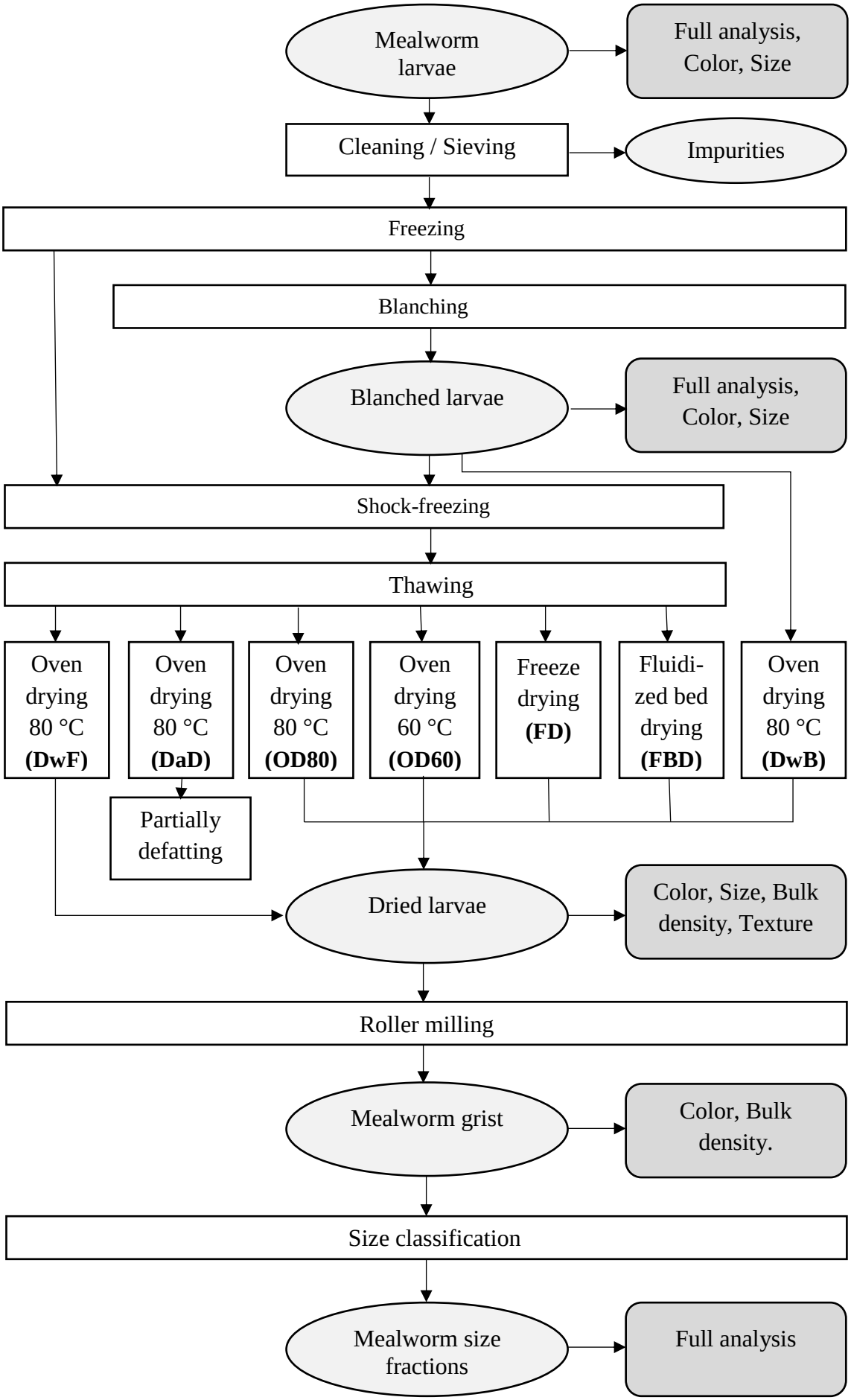

Figure 4: Flow diagram of the experimental procedure of the present thesis including process level (white, rectangle), material level (grey, ellipse) and analytical levels (dark grey, rounded corners). Sample codes are

5.1. Raw material and pre-treatment

Live mealworm (TML, *Tenebrio molitor*, Coleoptera) larvae were purchased by a pet food vendor (Dragon Terraristik, Duisburg, Germany). After delivery, the TML were cleaned by sieving through 3 mm screening size to remove substrate residues, frass and other impurities.

Further or fully developed life stages such as pupae or adults were manually removed as well. Aliquots of about 3.7 kg TML were transferred into a nylon bag which was closed tightly. Subsequently, the single aliquots were inactivated by blanching in boiling water for a period of 10 min at a larvae-water ratio of 1:12 (w/w). The T,t – profile was recorded using two thermos-couples placed in the water and in the thermal center of the bag. The blanched larvae were removed from the bags and evenly distributed on metal trays to cool down. Afterwards the trays were transferred into a shock freezer (blast chiller/freezer, IFR201R, SAGI S.p.a., Ascoli Piceno, Italy) and frozen for 30 min at -40 °C (program HARD -18 °C). Finally, each batch was packed into air-tight plastic bags and stored at 30 °C until further use.

5.2. Pretests

Currently no standard methods are available to test the physical abilities or characterize the TML. Small scale tests before the main analysis were necessary to select methods that obtain reproducible parameters. Additional to various textural analyses and optical measurements the kinetics of different drying methods were determined.

5.2.1. Drying kinetics

The selection of methods was based on the thermodynamic principle, the availability of machines and the practical use. Four processes with different conditions were analyzed including freeze drying, vacuum drying and oven drying (80 °C; 60 °C). No pretest was performed for the fluidized bed drying because the in-process weight control was not possible for the closed batch-mode. For each triple determination three aluminum containers were filled with 5 g thawed TML. Every 30 minutes the weight of each container was determined and documented. Aim was to reach a constant mass and thus determine a minimal drying time. Table 3 shows the different default settings.

Table 3: Default parameters [°C, bar, min] for pre-test drying kinetics

	temperature [°C]	pressure [bar]	time [min]
freeze drying (FD)	-49	0.1	1610
vacum drying (VD)	45	0.1 – 0.2	1640
oven drying (OD)	80	1.013	1610
	60	1.013	1391

5.2.2. Additional pretests

In addition to various TA-pretests, two experimental methods to characterize the physical abilities of the TML were tested.

The friabilimeter is used to analyze the friability and brittleness of malt by rotating it in a drum with a rough surface while applying physical stress. The resulting abrasion is measured and an indicator for the structure of the malt. Under certain circumstances the test showed reproducible results for whole dried TML but was not used for further analysis because the method needed more testing.

The second pretest followed the same principle. Whole dried TML as well as 40 glass beads were applied to a sieve (500 µm). After 10 minutes shaking the abrasion was measured. This method showed promising results and could be used for the characterization of various insects. However more research is necessary.

5.3. Drying methods

Drying is a highly complex process influenced by multiple factors. The dehydration changes the physical and chemical properties of the material, which in most cases is disadvantageous to quality of the product (Lewicki 2006). The drying methods for this study were selected based on availability, commercial use and their technical functionality. Drying kinetics were determined by pre-tests and upscaled for a larger quantity. Vacuum drying gave promising results on a small scale but was discarded because the used dryer (vacuum dryer, VD 23/53/115, Binder GmbH, Tuttlingen, Germany) was not suitable for larger amounts auf wet material. Table 4 gives an overview of the methods (triple determination).

Table 4: Overview of drying methods with abbreviation and default parameters [°C, h]

method	abbreviation	temperature [°C]	time [h]
Freeze drying	FD	-49	48
Oven drying	OD80	80	7
	OD60	60	24
Oven drying without blanching	DwB	80	7
Oven drying without freezing	DwF	80	17
Oven drying and defatting	DaD	80	7
Fluidized bed drying	FBD	60	3

5.3.1.1. Conventional oven drying

The oven drying process is the most common and simple way of drying substances. Hot air heats the sample, water diffuses to the surface and evaporates, the higher the temperature and circulation the faster the process.

In a first step 1730 g of the blanched, frozen (-30 °C) TML were spread out on three oven trays covered with aluminum foil and thawed at room temperature (~20°C) for 1 h. The oven (Universal oven dishwasher, UFP800DW, Memmert GmbH & Co. KG, Schwabach, Germany) was preheated to 83 °C for the methods OD80, DwB, DwF and DaD and 62 °C for method OD60. The settings resulted from a slight difference of target and actual internal temperature which was monitored with an external thermometer. Circulation level was set to 40 %.

The trays were evenly distributed in the oven and left to dry for 7 h (DwF 17 h). The trays were then removed from the oven and cooled at room temperature for 1 h. After weighing the dried TML were packed into a plastic bag and sealed air-tight.

5.3.1.2. Freeze drying

The process of freeze drying involves three steps (1) the freezing stage in which the sample is completely frozen, (2) the primary drying in which the water sublimates in a vacuum and (3) the secondary drying in which the remaining liquid water is vaporized (Duan et al. 2016).

Although this method is considered highly energy-consuming compared to conventional drying, it is used to dry whole insects because the structural integrity is not affected by physical stress. The settings and time needed to dry the TML was determined in pre-tests and upscaled.

In preparation 1730 g worms (-30 °C) were divided into 15 plastic containers and sealed with a paper. The containers were placed into the vacuum chambers and everything was sealed air-tight. Freeze dryer (Freezone 6, Labconco, Kansas City, USA) and vacuum pump (rotary vane pump, P122, Gardner Denver Thomas GmbH, Memmingen, Germany) were switched on. Pressure was set to 200 mbar and temperature to -49 °C. After 10 h and 24 h the pressure was released and the chambers were opened to remove the accumulated ice. After 48 hours, the process was stopped. The containers were removed and the content was bundled in a plastic bag. After weighing and air-tight sealing the sample was stored at room temperature.

5.3.1.3. Fluidized bed drying

The method of fluidized bed drying is commonly used for drying powder or granular products. A heated gas flows through the solid phase -in this case the larvae- and transfers the particles into a “fluid like” state at a certain gas velocity. The material is heated and the moisture is transported into the surrounding gas (Syahrul, Dincer and Hamdullahpur 2003).

A batch of 1730 g frozen larvae (-30 °C) was used for each determination. The fluidized bed dryer was set to an air velocity of 500 m³/h and a bed temperature of 60 °C. The entering air flow had a temperature of 95 °C but decreased with rising bed temperatures. Inlet temperature, velocity, bed temperature and outlet temperature was recorded. Velocity kept constant at 7.9 m^3/min – 8.1 m^3/min with a differential pressure in the bed of 15.7 bar in the beginning which decreased to 12.7 bar at the end of the process due to weight changes of the larvae.

5.4. Supercritical CO_2 defatting

Approximately 22 – 23 % of the TML dry matter is fat. Insect lipids have a very specific and unique composition (Tzompa-Sosa et al. 2014) and could be used in various food applications. The processing quality is highly influenced by the fat content. Therefor a significant removal of lipids could improve milling and size fractionation. To compare milling behavior of different drying methods the extraction was performed before the milling step. Defatting mealworms in their original shape is not beneficial to the process because a decreasing particle size leads to a high oil yield, whereas big particles impede the extraction (del Valle and Uquiche 2002).

Whole mealworms were hot-air dried at 80 °C for 7 h. After cooling down to room temperature two batches (252 g, 218 g) were applied to the extraction tube. Following parameter (Table 5) were used to achieve the highest yield (Purschke et al. 2017).

Table 5: Default temperature [°C], pressure [bar], flow rate [kg/h] and time for supercritical CO_2 defatting

Extractor pressure	**Separator pressure**	**Extractor temp.**	**Separator temp.**	**CO_2 flow rate**	**time**
[bar]	[bar]	[°C]	[°C]	[kg/h]	[min]
400	60	45	40	16–19	360

The scheme in Figure 5 shows a supercritical CO_2 pilot-plant. CO_2 is pumped from the storage tank into the pressurized extraction vessel where the fat is extracted from the mealworm larvae. The supercritical gas with the extracted fat is then pumped into to separator where a lower pressure induces a separation of the two phases. An optional second separator (11) was not used. Subsequently the CO_2 is cooled and pumped back into the storage tank.

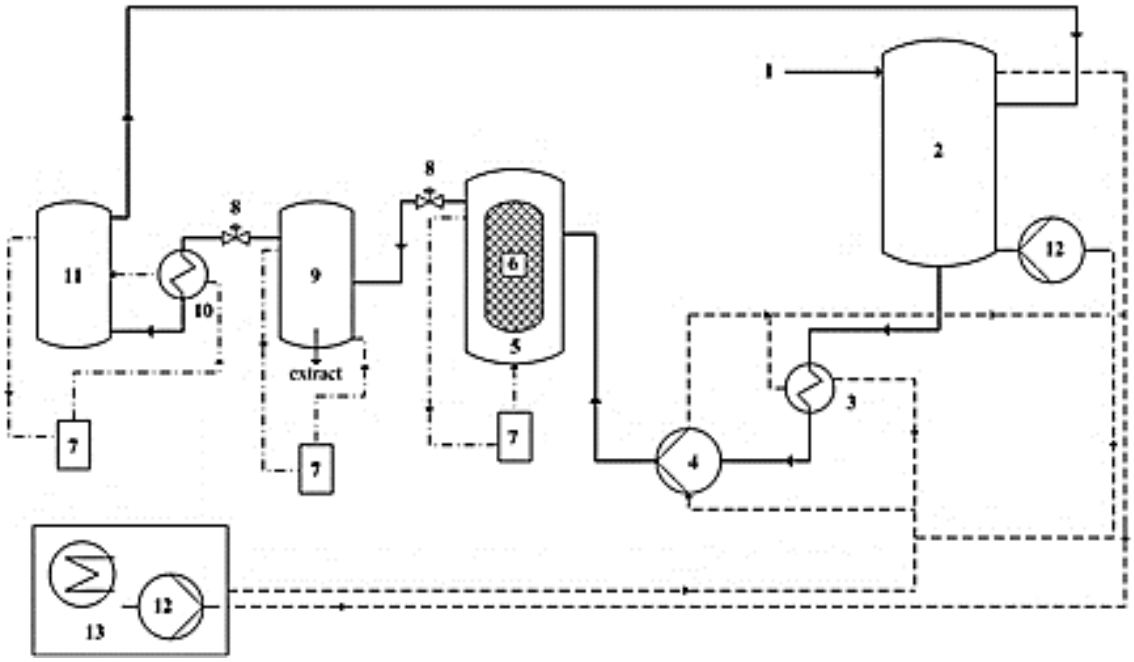

Figure 5: Scheme of pilot-scale SFE apparatus (1) CO_2 bottles, (2) CO_2 storage tank, (3) cooler, (4) piston pump, (5) extractor, (6) extraction vessel, (7) heating unit, (8) automatic pressure control valve, (9) separator 1, (10) heat exchanger, (11) separator 2, (12) centrifugal pump, and (13) cooling unit. CO_2 circuit (solid line), heating circuit (dash-dot line), and cooling circuit (dashed line) (Purschke et al. 2017).

5.5. Production of mealworm fractions

A key objective of this thesis is to determine if different drying methods influence the physical properties of the TML. Therefor the dried insects were milled and classified to obtain different size fractions.

5.5.1. Roller milling

The milling conditions of this process were determined by pretest using dry frozen TML. An optical assessment of the meal, produced by different milling gaps (0.5 mm, 0.4 mm, 0.3 mm; 0.2 mm) roller mill, Viktring, 1954), was performed. The 0.3 mm gap produced the best result. Bigger gaps resulted in too many unprocessed TML while smaller gaps put too much stress on the product and the mill. Approximately 300 g of each batch was milled. Color and bulk density of the meal was determined before the sieve analysis.

5.5.2. Sieve analysis

Five different sieves (test sieves, HAVER & BOECKER OHG, Oelde, Germany) were used to classify six size fractions which are presented in the following Table 6. The analysis was performed according to ICC Standard methods ICC Recommendation No. 207 (Determination of the Particle Size of Milling Products using Sieve Analysis).

The empty weight of each was determined (Pecisa, 3000D, OC Oerlikon AG, Pfäffikon, Switzerland) before applying the stacked sieves to the test screening machine (Analysensiebmaschine, EML 200-80, HAVER & BOECKER OHG, Oelde, Germany). 150 g of each batch was applied to the upper sieve (1400 µm) in a duplicate. Pretests were used to determine time and intensity of the process (total time = 20 min, intensity = 4). After 10 and 15 minutes the process was interrupted to loosen surface layers which occurred due to high fat content. In the end the mass of each fraction was determined and sealed air-tight for further analysis.

Table 6: Mesh size ranges [µm] for Sieve Analysis

Simplified term	**0**	**355**	**500**	**710**	**1000**	**1400**
Mesh size range [µm]	0-355	355-500	500-710	710-1000	1000-1400	>1400

5.6. Analytics

This part describes all analytical methods that were used to describe the physical and chemical properties of the samples. These tests were executed in the laboratories of the Institut für Lebensmitteltechnologie, Universität für Bodenkultur, Wien.

Following equipment was used for all analytics:

- Analytical scale, LA6314i, VWR international, Vienna, Austria
- Drying oven, 100-800, memmert GmbH & Co. KG, Schwabach, Germany
- Mixer, moulinette mini multi deluxe 350W, Moulinex, Alencon, France

5.6.1. Composition

The quantitative composition consists of the main components water, protein, fat, chitin and ash. In the following subchapters, the physical and chemical methods to determine their amounts are described.

5.6.1.1. Gravimetric methods

For the analysis of the fat content the Soxhlet extraction was used (ICC Standard methods ICC Recommendation No. 136. Determination of total fat content) The principle of this method is the evaporation of a nonpolar solvent (petroleum ether) and condensation over the sample tube (Serial Heating System, Foodalyt RS 60, Bremen, Germany). Solvent and extracted fat flows back into the evaporation chamber and another circle begins. With each circle (total of 30 circles or 4 h), more fat is removed from the sample.

For the determination of ash 2 g dried and defatted sample were pre-incinerated (Schnellverascher, SVR/E, Harry Gestigkeit GmbH, Düsseldorf, Germany) in a crucible and incinerated at 600 °C in a muffle furnace (chamber furnace, ELF 11/6b, Carbolite Gero GmbH & Co. KG, Neuhausen, Germany) for 5 h (based on AOAC 923.03).

Dry matter was determined by heating 5 g homogenized sample for approximately 3 h in a drying oven (based on AOAC 950.46).

5.6.1.2. Kjeldahl method

The crude protein and chitin were determined using the Kjeldahl method (AOAC Official Method 2001.11). In this process the nitrogen in the sample is converted to ammonium sulfate by an oxidative digestion and as ammonia transferred to a boric acid solution through steam distillation. The amount of ammonia can be determined by the titration of the solution with hydrochloric acid (Matissek, Steiner and Fischer 2014). A factor is used to calculate the protein or chitin from the total nitrogen as displayed in Equation 1.

$$Specific\ factor\ F = \frac{100}{Nitrogencontent\ [\%]} \qquad \textit{Equation 1}$$

For example: One unit of the chitin molecule (M = 203.19 g/mol) contains one N molecule (M = 14.01 g/mol) which results in a specific factor of 14.5. Proteins are composed of different amino acids. The average N-content is 16 % which results in a specific factor of 6.25.

For this analysis 0.2 g of the dried sample (0.3 g for chitin) was weighed using an analysis scale and transferred into a Kjeldahl flask. As a verification for the method one flask was filled with 0.15 g of glycin. Two flasks were used as blanks. To each flask two glass beads and two catalyst tablets were added. Under a fume hood 15 mL concentrated sulfuric acid was pipetted into each flask which then were transferred to a rack (capacity: 17 samples; 2 blanks, 1 glycin). The rack was then mounted to the digestion apparatus (KjelDigester, K-449, Büchi Labortechnik AG, Flawil, Switzerland) and connected to the gas scrubber (Scrubber, B-414, Büchi Labortechnik AG, Flawil, Switzerland). Then the automatic heating program was started (Preheating to 180 °C, heating to 280 °C, 20 min at 320 °C, 120 min at 420 °C, 36 min cooling).

After the digestion, the Kjeldahl flasks were transferred to the distillation unit (Kjelflex, K-360, Büchi Labortechnik AG, Flawil, Switzerland). 32%-NaOH was added to transfer the ammonia to a boric acid solution in a 250 mL Erlenmeyer flask. Two drops of Sher-indicator were added to the solution. Under constant stirring 0.1M hydrochloric acid was added (Dosimat, 775, Metrohm Inula GmbH, Vienna, Austria) until the indicator reached its turning point. The amount of HCl is equivalent to the amount of ammonia. The following Equation 2 is used to calculate the content of chitin or protein.

$$Content\ in\ dry\ matter[\%] = \frac{1.4008 * 0.1 * (V_{HCl} - V_B) * F}{m_S} \qquad \textit{Equation 2}$$

V_{HCl} = Amount of 0.1M HCl used for titration[mL]
V_B = Amount of 0.1M HCl used for blank [mL]
F = Specific factor
m_s = Weight of sample [g]

5.6.2. Determination of chitin

The quantitative analysis of chitin in insects is still a field without standardized methods. The complex and strong bond between protein and chitin varies a lot between different species and their stage of development. This makes the isolation of pure chitin a challenging task. For this thesis, the two following methods were used.

5.6.2.1. Total Dietary Fiber

The term crude or dietary fiber is used for a wide range of complex organic substances that are non-digestable. FINKE (2007) assumed that insect fiber can be analyzed as dietary fiber because of the structural similarities of cellulose and chitin. The basic principle of this method is the enzymatic digestion of starch and protein to obtain the dietary fiber. For this procedure, a "Total Dietary Fiber Assay Kit" (K-TDFR-200A, Megazyme Inc., Bray, Ireland) was used which is based on the methods AOAC 991.43 and AACC 32-05.01.

For each analysis 1 g (± 0.005 g) duplicate defatted samples were weighed into 300 mL Schott flasks. 40 mL MES-TRIS buffer (pH 8.2) and a stirrer were added to each flask including two blanks. Under constant stirring 50 µl heat-stable α-Amylase (3000 Ceralpha Units/mL) was added. The flasks were covered and put in a slowly shaking water bath (shaking water bath, 1092, GFL mbH, Burgwedel, Germany) at 98 – 100 °C for 30 min. After removing the flasks 10 mL distilled was used to clean the side walls. 100 µl protease (50 mg/mL; 350 Tyrosine Units/mL) was added and the flasks were incubated for 30 min at 60 °C under constant agitation. After the 30 min the pH of the solutions was adjusted with 0.56M HCl to 4.1 – 4.8 on a magnetic stirrer. After adding 200 µl amyloglucosidase (200 pNP β-maltoside Units/mL) the flasks were incubated for 30 min at 60 °C. Then 225 mL 95%-EtOH pre-heated to 60 °C was added to each flask and the samples were left to precipitate for 60 min. A dry filtration crucible with 0.5 g Celite was inserted into the filtration setup (vacuum pump, CVC 2, Vacuubrand GmbH, Wertheim, Germany) and washed with 10 mL EtOH. The mixture was filtered through the crucible and washed twice with 10 mL EtOH. After that the crucible was dried overnight at 105 °C and cooled for 30 min in a desiccator. The crucible was weighed and the residue was used for an ash and Kjeldahl analysis. The Dietary Fiber was calculated using the following Equation 3.

$$Dietary\ Fiber\ [\%] = \frac{\frac{R1 + R2}{2} - P - A - B}{\frac{m1 + m2}{2}} * 100$$

Equation 3

R1 / R2 = Residue weight [g]
P = Protein weight [g]
A = Ash weight [g]
B = Blank residue [g]
m1 / m2 = Sample weight [g]

5.6.2.2. Alkaline hydrolysis

The alkaline hydrolysis to determine the chitin content in insect samples is based on the same principle as the TDF-method only that an alkaline solution is used to digest all components but the chitin. This method was conducted according to the purification of chitin from crustacean waste (Daum 2006).

For four samples 1.2 L of 1M sodium hydroxide solution was prepared by diluting 112 mL of 32%-NaOH-solution (Natronlauge 32%-reinst., Carl Roth GmbH & Co. KG, Karlsruhe, Germany) with 1088 mL of distilled water. 5 g (± 0.05 g) of homogenized sample was weight into a 500 mL Schott flask with an analysis scale. 300 mL 1M-NaOH solution was added to each flask which then were tightly sealed with a lid. A water bath (shaking water bath, 1092, GFL mbH, Burgwedel, Germany) with appropriate insets was preheated to 65 °C. The flasks were put into to water bath for 24 h with constant shaking at a high frequency (151). The paper filters (Qualitative filter paper, 415, Size 330 mm, Particle retention 12 – 15 µm, VWR international, Vienna, Austria) were dried for 2 h at 105 °C and then cooled for 30 min in the desiccator. The empty weight of each filter was determined and then folded two times to fit into the funnel.After the hydrolysis, the flasks were removed from the water bath and left to rest for 10 min so the remaining chitin could sediment to the bottom. At this point an optional step of centrifugation was tested (Centrifuge, 5810, Eppendorf, Hamburg, Germany; 20 min at 4000 rpm) but was deemed unnecessary for these samples. After that two thirds of the solution were carefully decanted into the paper filter in the funnel so the chitin remained in the one third of solution in the Schott flask.

The flask was then filled with distilled water to the 400 mL mark and left to rest for 10 min. The decanting and filling step was repeated six times until the pH value of the solution was nearly neutral (pH 7 – 8). After the seventh dilution step the flask was emptied into the filter and cleaned with a washing bottle with distilled water to transfer the remaining chitin into the filter.

The filter with the remaining chitin were dried overnight (or at least 5 h), cooled for 30 min in the desiccator and the weight of the filter with the chitin was determined. Then 0.3 g of the chitin was analyzed with the Kjeldahl method to identify the nitrogen obtained from the chitin.

$$Residue\ [g] = m_{FS} - m_{FE} \qquad \textit{Equation 4}$$

$$Residue\ [\%] = \frac{Residue\ [g] * 100}{m_S\ [g]} \qquad \textit{Equation 5}$$

m_{FS} = Filter + sample [g]
m_{FE} = Empty filter [g]
m_S = Weight of sample [g]

5.6.3. Physico-chemical methods

Appearance, texture and other physico-chemical properties were analyzed as described in the following subchapters.

5.6.3.1. Color measurement

Ten to twenty whole TML were evenly separated on a white background. The processed insect meal was measured in a petri dish on a white background. Both pictures were taken with a Nikon DX using a digital imaging system (DigiEye, VeriVide, Leicester, UK) and analyzed with the program DigiEye 2.7.2 by digitally removing the background (intensity 151) and measuring a representative square. Color differences to the unprocessed TML were calculated with the following Equation 6.

$$\Delta E_{S,R} = \sqrt{\left(L_S - L_{R)}\right)^2 + (a_S - a_R)^2 + (b_S - b_R)^2} \qquad \textit{Equation 6}$$

$\Delta E_{S,O}$ = Color difference
L_S, a_S, b_S = Color sample
L_R, a_R, b_R = Color reference / unprocessed sample

5.6.3.2. Size distribution of whole dried TML

After the drying process and before the milling the size distribution was measured to compare the influence of each drying method on the length and thickness of the whole insect. For this purpose, 80 – 120 TML were spread out on a white background and a size standard (set square) was added. The DigiEye was used to take a picture and the program CellSens Dimensions was used to measure the size of each individual worm. The acquired data was categorized in clusters to visualize a size distribution.

Figure 6: Sample picture for color and size measurement.

5.6.3.3. Texture analysis

The physical properties of the whole dried mealworms were analyzed using a Texture Analyzer (TA-XT plus, 11455, Stable Micro Systems Ltd., Godalming, UK). The pre-testing of different methods showed that the compression over a set length of a defined quantity resulted in the most reproductive values. As shown in Figure 7 a cylinder (d = 50 mm) was filled with the sample (h = 40 mm). A 50 kg measuring cell was used to push down a stamp (d = 45 mm) at a speed of 3 mm/s. As it touched the surface (release force = 5 g) the speed lowered to 2 mm/s.

Over a length of 15 mm the force needed to compress the samples was measured. For a comparison of the different drying methods the maximum force at 15 mm and the force over the whole distance were evaluated.

Figure 7: Cylinder with defined volume of TML for Texture Analysis

5.6.3.4. Bulk density

The bulk density was measured in two different ways depending on the condition of the material. The whole dried TML were analyzed by filling a 500 mL measuring cylinder at a constant speed. Processed insect meal was measured according to ISO 7971-2:2009 by filling a cylinder with the sample and placing it over a cylinder with a known volume. After removing the bottom part of the upper cylinder, the meal falls into the lower cylinder and the weight can be measured.

5.7. Statistical Evaluation and accuracy of generated results

To ensure comparability of the results, the same raw material was used for all processes. Each drying and pre-treatment method was conducted as a triple determination using the same machines and default parameters. Sieve analysis was performed in duplicates. The chemical assessment of the composition was conducted in triplicates for each size fraction resulting in nine independent values for any parameter (chitin, protein, fat, ash). Size and diameter was determined by analyzing 800 – 1000 larvae per method.

For every set of values the mean and standard deviation (SD) was calculated using the following Equation 7 and Equation 8

$$s^2 = \frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2 \qquad \textit{Equation 7}$$

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i \qquad \textit{Equation 8}$$

s^2 = Standard deviation (SD)
n = Total number of values
x_i = Individual value
$\bar{x}$ = Mean value

The statistical significance and correlation of measured values was assessed using a one-way analysis of variances (ANOVA) with a statistical significance at $p \leq 0.05$ (XL Toolbox NG Add-in Microsoft® Excel®). All other statistical evaluation was performed using Microsoft® Excel® 2013.

6. Results and discussion

The change of physical and chemical properties of the larvae during the process of blanching, drying and milling was monitored by various analytical methods. Introduced by the composition of the raw material the results will first display the kinetics of the pre-treatments and drying processes followed by the comparison of the physical and optical properties of fresh and dried larvae. Then all processes after the milling step are evaluated including the sieve classification and the chemical analysis of the different size fractions

6.1. Temperature profile during blanching

At the outset of the processing the blanching process is used to inactivate all larvae (except DwB, inactivation by freezing) and reduce the microbial contamination. It was reported that boiling whole mealworm larvae for ten minutes reduces the total viable count, Enterobacteriaceae and bacterial spores to <1 cfu/g (Klunder et al. 2012). To assure the success of this treatment, data loggers are applied to record the temperature over time. An exemplary T,t – profile is depicted in Figure 8. It displays the water temperature as well as the temperature in the core of the nylon nets which contained the larvae.

As seen in the diagram the temperature of the blanching water is at the boiling point (~100 °C). After adding the TML the temperature drops by 5 °C and slowly rises back up. At the beginning, the core temperature of the larvae net is approximately 62 °C. It steadily increases within four minutes to a maximum of around 90 °C which is kept constant until the end of the process.

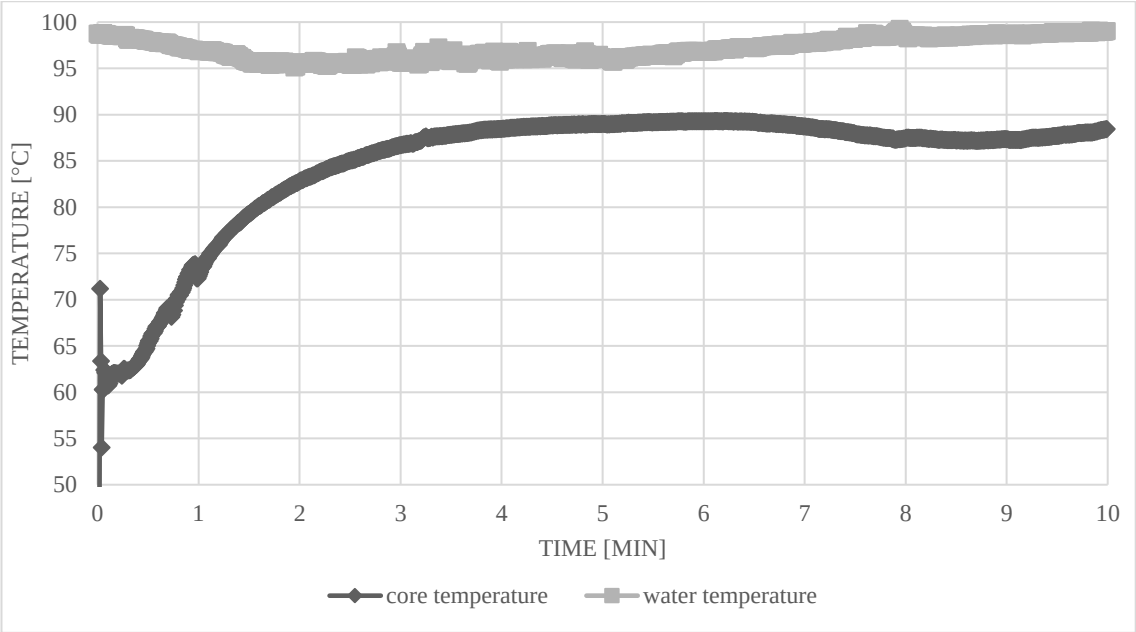

Figure 8: Core temperature in the treatment net and water temperature [°C] over time [min] during blanching

6.2. Composition of raw material

The composition of the fresh mealworm larvae is displayed in the pie chart (Figure 9). The dry matter contains 53 %db protein, 22 %db fat, 13 %db chitin, 4 %db ash and 8 %db Nitrogen-free-Extract (NfE). The results correspond with the analysis of (Zhao et al. 2016, Nowak et al. 2016) for protein content (51.5 / 47 %db), ash (4.9 / 5 %db) and NfE (10.7 %db) whereas a considerable difference is found in fat (32.9 / 34 %db) and chitin (5.5 %db). Species, stage of life, habitat, and diet of the insect have a big influence on the composition, which can result in the variation of the composition (Kinyuru 2015).

The water content increases during the blanching process as seen in Table 7. 68.36 % (m/m) total water compared to 73.98 % (m/m) after blanching. These values are corresponding to other studies (Azzollini, Derossi and Severini 2016). This increase by 5.62 % can be considered an influencing factor on the drying process. The less time needed for drying, the better the preservation of the natural functional abilities of the protein and the lower the negative effect on the structure as elaborated in following chapters. Differences in protein (F: 58.77 %db, B: 58.48 %db), fat (F: 22.72 %db, B: 22.14 %db) and ash (F: 4.5 %db, B: 4.36 %db) contents between blanched and fresh larvae are not significant.

This shows, that the composition of the dry matter is not affected by a blanching step as similarly shown for yellow mealworm after boiling for three minutes (Azzollini et al. 2016).

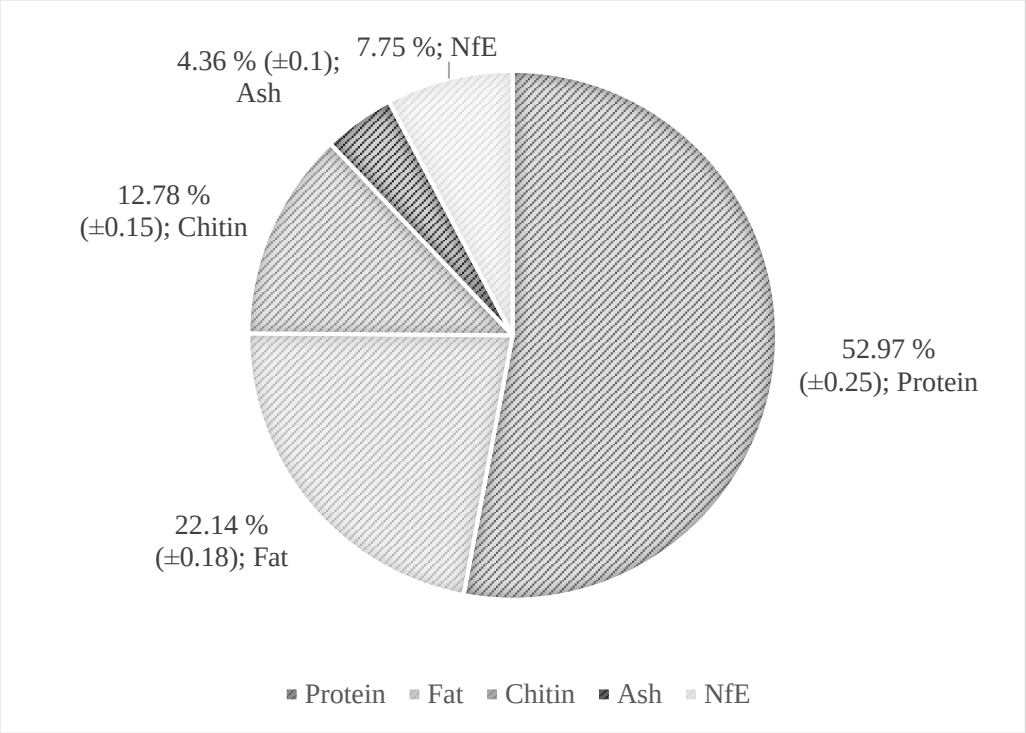

Figure 9: Composition of fresh TML based on dry matter [%db.]

Table 7: Water content of fresh and blanched TML [%db]

	[%]	SD
Water content blanched	73.98	0.06
Water content fresh	68.36	0.81

6.3. Pre-tests and pre-treatments

6.3.1. Drying kinetics

To assure the comparability of differently dried TML, the water content is standardized (<5 %db). The kinetics of four drying methods (freeze drying (FD), hot air drying 80°C and 60°C (OD80, OD60) and vacuum drying (VD)) were determined by pre-tests. Figure 10 shows the water losses over a period of 27 h. All kinetics have an even rate of water loss until they reach about 75 % mass loss after which the rate begins to decrease.

The main difference between the various methods is the time needed to reach the critical point (OD80: 4 h; OD60: 6.5 h; FD: 20 h; VD: 24 h). Increasing the temperature by 20°C reduces the drying time by 40 %. This could preserve the natural structure but also increases thermal stress and damage to nutrients (Azzollini et al. 2016).

The drying kinetics of the main tests are shown in Figure 11. Each batch contained 1730 g blanched and thawed TML. Contrary to the promising results of the pre-tests, the vacuum drying shows a reduced water removal rate. This resulted in a pronounced spoilage of the mealworms and eventually to the termination of the process. Main factor is the higher quantity of drying good. The dryer was not able to remove the water from the vacuum chamber which leads to a longer drying period and consequently to microbial growth.

In accordance to the pretests the hot air drying method OD80 reaches the desired water loss (70 % (m/m) water removed) after 6 – 7 h, OD60 and FD after 24 h (Figure 10). Azzollini et al (2016) showed that an increase by 20 °C decreased the oven drying time to reach similar water contents by a third. The control material for freeze-drying is not representative for the whole batch which results in an increased drying time (48 h) to assure a water content below five percent.

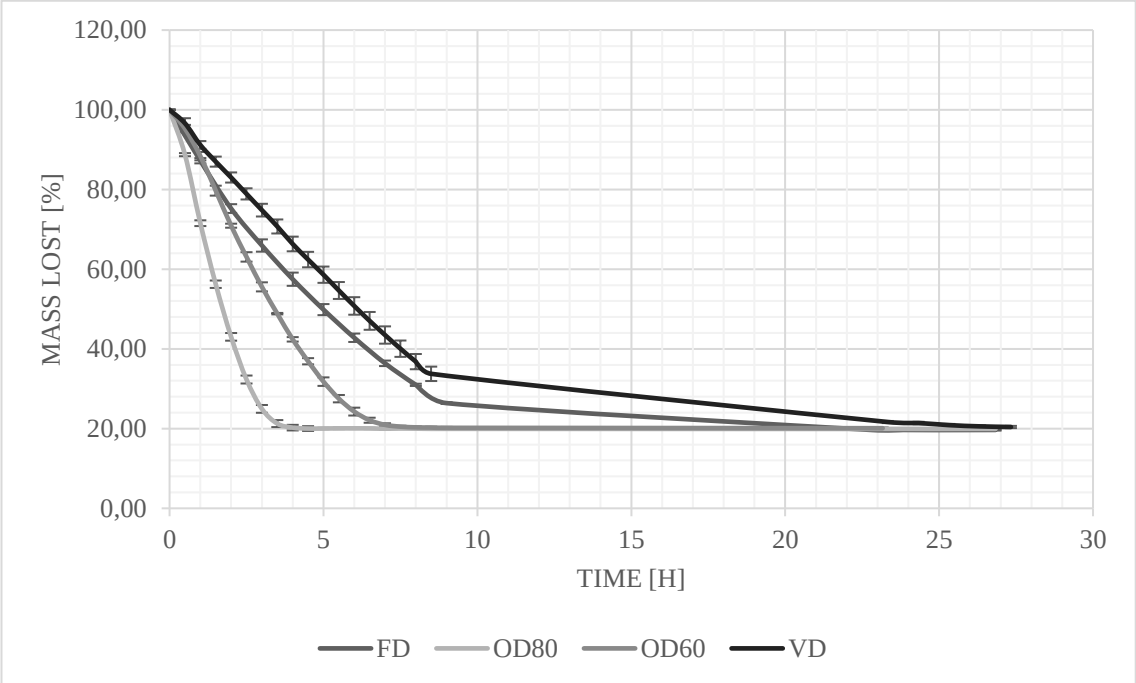

Figure 10: Drying kinetics for Freeze Drying (FD), Oven Drying (OD80, OD60) and Vacuum Drying (VD)

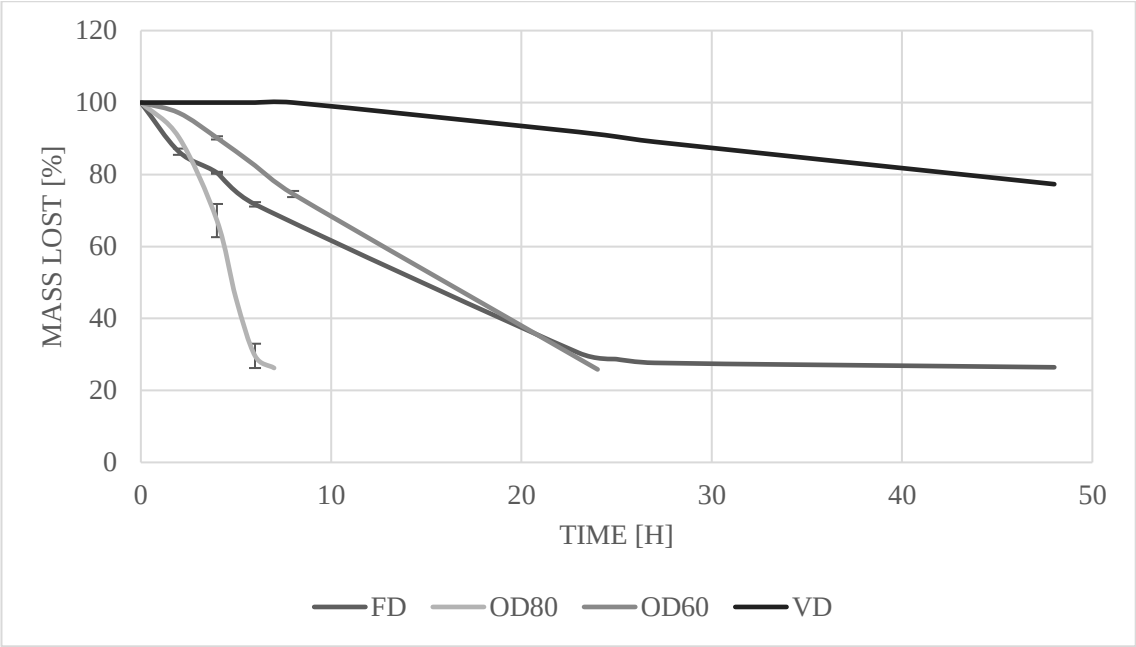

Figure 11: Drying kinetics of main test including Freeze Drying (FD), Oven Drying (OD80, OD60) and Vacuum Drying (VD)

6.3.2. Fluidized bed drying

Over a time of 120 minutes the blanched larvae were dried in a fluidized bed. The profile of inlet-, outlet- and bed-temperature is displayed in Figure 12. At the beginning a high inlet-temperature (90 °C) is necessary to transfer heat into the frozen TML. 20 minutes after starting the process the desired bed-temperature of 60 °C is reached and the incoming air temperature drops by 10 °C to prevent overheating of the material.

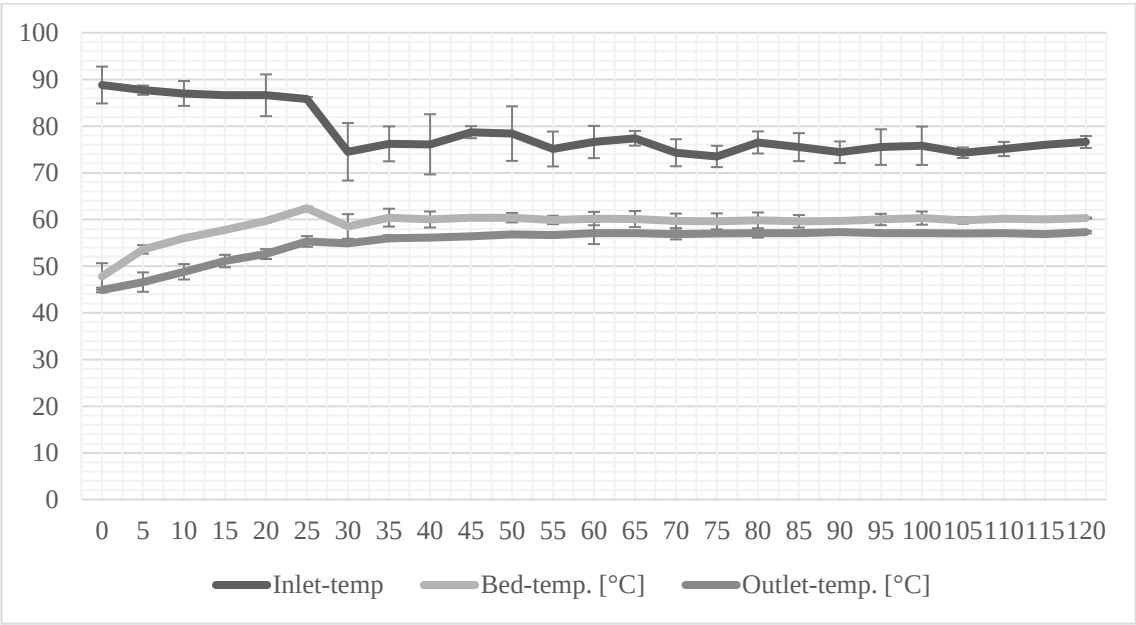

Figure 12: Fluidized Bed Drying temperature course [°C, min] of inlet, outlet- and bed-temperature over time [min]

6.3.3. Defatting

Aim of this pre-treatment is to lower the fat content prior to milling to obtain a better processability and prevent stickiness of the ground TML flour. After hot air drying the mealworms at 80 °C, a defatting step is performed to extract as much fat as possible. Lipids from the whole insects are extracted by supercritical CO_2 over a period of six hours using the default parameters described in Table 5. Every 15 minutes the extracted fat is taken from the separator and weighed. Figure 13 shows the rate at which the fat accumulates over six hours. 100 % (m/m) removed fat refers to the total quantity of fat which is taken from the separation vessel during the extraction. The logarithmic progression of the curve shows a rapid extraction at the beginning of the process. After 50 minutes the rate begins to decline. Half of the fat is extracted in the first 90 minutes, the other half in the remaining 250 minutes. Research shows that the maximum yield when extracting insect grist is reached at 100 – 120 minutes (Wei et al. 2009).

A quantitative analysis of the defatted mealworms reveals a remaining fat content of 9.47 %db (±0.14). A comparison to unprocessed TML (22.14 %db) shows that 57.23 % (m/m) of the total lipids are removed by the supercritical CO_2 extraction. Research on the defatting of milled Lepidoptera shows a maximum yield of 97 % (m/m) after 100 minutes (Wu 2012). This emphasizes that the efficiency of extracting oil from whole insects is distinctively lower than using milled material. The intended usability of this costly process for this specific study can be considered low.

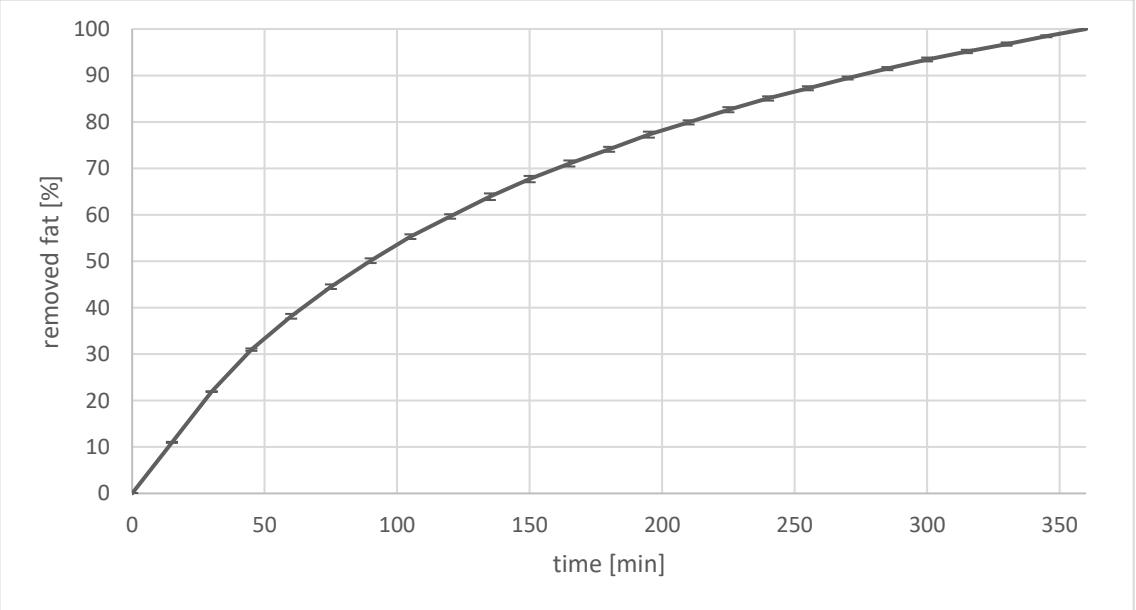

Figure 13: Removed fat [%] over time [min] during supercritical CO2 defatting

6.4. Comparison of physico-chemical properties of different drying methods

6.4.1. Changes in color

The changes in color after heat treatment can be used as an indicator for thermal stress on the insect. Figure 14 shows the color changes of whole larvae after drying and defatting. Fresh and blanched TML are used as a reference.

The color deviation can be classified as "very distinct ($\Delta E > 3$) or "distinct" ($1.5 < \Delta E < 3$) (Adekunte et al. 2010). All procedures lead to a "very distinct" difference to the reference. The most significant color change can be seen in DwB. With an ΔE of 18.3 to the fresh TML and a L* of 39.51 (fresh L* = 54.26) drying without blanching applied the most thermal stress. This is induced by the lower water content of the fresh -not blanched- larvae. Similar behavior but less distinct is seen at OD80 ($\Delta E = 4.76$), OD60 ($\Delta E = 3.62$), FBD ($\Delta E = 4.79$) and DwF ($\Delta E = 7.14$) due to non-enzymatic browning of the outer shell (Martins, Jongen and van Boekel 2000).

The freeze drying (FD) and partially defatting (DaD) process increased the brightness (L*= 59.73 (FD), 56.03 (DaD)) in comparison to the reference (L*= 54.26 (fresh), 51.19 (blanched)). Despite the hot air drying at 80 °C before the defatting the brightness is higher than before the blanching. This can be explained by co-extraction of brown lipophilic melanoidins during defatting (Jeon et al. 2016).

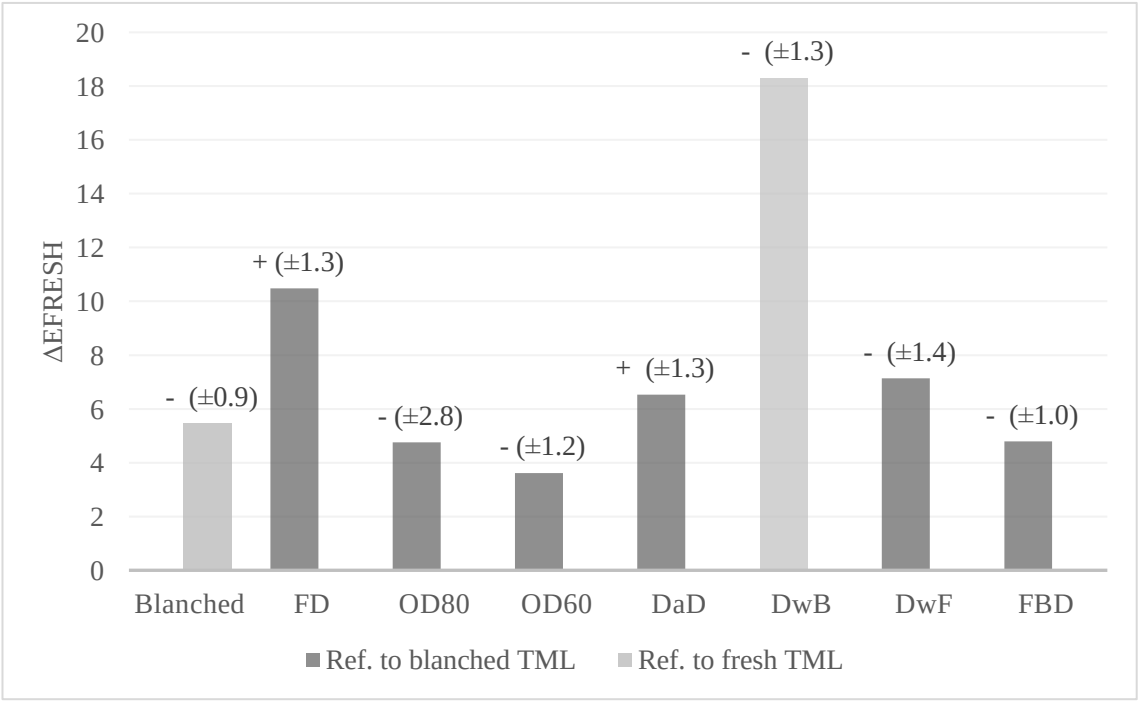

Figure 14: Color differences [ΔE] of drying methods Freeze Drying (FD), Oven Drying (OD80, OD60, DaD, DwB, DwF) and Fluidized Bed Drying (FBD) with reference to fresh and blanched TML. "+" illustrates increased brightness, "-" illustrates decreased brightness.

6.4.2. Changes in the size of the whole mealworm

The changes in size after the heat- and pre-treatment are shown in Figure 15 and Figure 16. For each of the procedures the length and diameter of 900 – 1100 individual larvae are measured and classified into size ranges (length: 5 mm; diameter: 0.5 mm).

The hot air drying processes (OD80, OD60, DaD, DwF) as well as the fluidized bed drying show similar size distribution with a peak at 11 – 15 mm. With 70 % of the larvae in the same range (11 – 15 mm) and less longer individuals the drying without blanching method has the biggest shortening effect. In contrast, the freeze drying has minimal effect on the size when compared to the fresh reference.

Figure 15 and Figure 16 show the average size of each treatment as well as the size of the fresh TML. All procedures have an impact on the size of the larvae. The freeze-drying process increases the length and diameter slightly. This could be caused by the low pressure in the vacuum chamber which not only increases porosity and volume but also preserves the structural integrity during the process. Hot air drying at 80 °C (OD80) shortens the larvae by 10.2 % (OD60: 13.6 %). The increased size reduction at lower temperatures can be explained by the expanded time needed for drying at 60 °C. The additional defatting step causes a minor reduction in length (-0.15 % compared to OD80) which shows that the supercritical CO_2 treatment has no significant impact on the size. The biggest impact is caused by the DwB (without blanching). The 20 % shrinkage can be explained by the reduced water content of non-blanched TML. With less water to remove at the same drying time the physical stress on the larvae is higher which causes a further tissue collapse. The FBD-process decreases length and diameter because the turbulence and the resulting collision breaks the whole insects which leads to more fragments with smaller size.

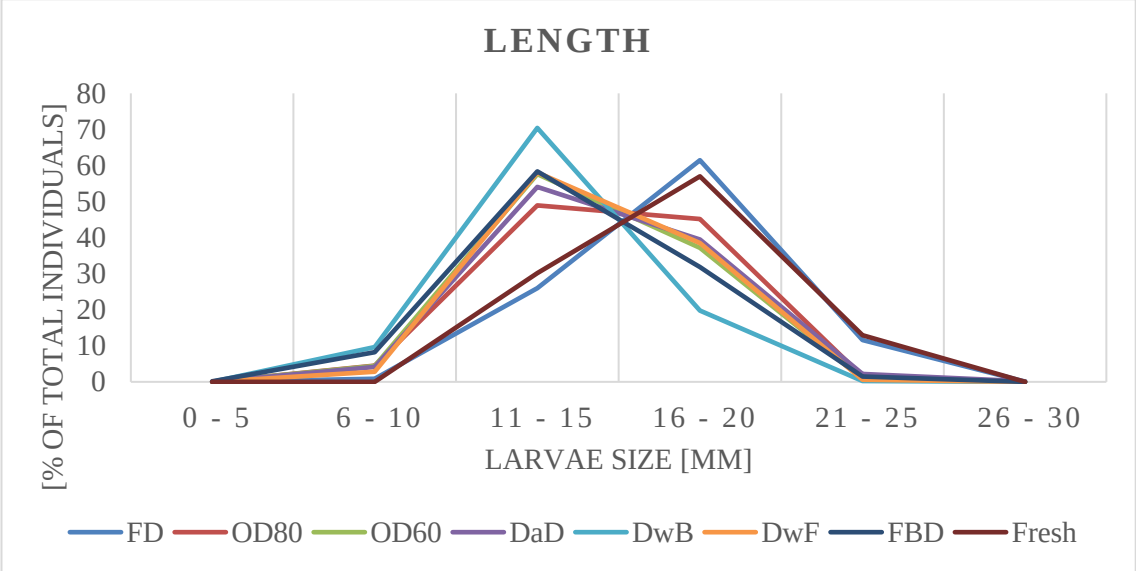

Figure 15: Length of whole TML after drying classified into six larvae lenth classes [mm]

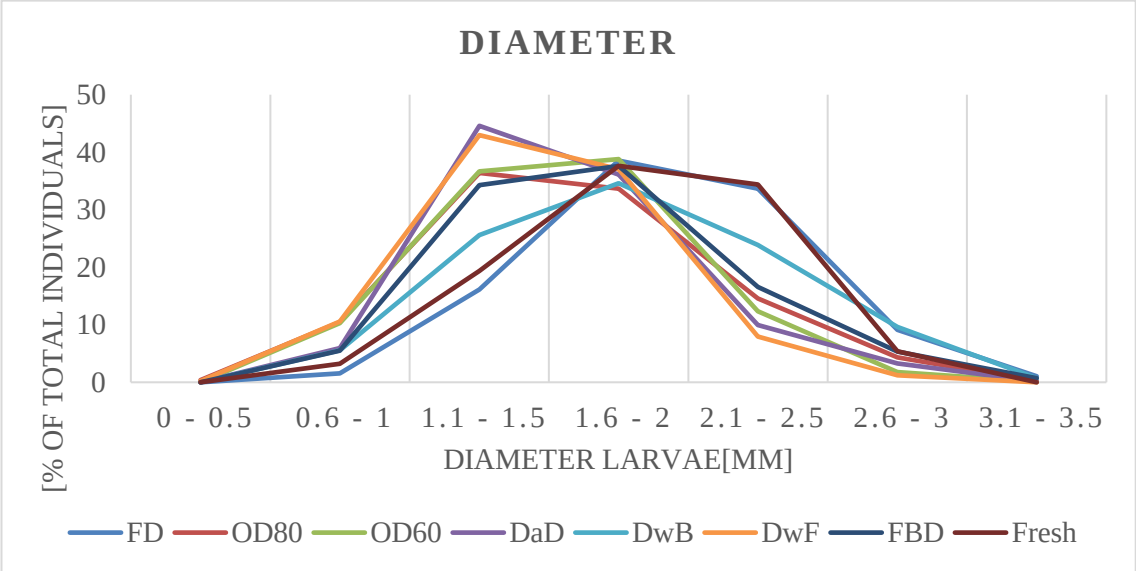

Figure 16: Diameter of whole TML after drying classified into seven larvae diameter classes [mm].

Table 8: Comparison of the average length and diameter of whole TML after drying [mm] and the shrinkage in reference to dresh TML

	Fresh	FD	OD80	OD60	DaD	DwB	DwF	FBD
Length [mm]	16.95	17.19	15.22	14.65	15.03	13.56	14.90	14.35
SD	0.37	0.35	0.41	0.15	0.07	0.12	0.32	0.18
Diameter [mm]	1.89	1.98	1.64	1.59	1.68	1.84	1.54	1.71
SD	0.03	0.05	0.12	0.03	0.03	0.02	0.02	0.05
Diff. to fresh [%]		+1.41	-10.20	-13.59	-11.35	-20.02	-12.09	-15.38

6.4.3. Influence of pre-treatments on the texture and bulk density

Further information about the textural influence on the milling behavior is obtained by comparing the maximal force [N] needed to compress a volume of whole dried TML over a defined length. The structural change during the drying process is caused by thermal or physical stress. Higher numbers imply a hard, brittle and compact structure whereas a low force indicates a porous and fragile structure. Figure 17 shows the results of the analysis. A reference to the raw material is not possible since only dried samples could be analyzed.

TA of the freeze-dried samples results in the lowest max. force (64.4 N) of all methods, which is 33.1 % of the highest force (DwF: 194.7 N). OD80 produces a max. force of 142 N. These three processes need significantly lower (FD) or higher (OD80, DwF) force than the remaining processes. Variance analysis shows no significant differences between OD60, DaD, DwB and FBD. These results show that more thermal stress leads to an increased hardening of the structure. In addition, non-heat drying (FD) produces a brittle and porous larvae structure which is beneficial to the following grinding process as elaborated in later discussion. A similar increase of fracturability is found in freeze-dried button mushrooms (Pei et al. 2014). Despite the same oven drying at 80 °C, the defatted samples have a 36.3 % lower max. force than OD80. This means that the supercritical CO_2 treatment loosens the hard structure of the larvae. Carrots dried in a supercritical fluid environment possess a similar low dense structure (Brown et al. 2008).

Figure 18 gives on overview over the bulk density [g/L] of whole dried larvae in comparison to the density of the grist. In compliance to the results of the TA (Figure 17), the freeze-dried samples have the lowest density (135.6 g/L) and the DwF process produces the highest density (245.3 g/L). Contrary to the TA, the bulk density of OD80 is 6.5 % lower than the density of non-blanched larvae (DwB). These observations show that in most cases a lower bulk density is related to a lower textural hardness. As illustrated in Figure 18 roller-milling caused an increased bulk density in all processes. DwB and FBD have a noticeable smaller increase (DwB: 19.9 %, FBD: 13.4 %) than the remaining processes. These differences in bulk density and the structural changes are expected to have an impact on the milling characteristics and consequently on the fractionation.

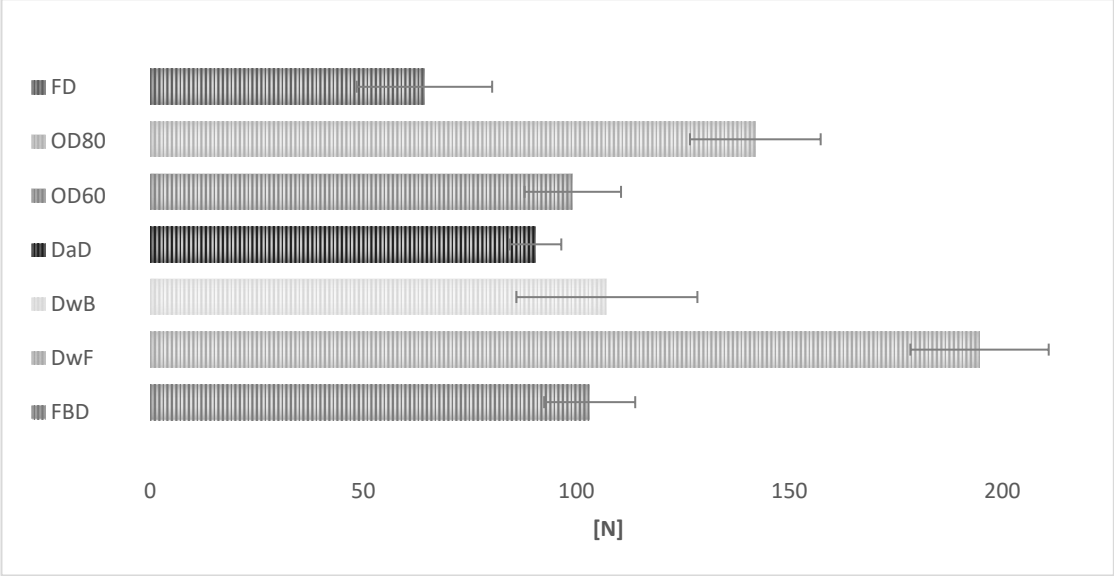

Figure 17: Maximal force [N] needed to compress a defined volume of dried TML with SD

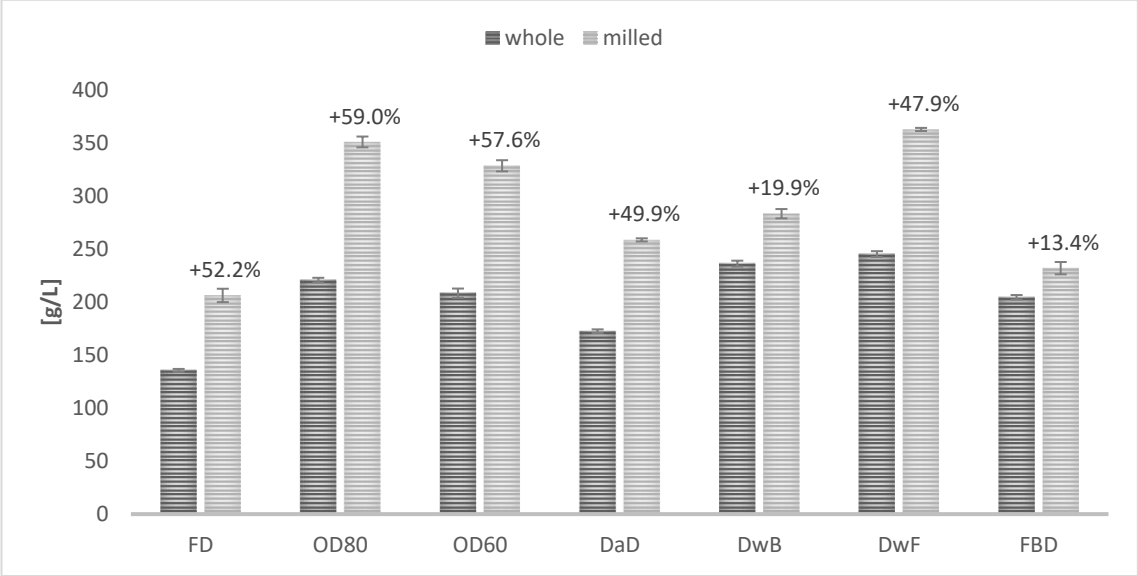

Figure 18: Comparison of bulk density [g/L] of whole TML after drying and milling

6.5. Distribution of size fractions after sieve analysis

Figure 19 and Table 9 present the results of the sieve analysis after roller milling the dried larvae. The data shows that there is a considerable difference between the differently dried and pre-treated TML.

A correlation between the curves of oven dried (OD80, OD60, DwB, DwF) TML can be seen in Figure 19. Less than 0.5 % (m/m) of the total grist mass has a particle size below 355 µm.

Most of the material can be found in the range 500 – 1000 µm with 87 – 89 % (m/m) of the total mass in this fraction (OD80: 89.32 % (m/m), OD60: 87.24 % (m/m), DwB: 87.09 % (m/m), DwF: 87.72 % (m/m)). Contrary to that, only 62.47 % (m/m) (FD) and 58.16 % (m/m) (DaD) of the grist mass have a particle size of 500 – 1000 µm. This observation shows that mealworm larvae which are treated without heat (FD) or defatted prior to milling (DaD) produce grist with a balanced particle size, whereas thermal stress leads to an accumulation of particles in a specific range. Fluidized bed drying shows characteristics of both sides. Three thirds of the total mass are in the range between 500 – 1000 µm but the curve is much lower than the rest.

Significant amounts of fine particles (<355 µm) can only be found in the freeze dried (FD) or defatted (DaD) samples. 23.08 % (m/m) of the defatted grist has a particle size below 355 µm, which can be ascribed to reduced stickiness which prevents agglomeration. This is similarly observed for defatted corn and oat flour (Sibakov et al. 2011). Only 0.5 % (m/m) of the grist of hot air dried or fluidized bed dried TML is in this size range. The hard and compact structure of the TML after being oven dried produces a coarse grist, whereas the larger size and porous structure of FD and DaD leads to a higher grinding degree induced by the pulverization of the larvae. Same observations are made for maize kernels. Increased hardness results in a higher amount of larger particles and a less fine material (Blandino et al.). Only small differences can be found in the size fraction above 1000 µm.

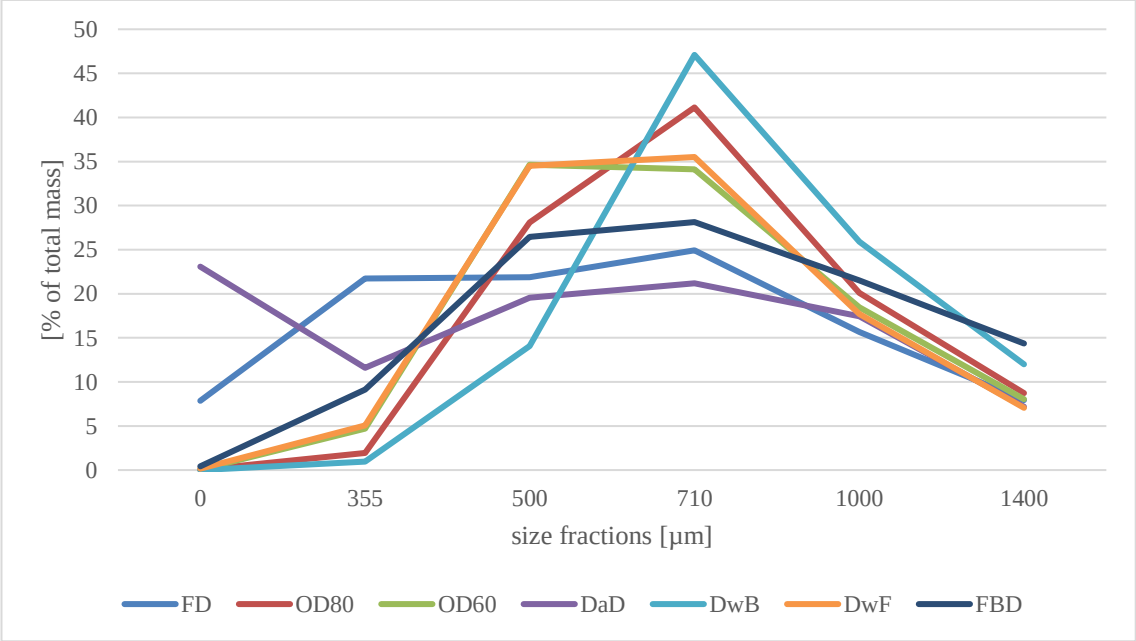

Figure 19: Size distribution after sieve analysis. Mass portion [%] in every size fraction of each drying method.

Table 9: Size distribution after sieve analysis. Mass portion [%] in every size fraction of each drying method with SD.

size fraction		0 µm	355 µm	500 µm	710 µm	1000 µm	1400 µm
FD		7.86	21.74	21.88	24.93	15.66	7.93
	SD	4.13	5.24	3.93	8.18	2.54	0.75
OD80		0.03	1.92	28.09	41.14	20.09	8.74
	SD	0.03	1.49	5.85	3.97	2.78	1.81
OD60		0.06	4.69	34.64	34.12	18.48	8.02
	SD	0.04	1.12	2.58	2.47	0.90	0.60
DaD		23.08	11.58	19.56	21.17	17.43	7.19
	SD	0.68	0.04	0.67	0.48	0.77	0.06
DwB		0.00	0.95	14.07	47.10	25.92	12.01
	SD	0.28	0.55	4.45	3.05	3.90	2.13
DwF		0.16	5.06	34.50	35.51	17.71	7.06
	SD	0.13	1.66	1.90	1.71	1.26	0.88
FBD		0.42	9.12	26.46	28.13	21.52	14.34
	SD	0.27	1.70	1.77	0.73	0.80	1.07

6.6. Composition of size fractions

These correlations show that the structural changes induced by thermal stress or defatting influence the particle size distribution. Further chemical analysis of the size fractions was performed to observe the possible impact on the composition.

6.6.1. Fat contents and ash

Figure 20 shows the distribution of fat in six different size fractions (0 – 1400 µm). The diagram displays the curves of four drying methods (FD, OD80, OD60 and FBD) and the defatted material (DaD).

The partial extraction of fat prior to milling results in a significantly lower percentage of fat in the dry matter. As mentioned in chapter 5.4, 57.23 % (m/m) of the total fat is extracted from the larvae, which is also represented in this data. With 11.26 %db in the smallest fraction (<355 µm) and 8.26 %db in the biggest fraction (>1400 µm) the fat content in the defatted material decreases with a larger particle size. A similar and even larger decrease (-4.3 %) can be determined for the freeze-dried samples. Roller milled fenugreek shows a similar behavior having the highest fat content in the coarse husk and the lowest in the flour (Sakhare, Inamdar and Prabhasankar 2015). The curves of OD80, OD60 and FBD show the opposite course.

Fat content increases with larger particle size (OD80: +2.8%, OD60: +1 %, FBD: +3.3 %). As elaborated before, this behavior can be explained by the agglomeration of fat resulting in larger particles (Sibakov et al. 2011).

It can be observed that there is an influence of the pre-treatment on the fat distribution. While heat involving processes lead to an accumulation of fat in larger particle sizes, defatting and non-heat processing lead to an increase of fat in smaller fractions. This knowledge in combination with the results of the chitin and protein analysis can be used to classify the optimal particle size at which the fat and chitin content is at a minimum and protein is enriched.

Ash content in the dry matter of each size fraction is determined by incinerating the defatted sample. The results of three drying methods are presented in Table 10. No significant differences could be found except a slightly decreased ash content in the 1000 µm freeze dried sample which might be explained by inaccuracies during the analysis.

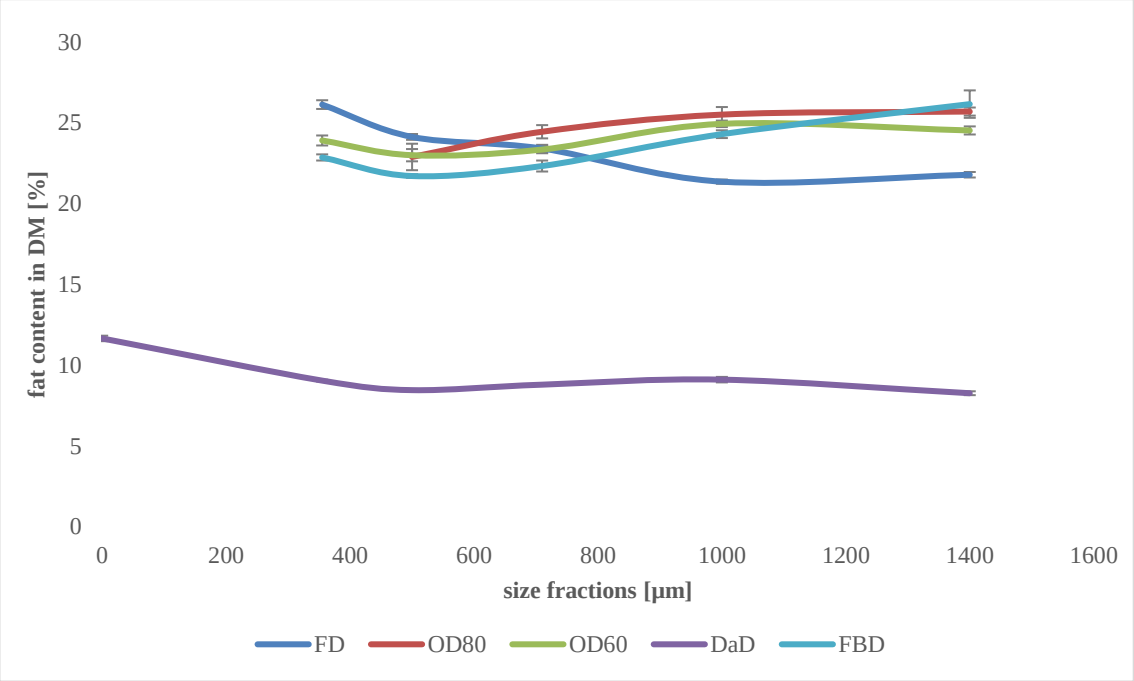

Figure 20: Fat content [%] of size fractions [µm] after sieve analysis of five drying and pre-treatment methods.

Table 10: Ash content [%] of size fractions (355 – 1400 μm) of three drying methods (FD, OD80, OD60)

		355 µm	500 µm	710 µm	1000 µm	1400 µm
FD		4.95	4.72	4.3	3.73	4.15
	SD	0.11	0.07	0.06	0.3	0.07
OD80			4.73	4.57	4.33	4.42
	SD		0.18	0.13	0.13	0.13
OD60		4.98	4.75	4.69	4.52	4.55
	SD	0.04	0.07	0.08	0.06	0.05

6.6.2. Protein contents

After sieve analysis, the protein content of each size fraction is determined by the Kjeldahl method. The following Figure 21 shows the results. Four to six fractions of each drying method are analyzed depending on the available material. A connection between the points gives a characteristic curve to display the protein accumulation in each particle size ranging from 0 – 1400 µm.

Overall the defatted sample (DaD) has a higher protein content than the other four (FD, OD80, OD60, FBD). Removing fat results in a lower dry matter content and a higher percentage of protein and other components. Another difference is the course of the curve. In the size range 0 – 500 µm the protein content is at a consistent level between 65.5 %db and 66.6 %db. Then a rapid drop to 60.3 %db at the size of 710 µm to 1000 µm occurs, followed by a slight increase by 2 %. This shows that there is a significant accumulation of protein in fractions with smaller particle size which is also reported for winter wheat (Tóth et al. 2006) or de-oiled rice bran (Jayadeep et al. 2009). Beneficial to this distribution is the reduced fat content which -as described before- lowers the agglomeration of particles (Sibakov et al. 2011). Although statistically not significant, the same observation can be made for the fluidized bed drying and oven drying at 80 °C which protein contents increase by 3.1 % (FBD) and 1 % (OD80) from the biggest to the smallest particle size. OD60 has a similar course between 500 µm and 1000 µm but drops by 2 % in the smallest fraction (355 µm). This is correlating to the chitin content (Figure 22) which also shows an anomaly at this particle size. The lowered protein content and the increased chitin in the smallest fraction shows that a temperature difference of 20 °C (oven drying) can influence the breakage behavior during the milling process.

While the increased drying time (OD60: 24 h, OD80: 7 h) reduces the hardness of the whole TML (-30 % max. force, Figure 17), the structure is affected in a way that a finer chitin dust is produced. Contrary to the heating processes, the curve of the freeze-dried samples shows a different behavior. Protein contents decrease from 1400 µm to 500 µm and increase below 500 µm by 2.3 %.

The most important observation from this data is the correlation between decreasing particle size and increasing protein content, significant in DaD and FBD.

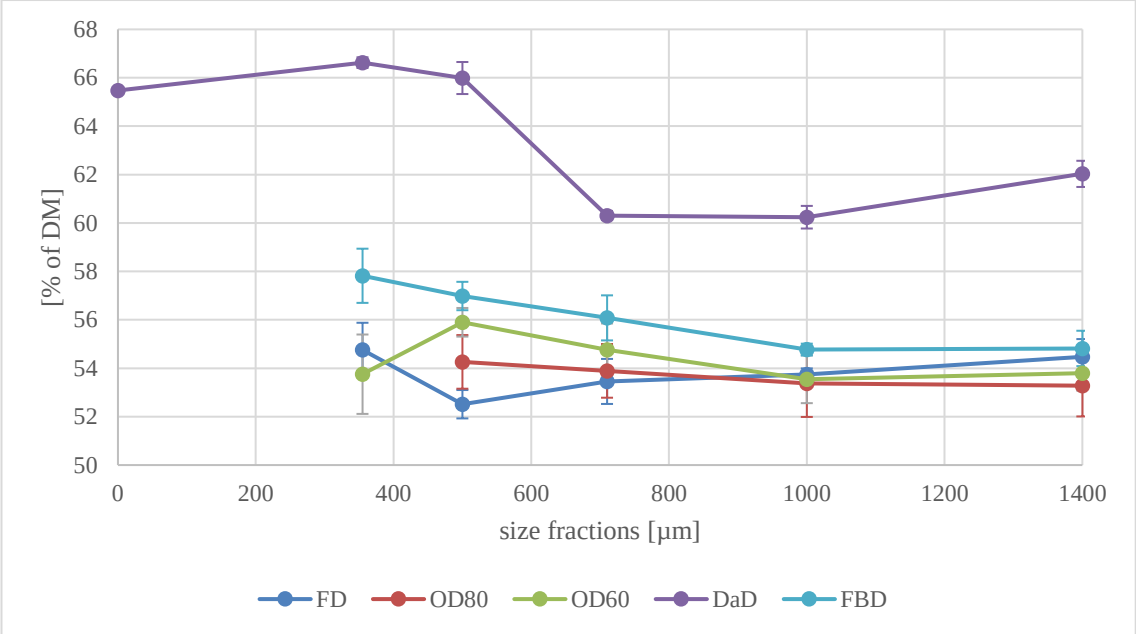

Figure 21: Protein distribution [% of DM] in the different size fractions.

6.6.3. Chitin contents

Four to six size fractions of each drying method are analyzed for their chitin content (%db). A minimum of 20 g is needed for the alkaline hydrolysis which results in a different quantity of size fractions. Each process gives a characteristic curve illustrated in Figure 22.

The most distinct course from small to large particle size can be seen at freeze drying, with the smallest fraction (<355 µm) only containing 3.6 %db chitin. With increasing particle size the chitin content triples to 11.2 %db (500 – 710 µm).

At an average size of 1000 µm the chitin peaks at 16.1 %db and then slightly decreases to 13.43 %db (>1400 µm). A comparable course can be seen at DaD (defatted). In this case, the whole size range (0 – >1400 µm) is analyzed, starting with 8.9 %db chitin in the smallest fraction (<355 µm), increasing to a peak at 13.5 %db (1000 µm). Similar chitin contest can be seen at fluidized bed drying (FBD) with 8.1 %db in smallest fraction. The peak is at 710 µm with 12.9 %db. Both hot air drying methods produce mealworm grist with a constant chitin content in all size fractions (OD80: 9.9 – 11 %db; OD60: 10 – 11.9 %db).

A significant difference can be seen between the conventional oven drying and other methods. This can be correlated to the results of the Texture analysis (chapter 6.4.3) and the change in size (chapter 6.4.2). The long heating process leads to smaller larvae and a harder structure compared to non-heat methods. The outer chitin shell and the protein within the larvae forms a strong bond which cannot be destroyed by roller milling (Jin, Feng and Xu 2013). Therefore, bigger particles with a constant ratio of chitin and protein are produced. Contrary to that, processes using no heat (FD) or shorter heat exposure (FBD) produce smaller fractions with a significant difference between small and large particles. The porous structure and bigger size are advantageous for the mechanical comminution. Protein is separated from the outer chitin shell and enriched in smaller fractions (Chapter 6.6.2). Chitin naturally forms a strong composite which inhibits pulverization by roller milling and supports the accumulation in larger fractions. The decrease of chitin after the peak (FD and DaD) can be explained by a significant number of small larvae which are still intact after milling and accumulate in the sieve with the widest mesh size (1400 µm). Due to the high protein content in whole TML, the chitin content is lowered in the highest size fraction. Compared to freeze drying, the supercritical CO_2 defatting has a similar effect on the texture and chitin content. Although the same drying process (OD80) is performed before the defatting, the chitin content differs significantly from the sole dried larvae. It can be assumed that the CO_2 solvent and the high-pressure treatment changes the constitution of the mealworm in a way that the strong binding between protein and chitin is weakened and separated more easily.

Chitin is one of the key factors in the usability of protein extracts from insects. These results show that chitin accumulates in different size fractions, depending on the method used for drying. A removal from the mealworm grist by a pre-treatment would increase the protein content. This can be done by sieve classification, air sifting or triboelectrification (Hemery et al. 2009).

Although these methods are successfully implemented in the cereal technology, a similar treatment for insects can be difficult due to the strong binding between chitin and protein. Chitin rarely exists in a pure form but instead is usually in a complex matrix with other compounds (Finke 2007). This makes the dry fractionation of insects for protein concentrate or isolate productions a challenging task.

Another challenge is, that no standard methods are available to determine the chitin content of insects. Isolation and purification of chitin for structural characterization is established but it lacks the accuracy for quantitative analysis due to lower yields (Paulino et al. 2006). The most promising approach is the hydrolysis of protein at low or high pH, as shown by the analysis of cicada (Sajomsang and Gonil 2010). A variant of this method (as described in chapter 5.6.2.2) is selected for this thesis. Another possible technique uses the similarity of chitin to cellulose. Dietary fiber can be purified by an enzymatic degradation (TDF) and determined gravimetrically. The following Figure 23 shows a comparison of these two methods (TDF, Alkaline hydrolysis), used to purify chitin for gravimetric analysis. The bars for each size fractions contrast the chitin content determined for three drying methods (FD, OD80 and OD60).

A significant difference can be seen in every size fraction except FD 355 µm. The Alkaline method doubles the percentage of chitin in the dry mass compared to the TDF results. This shows that the selected analysis has a big influence on the resulting data. In this case the TDF method was discarded for further analysis due to worse reproducibility and many potential error sources. Similar studies also suggested that the determination of chitin as ADF (acid detergent fiber) is altered by the presence of cuticular protein (Finke 2007).

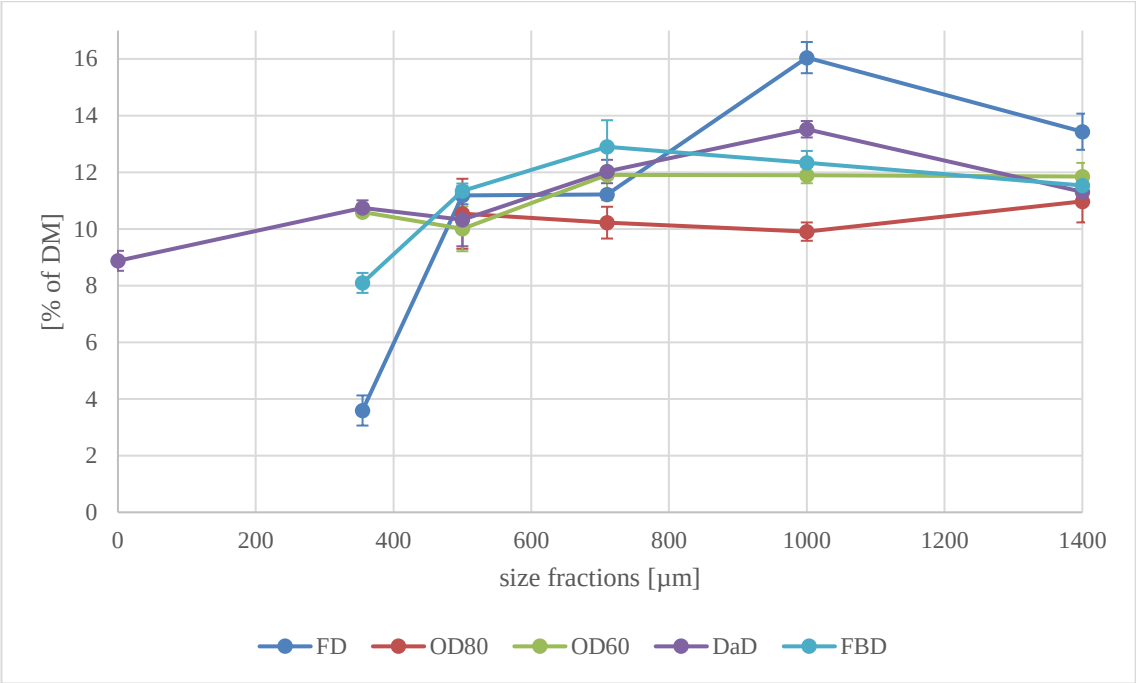

Figure 22: Chitin distribution [% of DM] in the different size fractions.

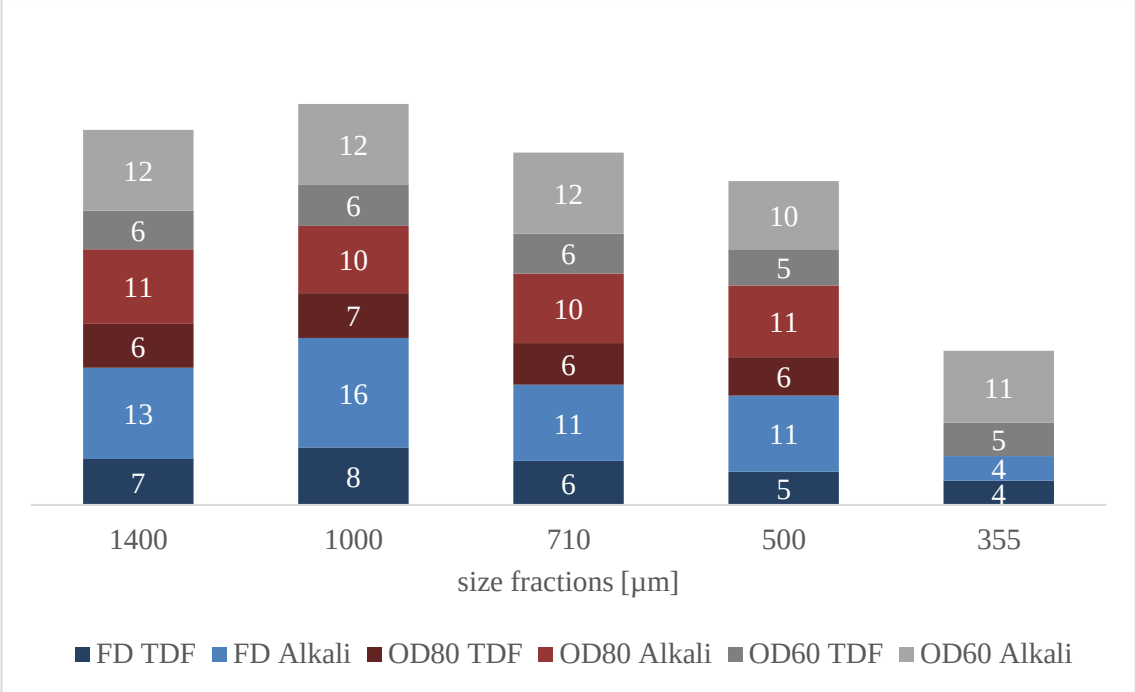

Figure 23: Comparison between the TDF method and alkaline hydrolysis (Alkali) method for the chitin analysis. The numbers within the bars show the chitin contents [% of DM] determined by each method.

6.7. Summary

Table 11 provides a simplified overview of the results discussed in previous chapters. All relevant properties are classified in three categories depending on their influence on the specific attribute: 1) "++" big influence, 2) "+" small influence, 3) "-" no influence. This assessment can be considered positive or negative and is explained by a short description.

Pre-treatments: freeze drying (FD), oven drying (OD80, OD60), defatting (DaD), fluidized bed drying (FBD).

Assessed criteria: Enriching of protein and chitin, influence on texture, color and size and separation properties by sieve fractionation

The highest enrichment of protein is determined in the defatted samples. Smaller particles contain significantly more protein than larger particles. Fluidized bed drying shows a similar distribution but less distinct. Even though a certain tendency is observed in the oven dried and freeze-dried larvae, the differences within the fractions are not significant. An accumulation of chitin is observed in the freeze-dried TML with 13 % more chitin in the 1000 µm fraction compared to the lowest particle size. Equal but less distinct behavior is found for DaD and FBD.

Changes in texture are determined in all treatments. FD and -to a lower degree- DaD lead to a weaker, more brittle structure whereas the thermally dried goods possess a hard structure. Oven drying at a higher temperature produces the most rigid larvae. All processes show either a brightening (FD, DaD) or a darkening (OD80, OD60, FBD) effect on the color of the outer shell. Especially freeze-drying has a strong effect on the larvae ($\Delta E = 10.5$ reference to fresh). Except FD, which maintains the original size, a shortening effect is observed in all processes. OD60 and FBD produces the smallest TML.

The fractionation by sieve analysis is evaluated in the last column of Table 11. An even distribution throughout all size fractions results in a better separability. High concentrations of material in one particle size and a low quantity of fractions result in a poor separability. The fractionation of defatted and freeze-dried mealworm larvae produces the best distribution and the most evaluable fractions (DaD: 6, FD: 5). Although grist of OD60 and FBD is also separated in five size fractions, the higher concentration of material in one section leads to worse separability. With only four groups and a distinct peak the OD80 shows a poor separability.

These differences between the various processes show that there is an influence on the milling and fractionation behavior. The consequences and use of this information is elaborated in more detail in the following chapter.

Table 11: Simplified comparison of the influence of the pre-treatment and drying methods on protein, chitin, texture, color, size and fractionation. "++" big influence. "+" small influence, "-" no influence. “C” chitin. “P” protein.

		Enriching of		Influence on			Fractionation
		Protein	**Chitin**	**Texture**	**Color**	**Size**	
Freeze drying (FD)		-	++ More C in larger particles	++ Porous structure	++ Brightening	-	++ Very good seperability
Oven drying (OD)	**80**	-	-	++ Very hard structure	+ Darkening	+ Shortening	- Poor separability
	60	-	-	+ Hard structure	+ Darkening	++ Shortening	+ Good separability
Defatting (DaD)		++ More P in smaller particles	+ More C in larger particles	+ Porous structure	++ Brightening	+ Shortening	++ Very good separability
Fluidized bed drying (FBD)		+ More P in smaller particles	+ More C in larger particles	+ Hard structure	+ Darkening	++ Shortening	+ Good separability

7. Conclusion and outlook

The assessment of the alterations caused by the different processes shows a variety of interesting observations. This information could be useful to establish the dry fractionation approach as an alternative to the commonly used wet extraction. Since this study is the first research on the influence of pre-treatments and drying methods on the dry fractionation behavior of mealworm larvae, it is contributing to the fundamental knowledge about this novel raw material.

Protein (P), as the most valuable fraction, was significantly enriched by the partial defatting process and the fluidized bed drying. Especially the smaller particle fraction contained more protein. Correlating to these observations, the chitin (C) content was reduced in larger particles. Freeze dried samples showed a chitin reduction in smaller fractions but had an evenly distributed protein concentration. On the other hand, no evidence for an accumulation was found for oven dried larvae. This information can be used to select the optimal parameters for obtaining protein and chitin. Since this polymer can be utilized for many different applications, further research on the nutritious usability (e.g. dietary fiber) should be conducted. Fluidized bed dried and/or partially defatted mealworms produced flours which could be separated in a high P / low C fraction (<500 µm) and a high C / low P fraction (>500 µm). Although these technologies are neither energy-efficient nor inexpensive, this knowledge could be used to establish new processes that use similar principles. A wet extraction of lipids prior to milling could be used to lower the fat content and improve the milling behavior.

It can be assumed, that the physio-chemical properties had a big influence on the fractionation behavior. The freeze drying and defatting process created a porous and brittle structure because the escaping water and lipids loosened the rigid exoskeleton while weakening the bonding between chitin and protein. This consequently led to an improvement of the breakage behavior during roller milling and a better separability of chitin and protein. Contrary to that, the convection drying methods led to a hardening of the structure and a stronger bonding of chitin and protein. Higher temperatures combined with longer drying time led to an increased hardening of the texture and reduced the separability. Although the freeze-dried samples showed the lowest structural integrity, the separation of chitin and protein was worse than for FBD and DaD. An explanation could be that a certain exposure to heat is needed to loosen the binding between the chitin and protein, which is not applied by the low temperatures of freeze-drying.

Further research on the heat stability of this connection between the protein and the polymer would be necessary. This information could also be useful for the vacuum drying process, which showed promising drying kinetics at relatively low temperatures (~45 °C).

A side effect worth mentioning is the color changes that occurred during this processes. Even though it most likely has small impact on the usability of the flour, it can be utilized as a simple indicator for the thermal stress the larvae went through. Increased brightness is linked to a better separability. However, when reaching a certain browning point, it can be assumed that an isolation of protein or chitin by dry fractionation is not possible anymore. A more detailed classification of these color changes could help to easily determine the processability.

Another field of interest are the methods used for the fractionation. The sieve classification showed acceptable and reproducible results but had limited applicability. A high fat content combined with the agglomeration of lipid particles led to a partial blockage of the sieves. A different design or another selection of mesh sizes could reduce this issue. Moreover, various classification methods like air classification or tribo-electric classification which are established in the cereal technology, could be promising alternatives. A high static charge of crystallized chitin was observed during the study. This could be utilized to separate the polymer with electrostatic processes.

Additional research is necessary on the functional properties of insect proteins. Dry fractionation is much less harsh than the wet alternative. It is suspected that proteins keep their native state during the fractionation and thus their functional abilities. More studies on the water binding, gelatinizing or foaming capacity are needed to support this theory.

To conclude these observations, the thesis has elucidated that there is a distinct influence on the dry fractionation behavior by the pre-treatments. This shows, that this otherwise established technology can be used as a viable alternative to the conventional wet fractionation of edible insects. Protein can be enriched and chitin can be separated by a simple classification. Optimizing and further researching these findings is still necessary to enlarge our knowledge on this novel material, which will consequently be introduced into our diet either as food, or indirectly as feed. It must be our objective to establish a sustainable supply of food for the growing human population and insects will do their part.

8. References

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9. Appendix

Table 12: Conductivity [mS] and degree Brix [%] of the water before blanching and after blanching.

	CD [mS]	Brix [%]
	Fresh water	
1	0.282	0.1
2	0.281	0
3	0.279	0.1
4	0.28	0.1
5	0.278	0.1
6	0.284	0.1
7	0.28	0.1
8	0.283	0.1
9	0.279	0.1
Mean [mS]	0.281	
	Blanching water	
1	0.472	0.1
2	0.602	0
3	0.548	0.1
4	0.508	0.1
5	0.569	0
6	0.705	0.1
7	0.651	0.2
8	0.701	0.1
mean	0.595	

Table 13: Weight gain [%] of the TML during the blanching process.

	Weight before blanching [g]	Weight after blanching [g]	Increase [%]
1	3757	4581	121.93
2	3758	4716	125.49
3	3757	4537	120.76
9	3759	4513	120.06
4	3756	4656	123.96
5	3826	4899	128.04
6	3756	4714	125.51
7	3756	4555	121.27
8	710	994	140.00
	30835	38165	123.77

Table 14:Color measurements [L,a,b] of the raw material, the dried larvae and the grist with the differences [ΔE] to the fresh and blanched larvae.

	L	a	b	ΔE_{fresh}	ΔE_{blanch}
Fresh	54.26	10.04	23.15	0.00	5.46
Blanched	51.19	7.38	19.50	5.46	0.00
Dried					
FD	59.73	8.94	25.37	6.01	10.48
OD80	48.04	7.47	15.94	9.86	4.76
OD60	48.01	7.93	17.87	8.44	3.62
DaD	56.03	8.58	23.72	2.37	6.53
DwB	39.51	6.90	12.78	18.30	13.49
DwF	45.35	6.98	15.42	12.18	7.14
FBD	46.61	8.76	19.19	8.70	4.80
Milled					
FD	46.84	8.02	19.44	8.53	4.40
OD80	28.87	6.26	21.77	25.70	22.46
OD60	28.99	6.36	20.69	25.65	22.25
DaD	46.40	7.27	19.74	9.00	4.80
DwB	16.44	6.54	12.81	39.36	35.40
DwF	24.36	7.52	17.52	30.53	26.91
FBD	29.45	7.15	18.93	25.32	21.75

Table 15: Composition (%db protein, fat and chitin) of different size fractions (0-1400 µm). Six methods (DwB, DaD, OD60,OD80, FD and FBD) are compared.

Sample ID	Composition [%db]					
	< 355 µm	**355 - 500 µm**	**500 - 710 µm**	**710 - 1000 µm**	**1000 - 1400 µm**	**> 1400 µm**
DwB						
Crude protein		56.84 ± 0.33		55.12 ± 0.57	53.36 ± 0.38	54.10 ± 0.69
Crude fat		24.06 ± 0.43		24.39 ± 0.18	26.62 ± 0.27	25.57 ± 0.31
Chitin		7.71 ± 0.46		10.28 ± 0.68	11.88 ± 1.33	10.39 ± 0.16
DaD						
Crude protein	65.55 ± 0.09	66.47 ± 0.09	65.99 ± 0.81	60.17 ± 0.01	60.24 ± 0.57	62.03 ± 0.66
Crude fat	11.65 ± 0.17	9.04 ± 0.06	8.45 ± 0.03	8.79 ± 0.02	9.10 ± 0.17	8.26 ± 0.12
Chitin	8.72 ± 0.23	10.75 ± 0.36	10.33 ± 0.44	12.04 ± 0.66	13.53 ± 1.20	11.64 ± 0.22
OD60						
Crude protein		53.39 ± 1.78	55.90 ± 0.69	54.77 ± 1.17	53.55 ± 1.11	54.59 ± 0.85
Crude fat		23.90 ± 0.31	22.99 ± 0.38	23.34 ± 0.23	24.93 ± 0.20	24.52 ± 0.25
Chitin		10.60 ± 0.00	10.01 ± 0.97	11.92 ± 1.10	11.91 ± 0.35	11.86 ± 0.59
OD80						
Crude protein		55.04 ± 1.40		53.89 ± 1.19	53.37 ± 1.54	53.21 ± 1.47
Crude fat		22.88 ± 0.82		24.44 ± 0.41	25.50 ± 0.47	25.69 ± 0.25
Chitin		10.54 ± 1.51		10.23 ± 0.69	9.91 ± 0.40	10.98 ± 0.91
FD						
Crude protein		55.05 ± 1.07	52.51 ± 0.78	53.45 ± 1.03	53.75 ± 0.86	54.48 ± 0.91
Crude fat		26.12 ± 0.27	24.12 ± 0.18	23.40 ± 0.23	21.35 ± 0.13	21.78 ± 0.17
Chitin		3.60 ± 0.65	11.20 ± 0.45	11.23 ± 0.19	16.06 ± 0.67	13.44 ± 0.90
FBD						
Crude protein		58.24 ± 0.13	56.61 ± 0.36	55.95 ± 0.59	54.57 ± 0.65	53.75 ± 0.48
Crude fat		22.85 ± 0.19	21.70 ± 0.11	22.32 ± 0.34	24.29 ± 0.24	26.15 ± 0.85
Chitin		8.10 ± 0.72	11.35 ± 1.30	13.02 ± 0.65	12.35 ± 0.73	12.46 ± 0.87