



Instituto Universitario de Investigación Mixto
Agroalimentario de Aragón
Universidad Zaragoza



METU

Insects as New Generation Protein Sources: Novel Production and Characterization Approaches

Hami Alpas, Mecit Halil Öztop, Berkay Bolat, Ahmet Erdem Uğur

Department of Food Engineering, Middle East Technical University, 06800, Ankara, Turkey



Outline

- Edible Insects
- Insects in Food Industry
- Insects in Literature
- International Conferences Around the World
- High Hydrostatic Pressure (HHP)
- Nuclear Magnetic Resonance (NMR)
- Characterization of Insect Proteins and Fats
- Preliminary Results
- Conclusion
- Ongoing Work



Edible Insects

Insects: Sustainable protein and nutrient source as food and feed
>2000 known to be edible

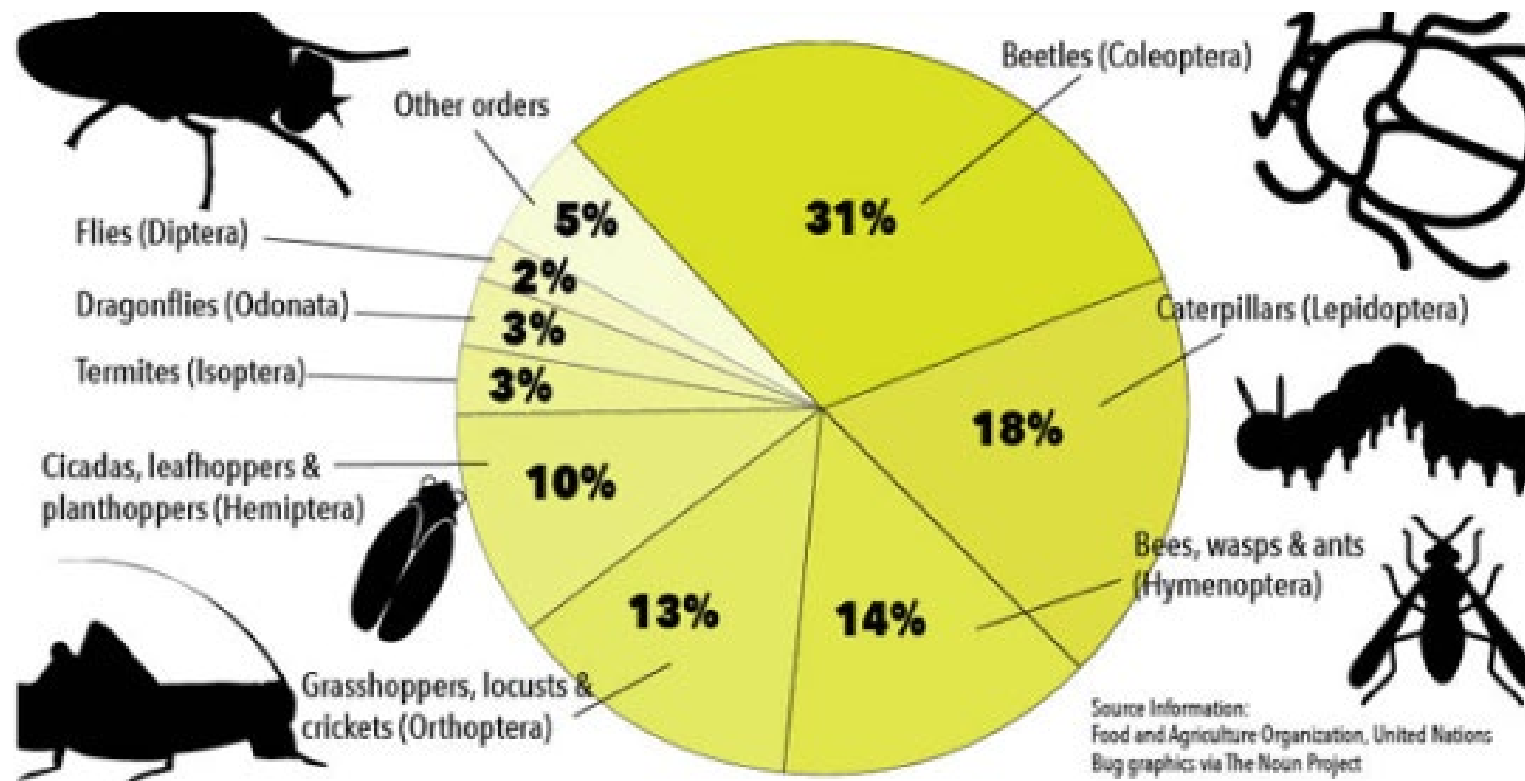


Figure. Most commonly eaten insects worldwide. Adapted from Insects for food and feed in *FAO*, Retrieved from <http://www.fao.org/edible-insects/84627/en/>

Edible Insects

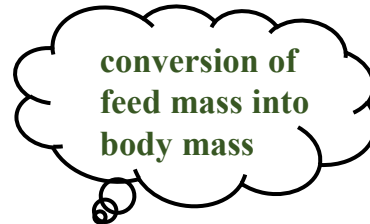


Green Benefits

- Environmentally friendly breeding
 - ✓ Generate less amount of waste product and greenhouse gases
 - ✓ Result in less water pollution & usage
 - ✓ Require less land
 - ✓ Play a vital role in waste biodegradation

- Higher feed conversion efficiency

- ✓ e.g *Acheta domesticus*
 - 2 x chicken
 - 4 x pigs
 - 12 x cattle



Health Benefits

- Higher nutritional value
 - ✓ **Protein content:** 20 to 76% of dry matter
 - ✓ **Fat content:** 2 to 50% of dry matter
 - ✓ Carbohydrates: 2.7 mg to 49.8 mg per kg of fresh matter
 - ✓ Mostly **rich in polyunsaturated fatty acids** & frequently contain the essential **linoleic & α -linolenic acids**.
 - ✓ Contain reasonable amount of minerals; K, Na, Ca, Cu, Fe, Zn, Mn, P
vitamins; thiamine, riboflavin, B12, retinol

Edible Insects



Green Benefits

Greenhouse Gasses

Live stock production creates massive amounts of greenhouse gas emissions (GHG). Globally it amounts to more than automobile emissions. Insect farming is more efficient and environmentally sound.

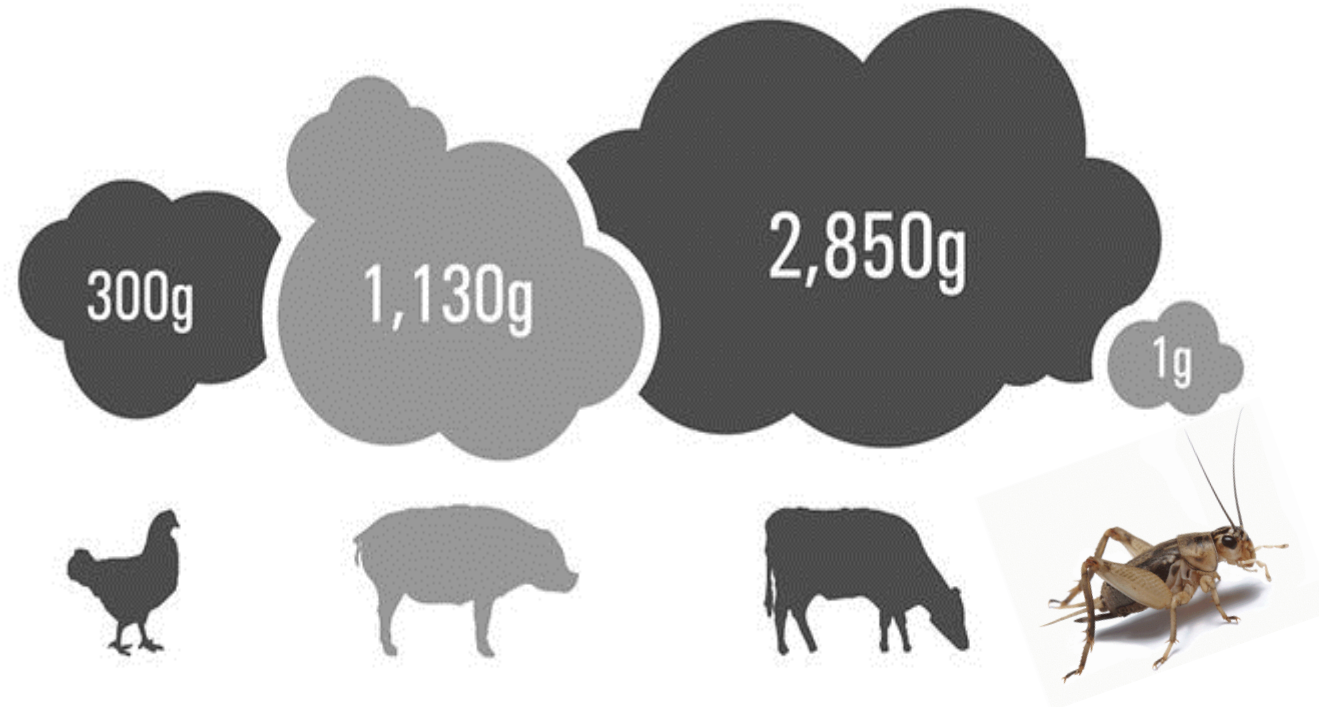


Figure. Average greenhouse gasses from the production of 1 kg of protein.

Adapted from Crickers Infographics in *JLF Design*, Retrieved from <http://cargocollective.com/jlfdesign/Crickers-Infographics>

Edible Insects



Green Benefits

Greenhouse Gasses and Ammonia

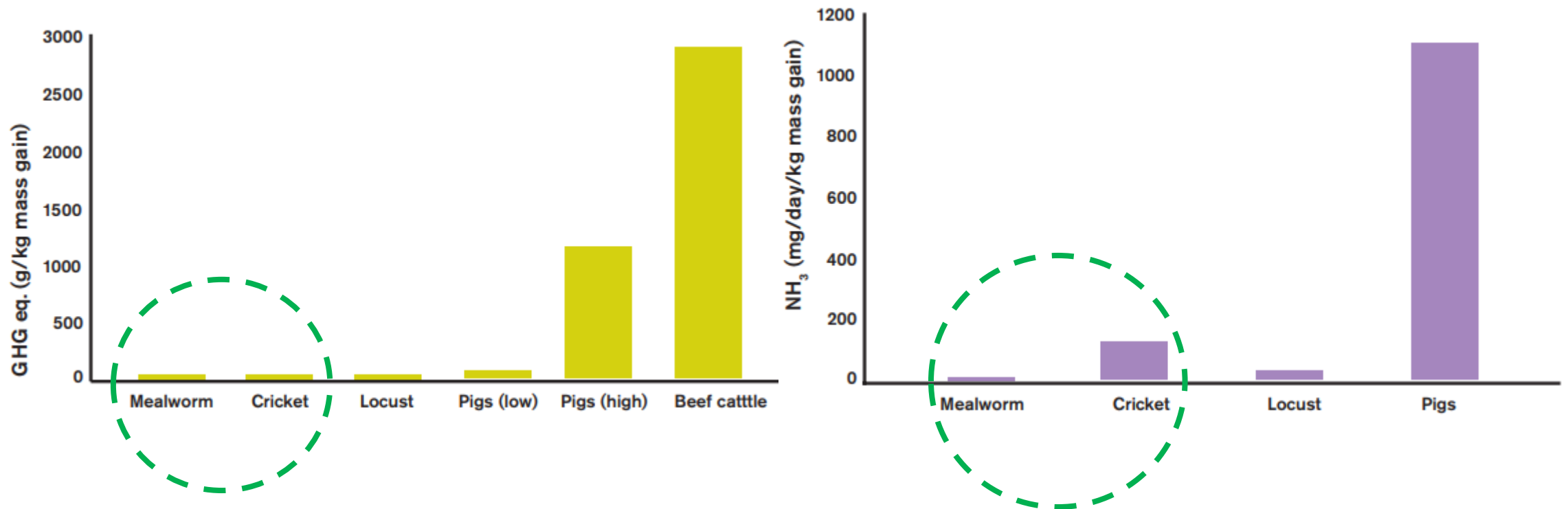


Figure. Adapted from *Insects as Food – Something for Future?*, by A.Jansson and A.Berggren, 2015 by the *Swedish University of Agricultural Sciences*

Edible Insects



Green Benefits

Smart Land Utilization

- 70% of agricultural land and 30% of all land on earth are utilized to raise animals.
- Insect farming on average requires much less land due to many innovations including vertical farming techniques

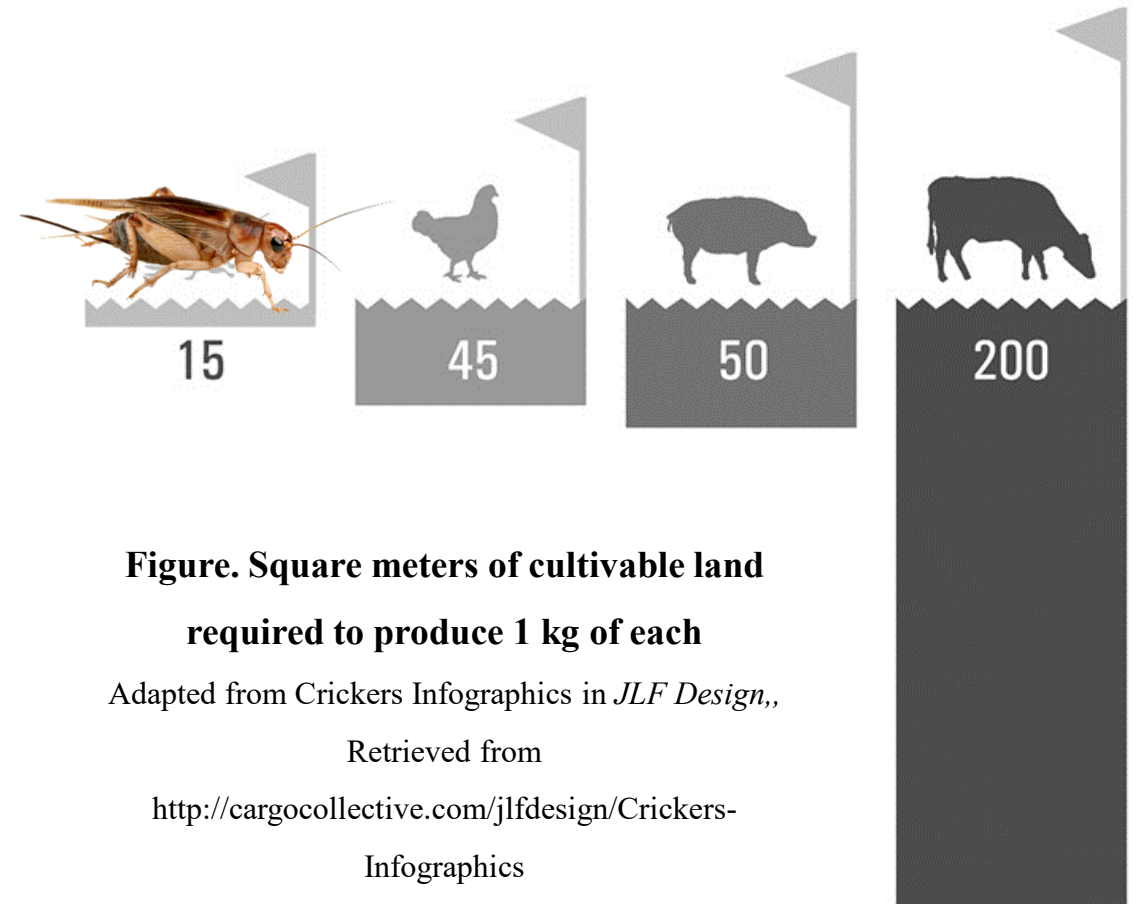


Figure. Square meters of cultivable land required to produce 1 kg of each

Adapted from Crickers Infographics in *JLF Design*,

Retrieved from

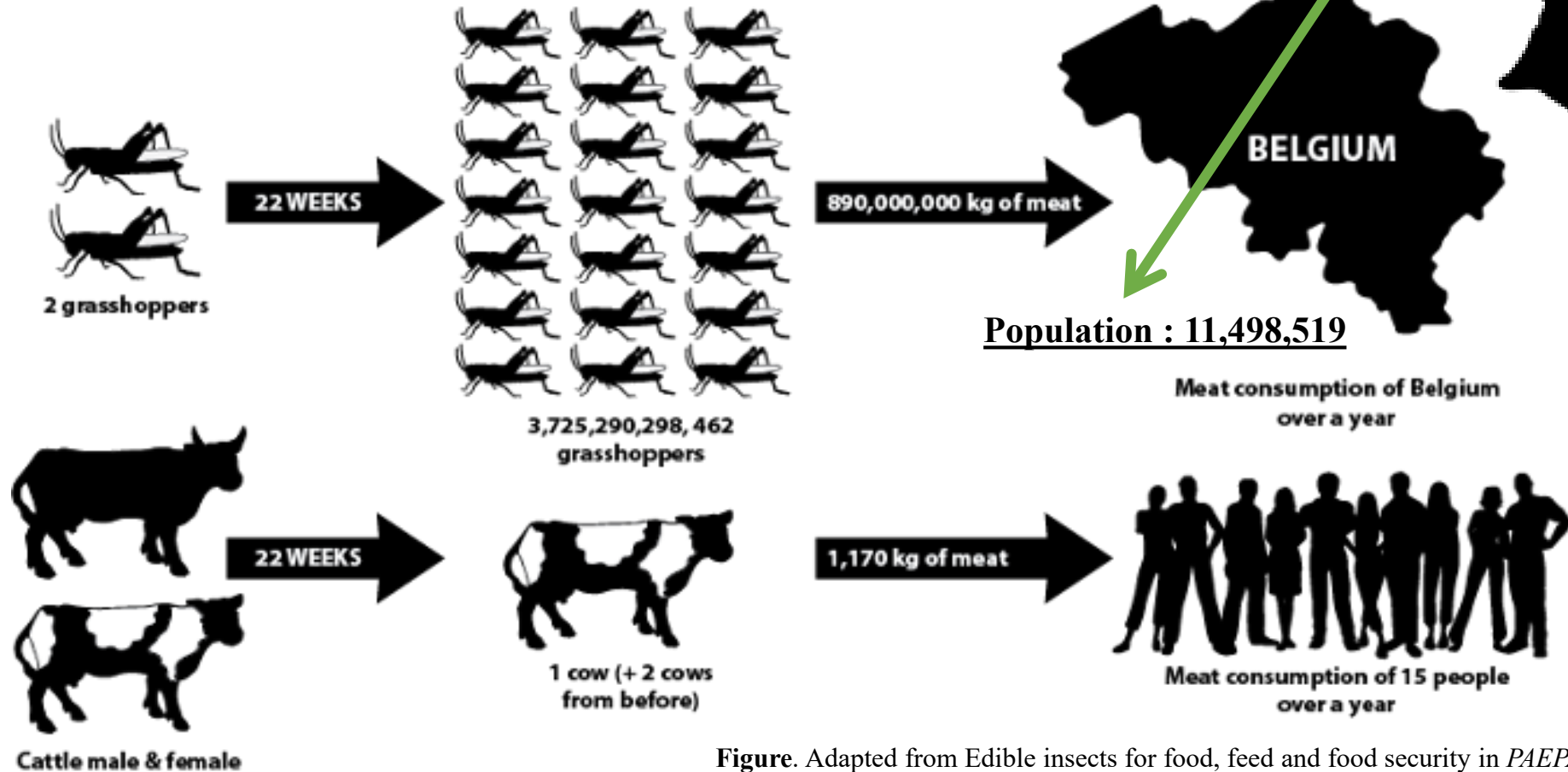
<http://cargocollective.com/jlfdesign/Crickers-Infographics>

Edible Insects



Green Benefits

Smart Land Utilization & Yield



Also, meat consumption of Portugal over a year!!!

PORTUGAL

BELGIUM

Figure. Adapted from Edible insects for food, feed and food security in *PAEPARD*, Retrieved from <http://paepard.blogspot.com.tr/2018/03/edible-insects-for-food-feed-and-food.html>

Edible Insects



Green Benefits

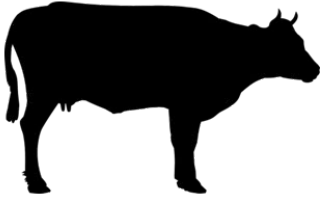
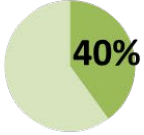




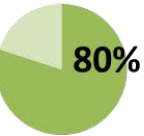



1kg Meat	Water	Feed	Emissions
  40% Edible	 20,000 litres	 25kg	 100x more
  80% Edible	 8 litres	 2kg	

Figure. Adapted from Why eat bugs? in *the BUGSHACK*, Retrieved from <http://thebugshack.co.uk/why-eat-bugs/>

Edible Insects



Green Benefits

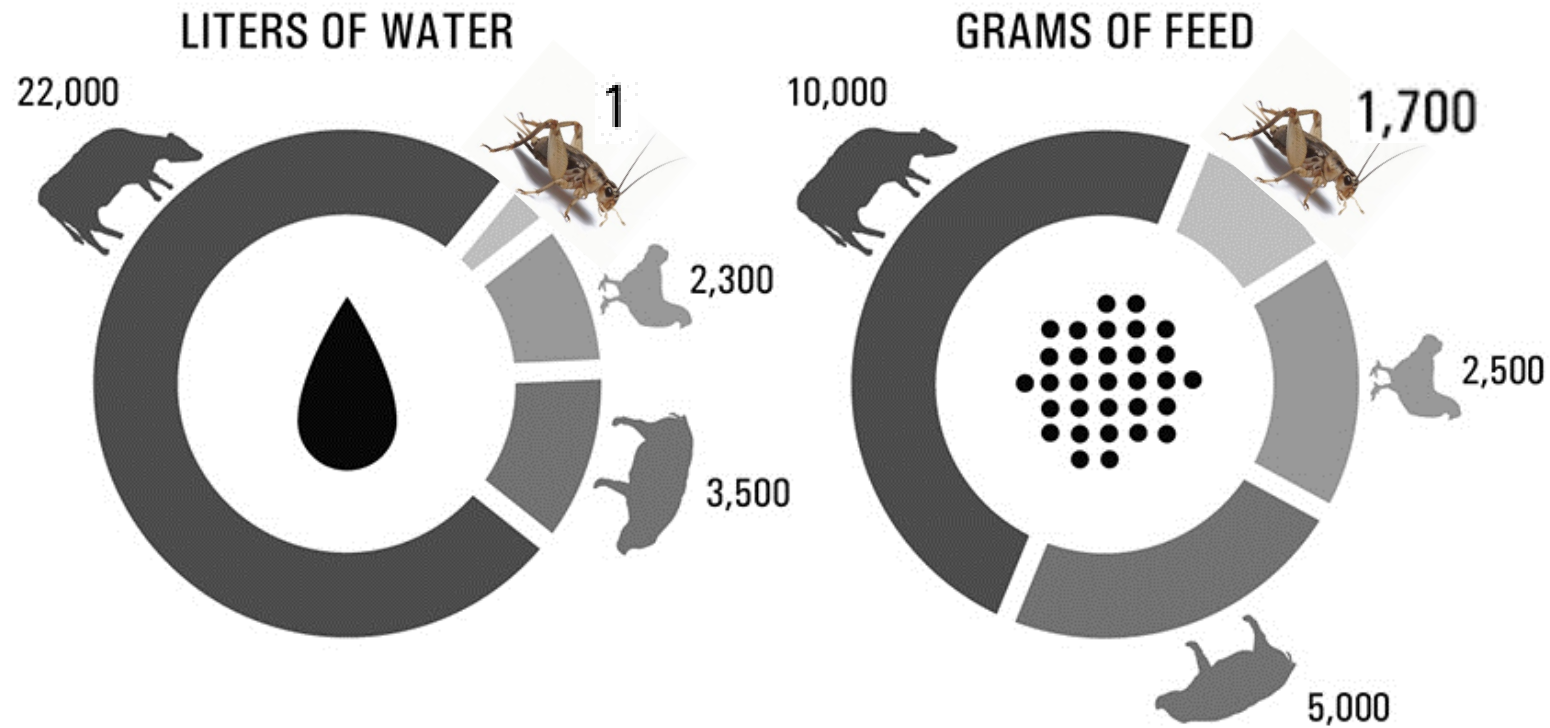


Figure: Resources needed to produce 1 kg of each protein source.

Adapted from Crickers Infographics in *JLF Design*, Retrieved from <http://cargocollective.com/jlfdesign/Crickers-Infographics>

Edible Insects



Green Benefits

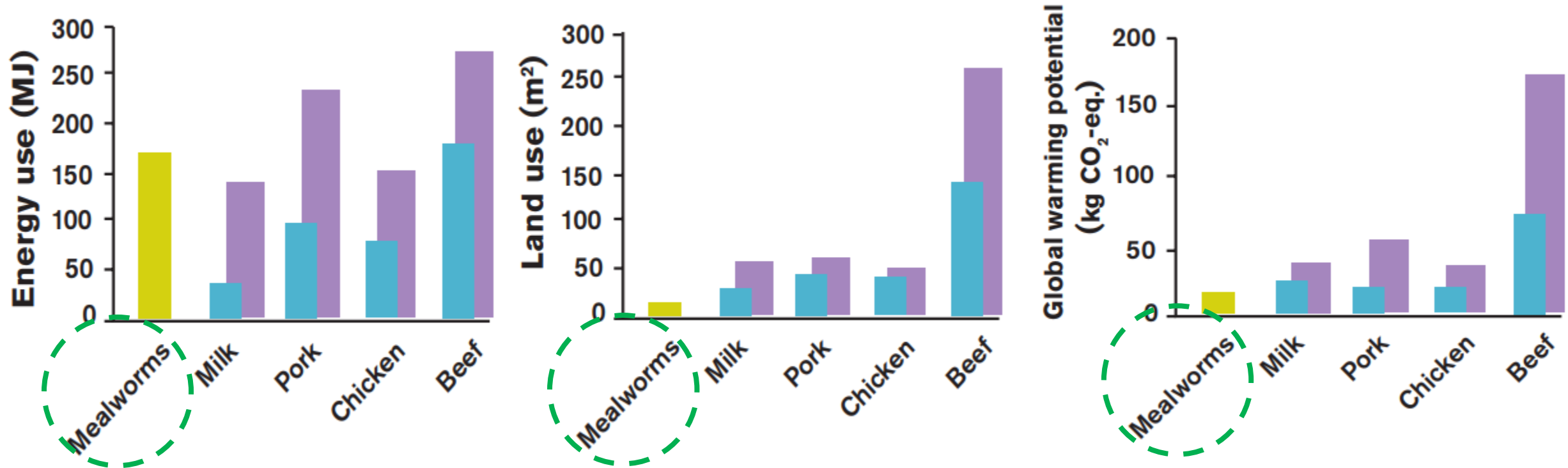


Figure. The production of one kg of edible protein. Adapted from *Insects as Food – Something for Future?*, by Berggren, A and Jansson, A, 2015 by the *Swedish University of Agricultural Sciences*

- The data was obtained from one farm in the Netherlands
- Purple and blue bars represent the maximum and minimum data from a literature survey

Edible Insects



Green Benefits

Feed Conversion Efficiency

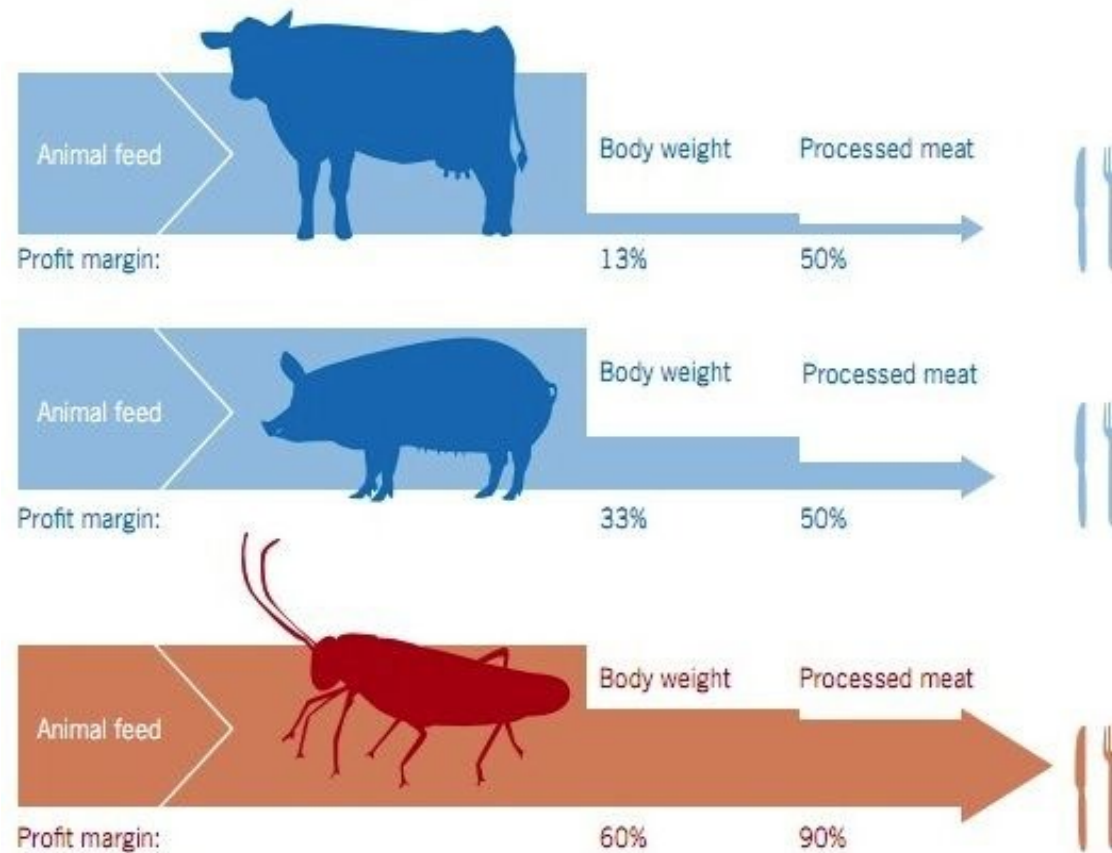


Figure. Adapted from Entomophagy Infographics in *Pinterest*, Retrieved from <https://tr.pinterest.com/bugvivant/entomophagy-infographics/>

Edible Insects

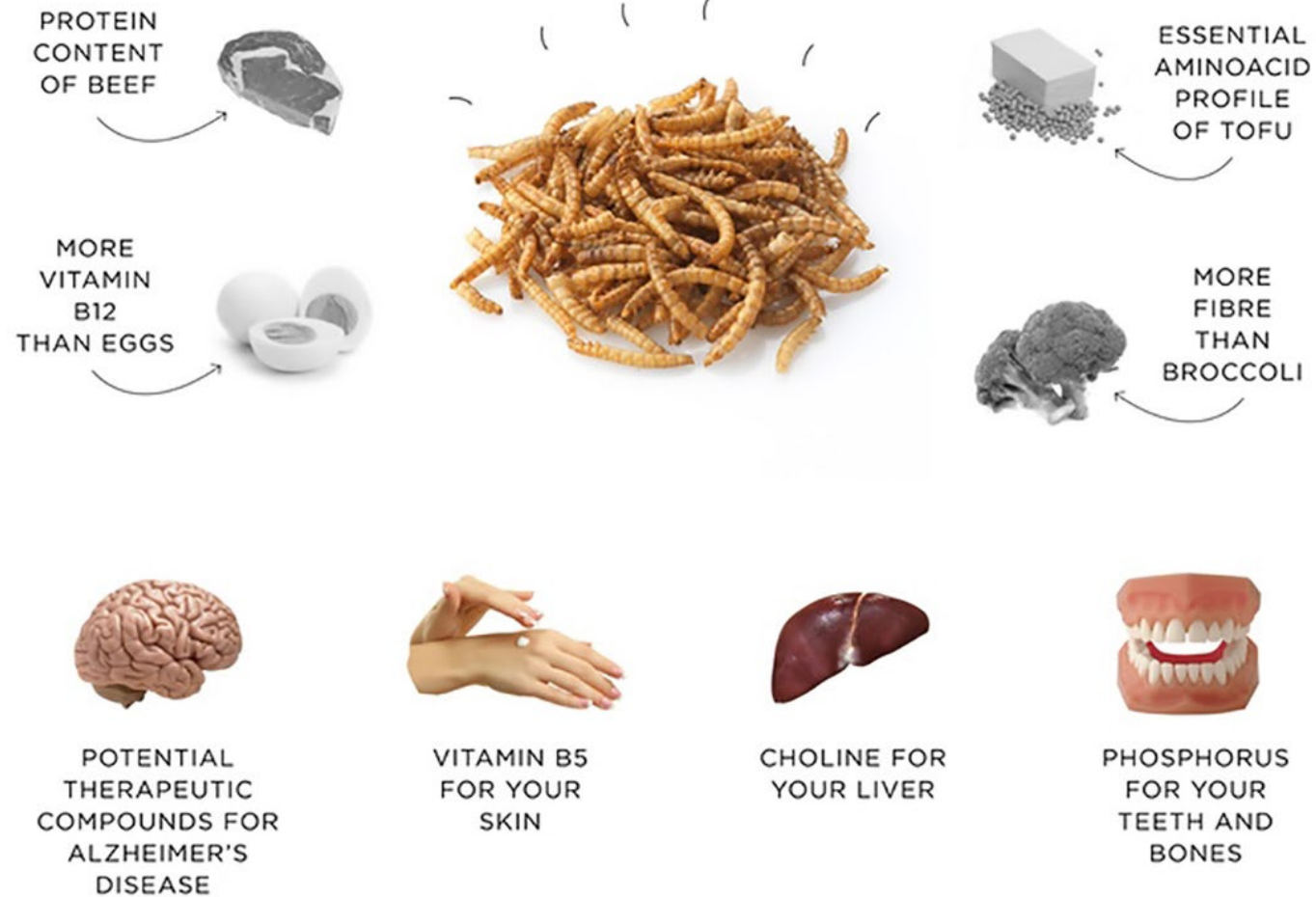


Figure. Adapted from Livin Farms make it easy to grow edible insects at home in *Inhabitat* ,Retrieved from <https://inhabitat.com/livin-farms-makes-growing-sustainable-and-healthy-protein-as-easy-as-compost/>

Edible Insects

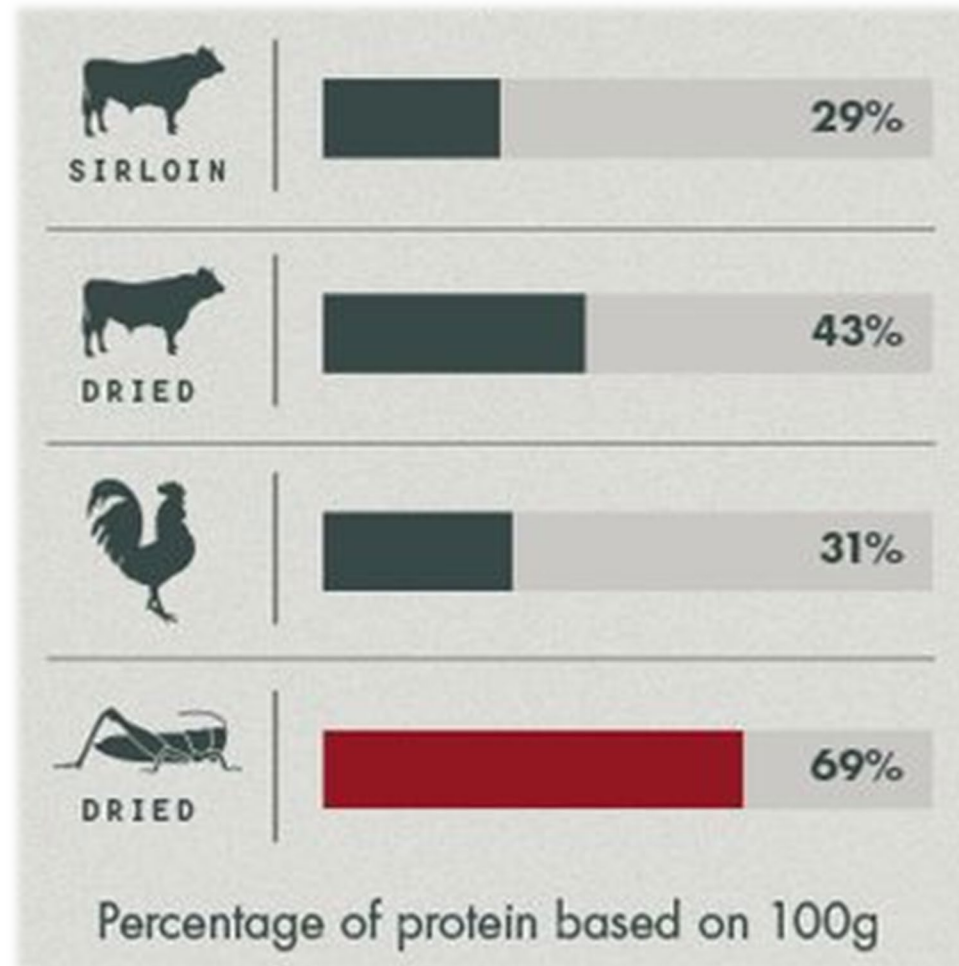


Figure. Adapted from Why Eat Insects or Crickets in *Seginus Farms*, Retrieved from <https://www.seginusfarms.com/blog/2017/1/11/why-eat-insects-or-cricket>

Edible Insects

Acheta domesticus & *Tenebrio molitor*

Acheta domesticus

- Commonly called as **house cricket**,
- Most likely native to Southwestern Asia, but has spread worldwide.
- Most commonly eaten as a deep-fried snack and are also sold in a powder form.



Tenebrio molitor

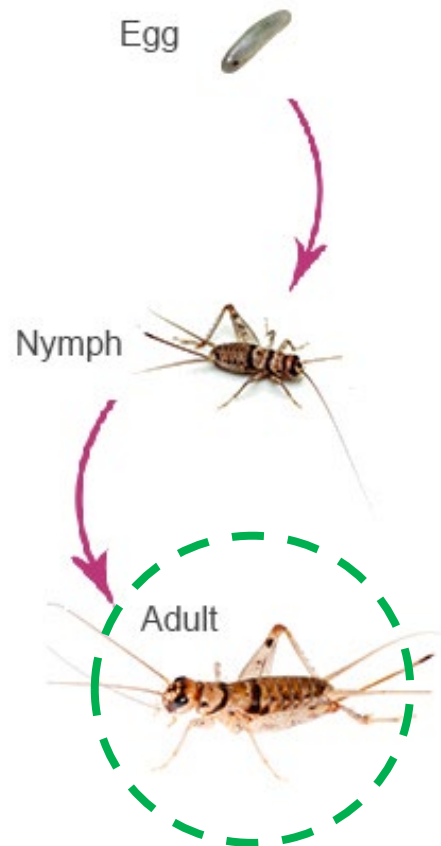
- Commonly referred as **mealworms**, yellow mealworms, mealworm beetles, darkling beetles, and darkening beetles,
- Mostly eaten in the larval stage.
- Easy to culture (often raised on oats, and females lay up to 500 eggs) and readily available commercially.



Edible Insects

Acheta domesticus & *Tenebrio molitor*

Acheta domesticus



Tenebrio molitor

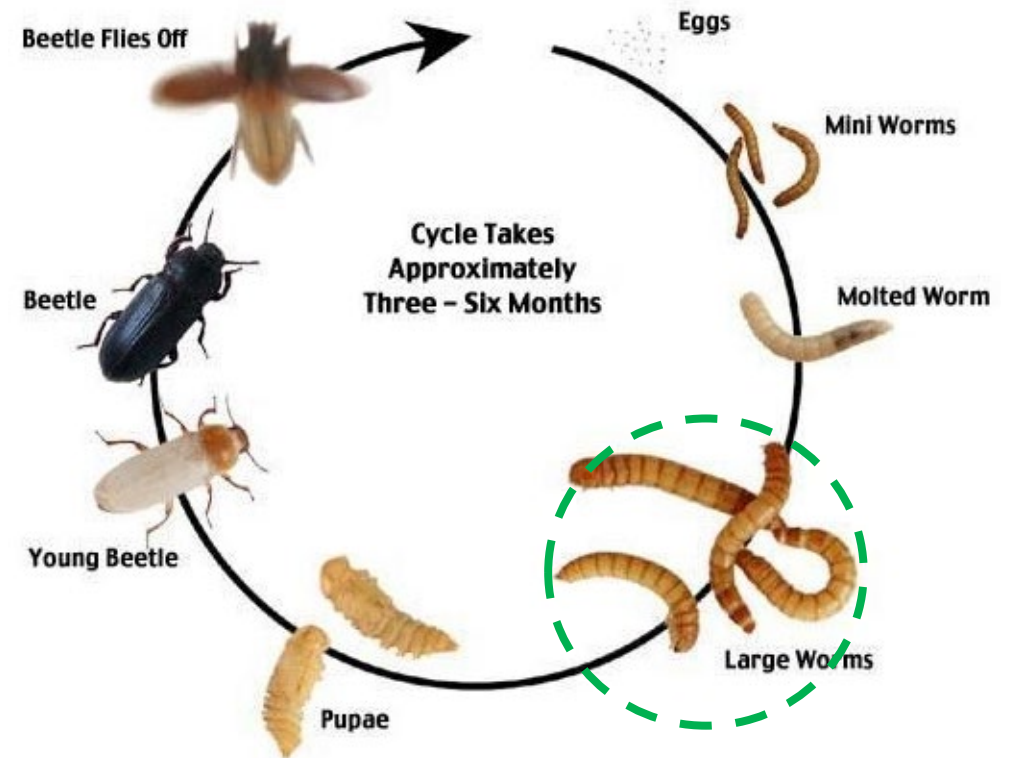


Figure. Adapted from Cricket Control in *Arrow Exterminators, Inc.*
Retrieved from <https://www.nomorebugs.com/crickets/>

Figure. Adapted from Livin Farms make it easy to grow edible insects at home in *Inhabitat*, Retrieved from <https://inhabitat.com/livin-farms-makes-growing-sustainable-and-healthy-protein-as-easy-as-compost/>

Edible Insects

Acheta domesticus & *Tenebrio molitor*

Proximate composition in DM (%)

Parameter	Insect Substrates		Reference Substrates		
	<i>Acheta domesticus</i>	<i>Tenebrio molitor</i>	Poultry meat meal	Fish meal	Soybean meal
Crude protein	70.6	52.0	69.1	71.0	51.6
Fat	17.7	33.9	12.8	9.2	2.5
Ash	5.3	3.9	15.4	19.9	6.8
AA Profile					
Arg	5.7	4.6	5.8	4.5	6.3
His	3.4	5.1	3.7	3.4	3.1
Ile	4.0	4.6	3.8	4.8	5.0
Leu	6.6	7.3	6.4	7.1	7.8
Lys	5.8	5.5	5.6	7.4	6.2
Met	1.6	1.4	1.0	1.9	2.0
Phe	3.2	3.4	3.3	3.5	5.2
Thr	3.6	4.0	3.6	4.0	3.9
Val	5.7	6.3	4.6	5.0	5.0
TIAA	39.6	42.3	37.8	41.5	44.4

Essential aminoacids:
histidine, isoleucine,
leucine, lysine,
methionine,
phenylalanine, threonine,
tryptophan, and valine.

Edible Insects

The FAO Perspective

FAO working on edible insects since 2003 to;

- Generate and share knowledge in the field through publications, expert meetings and a website on edible insects
- Raise awareness of insects as food through media collaborations
- Support networking and multidisciplinary interactions with various sectors within and outside FAO



The release the potential of insects as food, FAO (2013) has identified *four key* bottlenecks and challanges that must be addressed;

- Further documentation is needed on the nutritional value of insects, in order to efficiently promote insects as a healthy food source
- The environmental impacts of both harvesting and farming insects must be investigated, to allow comparisons with conventional livestock production
- The socio-economic benefits that insect harvesting and farming can offer, particularly in poor contries, must be clarified and communicated
- Clear and comprehensive legal frameworks at national and international level are needed to pave the way for investments, development of production and trade in insect production

Edible Insects

The FAO Perspective

The knowledge gaps;

- Sustainable harvest from nature
- Indigenous knowledge of edible insects
- Identification of edible insects
- *Standard methods for determination nutritional value*
- Mass-rearing techniques
- Trade and value chains
- Ethical issues (animal welfare)



Insects in Food Industry

Who eats bugs?

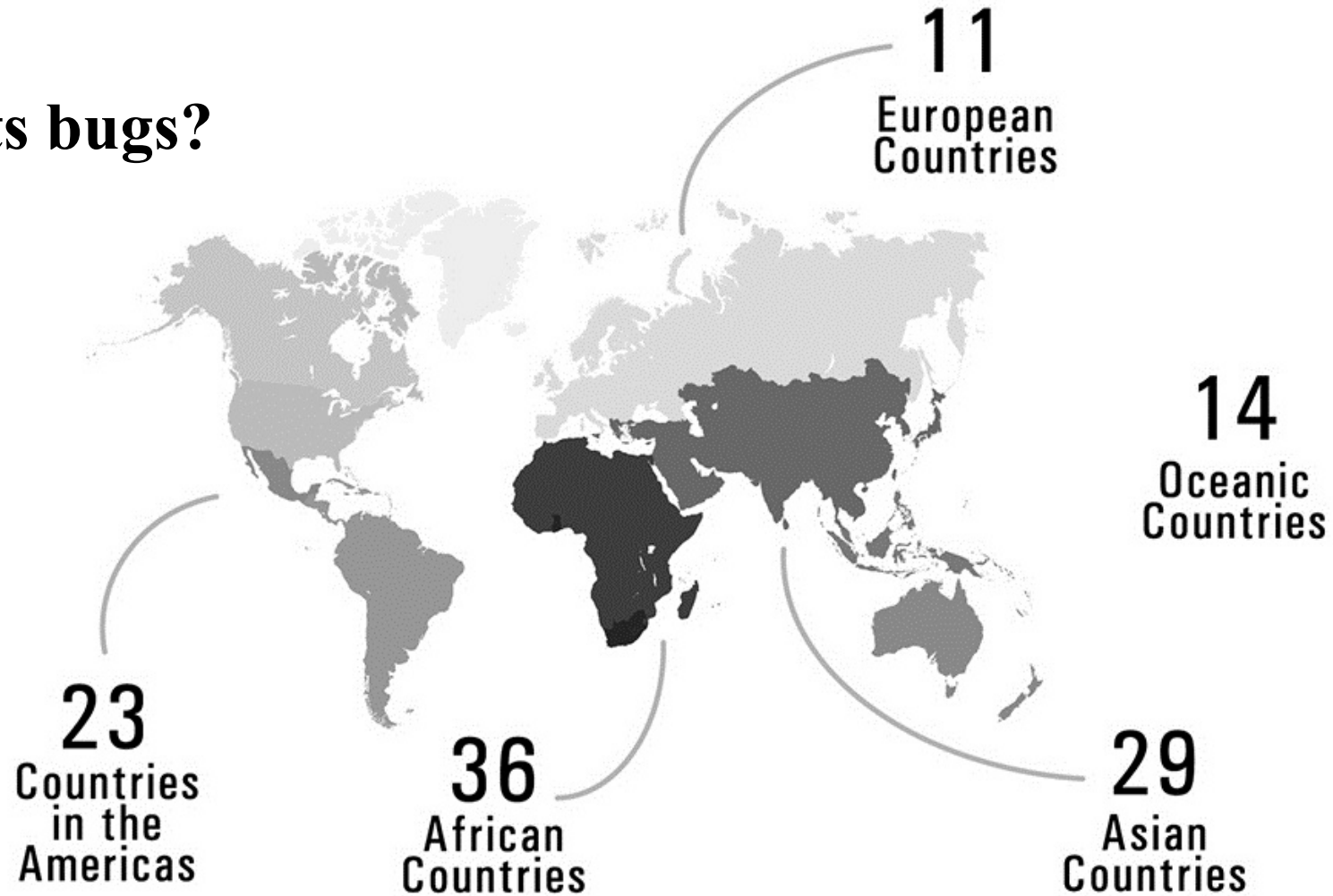


Figure. Adapted from Crickers Infographics in *JLF Design*, Retrieved from <http://cargocollective.com/jlfdesign/Crickers-Infographics>

Insects in Food Industry

*Edible insect market is expected to reach **1,18 billion \$** by 2023, supported by a CAGR* of **23.8%** during the forecast period of 2018 to 2023*

ASIA - PACIFIC

- Held the *lion's share* in global edible insect market with **13.0 million \$**,
- Led by **China, Vietnam and Thailand**
- In Thailand, over 200 bugs are consumed
 - Both cooked and fresh eatable bugs in wholesale and local markets.

EUROPE

- Forecasted to witness a robust growth in insect market and reach at **46.2 million \$** by 2023
- Dominated by **France, Belgium and Netherlands**
- Mostly use crickets and mealworms as pet food.

AMERICA

- **North America**, led by U.S.,
 - **45.3%** of beetles and caterpillars sales in the region in 2015
- **Latin America**, led by **Mexico and Brazil**,
 - Sales of bug-based flour reflected **40.6%** in Latin America in 2015

*The compound annual growth rate (CAGR) is the mean annual growth rate of an investment over a specified period of time longer than one year.

Insects in Food Industry

- **Major players;** *EnviroFlight, Kreca, AgriProtein, HaoCheng Mealworm Inc.*
- **Companies involved in insect farming and produces organic insect products;** *Thailand Unique, Entomo Farms, Proti-Farms*
- **Other notable industries;** *Chapul Inc., Exo Protein, Six Foods, Bitty Foods, Gathr Foods, Edible Inc, Bodhi, Nutribug*

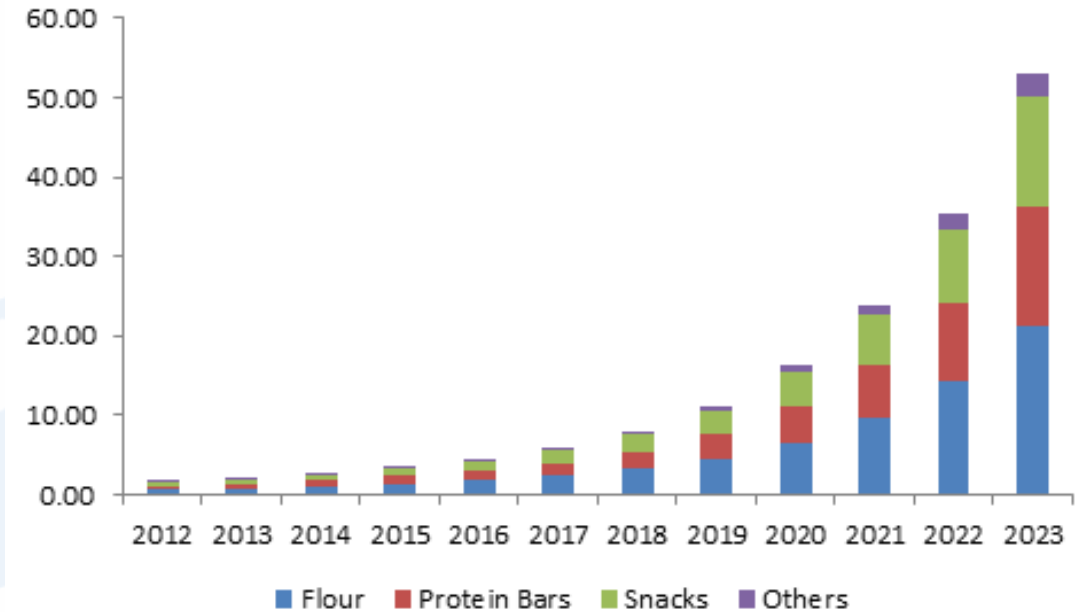


Figure. U.S. Edible Insects Market size, by application, 2012-2023 (Million \$)

Insects in Food Industry

Commercial Products

- *Company name:* **Chapul, USA**

✓ *Insect ingredient:* **Cricket**

✓ *Product:* **Tasty bars** in 4 different flavours;

- Aztec Bar; with cocoa and coffee beans
- Chaco Bar; with chocolate and peanuts
- Thai Bar; with coconut, ginger, and lime
- Matcha Bar; with Matcha tea, banana and organic tahini

Price: \$13.00/4 pack



- *Company name:* **Exo Protein, USA**

✓ *Insect ingredient:* **Cricket**

✓ *Product:* **Cricket Flour Based Protein Bars** in 4 different flavours;

- Cacao Nut
- Banana Bread
- Apple Cinnamon
- Blueberry Vanilla

Price \$36/12 pack



Insects in Food Industry

Commercial Products

- *Company name:* **Entosense - EntoVida, USA**

✓ *Insect ingredient:* **Cricket**

✓ *Product:* **Cricket crunch bar** with 2 flavours

- Dark Chocolate
- Milk Chocolate

Price: \$5.45/pack



- *Company name:* **HotLix, USA**

✓ *Insect ingredient:* **Ant, Cricket, Scorpion and Mealworm**

✓ *Product:* **Insect Lollipops** with various flavours

- Apple
- Banana
- Blueberry
- Strawberry
- Orange
- Grape
- Watermelon
- Tequila

Price: \$2.00/pack



Insects in Food Industry

Commercial Products

- *Company name:* **Micronutris, France**

✓ *Insect ingredient:* **Mealworm and Cricket**

✓ *Products*

- **Biscuits and Crackers**

➤ Price: 8,50€/pack

- **Insect Appetizers**

➤ Price: 9,90€ /pack

- **Pastas**

➤ Price: 10,60€/pack

- **Chocolates or Macorons with insects on top**

➤ Price: 13,90€/pack



- *Company name:* **Goffard Sisters, Belgium**

✓ *Insect ingredient:* **Mealworm**

✓ *Product:* **Aldento – Mealworm Flour Pasta**

Price: 4,69 € /pack



Insects in Food Industry

Commercial Products

- *Company name:* **Bitty Foods, USA**
- ✓ *Insect ingredient:* **Cricket**
- ✓ *Product:* **Chiridos Air-Puffed Chips** in 3 different flavours;
 - Salsa verde, with spices
 - Baja ranchero, with ranch seasoning
 - Spicy mole, with molé seasoning

Price: currently unavailable



- *Company name:* **Six Foods, USA**
- ✓ *Insect ingredient:* **Cricket**
- ✓ *Product:* **Chirps Cricket Chips** in 3 different flavours;
 - Sea salt
 - BBQ
 - Cheddar

Price: 12,99€/ 6 pack



Insects in Food Industry

Commercial Products

- *Company name:* **Bugfoundation, Germany**

✓ *Insect ingredient:* **Buffaloworm**

✓ *Product:* **The Bux Burger**

Price: 6 €



- *Company name:* **Essento, Switzerland**

✓ *Insect ingredient:* **Mealworm**

✓ *Product:* **Burger & Balls**



Insects in Food Industry

Commercial Products

- *Company name:* **Bite back, USA**
 - ✓ *Insect ingredient:* **Mealworm**
 - ✓ *Product:* **Butter & Cooking oil**



- *Company name:* **Flying SpArk, Israel**
 - ✓ *Insect ingredient:* **Fruit Fly**
 - ✓ *Product:* **Cooking oil**



Insects in Literature

- Less than 41,000 researches about **edible insects**
 - Less than 1,700 researches about **Acheta domesticus**
 - Less than 4,000 researches about **Tenebrio molitor**
- Less than 100,000 researches about **insect protein**
- Around 29,000 researches about **insect protein characterization**
- Less than 25,000 researches about **insect fat**
- Around 7,000 researches about **insect fat characterization**



High Hydrostatic Pressure (HHP)

What is HHP?

- ✓ Non-thermal food preservation method
- ✓ The application of extremely high pressures ($P > 2000$ atm) to foods submerged in a liquids for a desirable period of time(t) at a desirable temperature (T)

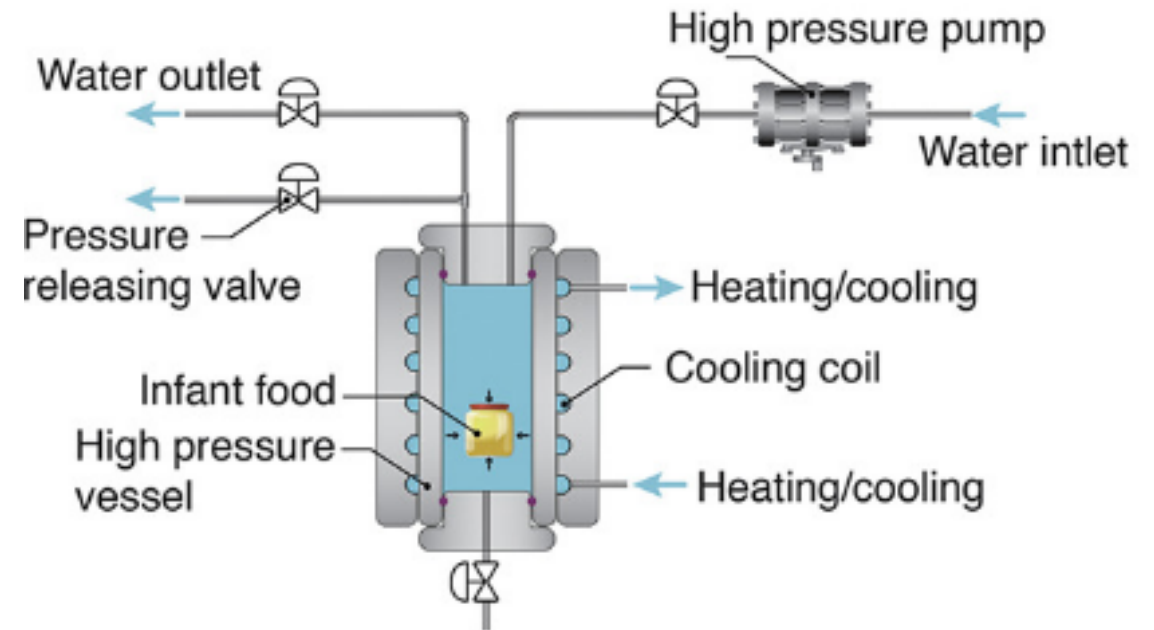
Advantages:

- Process independent of the mass, volume and the shape of the food so no edge or thickness effect takes place
- Sensory and nutritional attributes of the product remain virtually unaffected after the treatment
 - Do not adversely effect the food quality
 - Heat-labile nutrients and natural flavours are effectively remained
- Effectively destroys vegetative cells, enzymes, yeast and molds. Semi-effective on spores
 - Lengthen the shelf-life of food products
- **Can modify functional properties of components such as proteins**

High Hydrostatic Pressure (HHP)

Basic Components of Device

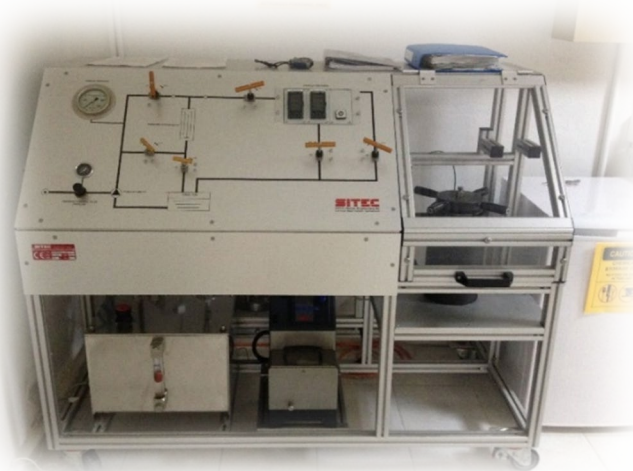
- Pressure making unit
- Pressure vessel
- Pressure transfer medium
 - ✓ Foodstuffs can be in the direct contact with medium, or over the flexible barrier.
 - ✓ Most common medium used for transfer of pressure is water, but with packaged foods ethanol, castor oil, glycol etc. can be used.



High Hydrostatic Pressure (HHP)

Critical Factors

- Greater pressure and longer time may cause changes in the appearance of some foods (raw high protein and structurally fragile foods)
- There is an increase of 3 °C for each 100 MPa of the product through adiabatic heating, therefore the achieve an uniform process temperature, uniform initial temperature is required
- Imposed pressure decrease the volume of the food and equal expansion occurs on decompression, therefore packaging material should be able to accommodate up to 15% reduction in volume, so that seal integrity and barrier properties are conserved



High Hydrostatic Pressure (HHP)

Effect on Proteins

- Pressure denaturation of protein is a complex phenomenon depending on;
 - ✓ protein structure, pressure range, temperature, pH, and solvent composition
- Oligomeric protein denaturation → at relatively low pressures (200 MPa)
- Monomeric protein denaturation → at pressures >300 Mpa
- Pressure greater than 100-200 MPa often causes:
 - ✓ dissociation of oligomeric structures into their subunits
 - ✓ partial unfolding and denaturation of monomeric structures
 - ✓ protein aggregation
 - ✓ protein gelation if protein concentration and pressure are high enough



High Hydrostatic Pressure (HHP)

Effect on Proteins

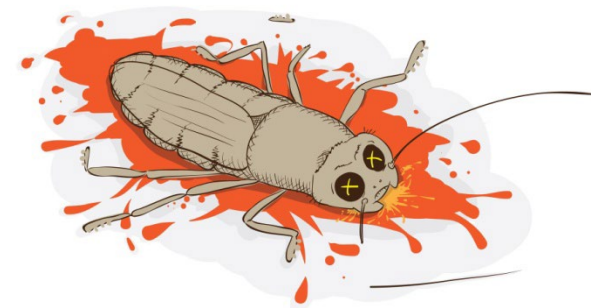
- Main targets of pressure;
 - ✓ electrostatic and hydrophobic interactions in protein molecules

CAUSES

- ✓ deprotonation of charged groups and disruption of salt bridges and hydrophobic interactions,
 - resulting in conformational and structural changes of proteins

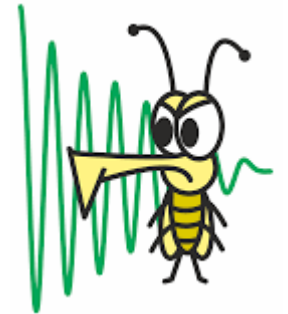
DO NOT AFFECT *covalent bonds*

- ✓ because the length of covalent bonds is already limited by the Born repulsion
 - small molecules such as vitamins, color, and flavor compounds will remain unaffected
 - resulting in retention of nutrients and more natural and “better” quality product



Nuclear Magnetic Resonance (NMR) Relaxometry

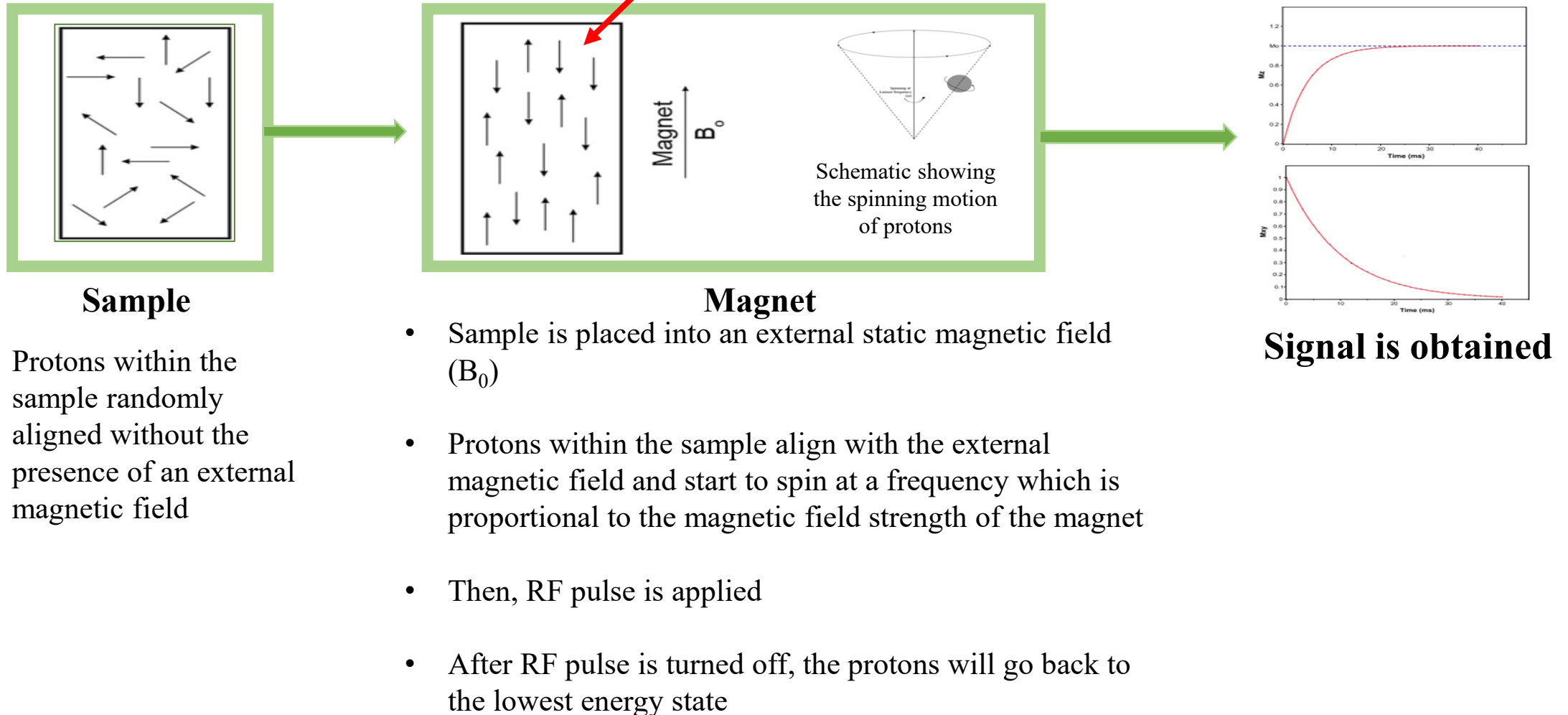
- Widely used in the analysis of physiological and biochemical changes in food samples
- Non-invasive, non-destructive method
- Feature the use of a radio frequency (RF) pulse in order to create a temporary disturbance on a sample placed into a magnetic field
- RF pulse is an electromagnetic wave in the radiofrequency range, used in combination with magnetic gradients to generate signal



Nuclear Magnetic Resonance (NMR) Relaxometry

How to get signal?

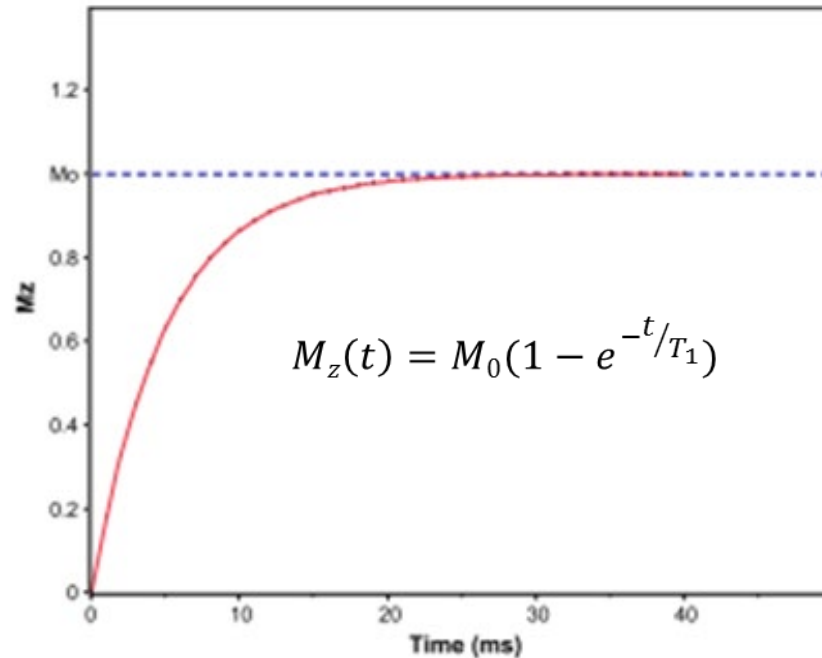
- Nuclear magnetism



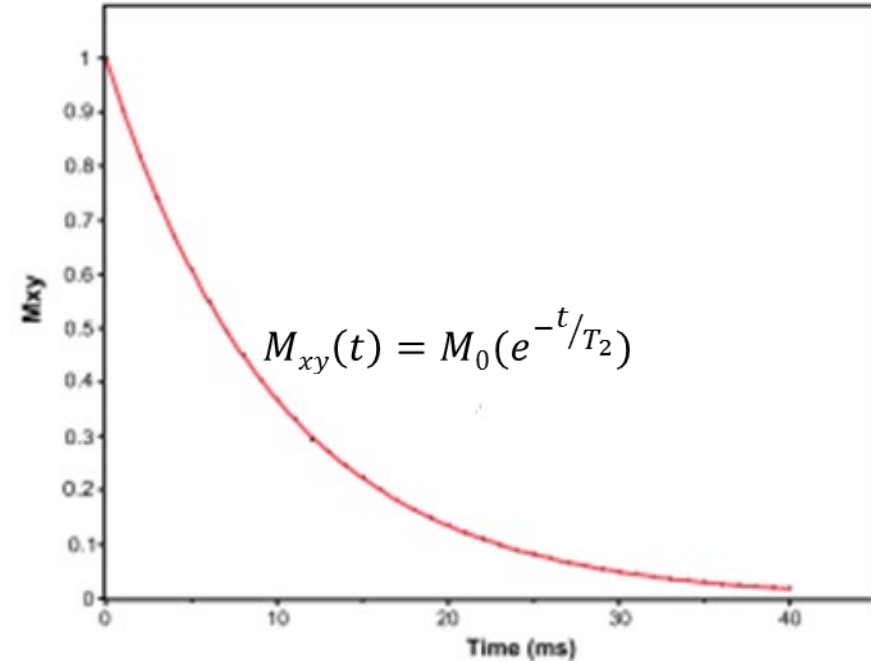
Nuclear Magnetic Resonance (NMR) Relaxometry

How to get signal?

- Owing to this RF pulse, some of the protons align themselves opposite to B_0 which causes a decline in longitudinal magnetization and precessional movement of protons gives rise to a transverse magnetization.
- When RF pulse is removed, the protons **turn back** to their previous state and a **relaxation** and a **recovery** signal is obtained.



Growth of magnetization in z-axis

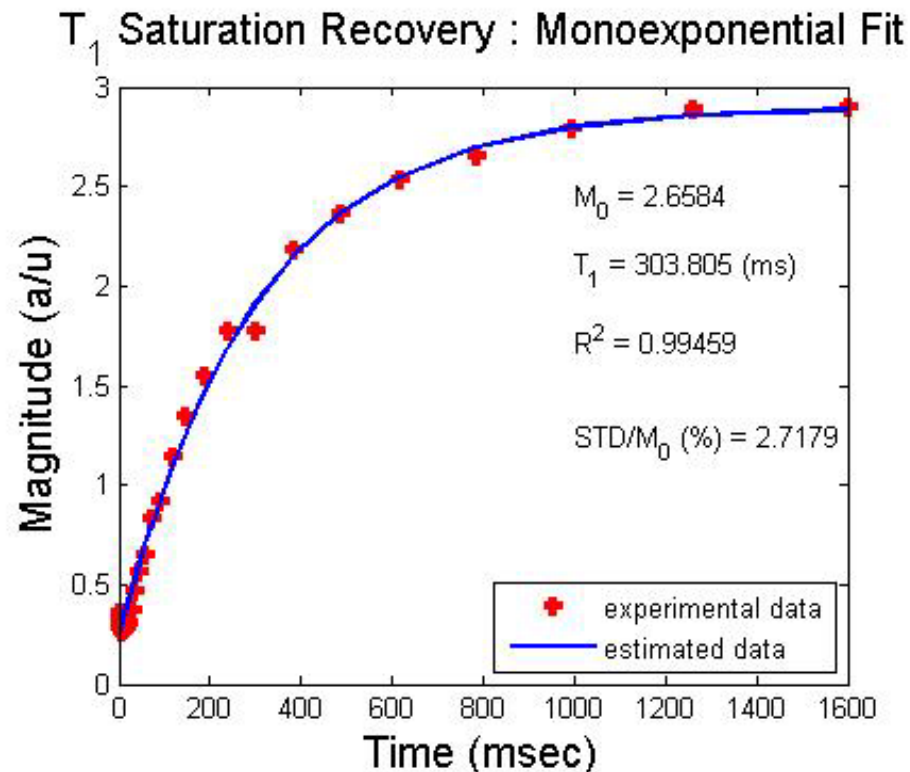


Decay of magnetization in x-y plane

Nuclear Magnetic Resonance (NMR) Relaxometry

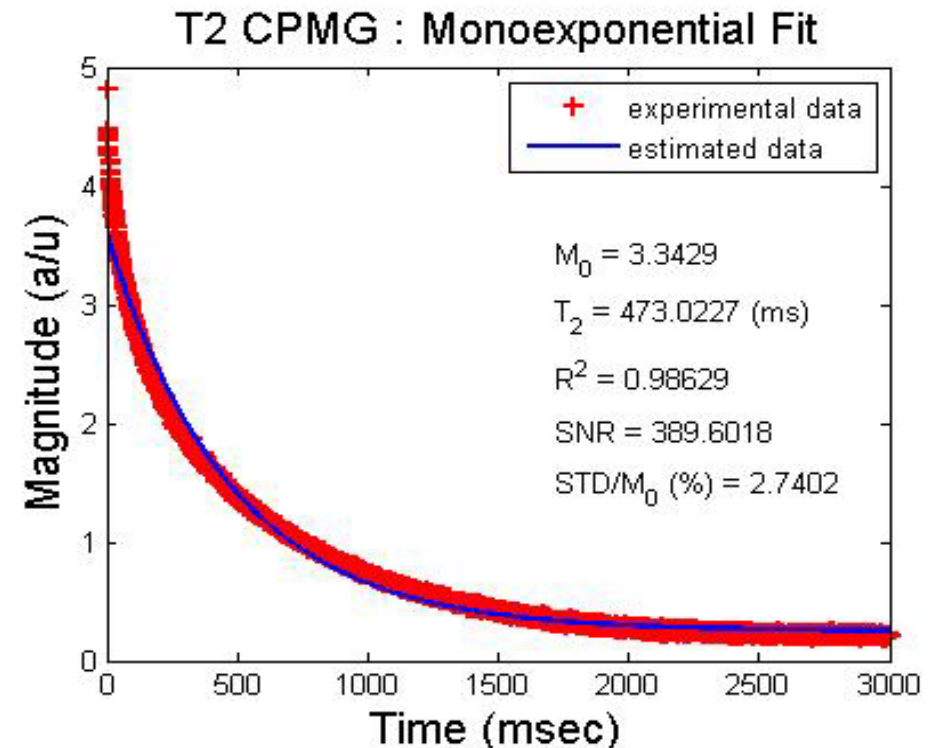
How to get signal?

Growth of magnetization in z-axis
Longitudinal relaxation time - T_1



The graph of recovery of longitudinal magnetization with the growth rate of T_1 for seed sample

Decay of magnetization in x-y plane
Transverse relaxation time - T_2

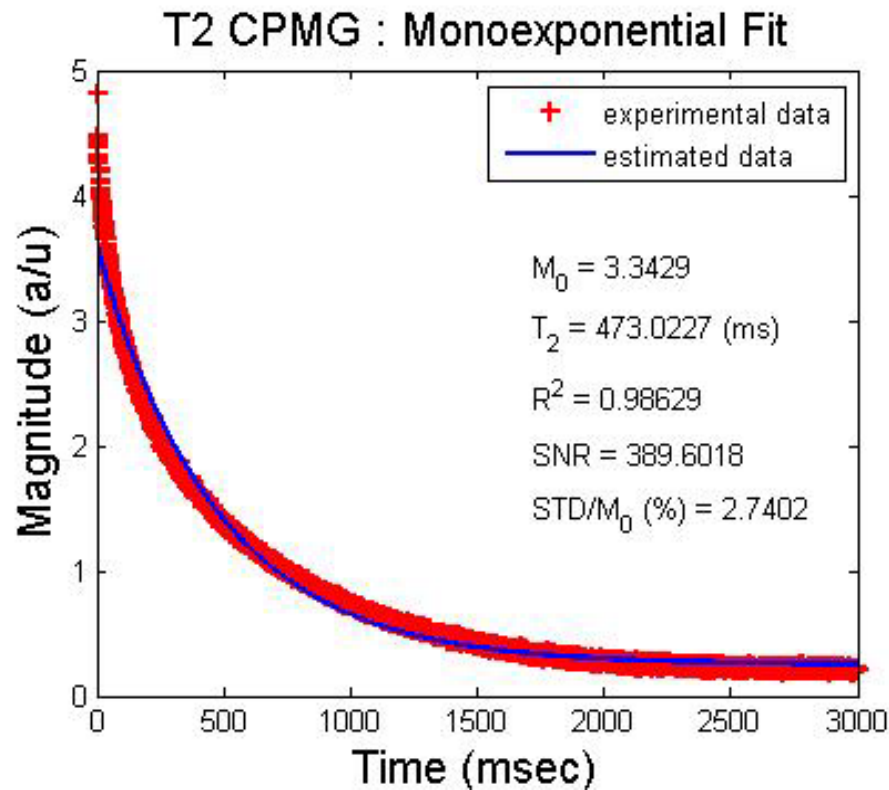


The graph of transverse magnetization with the decay rate of T_2 for seed sample

Nuclear Magnetic Resonance (NMR) Relaxometry

How to obtain a T_2 Relaxation Spectrum?

T_2 CPMG Decay



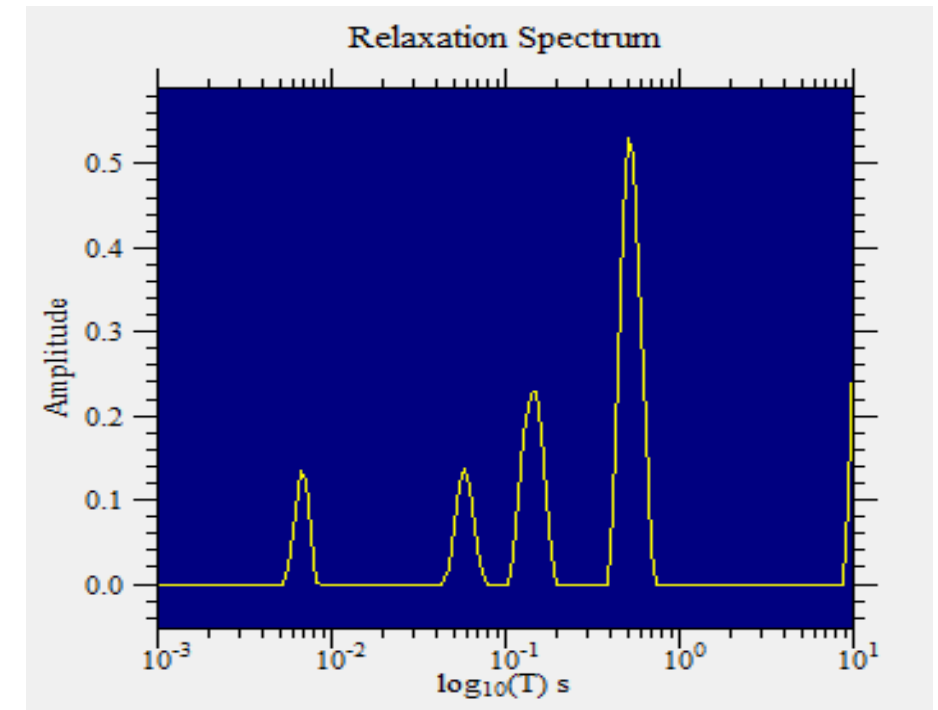
T_2 relaxation curve of seed soaked for about 4hr

→

NNLS
(Non-negative least square)

A mathematical transformation
Inverse Laplace Transform

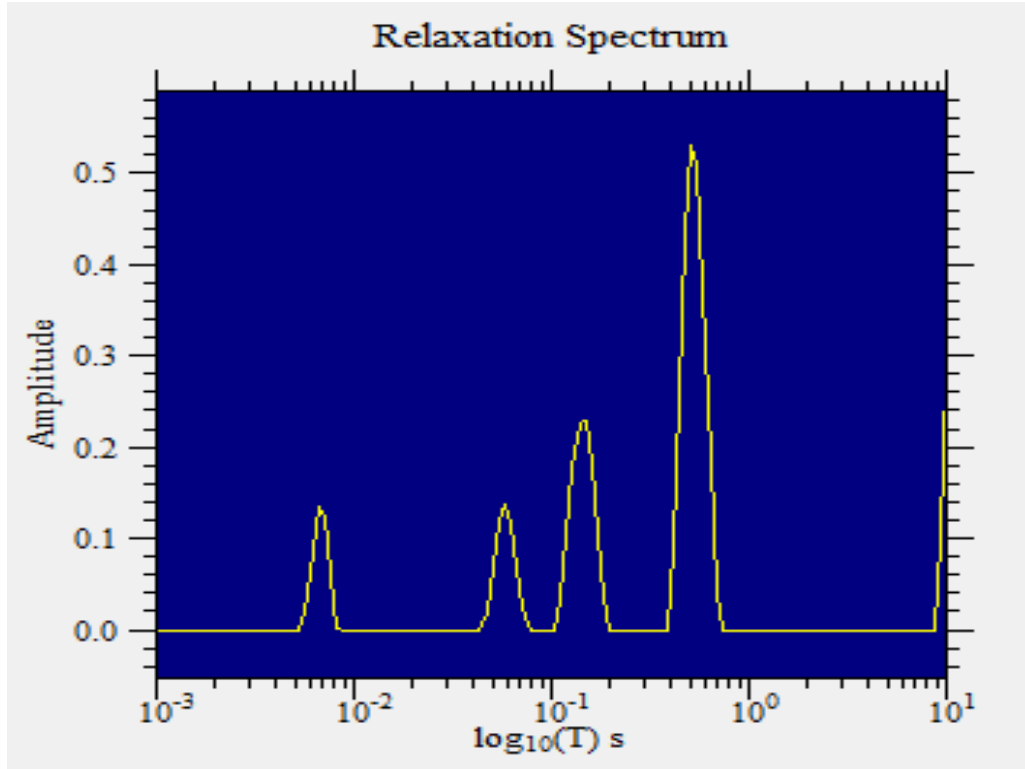
T_2 Relaxation spectrum



T_2 relaxation spectrum of seed soaked for about 4hr

Nuclear Magnetic Resonance (NMR) Relaxometry

T₂ Relaxation Spectrum



- This provides information about the compartments in the sample.
- Each peak in this multi-exponential relaxation indicates the presence of one compartment with different relaxation times.
- T₂ depends on proton mobility & affected by water uptake

Characterization of Insect Proteins and Fats

Mealworm powder

52.0% protein
33.9% fat



Cricket powder

70.6% protein
17.7% fat



Preliminary step:

- ✓ Defatting of insect powders;
 - Conventional extraction
 - HHP induced extraction
 - *followed by centrifugation

Characterization of Insect Proteins and Fats

Characterization of *protein*

- Protein Content
- Aminoacid composition
- Water Binding Capacity
- Oil Binding Capacity
- Particle Size of Emulsion (5%)
- Gelling Behavior (with NMR T2 Relaxometry)
- Fourier Transform Infrared Spectroscopy (FTIR)

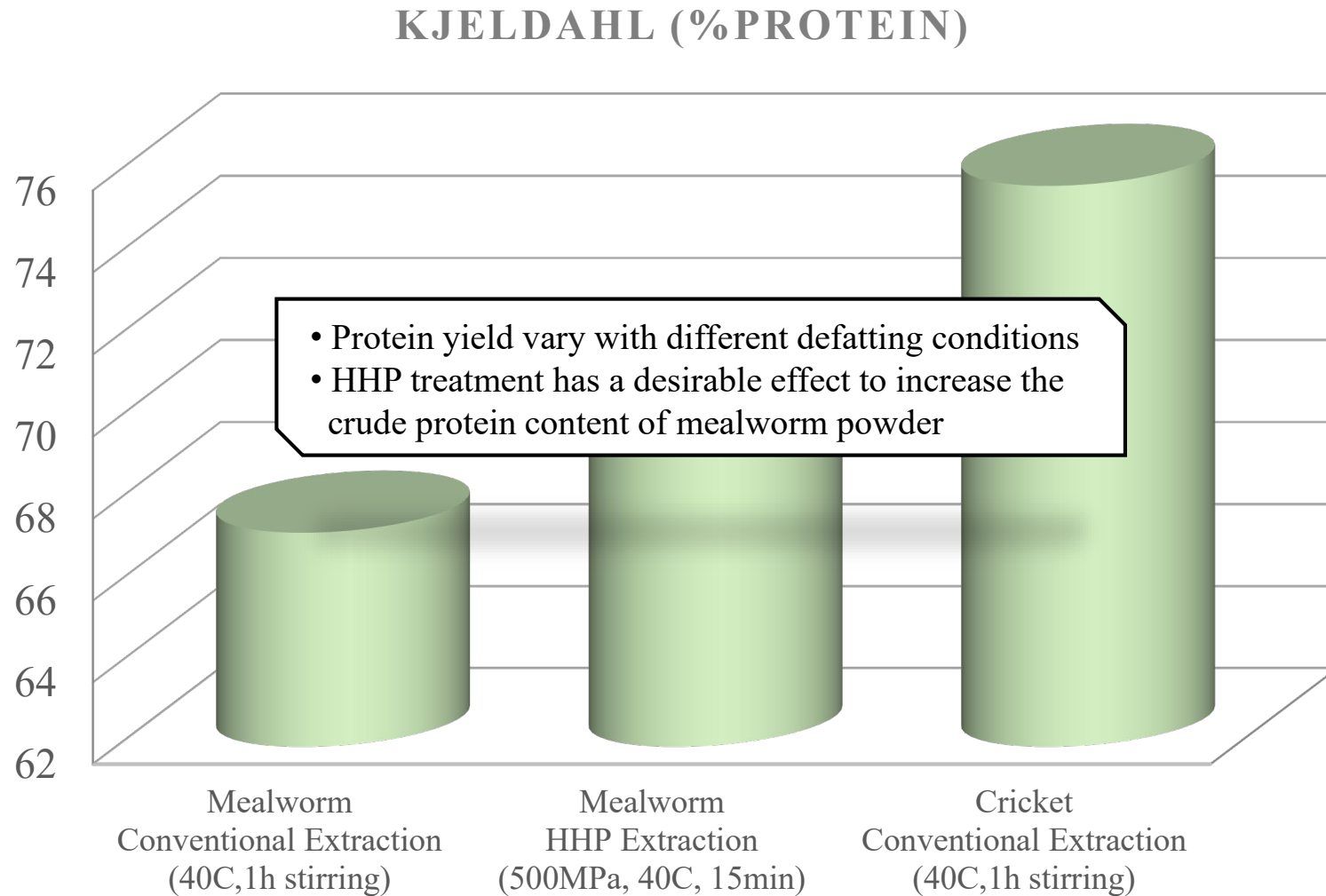
Characterization of *fat*

- Fat Content
- Fat Yield
- Fatty acid composition
- Antioxidant Capacity
- Phenolic content
- DSC

*with Conventional extraction & HHP induced extraction

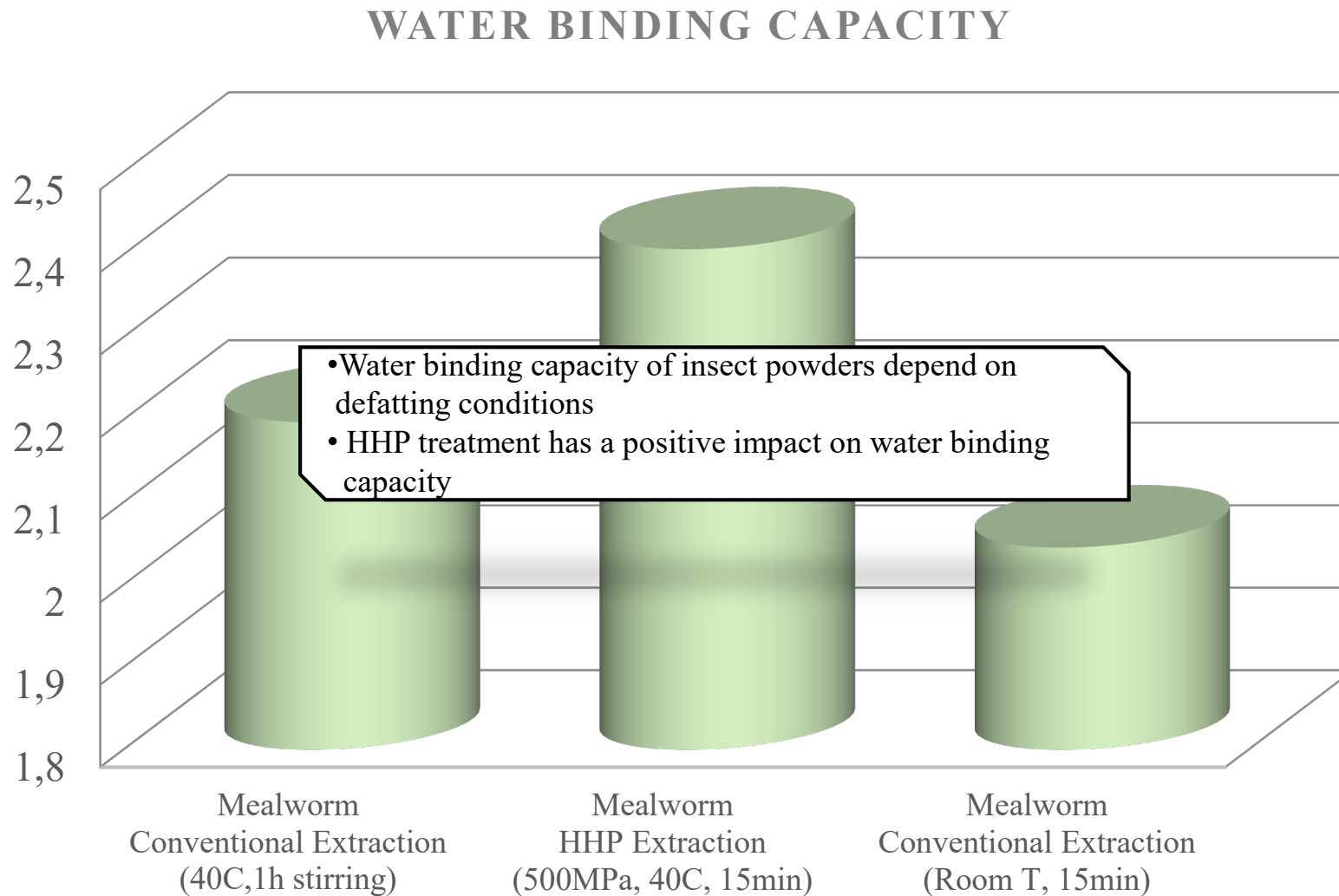
Characterization of Insect Proteins

Protein Content



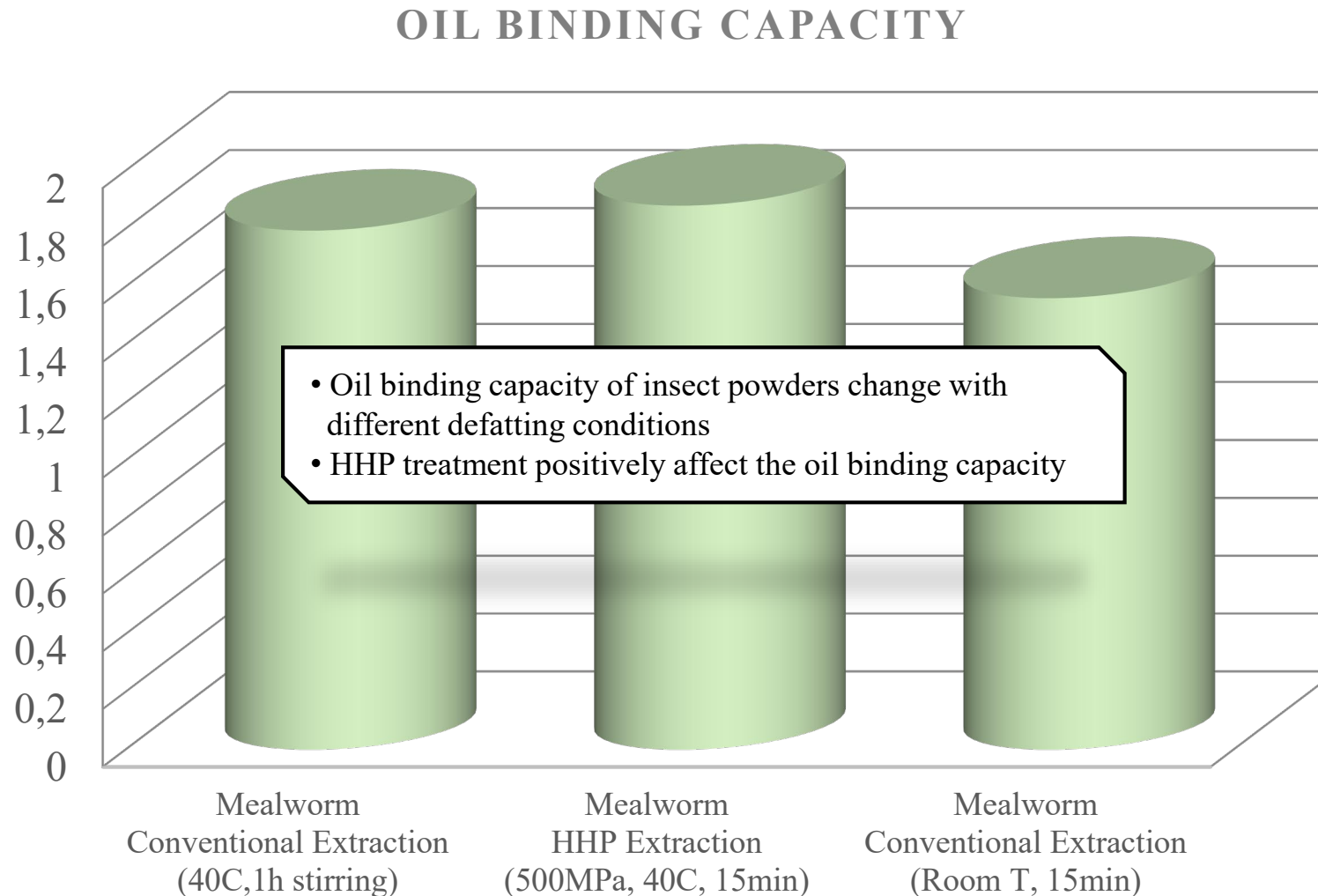
Characterization of Insect Proteins

Water Binding Capacity (WBC)



Characterization of Insect Proteins

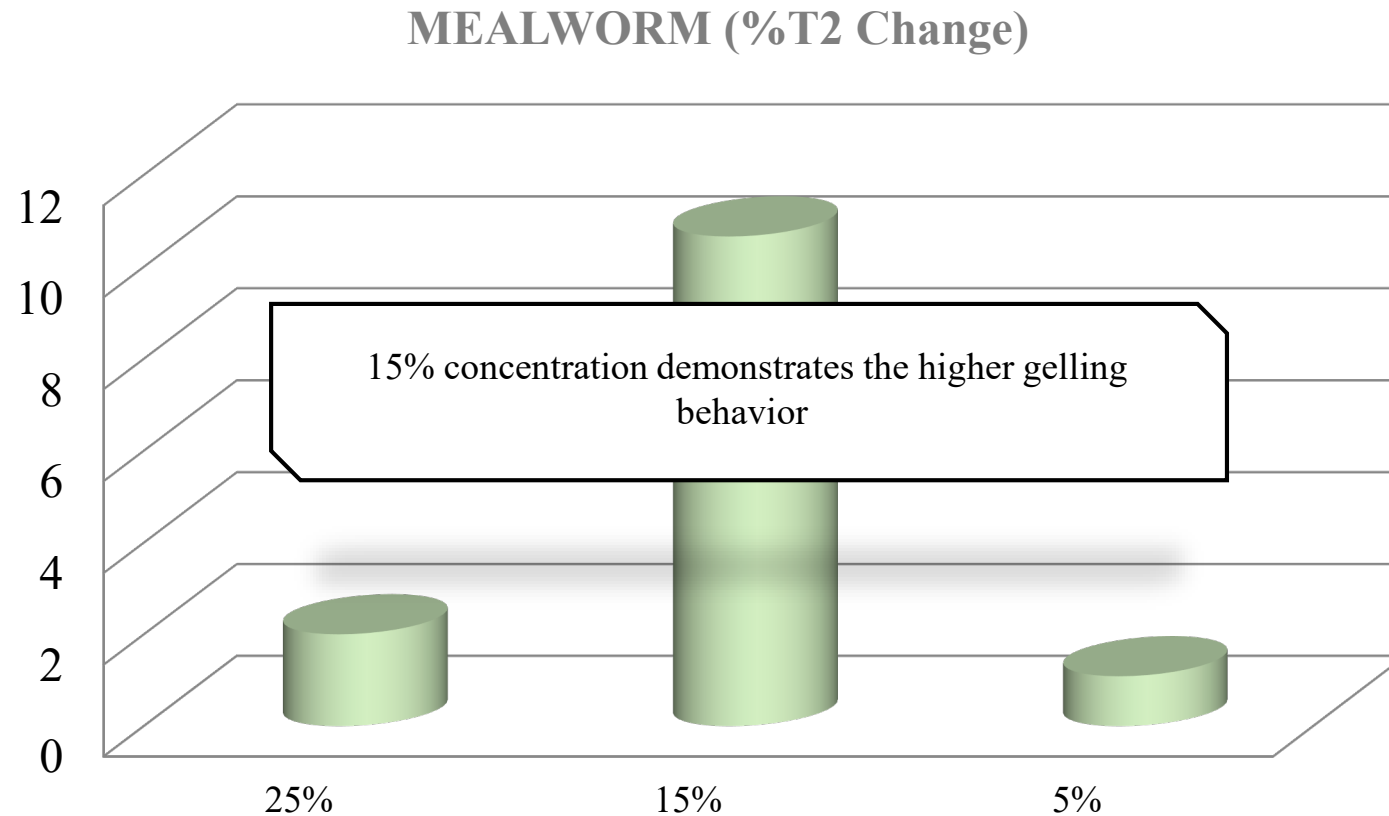
Oil Binding Capacity (OBC)



Characterization of Insect Proteins

Gelling Behaviour

The gelling ability of proteins is observed with NMR T2 Relaxometry,
Heating to 90 °C for 1 h, followed by cooling to 20 °C

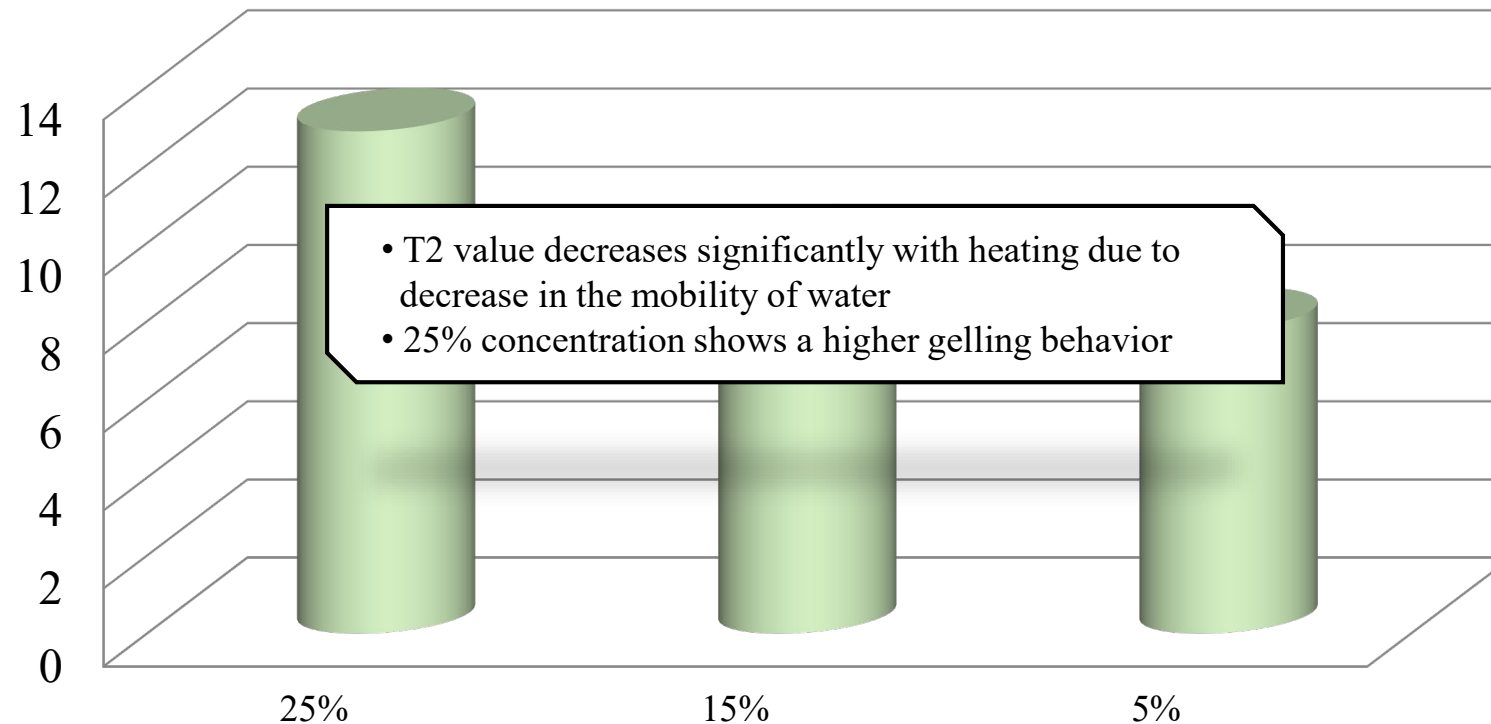


Characterization of Insect Proteins

Gelling Behaviour

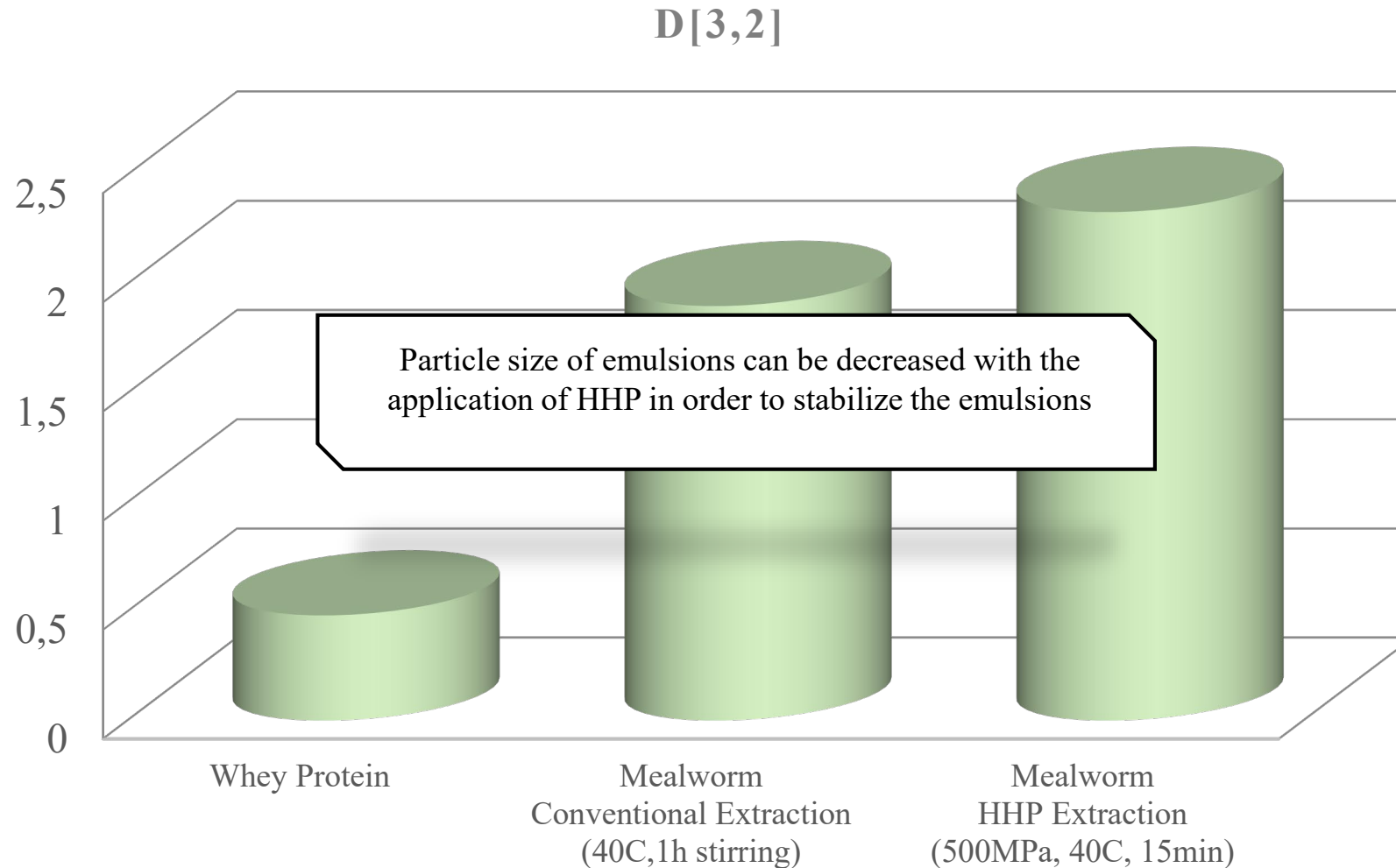
The gelling ability of proteins is observed with NMR T2 Relaxometry,
Heating to 90 °C for 1 h, followed by cooling to 20 °C

CRICKET (%T2 Change)



Characterization of Insect Proteins

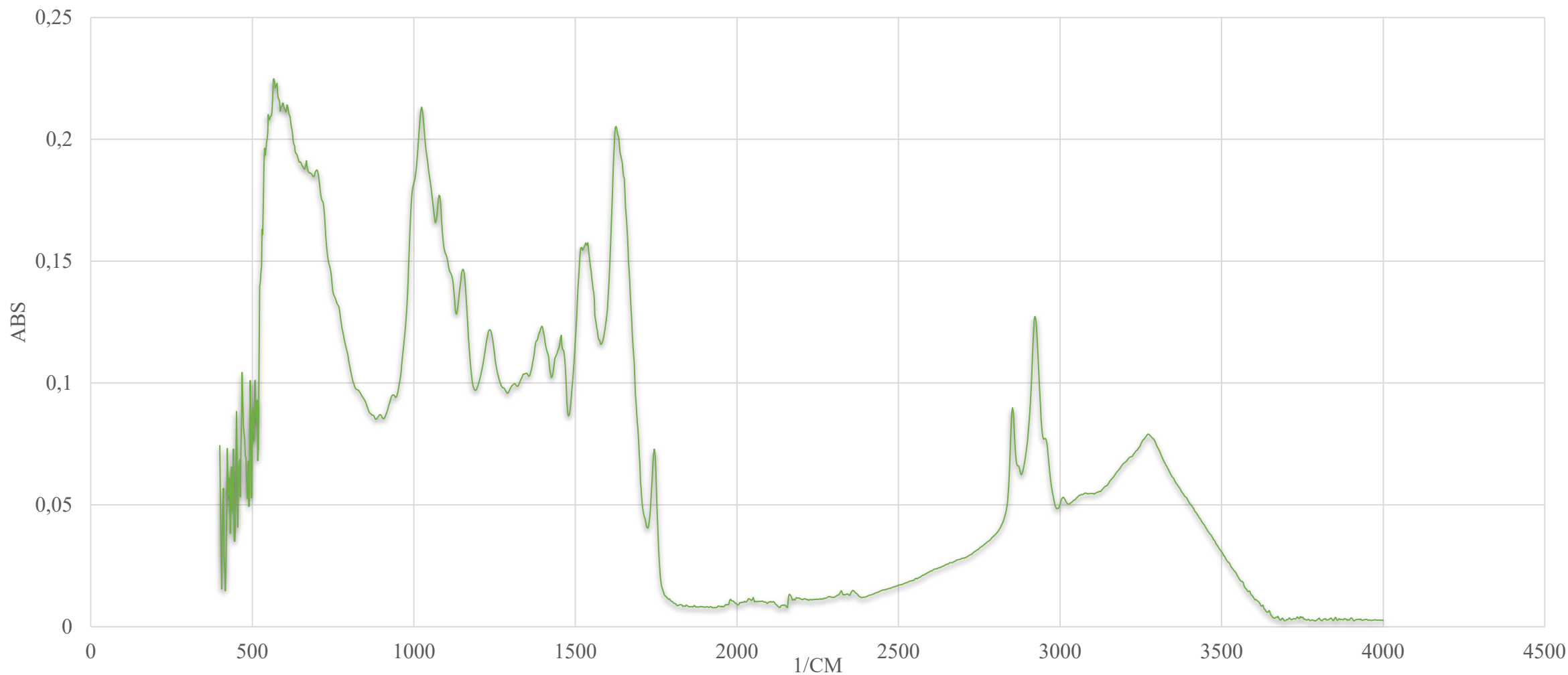
Particle Size of Emulsion (5%)



Characterization of Insect Proteins

FTIR

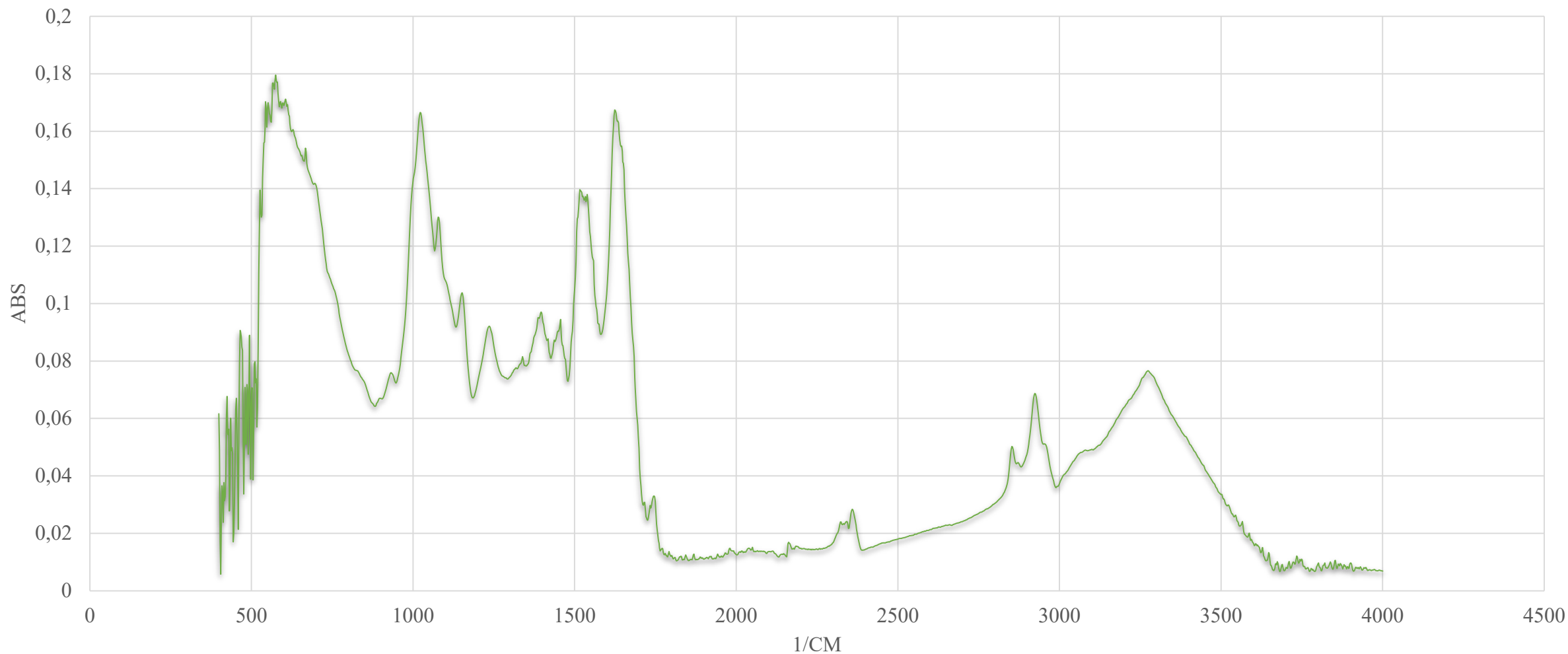
Non-defatted MW Powder



Characterization of Insect Proteins

FTIR

MW, Conventional Extraction, (40C, 1h stirring)



Characterization of Insect Proteins

FTIR

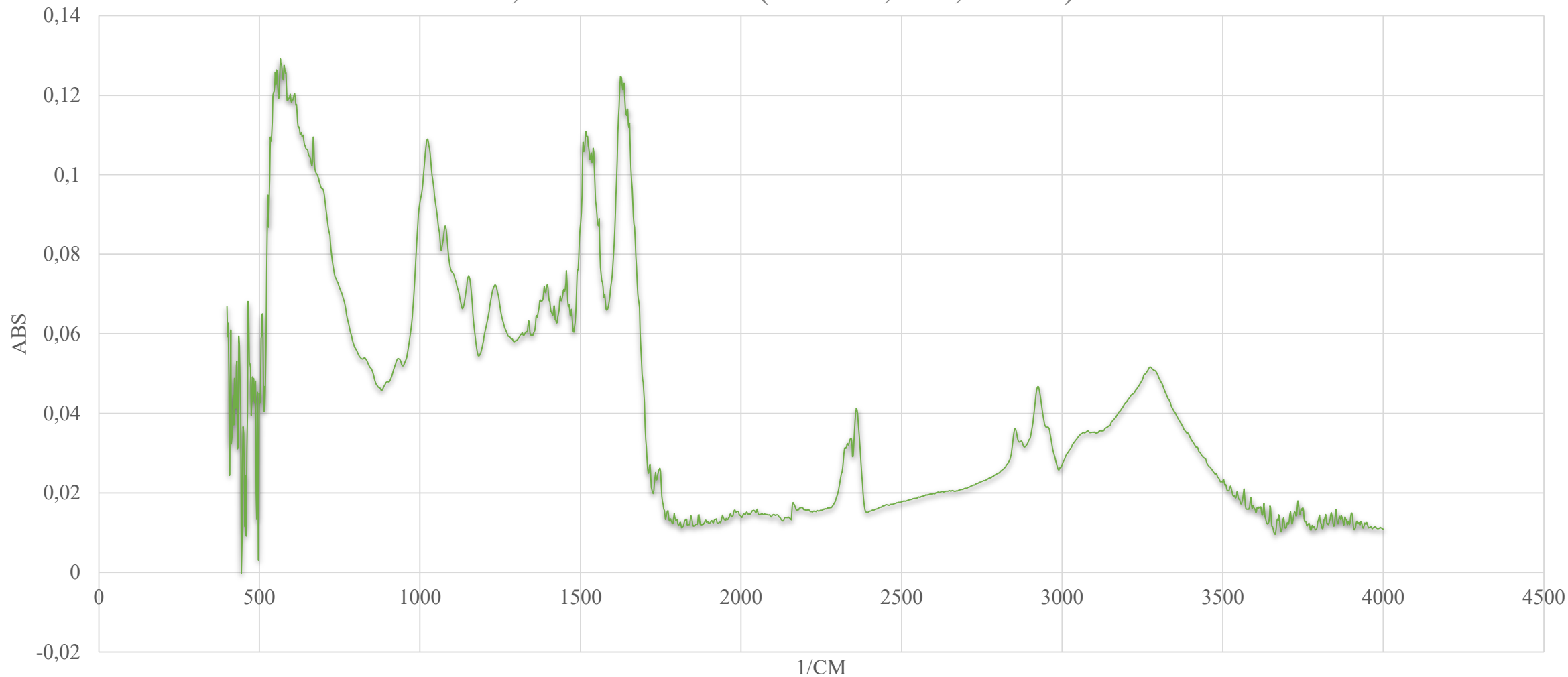
MW, HHP Extraction, (500MPa, 30C, 15 min)



Characterization of Insect Proteins

FTIR

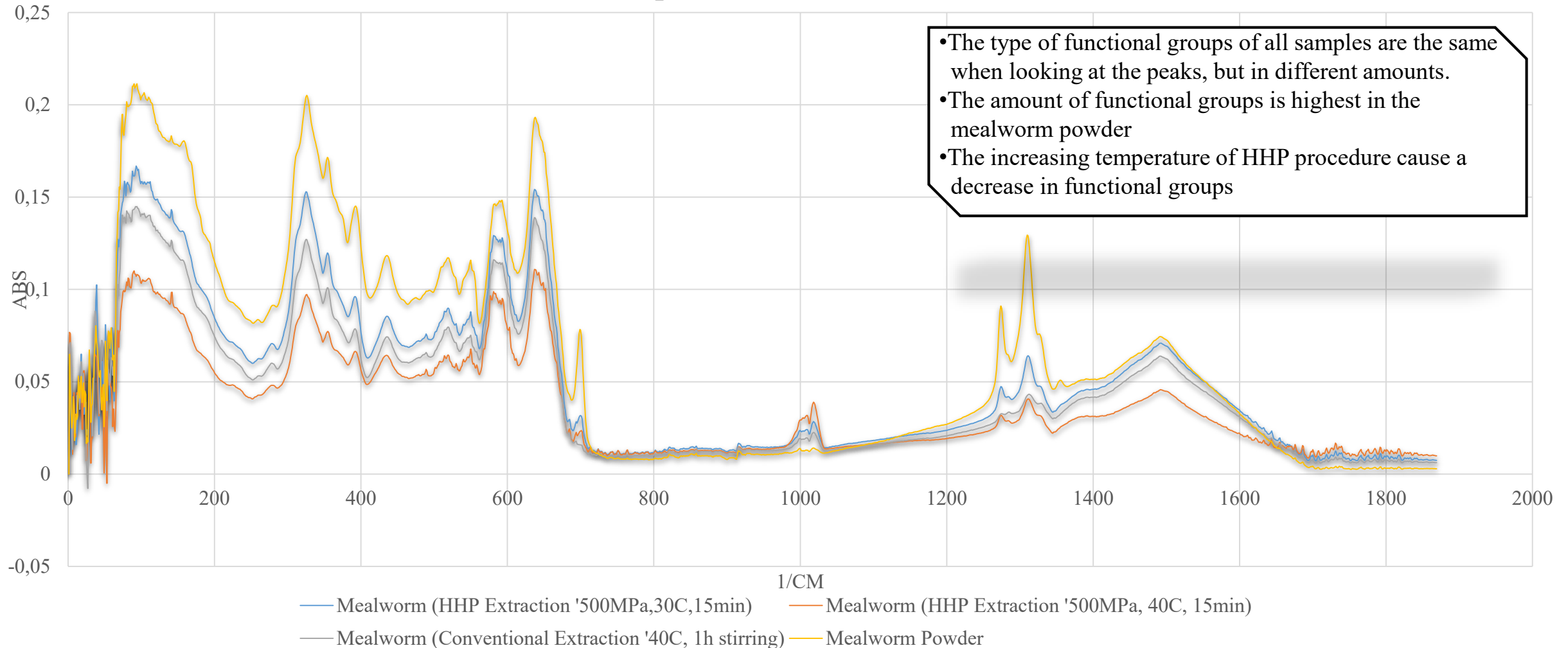
MW, HHP Extraction (500MPa, 40C, 15 min)



Characterization of Insect Proteins

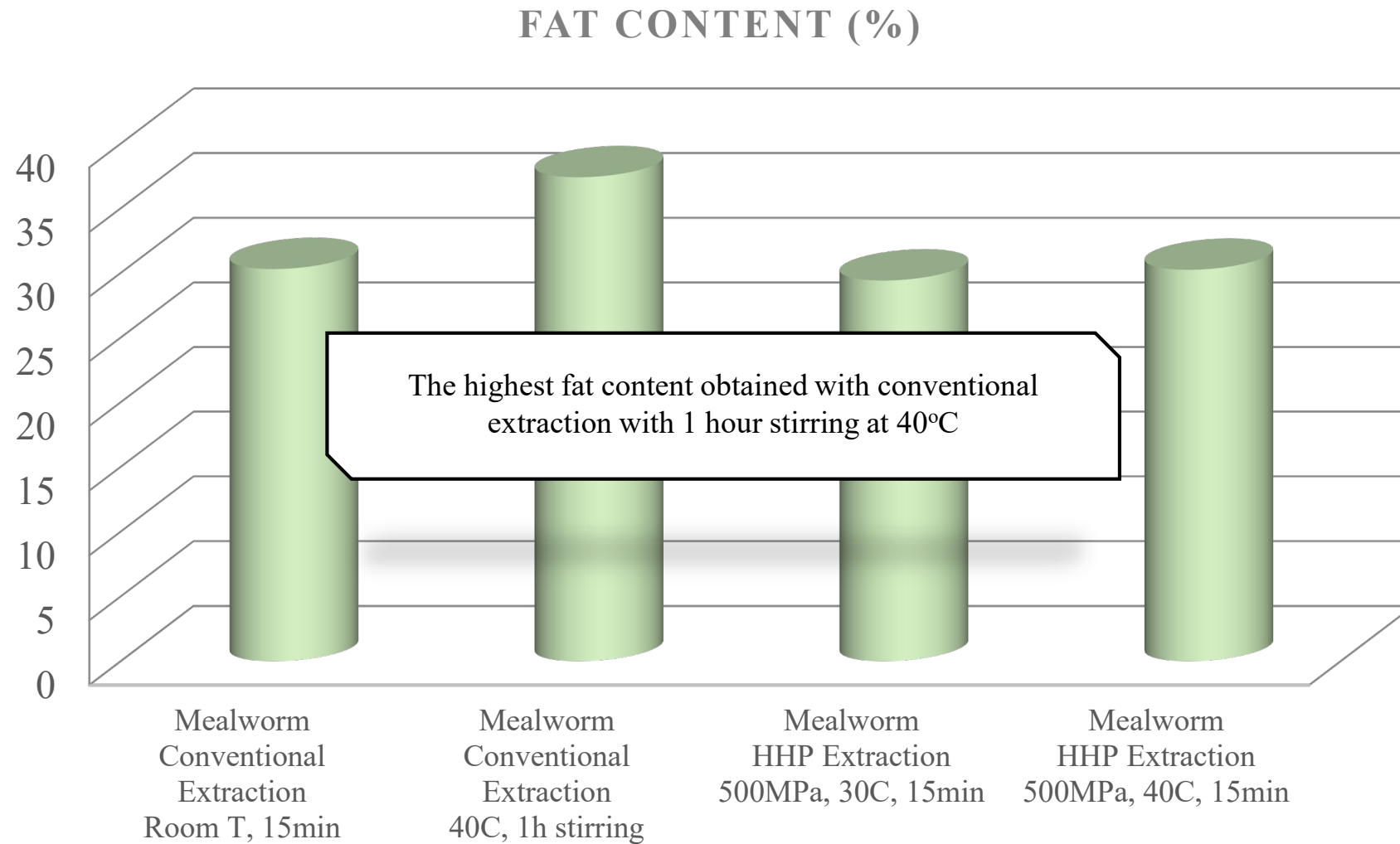
FTIR

Comparison of FTIR Results



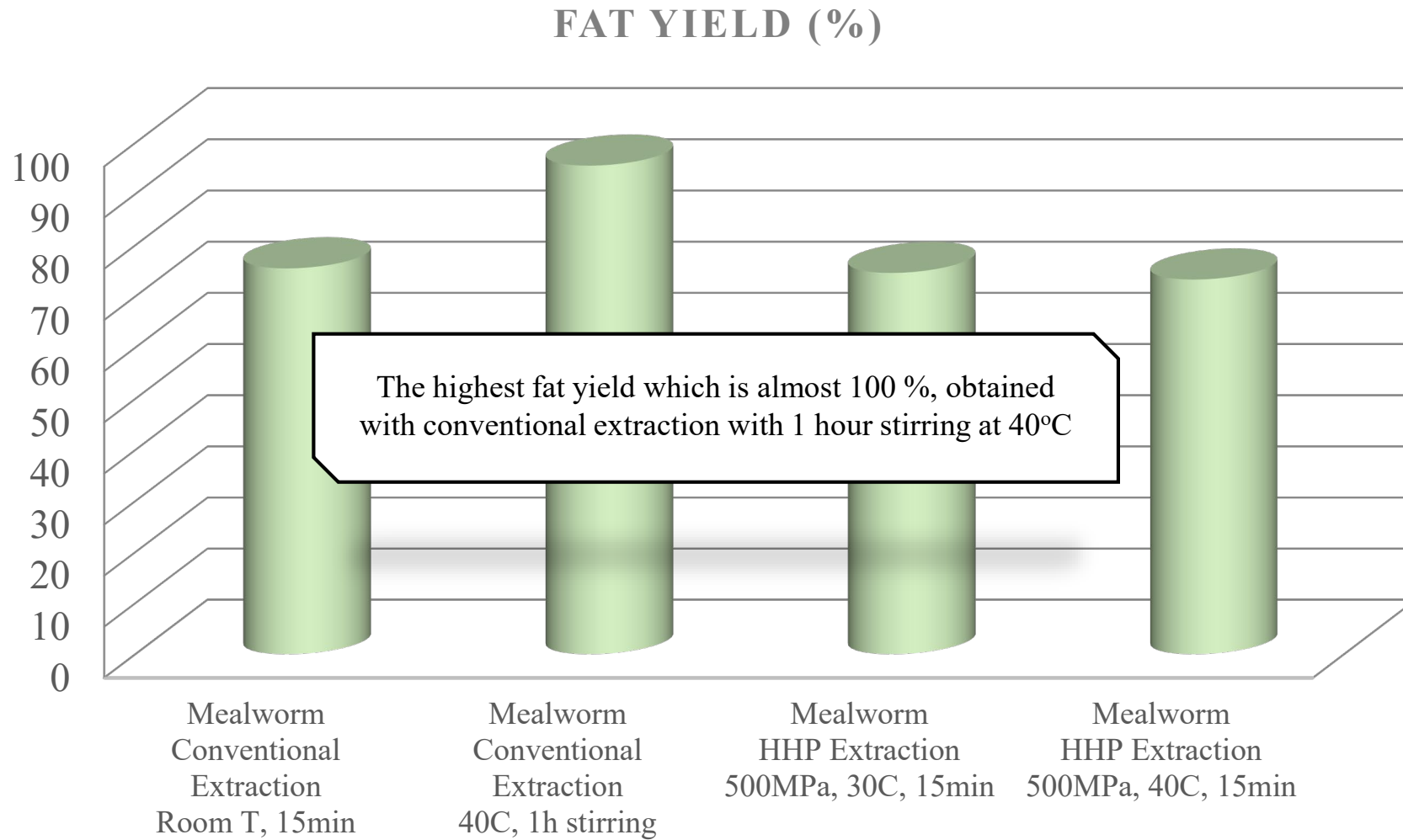
Characterization of Insect Fats

Fat Content (%)



Characterization of Insect Fats

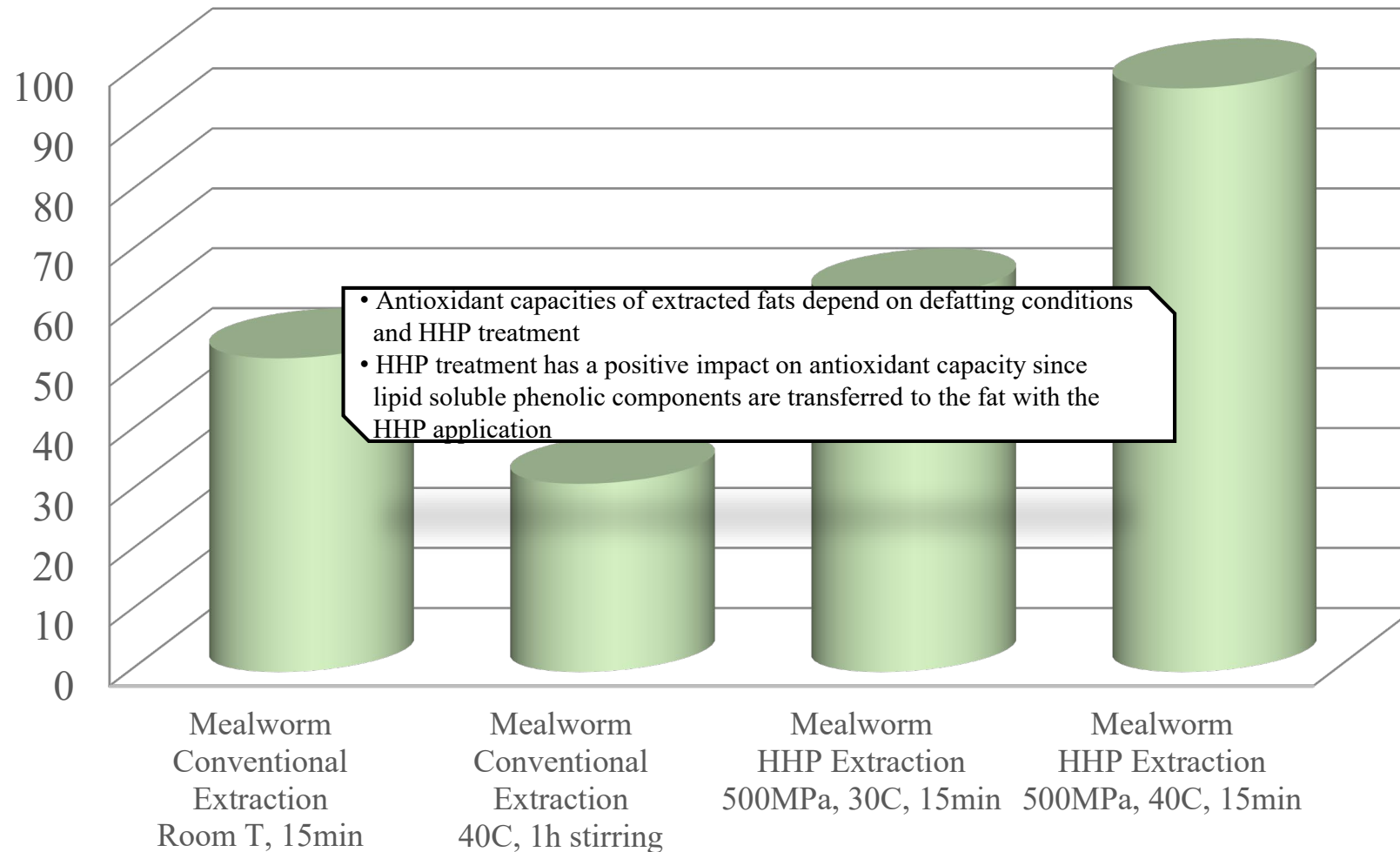
Fat Yield (%)



Characterization of Insect Fats

Antioxidant Capacity

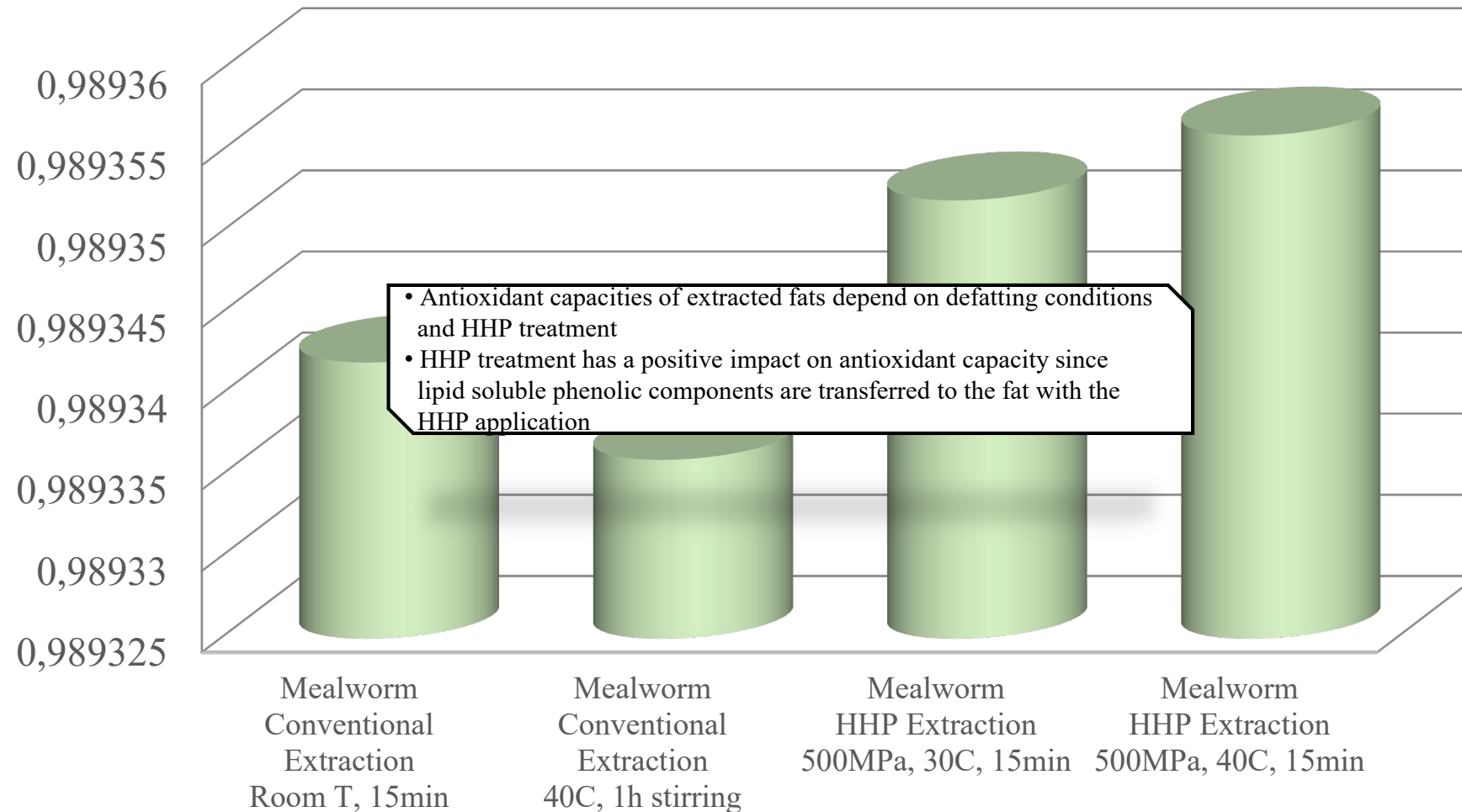
CUPRAC METHOD (MM TR)



Characterization of Insect Fats

Antioxidant Capacity

DPPH METHOD (PPM)



Conclusion

This study concerns not only today, any specific country or region in the world, but also aims to provide a comprehensive solution for more nutritious food due to possible shortage of food source and raw material



Research Group





Effects of High Hydrostatic Pressure assisted degreasing on the technological properties of insect powders obtained from *Acheta domesticus* & *Tenebrio molitor*

Berkay Bolat, Ahmet Erdem Ugur, Mecit Halil Oztop^{*}, Hami Alpas

Middle East Technical University, Department of Food Engineering, Ankara, 06800, Turkey

ARTICLE INFO

Keywords:
Yellow mealworm (*Tenebrio molitor*)
House cricket (*Acheta domesticus*)
Powder
High hydrostatic Pressure (HHP)
Nuclear Magnetic Resonance (NMR) relaxometry

ABSTRACT

In this study, two edible insect species; *Acheta domesticus* (house cricket) and *Tenebrio molitor* (yellow mealworm) were defatted using different extraction methods and characterized afterwards. The main goal of the study was to see the effect of High Hydrostatic Pressure (HHP) extraction at different temperatures on the functional properties of the insect powders. Protein content, solubility, water and oil binding capacity; total phenolic content and antioxidant activity were all effected from the extraction method. Results showed that, temperature increase from 30 to 40 °C caused a decrease in the protein solubility of both powders. NMR relaxometry was used to interpret the gelation behavior and FTIR spectroscopy showed absorbance peaks mainly in amide I, amide II and amide III regions for both species. Results confirmed that HHP can be used for defatting purposes and could improve the functional properties of the powders to be used as a food additive in formulations.

ORIGINAL PAPER



Effects of High Hydrostatic Pressure (HHP) Processing and Temperature on Physicochemical Characterization of Insect Oils Extracted from *Acheta domesticus* (House Cricket) and *Tenebrio molitor* (Yellow Mealworm)

Ahmet Erdem Ugur¹ · Berkay Bolat¹ · Mecit Halil Oztop¹ · Hami Alpas¹

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Abstract

Oil portion of *Tenebrio molitor* (yellow mealworm) and *Acheta domesticus* (house cricket) were examined and it was investigated how the physicochemical properties of the oils changed with High Hydrostatic Pressure Assisted Extraction (HHP-E) and conventional solvent extraction (CE) with hexane. The effect of HHP-E at 500 MPa and 30 and 40 °C for 15 min on the properties of oils was compared with the CE. Following the extraction of oil, fatty acid composition, peroxide value, crystallization and melting points, total phenolic content and antioxidant activities were determined. Oil yield was found in the range of 22.75–24.22% for mealworm and 16.17–18.09% for cricket with significant amount of Ω -3 and Ω -6 fatty acids. Fatty acid composition of insect oils was significantly affected from HHP-E and extraction temperature ($p < 0.05$). The difference between crystallization and melting point of mealworm were found to be higher than cricket ($p < 0.05$). HHP-E insect oil had desirable characteristics to be used as a food ingredient.

THANK YOU

Questions-Comments

imah@metu.edu.tr

