ProxIMed

Ex**p**loration and **I**mplementation of Products with Alternative **Pro**teins in **Med**iterranean Region

Transforming Protein Landscape in the Mediterranean

MANUELA PINTADO
(mpintado@ucp.pt)

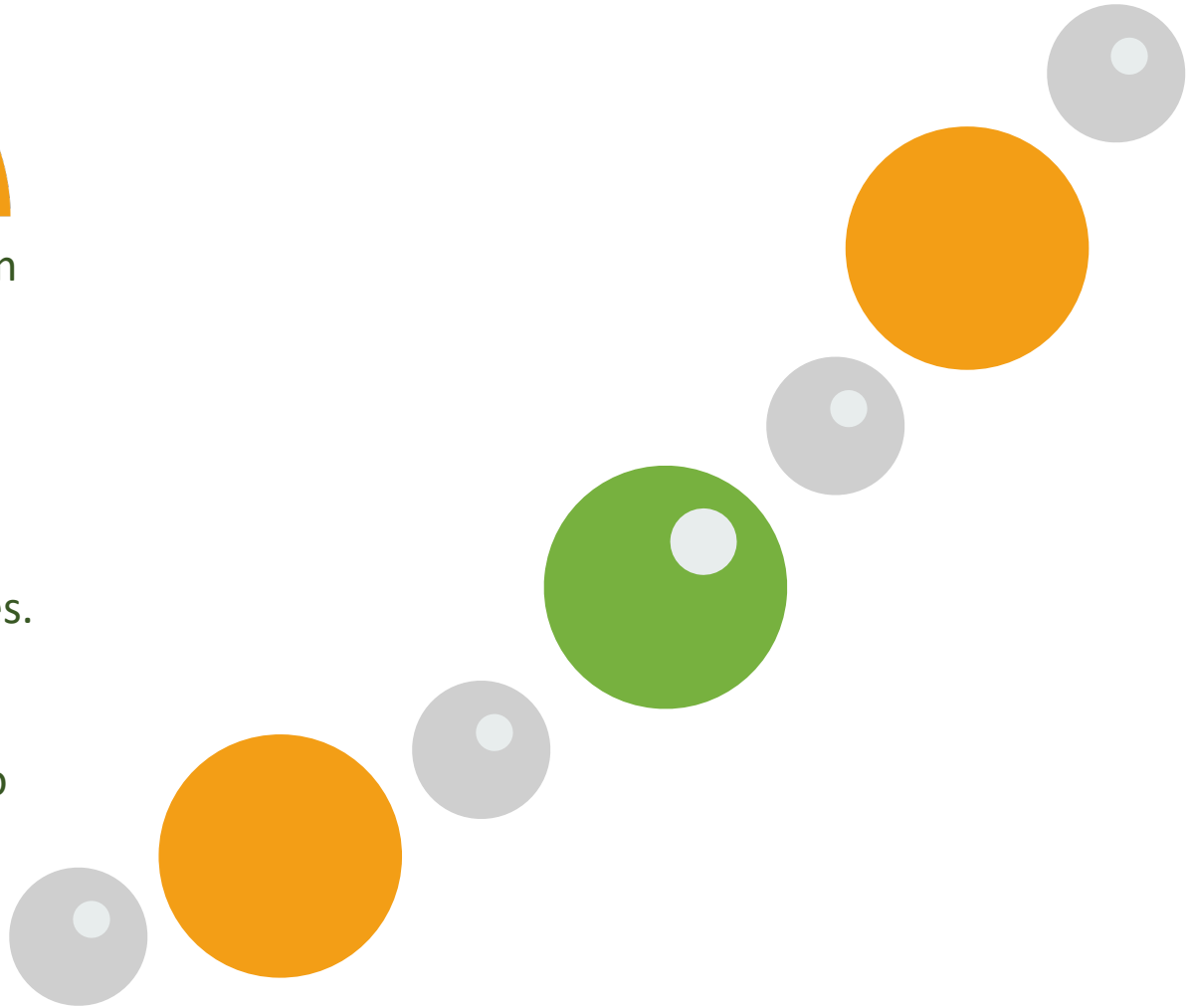
The PRIMA programme is supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation

Our Mission

- ProxIMed aims to revolutionize **protein consumption** in the **Mediterranean region** through **innovation** and **sustainability**.

- The project focuses on developing over **20 alternative protein products** with the active involvement of consumers, industry partners, and innovative technologies.

- By **reducing environmental impact, improving food security,** and **enhancing food choices**, ProxIMed strives to shape a **healthier and more sustainable future** for the Mediterranean population.



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PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA

Our Vision

"A future where alternative protein sources redefine diets, promoting health and sustainability."

- In this vision, ProxIMed envisions a shift towards protein sources that not only meet dietary needs but also **align with ecological and economic goals**.

- By integrating **low-input and available raw materials** with **innovative extraction processes**, ProxIMed seeks to create a **paradigm shift in the way people perceive and consume proteins** in the Mediterranean.

- The vision extends beyond product development, aiming to instill a **long-lasting positive impact on health, the environment, and the overall well-being of communities in the region**.

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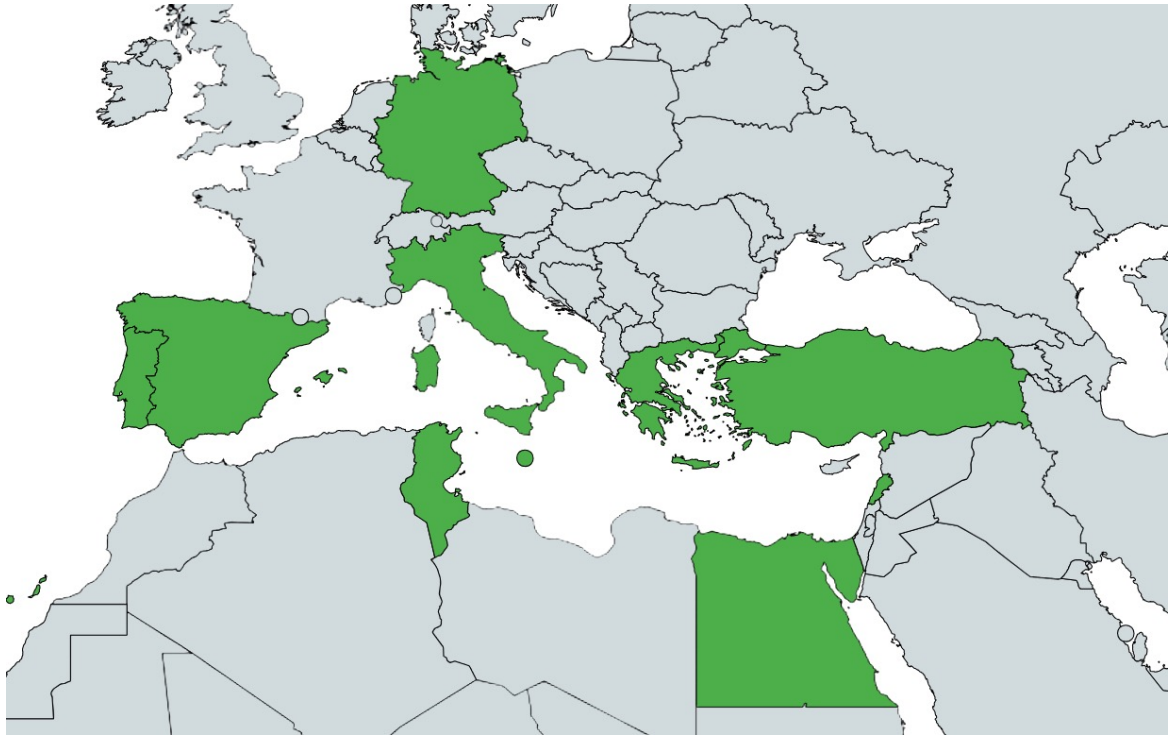
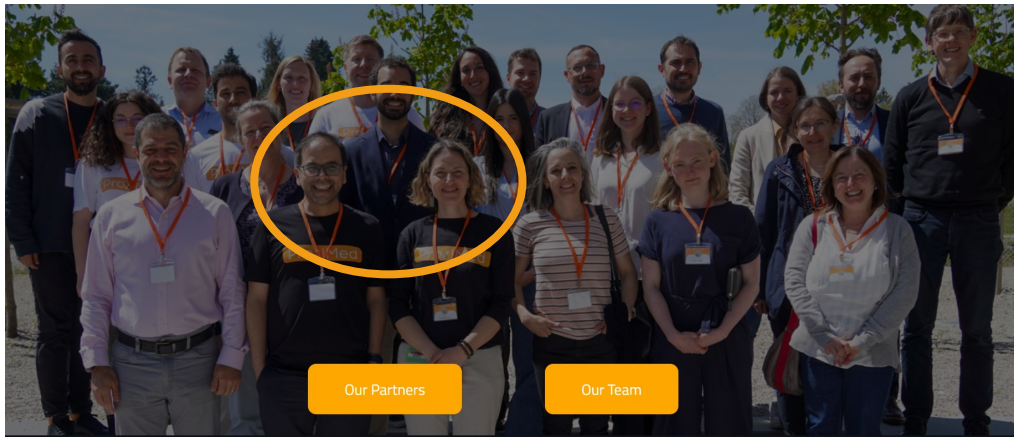
Our Reach





10 Countries

17 Partners

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Our Partners

WEIHENSTEPHAN · TRIESDORF
University of Applied Sciences

Hochschule Weihenstephan-Triesdorf (HSWT)
GERMANY

ODTÜ
METU

Middle East Technical University (METU)
TURKIYE

GreenSurvey
Institut für Marktforschung
Prof. Dr. Menrad GmbH

Green Survey (GS)
GERMANY

tat

TAT Gıda Inc.
TURKIYE

University of Sfax (UoS)
TUNISIA

AMERICAN UNIVERSITY OF BEIRUT
1866

American University of Berut (AUB)
LEBANON

ARISTOTLE UNIVERSITY OF THESSALONIKI

Aristotle University Thessaloniki (AUTh)
GREECE

focus

Focus Foodlabs (FFL)
GERMANY

Assiut University (AU)
EGYPT

ainia

AINIA
SPAIN

DIL

Deutsche Institut für Lebensmittel (DIL)
GERMANY

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Our Partners

Malta College of Arts, Science and Technology (MCAST)
MALTA

Uluova Dairy Company (UL)
TURKIYE

Proteinsecta (PS)
SPAIN

University of Parma (UNIPR)
ITALY

Universidade Catolica Portuguesa (UCP)
PORTUGAL

Arid Regions Institute (IRA)
TUNISIA

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Our Worklist

	Alt-protein Source	Products to be Developed	Partner
	Tomato Pomace	Fermented vegetable pickle in protein enriched solution Protein powder as an ingredient	TAT/METU
	Tomato Leaf	Fermented vegetable pickle in protein enriched solution Protein powder as an ingredient	TAT/METU
	Faba Beans	Easy mix vegan/vegetarian meatballs: A powder mix to prepare meat ball analogues Protein powder as an ingredient	HSWT/FFL
	Sesame Cake	Tahini enriched with sesame protein Protein powder as an ingredient	AUB Sonaco Al Rabih
	Date by-product	Protein powder as an ingredient	UoS/IRA
	Microalgae	Protein powder as an ingredient Capsules as supplement	UoS

	Alt-protein Source	Products to be Developed	Partner
	Mycoprotein	Powder as food ingredient Capsules as supplement Dessert / Snack	ANIA /UCP
	Algae/Date/ Sesame cake	Dairy substitutes	UL
	Insects	Protein powder as an ingredient Food products will be determined based on consumer studies (e.g. sport nutrition snack bars; meat and fish analogues) Animal feed	AINIA/PS/DIL
	Lentil	Protein powder as an ingredient	HSWT
	Chia Seed	Protein powder as an ingredient	HSWT
	Mallow	Protein powder as an ingredient Capsules as supplement	METU

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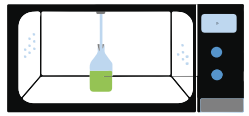
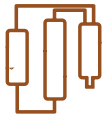
Pre-processing/Extraction/Fractionation Methods



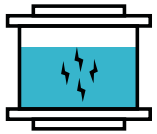
Microwave Heating

Microwave Vacuum Drying

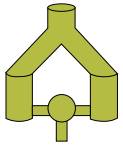


Supercritical Fluid Extraction

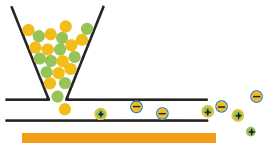
Ultrasonication(US)



In Liquid Plasma



Microfluidization



TriboTec

Product Nutrition, Safety & Health Aspects

Functionality

Allergenicity

Bio-accessibility

Bioavailability

Consumer

Quality

Sensory

Economic

Sustainability

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Visual Summary of ProxIMed

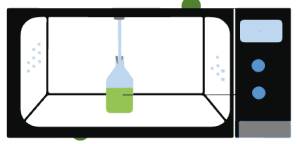
Protein Sources of ProxIMed

Traditional alternative protein sources			Terrestrial sources	
Lentil	Faba Bean	Chia Seed	Mallow	Tomato Leaves

Aquatic sources		Microbial sources	
Duckweed	Microalgae	Mycoprotein	Insects

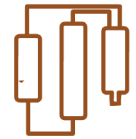
Agri-food by products		
Tomato pomace	Dates by products	Sesame cake

Pre-processing/Extraction/Fractionation Methods



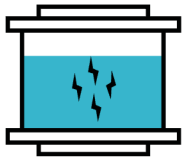
Microwave Heating

Microwave Vacuum Drying



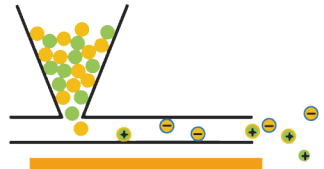
Supercritical Fluid Extraction

Ultrasonication(US)



In Liquid Plasma

Microfluidization



TriboTec

Product Nutrition, Safety & Health Aspects

Functionality

Allergenicity

Bio-accessibility

Bioavailability

Consumer

Quality

Sensory

Economic

Sustainability

Market Launch of the New Alt-Proteins

With the help of industrial partners (TAT, UL, FFL, PS), 2 selected products will be launched in Turkish and Tunisian markets.

Partnership for Research and Innovation in the Mediterranean Area

Exploration and Implementation of Products with Alternative Proteins in Mediterranean Region

UNIVERSIDADE CATÓLICA PORTUGUESA

RICARDO GARCIA

EZEQUIEL COSCUETA

M. JOÃO MONTEIRO

M. JOÃO MOREIRA

July 24th, 2024

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WP2: Consumers' acceptance

Task 2.1. Consumers' general interest in alternative protein products: (TAT, UoS, UCP)

WP3: Protein production through innovative technologies:

Task 3.6. Single cell mycoprotein production and characterization (*AINIA, UCP*)

Task 3.9. Technofunctional and nutritional properties of the proteins (METU, HSWT, AUB, UoS, IRA, AINIA, UCP, DILx)

WP4: Product development and sensorial evaluation:

Task 4.1. Mapping of the potential applications of the alternative protein ingredients (UCP, TAT, UL, METU, HSWT1, HSWT2, AUB)

Task 4.2. Product development with new sources of proteins (UCP, TAT, UL, METU, HSWT1, HSWT2, AUB)

WP6: Health, Nutrition and Safety Aspects

Task 6.4. Safety assessment including evaluation of allergenicity/toxicity of products

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Task 2.1. Consumers‘ general interest in alternative protein products: (TAT, UoS, UCP)

Focus group

11th and 12th June 2024

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Task 3.6 Single cell mycoprotein production (*AINIA, UCP*)

Four different species of yeast and molds (two yeast and two molds) by AINIA.

Characterization of biomasses in terms of protein content an amino acids profile

- Protein functional properties
- Molecular weight (MW) distribution using FPLC
- Quantification of protein
- Quantification of amino acid content.

Figure 1. FPLC

Figure 2. Micro Kjeldahl method

Task 3.6 Single cell mycoprotein production and characterization (*AINIA, UCP*)

Task 3.9 Technofunctional and nutritional properties of the proteins (METU, HSWT, AUB, UoS, IRA, AINIA, UCP, DIL)

Table 1 . Proximate chemical composition of the *Saccharomyces cerevisiae. Fusarium venenatum* biomass and two Quorn products used as comparison benchmark. 6.25 factor for protein conversion.

Component (%)*	*Saccharomyces cerevisiae*	*Fusarium venenatum*	Quorn Chicken	Quorn Mince
Total protein %	45.39 ± 0.55	32.25 ± 1.96	56.44 ± 1.20	50.91 ± 1.29
Fat%	0.62 ± 0.06	4.70 ± 0.56	4.68 ± 0.24	2.94 ± 0.35
Ash %	4.97 ± 0.83	7.75 ± 0.03	6.31 ± 0.06	4.42 ± 0.11
Total carbohydrates**	49.02 ± 0.76	55.3 ± 1.56	32.57 ± 1.48	41.73 ± 0.89
Total fiber %	45.20	39.87	29.50	35.38
Insoluble dietary fiber %	38.40 ± 0.82	36.71 ± 0.23	27.30 ± 0.90	33.35 ± 3.21
Soluble dietary fiber %	6.80 ± 1.45	3.16 ± 0.79	2.2 ± 0.31	2.03 ± 0.08
Total Glucan	24.09 ± 1.73	12.76 ± 0.61	17.54 ± 2.06	18.00 ± 0.09
Alpha Glucan	16.73 ± 1.19	7.52 ± 0.07	9.74 ± 0.83	7.40 ± 1.87
Beta Glucan	7.36 ± 0.54	5.24 ± 0.54	7.79 ± 2.88	10.60 ± 1.96

	Myco-*Rhizopus* (inactivated)	Myco-Pekilo	Myco-Quorn	Yeast-Rhodotorula	*P. maximae*
Total DF (% DW)	56,4	31,1	26,3	9,4	37,3
Insoluble dietary fibre (% dm)	53,2	23,5	21,1	7,8	36,2
Soluble dietary fibre (% dm)	3,2	7,6	3,2	1,6	1,1

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DOI:10.3390/foods11223621

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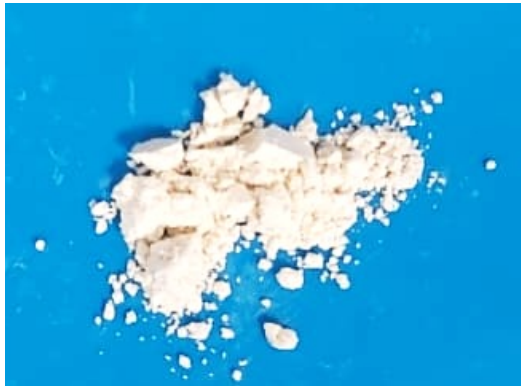
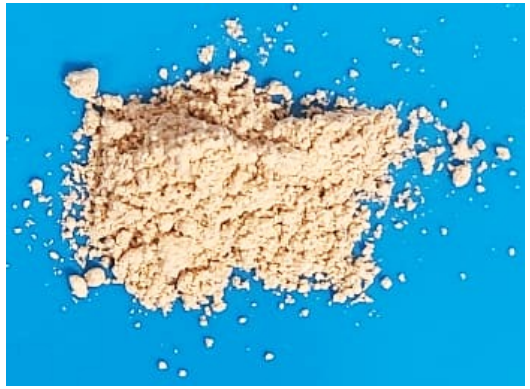
Future work

Monascus purpúreos biomass



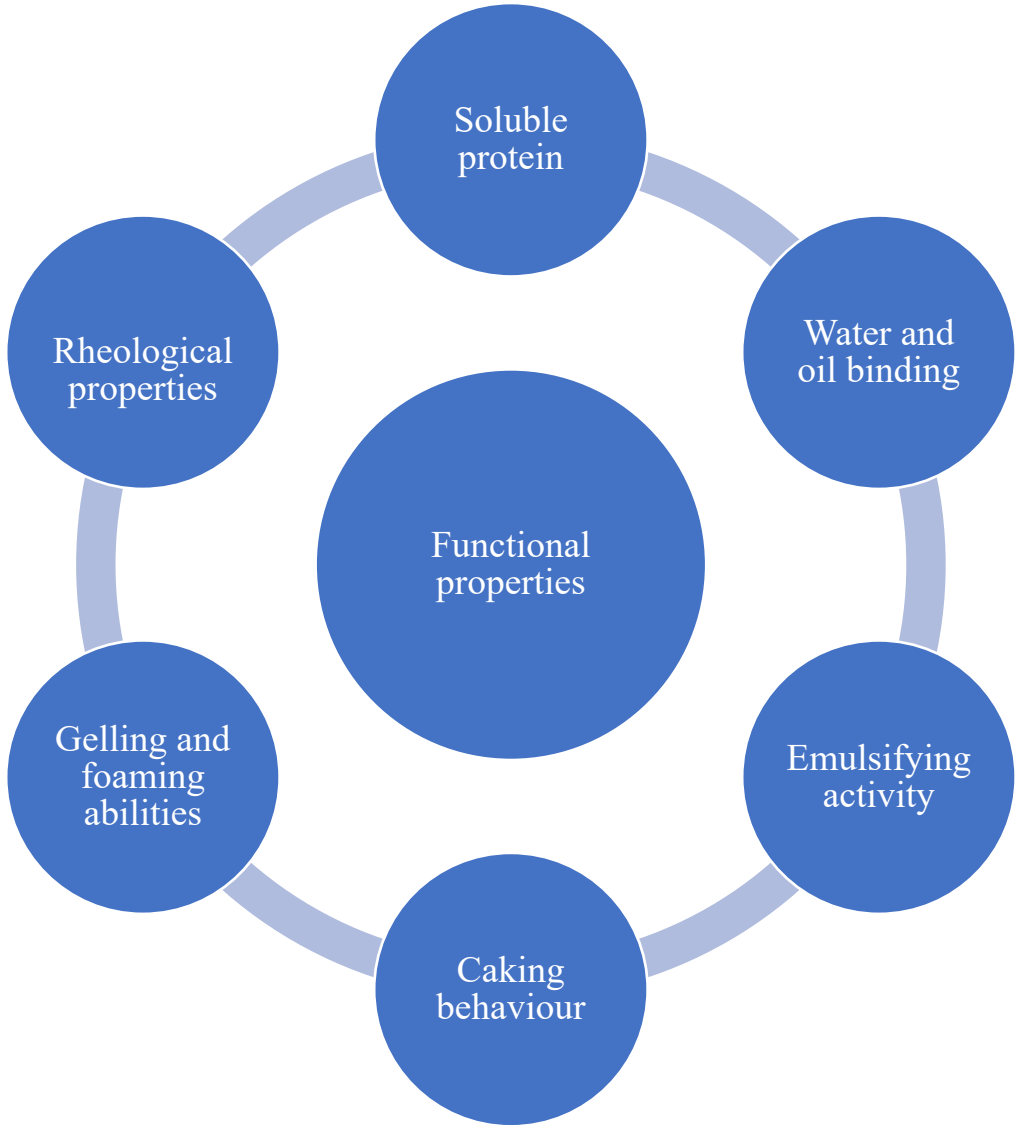
Cyberlindnera jadinii biomass



- Proximate analysis (Protein, fat, ash, fiber, glucan…)
- Analysis of Amino Acids by HPLC-Fluorescence Fatty acids by GC
- Protein functional properties
- Curve solubilization (BCA)
- Antioxidant activity (ABTS, ORAC, FRAP)

The production will begin on 29th july

Task 3.9 Technofunctional and nutritional properties of the proteins (METU, HSWT, AUB, UoS, IRA, AINIA, UCP, DIL)



Enhancing the texture, flavor, and sensory attributes of food items, making them suitable for widespread use in the food industry and offering various benefits.

Task 3.9 Technofunctional and nutritional properties of the proteins (METU, HSWT, AUB, UoS, IRA, AINIA, UCP, DIL)

Table 2. Functional properties of the *Saccharomyces cerevisiae*. *Fusarium venenatum* biomass and two Quorn products used as comparison benchmark

	Saccharomyces cerevisiae	*Fusarium venenatum*	Quorn chicken	Quorn mince
WBC (g/g)	0.65 ± 0.04	3.91 ± 0.51	5.00 ± 0.18	5.14 ± 0.07
OHC (g/g)	1.30 ± 0.11	4.44 ± 0.42	4.47 ± 0.21	4.87 ± 0.48
FA (cm)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.55 ± 0.00
FS (min)	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.00	13.64 ± 0.00
ES (%)	90.00 ± 0.00	87.5 ± 0.00	83.33 ± 0.00	80.00 ± 0.00

1: Water holding capacity (WBC); 2: Oil holding capacity (OHC); 3: Foaming ability; 4: Foaming stability (after 30s);.5: Emulsion capacity (after 30 min) (EC); 6: Emulsion stability (after 24h) (ES)

Functional properties	*P. maximae*
Water hydration capacity (g/g)	8.3
Oil holding capacity (g/g)	6.9
Foaming capacity (%)	No foam
Foaming stability (%)	No foam
Emulsion stability (%)	100

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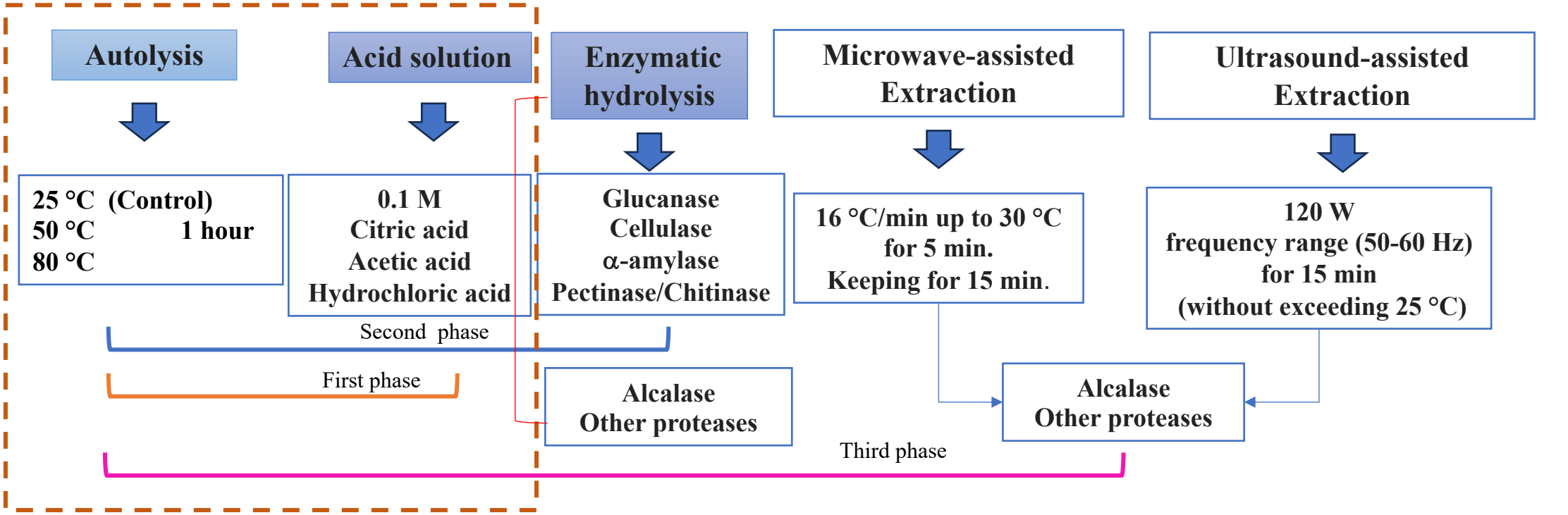
Task 3.6 Single cell mycoprotein production and characterization (*AINIA, UCP*)

Mycoprotein Hydrolysis – development of bioactive peptides



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Task 3.6 Single cell mycoprotein production and characterization (*AINIA, UCP*)

1st stage

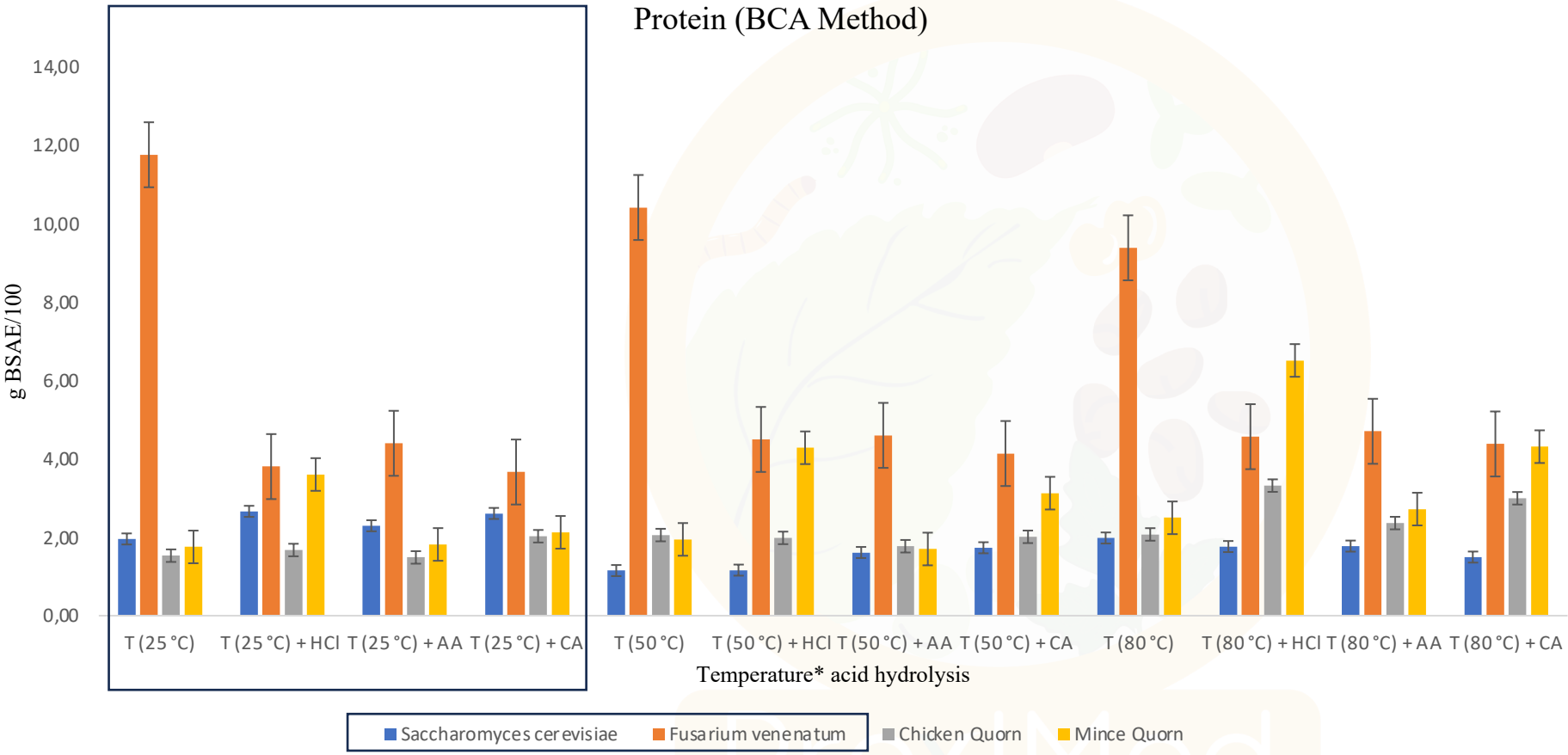

Fig. 17. Average relative protein determined with the BCA assay for the following temperatures (25, 50, and 80 °C) and acid treatment with 0.1 M Hydrochloric acid (HCl) acetic acid (AA) and citric acid (CA) of *Saccharomyces cerevisiae. Fusarium venenatum* biomass and two Quorn products (w/w).

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Task 3.6 Single cell mycoprotein production and characterization (*AINIA, UCP*)

1st stage

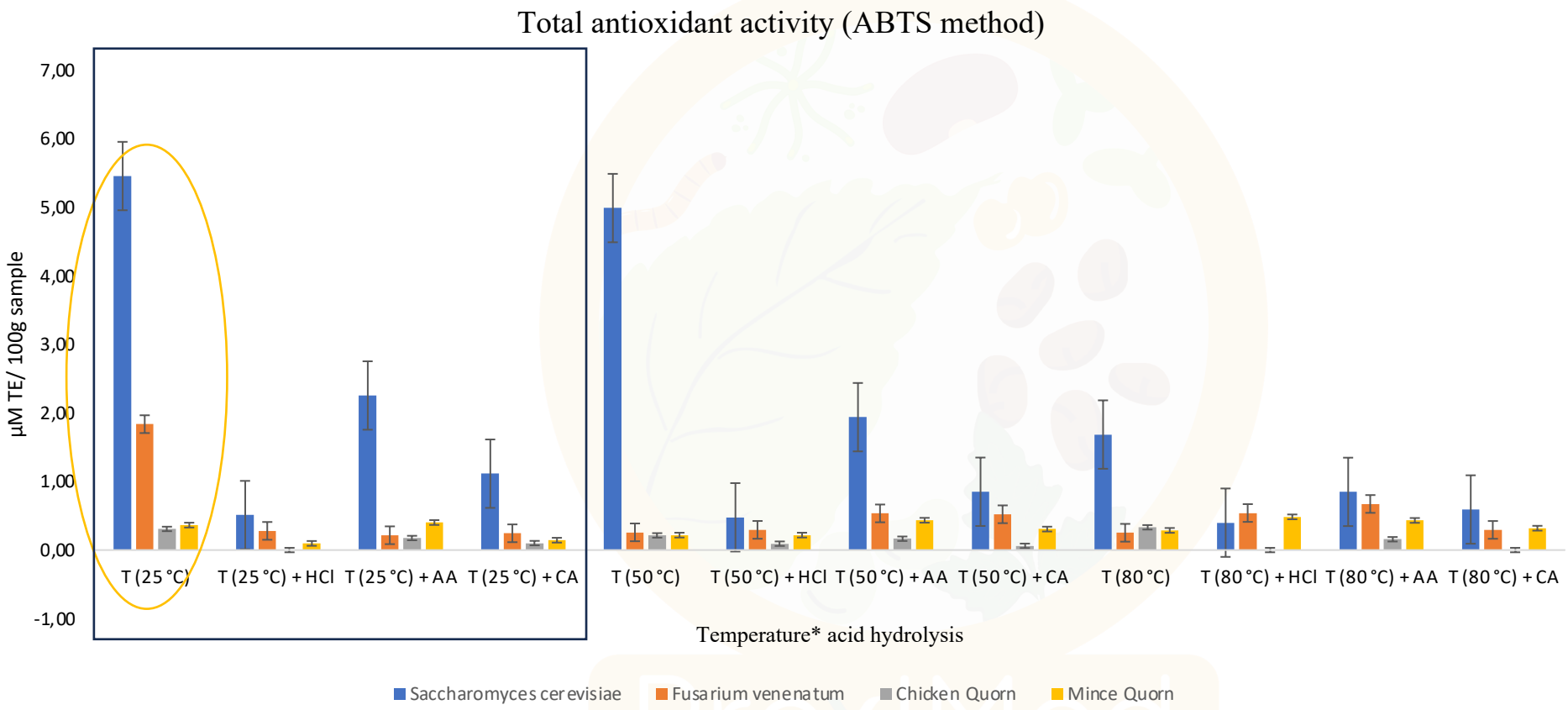

Figure 19. Total antioxidant activity (ABTS method) of the *Saccharomyces cerevisiae, Fusarium venenatum and* Quorn products fractions (supernatants). TE: Trolox equivalents; DM: dry matter

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Task 4.2. Product development with new sources of proteins (UCP, TAT, UL, METU, HSWT1, HSWT2, AUB)

- **Development of innovative/new mycoprotein products for the market**

Salty snack

Sustainable ingredients

Mycoprotein flour

Aromatic herbs

Salt flower

Pepper

Mycoprotein bar snack

Sustainable ingredients

Mycoprotein flour

Brewers spent grain

Pea or chickpea flour

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Task 4.2. Product development with new sources of proteins (UCP, TAT, UL, METU, HSWT1, HSWT2, AUB)

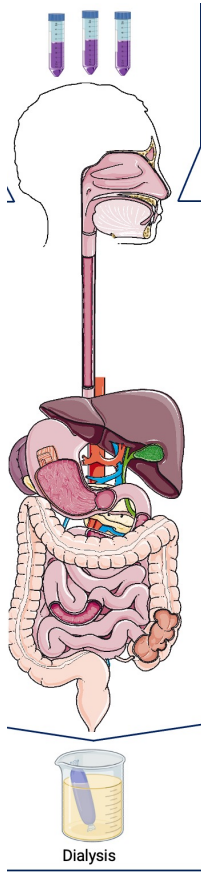
Ingredient (g/100 g)	PB00	PB10	PB20	PB30
Whey protein isolate or Pea or chickpea flour (Control)	40	36	32	28
Mycoprotein	-	10	20	30
Brewers spent grain	18	12	6	-
Glycerol	16	16	16	16
Liquid sorbitol	20	20	20	20
20% (w/w) salt solution	1	1	1	1
Peanut Butter	5	5	5	5

Table 3. The proximate composition of model high-protein nutrition bars with different formulations.

Improve the textural properties of the formulation and increase the level of fiber (second nutritional claim).

Proximate chemical composition and functional properties.

Task 6.4 Safety assessment including evaluation of allergenicity/toxicity of products



- **Bioactivities analysis before gastrointestinal tract simulation (INFOGEST 2.0)**

  - ✓ Antioxidant Activity - ABTS, ORAC and FRAP Method
  - ✓ Angiotensin-Converting Enzyme-I Inhibition Assay (iACE)
  - ✓ Mutagenicity Evaluation—AMES Assay
  - ✓ Antimicrobial Activity
  - ✓ Fatty Acid Permeability Assay
  - ✓ Tyrosinase Inhibition Assay
  - ✓ α-Glucosidase Inhibition Assay
  - ✓ Cytotoxicity Assay
  - ✓ Determination of heavy metals (Pb, Cd, Cu, Fe, Cr, Sr and Ni)

- **Bioactivities analysis during and after gastrointestinal tract simulation (INFOGEST 2.0)**

  - ✓ Antioxidant Activity - ABTS, ORAC and FRAP Method
  - ✓ Angiotensin-Converting Enzyme-I Inhibition Assay (iACE)
  - ✓ Mutagenicity Evaluation—AMES Assay
  - ✓ Antimicrobial Activity
  - ✓ Fatty Acid Permeability Assay
  - ✓ Tyrosinase Inhibition Assay
  - ✓ α-Glucosidase Inhibition Assay
  - ✓ Cytotoxicity Assay
  - ✓ Determination of heavy metals (Pb, Cd, Cu, Fe, Cr, Sr and Ni)

Proving their additional potential health benefits, as well as demonstrate safety, including the absence of allergenicity.

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Thank you.

We are ready for your questions.

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