75th EAAP Annual Meeting
1/5 September 2024 - Florence, Italy

DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

EAAP European Federation of Animal Science

PEPTIDOMIC PROFILE OF IN VITRO DIGESTED HEMP-BASED PRODUCTS AND THEIR EFFECT ON INTESTINAL EPITHELIAL CELLS

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INTRODUCTION

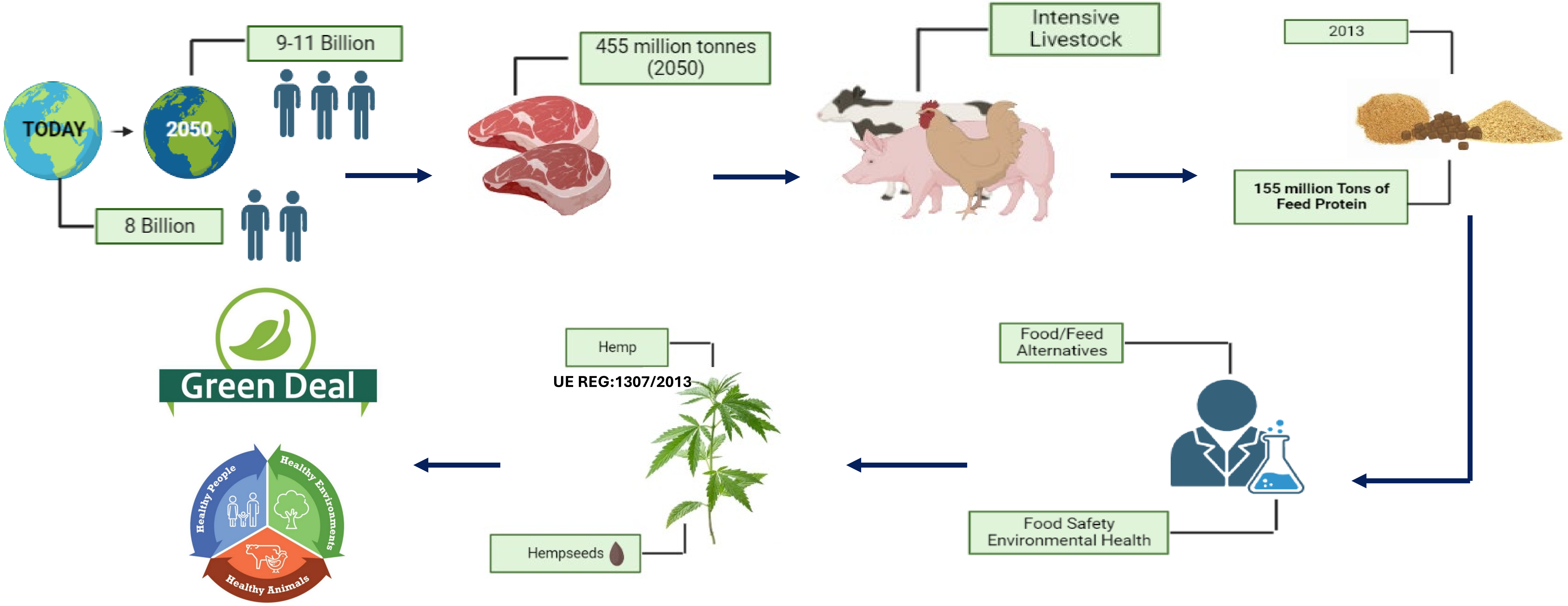
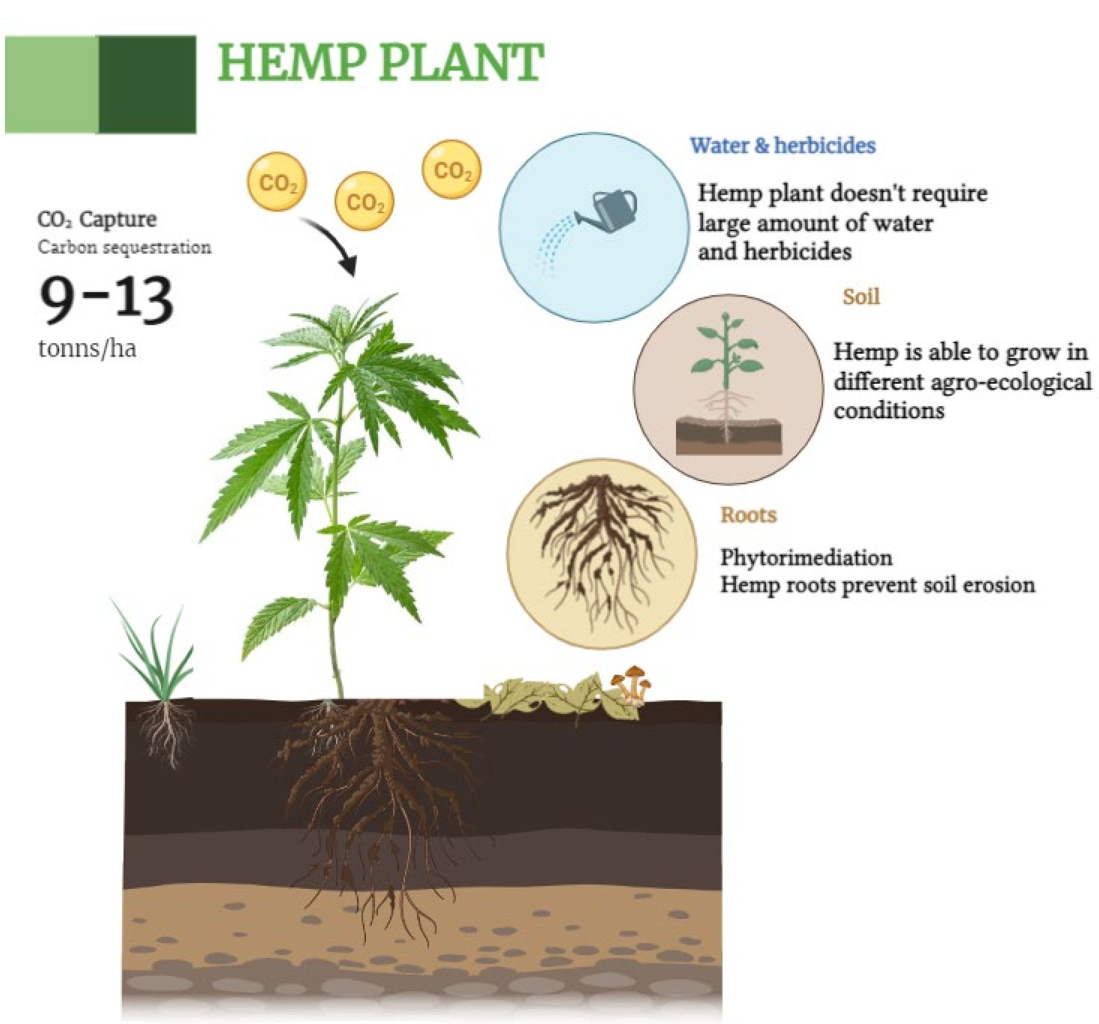


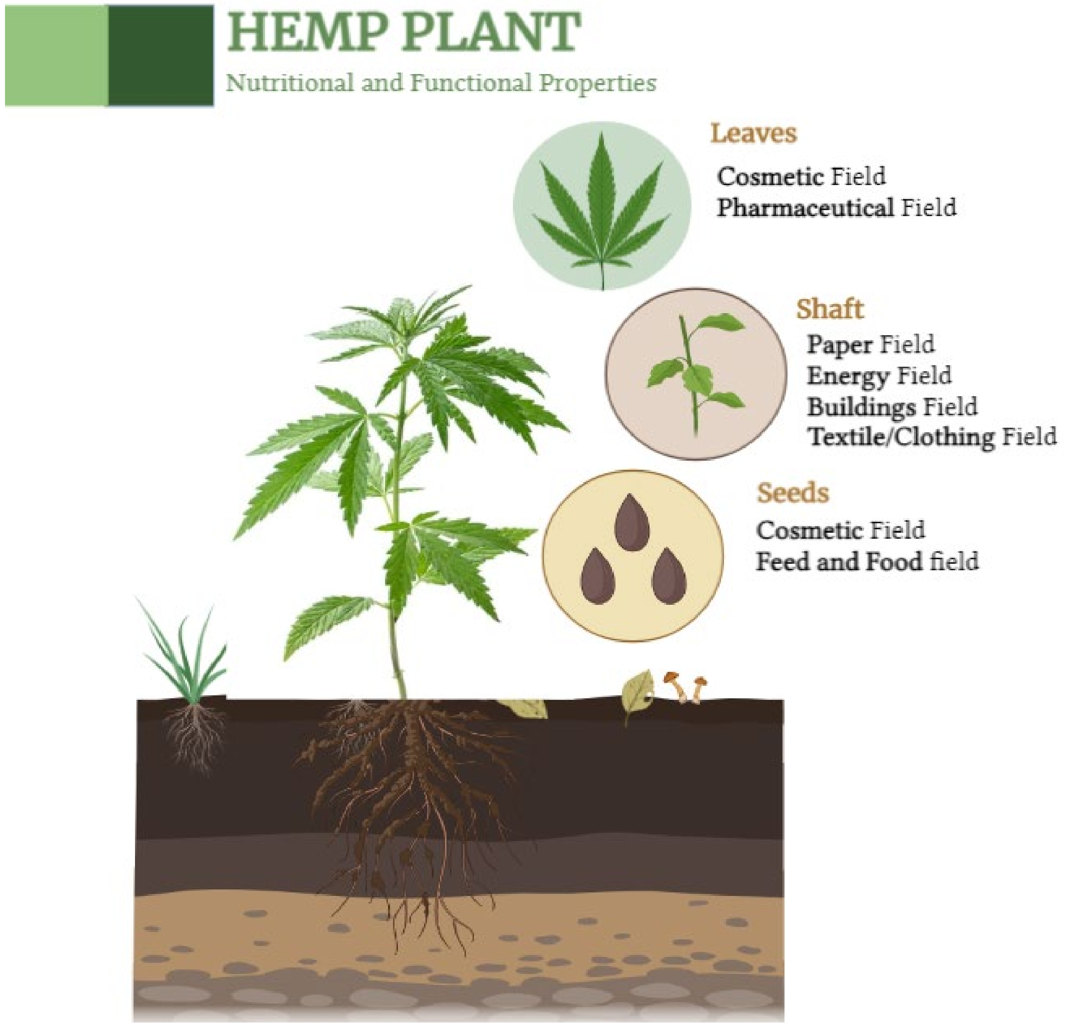
Figure created with Biorender.com

INTRODUCTION



ENVIRONMENT

APPLICATIONS



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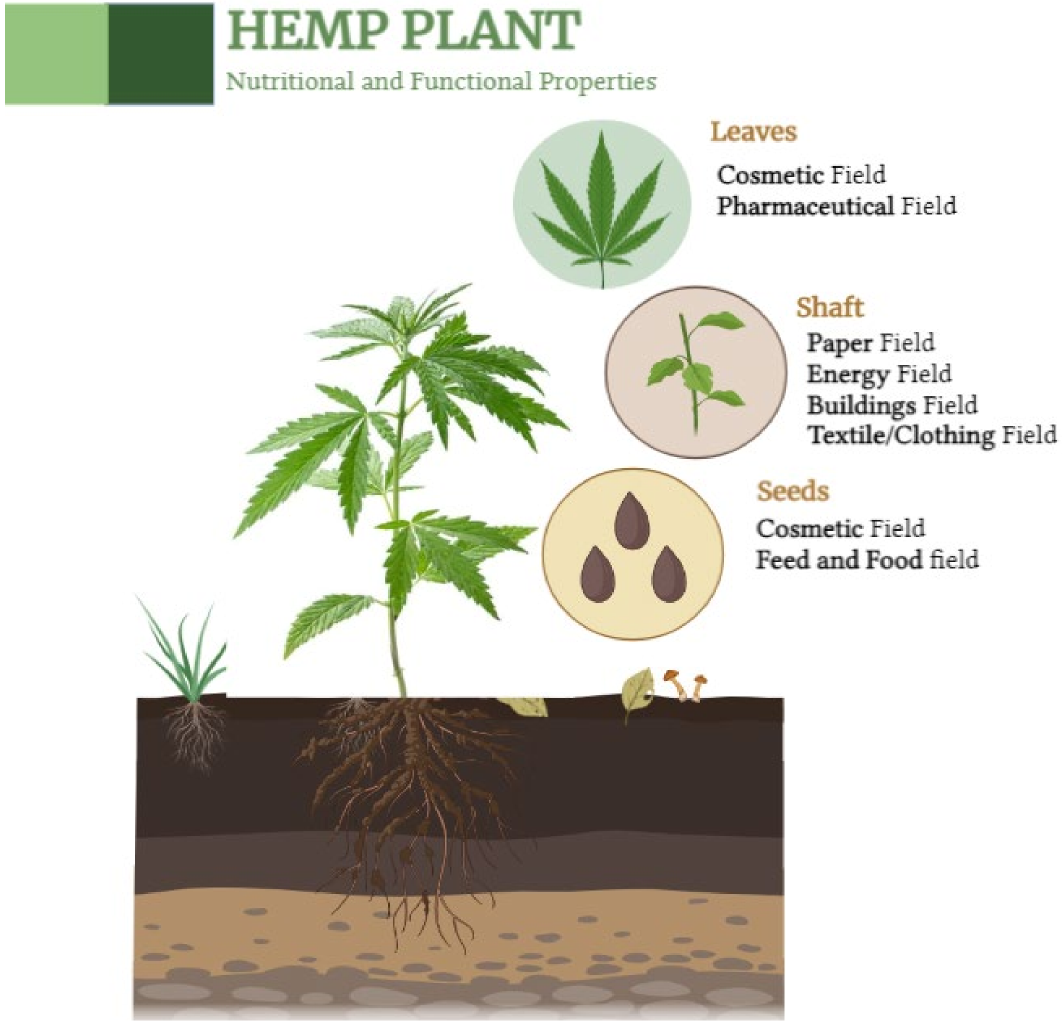
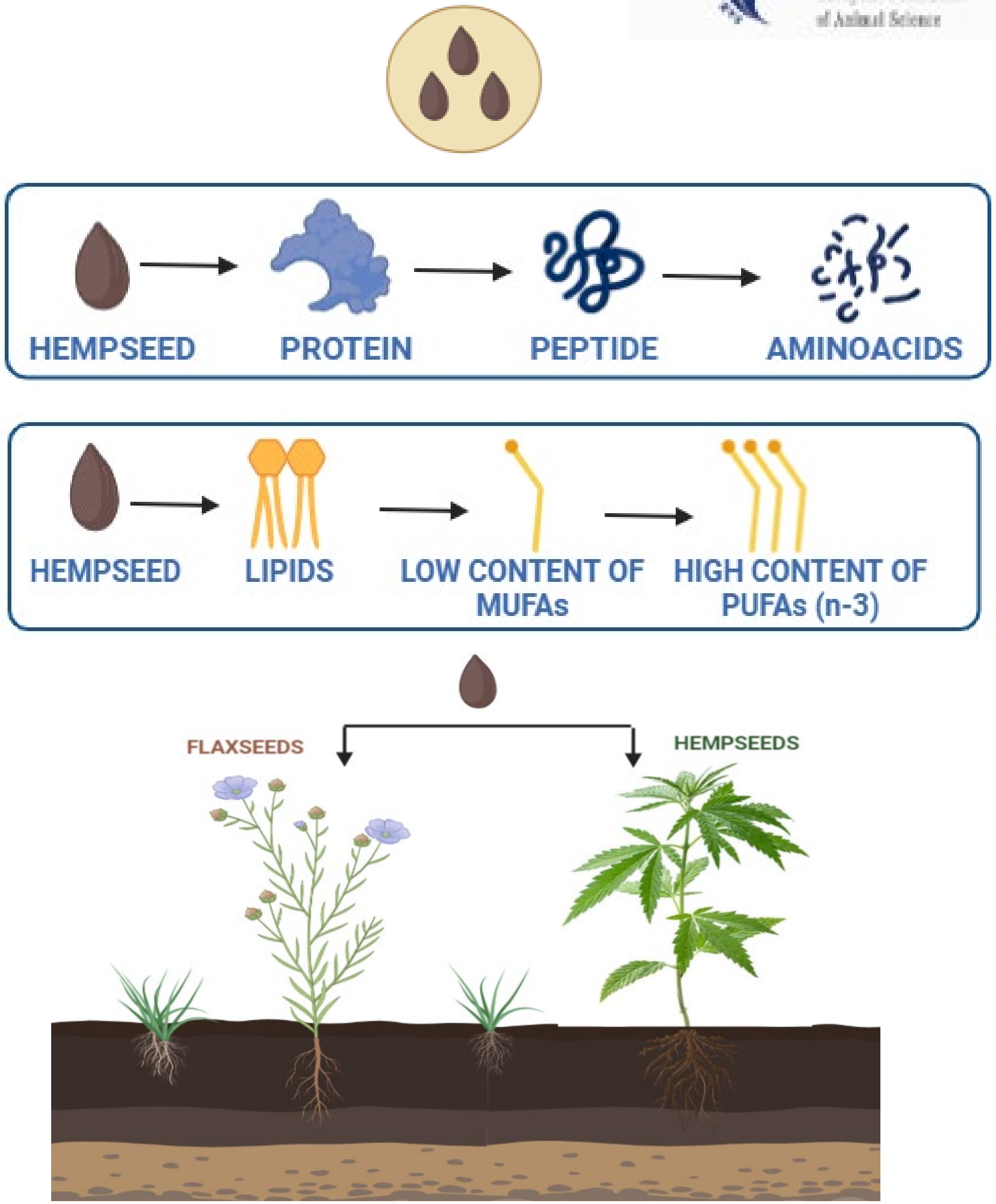
INTRODUCTION



NUTRITIONAL PROFILE



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AIM

1 Nutritional Properties

- HSs
- HSs protein
- Flaxseeds

2 Functional Properties

- Peptidomic profile
- Antioxidant activity
- Antimicrobial activity
- ACE-I activity

3 Cellular Effects

- C2C12
- HT29
- Alamar blue

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MATERIAL

Hempseeds

- *C. Sativa* L, variety Futura
- Czech company;
- THC= 0 ug/g;
- CBD=30 ug/g.

Hempseeds protein

- Commercial supplier
- *C. Sativa* L, variety Futura

Flaxseeds

- Commercial supplier
- *Linum usitatissimum* L.

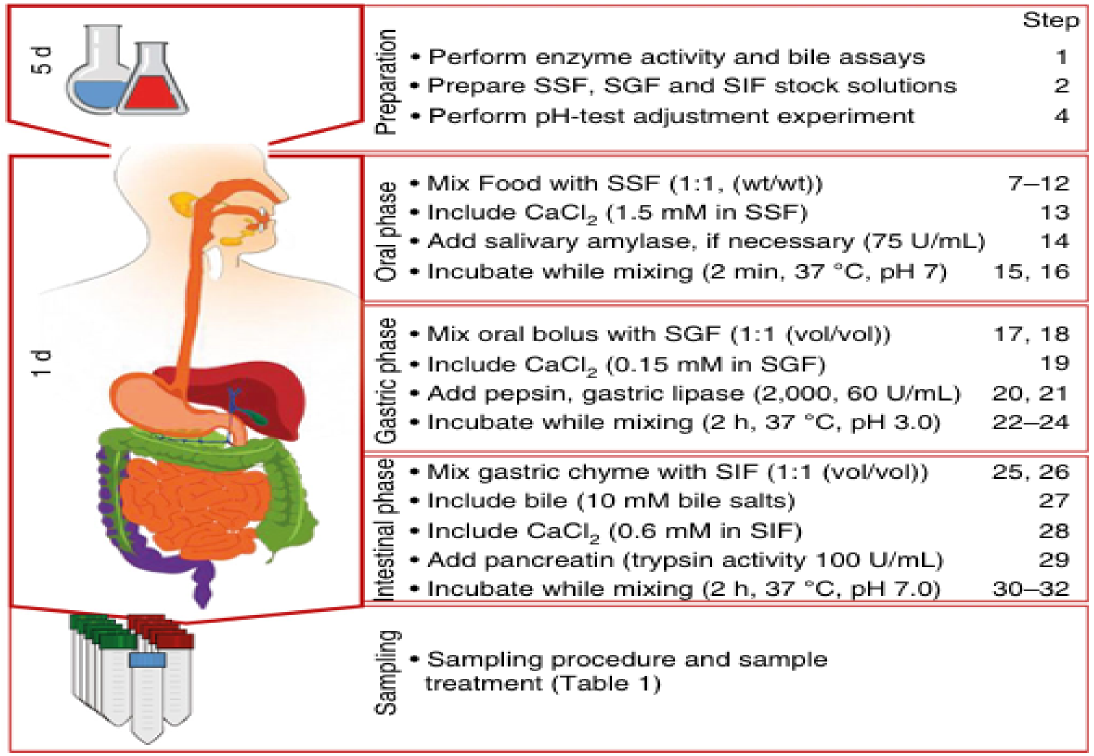
METHODS

Nutritional Profile

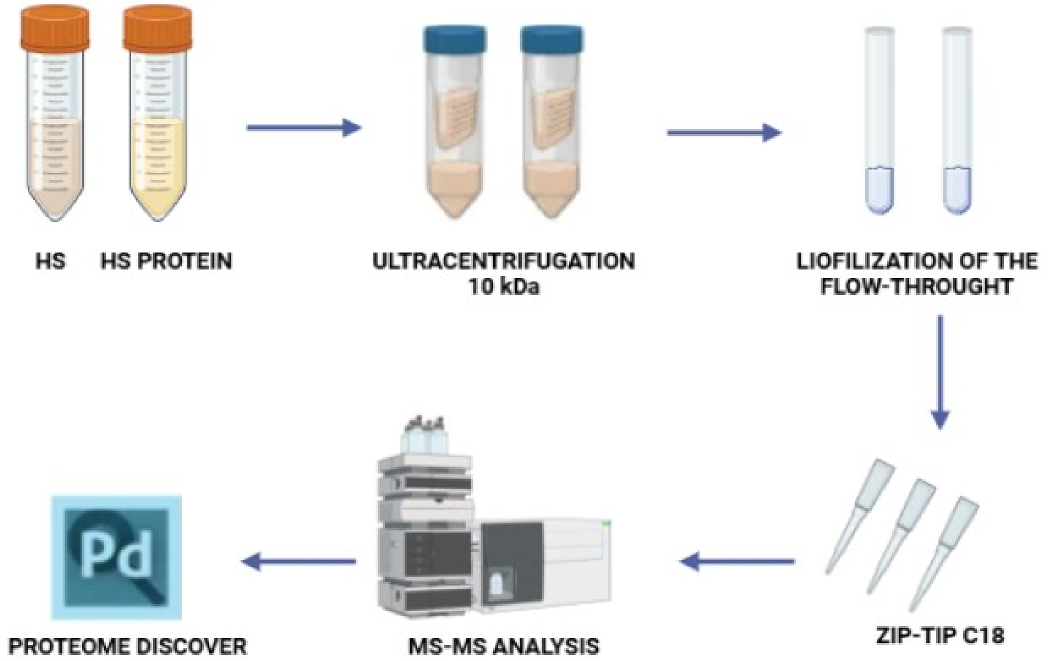
- AOAC (2005);
- Van Soest (1991) → NDF, ADF, ADL

METHODS

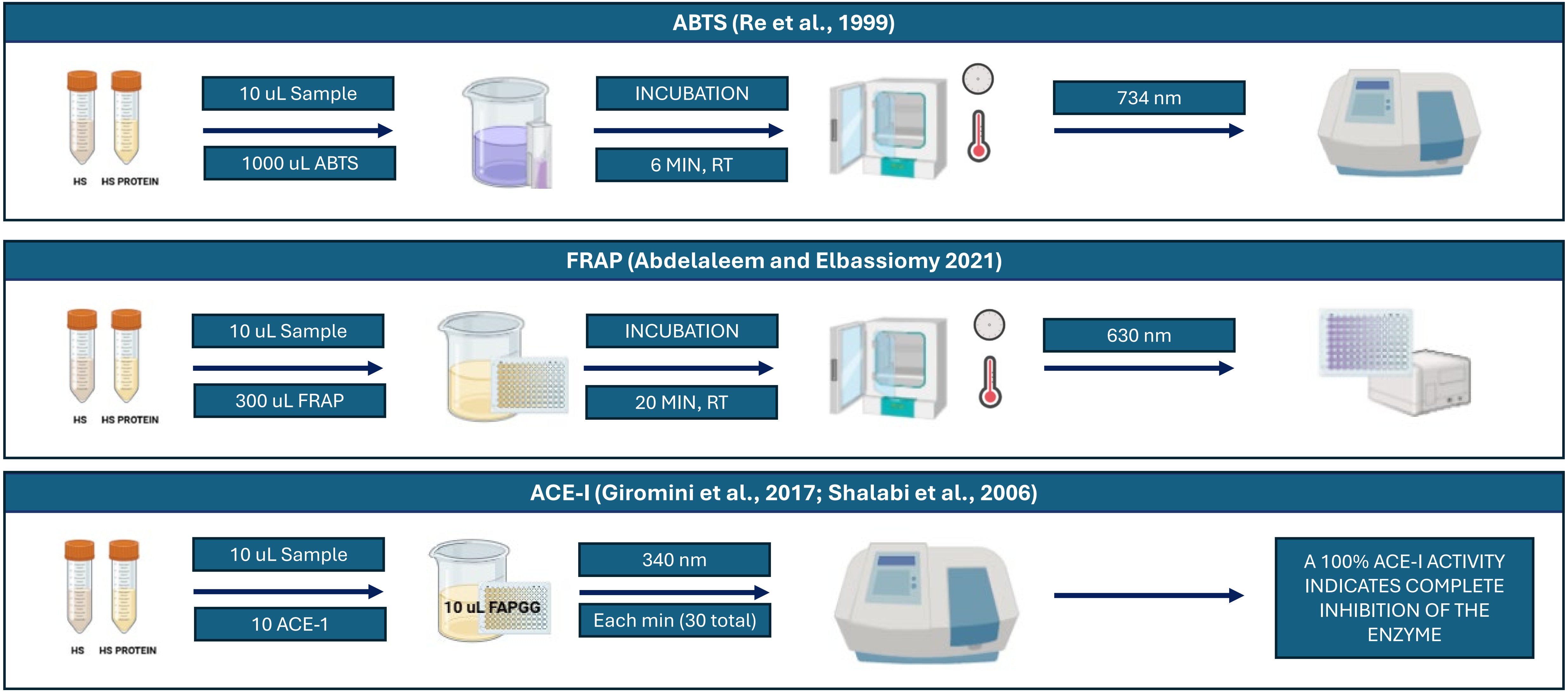
Infogest 2.0 (Brodkorb et al., 2019)



Peptidomic approach (Lanzoni et al., 2024)



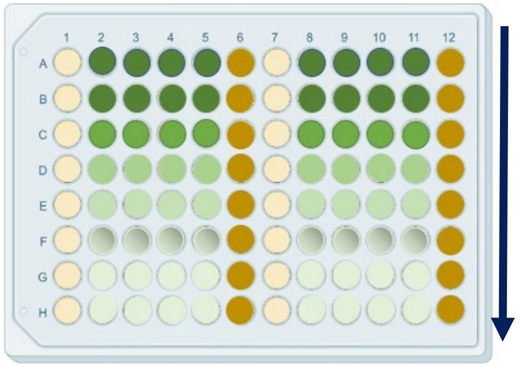
METHODS


ABTS (Re et al., 1999)
HS
HS PROTEIN
10 uL Sample
1000 uL ABTS
INCUBATION
6 MIN, RT
734 nm
FRAP (Abdelaleem and Elbassiomy 2021)
HS
HS PROTEIN
10 uL Sample
300 uL FRAP
INCUBATION
20 MIN, RT
630 nm
ACE-I (Giromini et al., 2017; Shalabi et al., 2006)
HS
HS PROTEIN
10 uL Sample
10 ACE-1
10 uL FAPGG
340 nm
Each min (30 total)
A 100% ACE-I ACTIVITY INDICATES COMPLETE INHIBITION OF THE ENZYME

METHODS

MINIMAL INHIBITORY CONCENTRATION (MIC) (Salmonella Enterica CCM420)

HS HS PROTEIN



NEGATIVE CONTROL (TRIPTON SOYA BROTH)

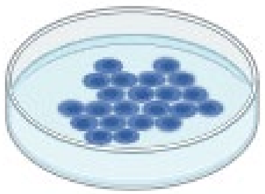
POSITIVE CONTROL

SAMPLES

PLATES INOCULATED BY SALMONELLA SUSPENSION AT A FINAL DENSITY 5 X 10^5 CFU/mL, INCUBATED A 37 C for 24 h

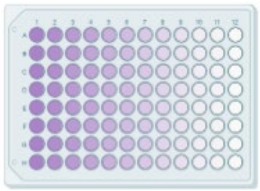
Alamar Blue (C2C12 cells)

HS



C2C12 CELLS

15 uL ALAMAR BLUE



570 nm

RESULTS: Nutritional Profile

SAMPLE	DM	CP	EE	NDF	ADF	ADL	DIG
HS	94.6±0.12[a]	23.1±0.57[a]	27.9±0.75[a]	44.6±0.21[a]	33.2±0.31[a]	14.4±0.32[a]	53.4±0.78[a]
HS Protein	95.7±0.02[b]	45.1±0.84[b]	9.2±0.09[b]	40.4±1.80[a]	20.2±1.02[b]	9.5±0.62[b]	65.5±0.67[b]
Flaxseeds	91.6±0.14[c]	23.0±0.45[a]	36.0±0.07[c]	41.5±1.27[a]	19.5±0.35[c]	9.2±0.41[b]	24.2±2.08c

Chemical composition and digestibility of study matrices (% *w*/*w* on DM basis). Data are presented as mean ± standard error of mean (SEM). (*n* = 3). DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; DIG = digestibility. Different superscript letters in columns indicate statistically significant differences ($p < 0.05$).

High nutritional value matrix

RESULTS: Peptidomic Profile of hempseed protein

	HEMPSEED PROTEIN	SATPdb	PROTEIN NAME
Antioxidative	DGIGNREFRFIGPPLPnDHTnFQVmADFCTRTVSTIW	FIGP	(R)-mandelonitrile lyase-like [Cannabis sativa]
	QKLGGIGDGGGQQINNEEEELVADDQILENNNASSSAAGGFF	AGGF	wall-associated receptor kinase-like 1 [Cannabis sativa]
Antihypertensive	LRENnEmDMFSTESASIANYYYRATLDFD	YYRA	G-type lectin S-receptor-like serine/threonine-protein kinase LECRK3 [Cannabis sativa]
	YNQQQQLQQLQQQQQQqqYMAMmMNQQRGN	LQQQ	heavy metal-associated isoprenylated plant protein 34 [Cannabis sativa]
Antibacterial	ANNTPPQSQQVLSNnDM	TPPQS	NAC domain-containing protein 17 isoform X1 [Cannabis sativa]
	LqQYNSFSARENGSVnVGESSA	FSAR	TOM1-like protein 6 [Cannabis sativa]
Antifungal	ANNTPPQSQQVLSNnDM	TPPQS	NAC domain-containing protein 17 isoform X1 [Cannabis sativa]
	LqQYNSFSARENGSVnVGESSA	FSAR	TOM1-like protein 6 [Cannabis sativa]
Antimicrobial	ANNTPPQSQQVLSNnDM	TPPQS	NAC domain-containing protein 17 isoform X1 [Cannabis sativa]
	LqQYNSFSARENGSVnVGESSA	FSAR	TOM1-like protein 6 [Cannabis sativa]
	NQGFmSRFNAETGTPmRPGPmDVY	RPGP	glucan endo-1,3-beta-glucosidase 5 [Cannabis sativa]
	qTPMDLSKHKSDYAmEPYSQDSSTHSDTNNNSNRRRRASnFRnSL	RRRR	potassium channel AKT1 [Cannabis sativa]
Antiparasitic	LqQYNSFSARENGSVnVGESSA	FSAR	TOM1-like protein 6 [Cannabis sativa]

	HEMPSEED PROTEIN	DFBP	PROTEIN NAME
Antioxidative	DGIGNREFRFIGPPLPnDHTnFQVmADFCTRTVSTIW	FIGP	(R)-mandelonitrile lyase-like [Cannabis sativa]
	QKLGGIGDGGGQQINNEEEELVADDQILENNNASSSAAGGFF	AGGF	wall-associated receptor kinase-like 1 [Cannabis sativa]
ACE inhibitors	LRENnEmDMFSTESASIANYYYRATLDFD	YYRA	G-type lectin S-receptor-like serine/threonine-protein kinase LECRK3 [Cannabis sativa]
	VAIGGSENFYSTFESEFAGVI	GSEN	eukaryotic translation initiation factor 6-2 [Cannabis sativa]
Antihypertensive	LRENnEmDMFSTESASIANYYYRATLDFD	YYRA	G-type lectin S-receptor-like serine/threonine-protein kinase LECRK3 [Cannabis sativa]
Antithrombotic	NQGFmSRFNAETGTPmRPGPmDVY	RPGP	glucan endo-1,3-beta-glucosidase 5 [Cannabis sativa]

Antioxidant activity

ACE inhibitors activity

Anthypertensive activity

Antimicrobial activity

RESULTS: Peptidomic Profile of hempseed protein

	HEMPSEED	SATPdb	PROTEIN NAME
Antioxidative	SCLRMEERLAYSSTEqDmVLLHHE	LLHH	alpha-aminoadipic semialdehyde synthase [Cannabis sativa]
	SLLPFqSPASTSSSSSSSSSSSSSSSSS	LLPF	protein trichome birefringence-like 12 isoform X1 [Cannabis sativa]
	VGGVQGNVGSSYHqNISLPEGDISLINDNDSVLqGmHSVNLSVDEDqF	LQGM	BEACH domain-containing protein C2 [Cannabis sativa]
ACE Inhibitors	RKGGTKIISLqTTmNDDNSEDGCDALPPPASILLPCGSCS	ALPP	DPH4 homolog [Cannabis sativa]
Antibacterial	PMmYARPPPAVNYmPAPYPYPPPPQYQ	YPYP	heavy metal-associated isoprenylated plant protein 33 [Cannabis sativa]
Antifungal	PMmYARPPPAVNYmPAPYPYPPPPQYQ	YPYP	heavy metal-associated isoprenylated plant protein 33 [Cannabis sativa]
Antimicrobial	PMmYARPPPAVNYmPAPYPYPPPPQYQ	YPYP	heavy metal-associated isoprenylated plant protein 33 [Cannabis sativa]
Antihypertensive	LEQLERSLSmLEKSFGDFIqqLNQTGWDTN	LEQL	protein root UVB sensitive 5 [Cannabis sativa]
	LLQQQQQQqqPNSSTTFTTATTTT	LQQQ	protein indeterminate-domain 4, chloroplastic [Cannabis sativa]
	NGGGPAmVDKGVDFANYFCTYAFLYHqKEmLCDRVRMDA	GPAM	protein arginine N-methyltransferase PRMT10 [Cannabis sativa]
	NTAITTQPSFGSTNIEKP	IEKP	aluminum-activated malate transporter 14 [Cannabis sativa]
	RKGGTKIISLqTTmNDDNSEDGCDALPPPASILLPCGSCS	ALPP	DPH4 homolog [Cannabis sativa]
	YSFSVKVEEGCqMNTVDqIAASVNqMMHRINAEL	TVDQ	transcription factor bHLH94 [Cannabis sativa]

	HEMPSEED	DFBP	PROTEIN NAME
Antioxidative	LVPKNPSEDVPLESm	LVPK	pentatricopeptide repeat-containing protein At5g25630 [Cannabis sativa]
	SCLRMEERLAYSSTEqDmVLLHHE	LLHH	BEACH domain-containing protein C2 [Cannabis sativa]
	SLLPFqSPASTSSSSSSSSSSSSSSSSS	LLPF	protein trichome birefringence-like 12 isoform X1 [Cannabis sativa]
	VGGVQGNVGSSYHqNISLPEGDISLINDNDSVLqGmHSVNLSVDEDqF	LQGM	BEACH domain-containing protein C2 [Cannabis sativa]
ACE inhibitors	RKGGTKIISLqTTmNDDNSEDGCDALPPPASILLPCGSCS	ALPP	DPH4 homolog [Cannabis sativa]
	VAIGGSENFYSTFESEFAGVI	GSEN	eukaryotic translation initiation factor 6-2 [Cannabis sativa]
	YSFSVKVEEGCqMNTVDqIAASVNqMMHRINAEL	TVDQ	transcription factor bHLH94 [Cannabis sativa]
Anticancer	LVPKNPSEDVPLESm	LVPK	pentatricopeptide repeat-containing protein At5g25630 [Cannabis sativa]
DPP IV-inhibitory	NVAAAQLGDPEKAEFFGLImACLNnSS	VAA	lipase-like PAD4 [Cannabis sativa]

Antioxidant activity

ACE inhibitors activity

Anthypertensive activity

Antimicrobial activity

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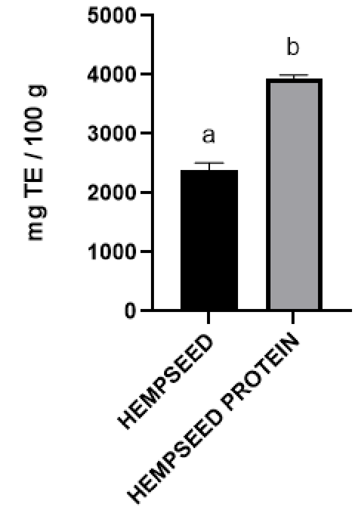
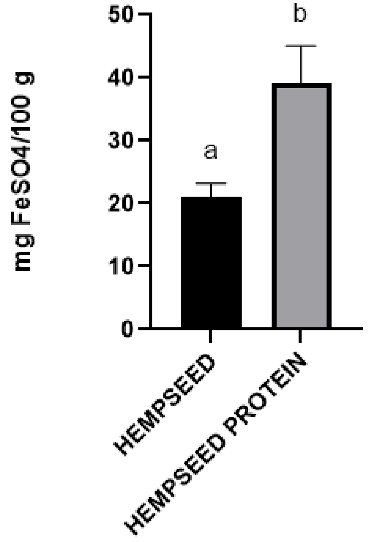
RESULTS: Functional activity of Hempseed and Hempseed protein

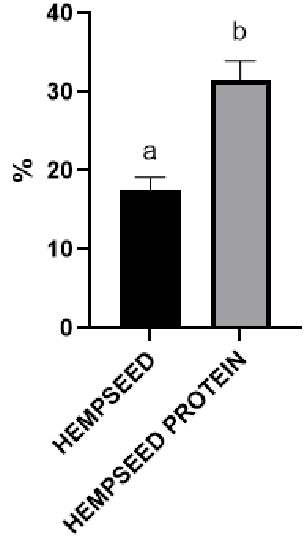
ABTS



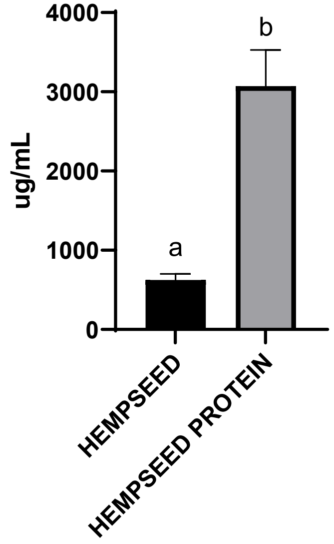
FRAP



ACE



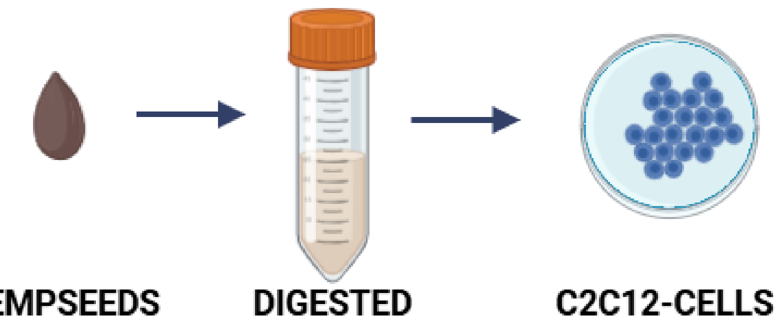
MIC (Salmonella CCM420)



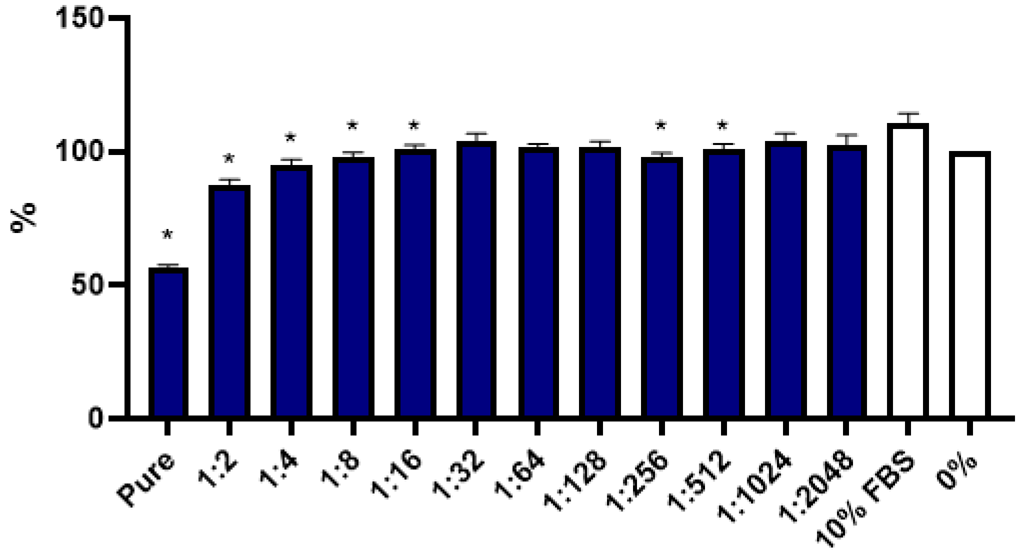
READY BIOAVAILABILITY OF HEMPSEED PROTEIN

PRESENCE OF PHYTOCANNABINODS

RESULTS: Cellular Effects

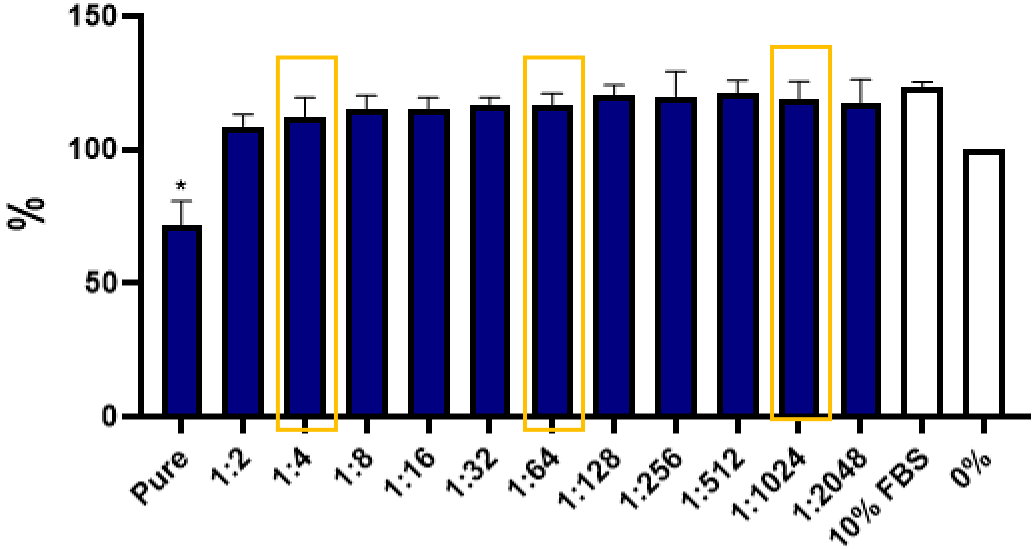


24 h



Effects of hemp seed digest on cell viability (c2c12) assayed with Alamar Blue. Results are expressed in % and standardised to control (0%FBS). * indicates statistically significant differences from 10%.

48 h



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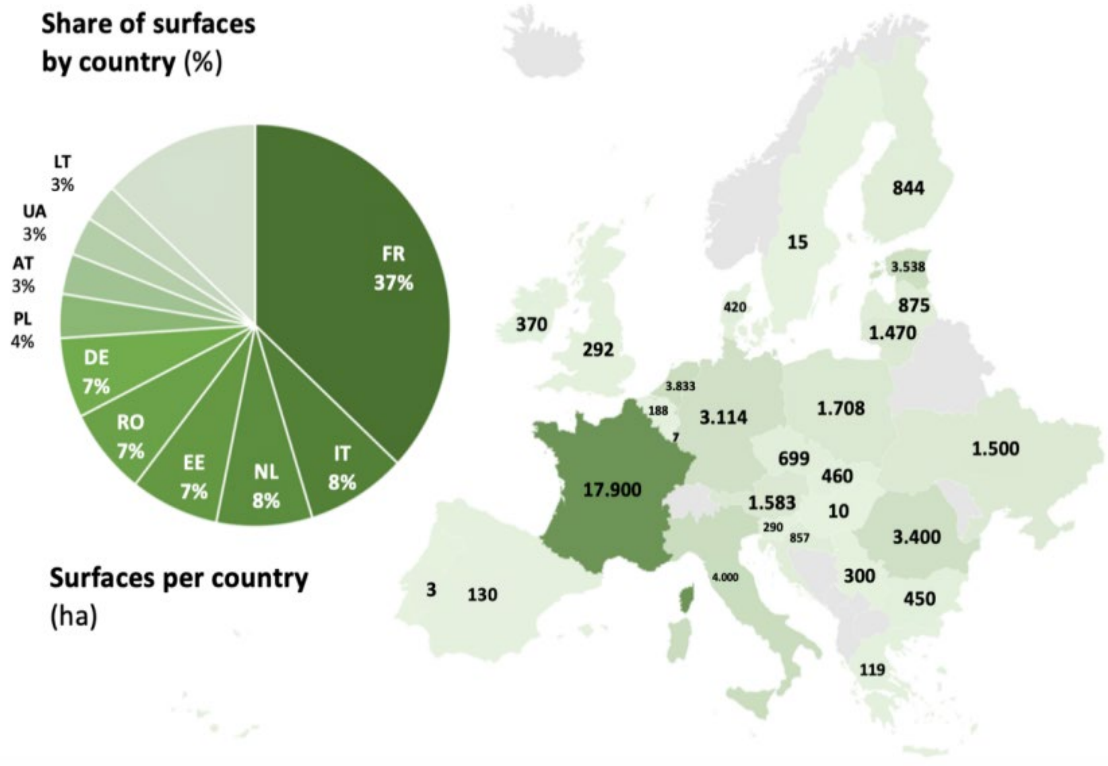
CONCLUSION



Italy is the second largest hemp producing country in Europe (4 000 hectares). Preceded only by France with 18 000 hectares (2018)

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FUTURE PROSPECTS

Università degli Studi di Palermo

POLITECNICO DI MILANO

1. Evaluation of viability, anti-inflammatory and antioxidant effects on animal (IPEC) and human intestinal cells (HT29).

2. Evaluation of hemp-based products for the creation of scaffolds capable of promoting the growth of cells in three-dimensional structures.

THANKS FOR YOUR ATTENTION

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