

PREDICTION APPROACH TO INTERPHASE CONTROL THROUGH PROTEIN PROFILE OF THE SUNFLOWER SEED (*HELIANTHUS ANNUS* L.) USED TO HALVA PROCESSING

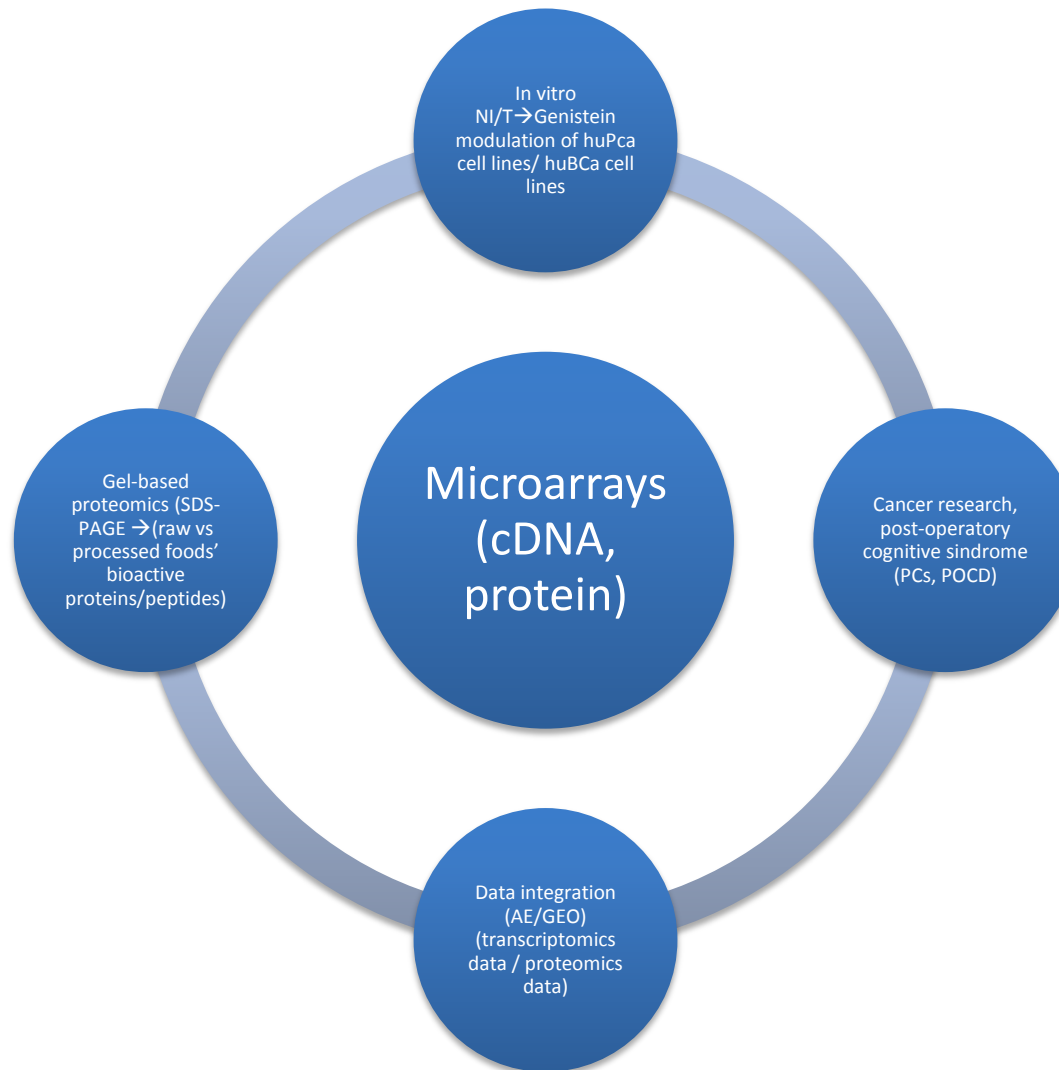


Ramona Suharoschi

Institute of Life Sciences – Molecular Nutrition Lab

Nitra, 25th May 2015







A PROPOSED CURATION PROTOCOL FOR DISCOVERY CANCER POTENTIAL BIOMARKER CANDIDATES

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IL Muntean⁵**

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Experiences in Designing a Distributed Service-Oriented Platform for In-Silico Biomarker Discovery and Validation

Ioan Lucian Muntean*, Bogdan Rotaru*, Ramona Suharoschi[†] and Cristina Iuga[‡]

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[†]Food Science and Technology Department

University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

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University of Medicine and Pharmacy, Cluj-Napoca, Romania

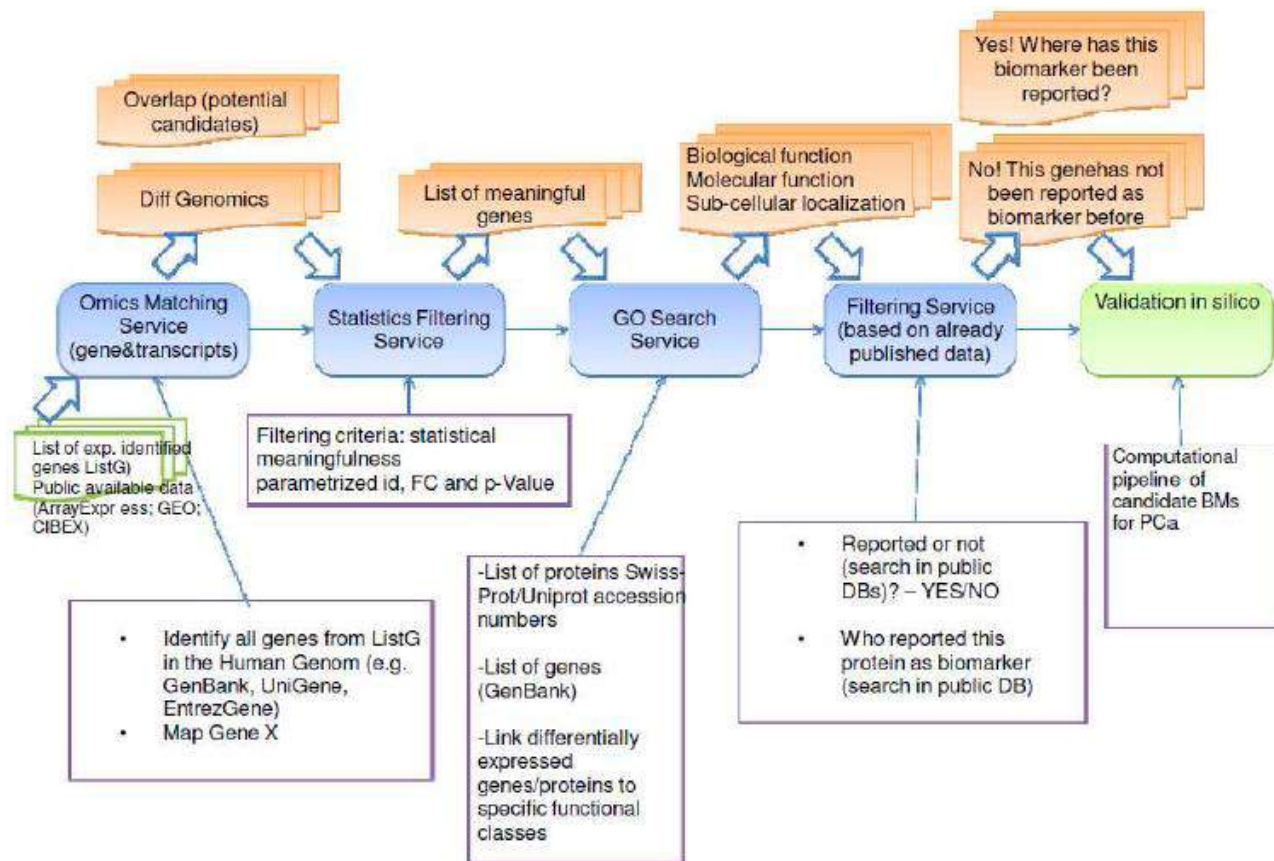
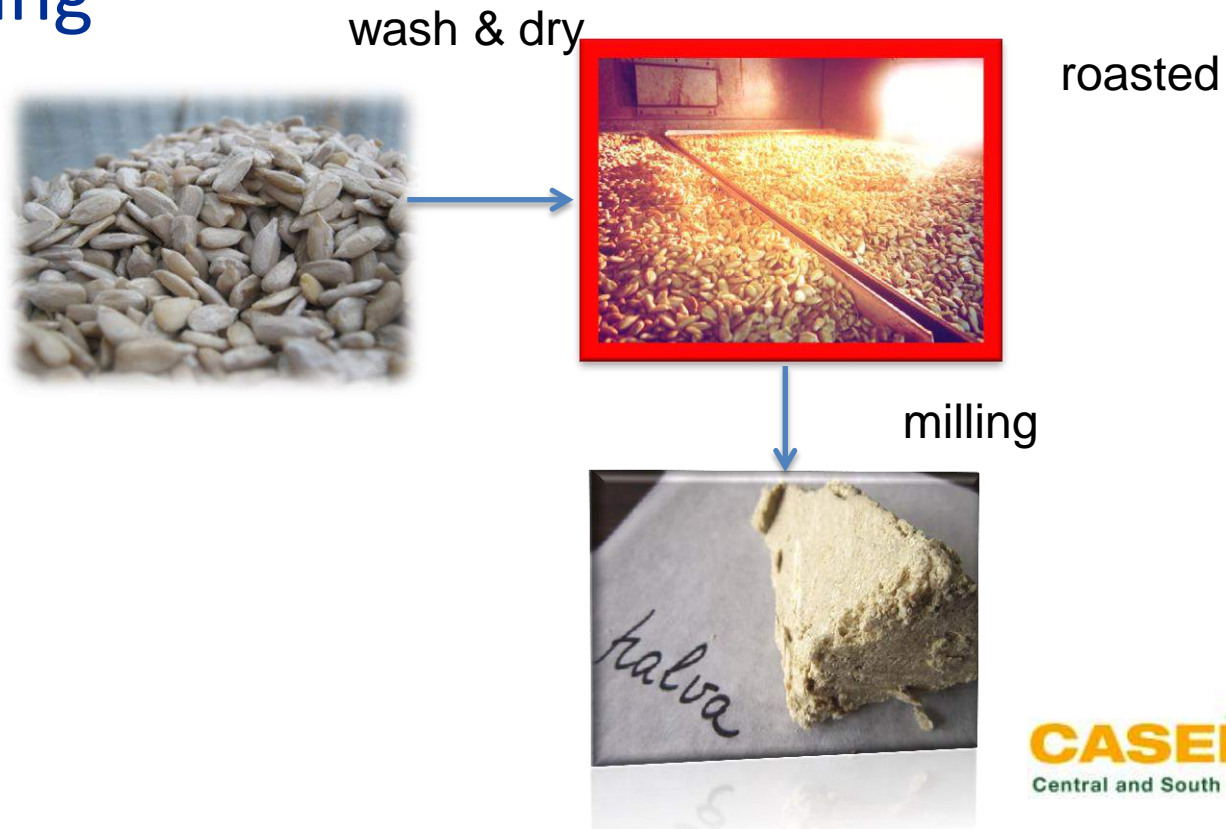


Fig. 1 Biomarker discovery and validation services provided through the intergative open software platform BioGenProtOMICS (developed by IL Muntean, C Iuga and R Suharoschi)

- AIM: approach the interphase control of the protein profile alteration during Halva processing

- HALVA



Lipids 47-65%

FA

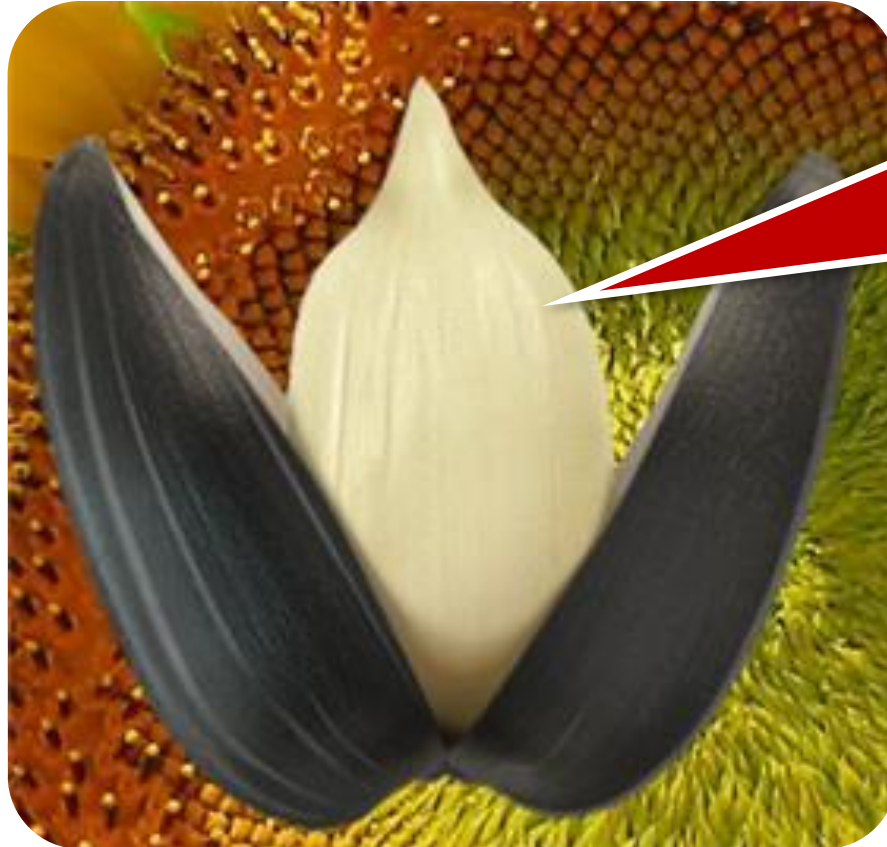
(PUFA/MUFA):

linoleic

51-73%

oleic 15-37%

linolenic 0,3%



20-40% Proteins
Globuline
(Helianthinin), Albumine
AA: Methionin, Cisteine,
Lisine

Minerals 3-4%

(P,K,Mg,Se,Na)

Vitamins

E, A, B₁, B₉

Organic acids:

a. Clorogenic

a. Quinic

Comparison of Sunflower Halva Products from Romanian Market

**Emil RACOLTA, Vlad MUREȘAN,
Sevastita MUSTE, Cristina Anamaria SEMENIUC**

University of Agricultural Sciences and Veterinary Medicine, Faculty of Agriculture,
Mănăștur Street, No.3-5, 400372, Cluj-Napoca, Romania; emil_racolta@yahoo.com

Abstract. Halva is a traditional confection, consists of tahini (sesame paste), cooked sugar and soapwort root



Biotechnol. Agron. Soc. Environ. 2013 **17**(4), 651-659

Focus on:

Confectionery products (halva type) obtained from sunflower: production technology and quality alterations. A review

Vlad Mureșan^(1,2), Christophe Blecker⁽¹⁾, Sabine Danthine⁽¹⁾, Emil Racolța⁽²⁾,
Sevastița Muste⁽²⁾

⁽¹⁾ Univ. Liege - Gembloux Agro-Bio Tech. Food Science and Formulation Department. Passage des Déportés, 2, B-5030 Gembloux (Belgium). E-mail: vlad_muressan@yahoo.com

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J Am Oil Chem Soc (2015) 92:669–683
DOI 10.1007/s11746-015-2622-7

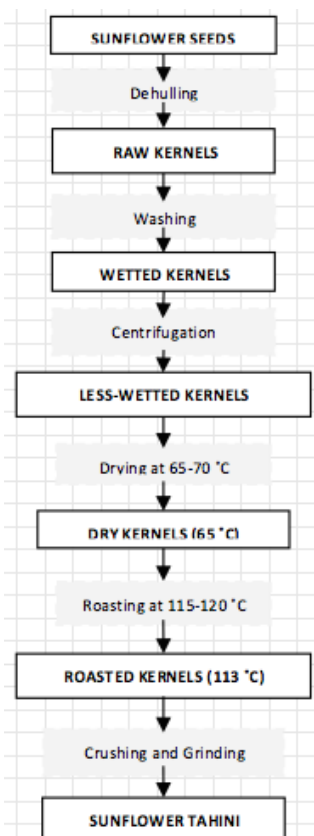


ORIGINAL PAPER

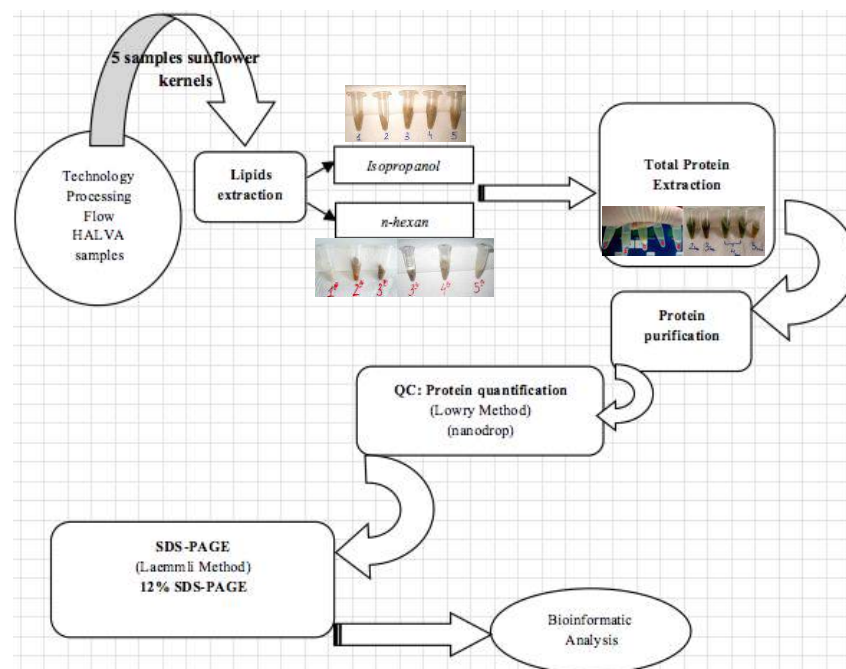
Roasted Sunflower Kernel Paste (Tahini) Stability: Storage Conditions and Particle Size Influence

Vlad Mureșan¹ · Sabine Danthine² · Sorana D. Bolboacă³ · Emil Racolța¹ ·
Sevastița Muste¹ · Carmen Socaciu⁴ · Christophe Blecker²

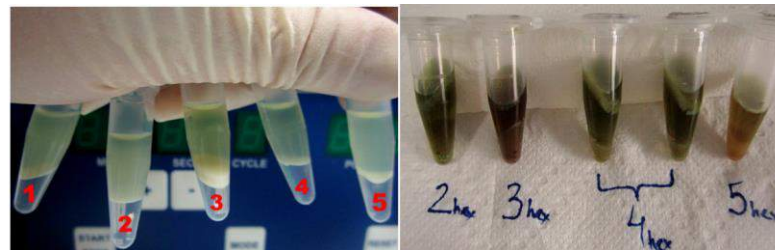
Technological flowchart of the sunflower tahini



Flowchart of the sunflower samples proteins assessment

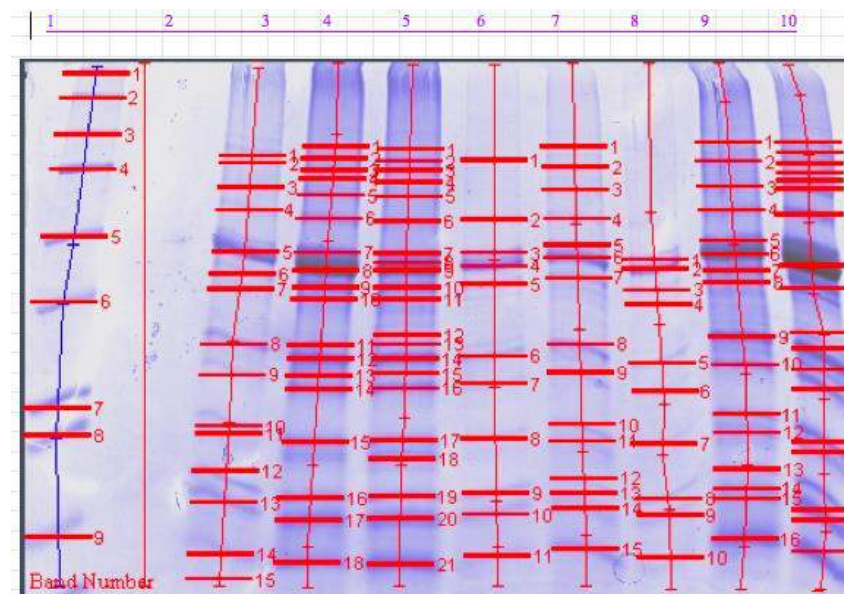
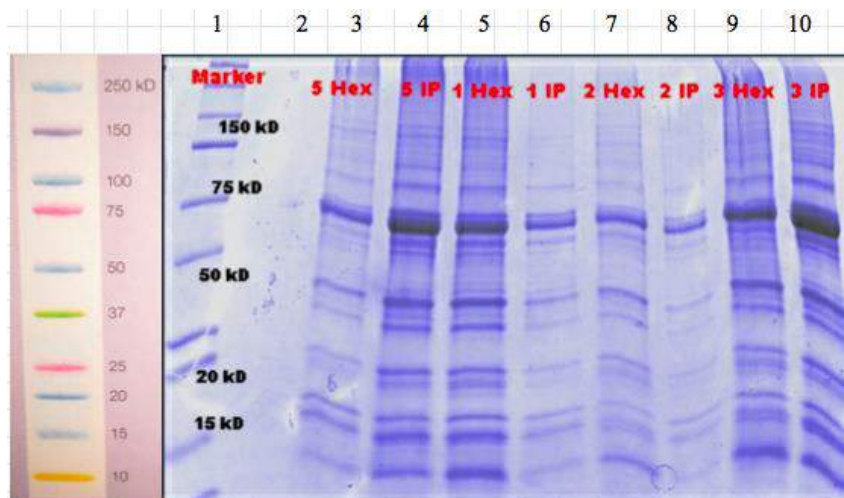


	Extraction phase	<i>Isopropanol</i> extraction sample	<i>n-hexane</i> extraction sample
Remove Lipids	Extraction time	2 hours	4 hours
	Centrifuge	10 min., 15000 rpm, 4°C	20 min., 5000 rpm, 4°C
	Drying	At drying oven, 31-32°C, 15 min.	At room temperature (RT)
	Groats color (fig. 2 and 3)	Pale-brown, p5-darker	p2-brown, p1-white
Total protein extraction	Centrifuge	25 min., 13000 rpm, 18°C	x3 times, 25 min. 18000 rpm, 4°C
	Protein extract color (fig. 5)	white, p3-pale green	Dark green, brown, p5-brown
Determination of protein concentration	Dilution	1:15	1:70
Protein purification	Laemmli reagent	50 µl	100µl



SDS-PAGE (Laemmli method)

SDS-PAGE gel analysis with QuantityOne® (v4.6.3) (Bio-Rad)



Roasted – 15 proteins (Lane3) (nH); 18 (IP) (Line 5: Mw:84-13kDa)
 Raw – 21 proteins (Line 4); 11 (IP) (Line 6: Mw:84-13kDa)



Raw vs roasted sunflower kernel seed proteins separated by SDS-PAGE

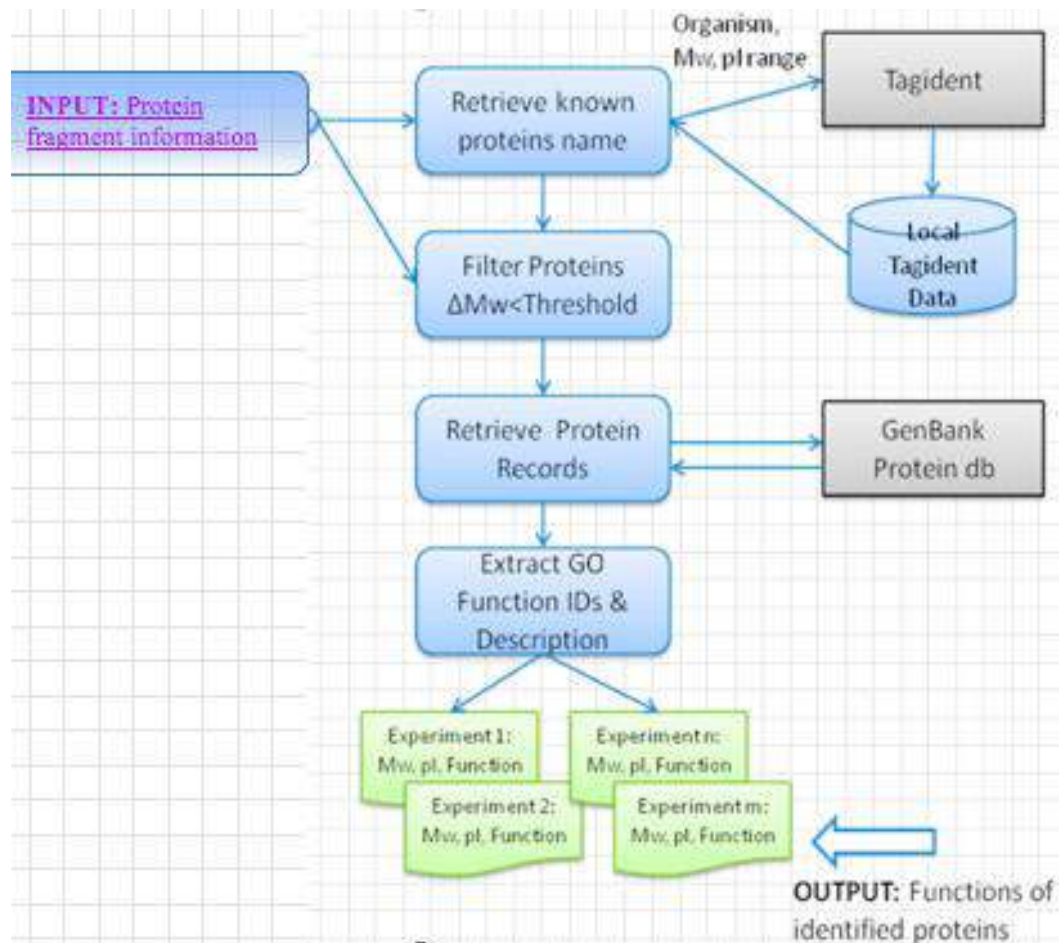
Sample 1(raw kernel), n-hexan (*Helianthus annus L.*)

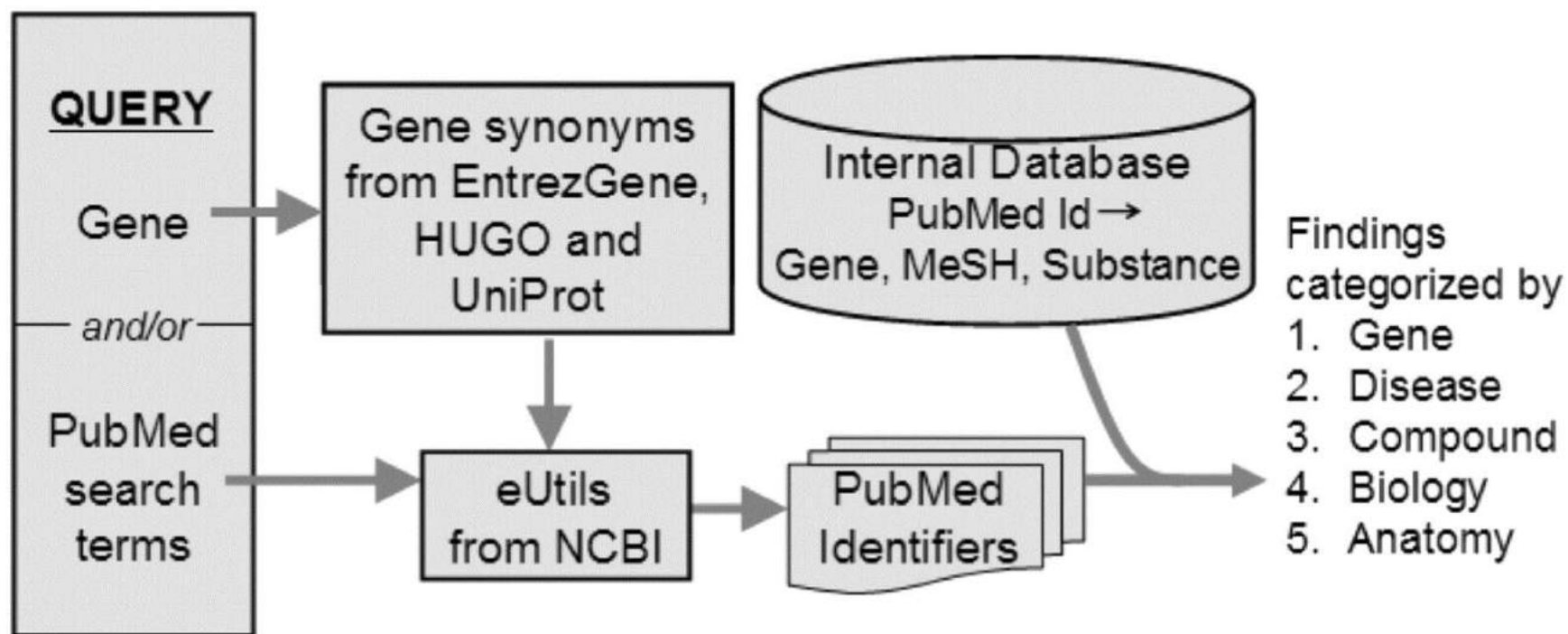
Line/Band	Mw (Da) prot.identif gel	Proteine TagIdent (pI=(3-10); Mw range=10%)
5-1	87,444	PSAA, PSAB, RPOC1, NU5C
5-2	79,346	PALY, PSAA, PSAB, RPOC1, NU5C
5-3	73,817	PALY, RPOC1
5-4	68,678	GAS2, PALY, CS, GAS1
5-5	63,209	GAS2, TCMO, MATK, CALX, CS, GAS1
5-6	54,518	CATA, TCMO, NU4C, PSBB, ACCD, ATPB, PSBC, ATPA, MATK, CALX, SLD
5-7	45,943	SYS, PRS6B, NDHH, PSBC
5-8	44,101	PRS6B, NU1C, NDHH, STAD
5-9	42,448	STAD, PSBD, NU1C, FPPS
5-10	39,327	SF21, FPPS
5-11	37,239	CCSA, NU1C, PSBD, PSBA, RPOA
5-12	32,674	RL5, DCAM, CYF, PP2A, 11S3
5-13	31,547	CYF, DCAM, 11S3
5-14	29,928	COX3, RS3A, CYF
5-15	28,33	1433, COX3, RS3A
5-16	26,817	PSBO, RR3, CEMA, ATPI, RR2
5-17	19,674	NDHI, YCF3, GPX4, GPX1, 11S3, YMF19, PSBP, UP1
5-18	18,652	GPX1, YMF19, NU6C, NDHJ, HSP21
5-19	16,82	CALM, NDK, PETD, 2SS5
5-20	15,814	2SS5, RBS, CALM
5-21	13,91	NU3C, NU3M, RK14, PROF, RBS

Sample 5 (roasted kernel), n-hexan (*Helianthus annus L.*)

Line/Band	Mw (Da) prot.identif gel	Protein- TagIdent (pI=(3-10); Mw range=10%)
3-1	84,583	PSAA, NU5C
3-2	79,766	PALY, PSAA, PSAB, RPOC1, NU5C
3-3	67,657	GAS2, CS, GAS1
3-4	58,943	MATX, CALX, TCMO, CATA, NU2C1, NU2C2, NU4C
3-5	46,73	PRS6B, NU1C
3-6	42,219	STAD, NU1C, SF21, PSBD
3-7	39,42	PSBD, PSBA, RPOA, NU1C, FPPS, SF21, STAD
3-8	31,709	11S3, CYF, DCAM
3-9	28,333	1433, CYF, COX3, RS3A
3-10	21,611	GPX4, CSPL, 11S3, PSBP, UP1, CLPP, YCF3, ATPF, NDHI
3-11	20,359	GPX4, GPX1, CSPL, 11S3, PSBP, UP1, NU6C, CLPP, YCF4, ATPF, NDHI
3-12	18,104	YMF19, NDHJ, NU6C, GPX1, RK22
3-13	16,565	2SS5, HSP11, YMF19, HSP21, CALM, RK22, PETD, NDK
3-14	14,317	PROF, RBS, NU3M, RK14, ATPE, NU3C
3-15	13,366	PROF, RBS, 2SS8, NU3M, RK14, ATPE, NU3C

Bio-Rad Quantity One Software (Version 4.6.3 for Windows)





EXPASY Search Resource Portal TagIdent

Name of your query (optional):

pI range: min 3 max 10

Mw (in Daltons, *not* kD) 55803

Mw range (in percent) 5 %

Check for protein sequences with cysteines in reduced form (-SH) with cysteines oxidized (-S-S-)

Organism name or classification or NCBI_TaxID (e.g. homo sapiens, eukaryota, 9606):
Helianthus

To run the search: Start TagIdent or Reset

TagIdent

Results from TagIdent

The search in UniProtKB/Swiss-Prot has been launched with the following values:
 pI range: 3 - 10
 Mw range: 53012.85 - 58593.15
 with cysteines in reduced form
 OS/OC/OX = Helianthus (corresponding to TaxID: 4231)
 KW keyword = ALL

Scan done on 9-Jun-2014.

UniProtKB/Swiss-Prot Release 2014_05 of 14-May-14: 545388 entries

Swiss-Prot
Number of proteins found in the specified pI/Mw ranges 15

Scan in UniProtKB/Swiss-Prot database (545388 entries)

15 proteins found in the specified pI/Mw range

GAO_HELAN (D5JBX0)

How To Sign in to NCBI

Protein helianthus annuus Search

Save search Advanced Help

Display Settings: Summary, 20 per page, Sorted by Default order Send to: Filters: Manage Filters

Results: 1 to 20 of 12500 << First < Prev Page 1 of 625 Next > Last >>

Results by taxon

Top Organisms [Tree]

- Helianthus annuus (12453)
- Tobacco streak virus (12)
- Arabidopsis thaliana (2)
- All other taxa (33)

Find related data

Database: (Select)

Find items

Search details

"Helianthus annuus"[Organism] OR helianthus annus[All Fields]

Search

- unnamed protein product [Helianthus annuus]

341 aa protein

Accession: CAC40001.1 GI: 14274048

GenPept FASTA Graphics Related Sequences Identical Proteins
- ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial (plastid) [Helianthus annuus]

469 aa protein

Accession: AKG25167.1 GI: 817992005

GenPept FASTA Graphics
- RecName: Full=Delta(8)-fatty-acid desaturase; AltName: Full=Delta(8)-sphingolipid desaturase [Helianthus annuus]

458 aa protein

Accession: Q43469.1 GI: 75319741

GenPept FASTA Graphics Related Sequences Identical Proteins
- RecName: Full=ATP synthase epsilon chain_chloroplastic; AltName: Full=ATP synthase F1 sector epsilon subunit; AltName: Full=F-ATPase epsilon subunit (chloroplast) [Helianthus annuus]

Results

Filter by

- Reviewed (152)
Swiss-Prot
- Unreviewed (2,660)
TrEMBL
- Popular organisms**
- HELAN (2,787)
- TuYV (6)
- BWYV (4)
- PSENA (2)
- Pseudois nayaur nayaur (13)

Search terms

- filter "helan" as:
- organism (2,787)
- strain (2)
- taxonomy (2,789)
- virus host (10)

View by

[BLAST](#) [Align](#) [Download](#) [Add to basket](#) [Columns](#) [>](#)
◀ 1 to 25 of 2,812 ▶ Show 25

<input type="checkbox"/>	Entry	Entry name	Protein names	Gene names	Organism	Length
<input type="checkbox"/>	Q4GWU5	SFTI1_HELAN	Trypsin inhibitor 1	sfti1	Helianthus annuus (Common sunflower)	56
<input type="checkbox"/>	P23110	2SS8_HELAN	Albumin-8		Helianthus annuus (Common sunflower)	141
<input type="checkbox"/>	Q4U3F6	CS_HELAN	Alpha-copaene synthase	CS	Helianthus annuus (Common sunflower)	555
<input type="checkbox"/>	Q4U3F7	GAS1_HELAN	Germacrene A synthase 1	GAS1	Helianthus annuus (Common sunflower)	559
<input type="checkbox"/>	B0FGA9	GAS2_HELAN	Germacrene A synthase 2	GAS2	Helianthus annuus (Common sunflower)	559
<input type="checkbox"/>	P82007	NLTP1_HELAN	Non-specific lipid-transfer protein...		Helianthus annuus (Common sunflower)	116
<input type="checkbox"/>	O04058	PALY_HELAN	Phenylalanine ammonia-lyase	PAL	Helianthus annuus (Common sunflower)	667
<input type="checkbox"/>	Q43469	SLD1_HELAN	Delta(8)-fatty-acid desaturase	sid1	Helianthus annuus (Common sunflower)	458
<input type="checkbox"/>	P18260	ATPAM_HELAN	ATP synthase subunit alpha, mitocho...	ATPA	Helianthus annuus (Common sunflower)	510
<input type="checkbox"/>	P45738	RBL_HELAN	Ribulose biphosphate carboxylase l...	rbcl	Helianthus annuus (Common sunflower)	485
<input type="checkbox"/>	P19084	11S3_HELAN	11S globulin seed storage protein G...	HAG3	Helianthus annuus (Common sunflower)	493

Protein name	ID (ID-UniProt)	Molecular weight (Mw) (Da)	pI	Aminoacid sequence length	Cellular component (GO)	Molecular function (GO)	Biological process (GO)	Gene
<i>Photosystem II CP47 chlorophyll apoprotein</i>	PSBB_HELAN	56176	6.27	508	chloroplast thylakoid membrane; photosystem II	chlorophyll binding	Photosynthesis	psbB
<i>ATP synthase subunit alpha</i>	ATPAM_HELAN	55487	6.02	510	mitochondrial inner membrane; proton-transporting ATP synthase complex, catalytic core F(1)	ATP binding; proton-transporting ATP synthase activity, rotational mechanism	ATP hydrolysis coupled proton transport	ATPA
<i>Germacrene A oxidase</i>	GAO_HELAN	55088	8.59	488	endoplasmic reticulum membrane	heme binding; monooxygenase activity; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; Monooxygenase, Oxidoreductase		NA
<i>Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta</i>	ACCD_HELAN	54284	4.92	480	acetyl-CoA carboxylase complex	acetyl-CoA carboxylase activity; zinc ion binding; Ligase	fatty acid biosynthetic process; Fatty acid biosynthesis, Fatty acid metabolism, Lipid biosynthesis, Lipid metabolism	accD
<i>Ribulose bisphosphate carboxylase large chain</i>	RBL_HELAN	53635	5.97	485	chloroplast	magnesium ion binding; ribulose-bisphosphate carboxylase activity	photorespiration	rbcL
<i>Delta(8)-fatty-acid desaturase</i>	SLD1_HELAN	52232	8.92	458	An integral component of the membrane	heme binding; oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water;	The fatty acid biosynthetic process	slD1
<i>Oxygen-evolving enhancer protein 1</i>	PSBO_HELAN	26525	5.01	324	cell outer membrane; extrinsic component of membrane; photosystem II oxygen evolving complex	calcium ion binding	photosynthesis	PSBO

Protein name	ID (ID-UniProt)	Molecular weight (Mw) (Da)	pI	Aminoacid sequence length	Cellular component (GO)	Molecular function (GO)	Biological process (GO)	Gene
<i>Photosystem II CP47 chlorophyll apoprotein</i>	PSBB_HELAN	56176	6.27	508	chloroplast thylakoid membrane; photosystem II	chlorophyll binding	Photosynthesis	psbB
<i>ATP synthase subunit alpha</i>	ATPAM_HELAN	55487	6.02	510	mitochondrial inner membrane; proton-transporting ATP synthase complex, catalytic core F(1)	ATP binding; proton-transporting ATP synthase activity, rotational mechanism	ATP hydrolysis coupled proton transport	ATPA
<i>Germacrene A oxidase</i>	GAO_HELAN	55088	8.59	488	endoplasmic reticulum membrane	heme binding; monooxygenase activity; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; Monooxygenase, Oxidoreductase		NA
<i>Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta</i>	ACCD_HELAN	54284	4.92	480	acetyl-CoA carboxylase complex	acetyl-CoA carboxylase activity; zinc ion binding; Ligase	fatty acid biosynthetic process; Fatty acid biosynthesis, Fatty acid metabolism, Lipid biosynthesis, Lipid metabolism	accD
<i>Ribulose bisphosphate carboxylase large chain</i>	RBL_HELAN	53635	5.97	485	chloroplast	magnesium ion binding; ribulose-bisphosphate carboxylase activity	photorespiration	rbcL
<i>Delta(8)-fatty-acid desaturase</i>	SLD1_HELAN	52232	8.92	458	An integral component of the membrane	heme binding; oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water;	The fatty acid biosynthetic process	slid1
<i>Oxygen-evolving enhancer protein 1</i>	PSBO_HELAN	26525	5.01	324	cell outer membrane; extrinsic component of membrane; photosystem II oxygen evolving complex	calcium ion binding	photosynthesis	PSBO

- to develop and optimize a new extraction method that should be characterized by the high amount of protein extraction with a high purity.
- integrate the low-cost molecular techniques with *in silico* methods.
 - the bioinformatic tools → predict a set of differentially expressed proteins between Halva processing interphases with a potential to develop a **interphase control kit** with product processing application.
- proteins identified by *in silico* approach need to be validated in wet experiments.
- Design of a healthier product (Mallard compounds → biotechnology processes)

Pros and Cons

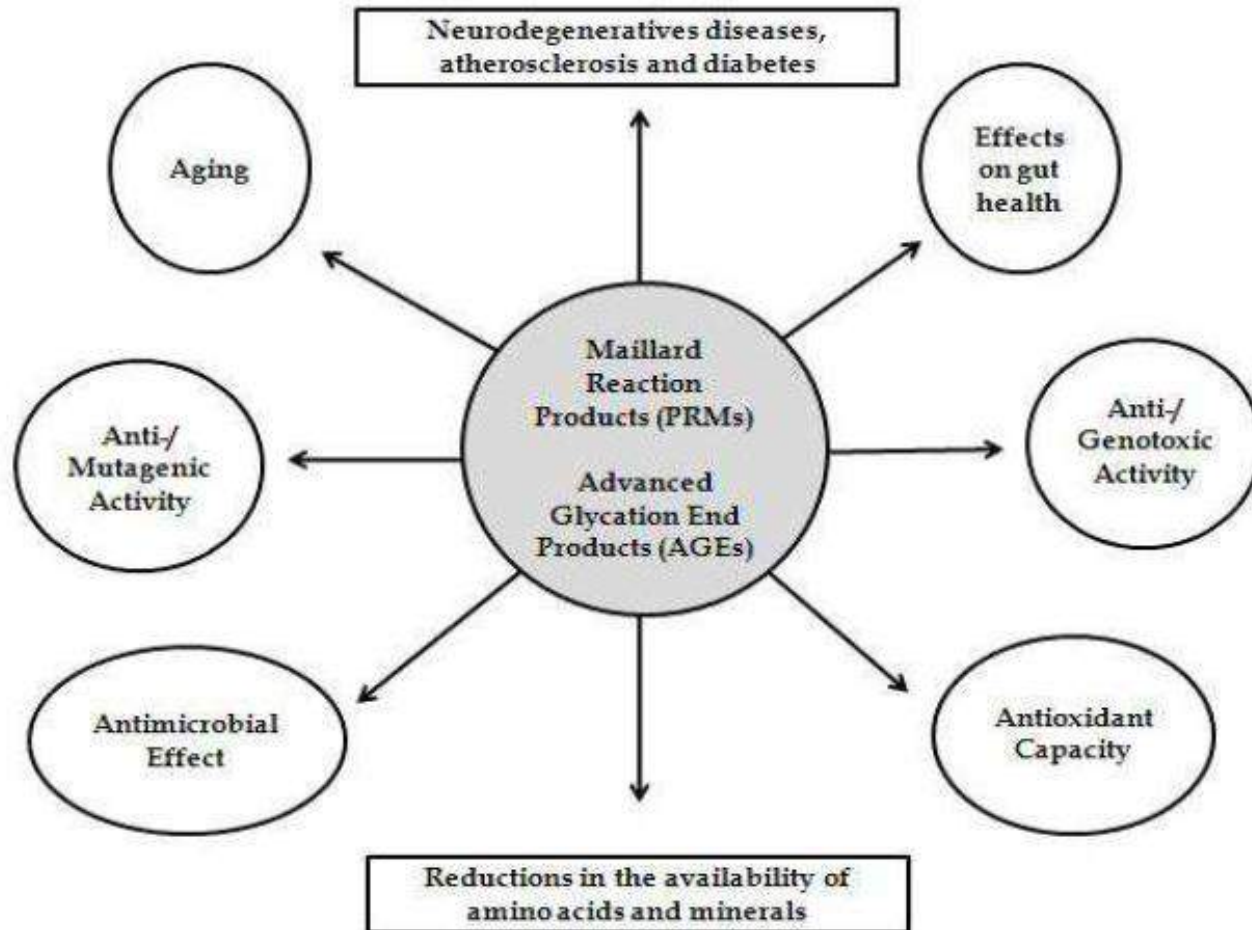
Deborah Markowicz Bastos, Érica Monaro,
Érica Siguemoto and Mariana Séfora
*Nutrition Department, School of Public Health, São Paulo University
Brazil*

1. Introduction

The Maillard reaction was first reported in 1912 by Louis-Camille Maillard, who described that upon gently heating sugars and amino acids in water, a yellow-brown color developed. The reaction that leads to these colorful compounds, firstly described from a simple observation, is actually the result of a complicated pathway of chemical reactions. The Maillard reaction is often described in food systems but it also occurs in living organisms, and in this case, it is called glycation. In biological systems, the ramifications of the Maillard reaction have been observed and analyzed, as this reaction has become important in the field of food science and medicine (Finot, 2005; Gerrard, 2002a).

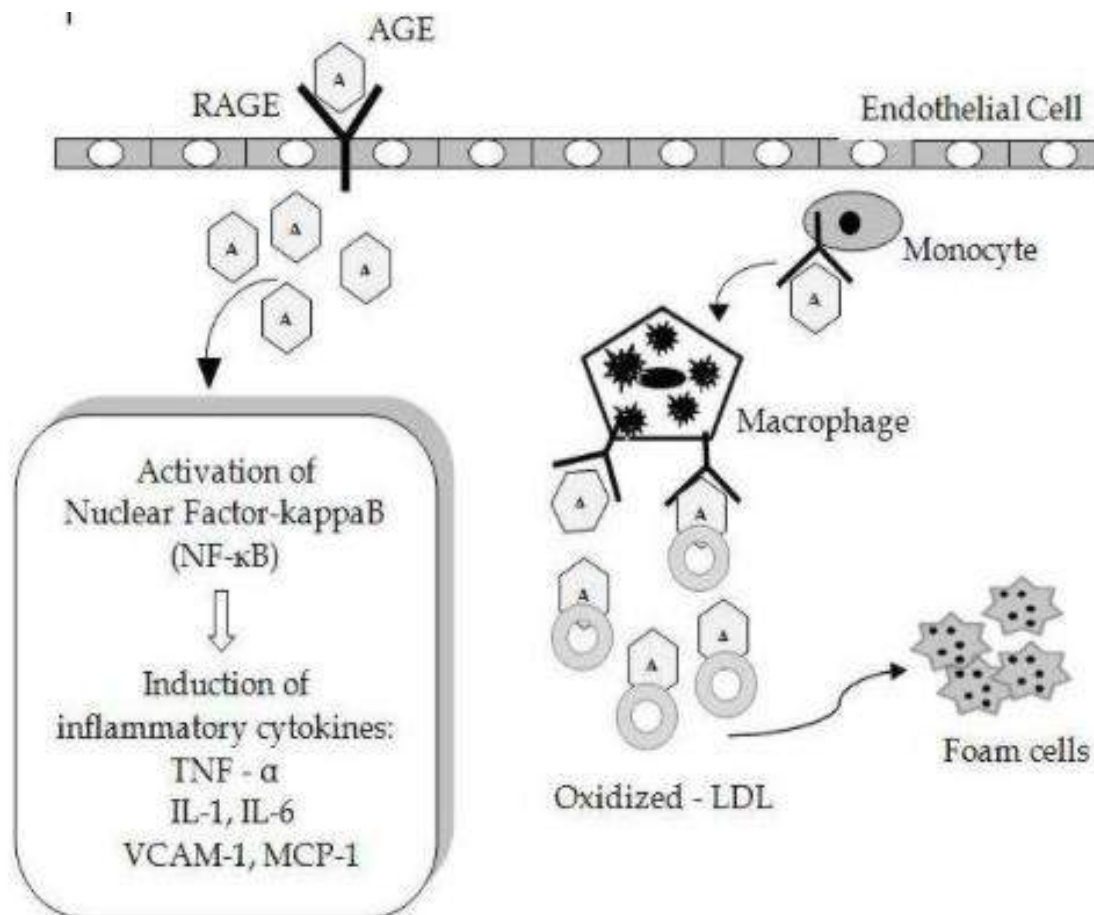
The consumption of Maillard Reaction Products (MRPs) has increased in recent decades and

(After: Bastos, 2012 INTECH, www.intechopen.com)



Possible biological effects of the Maillard Reaction Products (MRPs) and Advanced Glycation End Products (AGEs) (Based on Somoza 2005)

(After: Bastos, 2012 INTECH, www.intechopen.com)



AGE-RAGE interaction and its association with atherosclerosis (Based on Hartog et al., 2007)
 (After: Bastos, 2012 INTECH, www.intechopen.com)

- The study of the ***functional capacity of proteins*** from the raw sunflower kernels (expression, purification and characterization) as **new formulation of a healthier product** (raw sunflower seeds) → investigate its sustainability to food industry and human nutrition.
- Development of a kit with a set of differentially expressed proteins and common proteins → develop and validate as an interphase control kit for Halva processing.

+

MSc in Gastronomy, Nutrition and Dietetics



*MSc in Gastronomy, Nutrition and Dietetics
at the University of Agricultural Sciences and Veterinary*

SEMESTER ABROAD AT USAMV CLUJ – ROMANIA

This experience includes coursework and laboratory research experience, with the opportunity to tour food companies and attend the Institute of International annual meeting in Cluj. Students will interact with students, faculty, and food industry leaders in a variety of activities to develop a further understanding of food science and technology, food research and the food processing industry in the field of Gastronomy, Nutrition and Dietetics.

- Molecular gastronomy
- Personalized Nutrition and Dietetics
- Traditional Foods
- Food Nutrients and Ingredients
- Physiology
- Applied Research
- Applied Biostatistics

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Prof. D Haltrich, BOKU, AT

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