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Inhibitory Effect of Buckwheat Derived Bakery Products on the Formation of Advanced Glycation End Products – a Comparison Study





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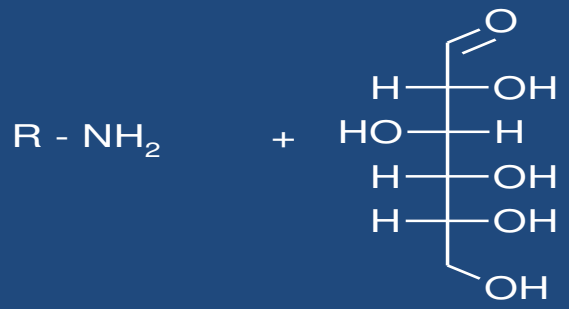
INTRODUCTION

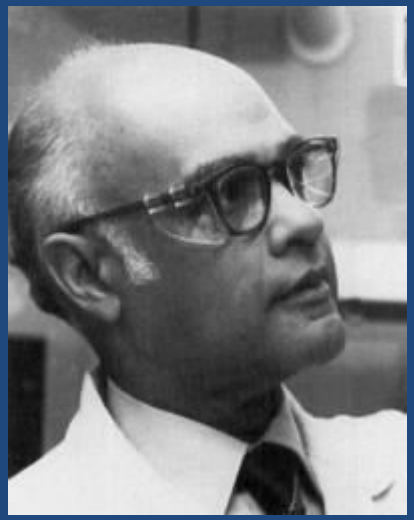


French chemist Louis-Camille Maillard described the reaction between amino acids and sugars in 1912.

His study did not offer much in the way of analysis on the reaction's impact on flavour and aroma in food.

PROTEIN GLICATION





In 1973, American chemist John E Hodge published a mechanism for the different steps of the reaction.

He identified the first stage as being the reaction between the sugar and the amino acid; this produced a glycosylamine compound, which in the second step rearranged to produce a ketosamine.

The final stage consists of this compound reacting in a number of ways to produce several different compounds, which can themselves react to produce further products.

Scheme of Maillard reaction

Initial stage — Amadori compounds



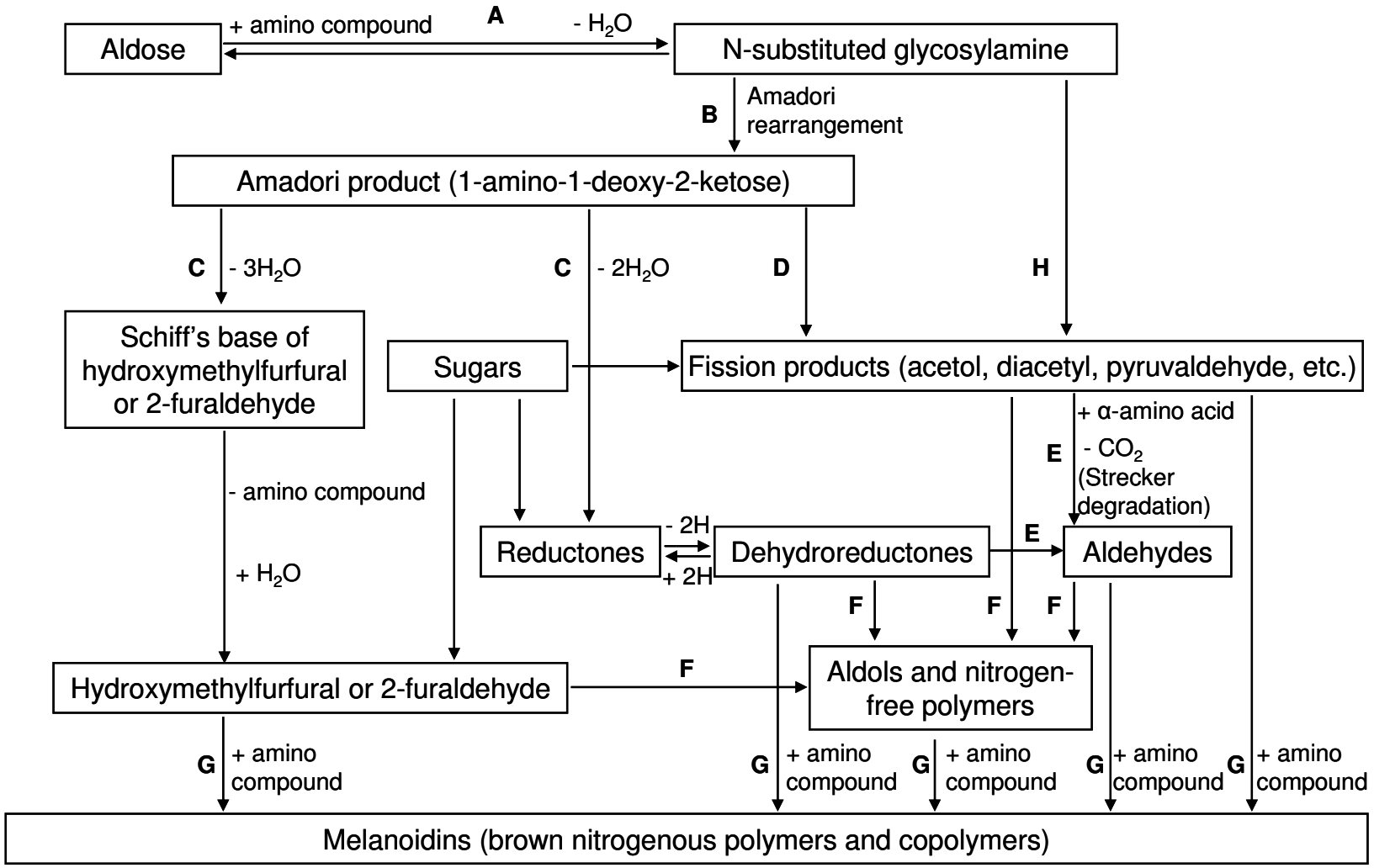
Advanced stage — Fluorescence compounds



- Carboxymethyllysine CML
- Pyralline
- Pentosidine

Final stage — Melanoidins

Hodge Diagram Summarising the MR

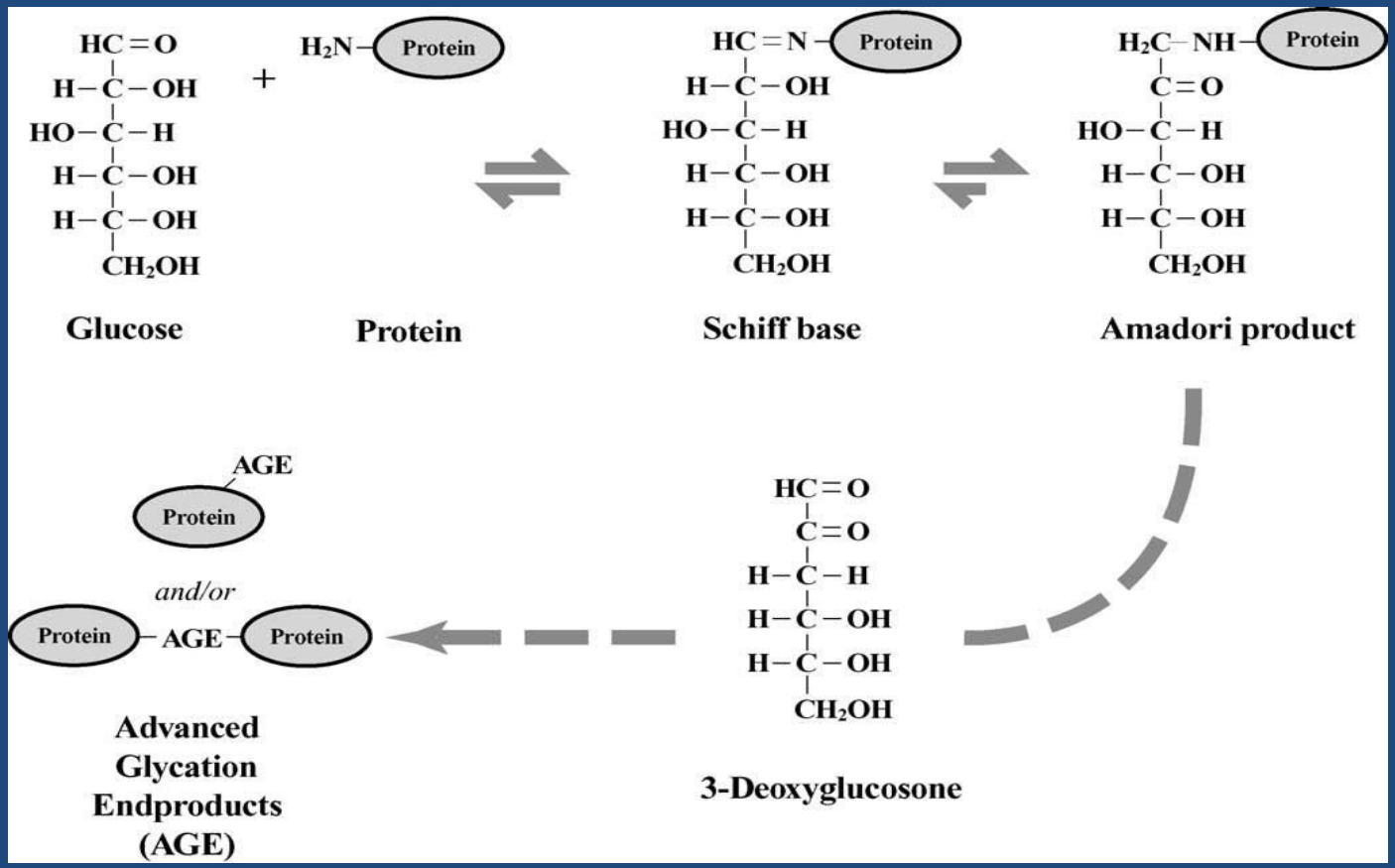


FOOD

Advanced glycation endproducts (AGEs) are produced in the advanced stage of nonenzymatic reaction between reducing sugars and amino groups, when the group of intermediate compounds produced during Amadori rearrangements react with amino groups either oxidatively or non-oxidatevely. AGEs formation is a comprehensive result of amine blockage by the oxidative degradation products of sugar or lipid.

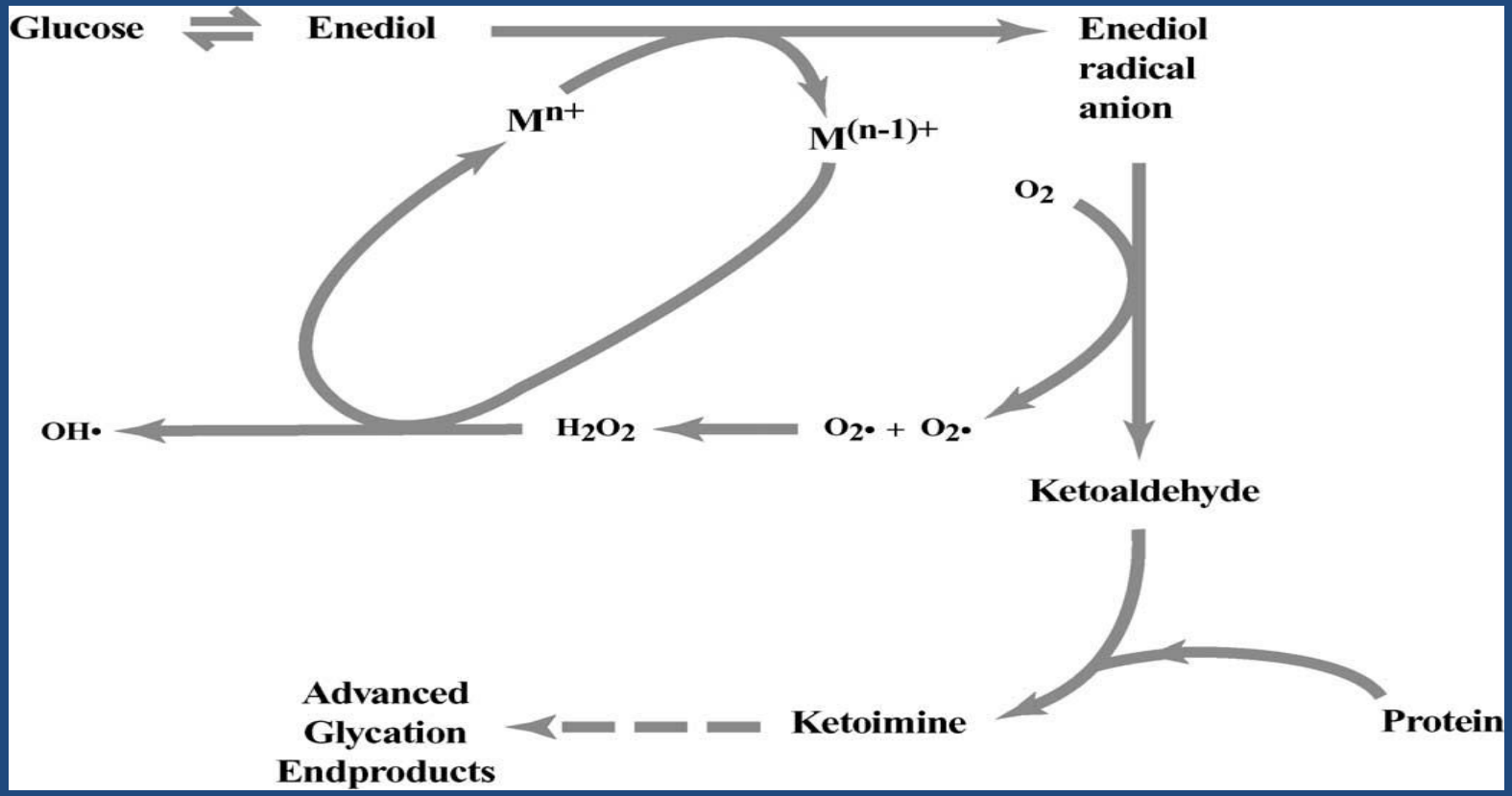
HUMAN ORGANISM

In addition to dietary sources where they are generated during food processing and storage, AGEs would also form and accumulate *in vivo* via sugar-protein interactions and cause pathogenic concequences such as diabetic complications.



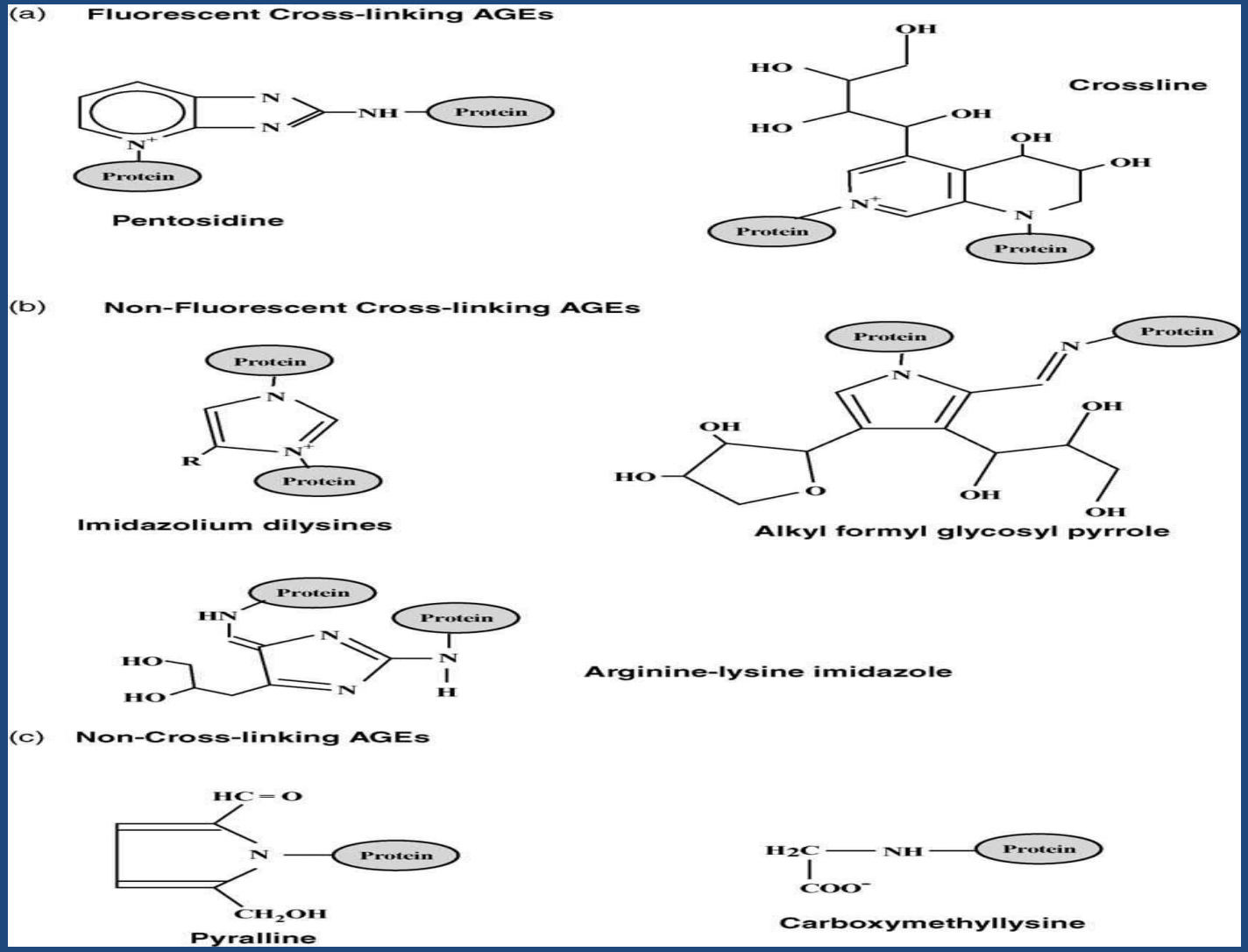
The initial reaction between glucose and protein amino groups forms a reversible Schiff base that rearranges to a ketoamine or Amadori product. With time, these Amadori products form AGEs via dicarbonyl intermediates such as 3-DG.

N. Ahmed /Diabetes Research and Clinical Practice 67 (2005) 3–21



Autoxidative glycation where glucose is converted to a dicarbonyl ketoaldehye via its enediol radical. This ketoaldehyde can react with a protein amino group to form a ketoimine capable of forming AGEs. These steps are catalysed by transition metals (M) and the superoxide radical generated can be converted to the hydroxyl radical via the Fenton reaction.

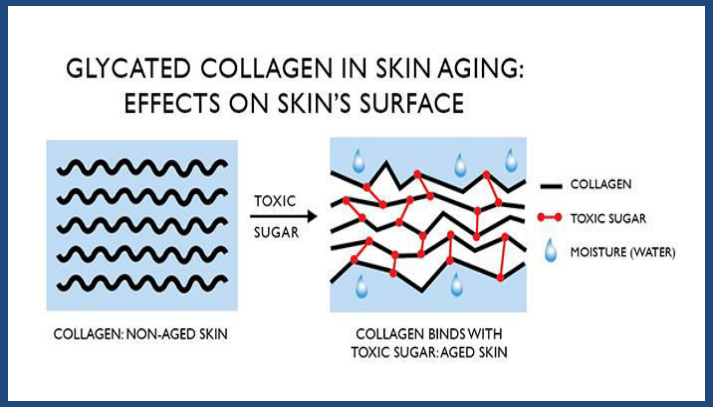
N. Ahmed /Diabetes Research and Clinical Practice 67 (2005) 3–21



Chemical structure of (a) fluorescent cross-linking AGEs such as pentosidine and crossline, (b) non-fluorescent cross-linking AGEs such as imadazolium dilysine cross-links, alkyl formyl glycosyl pyrroles and arginine–lysine imidazole cross-links, (c) non-cross-linking AGEs such as pyrraline and N-carboxymethyllysine.

N. Ahmed /Diabetes Research and Clinical Practice 67 (2005) 3–21

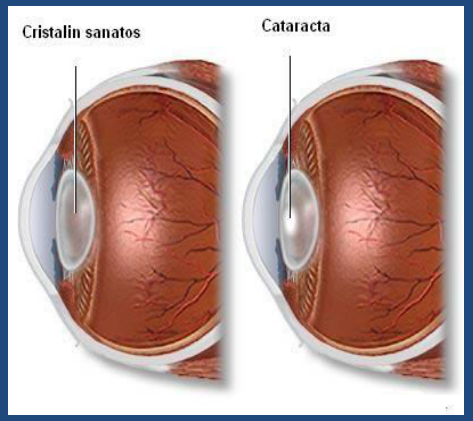
Medical consequences of protein glycation



Aging due to the colagen glycation



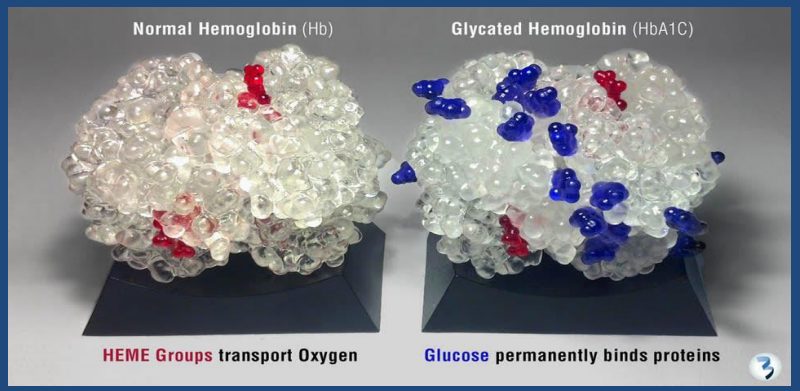
Skin aging



Cataract



Diabetic complications



Glycated hemoglobin

Enhanced formation and accumulation of AGEs have been implicated as a major pathogenesis process leading to diabetic complications, normal aging, atherosclerosis, and Alzheimer's Disease.

Studies for discovering and characteriying effective AGEs-inhibitors are valuable in exploring therapeutic approaches for treating AGEs associated diseases.

AGEs inhibitory mechanisms

- Blocking sugar attachment
- Scavenging reactive radicals and carbonyls from lipid or sugar oxidation
- Breaking sugar-protein cross-links

AGEs-inhibitors

Synthetic

Aminoguanidine

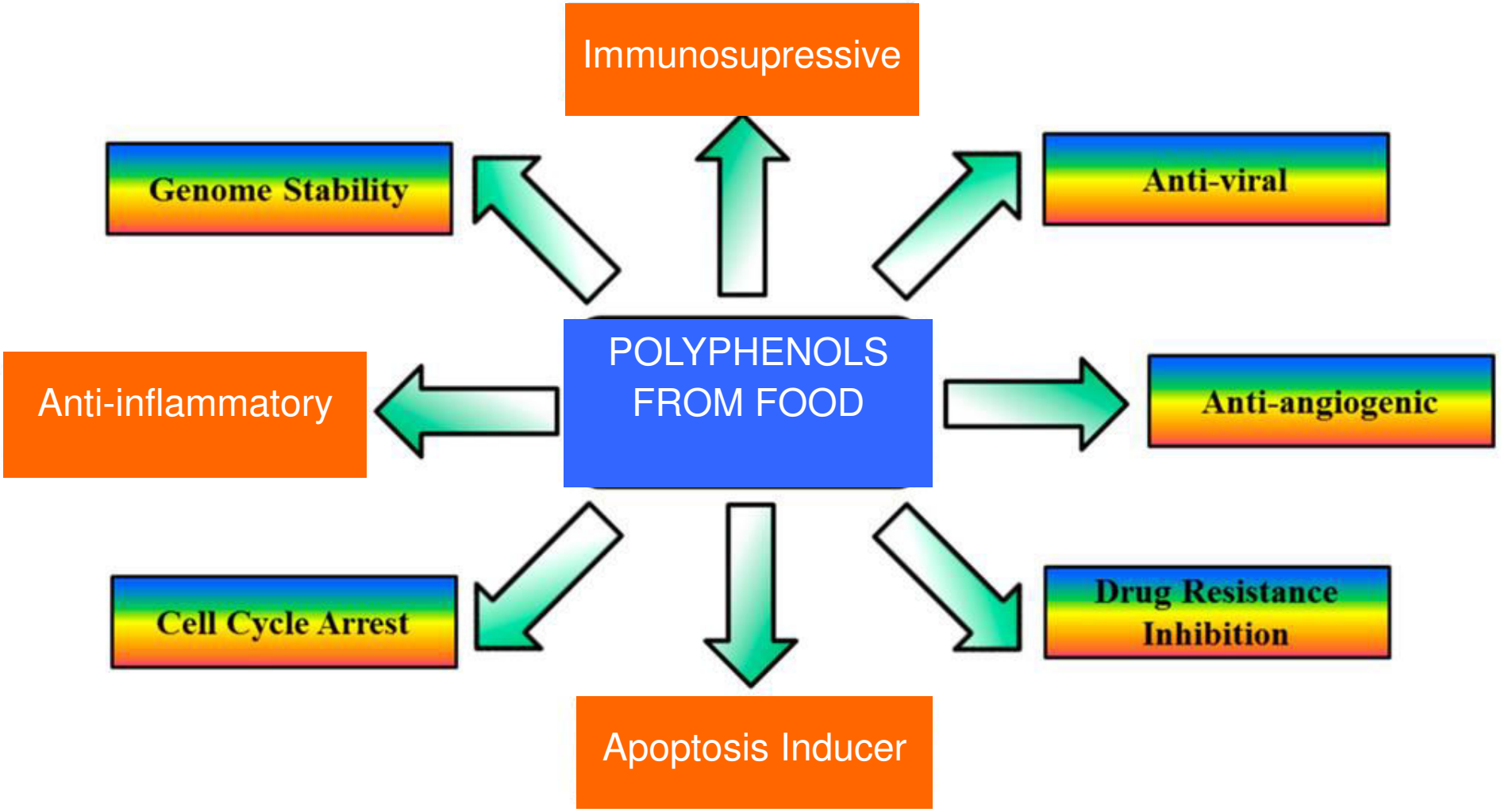


Metformin
Carnosine
Tenilsetam
Aspirin
Vitamin B1 and B6 derivatives

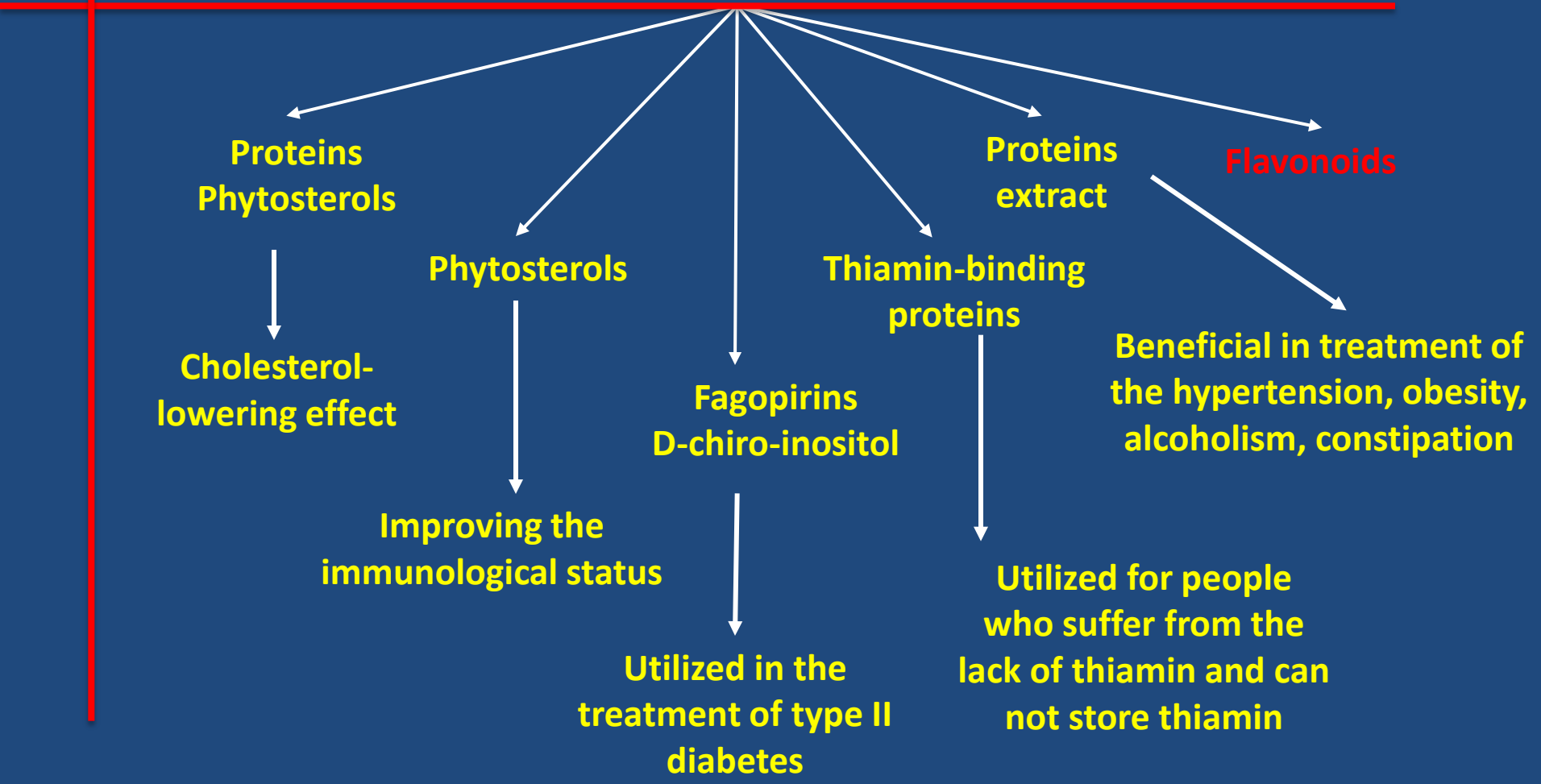
Natural

Phenolic acid and flavonoids:
Caffeic acid
Chlorogenic acid
Epigallocatechin
Flavone *C*-glucosides
Quercitin
Rutin

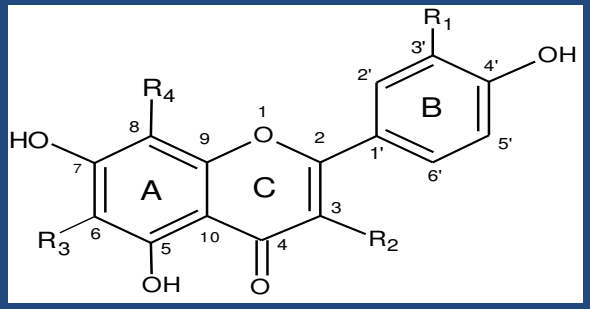
Food rich in polyphenols:
Green tea
Tea infused with selected herbs
Tomato paste
Spices


Immunosupressive
Genome Stability
Anti-viral
POLYPHENOLS FROM FOOD
Anti-inflammatory
Anti-angiogenic
Cell Cycle Arrest
Drug Resistance Inhibition
Apoptosis Inducer

Biologically active compounds of buckwheat with beneficial action on consumer's organism



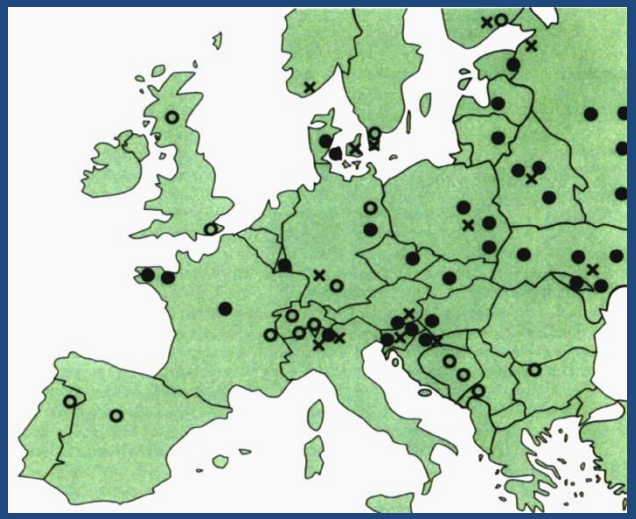
Buckwheat phytochemicals attracting attention due to their potential health beneficial action



Buckwheat flavonoids	R_1	R_2	R_3	R_4
rutin	OH	rutinose	H	H
quercetin	OH	OH	H	H
quercitrin	OH	ramnose	H	H
orientin	OH	H	H	glucose
homoorientin	OH	H	glucose	H
vitexin	H	H	H	glucose
isovitexin	H	H	glucose	H

BUCKWHEAT INDUSTRY IN POLAND





Raw buckwheat

Steaming
Roasting

Dehulling

Hulls – waste byproduct

Roasted groat

Milling

Novel buckwheat flour from roasted groats



Typical milling products from unhusked and husked buckwheat

Wholegrain buckwheat flour

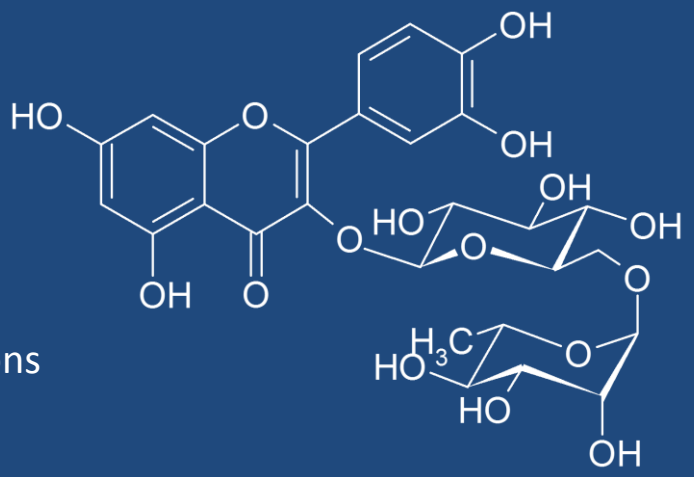


Flour from husked buckwheat



Potential health beneficial action of buckwheat flavonoids

Rutin (quercetin-3-rutinoside)

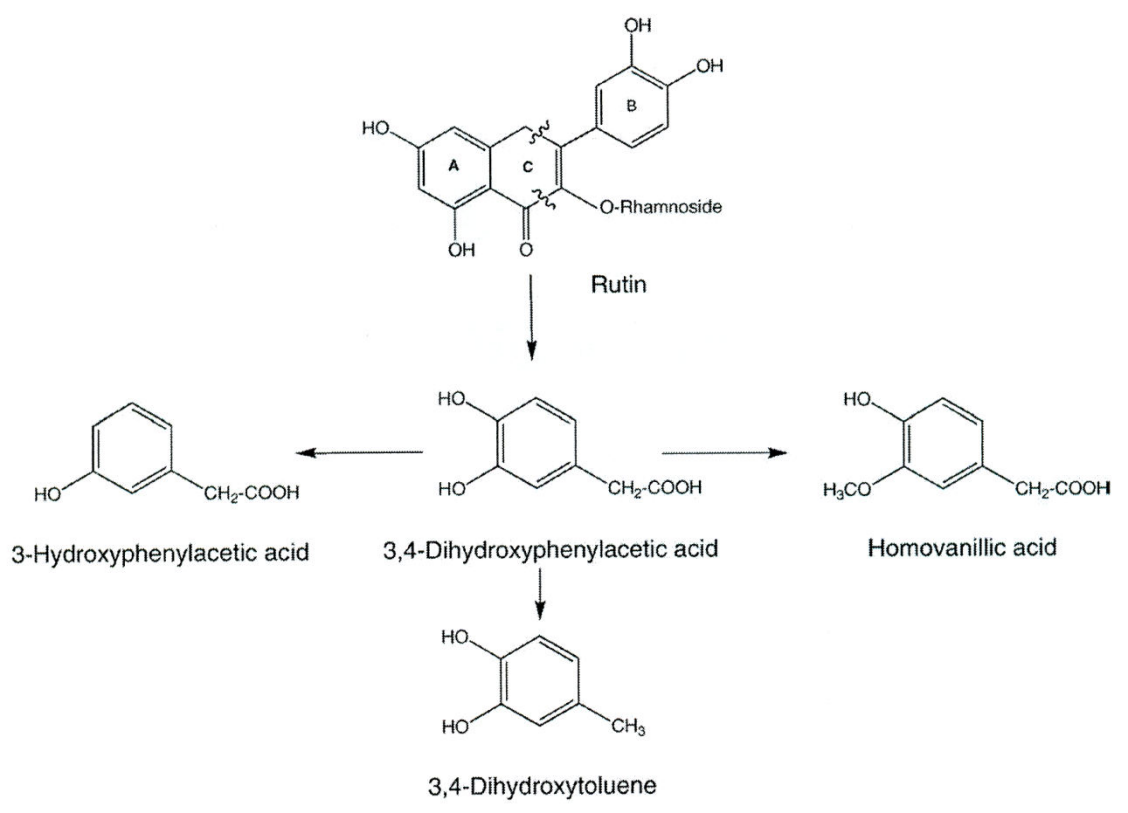


- anti-inflammatory and vasoactive properties
- capability to diminish capillary permeability
- reduce the risk of arteriosclerosis
- reducing coronary heart disease,
- diminishing of platelet aggregation
- inhibiting low-density lipoprotein (LDL) peroxidation
- protective effects against ethanol-induced gastric lesions
- against DNA damage
- protective agent against carcinogenesis
- **the most potent natural inhibitors of AGEs formation**
- hypocholesterolemic effect in humans after the intake of buckwheat products.

Orientin
Homoorientin
Isovitexin
Vitexin

- hypotensive properties
- anti-inflammatory
- antispasmodic
- antimicrobial
- radioprotective effects
- anti-glycation activity

Degradation of rutin in the colon



Pashikanti S., de Alba D.R., Boissonneault G.A., Cervanted-Laurean. Rutin metabolites: Novel inhibitors of nonoxidative advanced glycation end products. Free Radical Biology & Medicine 48 (2010) 656-663

THE AIM OF THE STUDY

To find out the potential non-pharmacologic prevention of buckwheat enhanced bakery products against formation of advanced glycation end products (AGEs) due to the presence of quercetin-3-*O*-rutinoside (rutin) – the main buckwheat flavonoid and other polyphenol sources.

MATERIAL

PART I. BUCKWHEAT ENHANCED WHEAT BREADS

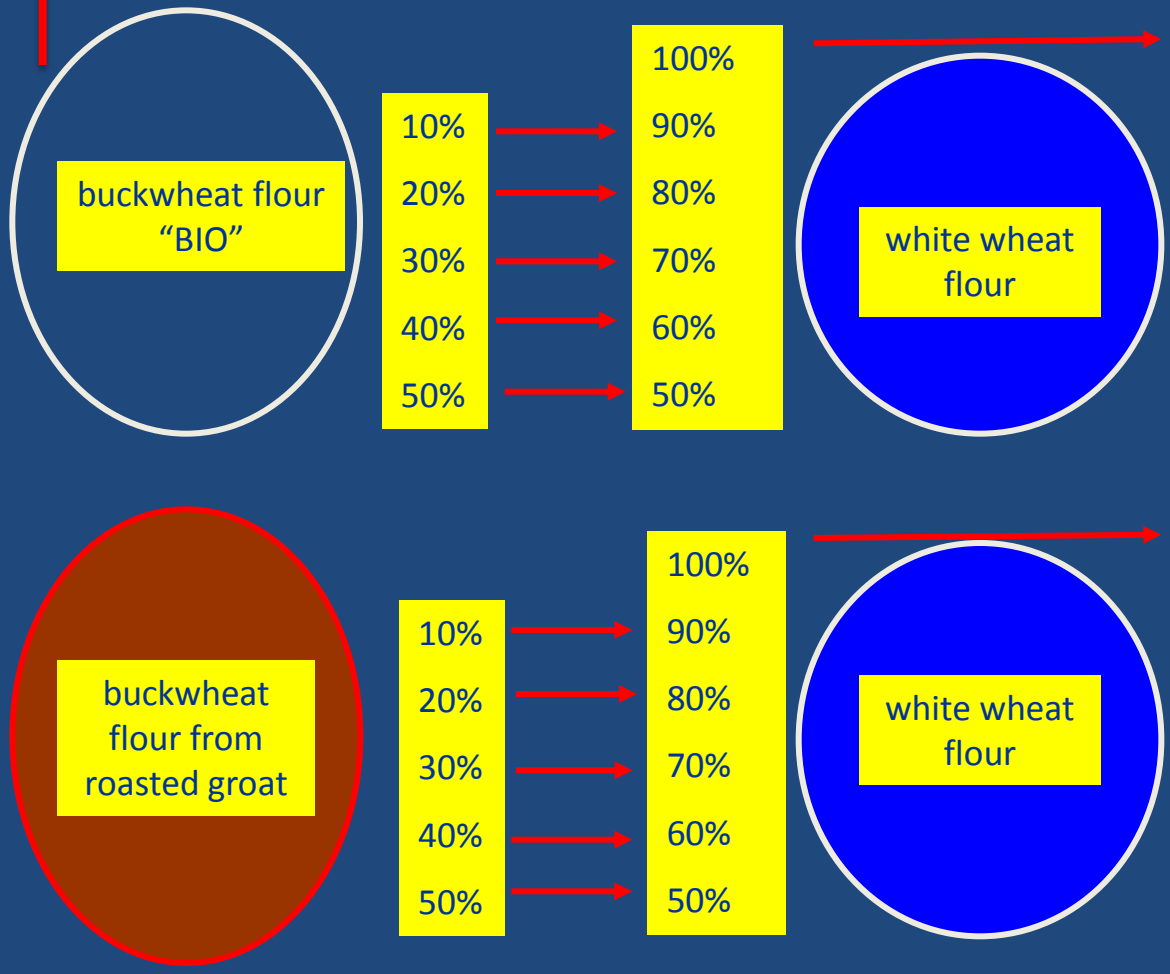
PART II. RYE-BUCKWHEAT GINGER CAKES ENRICHED WITH RUTIN

PART III. INNOVATIVE BREADS ENHANCED WITH NATURAL INGREDIENTS CONTAINING POLYPHENOLS

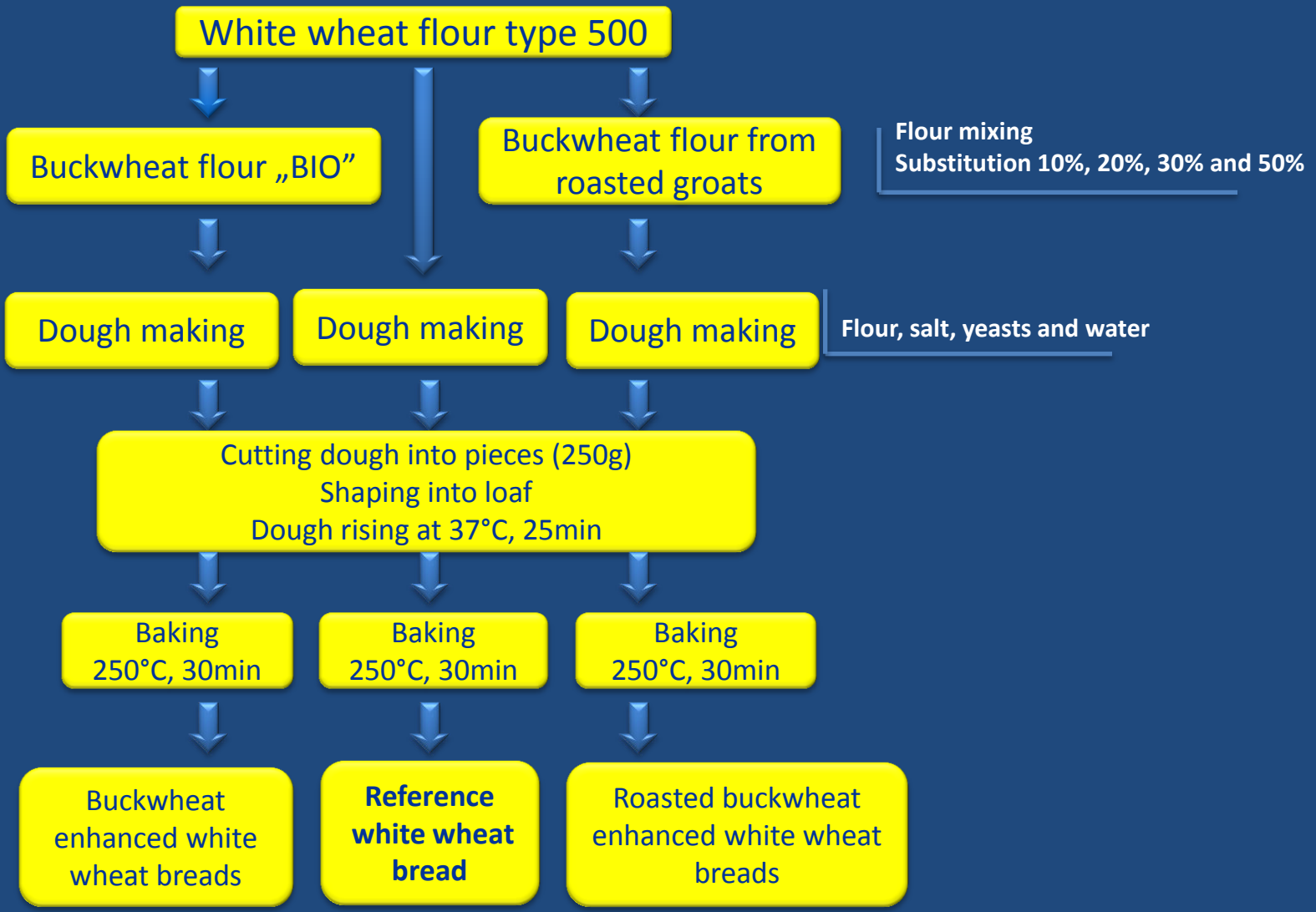
PART I. BUCKWHEAT ENHANCED WHEAT BREADS

- White wheat flour type 500,
- buckwheat flour “BIO” from whole grain of common buckwheat (variety Kora),
- novel flour from roasted buckwheat groats

Buckwheat enhanced wheat breads



Buckwheat enhanced white wheat breads formulation and baking conditions



PART II. RYE-BUCKWHEAT GINGER CAKES ENRICHED WITH RUTIN

flour from husked buckwheat

flour from roasted buckwheat groats



Rye-buckwheat ginger cakes with LOW addition of rutin
(2.5 mg of rutin in 50 g of product)



Rye-buckwheat ginger cakes with MEDIUM addition of rutin
(12.5 mg rutin in 50 g of product)



Rye-buckwheat ginger cakes with HIGH addition of rutin
(25 mg rutin in 50 g of product)

FORMULATION OF RYE-BUCKWHEAT GINGER CAKES WITH RUTIN

buckwheat honey 50 g
sugar 20 g
baking soda 3 g
butter 25 g
spice mix 2g

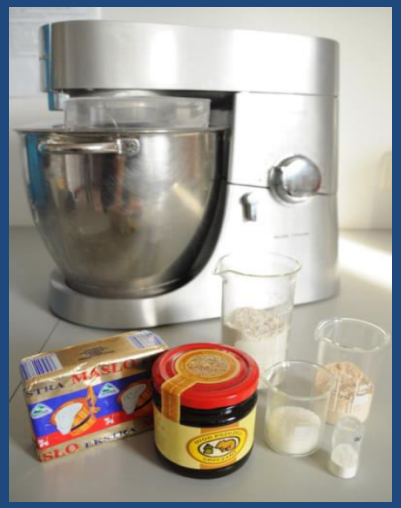
+ rye flour 100 g

CONTROL

buckwheat honey 50 g
sugar 20 g
baking soda 3 g
butter 25 g
spice mix 2g

+ rye flour 70 g + flour from roasted buckwheat groats 30 g + synthetic rutin 10 mg / 50 mg / 100 mg

rye flour 70 g + flour from husked buckwheat 30 g + synthetic rutin 10 mg / 50 mg / 100 mg



Dought making

Baking
180 °C 18 minutes



THE IDEA



=



=



weight: 50 g

6 pieces

1 tablet contains 25 mg of rutin

50 g of ginger cakes with high amount of rutin corresponds to one tablet of rutin

PART III. INNOVATIVE BREADS ENHANCED WITH NATURAL INGREDIENTS CONTAINING POLYPHENOLS

IN-1: spelt bread with cherry (P.410947, 2015, PL),

IN-2: mixed wheat/rye bread enhanced with roasted buckwheat flour (P.411732, 2015, PL)

IN-3: mixed wheat/rye bread enriched with dry onion skins (P.411555, 2015, PL)

IN-4: sourdough fermented rye bread enriched with roasted buckwheat hull (P.411731, 2015, PL)

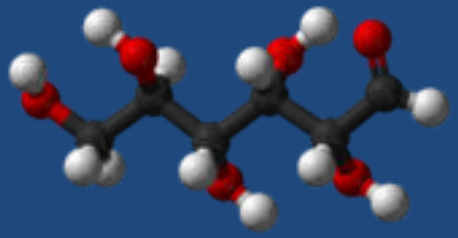
IN-5: roll type graham with raw buchwheat hull (P.410730, 2014, PL)

METHODS

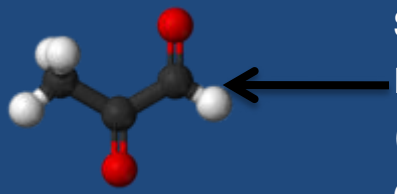
HOW DID WE PERFORM THE *IN VITRO* STUDY?

MODEL SYSTEMS:

BSA + glucose



BSA + methylglyoxal



side-product of several metabolic pathways (intermediate ot threonine catabolism, lipid peroxidation and glycolysis)

Methylglyoxal is proven to be the most important glycation agent (forming AGEs)

Glucose

Glycation

Protein-NH_2

Schiff base

Amadori products

Intermediary glycation products

Glycoxydation

Oxydative stress

Glyoxal

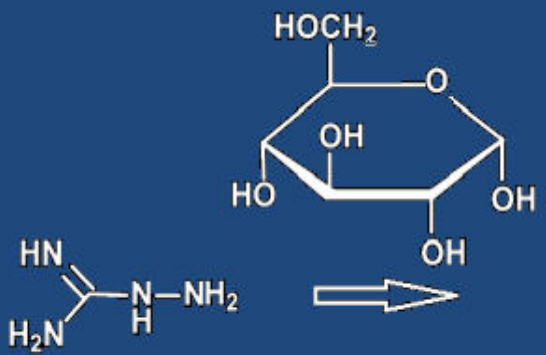
3-Deoxyglucosone

Protein-NH_2

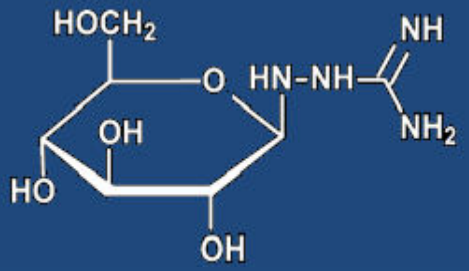
Methylglyoxal...

Protein-NH_2

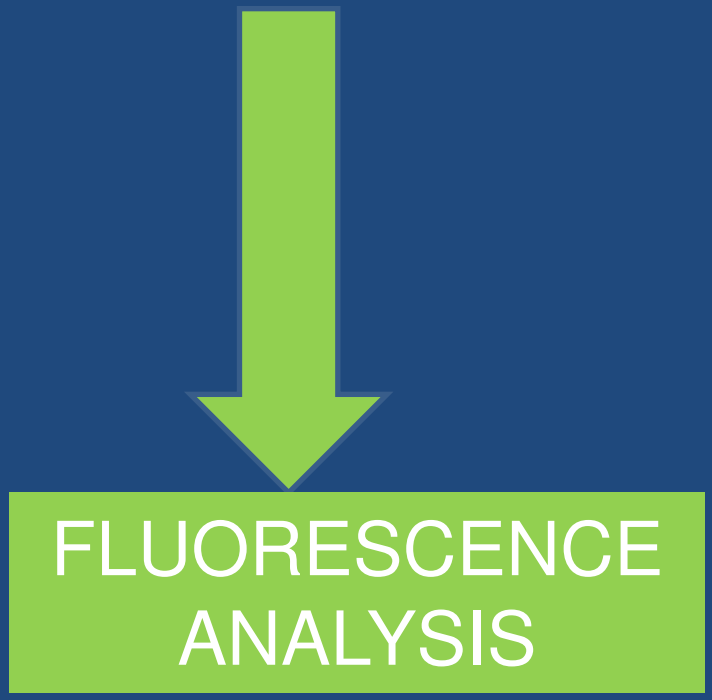
Advanced Glycation End-products



aminoguanidine



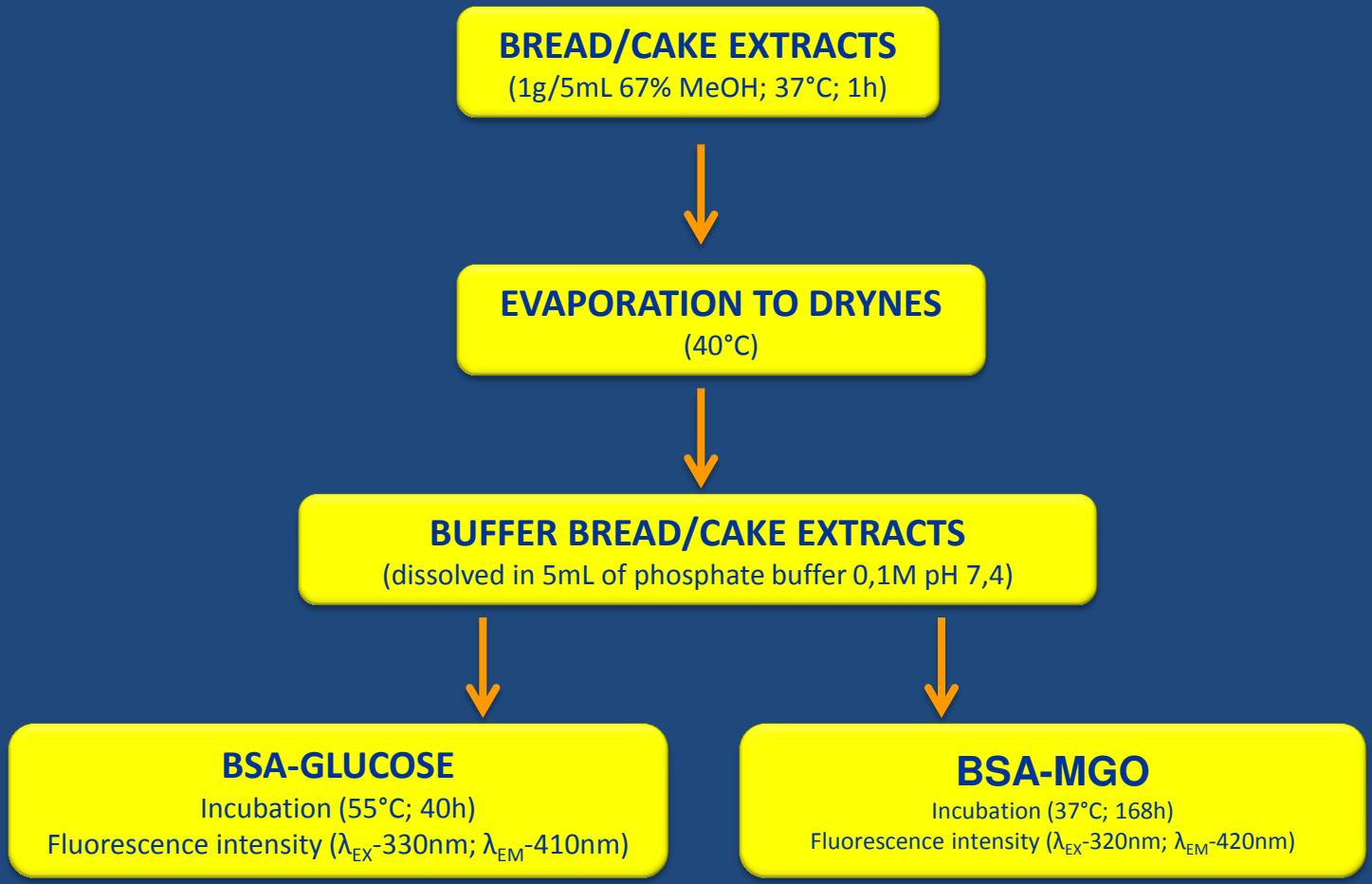
HOW DID WE DETERMINE AGES FORMATION/INHIBITION?



$$\% \text{ inhibition} = \left\{1 - \left(\frac{\text{fluorescence of the solution with inhibitors}}{\text{fluorescence of the solution without inhibitors}}\right)\right\} \times 100\%$$

aminoquanidine (AG) 1 mM was used as positive control

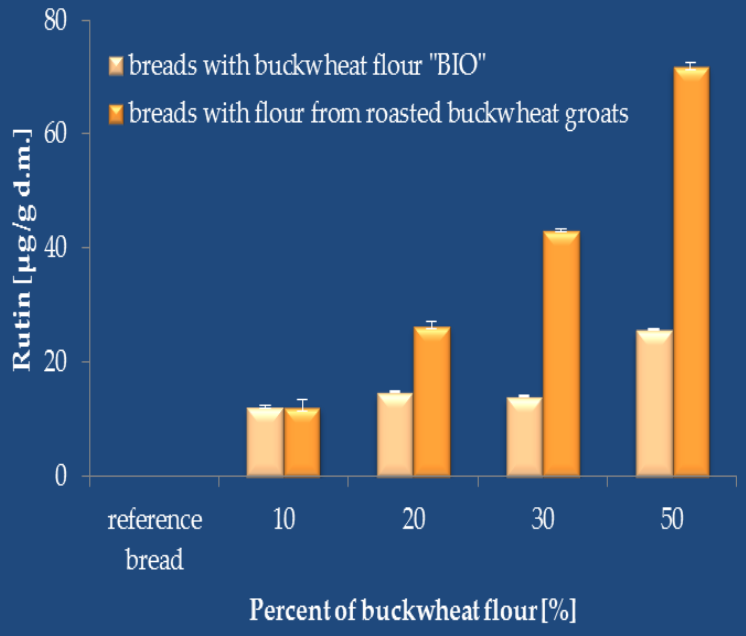
Measurement of the inhibitory effect of buckwheat enhanced bakery products on the formation of advanced glycation end products (AGEs)



RESULTS

PART I. BUCKWHEAT ENHANCED WHEAT BREADS

Rutin content

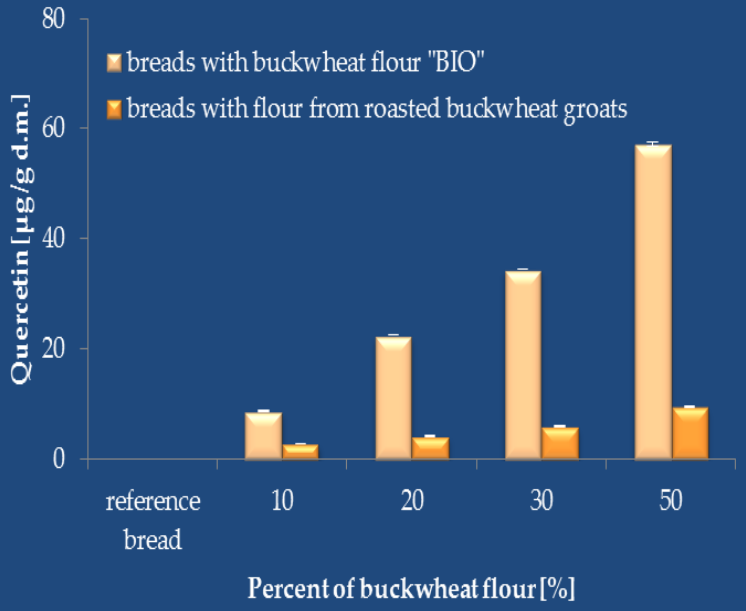


buckwheat flour "BIO"

white wheat flour

Ru and Q

Quercetin content



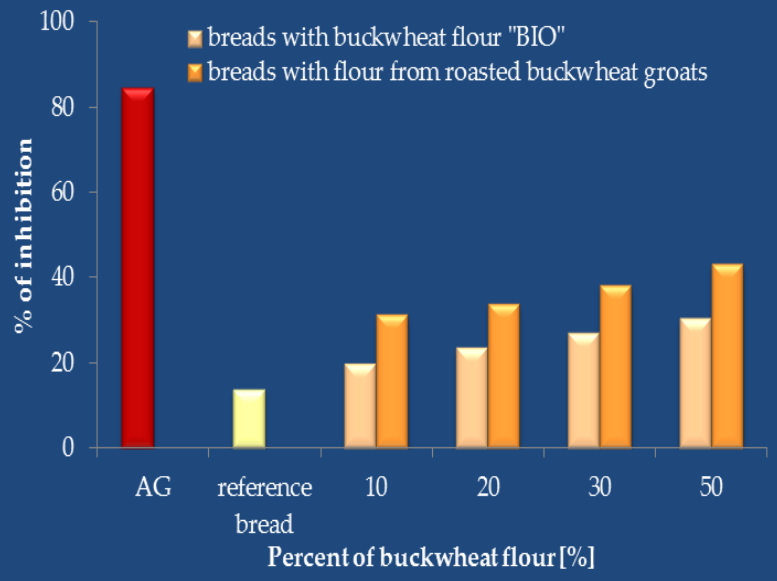
buckwheat flour from roasted groat

white wheat flour

Ru and Q

The inhibitory effect of buckwheat enhanced wheat bread extracts on the formation of AGEs

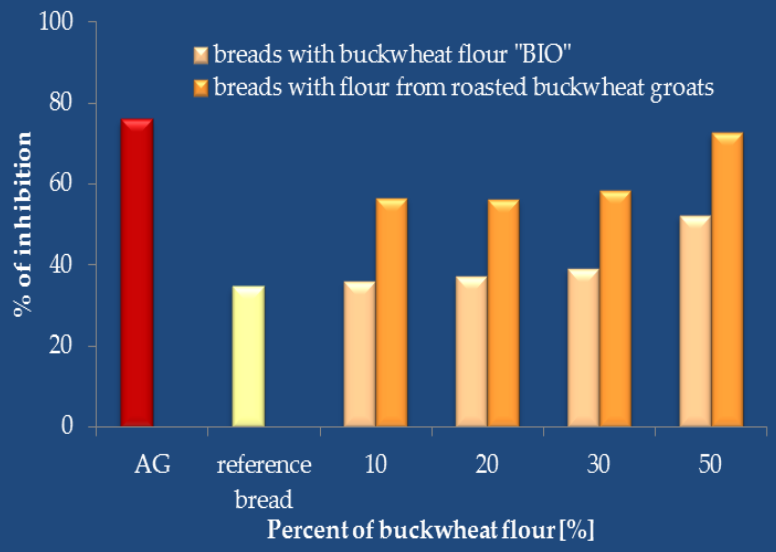
BSA-Glucose model system



Inhibition AGEs vs Ru r = 0.86 (BIO)

Inhibition AGEs vs Ru r = 0.89 (ROASTED)

BSA-MGO model system

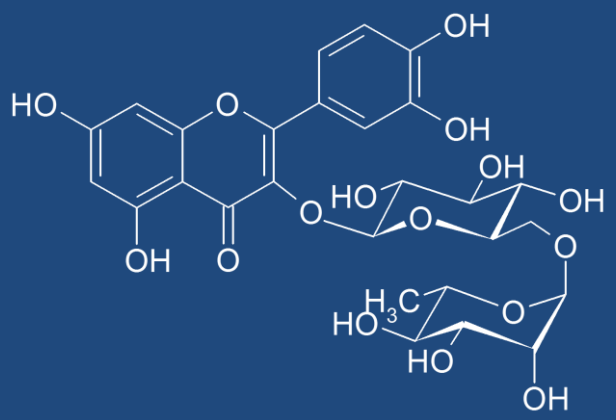


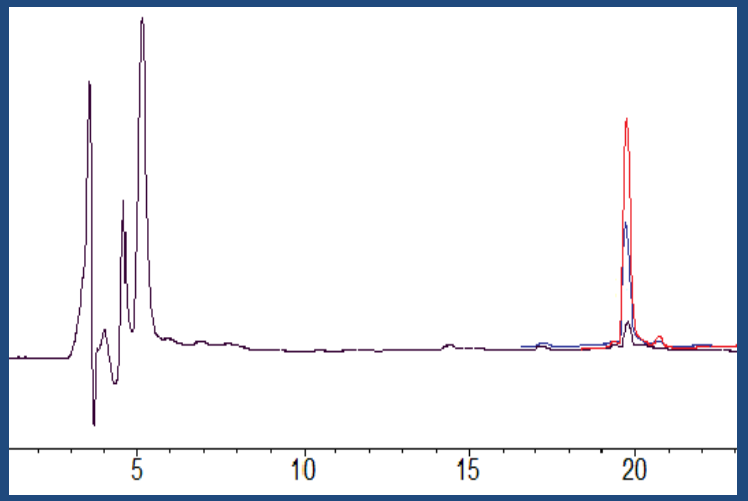
Inhibition AGEs vs Ru r = 0.94 (BIO)

Inhibition AGEs vs Ru r = 0.88 (ROASTED)

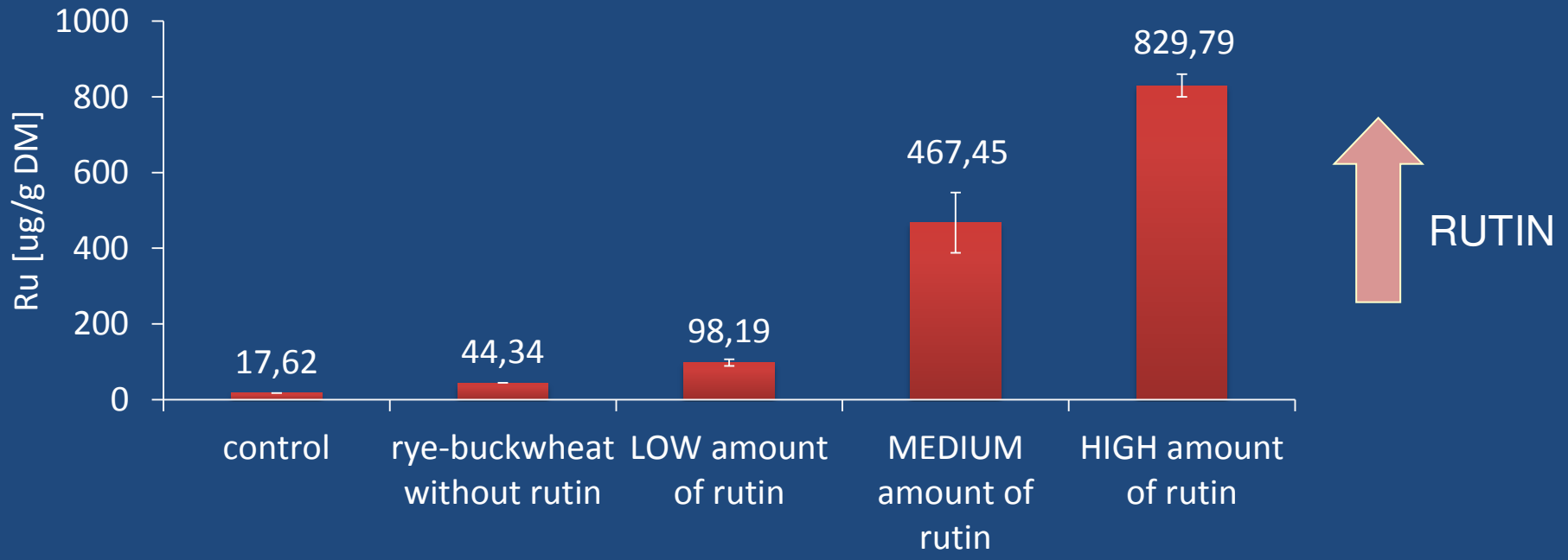
PART II. RYE-BUCKWHEAT GINGER CAKES ENRICHED WITH RUTIN

Determination of rutin content



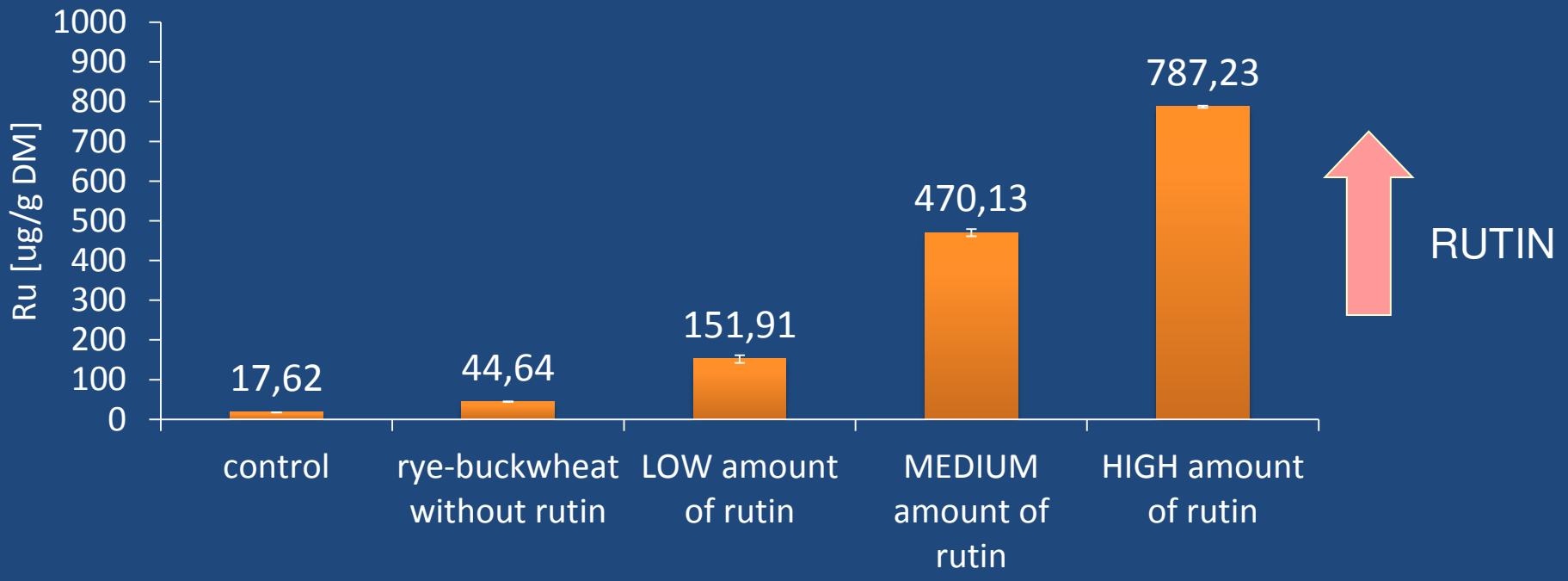


Ginger cakes based on flour from buckwheat flour



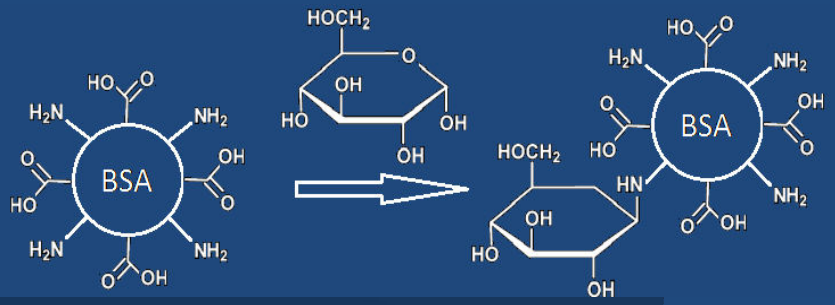
Determination of rutin content

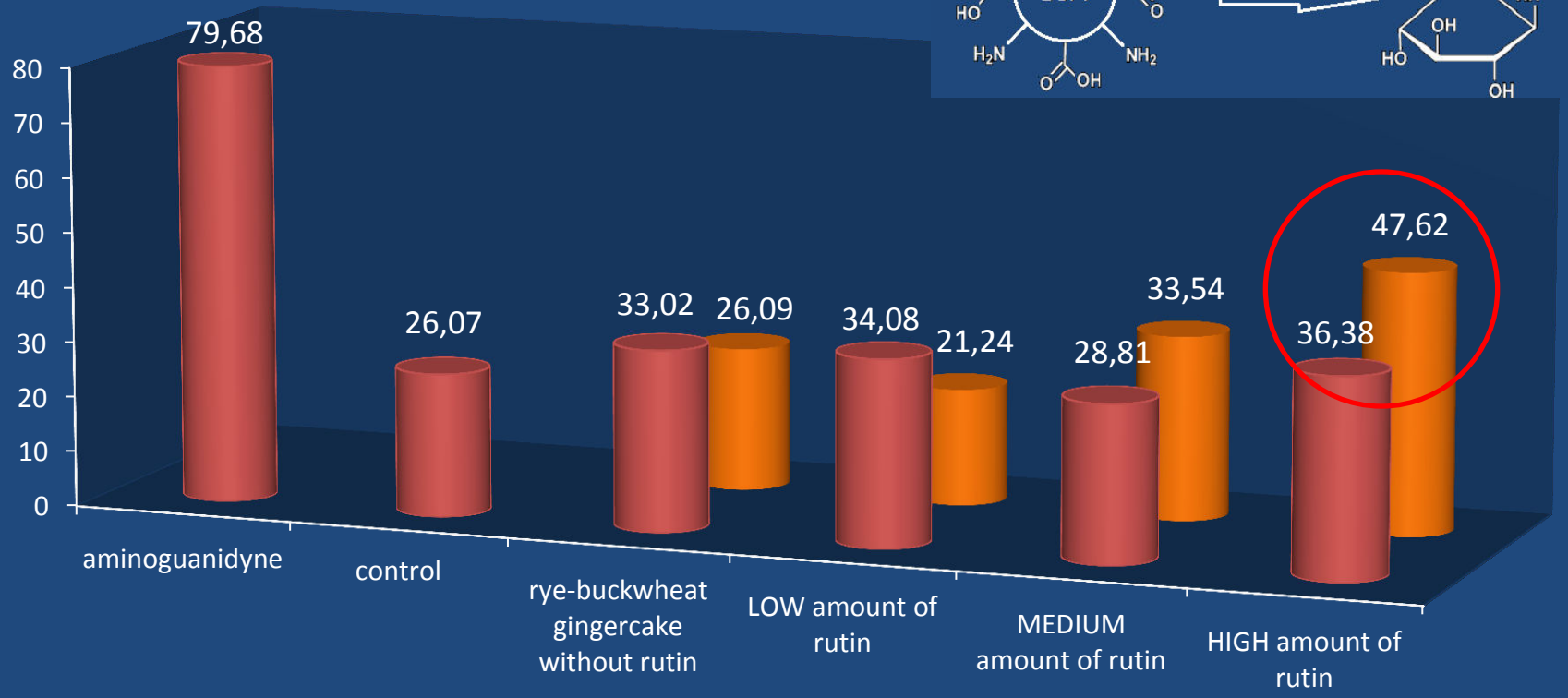
Ginger cakes formulated on flour from milled roasted buckwheat groats



The inhibitory effect of buckwheat ginger cake extracts on the formation of AGEs

Bovine serum albumin -glucose system (BSA/glucose)



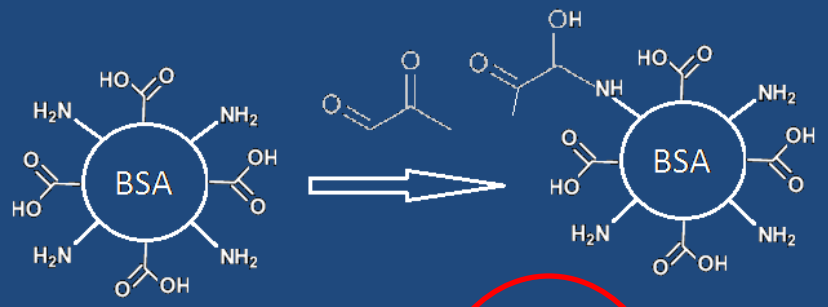


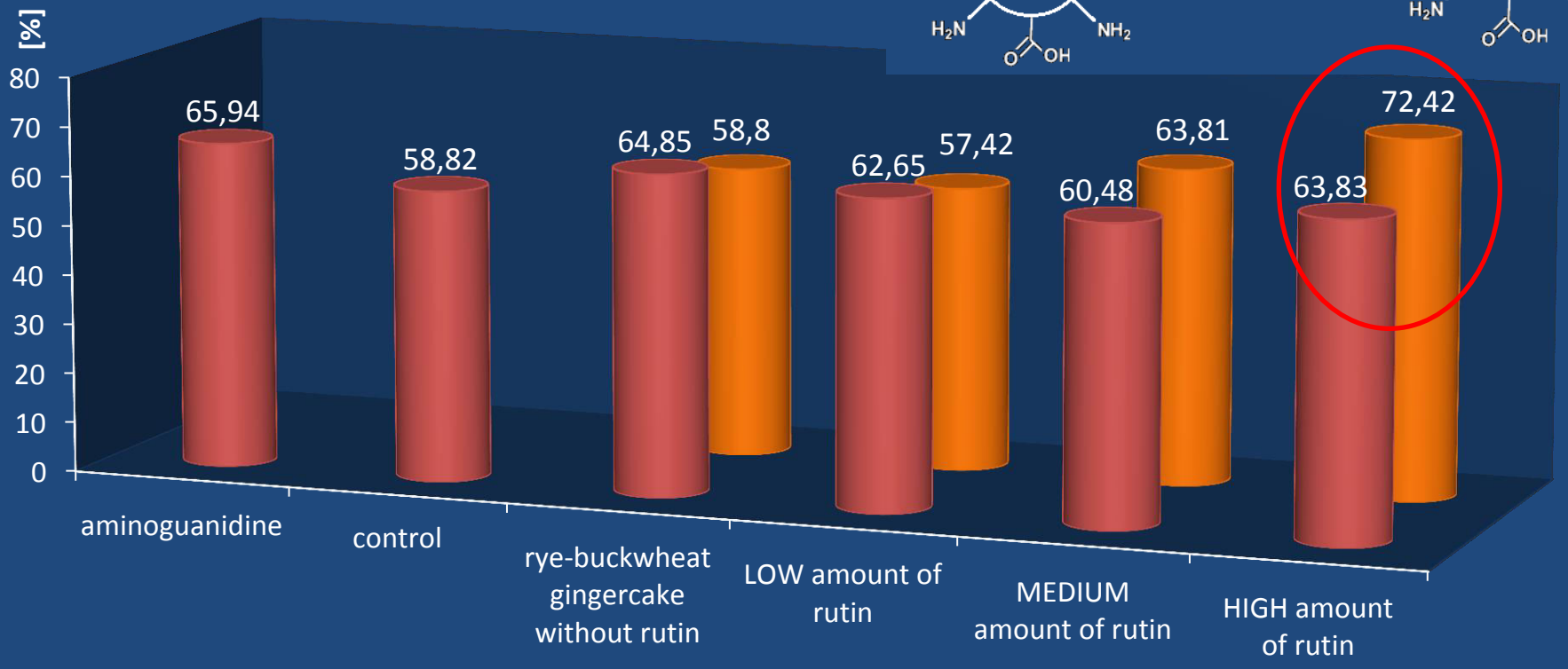
ginger cakes based on flour from husked buckwheat — Rutin vs BSA /Glu r = 0.47

ginger cakes formulated on flour from milled roasted buckwheat groats — Rutin vs BSA /Glu r = 0.93

The inhibitory effect of buckwheat ginger cake extracts on the formation of AGEs

Bovine serum albumin- methylglyoxal system (BSA/ MGO)





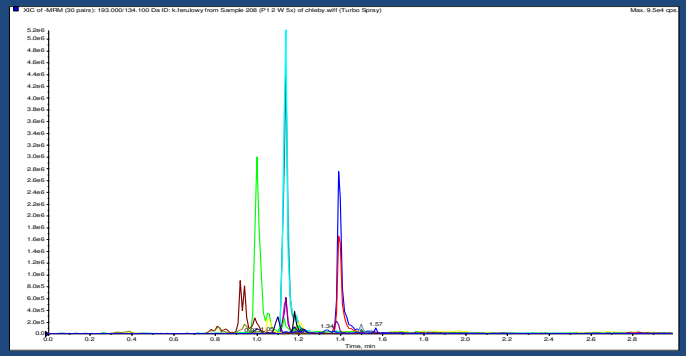
ginger cakes based on flour from husked buckwheat — Rutin vs BSA /MGO r= 0.22

ginger cakes formulated on flour from milled roasted buckwheat groats — Rutin vs BSA /MGO r= 0.96

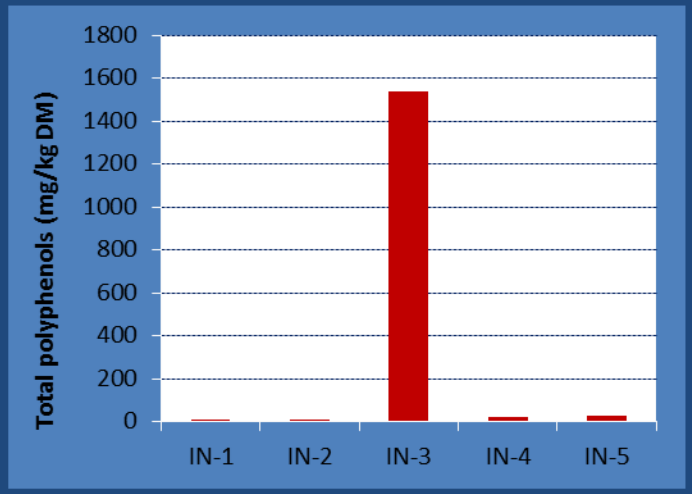
PART III. INNOVATIVE BREADS ENHANCED WITH NATURAL INGREDIENTS CONTAINING POLYPHENOLS

IN-1: spelt bread with cherry
IN-2: mixed wheat/rye bread enhanced with roasted buckwheat flour
IN-3: mixed wheat/rye bread enriched with dry onion skins
IN-4: sourdough fermented rye bread enriched with roasted buckwheat hull
IN-5: roll type graham with raw buchwheat hull

Total polyphenols

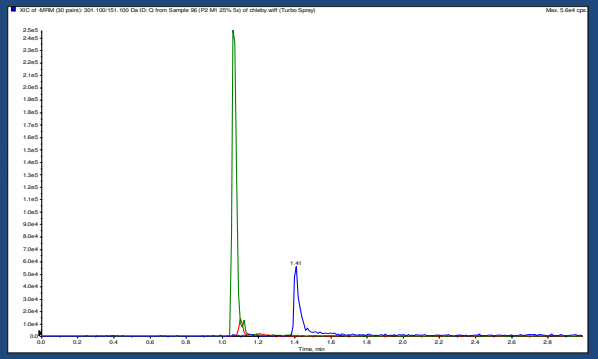


Profile of phenolics acids determined by HPLC-MS/MS



Content of phenolic acids and flavonoids

Quercetin content

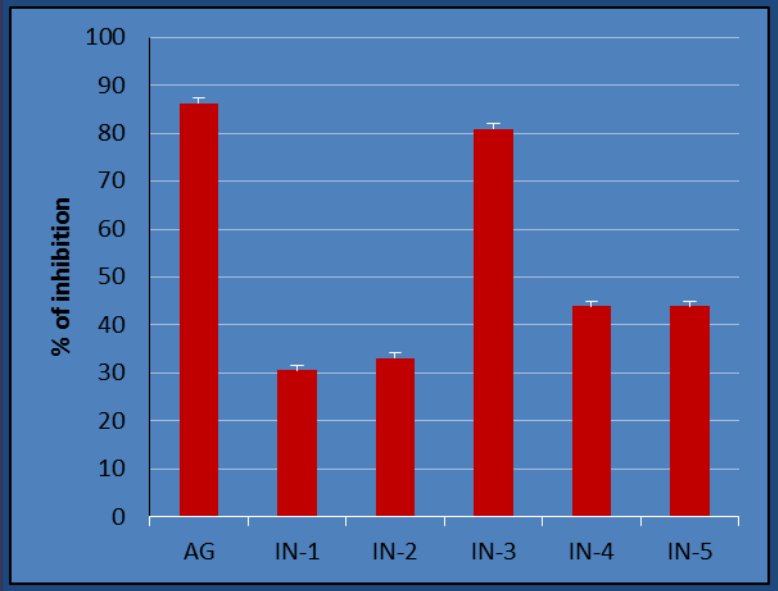


Profile of flavonoids determined by HPLC-MS/MS

sinapic acid
p-coumaric acid
m-coumaric acid
caffeic acid
Isoferrulic acid
quercetin
quercetin glycosides
rutin

The inhibitory effect of innovative breads enhanced with natiral ingriedients containing polyphenols on the formation of AGEs

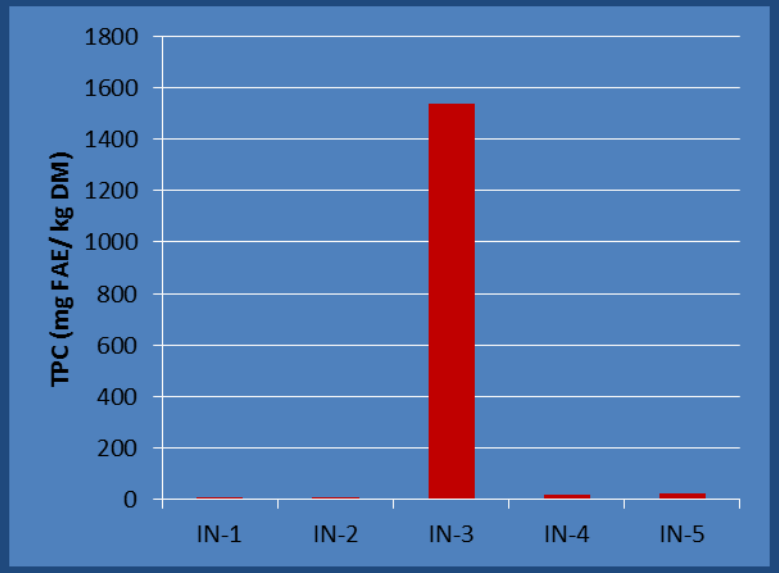
BSA-Glucose model system



(phenolic acids + flavonoids) vs BSA /Glu r= 0.96

Q vs BSA /Glu r= 0.95

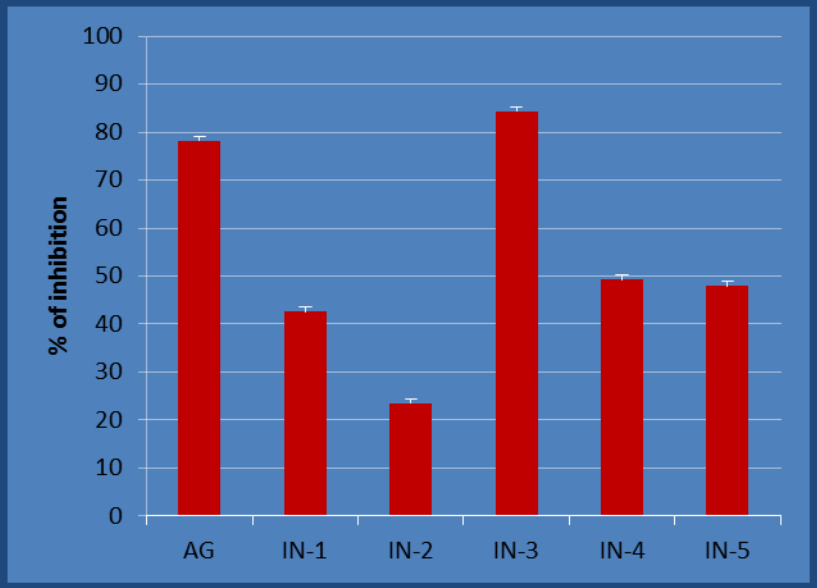
Total phenolic compounds by Follin reagent



TPC vs BSA /Glu r= 0.96

The inhibitory effect of innovative breads enhanced with natiral ingriedients containing polyphenols on the formation of AGEs

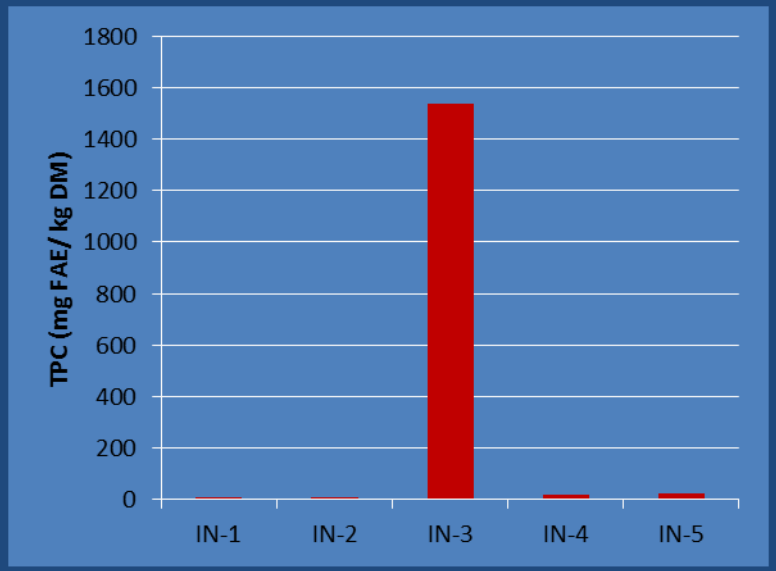
BSA-MGO model system



(phenolic acids + flavonoids) vs BSA /Glu r= 0,89

Q vs BSA /Glu r= 0,89

Total phenolic compounds by Follin reagent



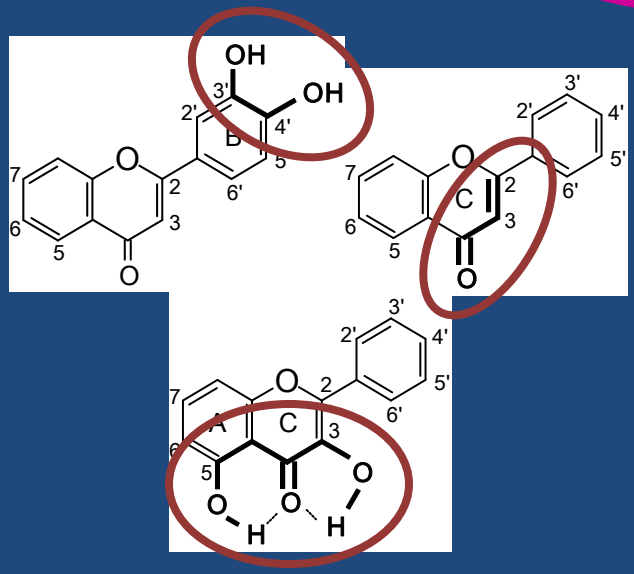
TPC vs BSA /Glu r= 0,89



quercetin - Q

3,3’,4’,5,7–pentahydroksyflavon

185 – 1917 mg Q/kg WM



SUMMARY

Extracts from buckwheat enhanced wheat breads, formulated on white wheat flour and flour from roasted buckwheat groats showed higher inhibitory effects against AGEs formation than those formulated on white wheat flour and buckwheat flour “BIO”.

Rye-buckwheat ginger cakes with high rutin addition showed the highest inhibitory activity against AGEs formation as compared to ginger cakes with low and medium rutin supplementation.

Mixed wheat/rye bread enriched with dry onion skins showed the highest inhibitory activity against AGEs formation amongs innovative breads.

CONCLUSIONS

This study showed possibility of formulation buckwheat derived bakery products with effective inhibition the formation of AGEs *in vitro*.

This further supports that buckwheat derived bakery products may be beneficial food choice for diabetics as AGEs have been implicated in the pathogenesis of various diabetic complications and other diseases.

The rich source of polyphenols such as buckwheat flours, buckwheat hull or dry onion skins should be considered as a new ingredients in innovative breads for diabetics.

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