Одеська національна академія харчових технологій

Odesa National Academy of Food Technologies

PLANT RAW MATERIAL BIOTRANSFORMATIONS ARE USEFUL METHODS FOR FOOD FUNCTIONAL INGREDIENTS PRODUCTION

БІОТРАНСФОРМАЦІЯ РОСЛИННОЇ СИРОВИНИ ЯК МЕТОД ОТРИМАННЯ ФІЗІОЛОГІЧНО-ФУНКЦІОНАЛЬНИХ ХАРЧОВИХ ІНГРЕДІЄНТІВ

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Production and exportation of cereals in UKRAINE in last years, *mln.t*

2016/17	66,1	43,8
2017/18	61,4	39,4
2018/19	70,1	44,9
2021/22	100	77

CHEMICAL COMPOSITION OF WHEAT POLYSACCHARIDS

Starch ……. ……………….63-65 %

Cellulose………………….. 3-4 %

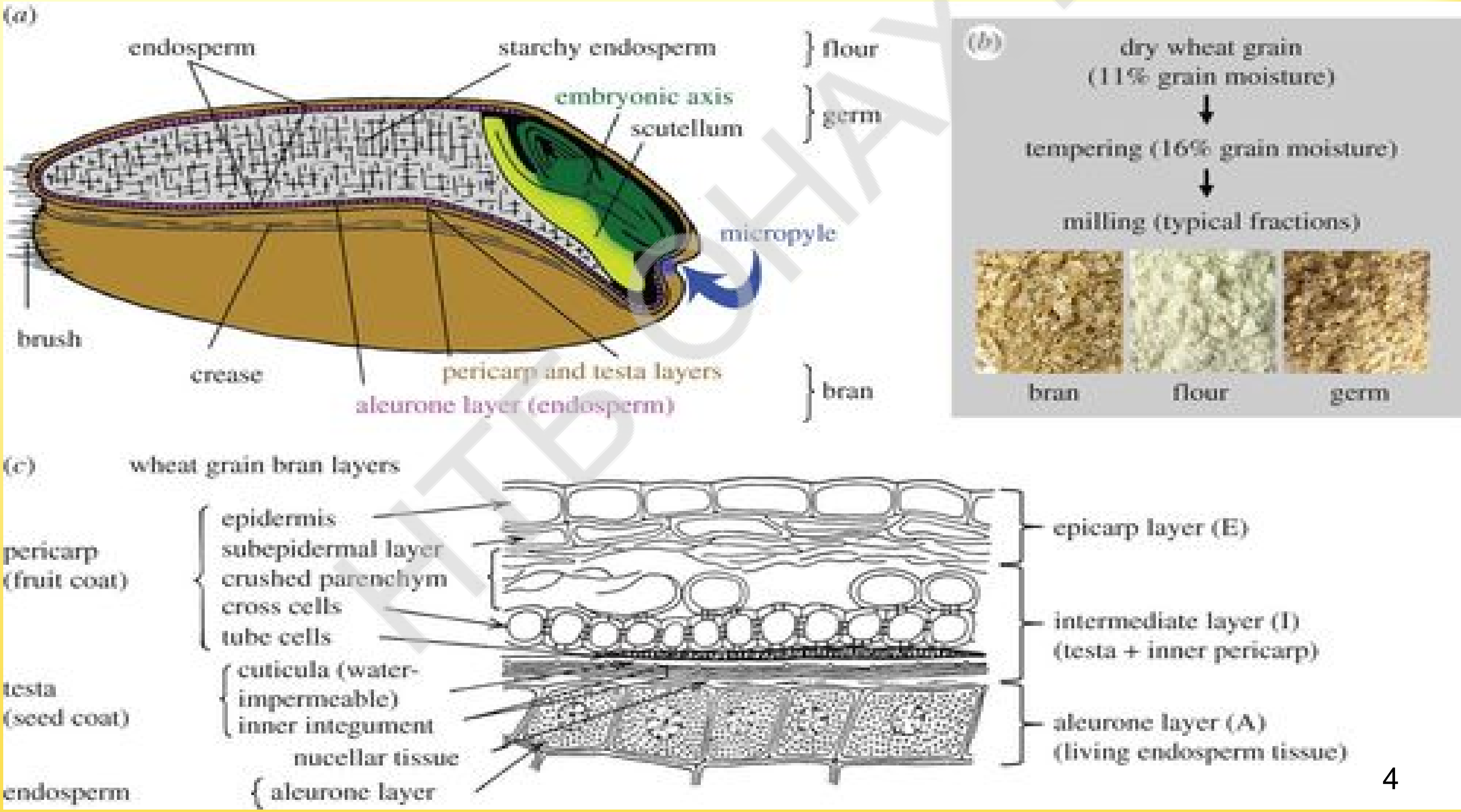
Hemicelluloses……………. 8-9 %

Pectic substances……….. 2-3 %

Beta-glucans……………. 1- 1,5%

Total polysaccharids…… 82%

Wheat kernel anatomic and biopolimers composition




20 µm
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Composition of various bran from cereals of Ukraine (% of dry matter)

Sample	Dietary fibers	Polysaccharides constituents of dietary fibers				Starch	Protein	Lipids	Ash
		Hemicelluloses	cellulose	pectin	lignin				
Wheat	49,8	26,3	8,8	5,3	9,4	26,4	16,4	3,3	4,1
Rye	57,7	25,1	14,3	8,0	10,3	16,0	17,9	3,1	5,3
Triticale	46,3	17,8	12,6	5,1	10,8	25,4	18,3	2,9	5,0
Oats	15,1	8,7	3,7	0,5	2,2	59,2	14,0	7,0	2,2
Barley	30,5	18,3	7,8	0,7	3,9	38,4	17,4	8,6	4,3
Corn	56,0	32,3	14,7	0,3	8,7	24,7	9,3	7,3	2,7

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MOTIVATION

Polysaccharides are used extensively in various industrial applications. For many of these applications, polysaccharides need structural modifications in order to improve their properties.

In this context ***enzymatic modification (biotransformation)*** of their structure is a way to improve their properties, produce bioactive oligosaccharides for biotechnological application. Mostly, this carbohydrate constate a nutritional sourse of “fiber” (prebiotic) that is beneficial for well-being and thus to enlarge the field of their potential applications.

DEFINITIONS

biotransformation (enzymatic modification)

is the process of modifying (chemical transformations) by the conversion of organic materials, such as plant or animal waste, into usable products or energy sources by biological processes or agents, such as certain microorganisms and enzymes.

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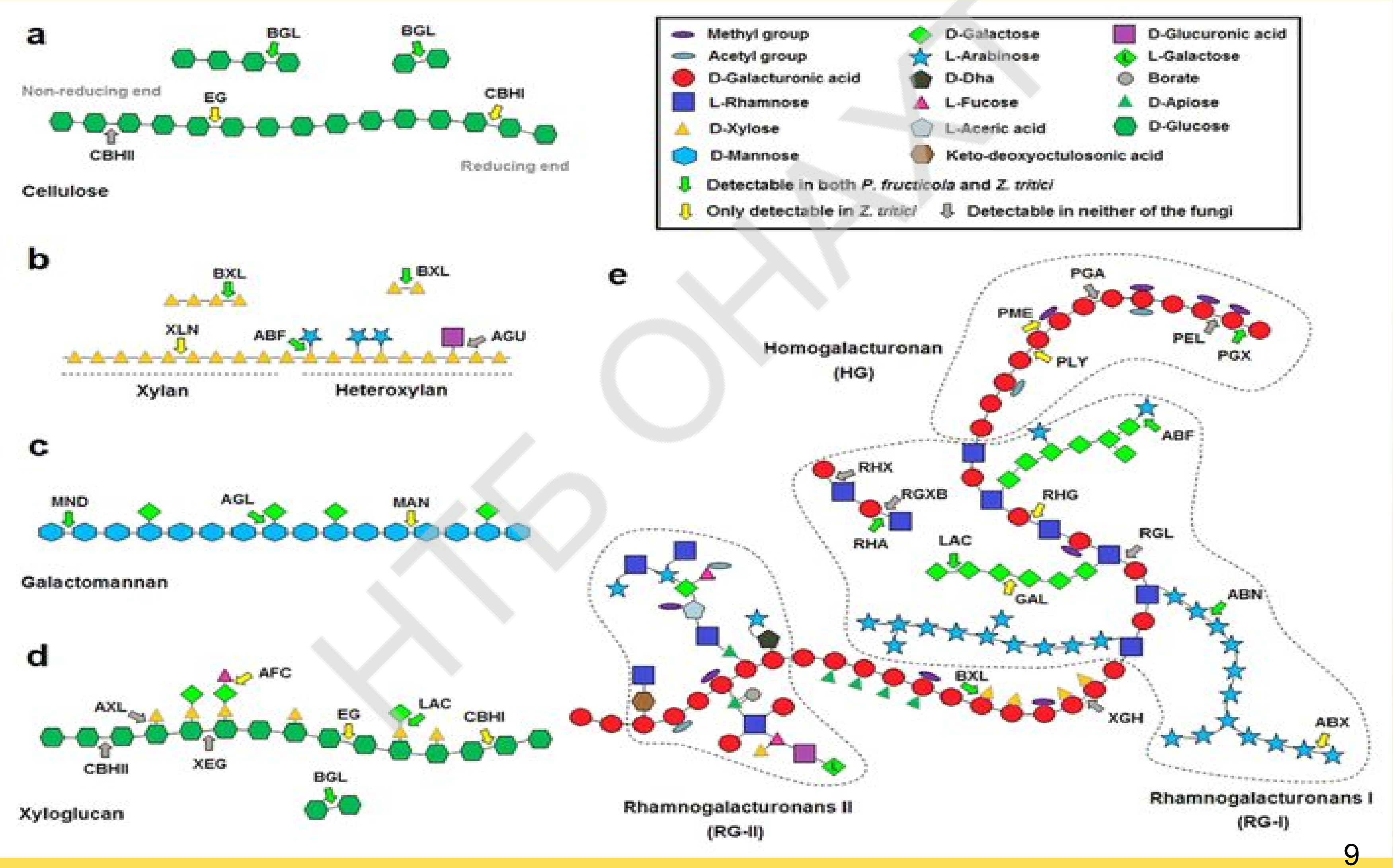
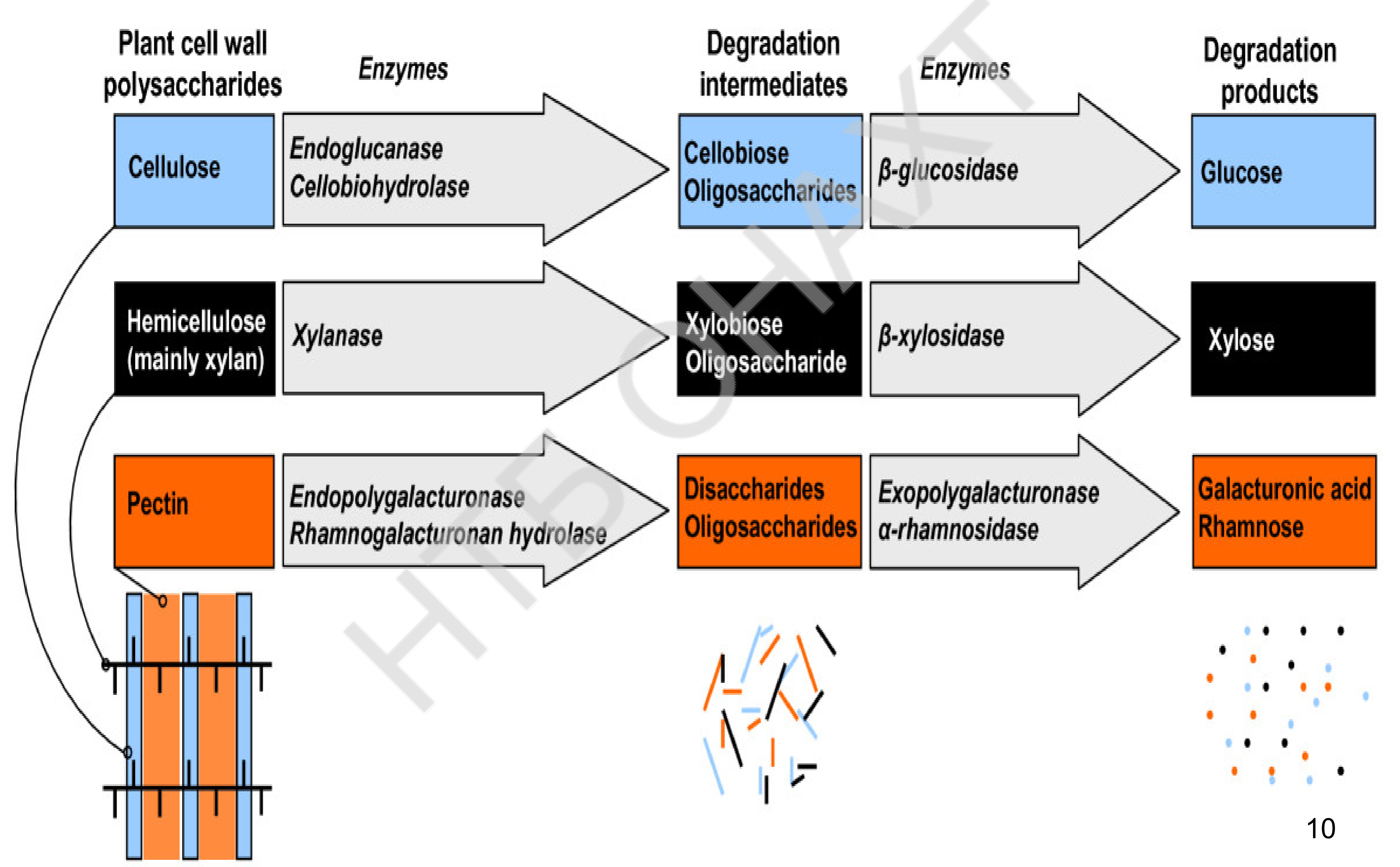
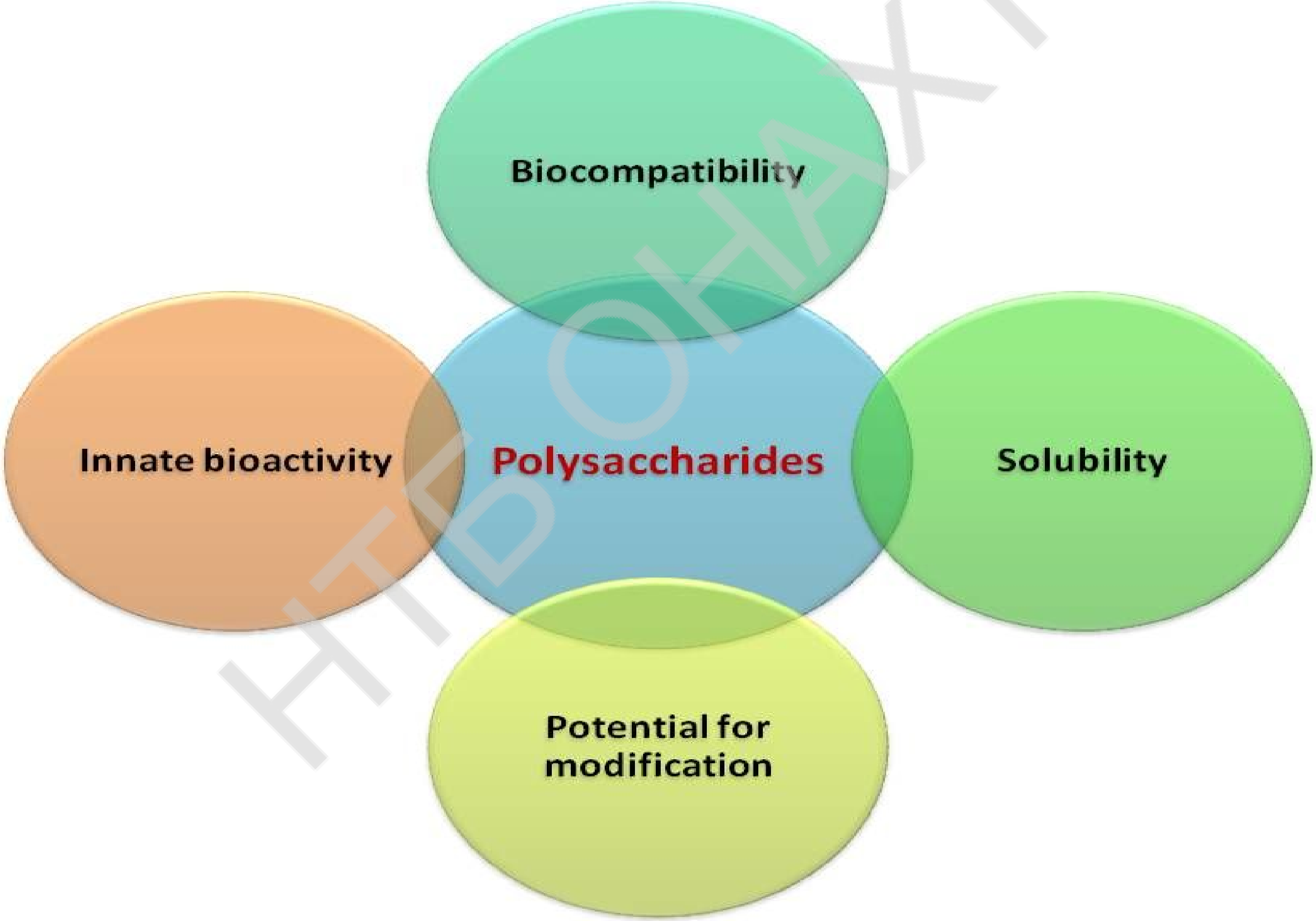
Plant polysaccharides structures



Directions of plant polysaccharides enzymatic modifications



Properties of polysaccharides for potential use in food systems



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Enzyme classes used in biopolymers modifications

1. *Oxidoreductases* (8%) $AH_2 + B = A + BH_2$
2. *Transferases* (9%) $AX + B = BX + A$
3. *Hydrolases* (58%) $A\text{-}B + H_2O = AH + BOH$
4. *Lyases* (4%) $B + X\text{-}Y = A\text{-}B + X + Y$
5. *Isomerases* (11%) $A = B$
6. *Ligases* $A + B + NTP = A\text{-}B + NDP + P$
7. *Translocases** $AX + B \,\|\, A + X \,\|\, B$

(side 1) *(2side)*

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**7th class translocases, was added in 2018 by Committee IUBNB: Tipton, K. Translocases (EC 7)*: Anew EC Class. *Enzyme Nomenclature News, 2018.*

Glycoside hydrolase GH-family classification

The CAZy (http://www.cazy.org/) server classified into *four main groups* of sequence-based families::

1. **GH, *glycoside hydrolases;***
2. **GT, *glycosyl transferases*;**
3. **PL, *polysaccharide lyases;***
4. **CE, *carbohydrate esterases*.**

***The most useful amylolytic enzymes are*:**

GH13 - α-amylase E(C 3.2.1.1), endo

GH14 - β-amylase (EC 3.2.1.2), exo

GH15 - glucoamylase (EC 3.2.1.3), endo

(CANTAREL et al.,2009, 2018)

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BIOACTIVE OLIGOSACCHARIDES: PRODUCTION, BIOLOGICAL FUNCTIONS AND POTENTIAL COMMERCIAL APPLICATIONS

Oligosaccharide	*Molecular formula*
• **Fructo-oligosaccharides**	β-Fruf-(2→[1)-β-Fruf-(2]n→1)-β-Fruf-(2→1)-αGlcp β- Fruf-(2→[1)-β-Fruf-(2]n→1)-β-Fruf
• **Xylo-oligosaccharides(XOS)**	β-Xylp-(1→[4)-β-Xylp-(1]n→4)-β-Xylp
• **Malto-oligosaccharides**	α-Glcp-(1→[4)-α-Glcp-(1]n→4)-α-Glcp
• **Isomalto-oligosaccharides (IMOS)**	α-Glcp-(1→[6)-α-Glcp-(1]n→6)-α-Glcpα-Glcp-(1→6) -α- Glcp-(1→[4)-α-Glcp-(1]n→4)-αGlcp
• **Cyclomaltodextrins(CD)**	Glcp-(1→[4)-α-Glcp-(1]n→4)-Glcp n = 4, 5 or 6
• **Galacto-oligosaccharides**	β-Galp-(1→[4)-β-Galp-(1]n→4)-β-Galp-(1→4)- Glcp
Transgalacto-oligosaccharides	β-Galp-(1→[4)-β-Galp-(1]n→4)-β-Galp-(1→4) - Glcp β-Galp-(1→[6)-β-Galp-(1]n→6)-β-Galp
• **Gentio-oligosaccharides**	β-Glcp-(1→[6)-β-Glcp-(1]n→6)-β-Glcp
• **Lactulose***	β-Galp-(1→4)-Fruf

**semi-synthetic compound Legend: Glcp, glucopyranoside; Fruf, fructofuranoside; Xylp, xylopyranoside; Galp, galactopyranoside; β-Galp-(1→4)-Glcp, lactose; β-Fruf- (2→1)-α-Glcp, sucrose.*

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Some microbial enzymes used in processes to produce oligosaccharides

Oligosaccharide	Enzyme	Microorganism
FOS	β-fructofuranosidase	*Aspergillus japonicus*
		Aureobasidium pullulans
	β-fructosyltransferase	*Aspergillus japonicus*
		Aspergillus aculeatus
XOS	xylanase	*Trichoderma harzianum*
		Thermotoga maritima
MOS	glucansucrase	*Strept. sobrinus*
	α-amylase	*Bacillus subtilis*
IMOS	trans-α-glucosidase	*Saccharomyces carlsbergensis*;

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Oligosaccharides as functional food ingredients: biological properties

Oligosaccharide	Function
Xylo-oligosaccharides	Promotes the formation of vitamin B
Fructo-oligosaccharides	Enhancement of organic acids production and inhibition of pathogen
Lactosucrose	Increases the absorption of calcium by the intestine
Manno-oligosaccharides	Reduction of blood pressure in hypertensive individuals
Isomalto-oligosaccharides	Prevents dental caries and relieves constipation in the elderly population
Galacto-oligosaccharides	Increases the resistance to fractures
Transgalactooligosaccharides	Promotes absorption of calcium in the post menopausal period of women

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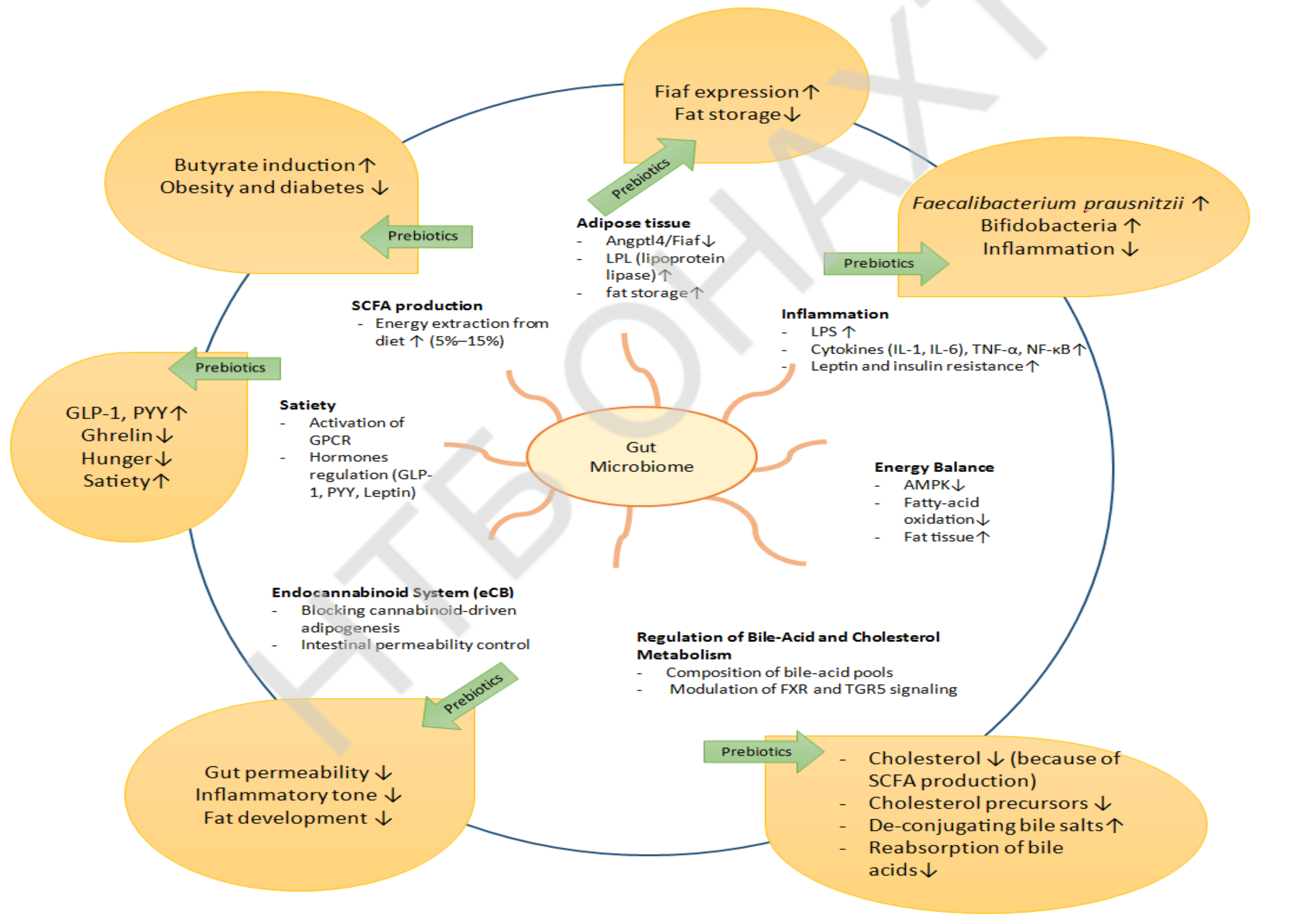
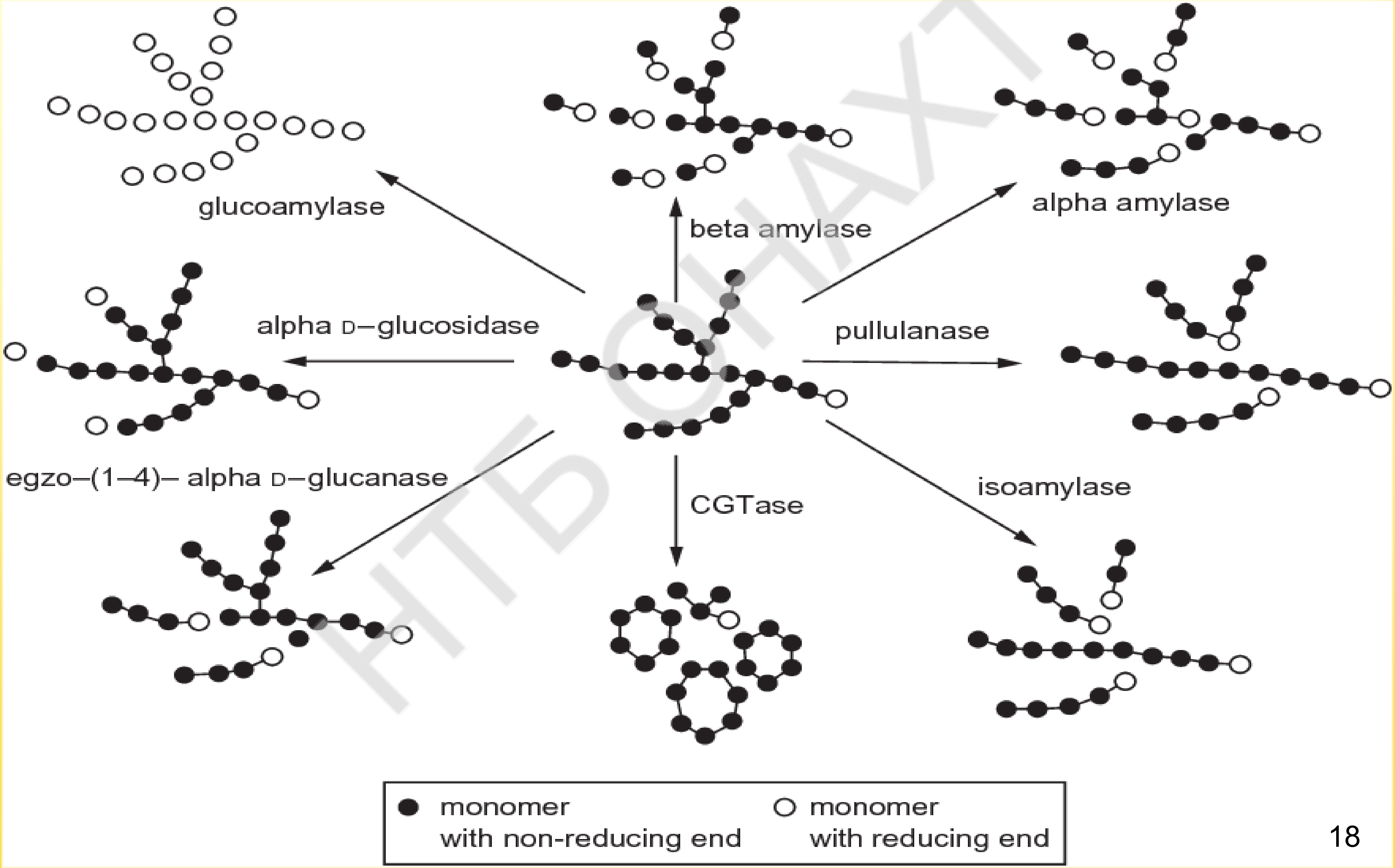
HELTH BENEFITS OF PREBIOTIC OLIGOSACCHARIDES

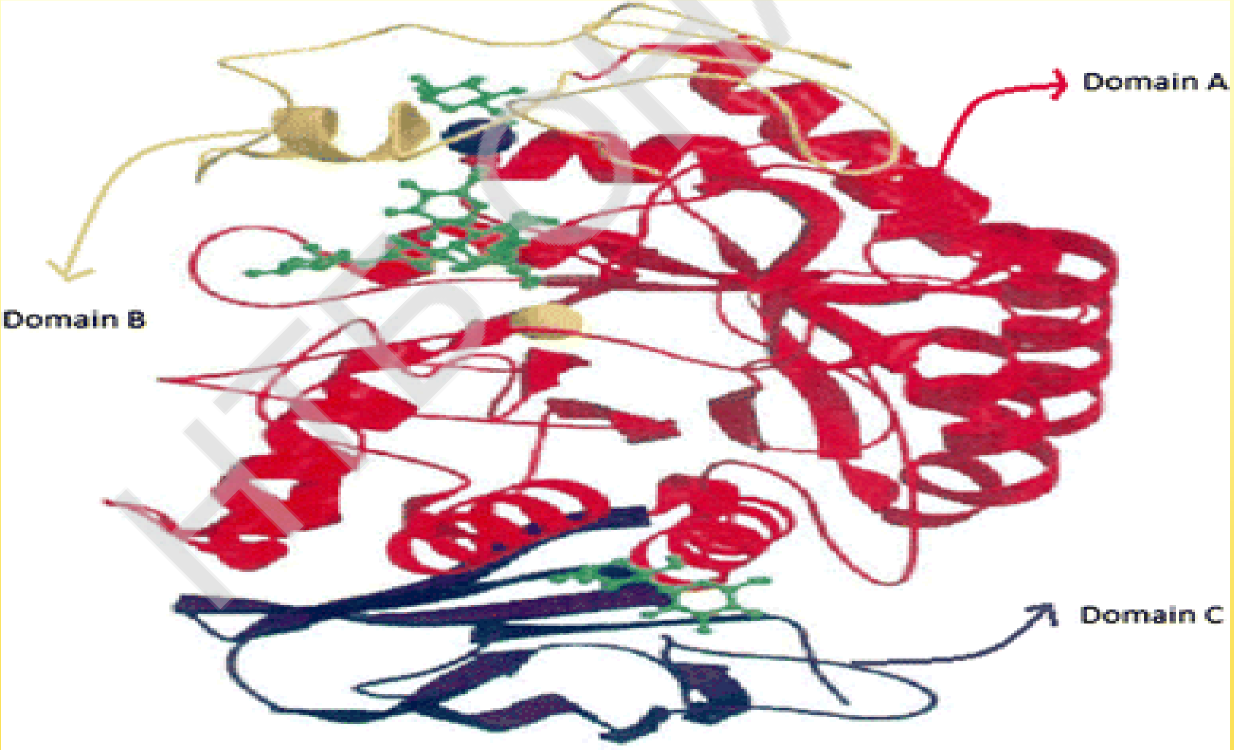


Amylase actions on starch polysaccharides

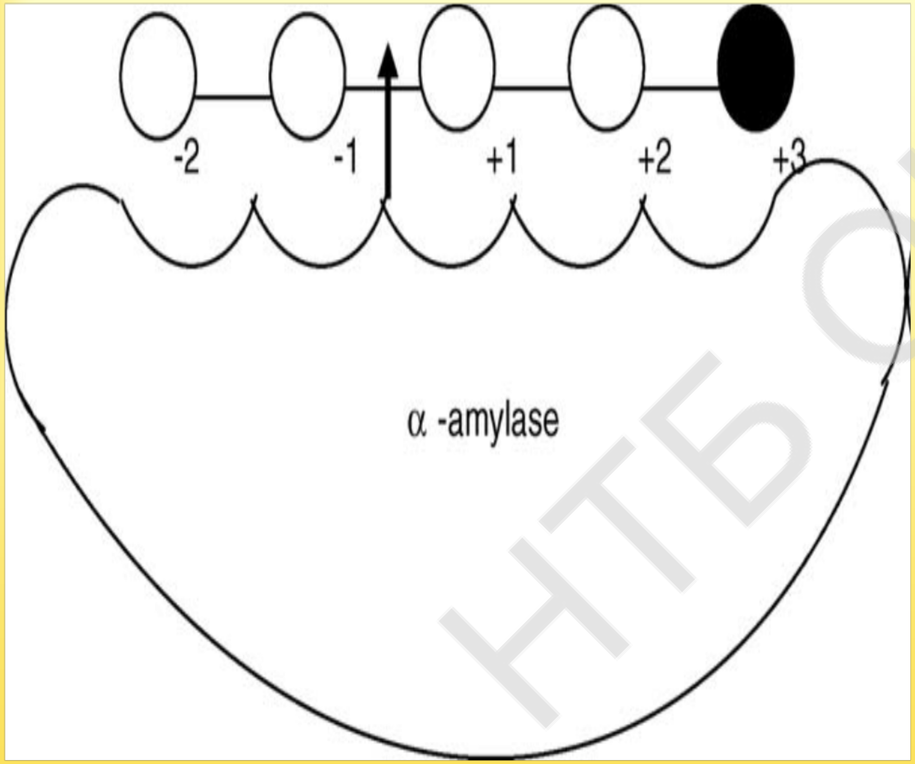


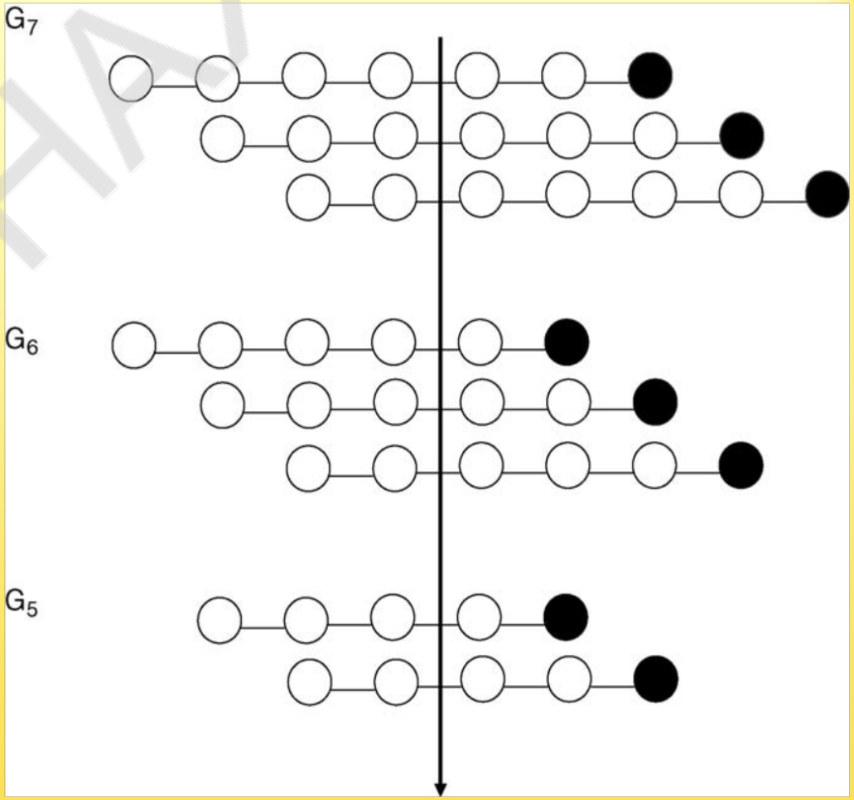
Three-dimensional structure of GH13 α-amylase from *Aspergillus oryzae*

The α-amylase family members are multidomain proteins: the main catalytic domain in the form of a parallel (β/α)8-barrel (domain A) that is interrupted by a small domain in the place of the loop 3 connecting the strand β3 with the helix α3 (domain B) and by the antiparallel β-sandwich domainC



Structural organization of subsites of α-amylase active center (*endo-enzyme*)





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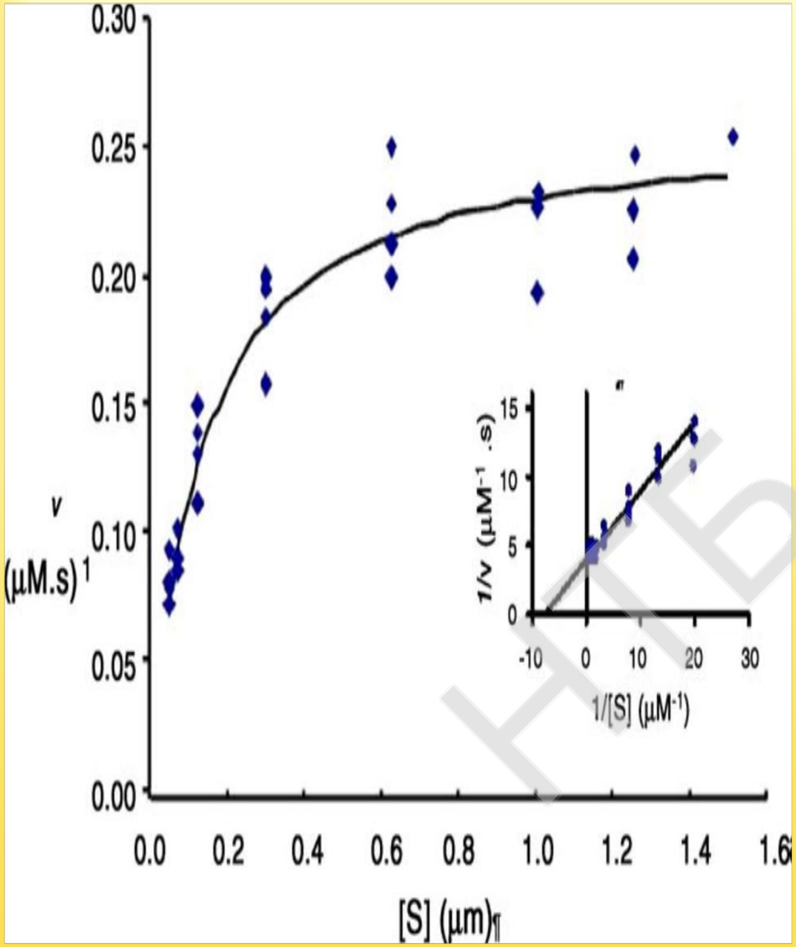
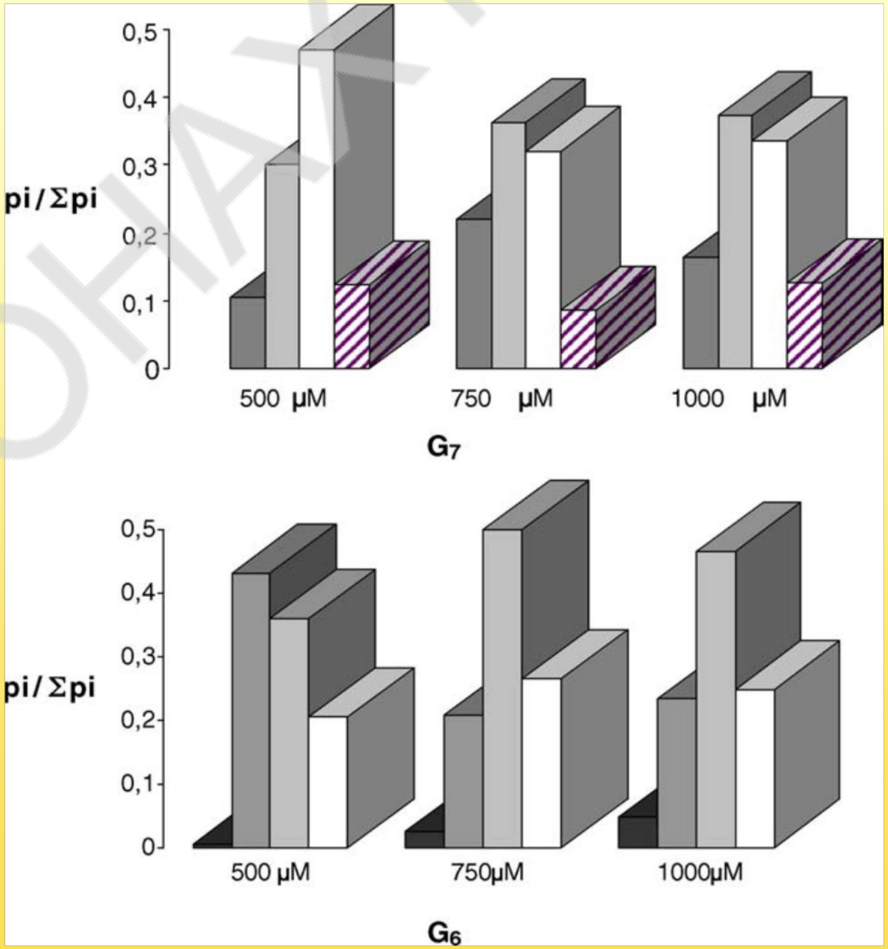
Kinetic experiments and products distribution of the olygosaccharides





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Physical states of the starch granules as enzymatic modification factor

State of the starch granules

heterogeneous
- α-amylase
- Modified starches (with granules structure)

homogeneuos – heterogeneuos
- α-amylase, β-amylase
- Thermo-reversible Maltodextrins +limited dextrins

homogeneous
- α - amylase, β - amylase, glucosidases, CD- transferase
- ·Cereal glucose syrups (CGS)
- ·Maltooligo-saccharides
- ·Cyclodextrins

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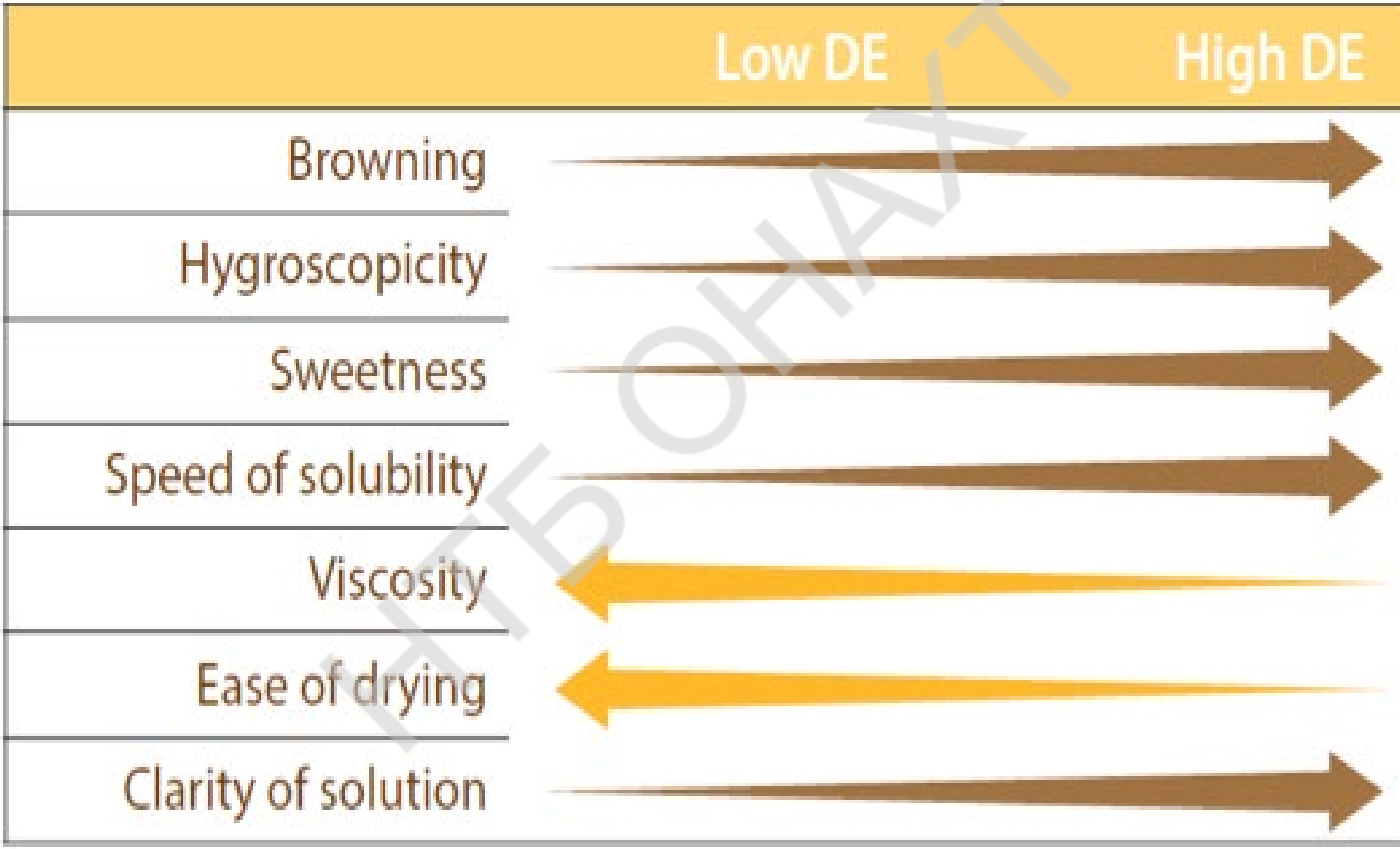
Maltodextrins properties and DE



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Relation of DE with properties of maltodextrins

As DE increases (DP decreases), so do the following characteristics:

1) browning (due to the increased level of reducing sugars);
2) hygroscopicity/humectant properties;
3) plasticity;
4) sweetness;
5) solubility;
6) osmolality.

As DE decreases (DP increases) , the following characteristics increase:

1) molecular weight;
2) viscosity;
3) cohesiveness;
4) film-forming properties;
5) prevention of large sugar-crystal formation.

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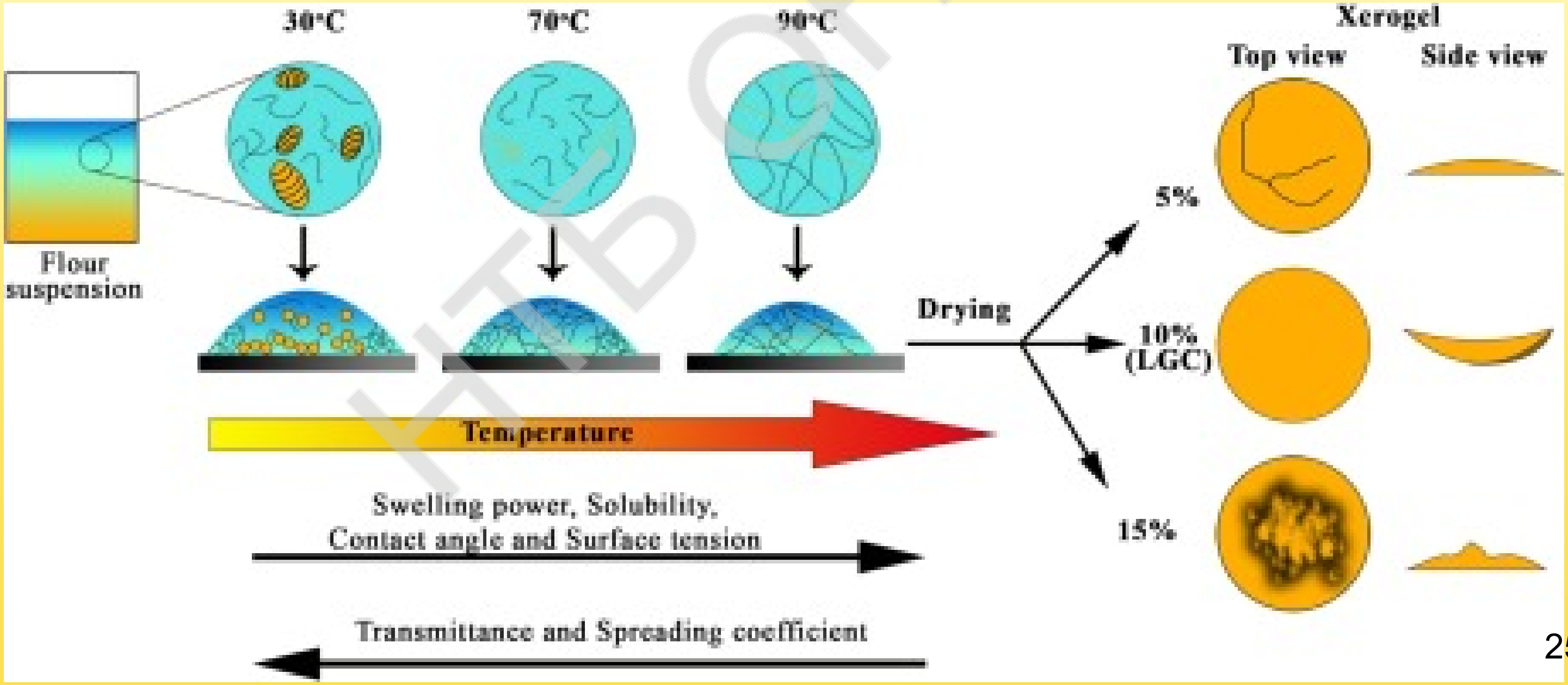
Directions of amylase modifications and state of starch polysaccharides:

- homogeneous (T=70-90 C)
- heterogeneous (T=25-30 C)
- homogeneous-heterogeneous(T=65-70C)



PROPERTIES OF MODIFIED STARCHES

Phisico-chemical properties of cereal starches modified by α-amylase in heterogeneous conditions				
starch		**viscosity [ŋ], ml/g**	T gel, °C	**fluidity, convent. units**
wheat	control	176	61	11
	modified	50	59	67
rye	control	155	58	10
	modified	49	56	55
corn	control	192	78	12
	modified	51	73	72

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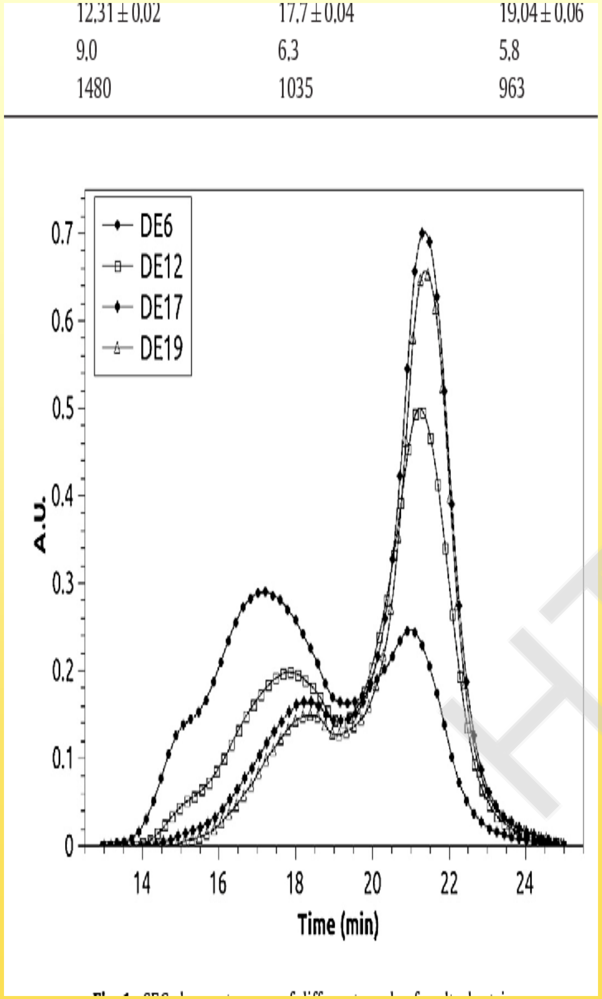
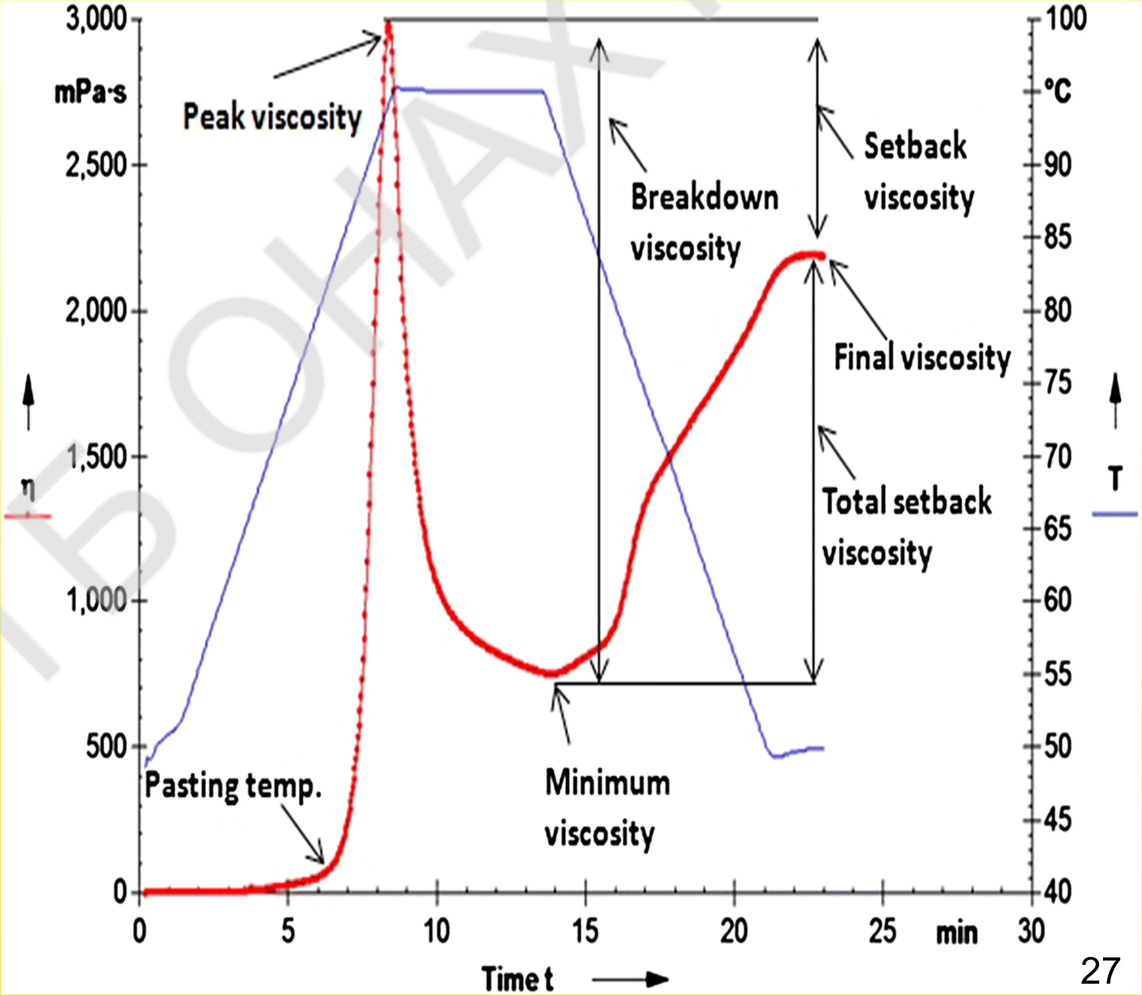
Physico-chemical and functional properties of native and modified by alfa-amylase wheat starch in homogeneous-heterogeneous conditions





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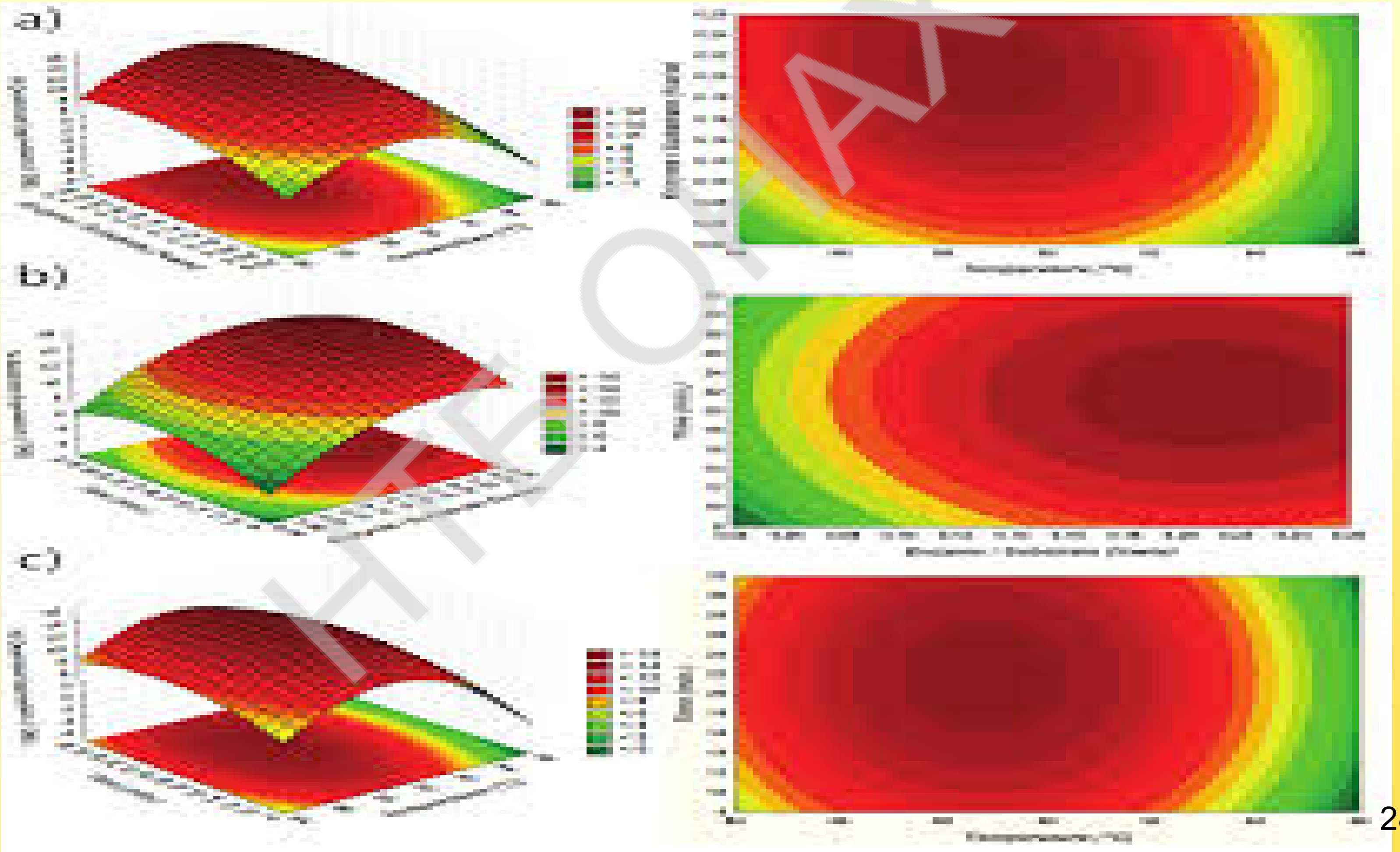
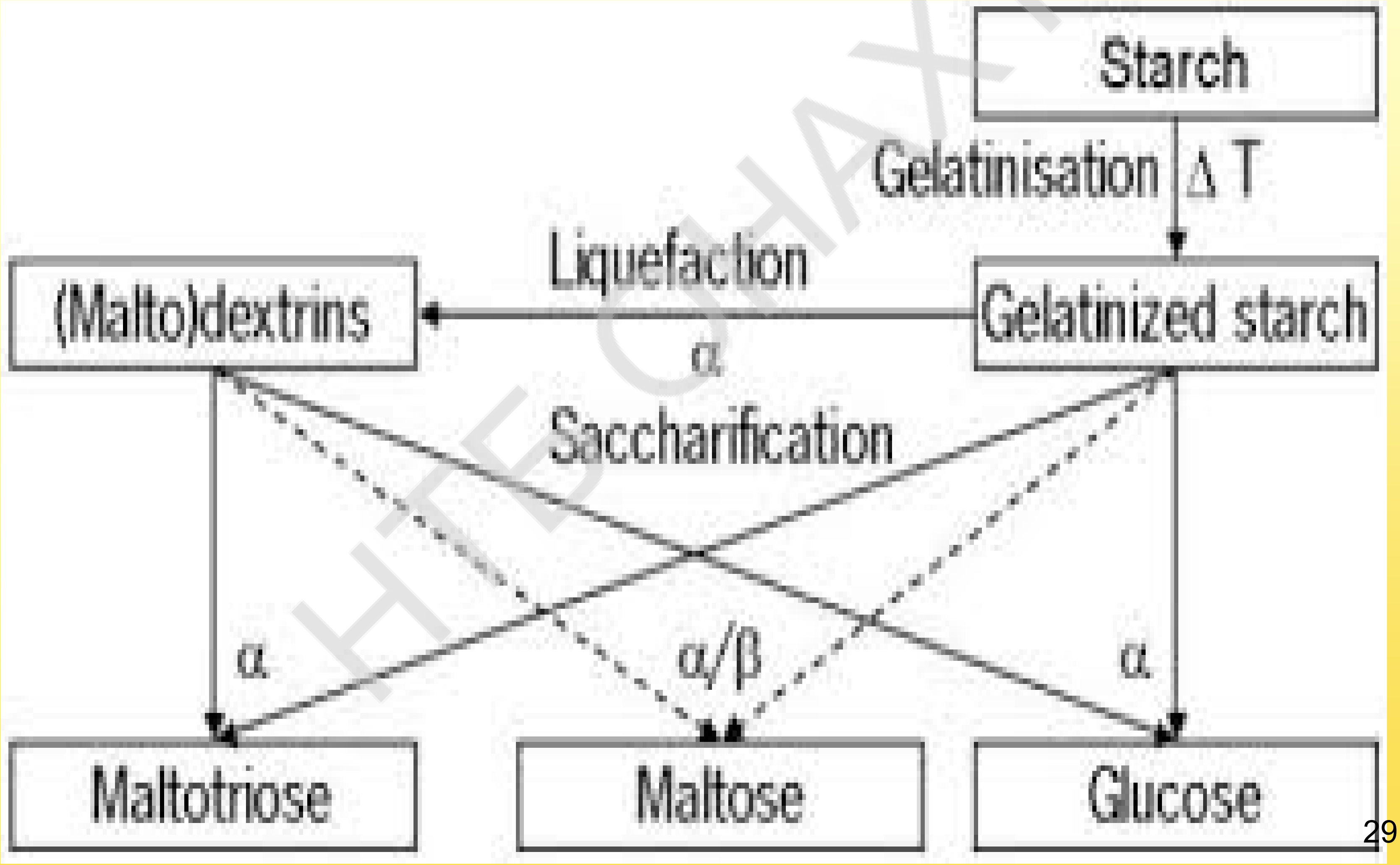
Syrup production via enzymatic conversion of a byproduct (wheat bran)

α-amylase (Termamyl 120L) and amyloglucosidase (A LMG)

a) DE:E/S:T, b) DE:time:E/S, c) DE:time:T

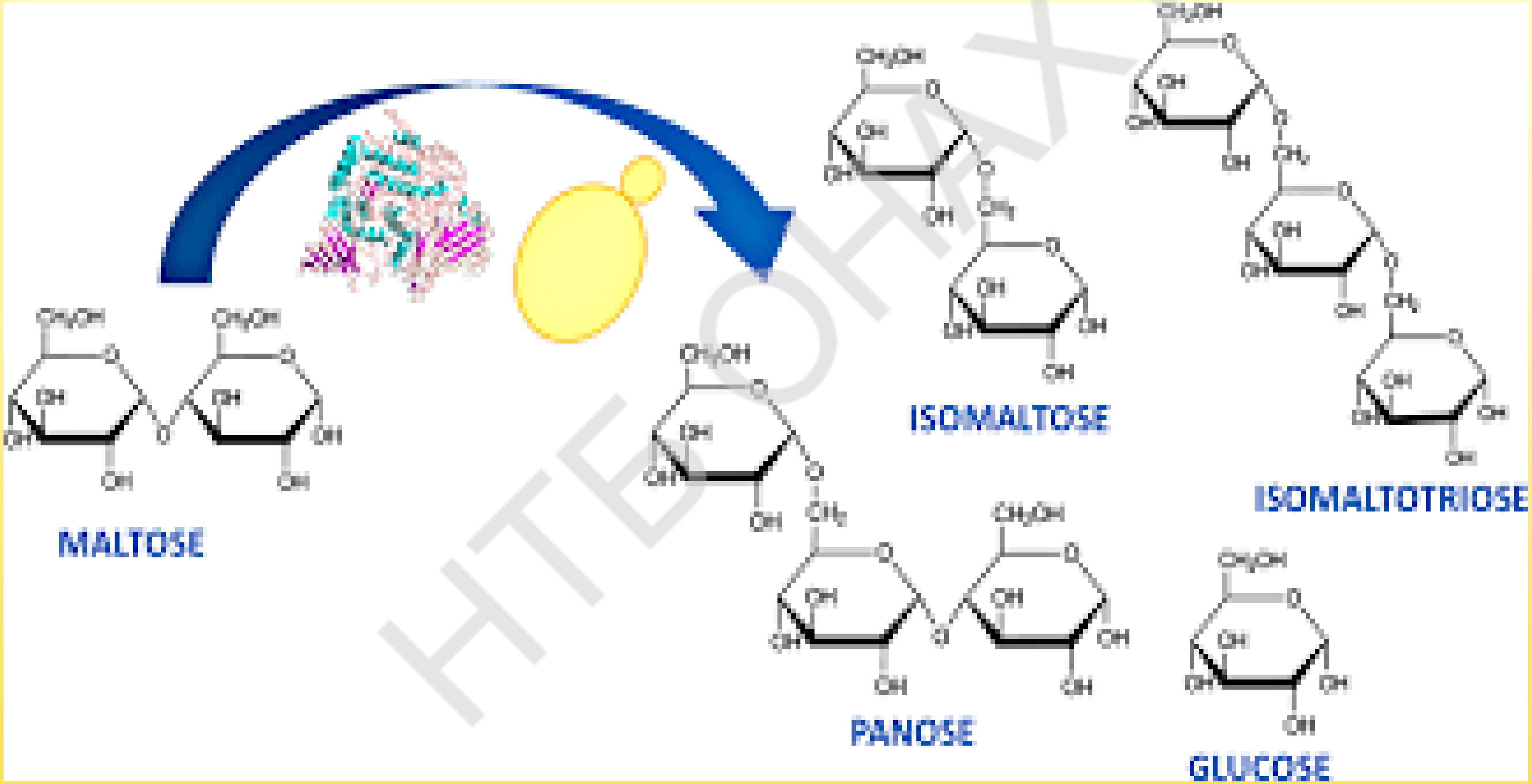


Enzymatic conversions of starch in highly concentrated reaction systems to syrups with high DE



HTE OHAXT

Isomaltooligosaccharide(IMOs) prebiotic structures



HTBOHAXT

Multi-enzyme compositions (cockteils) for starch modification have been created in this investigations

MEC-5: α-amylase and α-glucosidases,

MEC-7: amylomaltase and α-transglucosidases,

MEC-10: α-amylase and α-transglucosidases,

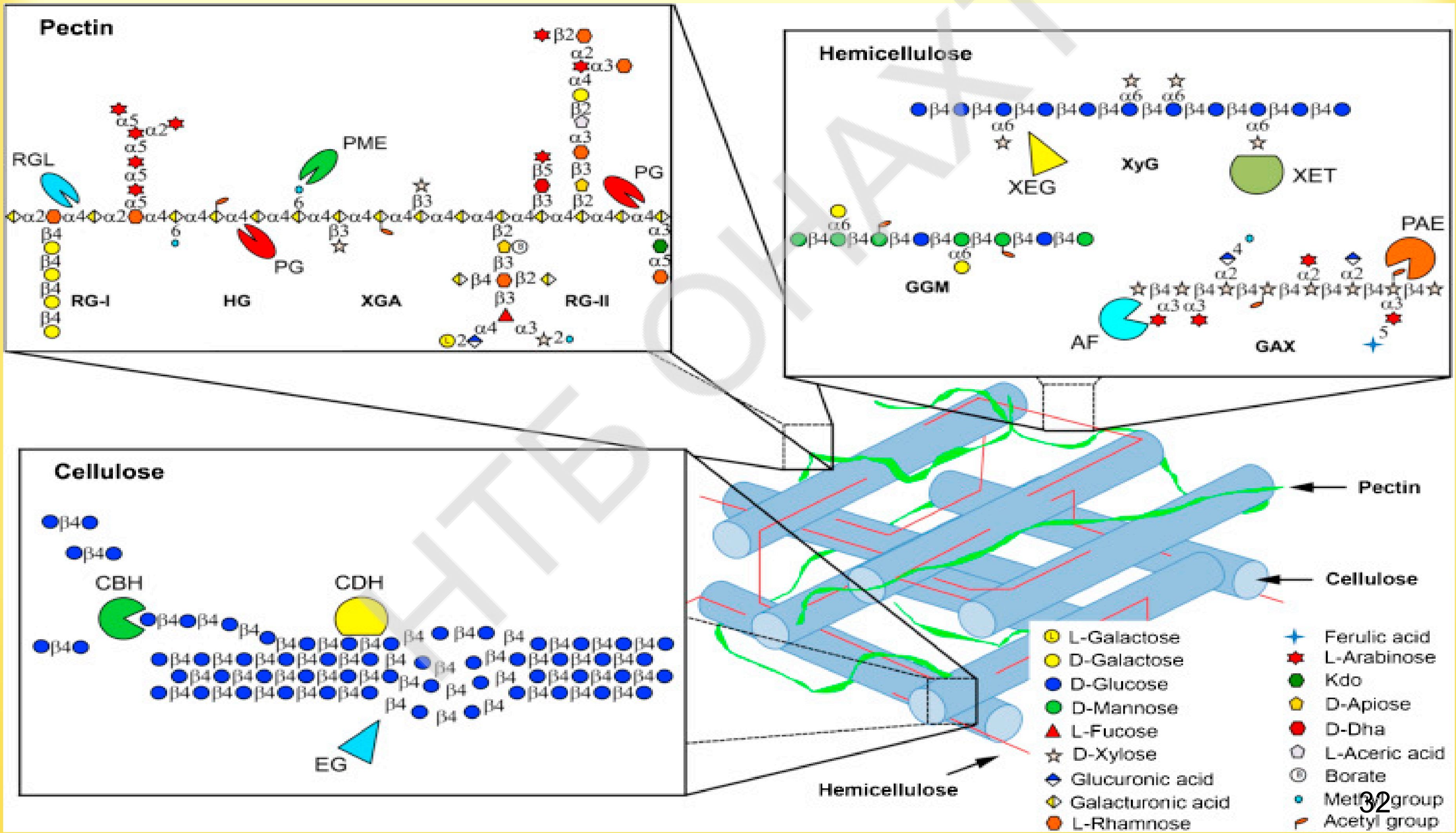
MEC 12: α-amylase, amylomaltase and maltogenic α-amylase,

MEC-15: α-amylase, maltogenic α-amylase and α-transglucosidases,

MEC-17: pullulanase, α-amylase, β-amylase and α-transglucosidases

MEC-18: maltogenic α-amylase, CDT-ase

Enzymatic modifications of cellulose, hemicelluloses and pectin polysaccharides



Classes of enzymes involved in cellulose hydrolise

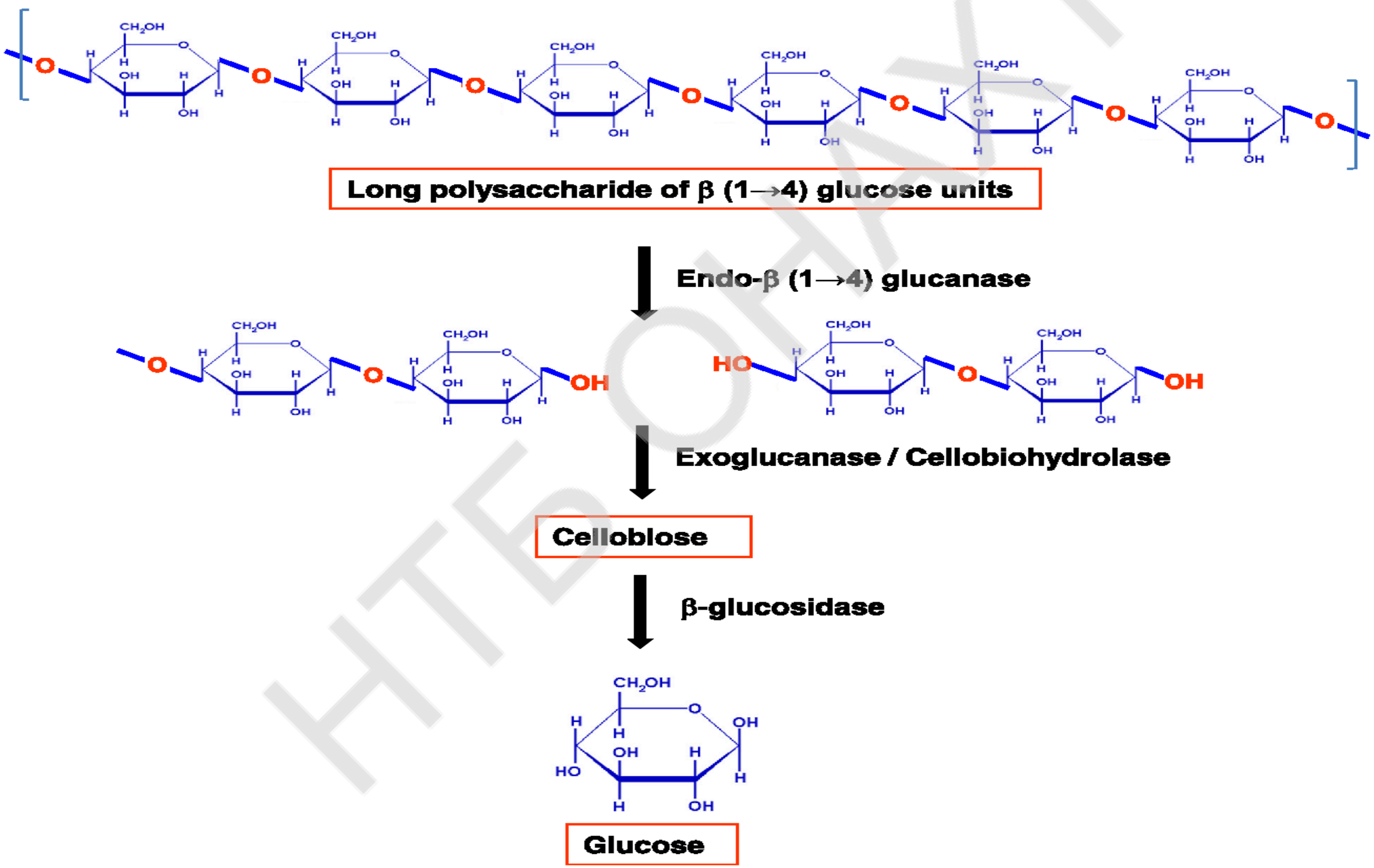
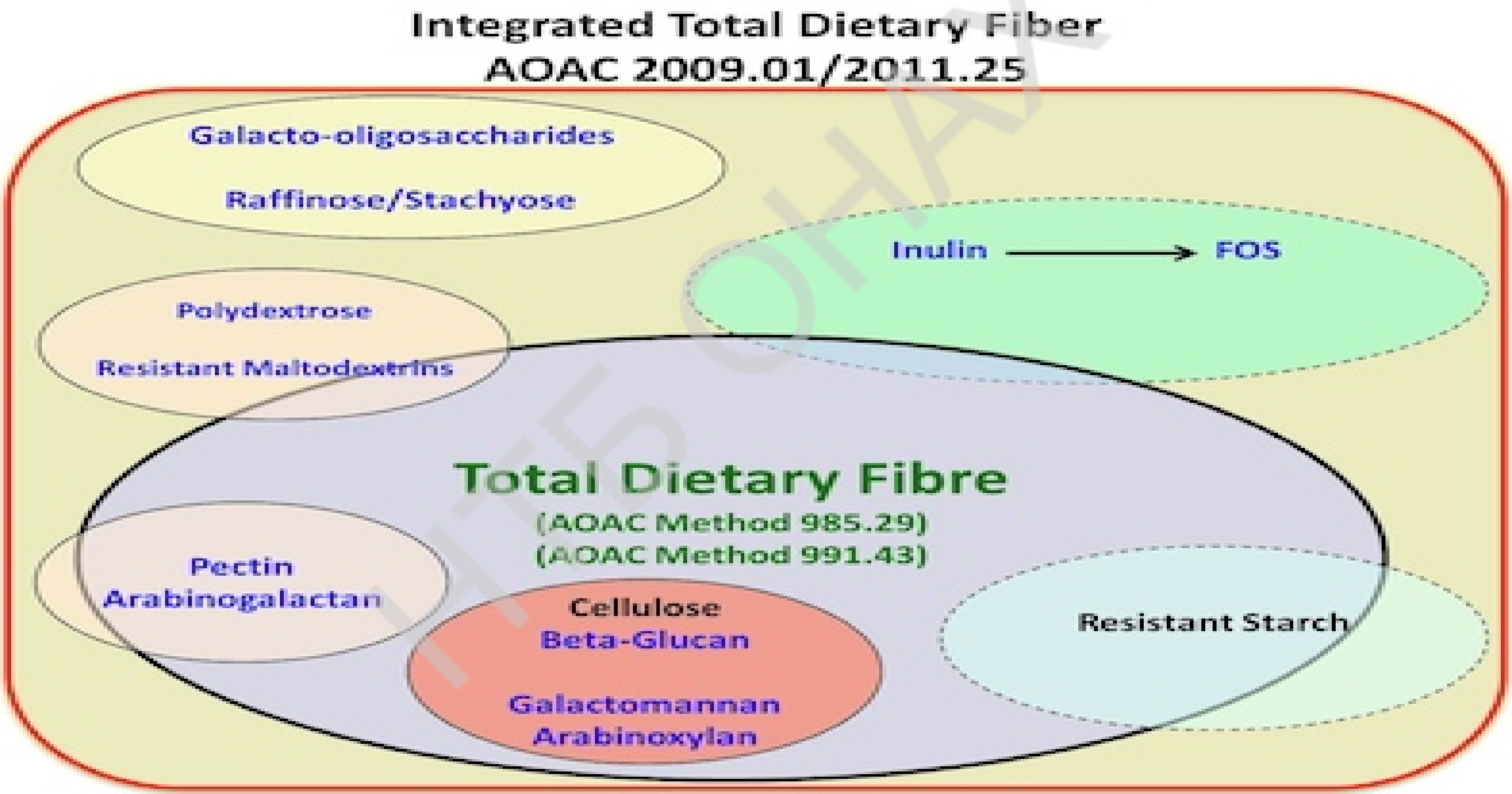
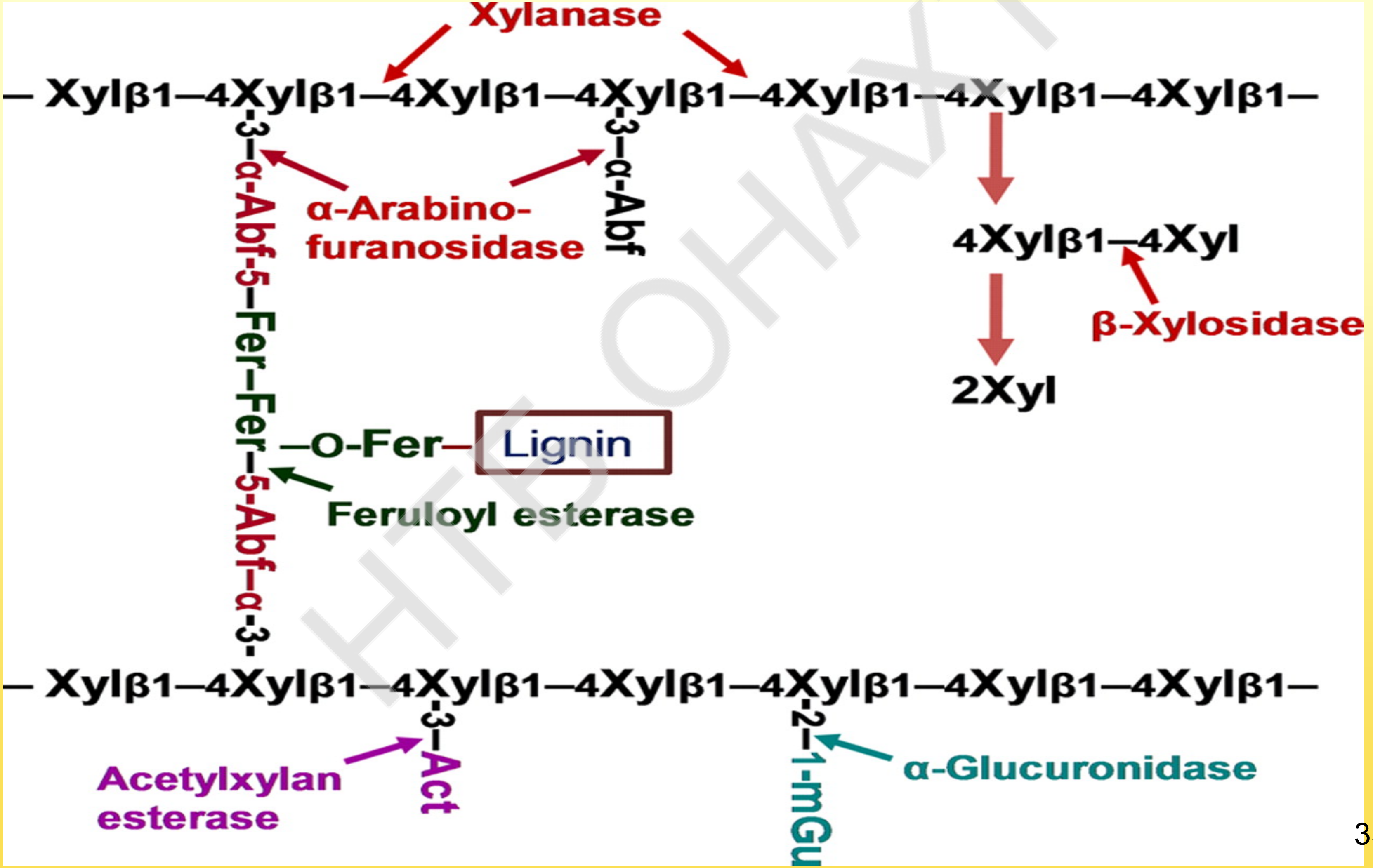


Figure 2 Classes of enzymes involved in cellulose breakdown. Endoglucanase produce random cuts at an internal position within cellulose

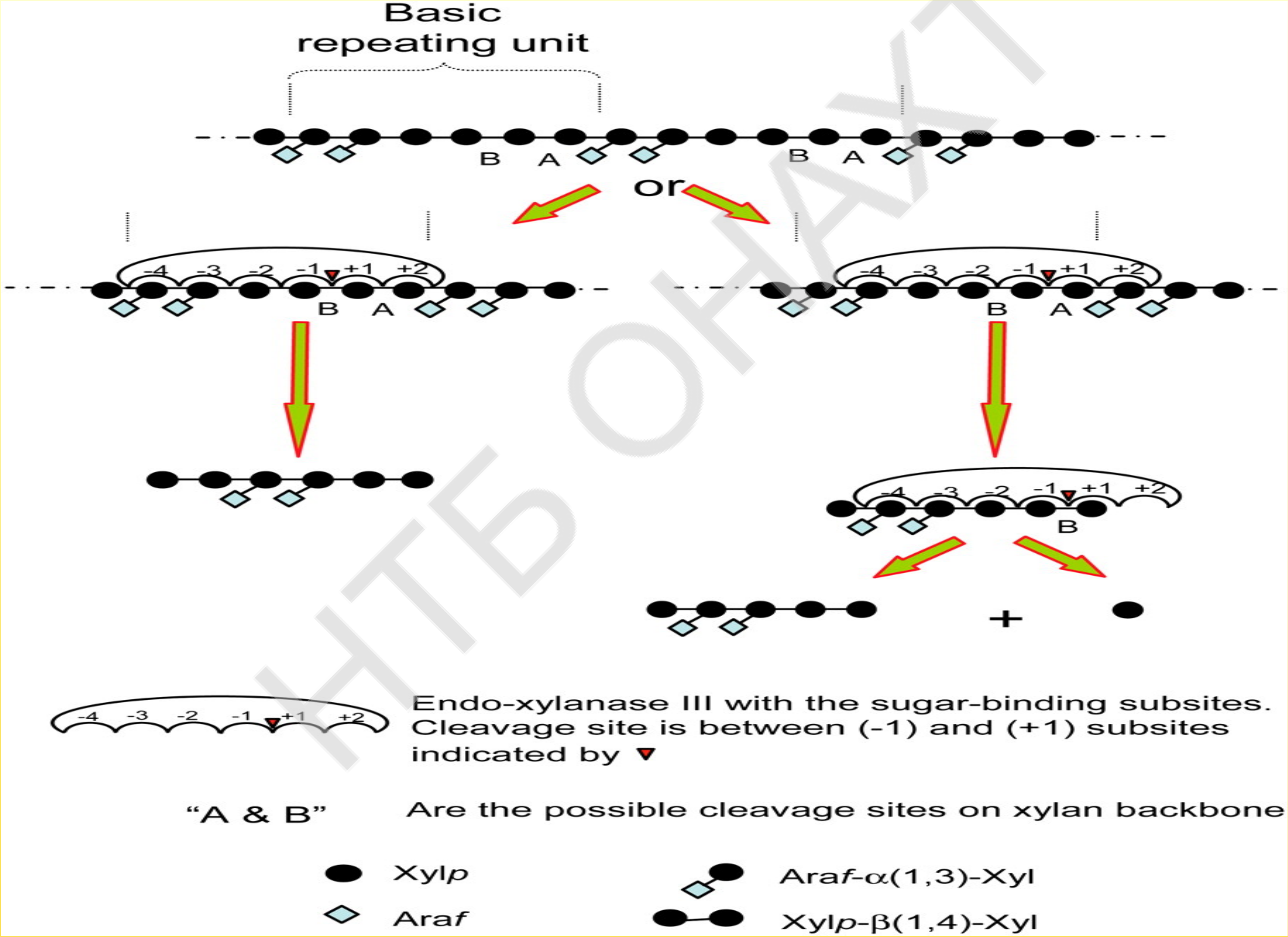
Composition of Modified dietary Fibers as functional ingredients



Enzymatic production of xylooligosaccharides (XOS)



Mechanisms of action of xylanases and formation of xylooligosaccharide profiles



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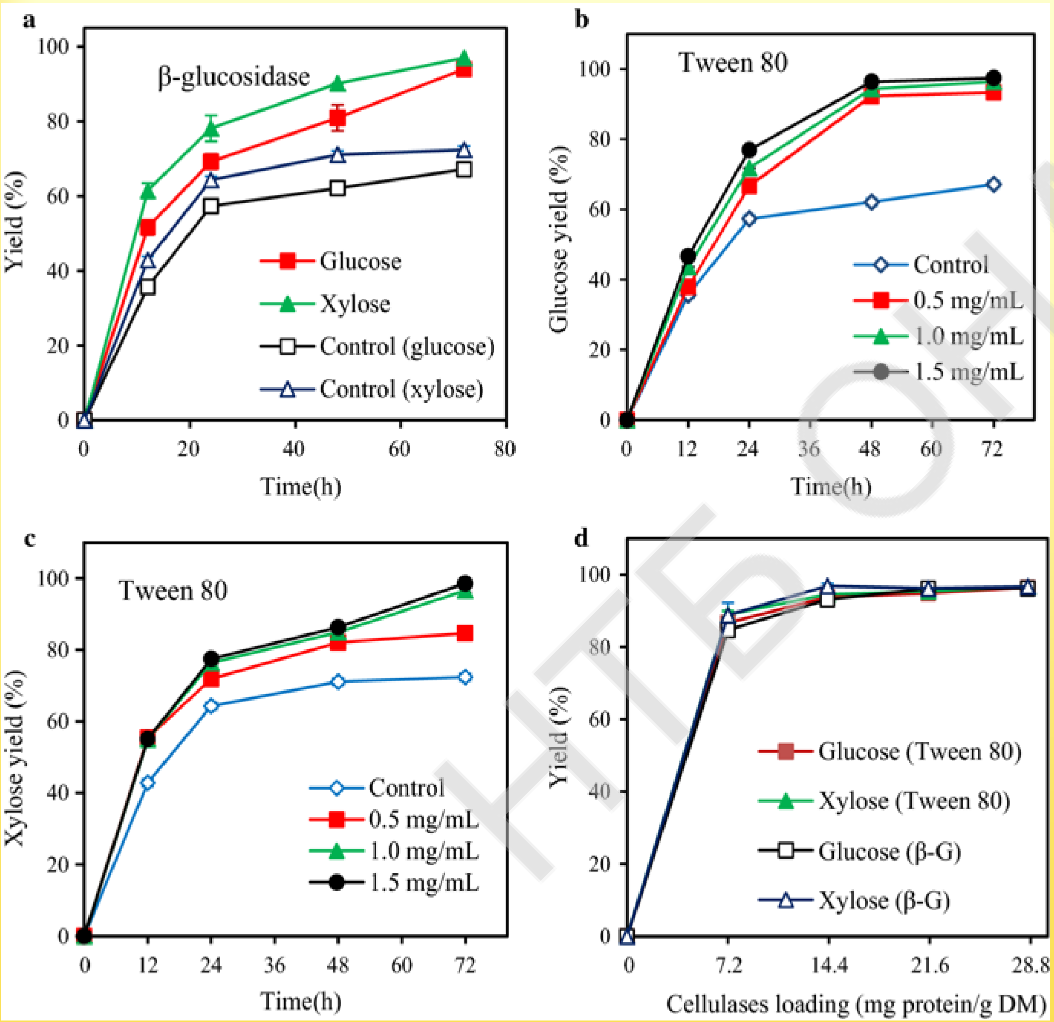
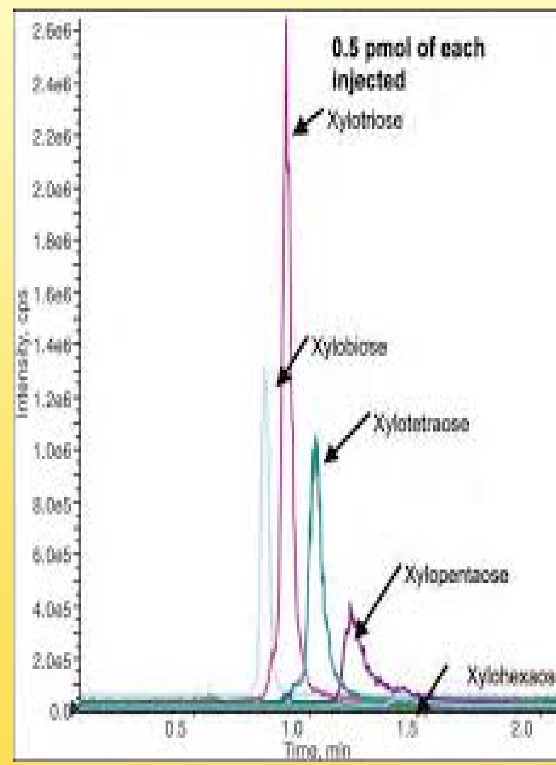
ENZYMATIC TREATMENT by *VISCOZYME* CEREALS HEMICELLULOSES



Xyloolygosacchyrides (XOS) composition



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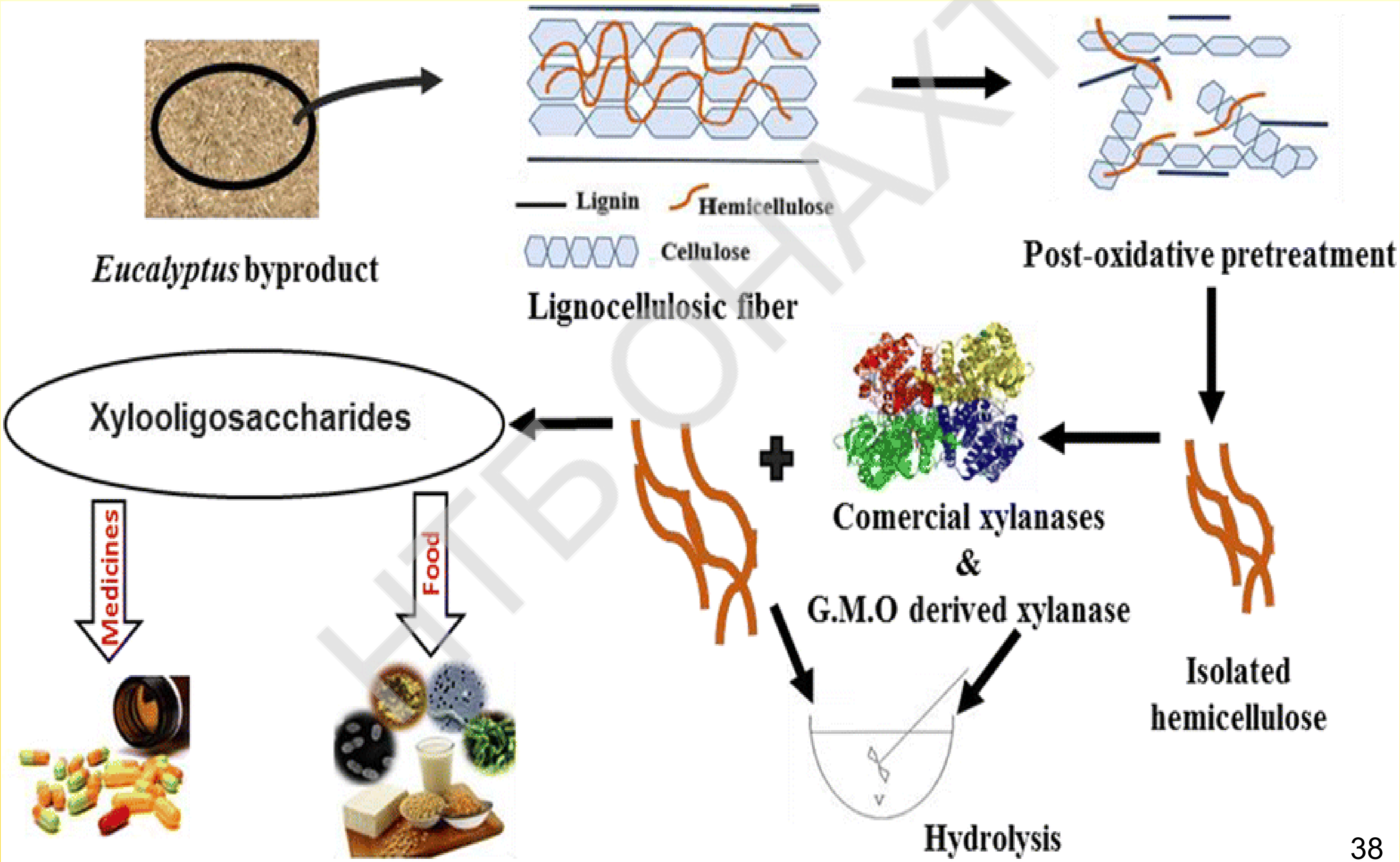
Xylooligosaccharides production process from lignocellulosic by-propducts



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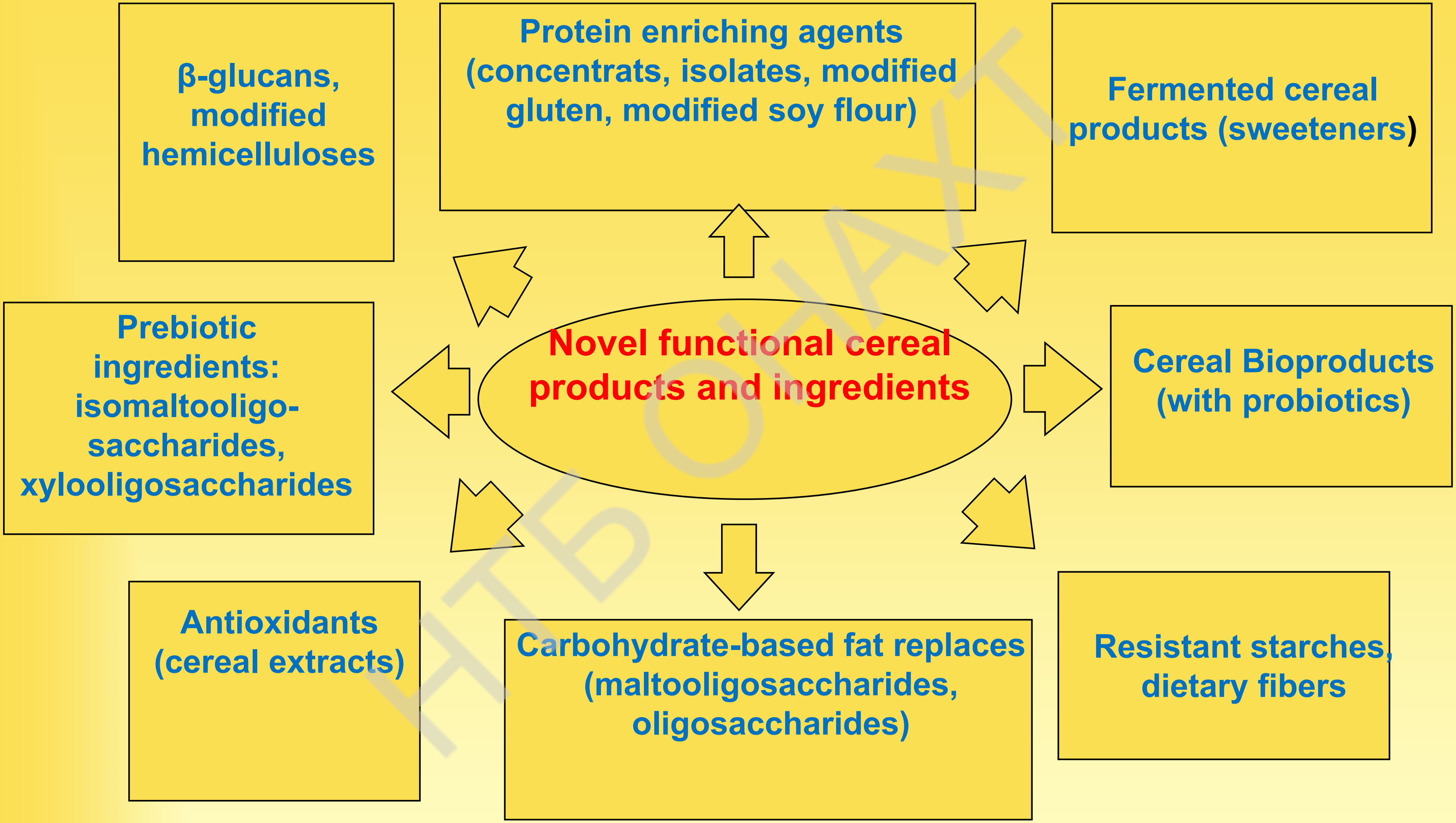
EXEMPLES OF ENZYMATIC TREATMENTS OF CEREALS RAW MATERIALS
TO THE FUNCTIONAL INGREDIENTS AND PRODUCTS

The aim of this part of the work was application of biotechnological enzymatic methods for reprocessing of grain raw materials and by-products in food and biologically active supplements, as well as enriching agents and functional products


β-glucans, modified hemicelluloses
Protein enriching agents (concentrats, isolates, modified gluten, modified soy flour)
Fermented cereal products (sweeteners)
Prebiotic ingredients: isomaltooligo-saccharides, xylooligosaccharides
Novel functional cereal products and ingredients
Cereal Bioproducts (with probiotics)
Antioxidants (cereal extracts)
Carbohydrate-based fat replaces (maltooligosaccharides, oligosaccharides)
Resistant starches, dietary fibers

Main enzymes used for production of cereal functional additives and products

Enzymes

Amylases: α-amylases, β-amylase,
glucoamylase, pullulanase

Cellulases and hemicellulases:
cellulase, laminarase, lechinase, α-L-arabinase

Proteinases, peptidases

Lipases and esterases

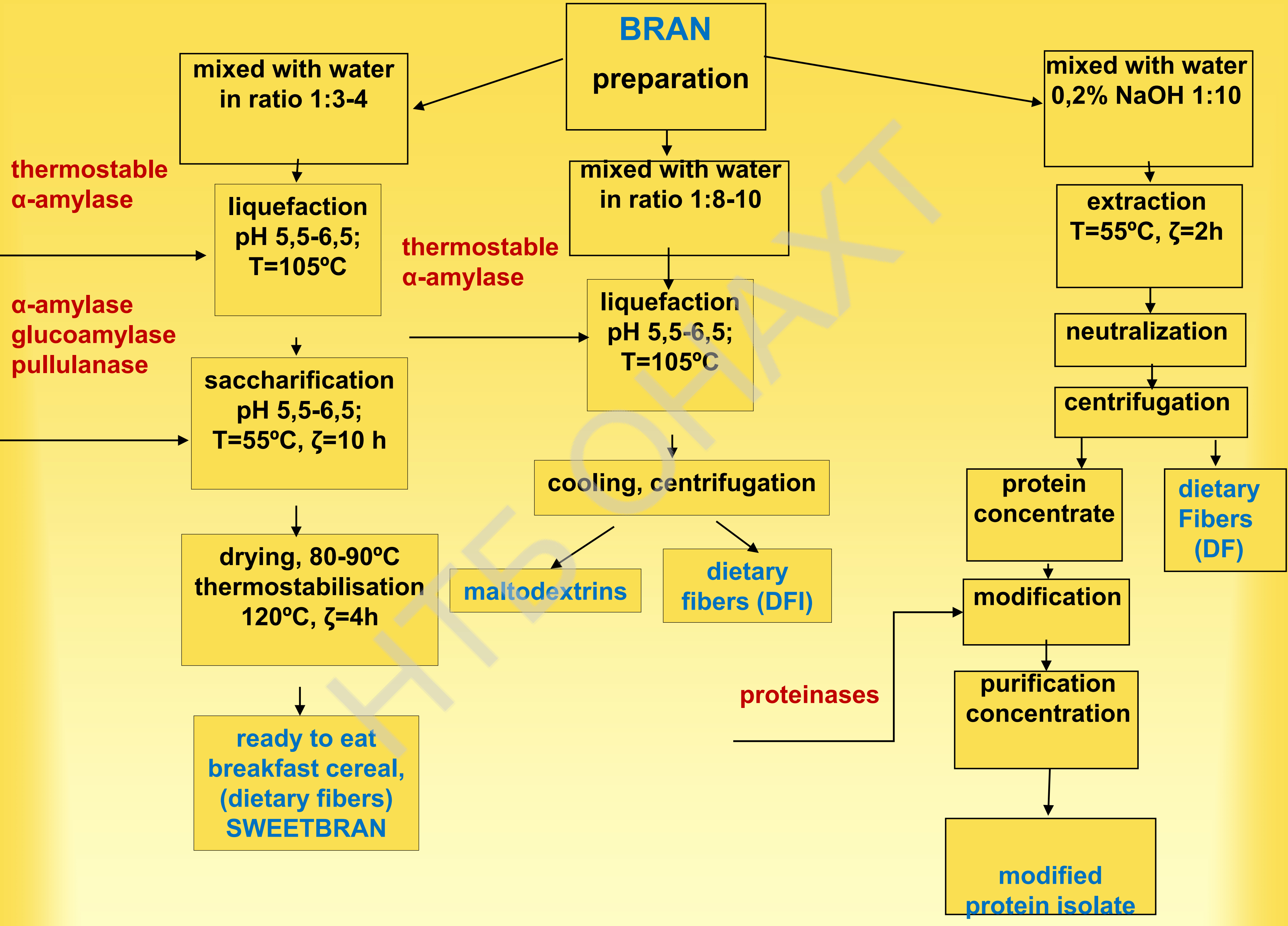
Lactobacillus, Bifidus

Cereal substates

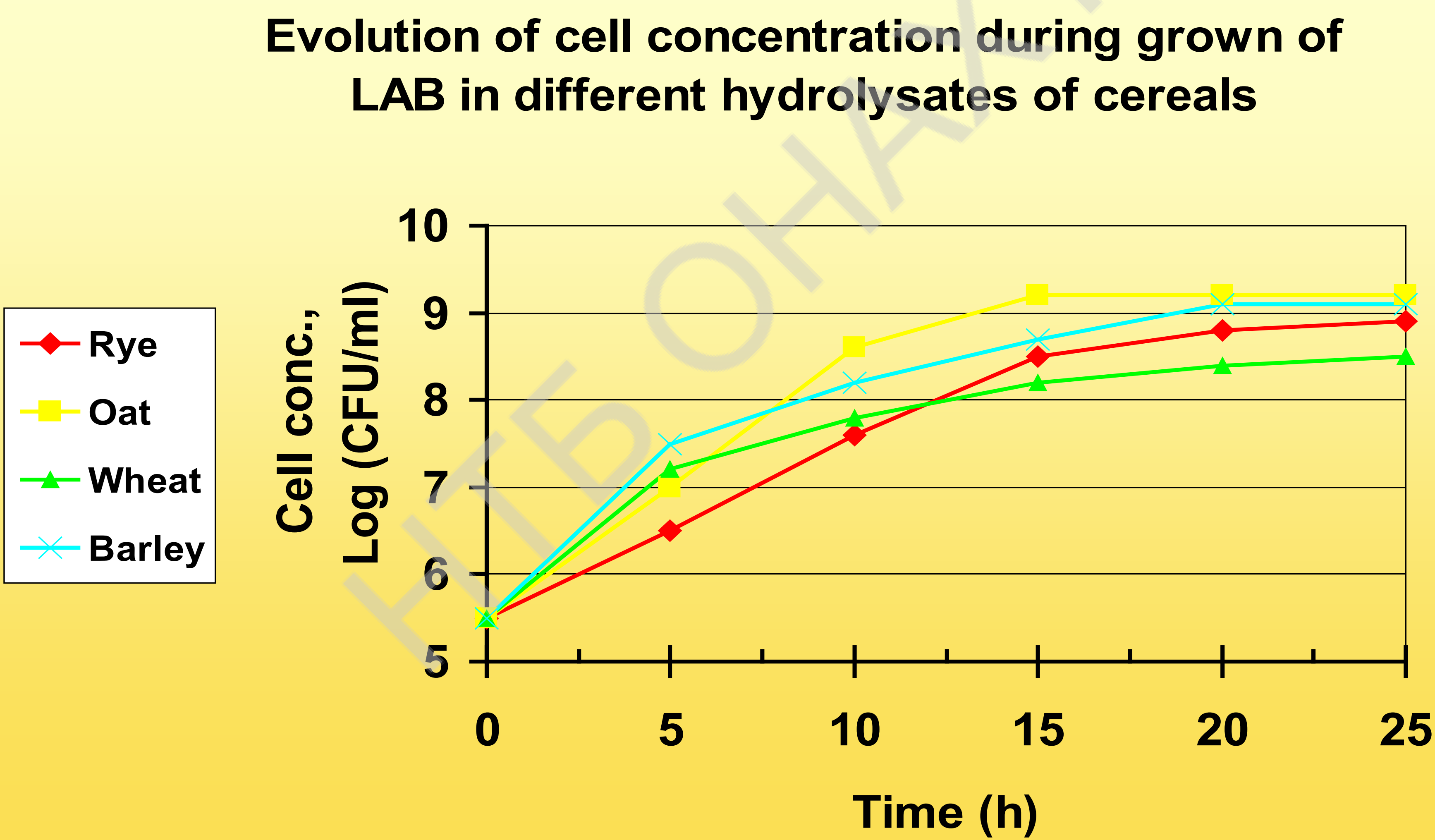
amylose, amylopectin,
cell wall polisaccharides:
β-glucan, pentosans,
arabinoxylans,
hemicelluloses;
proteins, peptides,
lipides and
phospholipides

Products

Glucose
maltose
maltodextrins
isomaltooligosaccharides
β-glucans
dietary fibers
modified hemicelluloses
resistant starch
antioxidants
modified proteins
fermented products


BRAN
preparation
mixed with water
in ratio 1:3-4
thermostable
α-amylase
liquefaction
pH 5,5-6,5;
T=105ºC
α-amylase
glucoamylase
pullulanase
saccharification
pH 5,5-6,5;
T=55ºC, ζ=10 h
drying, 80-90ºC
thermostabilisation
120ºC, ζ=4h
ready to eat
breakfast cereal,
(dietary fibers)
SWEETBRAN
mixed with water
in ratio 1:8-10
thermostable
α-amylase
liquefaction
pH 5,5-6,5;
T=105ºC
cooling, centrifugation
maltodextrins
dietary
fibers (DFI)
mixed with water
0,2% NaOH 1:10
extraction
T=55ºC, ζ=2h
neutralization
centrifugation
protein
concentrate
dietary
Fibers
(DF)
modification
proteinases
purification
concentration
modified
protein isolate

Fermentation of hydrolysates of cereal bran by *Lactic Acid Bacteria* (co-fermentation)



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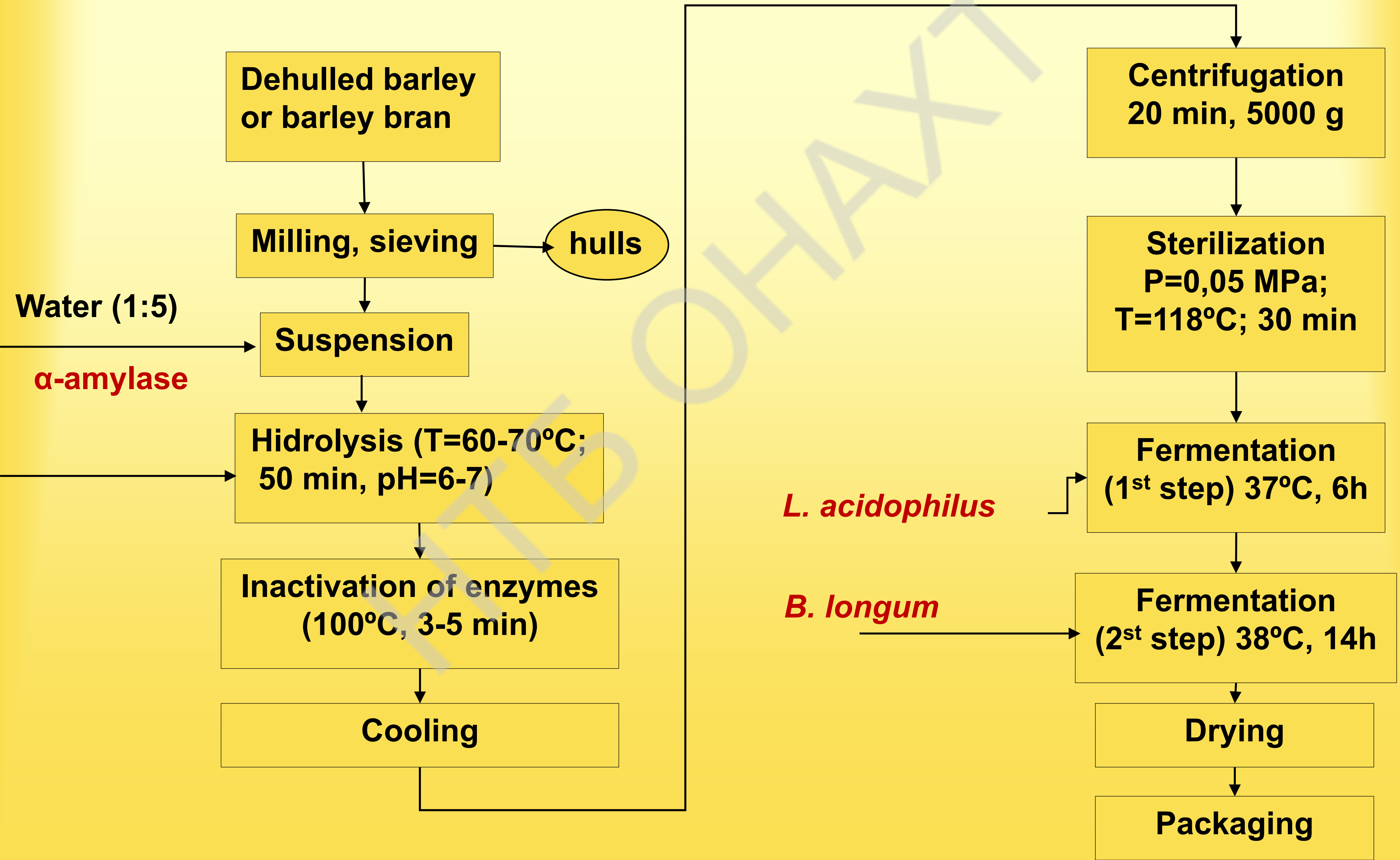
Process for making a functional cereal bioproduct “BILAMIN” from barley



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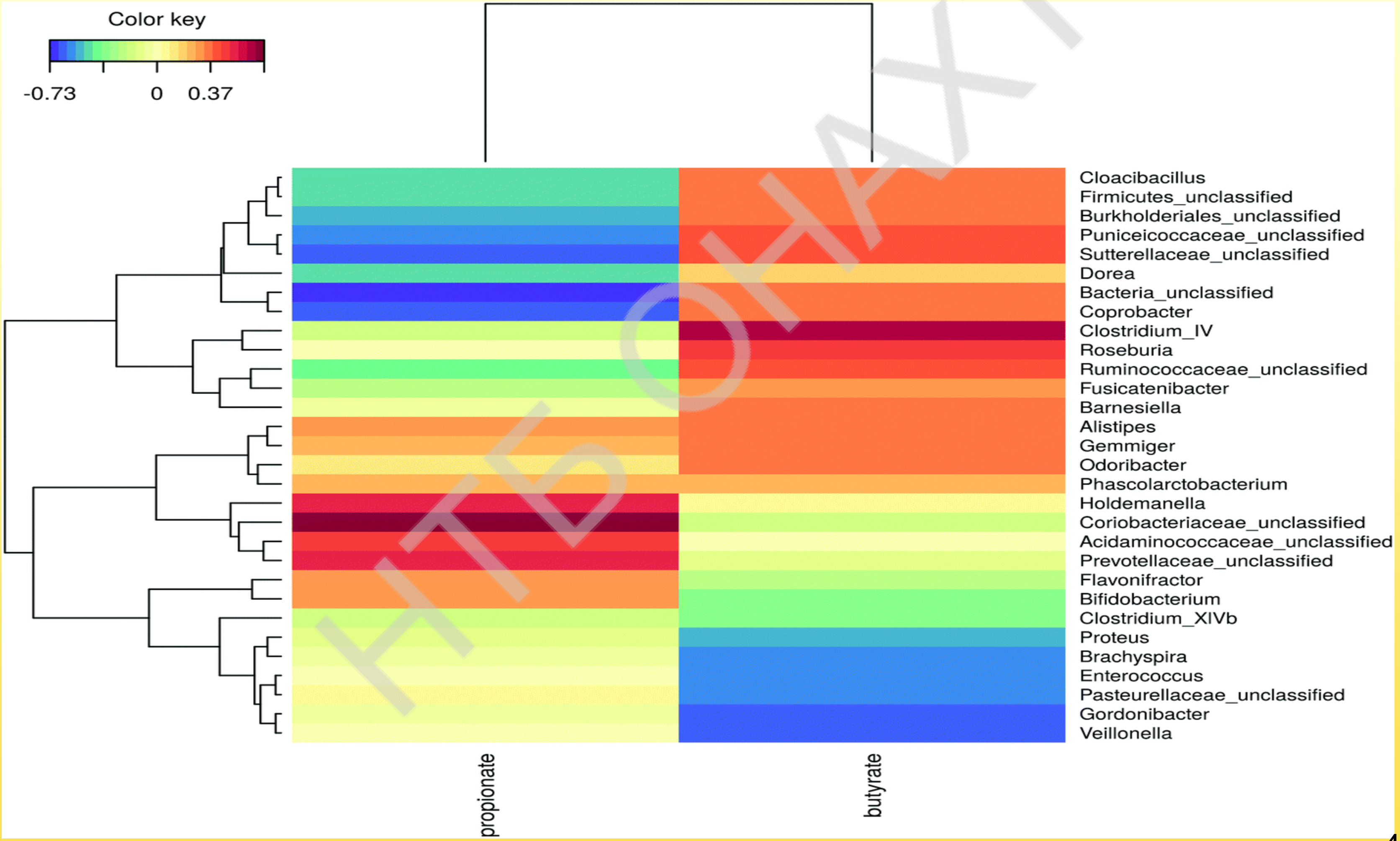
Color key
-0.73
0
0.37
Cloacibacillus
Firmicutes_unclassified
Burkholderiales_unclassified
Puniceicoccaceae_unclassified
Sutterellaceae_unclassified
Dorea
Bacteria_unclassified
Coprobacter
Clostridium_IV
Roseburia
Ruminococcaceae_unclassified
Fusicatenibacter
Barnesiella
Alistipes
Gemmiger
Odoribacter
Phascolarctobacterium
Holdemanella
Coriobacteriaceae_unclassified
Acidaminococcaceae_unclassified
Prevotellaceae_unclassified
Flavonifractor
Bifidobacterium
Clostridium_XIVb
Proteus
Brachyspira
Enterococcus
Pasteurellaceae_unclassified
Gordonibacter
Veillonella
propionate
butyrate

Characteristics of some cereal bioproducts

Product types and cereal material used for the production	Proteins %	Carbohydrates, g/100 g		Probiotic cultures
		digestible	dietary fibers	
"Bilamin" (bran or flour of barley)	17 ± 2	31,7	29,3	L. acidophilus B. adolesentis
"Avena" (oat bran or flour)	19 ± 1	31,3	27,7	L. acidophilus B. longum
"Tonus" (flour of backwheat)	15 ± 1	44,3	23,8	L. acidophilus B. adolesentis L. casei
"Multisyrup" (25% of flour barley, 25% soy flour, 25% backwheat flour)	24 ± 2	27,3	21,4	L. acidophilus L.termophilus L. bulgaricus
"Trisan"(wheat bran)	15,2 ± 1	24,6	33,5	L. acidophilus B. longum

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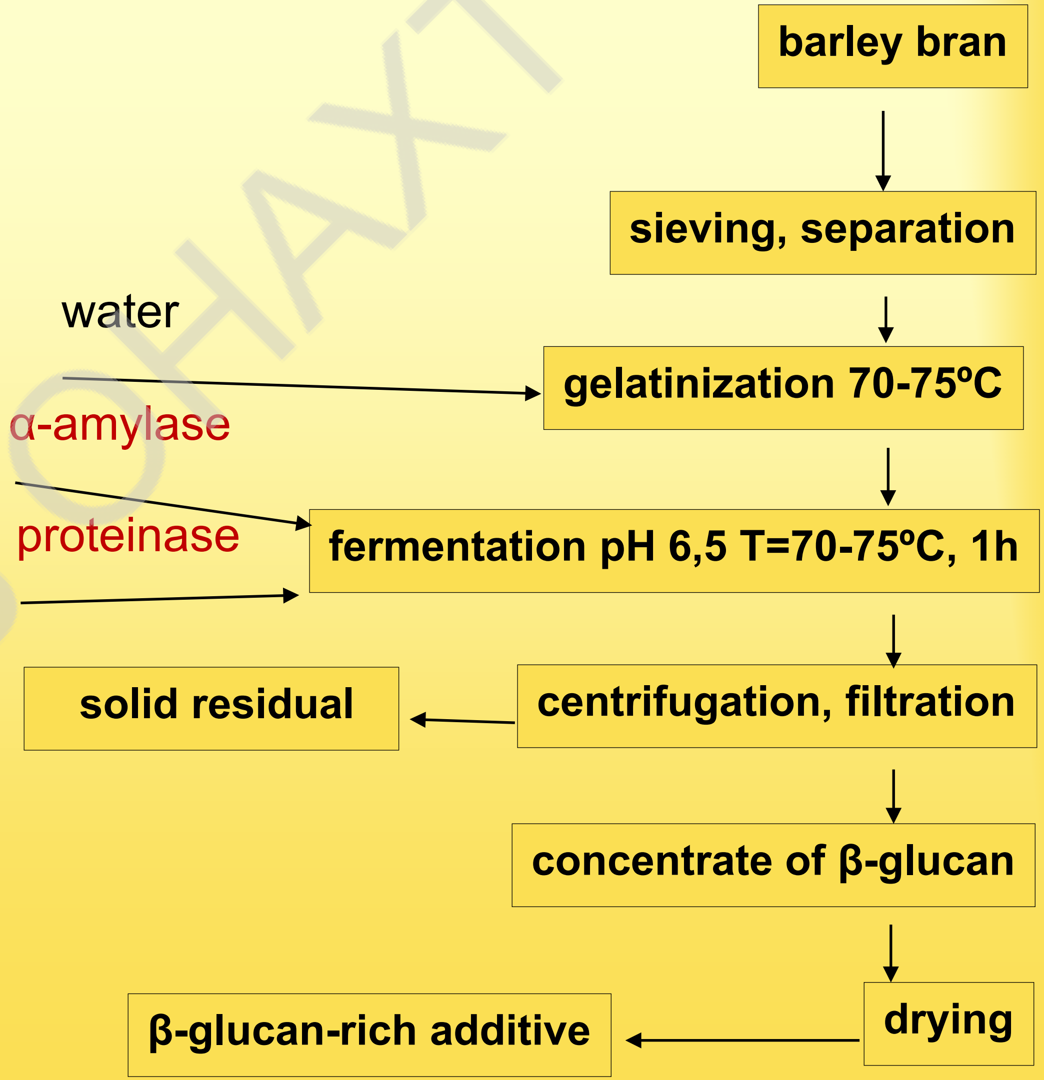
Preparation of β-glucan-rich additives using enzymatic methods

β-D-Glucan content of cereals of Ukraine (% dry weight basis)

Cereal	β-D-Glucan	
	Whole grain	Bran
Barley	8-10	10-16
Oats	5-8	7-9
Rye	2-3	4-5
Wheat	0,5-0,8	0,9-1,5
Corn	0,2-0,3	0,6-0,9
Rice	0,1-0,3	0,4-0,7



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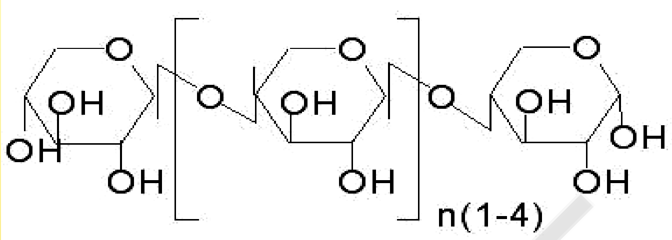
Enzymatic production of prebiotic oligosaccharides (XOS, IMOs) from bran hemicelluloses

Prebiotics are prodused from cereals e.g. xylooligosaccharides (XOS) and isomaltooligosaccharides (IMOS) by enzymatic biotransformations of polysaccharides

Hulls, bran alkaline extraction and purification → **Xylan**

↓ **hydrolysis β-xylanase**



xylo-oligosaccharides

Components	XOS	Components	IMOS
Dry subst.	92,0	Dry subst.	80,4
Xylose	5,7	Panose	34,9
Xylobiose	16,3	Isomaltose	8,4
Xylotriose	22,1	Maltose	14,9
Xylotetraose	45,6	Glucose	24,7
Protein	3,2	Protein	14,2
Ash	2,7	Ash	1,3

Bran → **starch**

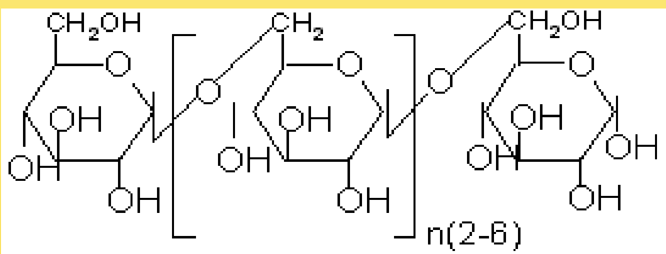
↓ **liquefaction** **α-amylase**

dextrins

↓ **hydrolysis** **β-amylase**

maltose

↓ **transglucosilation** **α-glucosidase**



isomalto-oligosaccharides

From 1000 kg of wheat bran could be obtained:

modified starch	*250 kg*
cereal sweeteners	*75 kg*
dietary fibers (DF)	*150-200 kg*
prebiotics (IMOS, KOS	*60-70 kg*
modified hemicelluloses	*75-90 kg*
fermented bioproducts	*100 kg*
protein concentrate	*60-70 kg*

Одеська національна академія харчових технологій

Odessa National Academia of Food Technologies

Thank you for your attention

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