TORTILLA INDUSTRY ASSOCIATION
TIA

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Clean Label Tortilla Improvers

From dough development to softness

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R&D

Agenda

- Clean label improvers
- Ingredients
- Enzymes
- Conclusions

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STERNWYWIOL GRUPPE

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Clean label improvers

Hopes and Fears

No Aluminium (SAS)
No Emulsifiers
No GMO
No Gums
No Soy
No Lard
No Bleach
No L-cystine
No Metabisulfite
No Calcium Propionate
No Sorbic acid
No Potassium Sorbate
No Fumaric Acid

Fears

Hope

USDA Organic
Natural
FDA Health Clames
5/6 Ingredientes or less

Pronounceable ingredients

Benefits of clean label solutions

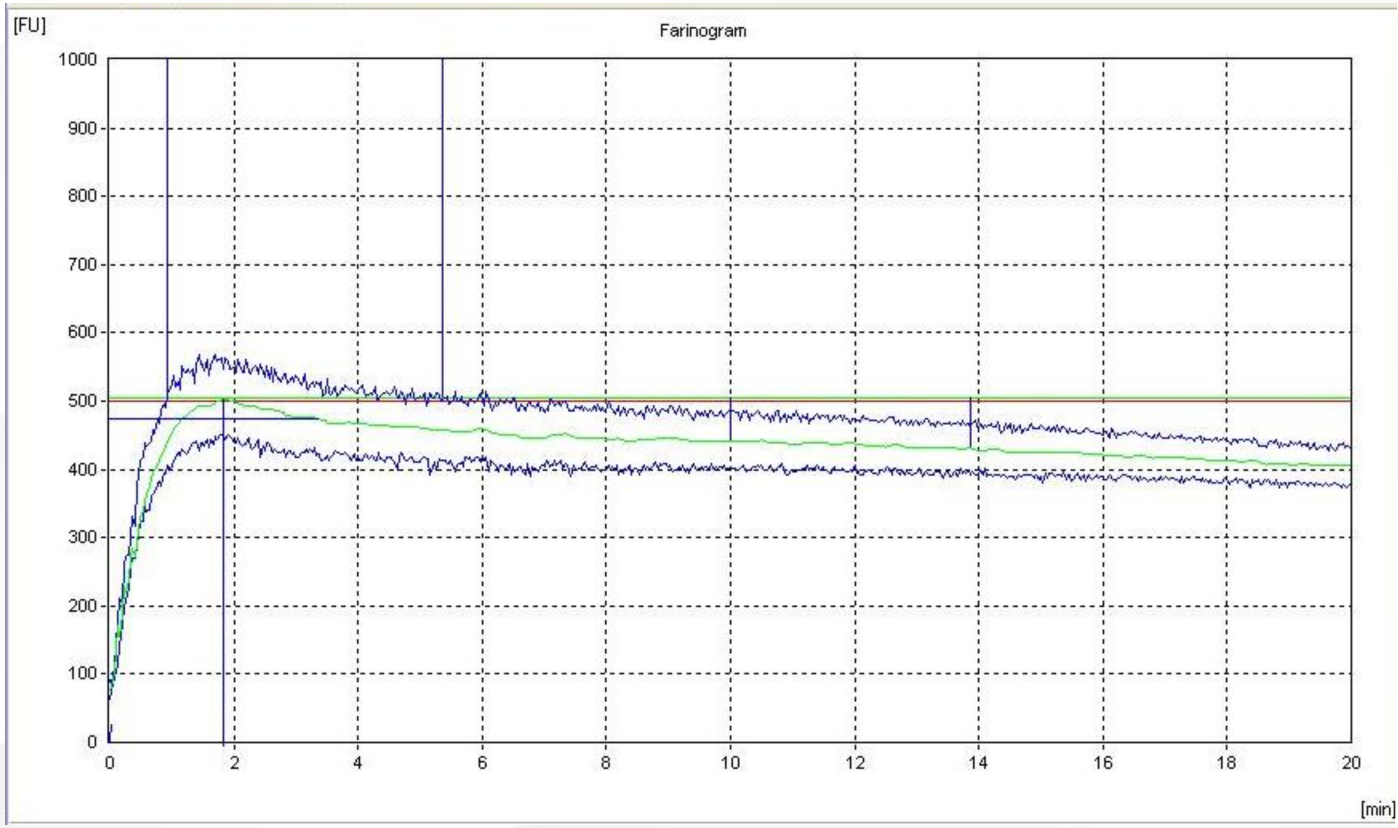
- Cost savings
    - 1 kg enzyme replaces 100 – 1000 kg chemicals
    - Cost savings in storage, transportation and handling
    - Reduced downstream-processing (e.g. for separation of stereoisomers)
- Eco-friendly
    - Clean label
    - Smaller quantities required → smaller carbon dioxide foot print
- Easy implementation
    - No need for special equipment and control systems
- Safer
- Less harsh and/or hazardous process conditions

Ingredients

Effect of Fibers on Dough & Baked Good Properties

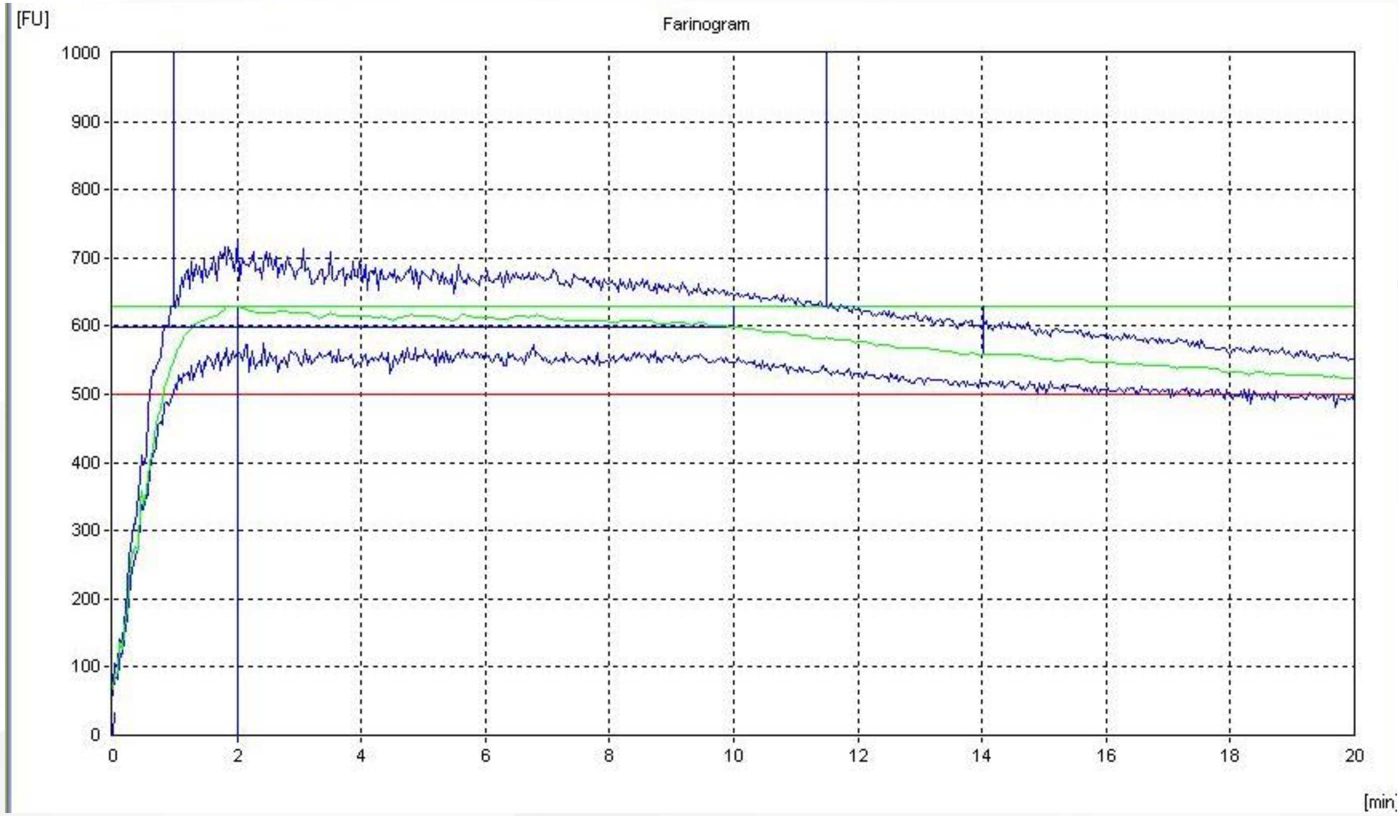
- Increased water absorption
- Improved Handling
    - Reduction of sticking of dough
    - Enhanced or degreased extensibility
- Reduction of pull back
- Reduction of shrinkage upon baking

Base Flour



Water absorption (500FU)	55,1%
Development time	1,9 min
Stability	4,4 min

2% Fibers

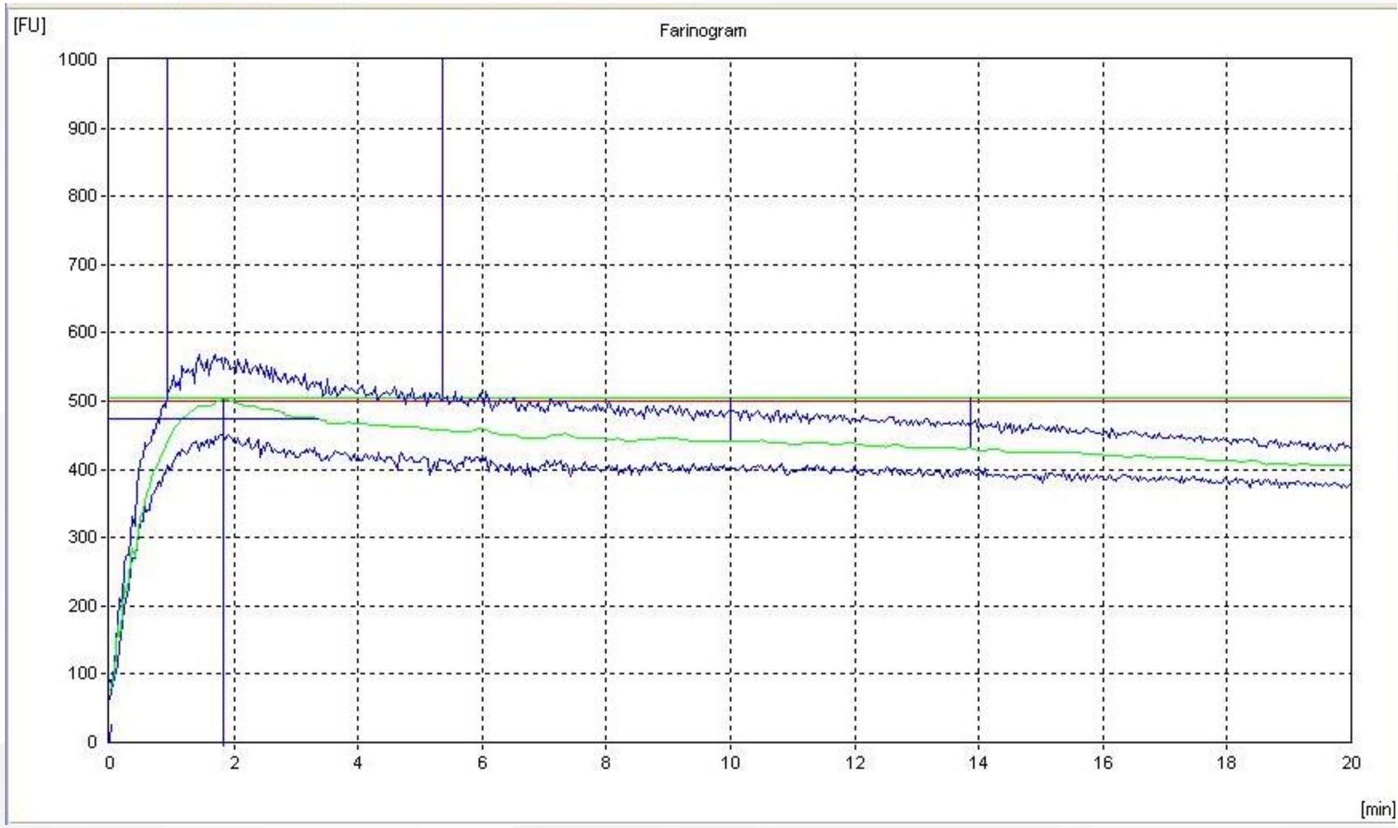


Waterabsorption (500FU)	58,5%
Development time	2 min
Stability	10,5 min

Effects of gluten on dough

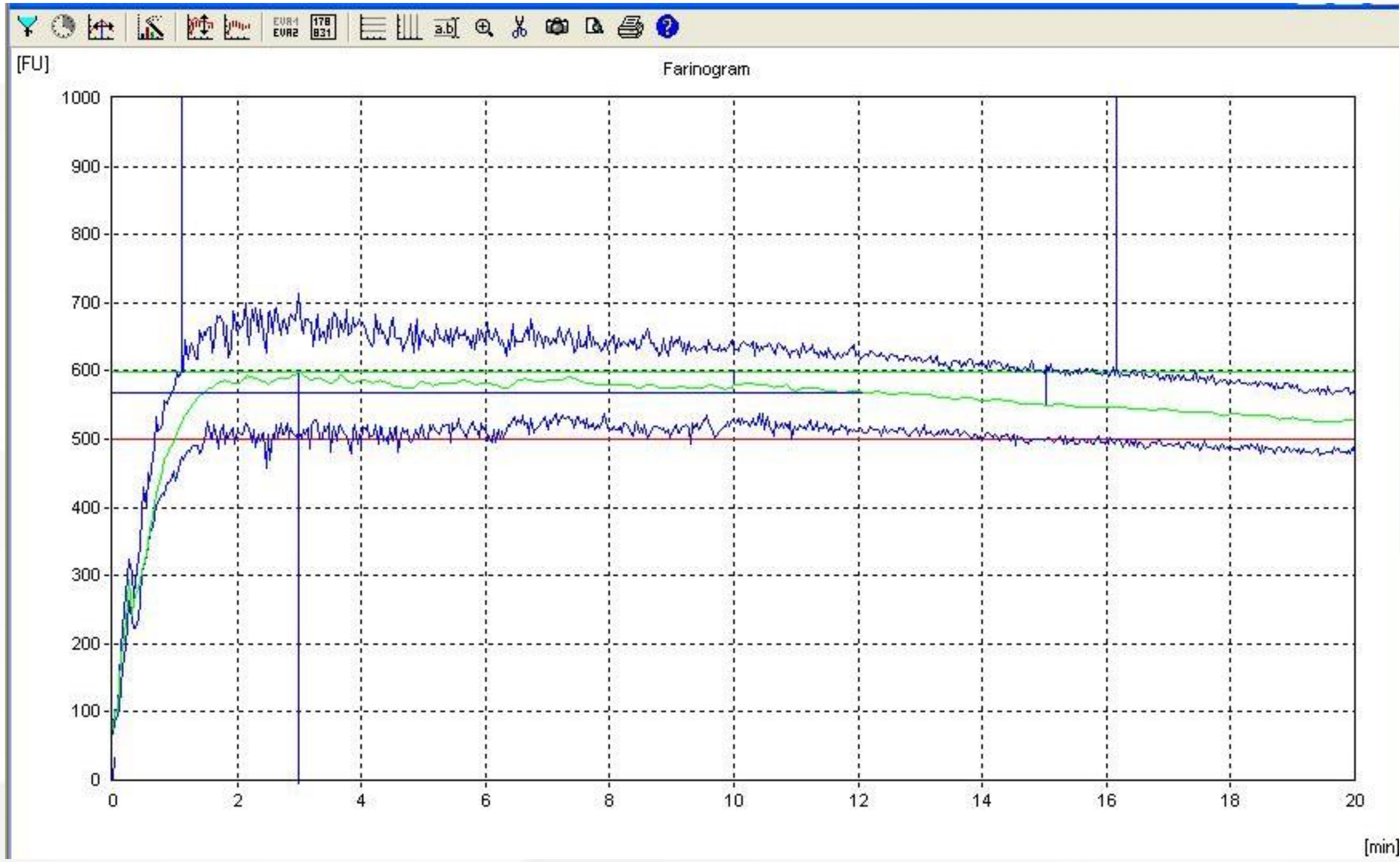
- Increased water absorption
- Improved Handling
  - Reduction of sticking of dough
  - Enhancing of dough stability
- Better stability
- Better Product handling

Base Flour



Water absorption (500FU)	55,1%
Development time	1,9 min
Stability	4,4 min

3% Gluten



Water absorption (500FU)	57,5%
Development time	3 min
Stability	15,1 min

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Enzymes

Enzyme History

1833 Anselme Payen and Jean Persoz isolate an enzyme complex from malt and call it “diastase”

1835 Jon Jakob Berzelius terms the action of ferments “catalytic”.

1874 Christian Hansen extracts rennet from dried calves’ stomach.

1877 Wilhelm Kühne proposes the name “enzyme”, from Greek *en* = in, and *zyme* = yeast/sourdough.

1894 Jokichi Takamine develops a fermentation process using *A. oryzae* grown on wet rice or wheat bran as surface culture to produce an amylase (“Koji culture”) → first U.S. patent for microbial enzymes

1897 Eduard Buchner proves that cell-free extracts from yeast convert glucose into ethanol and CO_2; he calls the enzyme “zymase” → Nobel prize in 1907

1922 Alexander Fleming published his work on the anti-microbial enzyme lysozyme (→ penicillin in 1928 → Nobel prize in 1945)

1926 James Batcheller Sumner crystallizes urease from jack bean proving enzymes to be single molecules. → Nobel prize 1946

1930 John Howard Northrop and Wendell Meredith Stanley demonstrate on pepsin that enzymes are pure proteins → Nobel prize in 1946

1930s Otto Röhm uses digestive enzymes from pancreas for bating of *licheniformis* leather (previously, animal excrements were used); trypsin from pancreatin is introduced as first detergent enzyme

1934 O. Röhm develops pectinase for fruit processing

1937 J. B. Sumner crystallizes catalase

1950s Fungal amylase for sugar syrup from starch

1956 E. Jaag develops bacterial protease for bating → production by Gebrüder Schnyder & Swiss Ferment AG

1958 Launch of the first baking enzyme by Röhm

1960 Novo Industries introduces alcalase, alkaline bacterial protease from *B. licheniformis*

1960s Glucoamylase is introduced to the starch industry

1965 David Chilton Phillips elucidates the structure of lysozyme by X-ray crystallography

1973 Invention of xylanase for baking

1983 PCR by Kary Banks Mullis → Nobel prize in 1983

2020 Emmanuelle Charpentier and Jennifer A. Doudna developed the Crispr CAS tool, which can change the DNA of animals, plants and microorganisms with high precision → Nobel prize in 2020

Enzymes in Food Applications, Examples

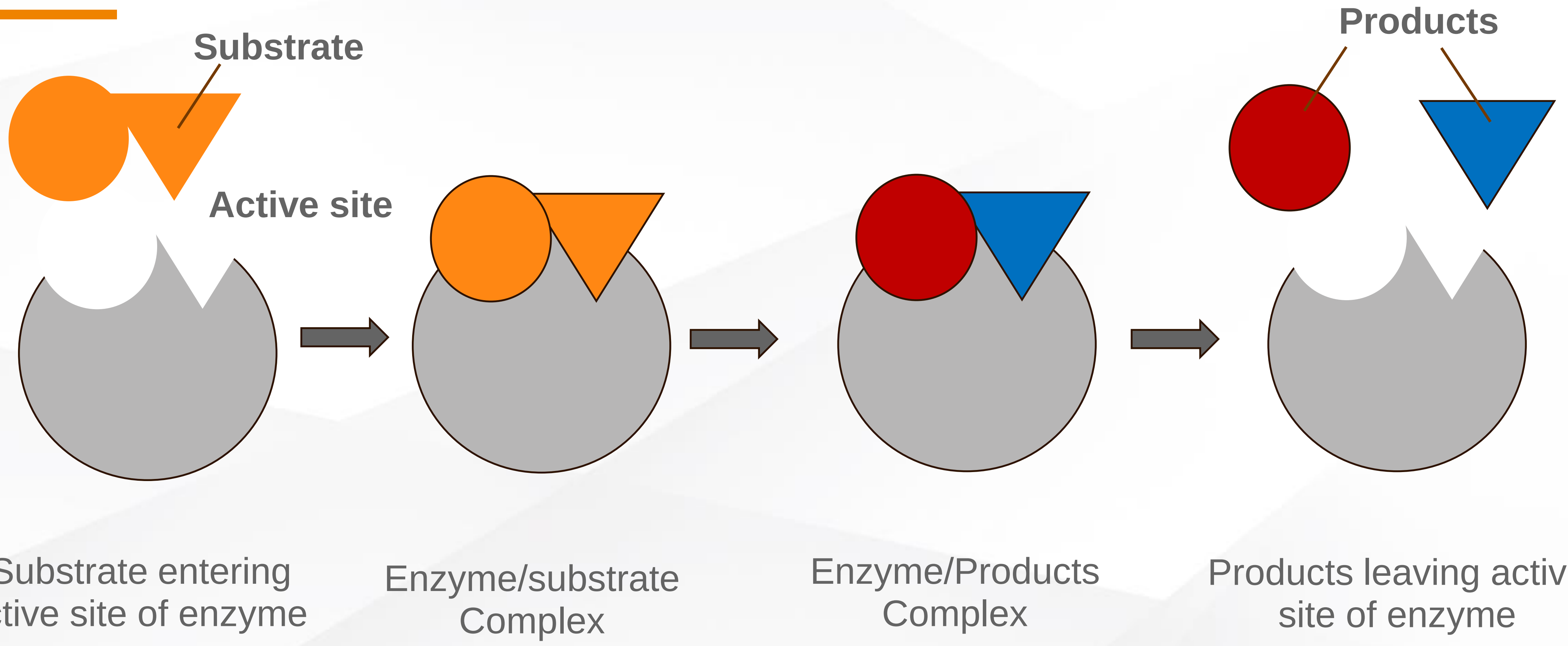
Application	Enzyme examples	Purpose
Baking	Amylase, xylanase, protease, carboxyl esterase, lipoxygenase, oxidase	Volume yield, processing properties, dough stability, bleaching, shelf-life
Brewing	Amylase, glucanase, protease	Fermentation, stability
Cheese	Protease, lipase, phospholipase, peroxidase, lysozyme	Structure, flavor, yield, bleaching, preservation
Confectionery	Invertase	Structure, shelf-life
Egg	Glucose-oxidase, phospholipase	Glucose removal, heat stability, emulsifying properties
Flavors	Lipase, lipoxygenase	Formation of free fatty acids, aldehydes
Fruit & vegetables	Pectinase, pectinmethyl esterase	Softening, firming
Juices	Pectinase, arabinase, amylase	Yield, clarification, stabilization
Lipids	Lipase, phospholipase	Transesterification, hydrolysis, degumming
Meat & fish	Protease, transglutaminase	Softening, firming
Milk	Lactase	Lactose removal
Sugar	Dextranase, amylase	Viscosity reduction, clarification
Wine	Pectinase, protease, laccase, lysozyme	Clarification, stabilization, flavor, removal of off-flavors, preservation

Estimated Enzyme Consumption via Baked Goods

- USA: approx. 133Lbs (60 kg) flour per person
- Assumed treatment: 0.25 g enzyme protein on 100 kg flour
  - → 0.15 g amylase in 100 kg bread
  - → 6 – 7 years for uptake of 1 g enzyme protein (amylase)
  - → 189 years for uptake of 1 oz enzyme protein (amylase)

- Other enzymes in baking used less frequently and at even lower dosage

Enzymes are Biocataclysts



Substrate entering active site of enzyme

Enzyme/substrate Complex

Enzyme/Products Complex

Products leaving active site of enzyme

Enzyme Activity Depends on Many Factors

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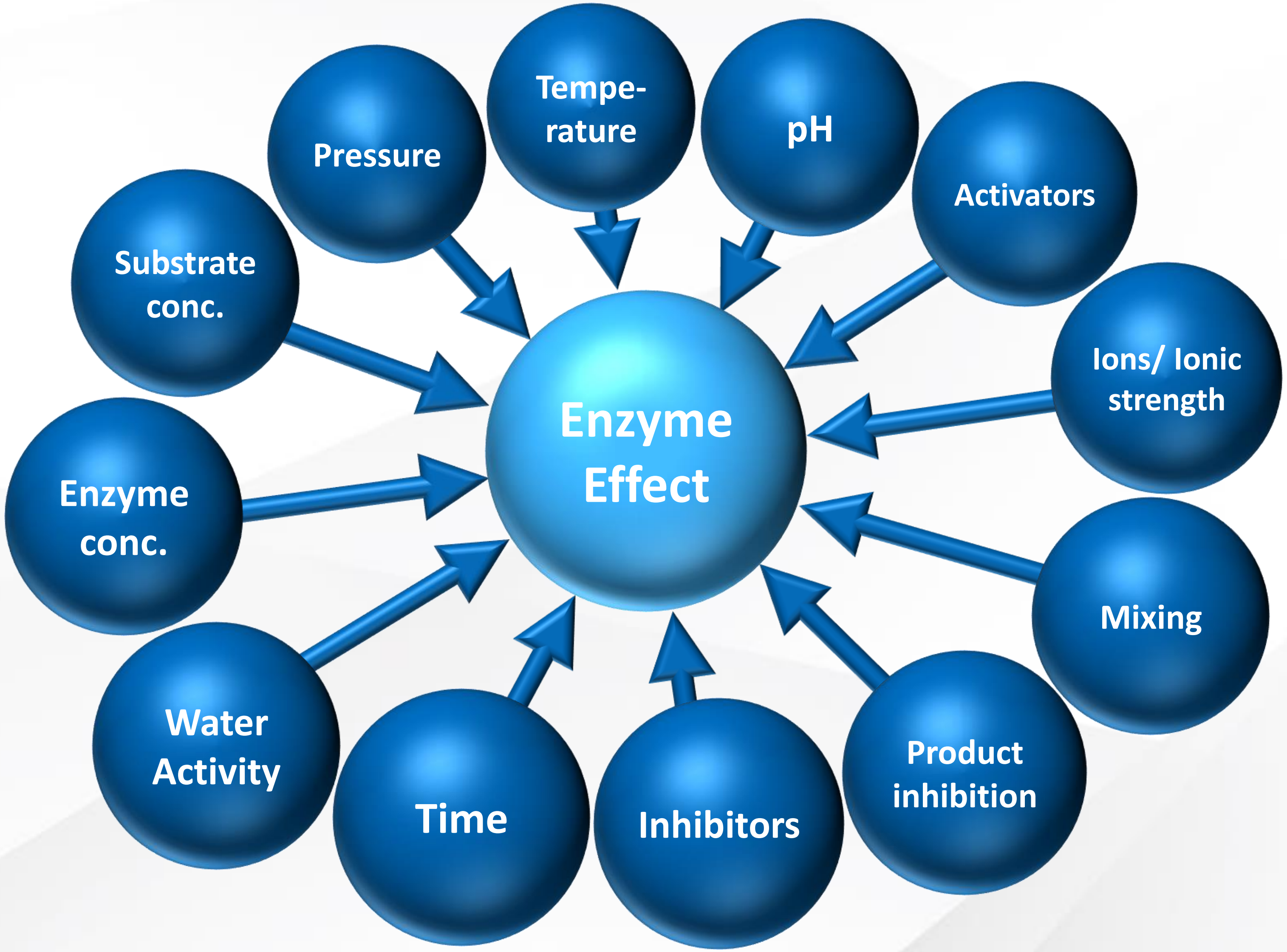

Enzyme Effect

Pressure

Tempe-rature

pH

Activators

Substrate conc.

Ions/ Ionic strength

Enzyme conc.

Mixing

Water Activity

Time

Inhibitors

Product inhibition

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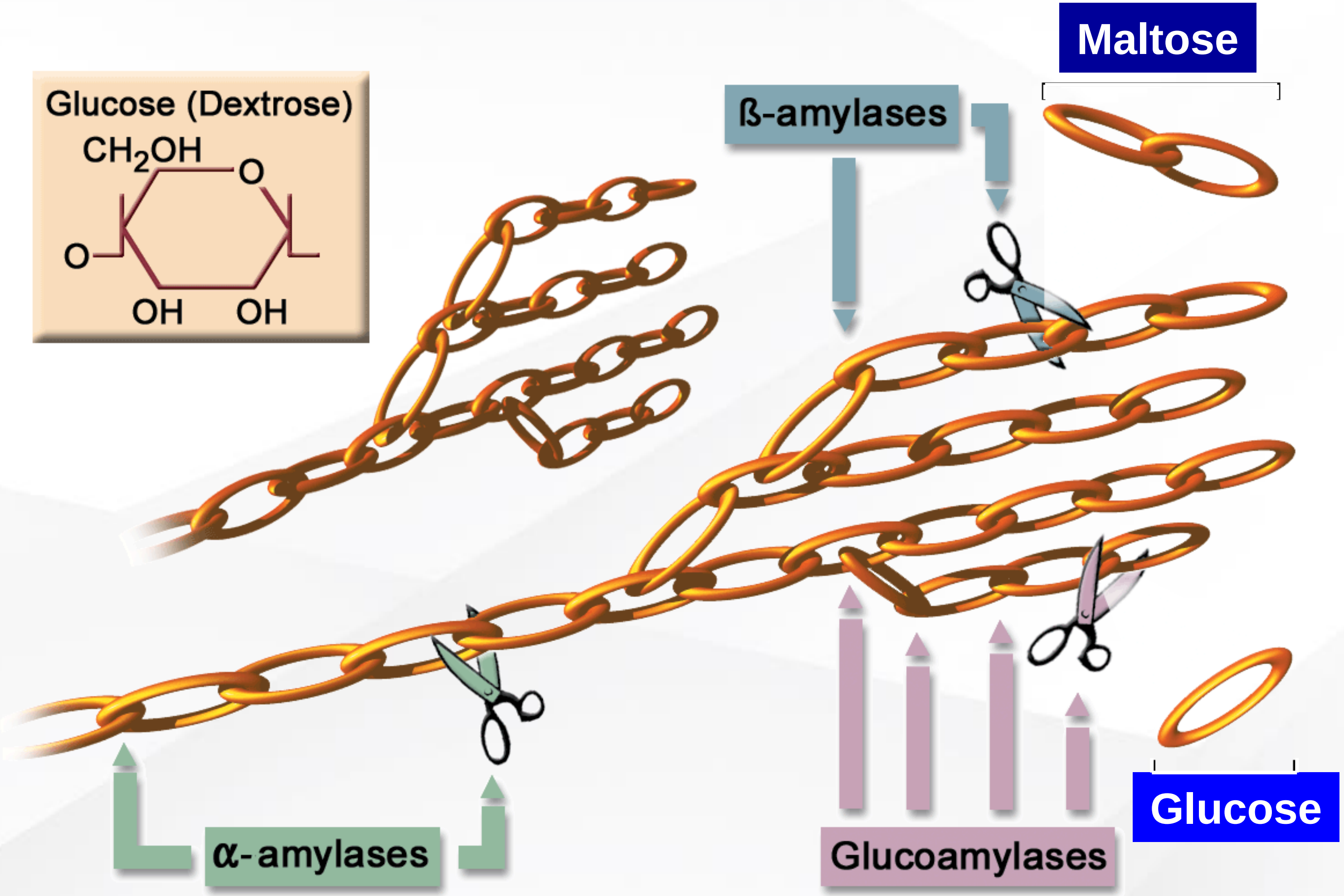
Amylolytic Enzymes

Amylolytic Enzymes used in Baking



Amylolytic Enzymes used in Baking

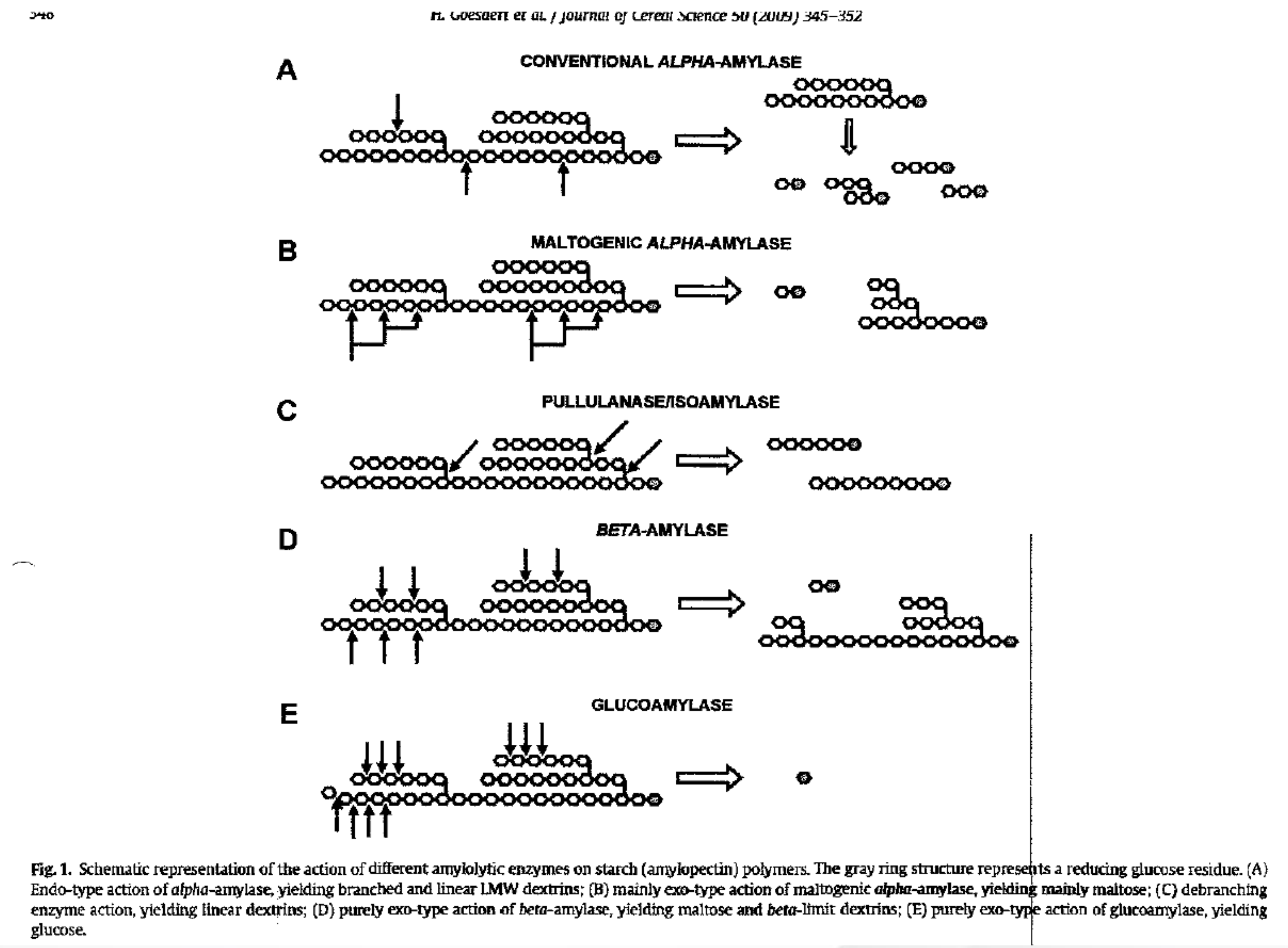
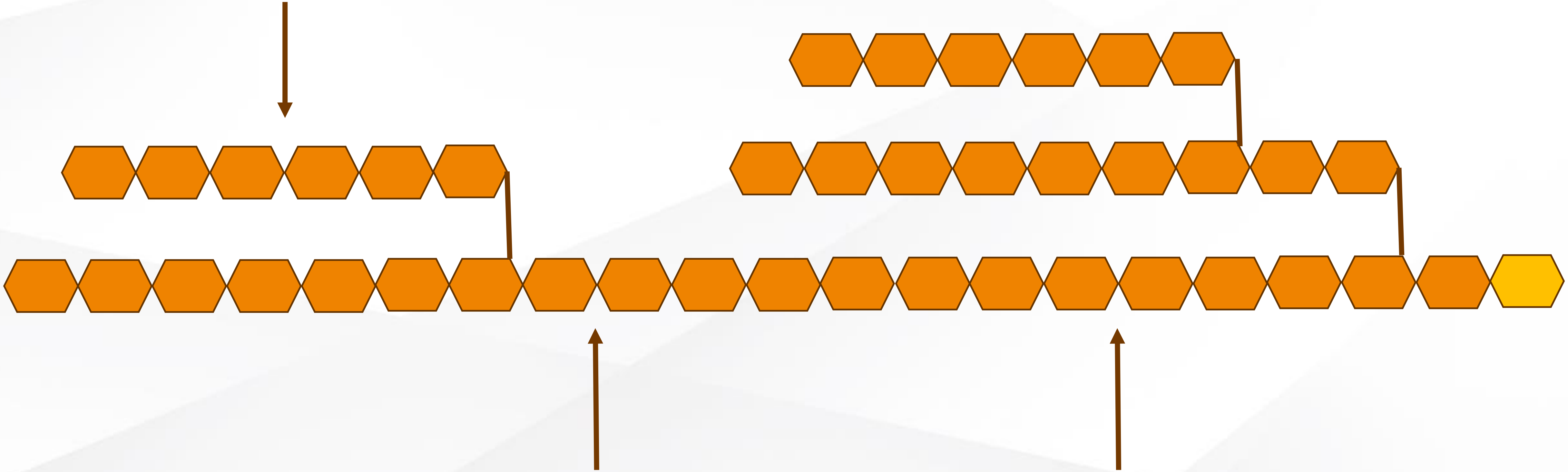
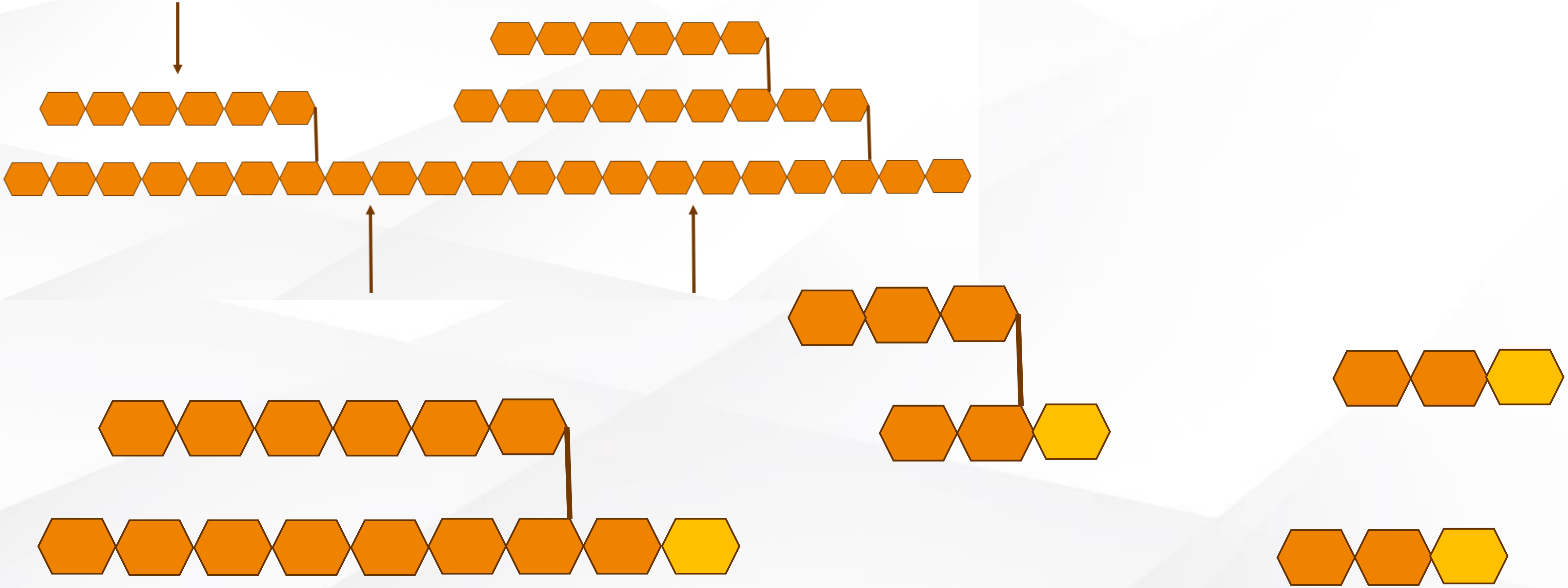


Fig. 1. Schematic representation of the action of different amylolytic enzymes on starch (amylopectin) polymers. The gray ring structure represents a reducing glucose residue. (A) Endo-type action of *alpha*-amylase, yielding branched and linear LMW dextrins; (B) mainly exo-type action of maltogenic *alpha*-amylase, yielding mainly maltose; (C) debranching enzyme action, yielding linear dextrins; (D) purely exo-type action of *beta*-amylase, yielding maltose and *beta*-limit dextrins; (E) purely exo-type action of glucoamylase, yielding glucose.

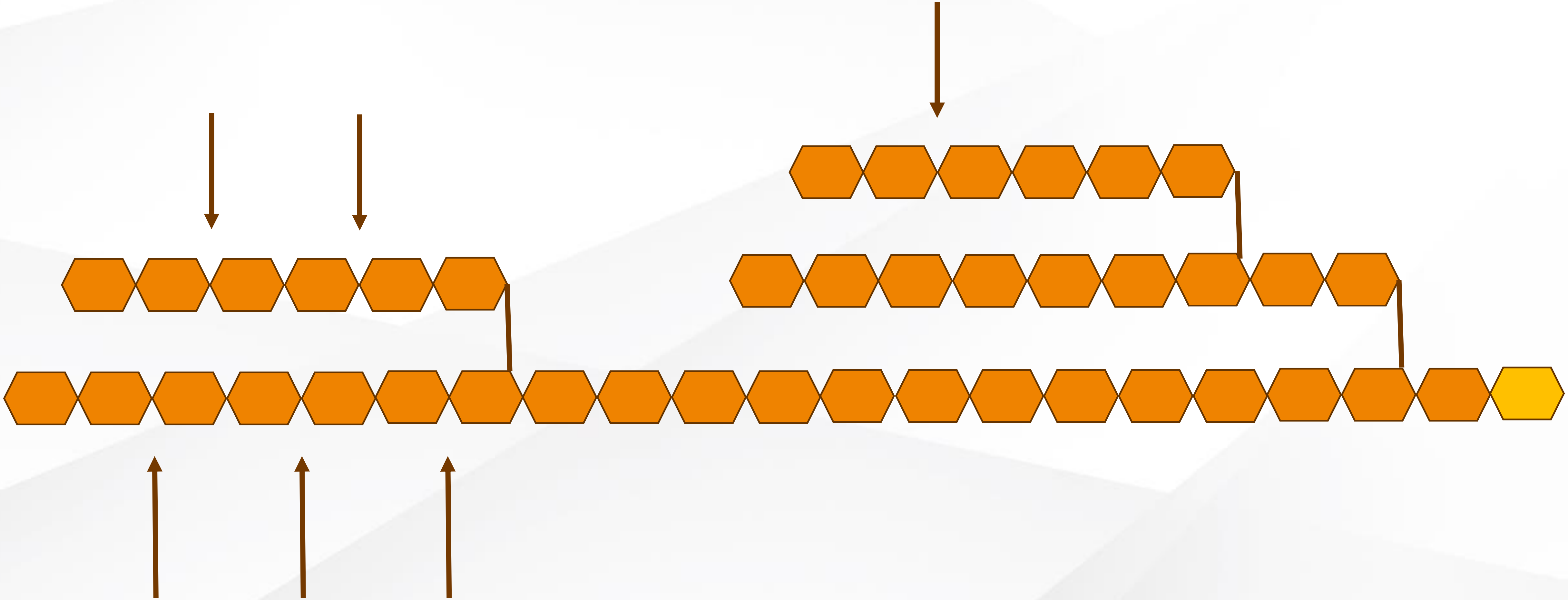
Alpha-amylase



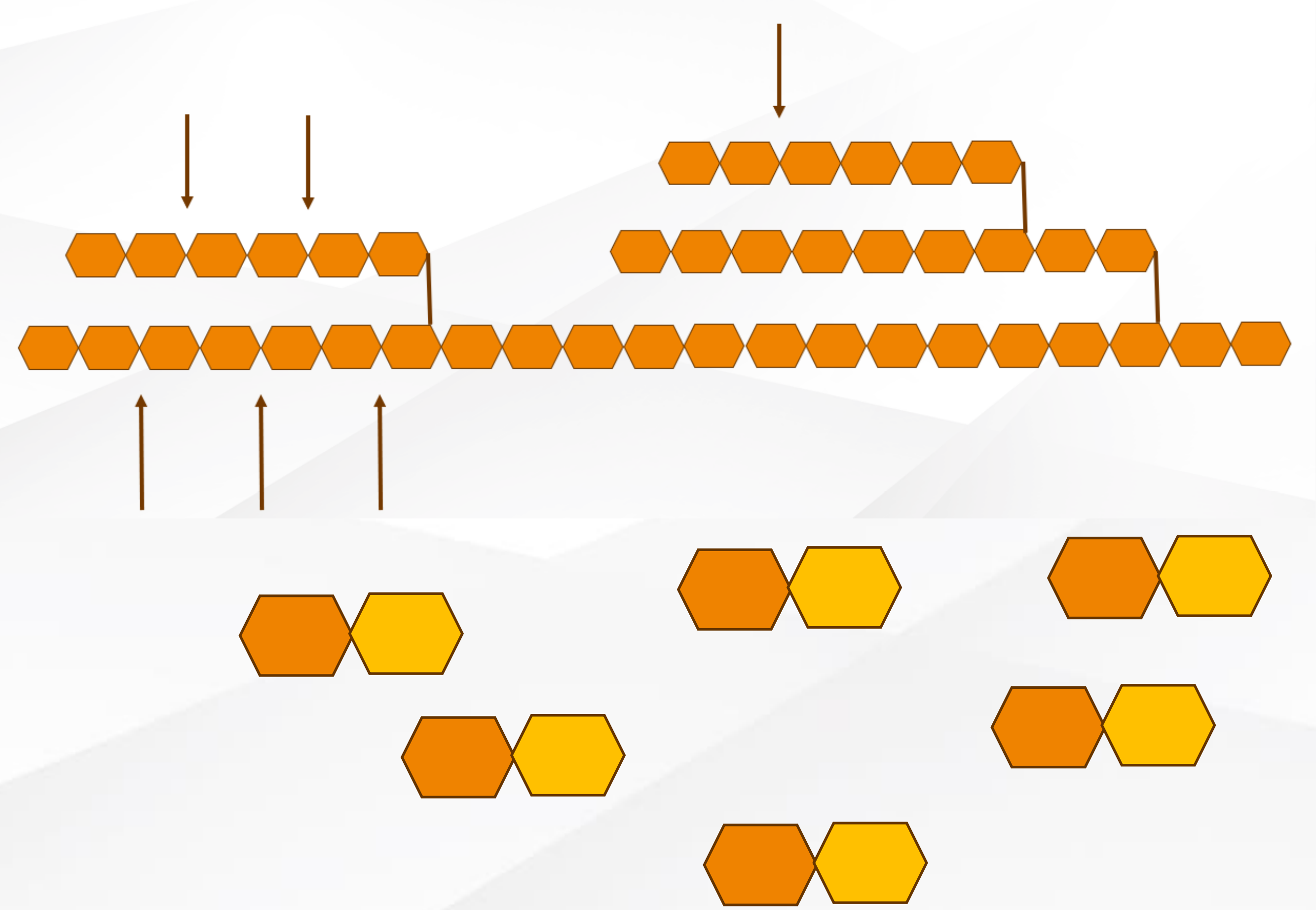
Alpha-amylase



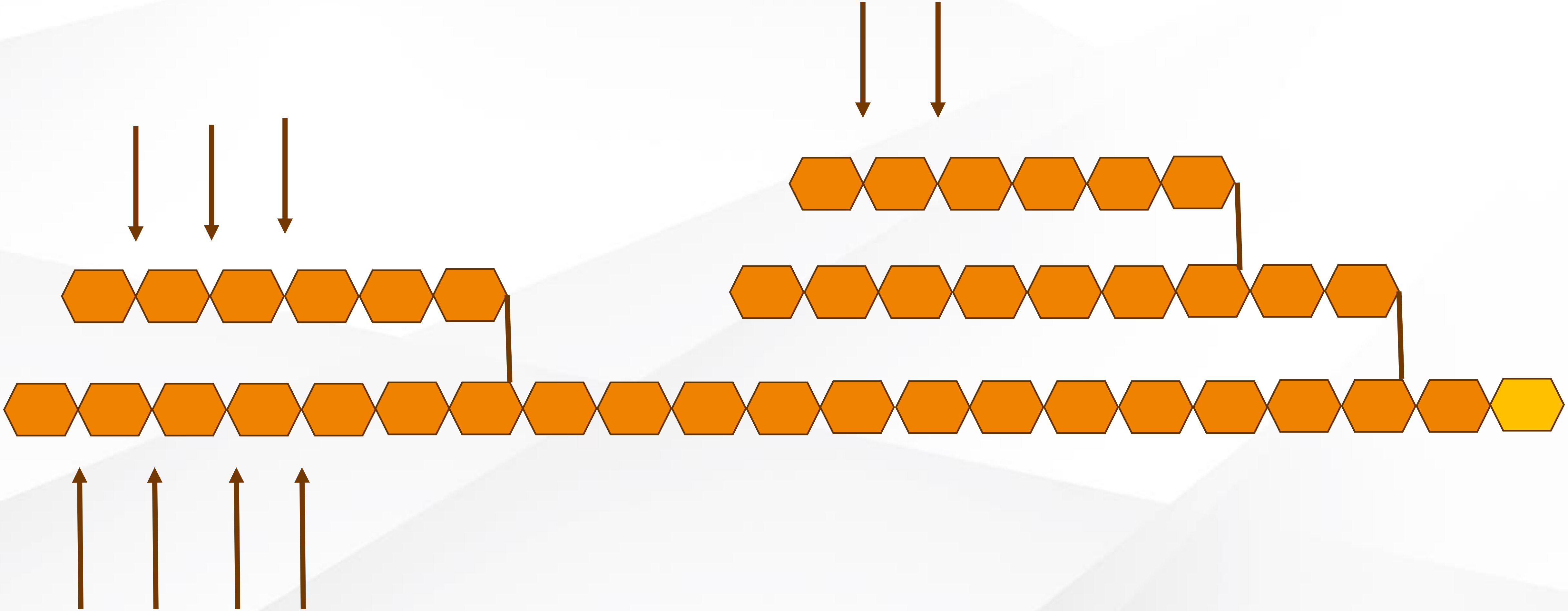
Beta-amylase



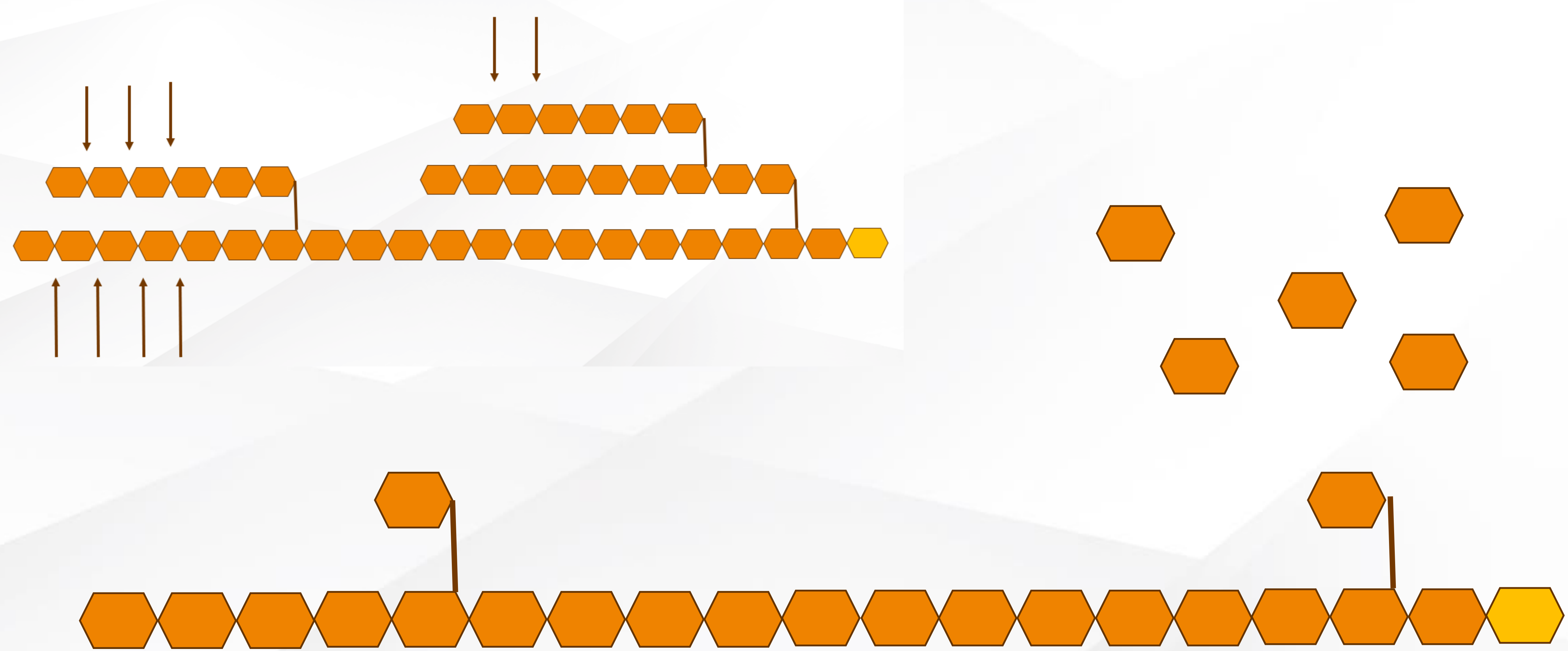
Beta-amylase



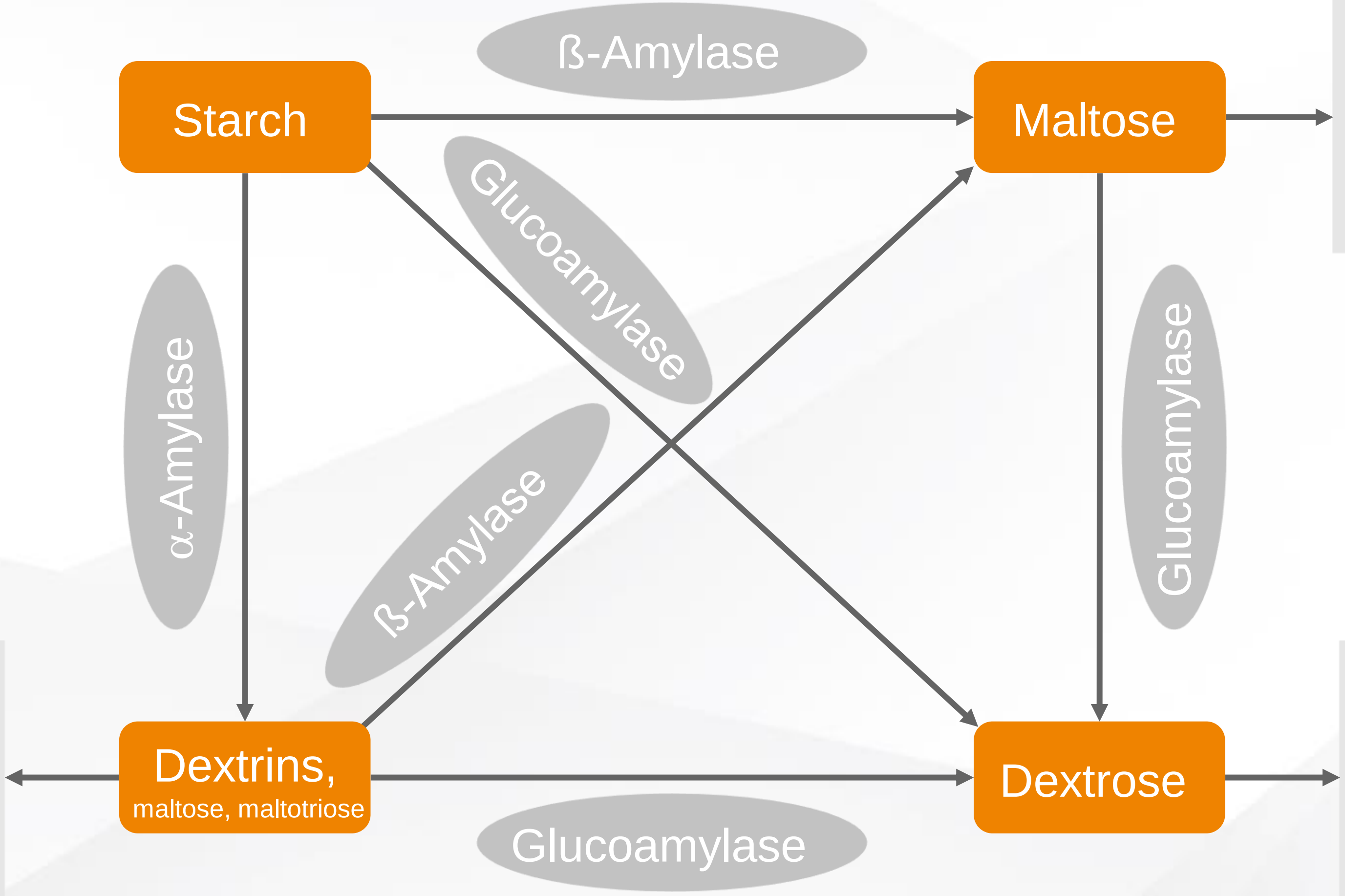
Glucoamylase



Glucoamylase



Effects of Amylases on dough and baked goods

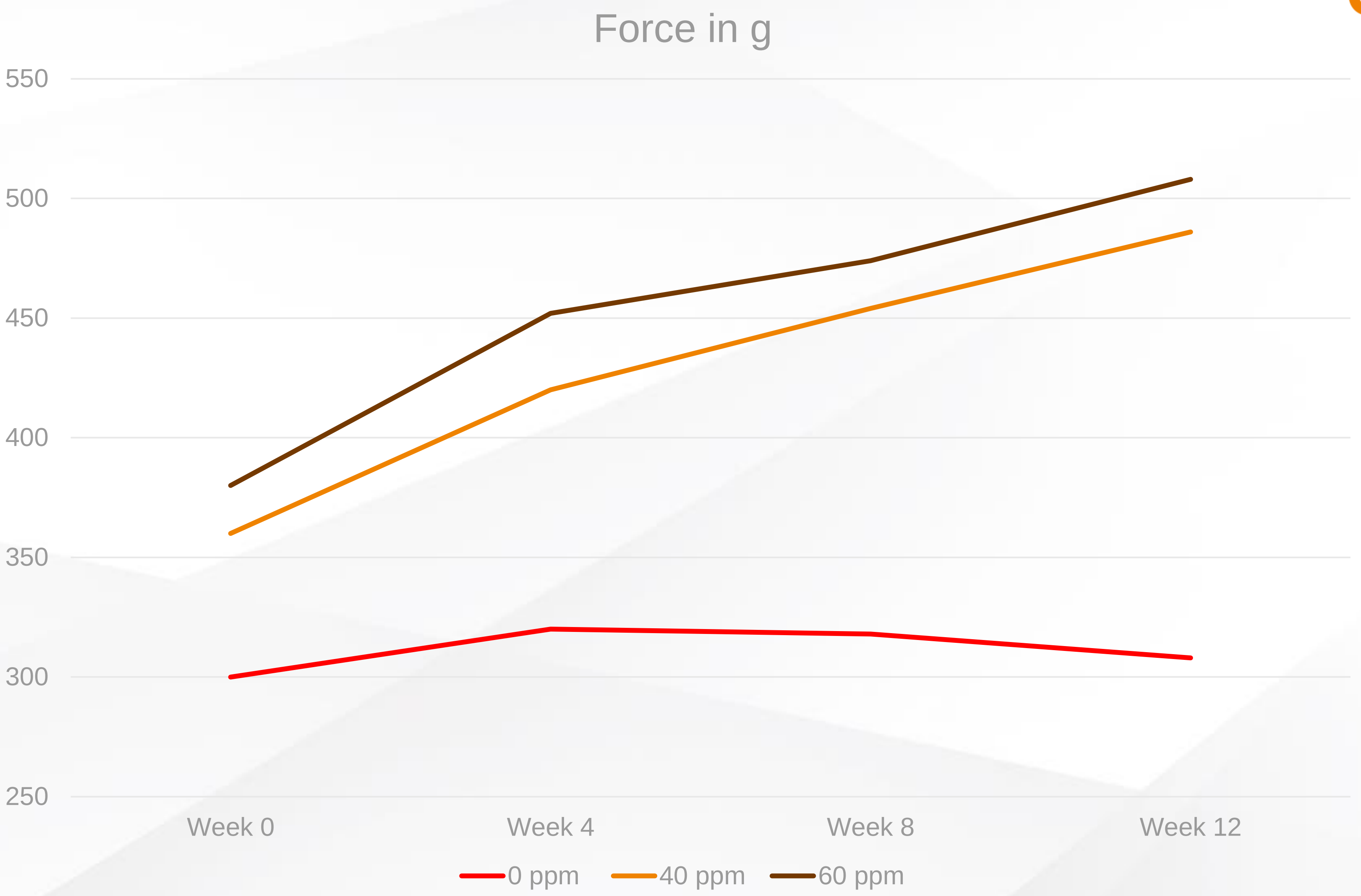


- yeast food
- gas formation
- dough aeration
- bread volume
- flavor, color

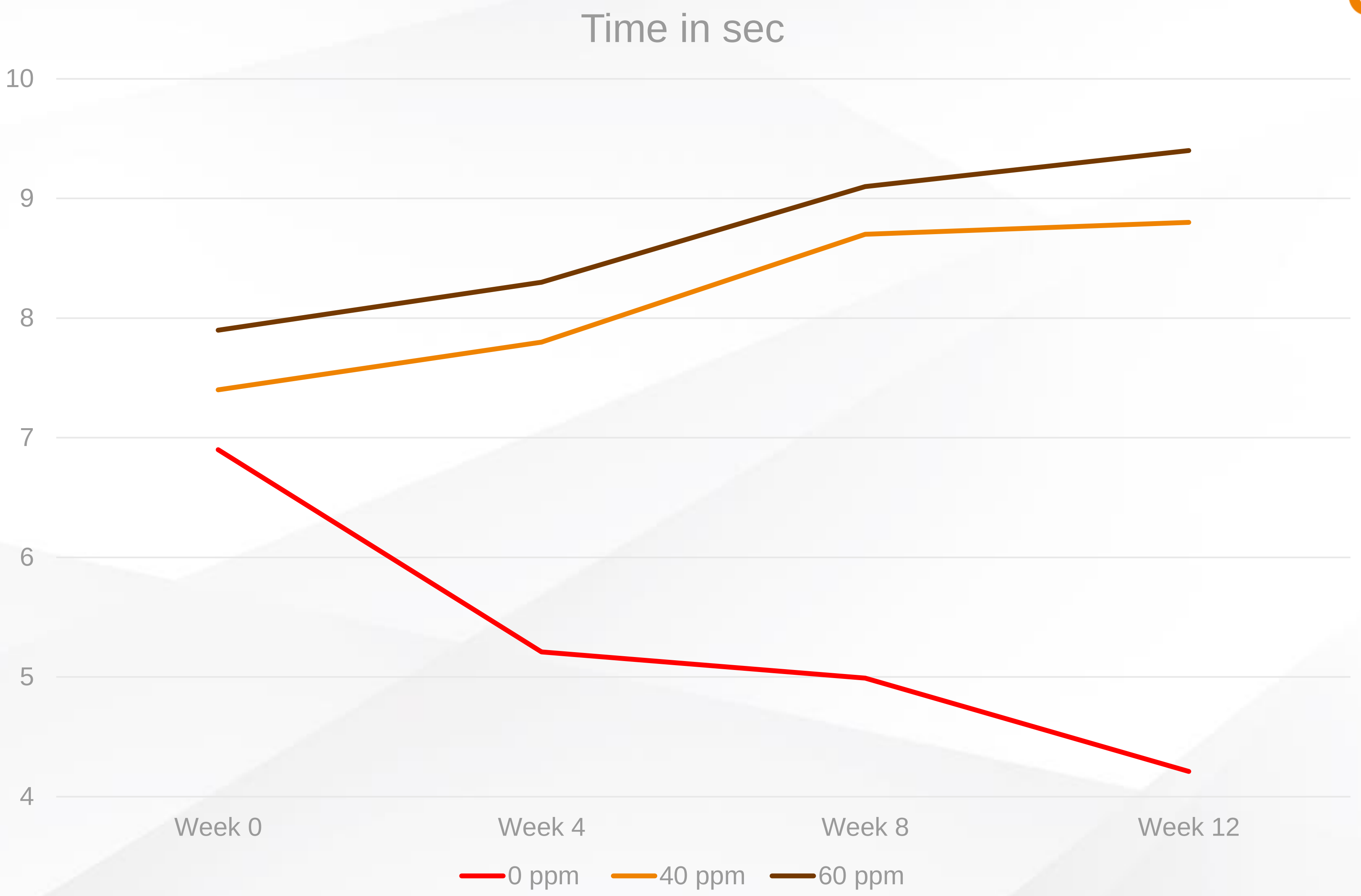
- water release
- viscosity decrease
- dough aeration
- crumb softness
- bread structure

- yeast food
- *fast* gas formation
- dough aeration
- bread volume
- flavor, color

Amylases


Force in g
550
500
450
400
350
300
250
Week 0
Week 4
Week 8
Week 12
0 ppm
40 ppm
60 ppm

Amylases



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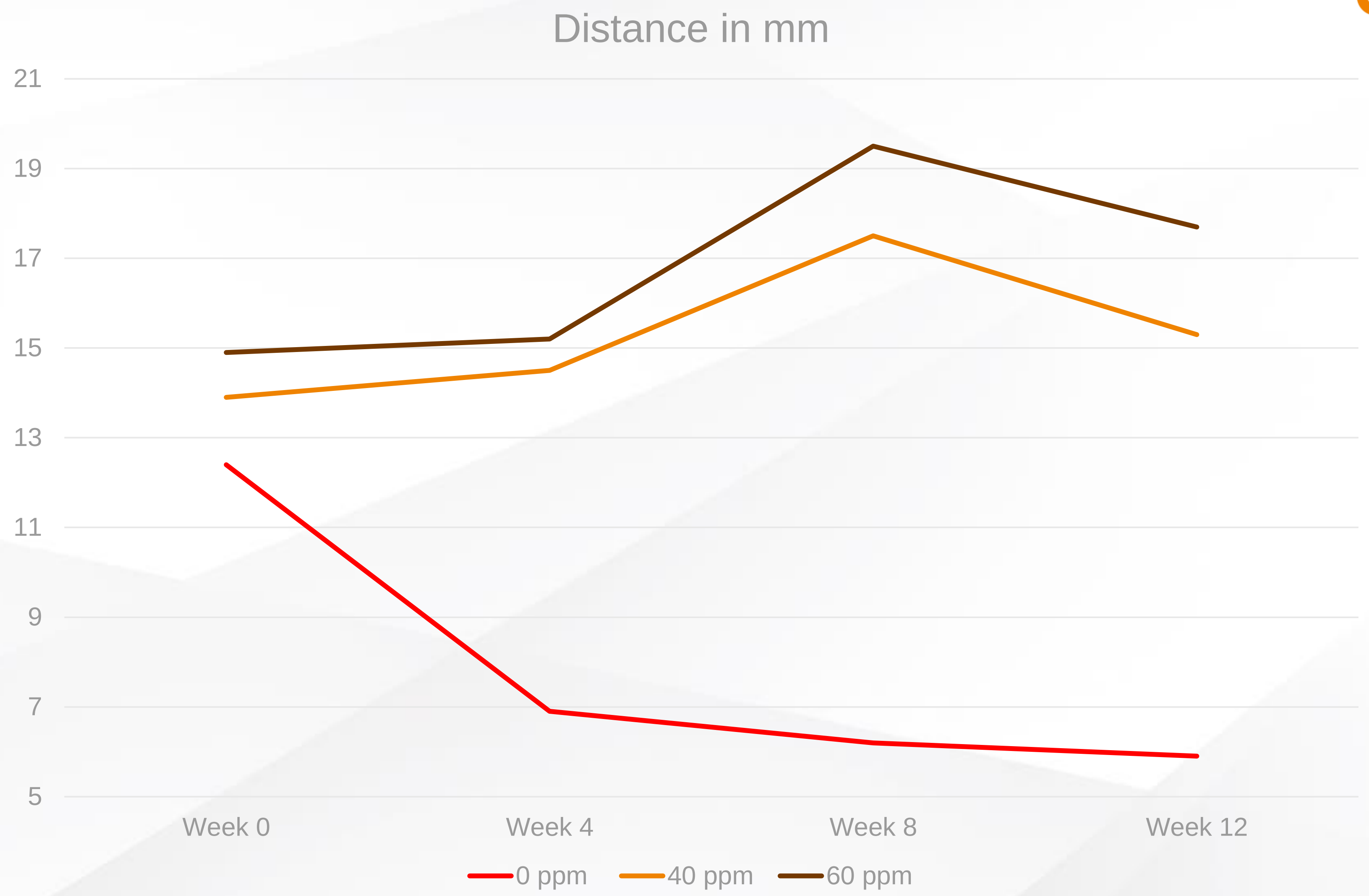
Amylases


Distance in mm
21
19
17
15
13
11
9
7
5
Week 0
Week 4
Week 8
Week 12
0 ppm
40 ppm
60 ppm

Retrogradation in Tortillas

- Loss of Foldability
- Loss of Freshness and Softness
- Hardness
- Cracking

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Proteolyic Enzymes

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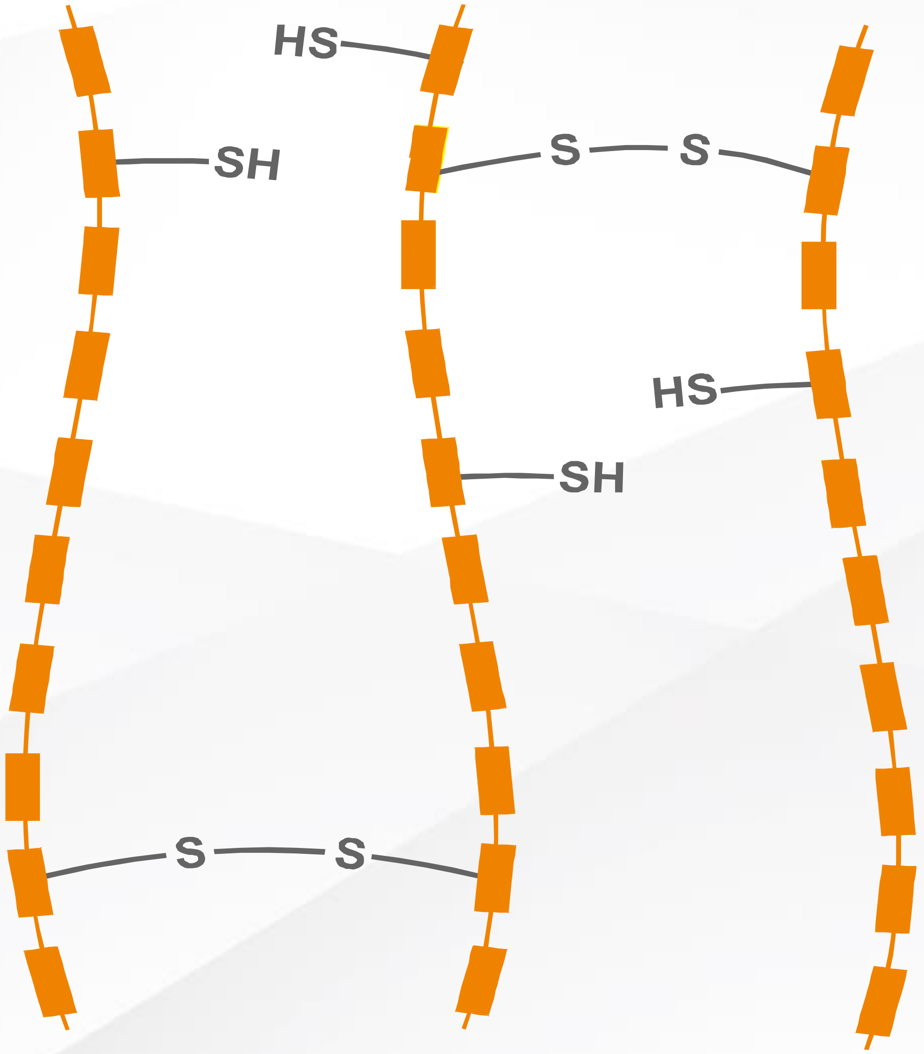
Disulfide Bonds in Gluten

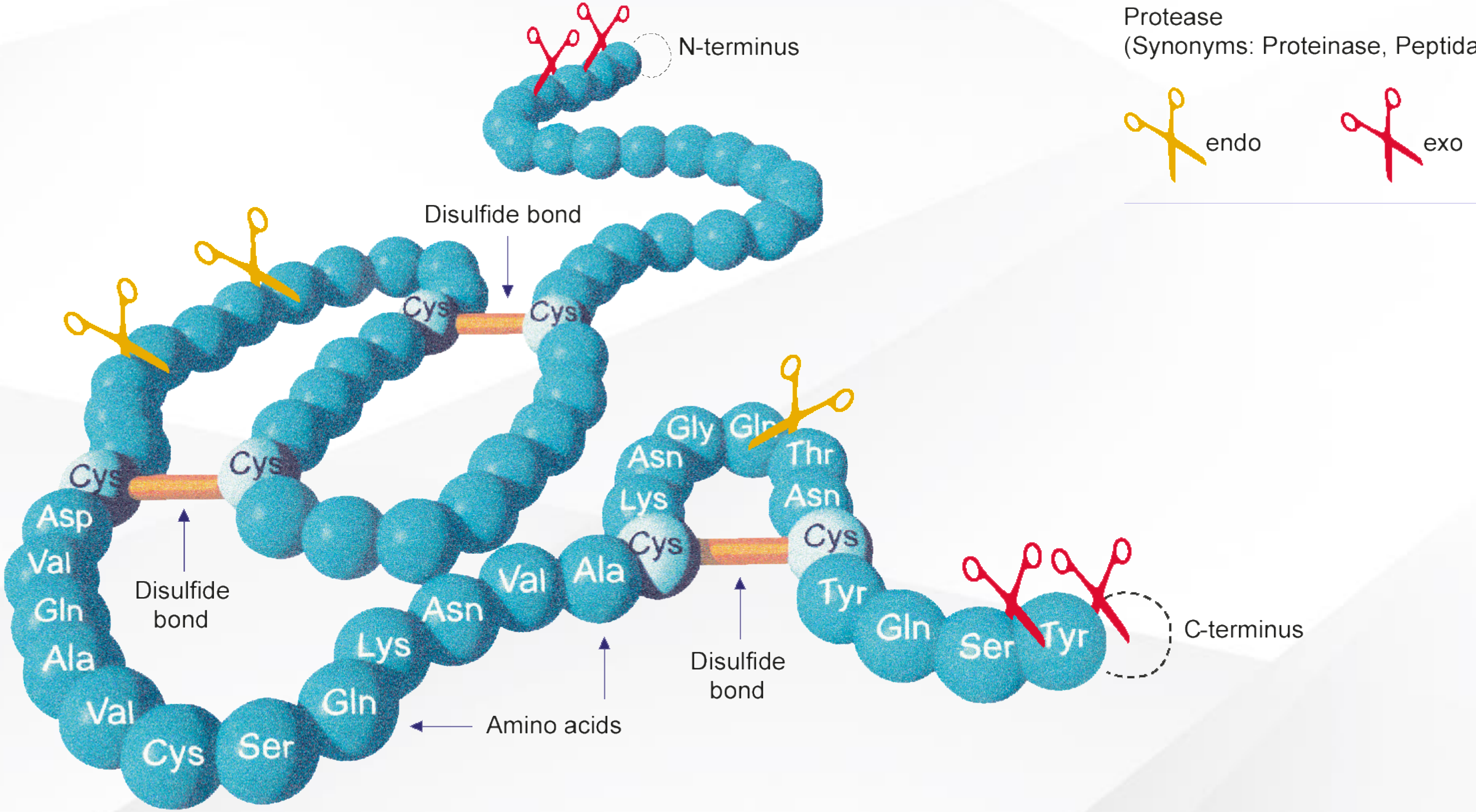


– SH = Sulfhydryl groups

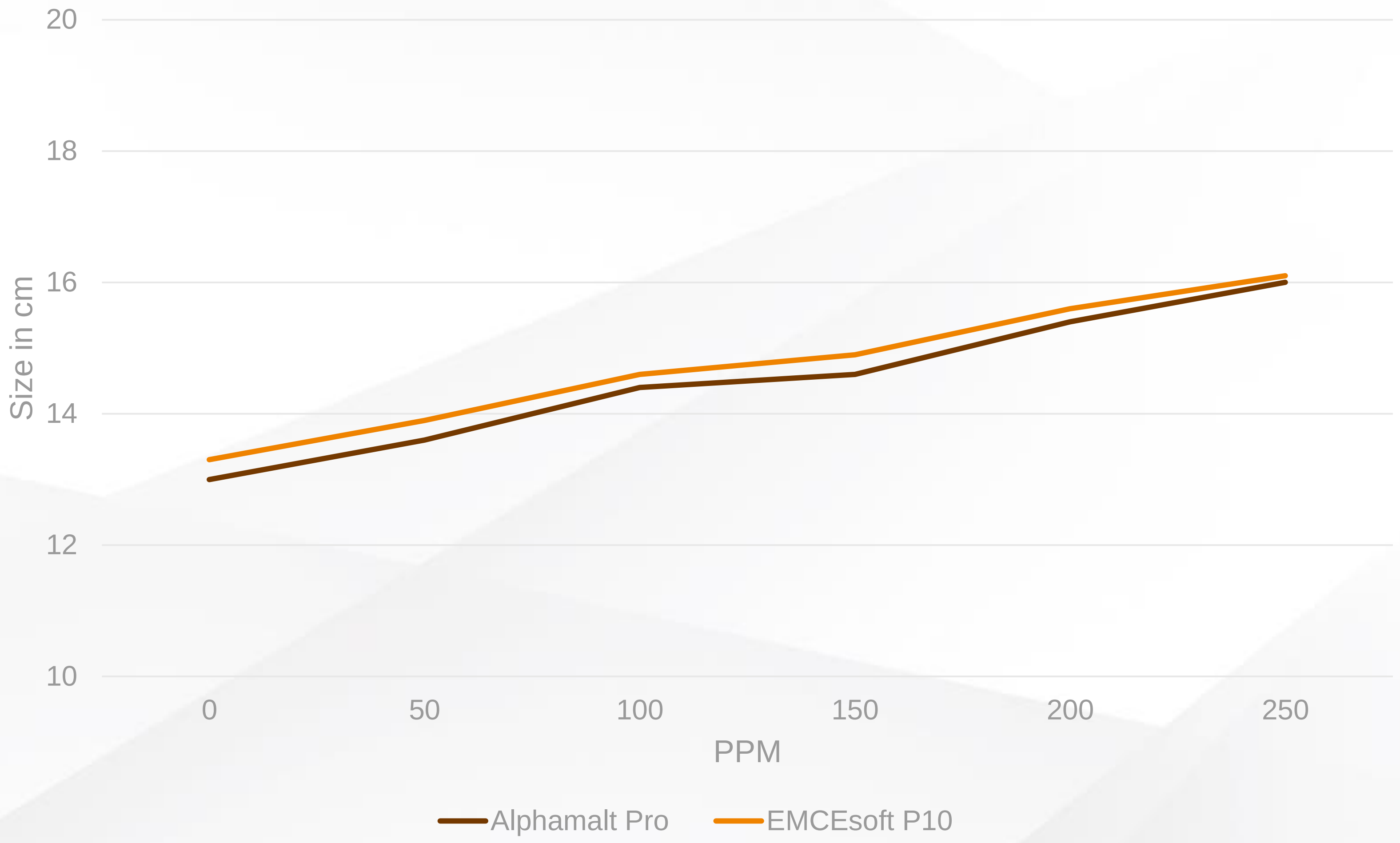
▮ = Amino acids

Proteolytic Enzymes

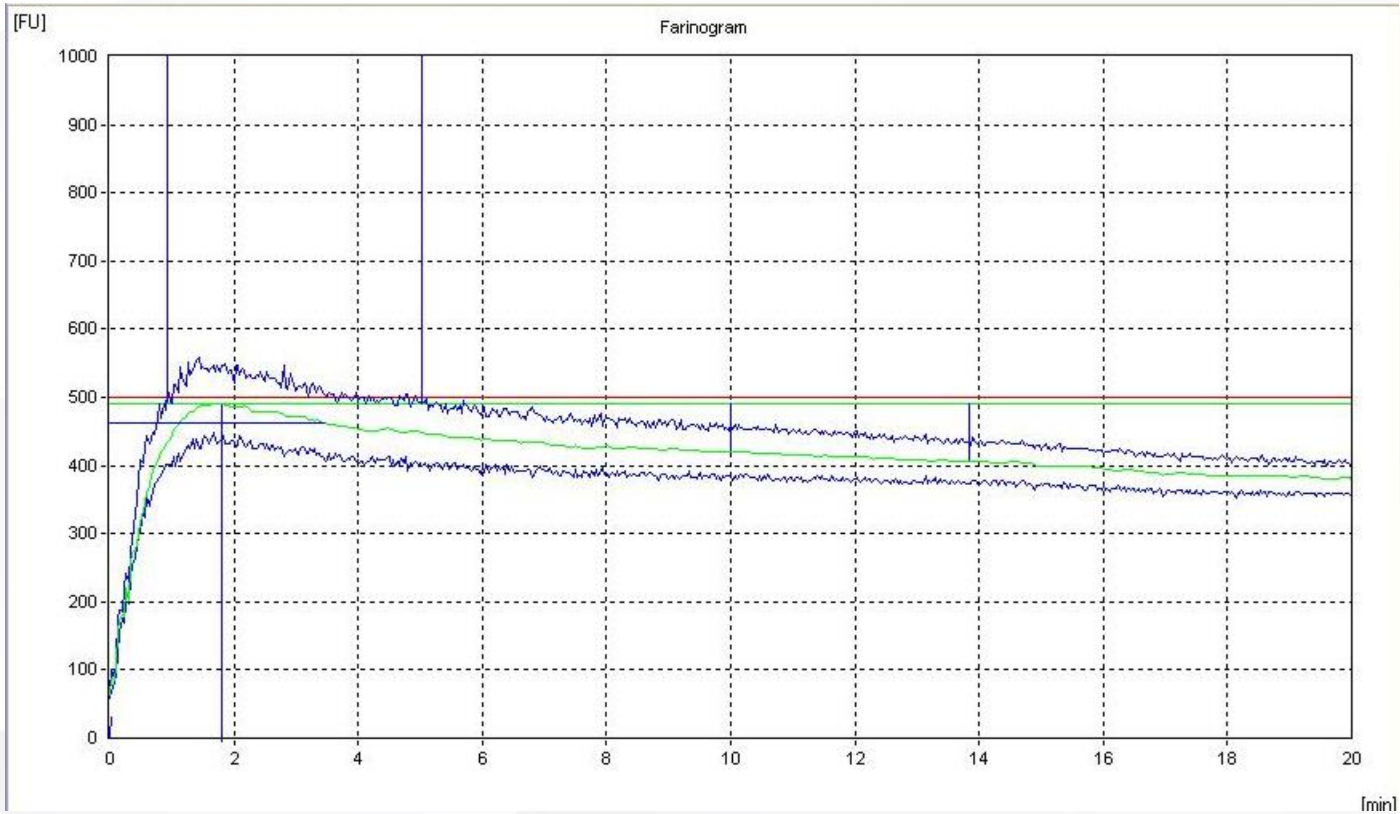
Protease
(Synonyms: Proteinase, Peptidase)



Protease against L-Cystein

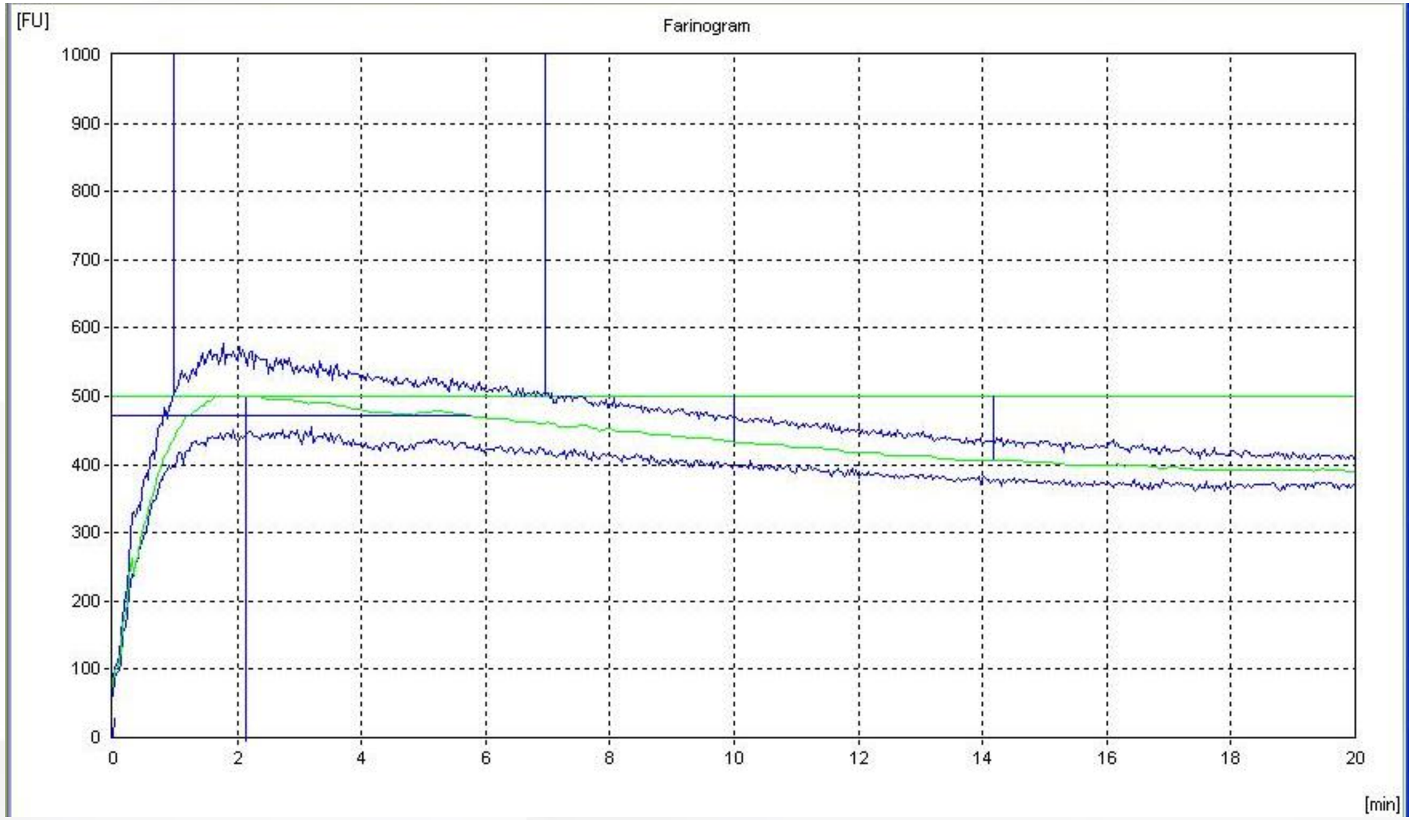


Protease



Water absorption (500FU)	54,8%
Development time	1,8 min
Stability	4,1 min

EMCE Soft P 10



Waterabsorption (500FU)	55%
Development time	2,2 min
Stability	6 min

Effects of Proteases on Dough & Baked Good Properties

- Softening and relaxation of the dough
- Reduction of spring-back
- Reduction of shrinkage upon baking
- Accelerated Maillard reaction

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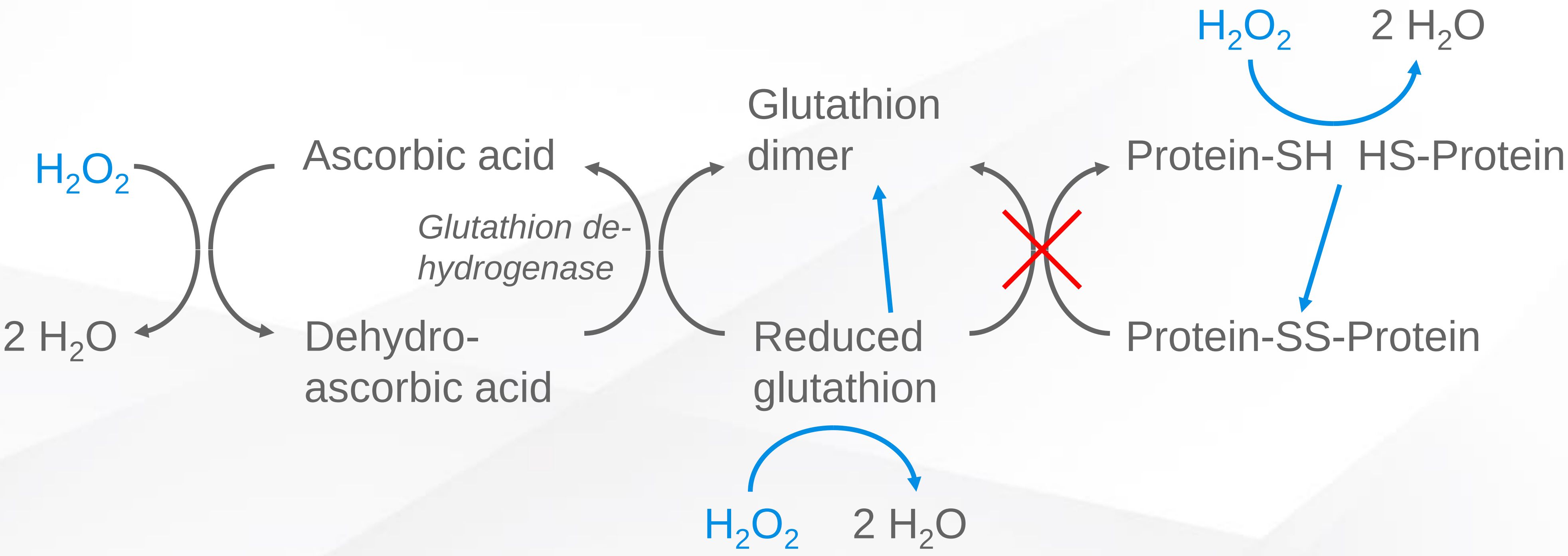
Oxidases

Effects of Glucose Oxidase in Dough

Glucose + O_2 + H_2O $\xrightarrow{\text{GOD}}$ Gluconic acid + H_2O_2



H_2O_2

$2\ H_2O$

Ascorbic acid

Glutathion de-hydrogenase

Dehydro-ascorbic acid

Glutathion dimer

Reduced glutathion

H_2O_2 $2\ H_2O$

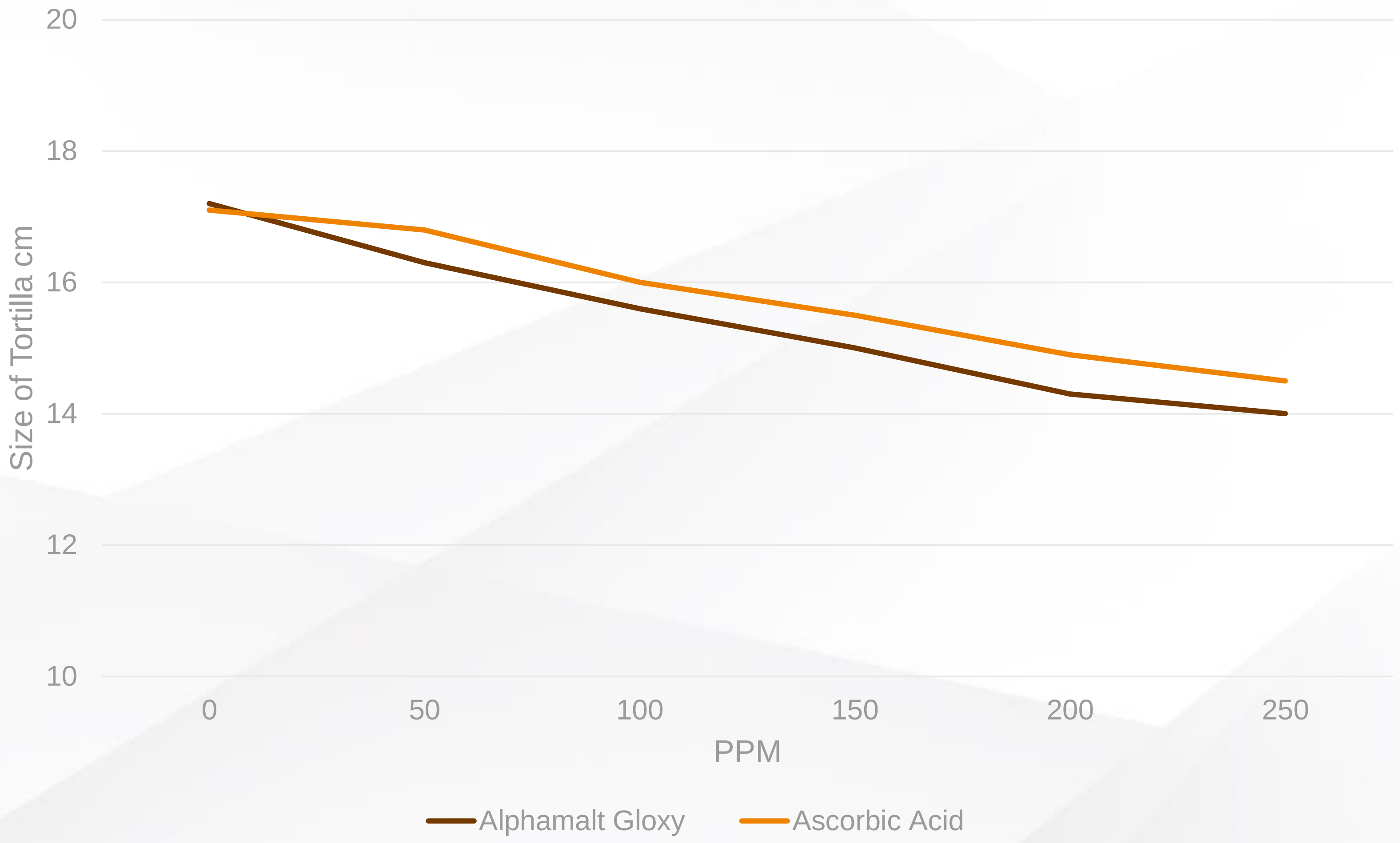
H_2O_2 $2\ H_2O$

Protein-SH HS-Protein

Protein-SS-Protein

Pentosan + H_2O_2 $\xrightarrow{\text{(Peroxidase)}}$ Oxidative gelation

Glucoxidas against Ascorbic acid



Summary of the effects of Oxidases

- Create hydrogen peroxide
- Cause cross-linking of proteins and pentosans
- Increase water absorption
- Result in dryer dough surfaces and hence better handling properties
- Improve dough stability

Typical Effects of Enzymes on Bread Quality

Enzyme	WA(1)	Volume(2)	Stability(3)	Cut(4)	Colour(5)	Crumb(6)	Shelf-life(7)
α-Amylase, fungal	o	++	-	+	+	-	+
α-Amylase, cereal	-	+	--	-	++	--	+
α-Amylase, bacterial	-	(+)	(-)	o	o	-	+
α-Amylase, maltogenic	o	o	o	o	o	o	++
Xylanase$_{\text{WUX}}$	+	++	+	+	o	+	(+)
Xylanase$_{\text{WEX}}$	-	+	-	-	o	-	o
Protease	o	(+)	(+)/-	+	o	(-)	o
Oxidase	++	+	++	++	o	+	(+)
Carboxylesterases	+	++	+	+	o	++	+
Transglutaminase	o	o	+	+	o	o	o

(1) Water absorption (2) Baking volume yield (3) Shape stabilty (4) Opening of the cut, shred (5) Crust colour (6) Crumb fineness (7) Non-microbial shelf-life

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Conclusion

Conclusion

- Clean label as few ingredients as possible
- Lower storage and transport amounts
- Choosing the correct solution
- Ingredients like Fibers and Proteins can be used
- Finding the correct enzyme blend for the application

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Thank you very much for your attention

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R&D

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