

# Amylolytic Enzymes and Bread Firming

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# Outline

- Introduction
- Starch and gluten in breadmaking
- Networks in dough and bread
- Amylases in breadmaking
- Experimental work
- Conclusions

# Outline

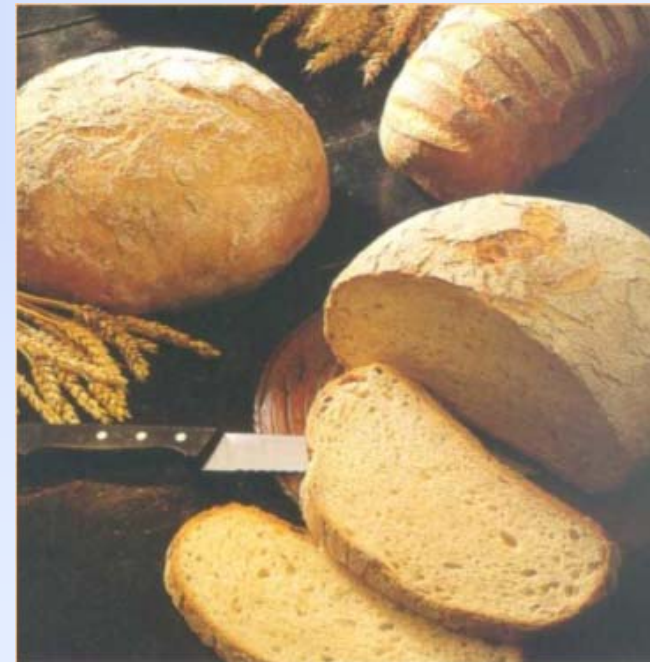
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# Introduction

- Quality and freshness are very important for bakery products
- Bread is an important constituent of an equilibrated diet
- Bread staling is an important phenomenon



- Loss of aroma
- Loss of crust crispness
- Crumb firming



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# Starch and gluten in breadmaking

- From wheat (100 %) to flour (ca 70 %)
  - Wheat flour constituents
    - starch (ca. 70 %)
    - protein (ca. 12 %)
    - nonstarch polysaccharides (ca. 2 %)
    - lipids (ca. 1%)
    - water (ca. 14%)

# Starch and gluten in breadmaking

## ■ Starch

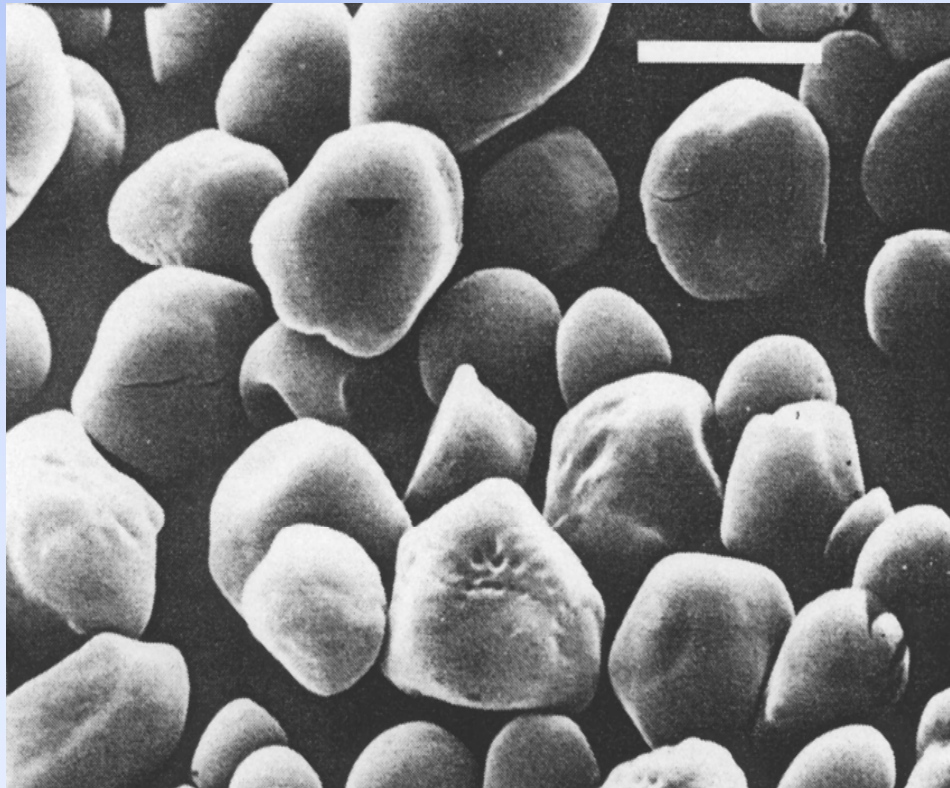
- Reserve polysaccharide
- Granular structure

- sizes: 2 to 10  $\mu$ , 20 to 35  $\mu$
- shape: round and lenticular

- Milling causes some starch damage

# Starch and gluten in breadmaking

- Starch granular structure



# Starch and gluten in breadmaking

## ■ Starch composition

- Poly- $\alpha$ -D-glucose:
  - amylose (ca 25 %)
  - amylopectin (ca 75 %)
- Amylose :
  - linkages:
    - $\alpha$ -1,4
    - hardly  $\alpha$ -1,6
  - ca 2,000 glucose units
  - essentially linear
  
  - **amorphous**

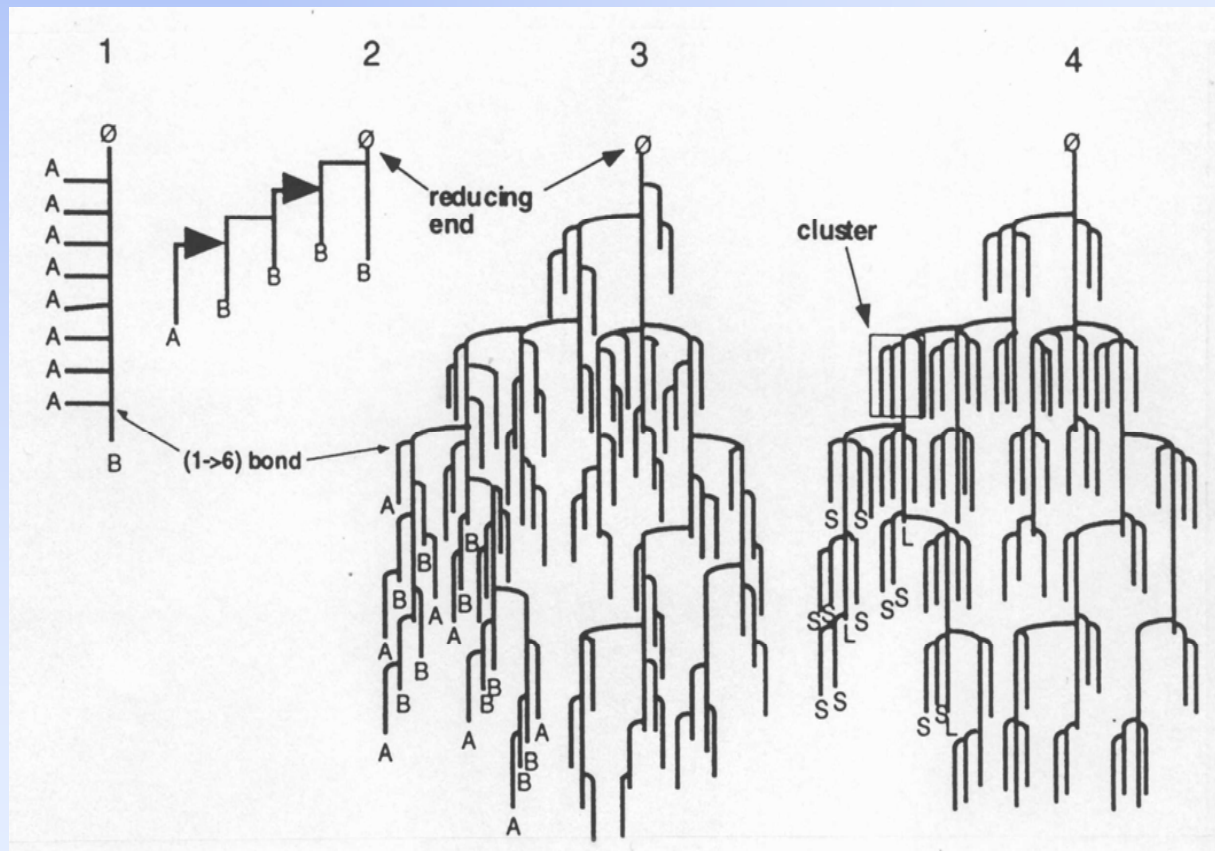
# Starch and gluten in breadmaking

## ■ Starch composition

- Amylopectin:
  - linkages:
    - ca 5%  $\alpha$ -1,6
    - ca 95%  $\alpha$ -1,4
  - ca 500,000 glucose units
  - heavily branched
  - **amorphous/crystalline**  
**A-type crystals**

# Starch and gluten in breadmaking

## ■ Amylopectin structure



# Starch and gluten in breadmaking

- Starch related phenomena occurring at dough stage
  - Native starch: water uptake
    - 30% water
    - 5% volume increase
  - Damaged starch:
    - huge water uptake
    - amylose and amylopectin entanglement

# Starch and gluten in breadmaking

- Starch related phenomena occurring during baking
  - Irreversible water uptake
    - swelling
    - (limited) amylose leaching
    - granule remnants
      - swollen
      - enriched in amylopectin
    - **loss of crystallinity**
  - Increase in viscosity

# Starch and gluten in breadmaking

- Gluten and protein Osborne fractions:
  - Albumins
  - Globulins
  - Prolamins (gliadins): 70% ethanol extractable
  - Glutelins (glutenins): dilute acid extractable
  - Unextractable glutenin
  - Prolamin + glutenin - majority of protein  
- together form gluten

# Starch and gluten in breadmaking

- Gliadin:
  - Monomeric protein:
    - MM 30 - 80 kDa
    - intramolecular S-S
  - $\alpha$ -,  $\gamma$ -,  $\omega$ - Types
  - Form gluten together with glutenin

# Starch and gluten in breadmaking

- Glutenin:
  - Polymeric protein consisting of subunits
    - HMW - GS (ca 15%): MM 65 - 90 kDa
    - LMW - GS (ca 85%): MM 30 - 60 kDa
  - Intermolecular and intramolecular S-S
  - Form gluten together with gliadin

# Starch and gluten in breadmaking

- Gluten:
  - Gluten - forms transient network during dough mixing
    - responsible for dough
      - visco-elastic properties
      - gas holding properties
  - Gluten functionality impacted by its size
  - Gluten polymerises during baking and forms permanent network

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# Networks in dough and bread

- Networks necessary for coherent dough/crumb structures
- Networks can be
  - transient , based on
    - entanglement
    - dynamic exchange covalent bonds
  - permanent, based on
    - covalent linkages
    - (partial) crystallinity

# Networks in dough and bread

- Networks in dough are of transient type and consist of
  - Amylose and amylopectin from damaged starch
  - Glutenin
    - dynamic exchange of disulfide bonds
    - entanglement
- Gliadin does not entangle (globular)

# Networks in dough and bread

- Networks in baked fresh warm bread are of transient and permanent types:
  - Gelatinized starch is entangled
  - Glutenin network is permanent as a result of thermosetting and has (some) gliadin incorporated

# Networks in dough and bread

- Networks in baked fresh cooled bread are of transient and permanent types:
  - Gelatinized starch is entangled
  - Glutenin network is permanent as a result of thermosetting and has (some) gliadin incorporated
  - Amylose network is permanent as a result of chain folded crystallisation leading to partial crystallinity

# Networks in dough and bread

- Networks in baked stored bread are

- Networks in baked fresh bread

+

- Permanent amylopectin network which itself is

the result of  
amylopectin intermolecular crystallisation

# Networks in dough and bread

- Networks in baked stored cool bread are increasingly of the permanent type:
  - permanent amylopectin **B-type crystals incorporate water** from the amorphous phase, making gluten *e.g.* less plasticized
- The net result is bread firming and loss of resilience

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# Amylases in breadmaking

- $\alpha$ -Amylase
- $\beta$ -Amylase
- Maltogenic amylase

# Amylases in breadmaking

- $\alpha$ -Amylase
  - active on native but much more on gelatinized starch
  - virtually absent in sound flour
  - generates maltose for fermentation
  - reduces viscosity during starch gelatinization and thus increases volume
  - antifirming effects, many times poor crumb resilience
  - malt, fungal or bacterial origin
- $\beta$ -Amylase
  - active on gelatinized starch
  - abundantly present in sound flour
  - inactivated before starch gelatinization
- Maltogenic amylase
  - active on gelatinized starch
  - antifirming effects, good crumb resilience

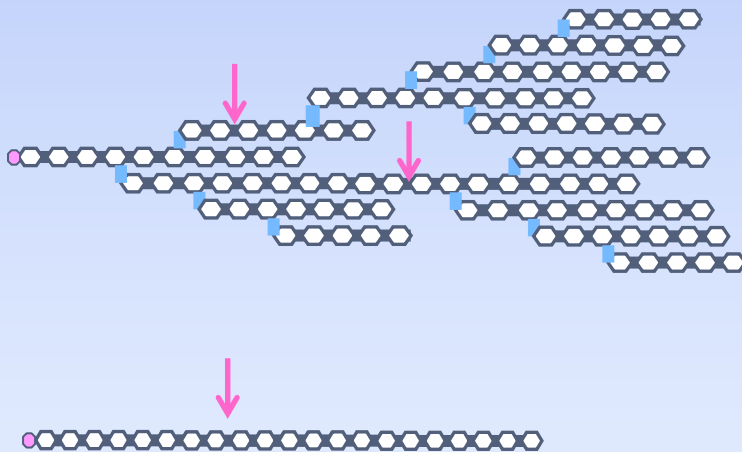
# Amylases in breadmaking

- The ideal antifirming amylases have a right window of activity, *i.e.* they
  - are not inactivated before starch gelatinization
  - are active during/after starch gelatinization
  - are denatured during further baking and/or are no longer active when the bread is stored
  - lead to soft crumb structures
  - lead to resilient crumb structures by ensuring water retention by gluten

# Amylases in breadmaking

## $\alpha$ -amylases

hydrolyze  $\alpha$ -(1 $\rightarrow$ 4)-bonds of starch internally

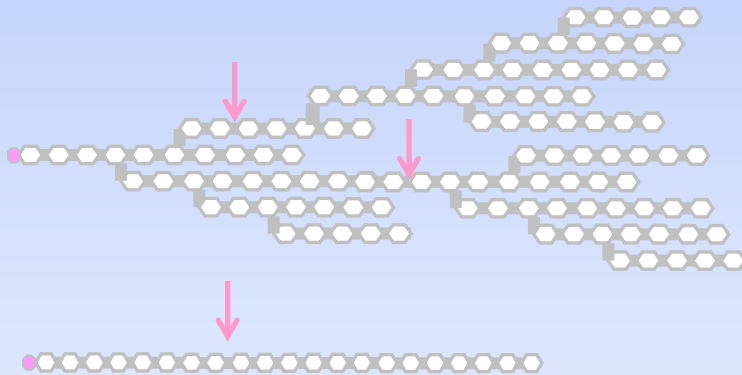


# Amylases in breadmaking

## $\alpha$ -amylases

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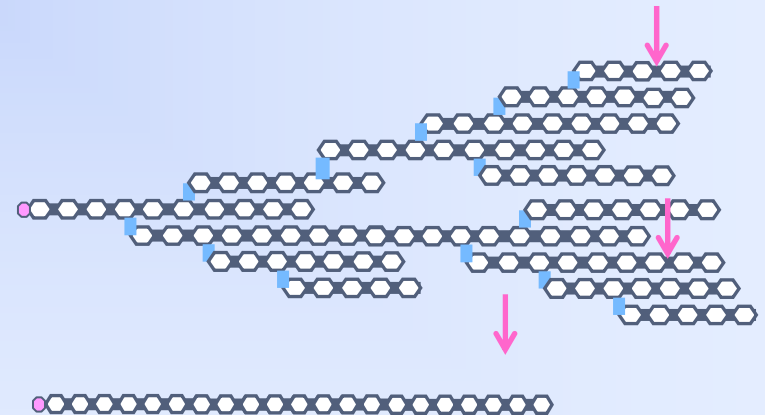
internally



maltogenic amylase

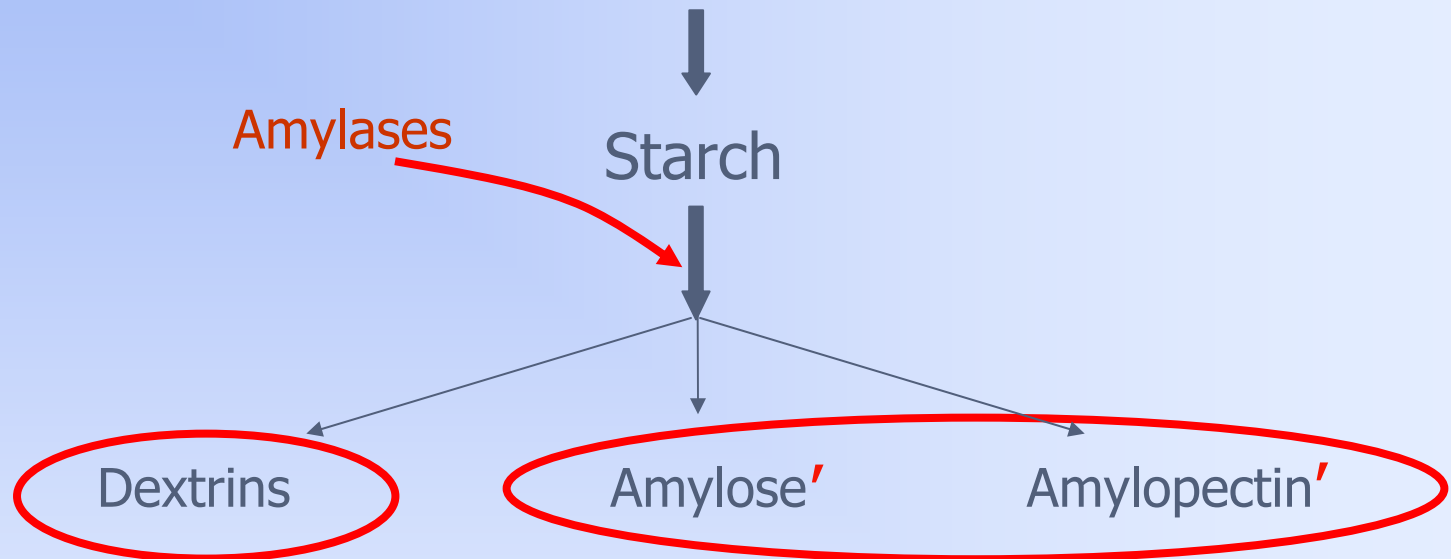
## $\beta$ -amylase

from the non-reducing ends



# Amylases in breadmaking

**BREAD:** water, flour, yeast, sugar and salt

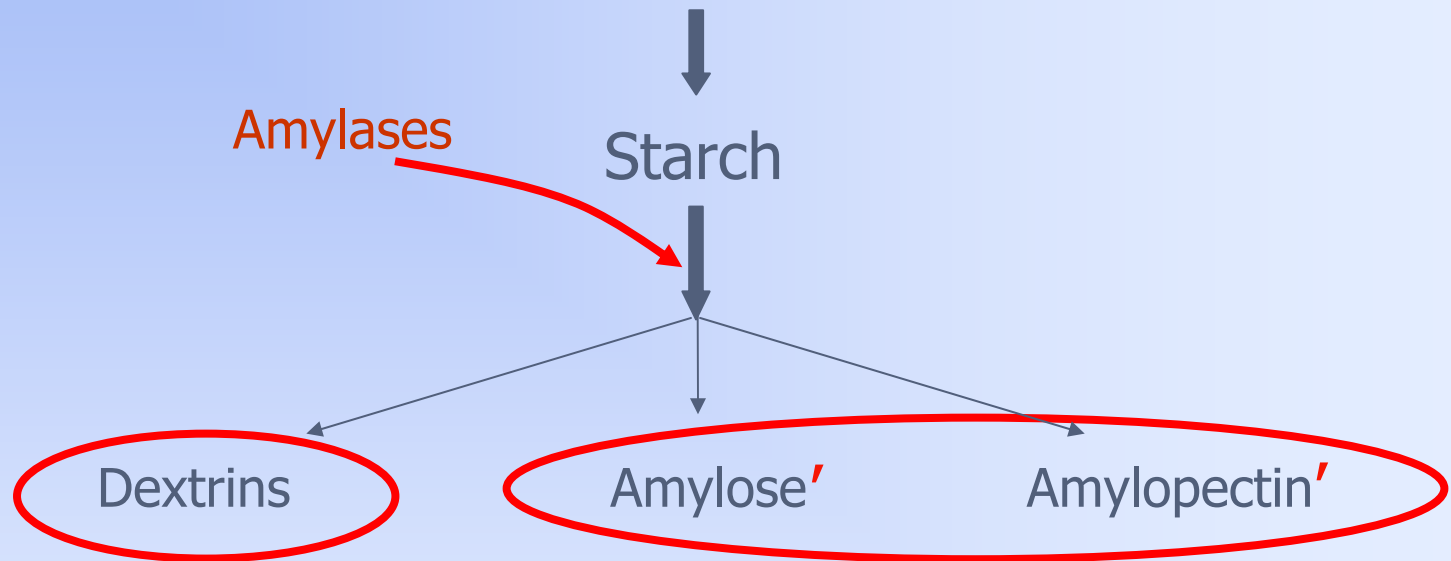


Earlier hypothesis 1: The formed dextrins

- interfere with gluten-starch-interactions
- influence retrogradation of the residual starch fraction

# Amylases in breadmaking

**BREAD:** water, flour, yeast, sugar and salt



Earlier Hypothesis 2: The resulting amylose and amylopectin have a changed retrogradation behaviour

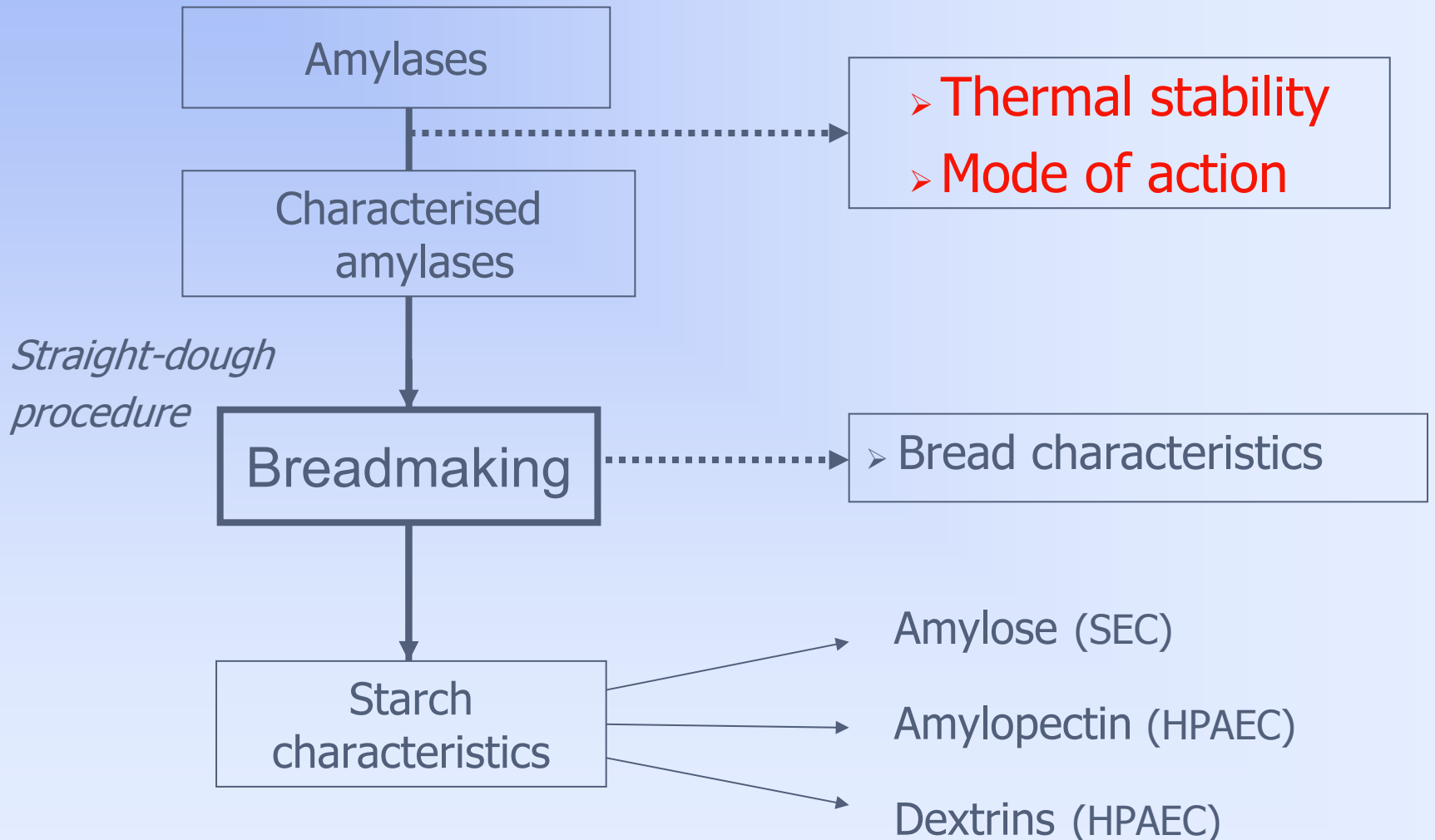
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# Experimental work

- Experiments were designed to explain the
  - Positive impact of  $\alpha$ -amylase on crumb firming and the negative impact on resilience
  - Positive impacts of BStA (maltogenic amylase) on both crumb firming and resilience

# Experimental work

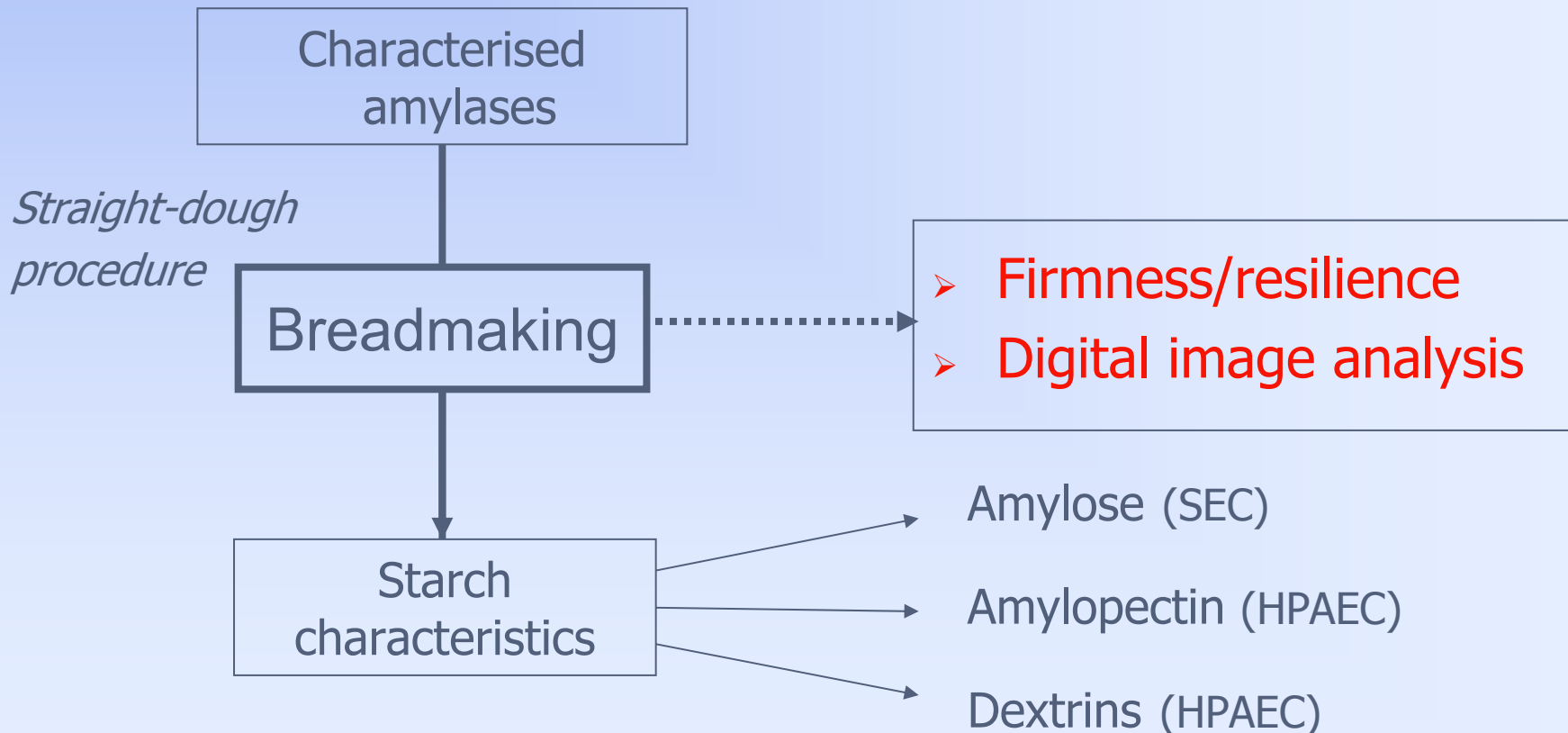


# Experimental work

- Amylase characteristics

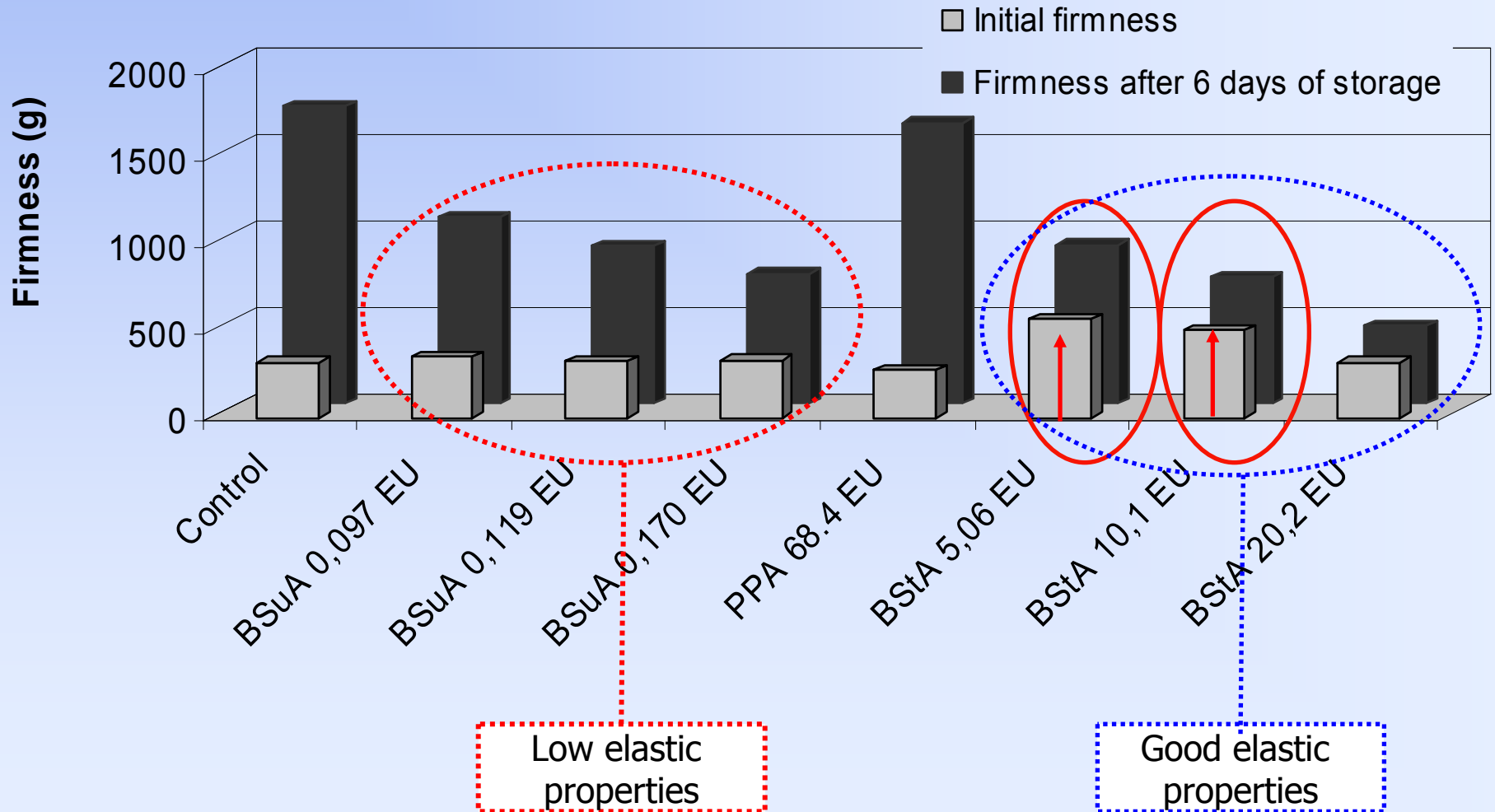
	Amylase	Mode of action	Thermal stability
BSuA	<i>Bacillus subtilis</i>	endo	intermediate
PPA	Porcine pancreatic	endo	low
BStA	<i>Bacillus stearothermophilus</i>	maltogenic	intermediate

# Experimental work



# Experimental work

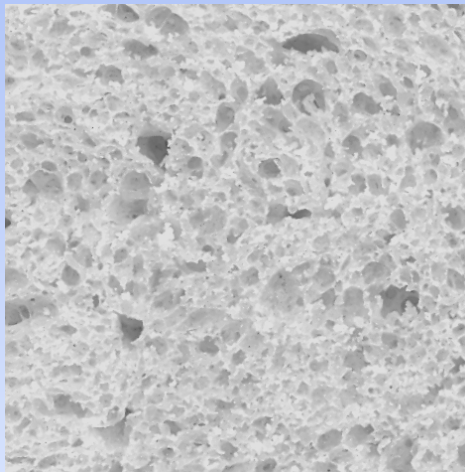
- Bread characteristics: Firmness/resilience



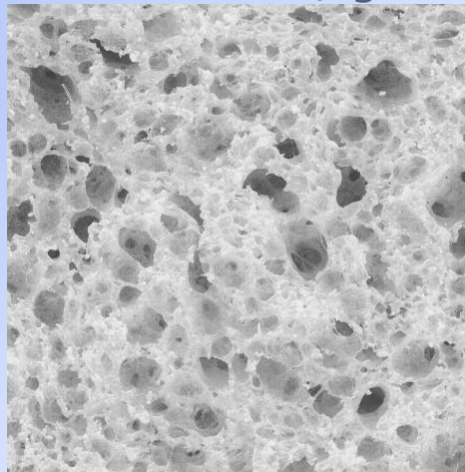
# Experimental work

- Bread characteristics: digital image analysis

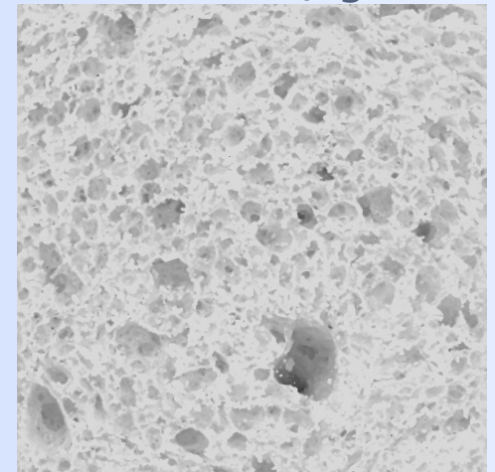
Control



BSuA 0.119 EU/ g flour

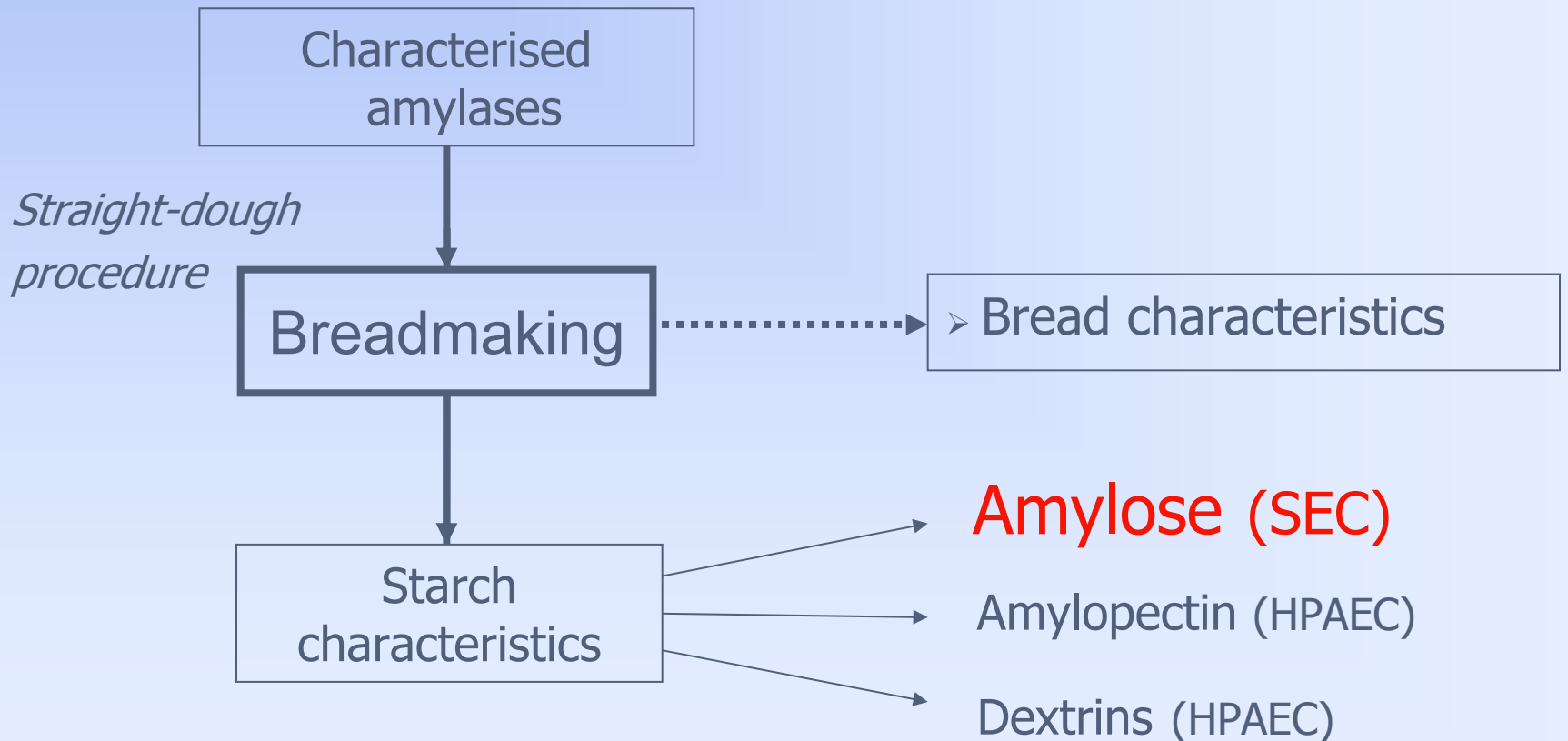


BStA 5.06 EU/ g flour



	Mean Cell Area (mm <sup>2</sup> )	Cells/ cm <sup>2</sup>	Cells to total area ratio
Control	0.31	74.7	27.7
BSuA 0.119 EU/ g flour	0.41	56.2	25.9
BStA 5.06 EU/ g flour	0.33	72.9	27.5

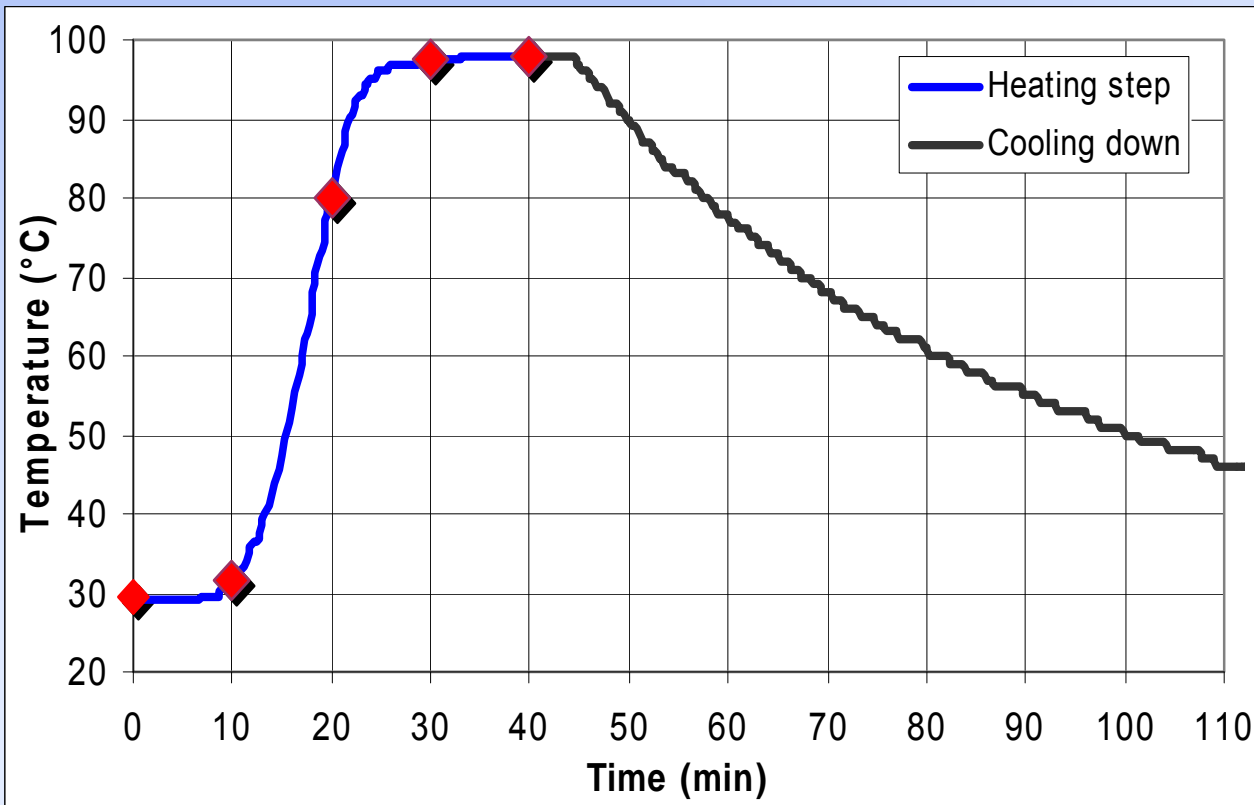
# Experimental work



# Experimental work

## ■ Samples withdrawn

The centre of bread loaf (oven temp. 210°C)



## Samples

00' (30°C)

10' (35°C)

20' (80°C)

30' (97°C)

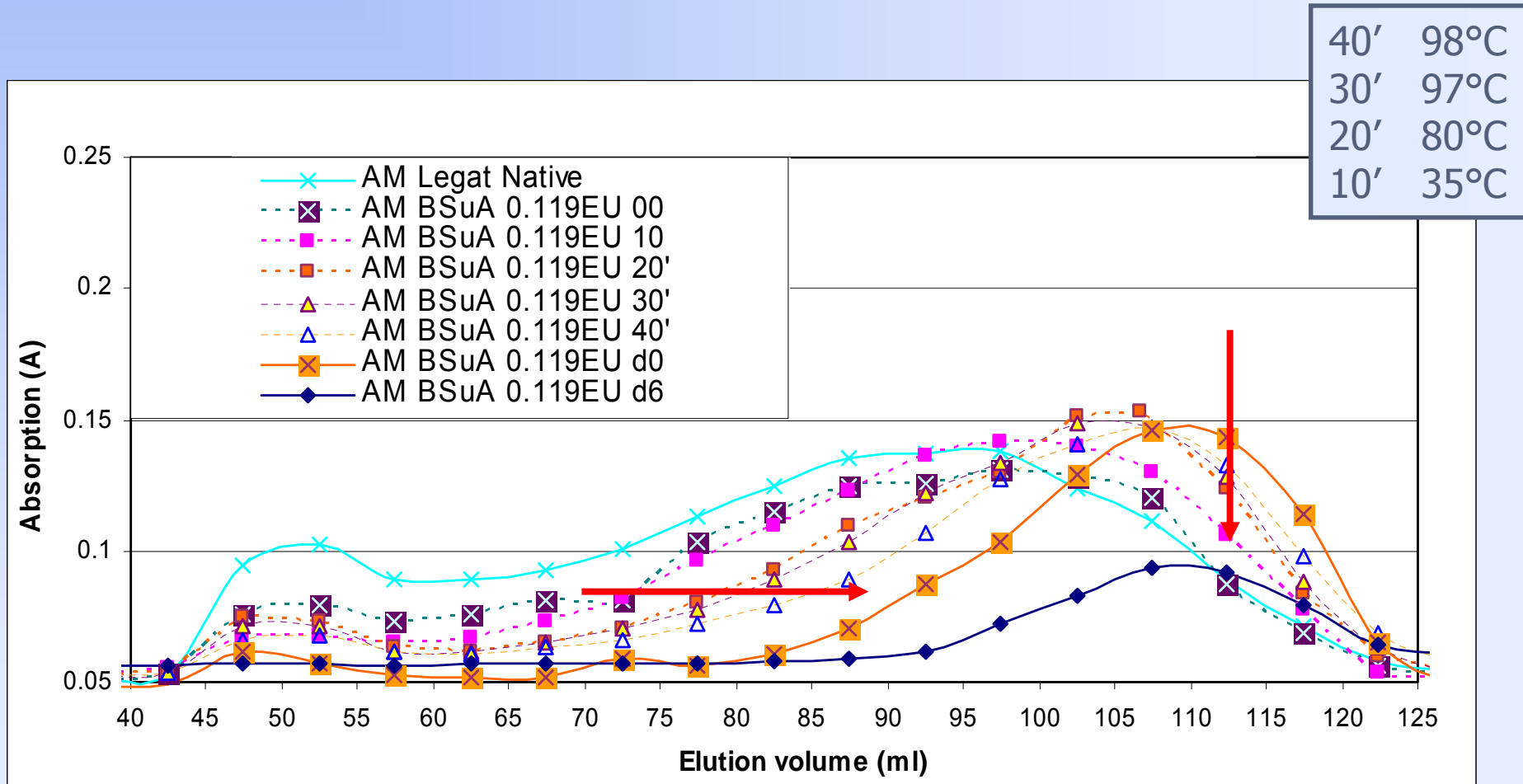
40' (98°C)

d0 after 4h of cooling

d6 after 6 days of storage

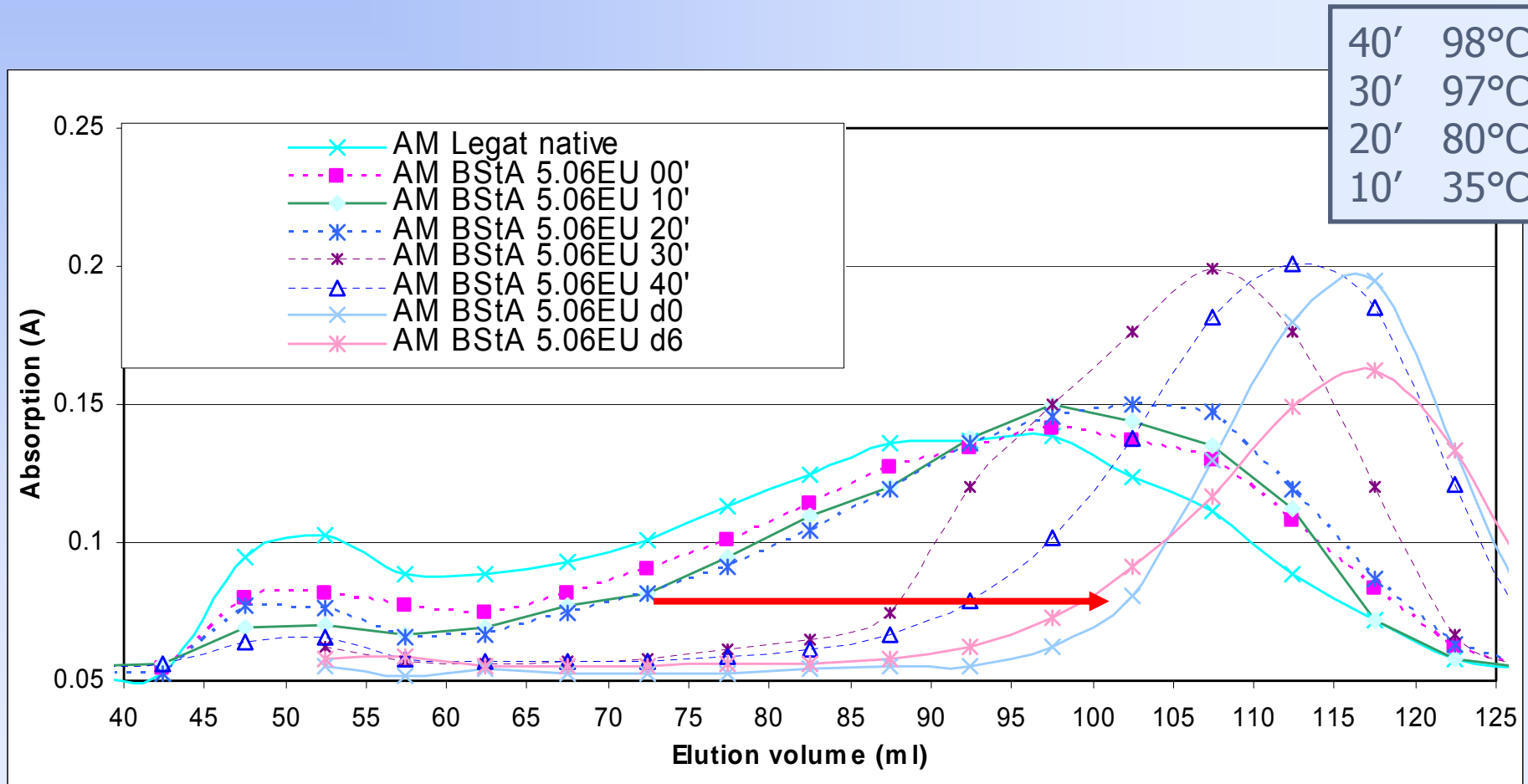
# Experimental work

- Amylose MW distribution during bread making and storage

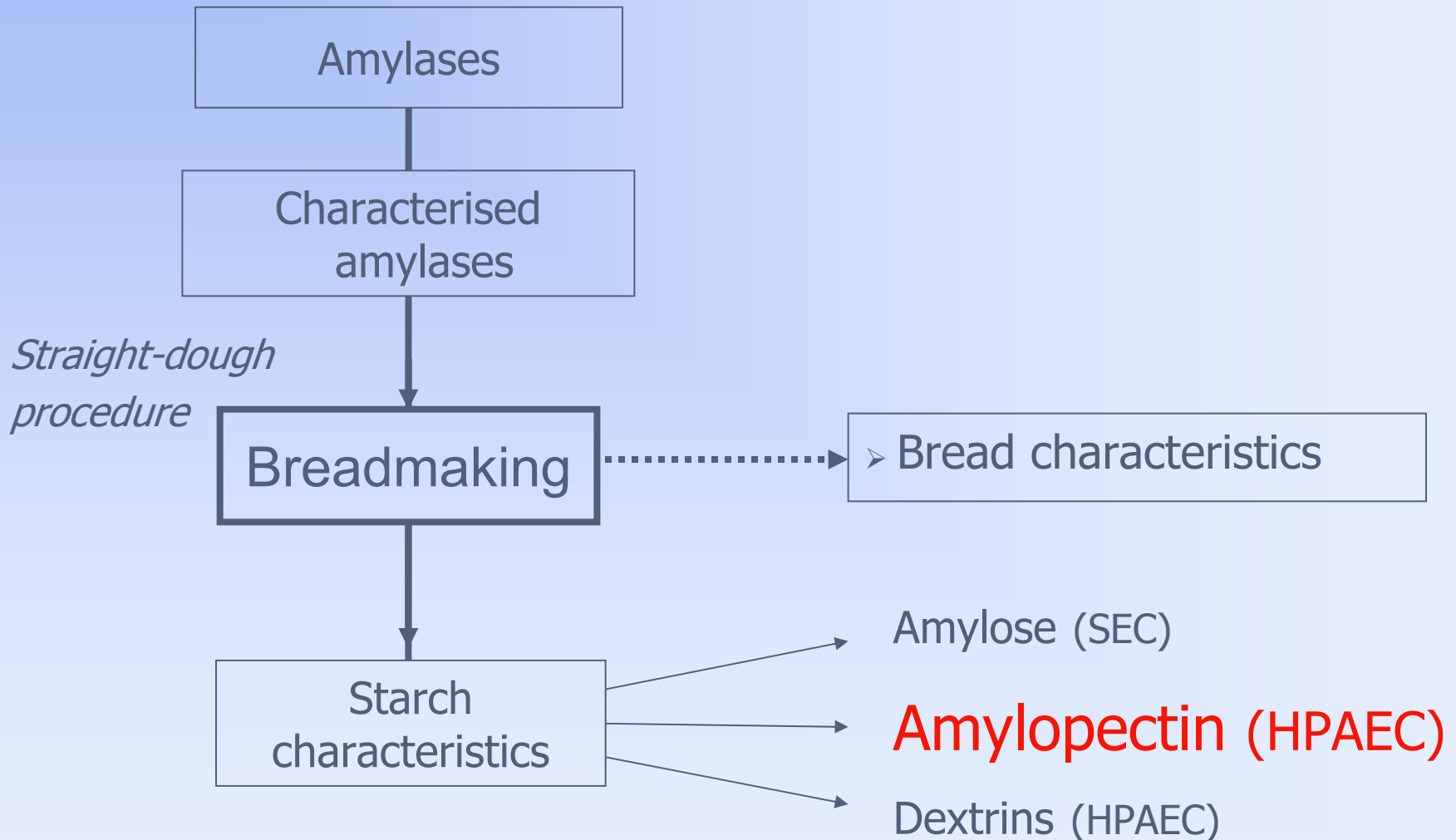


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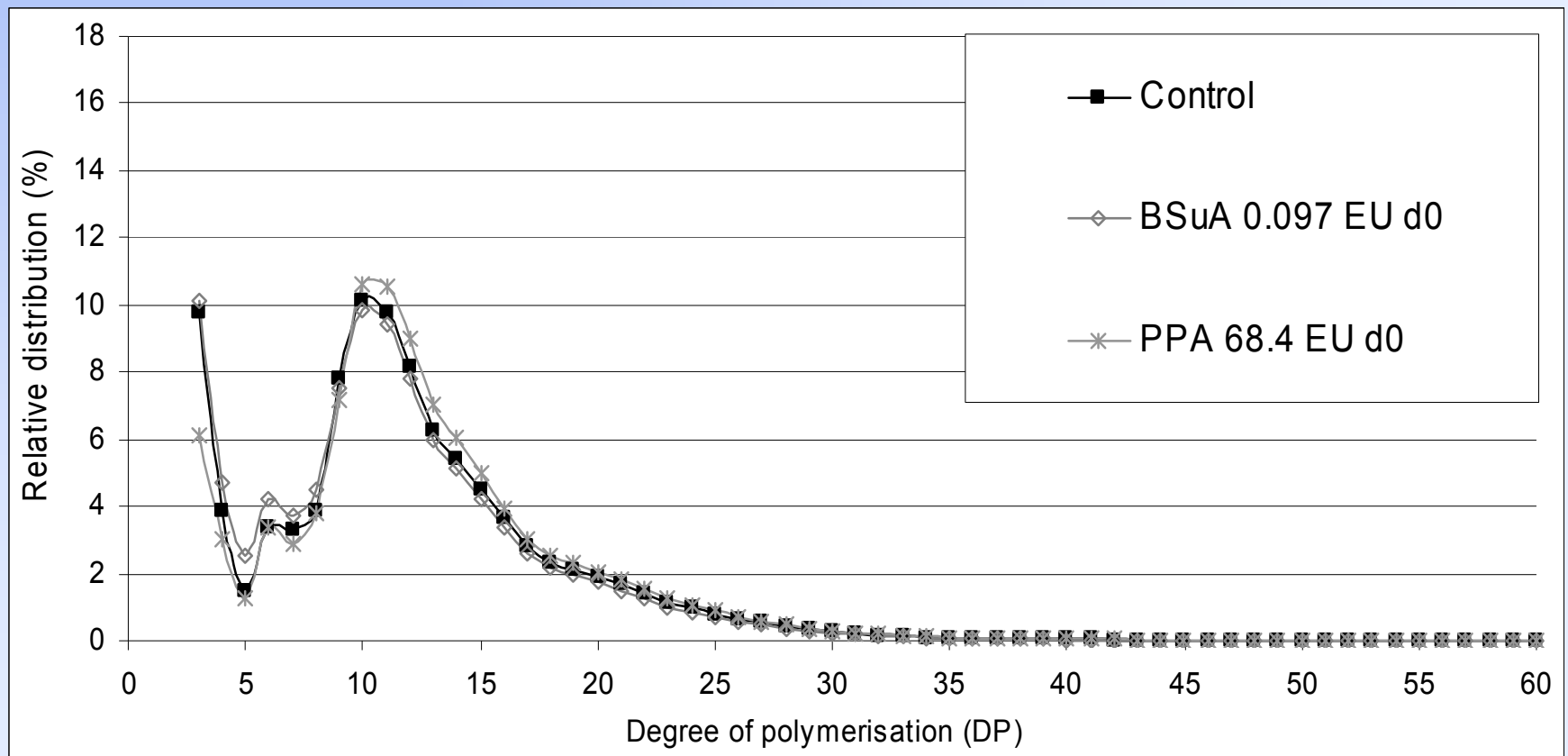


# Experimental work



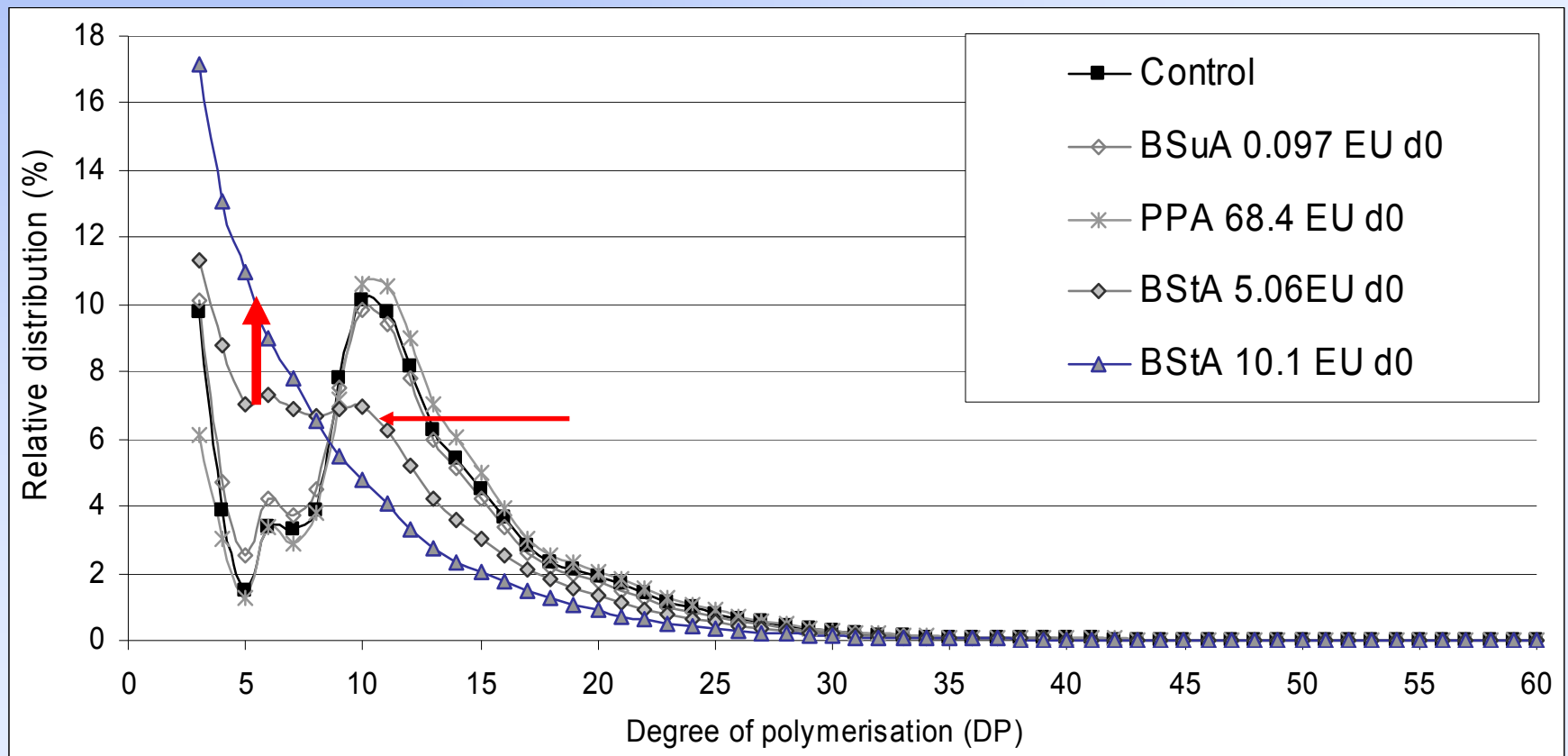
# Experimental work

- Molecular properties of amylopectin: side chain distribution



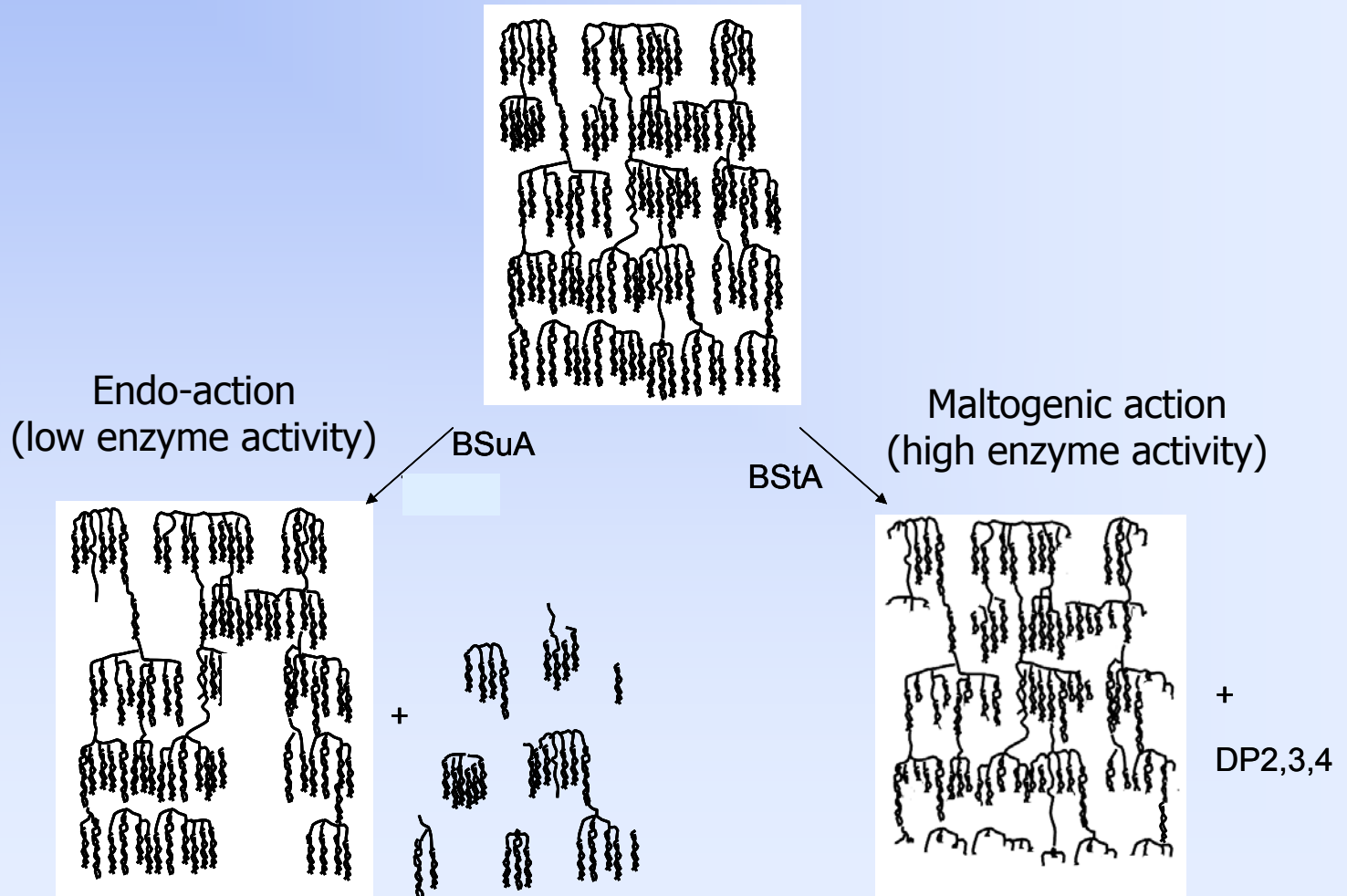
# Experimental work

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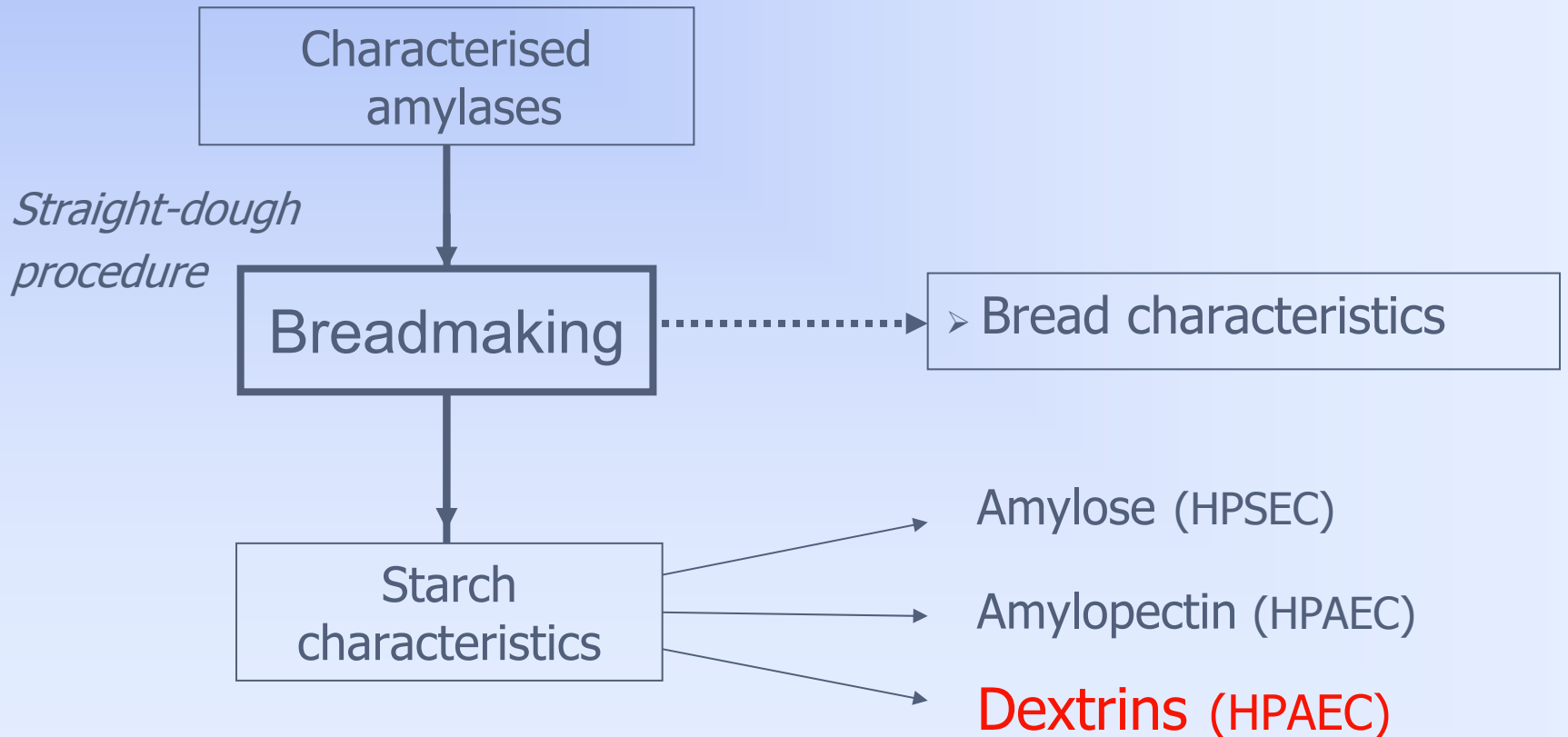


# Experimental work

- Molecular properties of AP (general concept)



# Experimental work



# Experimental work

- Molecular properties of dextrans & residual amylase activity

Amylase concentration (EU/ g flour)	Average DP		Soluble carbohydrate (%)	
	Day 0	Day 6	Day 0	Day 6
Control	2.1	2.0	9.7	10.0
BSuA 0.097 EU	5.1	5.4	17.9	22.9
BSuA 0.119 EU	6.2	nd	21.2	27.1
PPA 68.4 EU	5.5	5.3	14.2	14.3
BStA 5.06 EU	2.8	2.8	21.6	21.9
BStA 10.1 EU	2.6	2.7	22.3	22.2

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# Conclusions

- BSuA
  - not active on crystallisable outer amylopectin branches
  - loss of starch entanglement network because of endo action and hence antifirming effect
  - continues action after baking
  - crystallization of outer branches and moisture loss from gluten amorphous phase to crystals continues
  - poor resilience of resultant bread

# Conclusions

- BStA (maltogenic amylase)
  - active on crystallisable outer amylopectin branches
  - loss of amylopectin crystallisation network because of exo action and hence antifirming effect
  - does not continue action after baking
  - prevents moisture loss from gluten
  - excellent resilience of resultant bread

# Conclusions

- BStA action during bread making:
  - BStA is mainly active after the structure is 'set' (~70-80°C)
    - no influence on loaf volume and crumb structure
  - A significant portion of starch fraction is degraded
    - *in situ* formation of high levels of dextrans (DP 2-3)
    - significant reduction of amylose MW
      - mainly hydrolysed at high temperature and during cooling
    - extensive degradation of external amylopectin chains
      - mainly exo-type degradation

# Conclusions

- BStA increases initial firmness
  - increased mobility of the monodisperse amylose population of lower MW may lead to enhanced network formation
- BStA is an efficient antistaling enzyme
  - specific amylopectin degradation inhibits the formation of double helices and hence amylopectin recrystallization
  - formation of high levels of maltose
    - reduces the mobility of amylopectin chains
    - hinders formation of double helices (antiplasticizing effect)
    - reduces crumb RH and water migration to the crust