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#ElevateBeer

Secondary Structural Changes in Protein Z During Mashing and Boiling Processes

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The lab of brewing science and technology:

- Since 1980s
- Research interest:
  - Beer brewing engineering (material and adjunct, yeast, beer quality, …)
  - Brewing enzyme preparations
  - Novel alcoholic beverage product
- Six research fellows

Jiangnan University

Excellent discipline :

1. Food Science and Technology
2. Light Industry Technology and Engineering
3. Textile Science and Engineering.
4. Design

Beer foam

- Beer foam is one of the most appealing beer qualities
  - White, fine and smooth foam is tantalizing.
  - Gas-pitching of beer aroma flavor
  - Looks fresh
- What are the good features of beautiful beer foam?

Beer foam quality:

- Foam appearance (whiteness and size)
- Bubble formation and creaminess
- Foam stability
- Cup-hanging

The role of beer foam

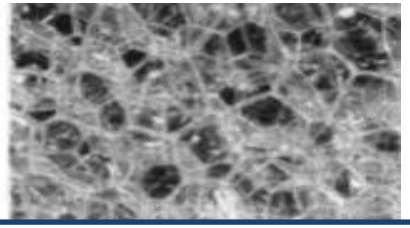
Foam formation ≠ Foam stability

- Form foam formation to foam collapse:

bubble nucleation →bubble growth → bubble rise → foam formation → drainage (wet foam shift to dry foam) → bubbles continue to rise meaning creaming → coalescence (from small size bubble to large size bubble), bubble collapse.

Beer foam **formation**:

1. Supersaturated gas (CO_2);
2. Nucleation activity (a particle, scratch
3. Surface
4. Viscosi



Foam stability： **involving all steps after formation, the bubbles need stably maintains.**

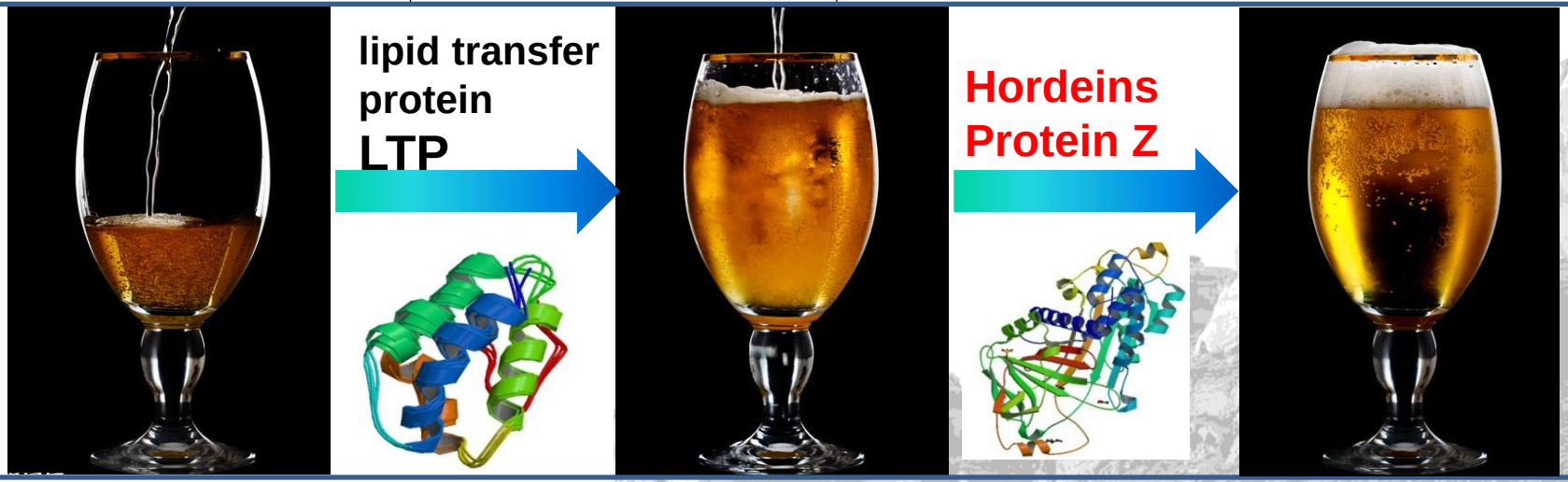
It is affected by various components in beer

Beer-foam stabilization factors

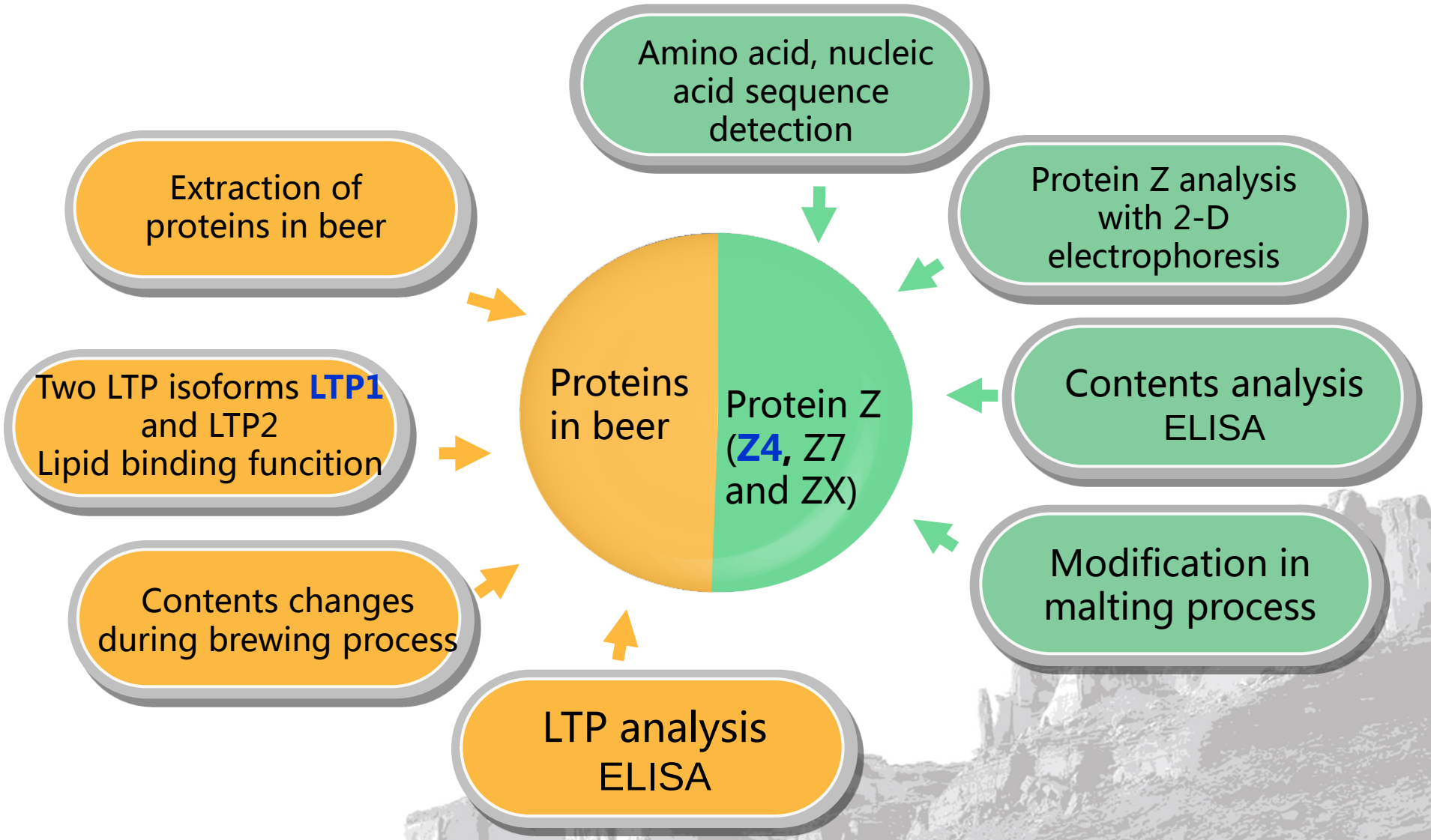
Positive(+)	Negative(-)
Protein、Polypeptide	Free amino acid
Iso-α-acid	Protease A
Melanoid	Higher alcohols
Polysaccharide	Lipid
Metal ion	
CO_2	

Protein is the key factor

Protein can be the foam framework with surface activity, and it can affect the foam stability.



State-of-the-art of foam-promoting proteins research



Interesting question about structure dynamic changes of beer-foam proteins during mashing process

Do the brewing processes modify structural transformation of malt protein Z ?

Are modifications of protein Z important for the stability of beer foam ?

What kind of modification happen to protein Z during mashing ?

Protein Z analysis ⇨ Structural dynamic changes during mashing process

Research content

Purification of protein Z

- Establishing purification method of protein Z.

CD analysis of protein Z during mashing and boiling processes

- Detecting the secondary structure transformation of protein Z using **circular dichroism (CD) analysis of protein backbone**.

FTIR analysis of protein Z during mashing and boiling processes

- Monitoring the **functional groups changes** and secondary structure using **Fourier transform infrared spectroscopy (FITR)**.

Purification of protein Z

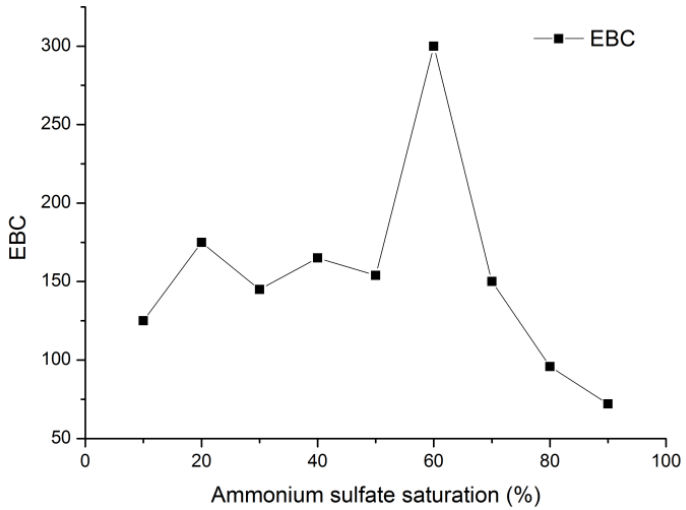


Fig. 1 Salting-out curve using $(NH_4)_2SO_4$.

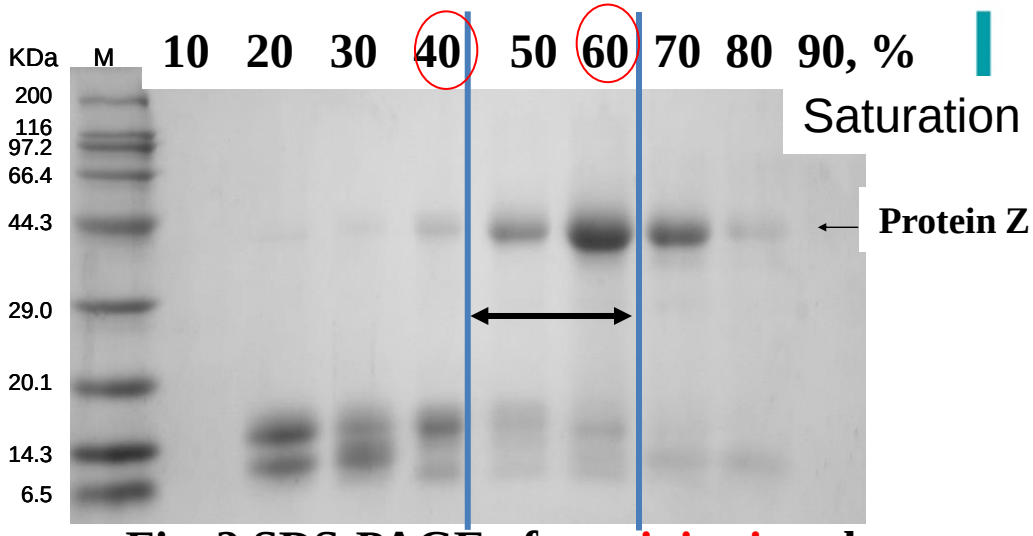


Fig. 2 SDS-PAGE of precipitations by different concentration of （$(NH_4)_2SO_4$.

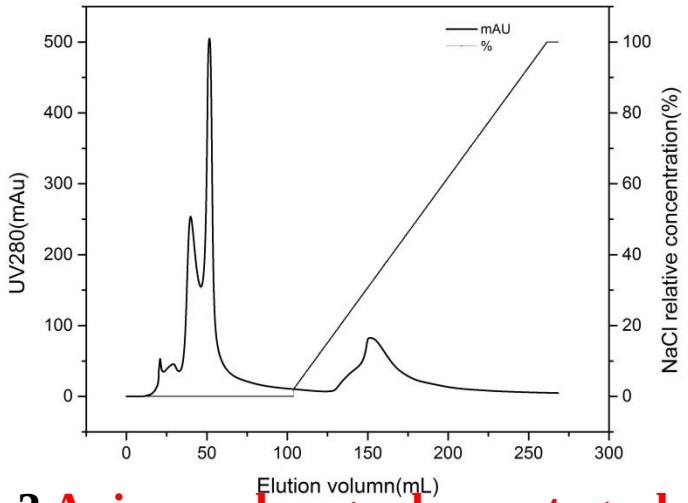


Fig. 3 Anion exchange chromatograhpy (HiPrep DEAE FF 16/10 column).

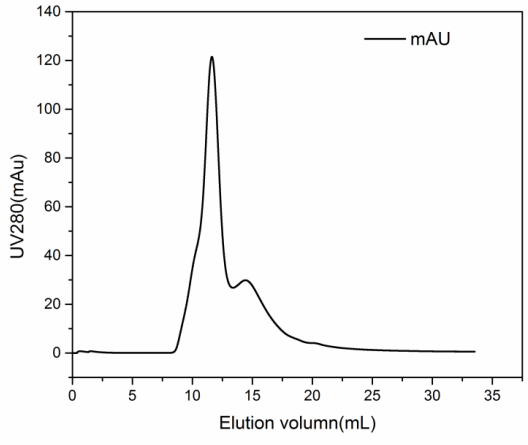
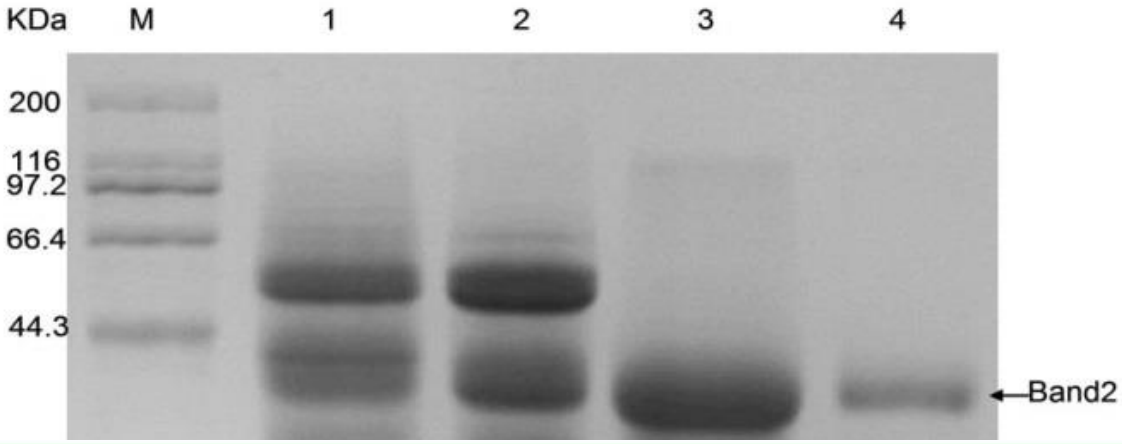


Fig. 4 Gel filtration chromatography (Sephadex G75 column)

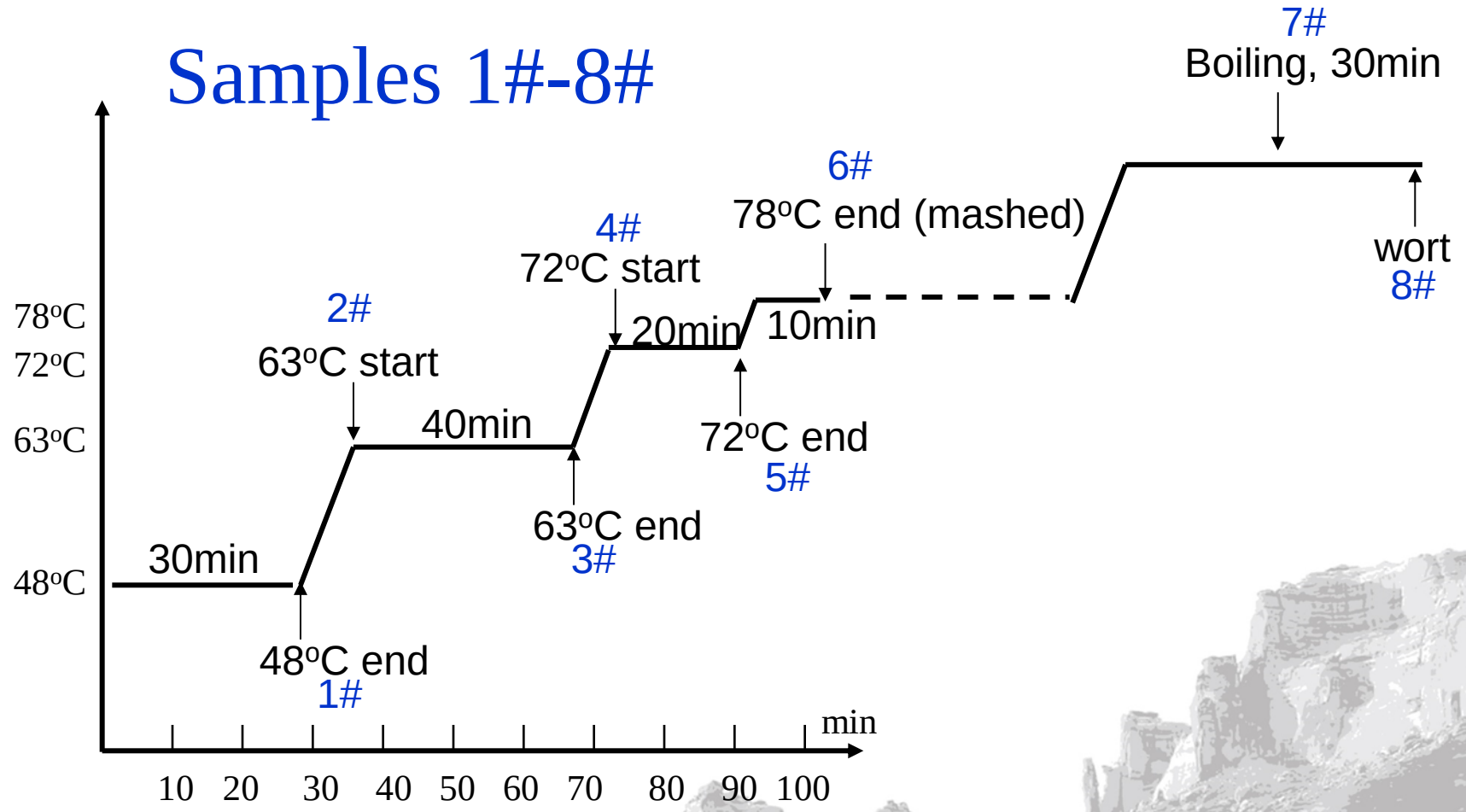
Identification of protein Z



Band number	Protein names	Accession number in NCBI	Theoretical Mr (Da)	Theoretical pI
Band2	protein z-type serpin	gi|1310677	43220	5.61

Mixed soluble protein
Crude precipitates
Anion exchange
Gel filtration

Samples for purifying protein Z from mashing and boiling process



Mashing procedure and sampling sites

CD profiles of protein Z

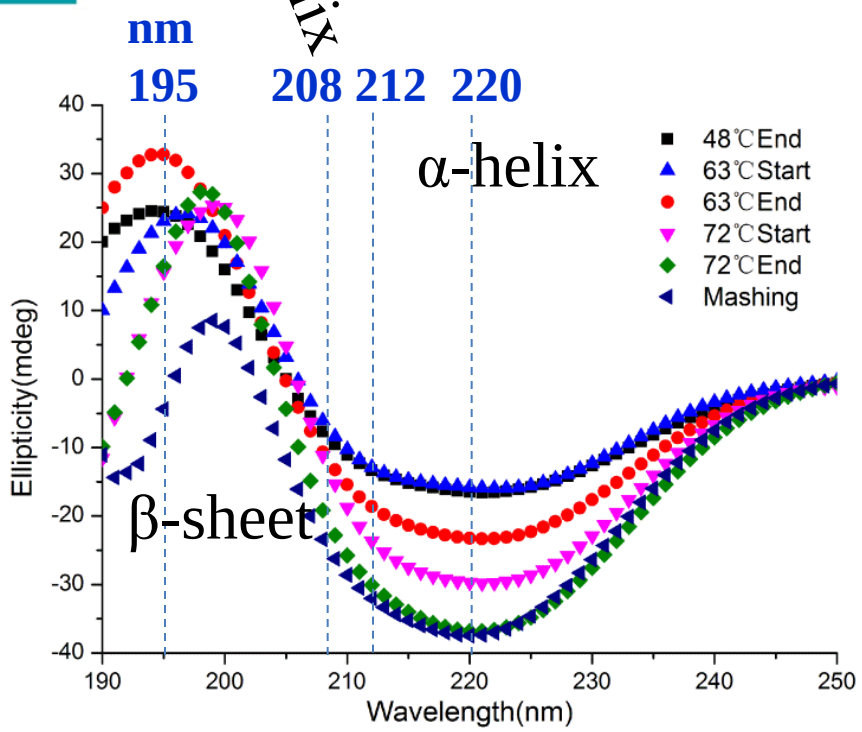


Fig. 6 Circular dichroism spectra of the purified protein Z during mashing process.

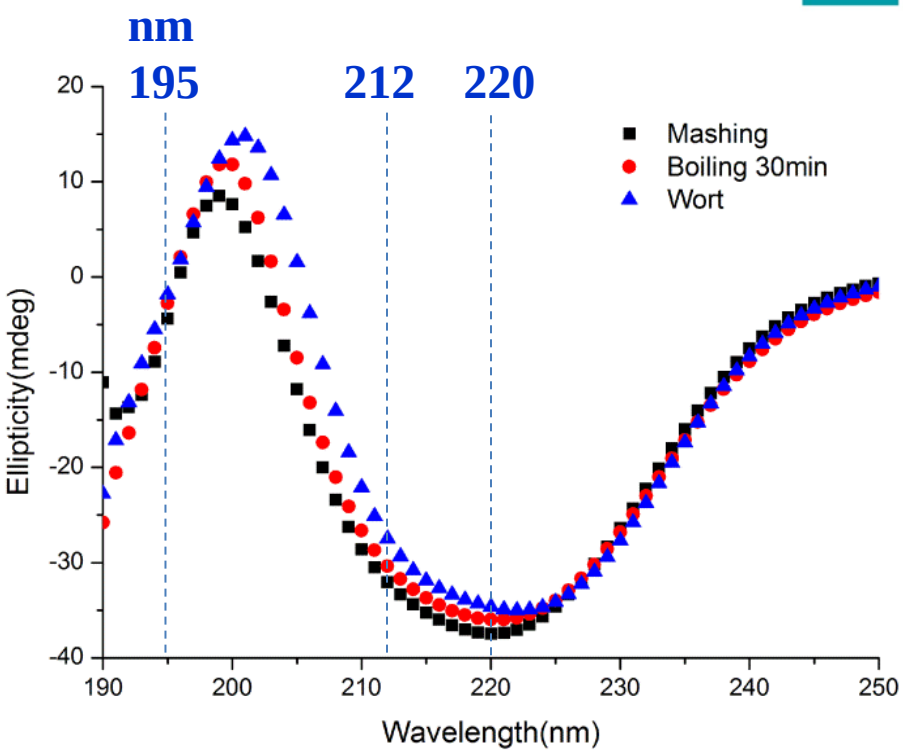
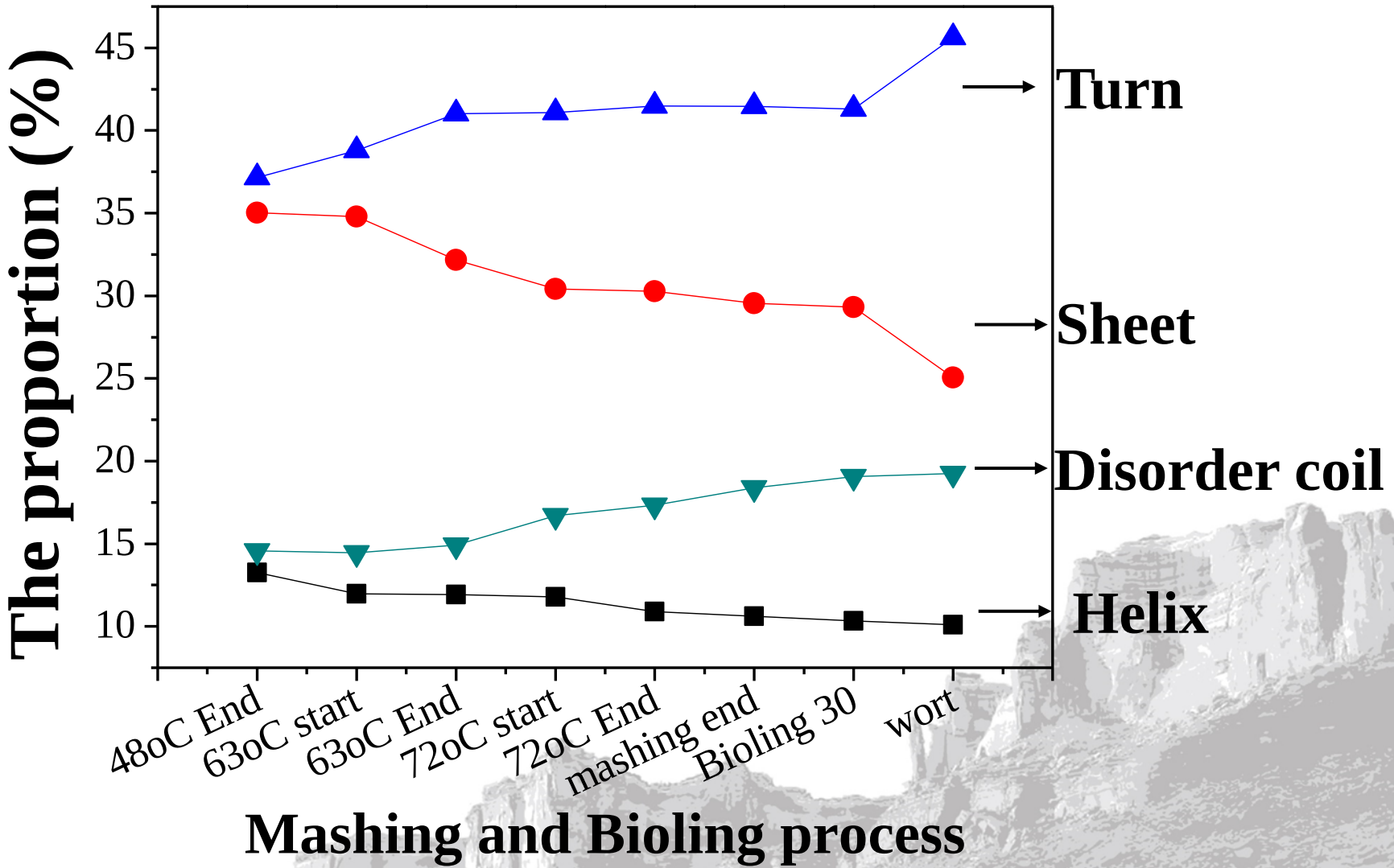


Fig. 7 Circular dichroism spectra of the purified protein Z during the boiling process.

The dynamic changes of secondary structure proportion



FTIR analysis Mashing

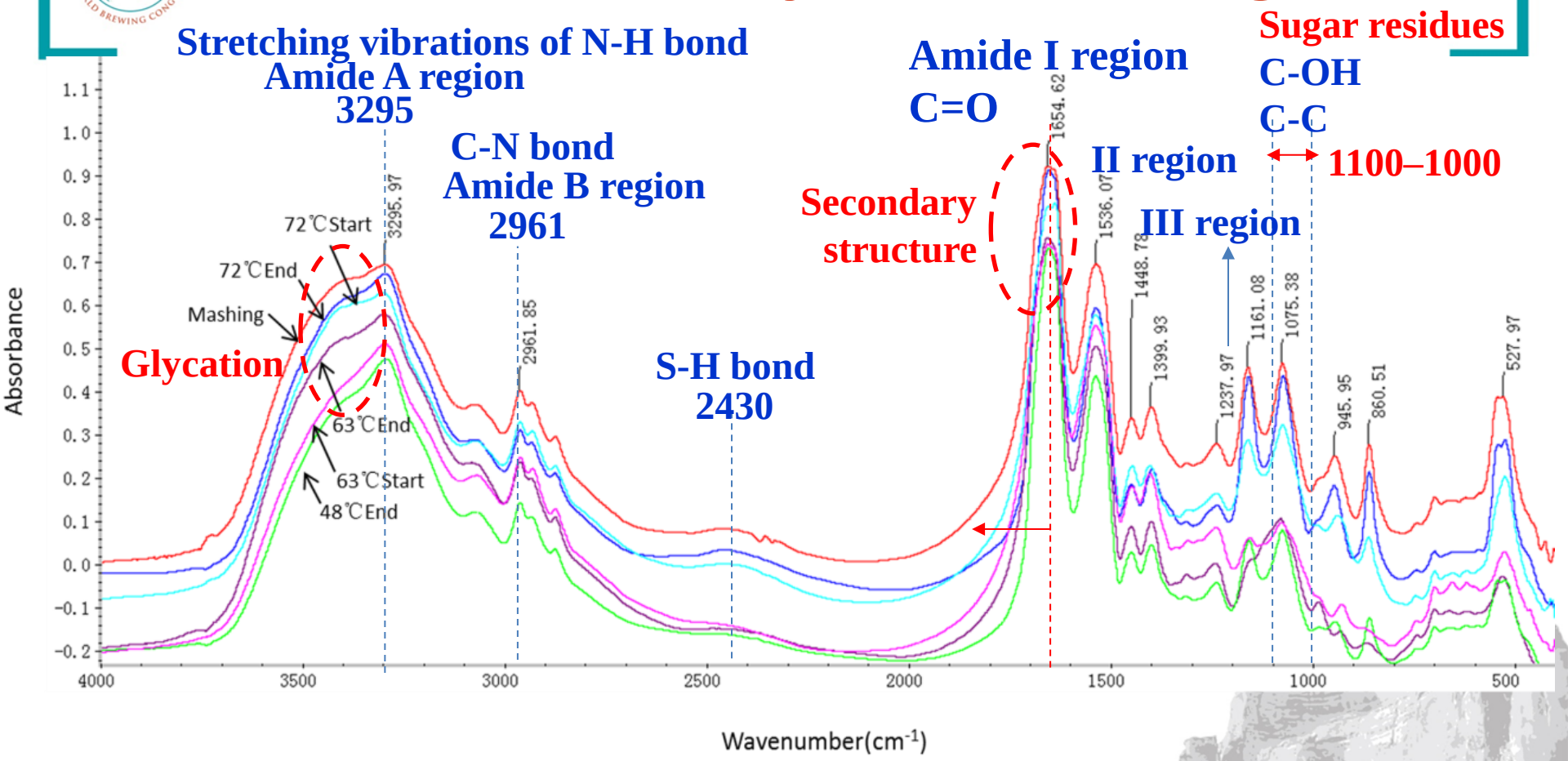


Fig. 8 The FTIR spectra of the purified protein Z during mashing process after baseline correction and vectorial normalization.

Numbers indicate the mian peak positions.

FTIR analysis (Boiling)

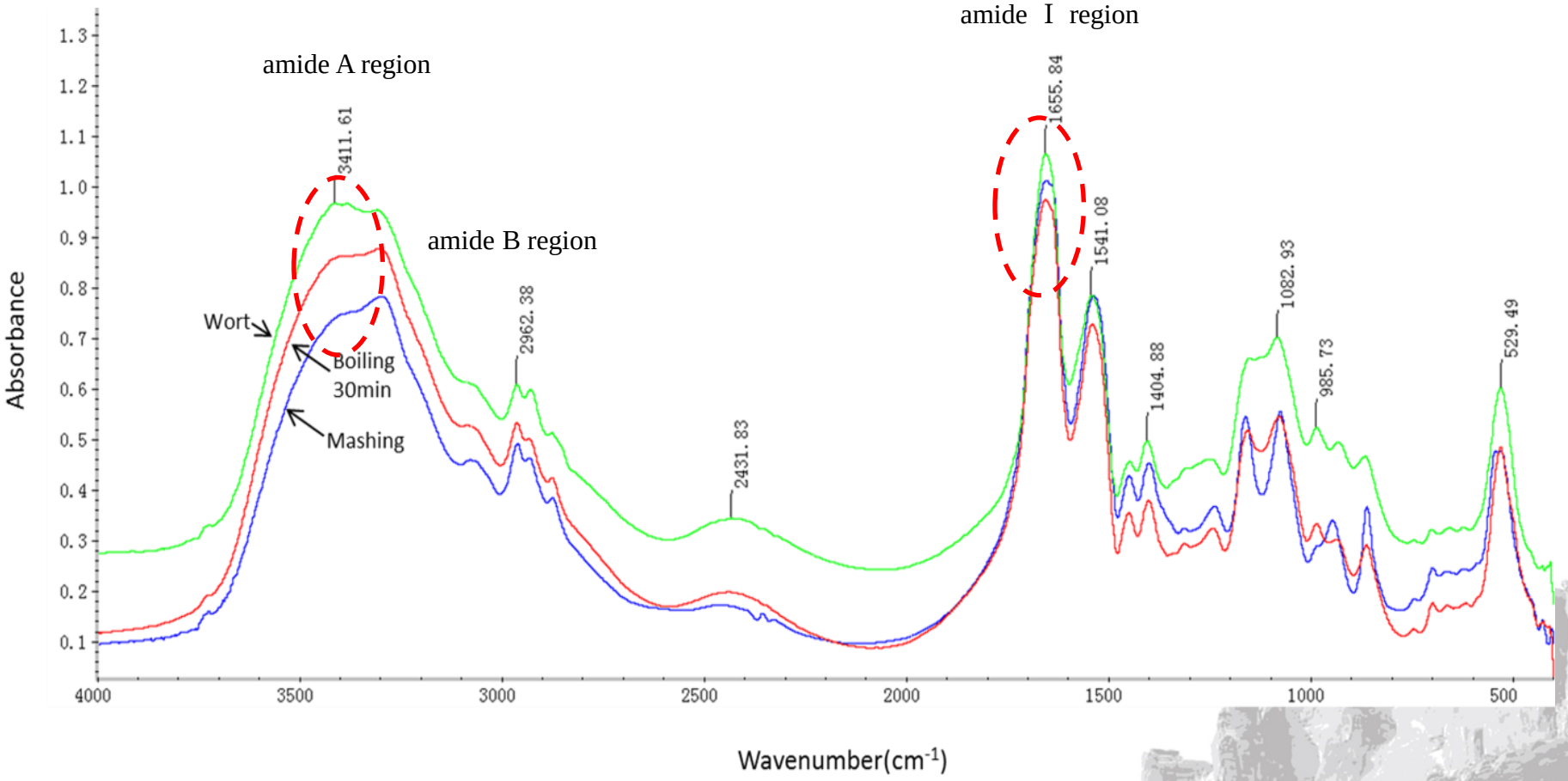
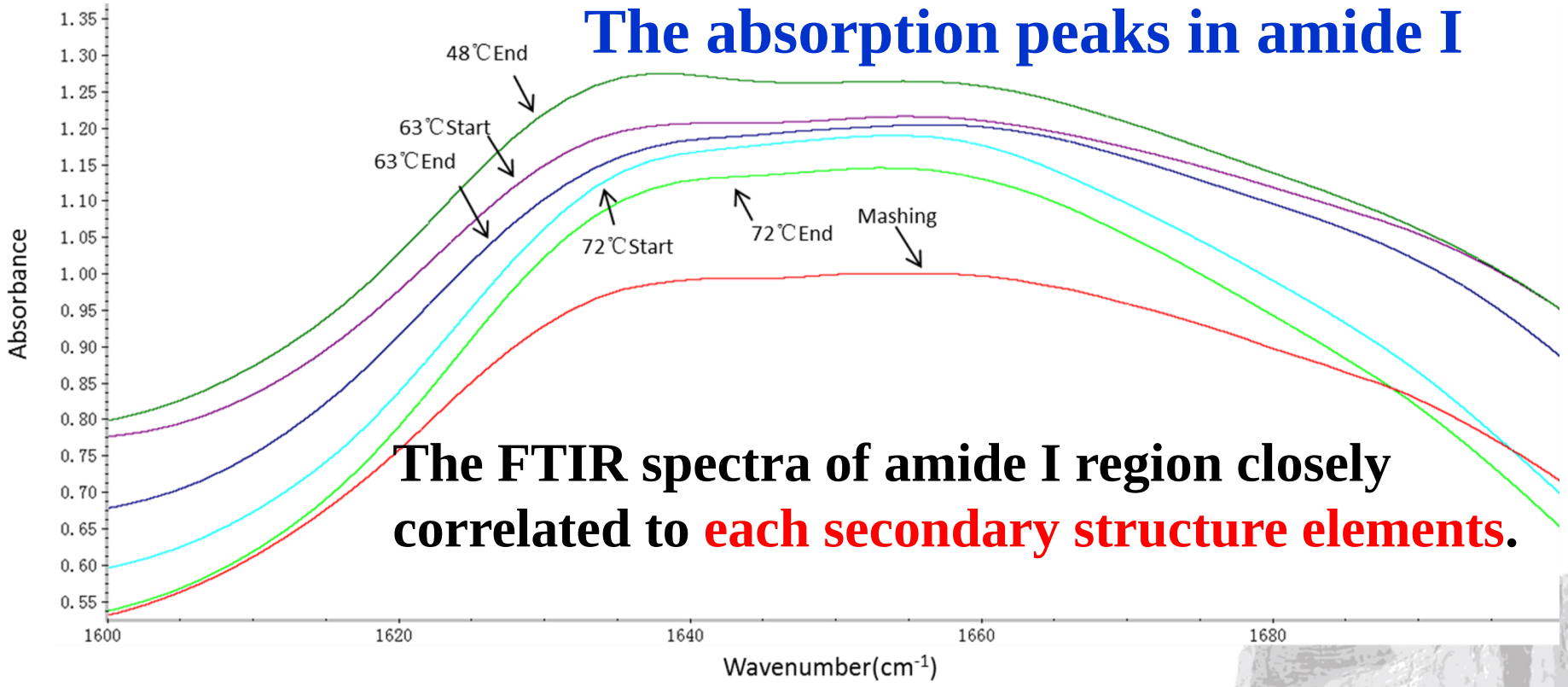


Fig . 9 The FTIR spectra of the purified protein Z during boiling process after baseline correction and vectorial normalization.

Numbers indicate the main peak positions.

Amide I region analysis (mashing)

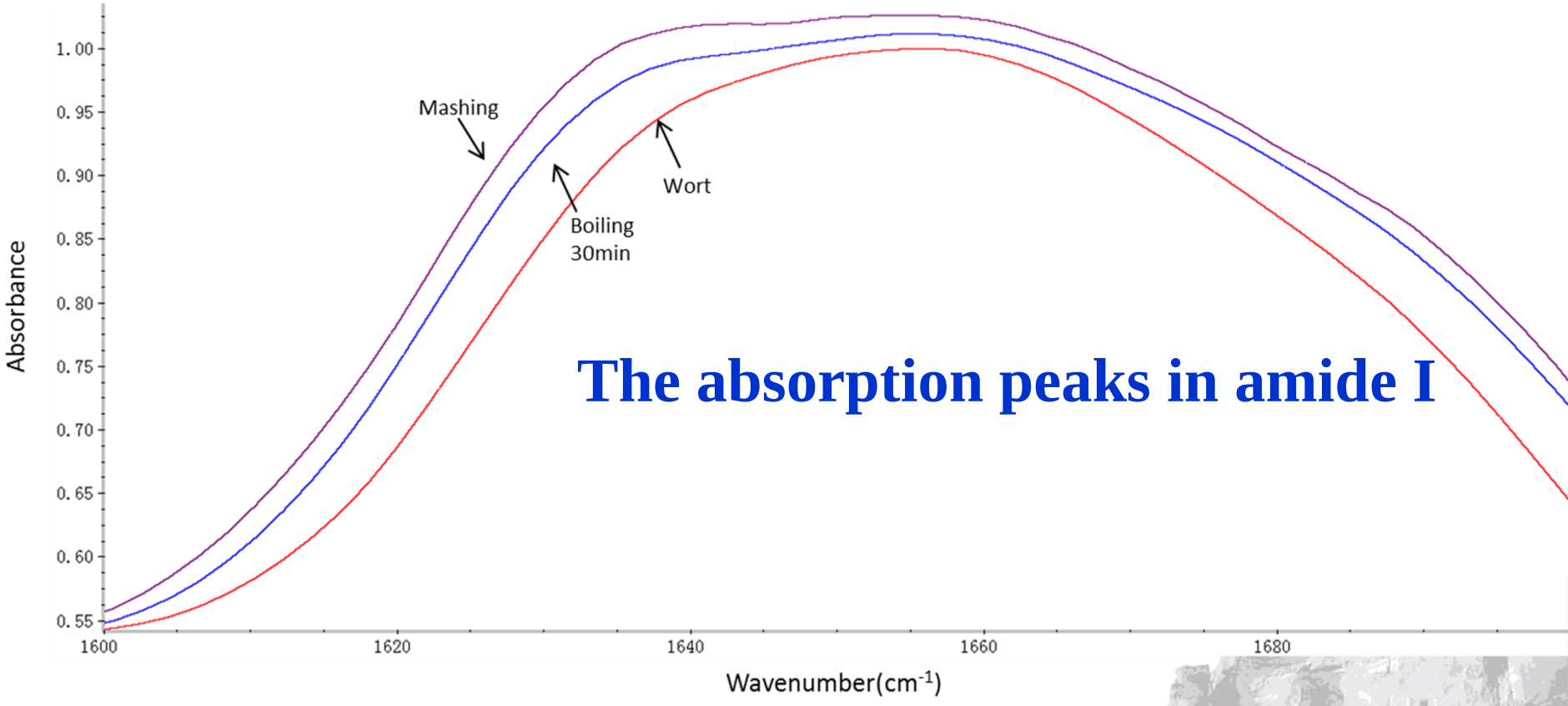
The absorption peaks in amide I



The FTIR spectra of amide I region closely correlated to each secondary structure elements.

The absorption peaks at 1635 cm^{-1} gradually decreased and the absorption of the amide I region moved towards high wavenumbers.

Amide I region analysis (boiling)



To reveal the changes in internal structure of protein Z, deconvolution, curve fitting and peak resolution of the amide I region based on Gauss formula were carried out

Curve fitting of amide I region and 10-peak resolution (mashing)

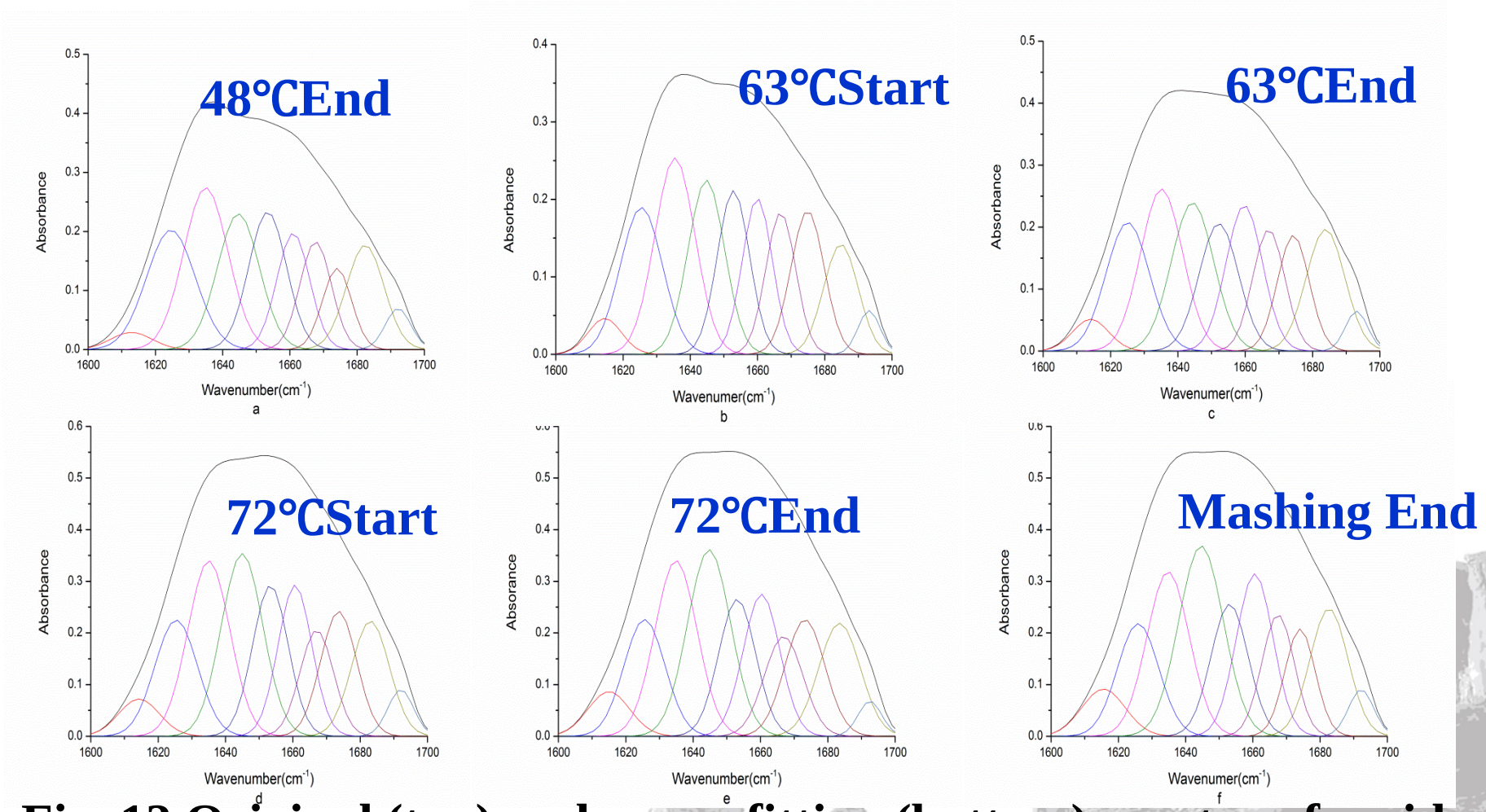


Fig. 12 Original (top) and curve-fitting (bottom) spectra of amide I region of protein Z during mashing process.

Curve fitting of amide I region and 10-peak resolution (Boiling)

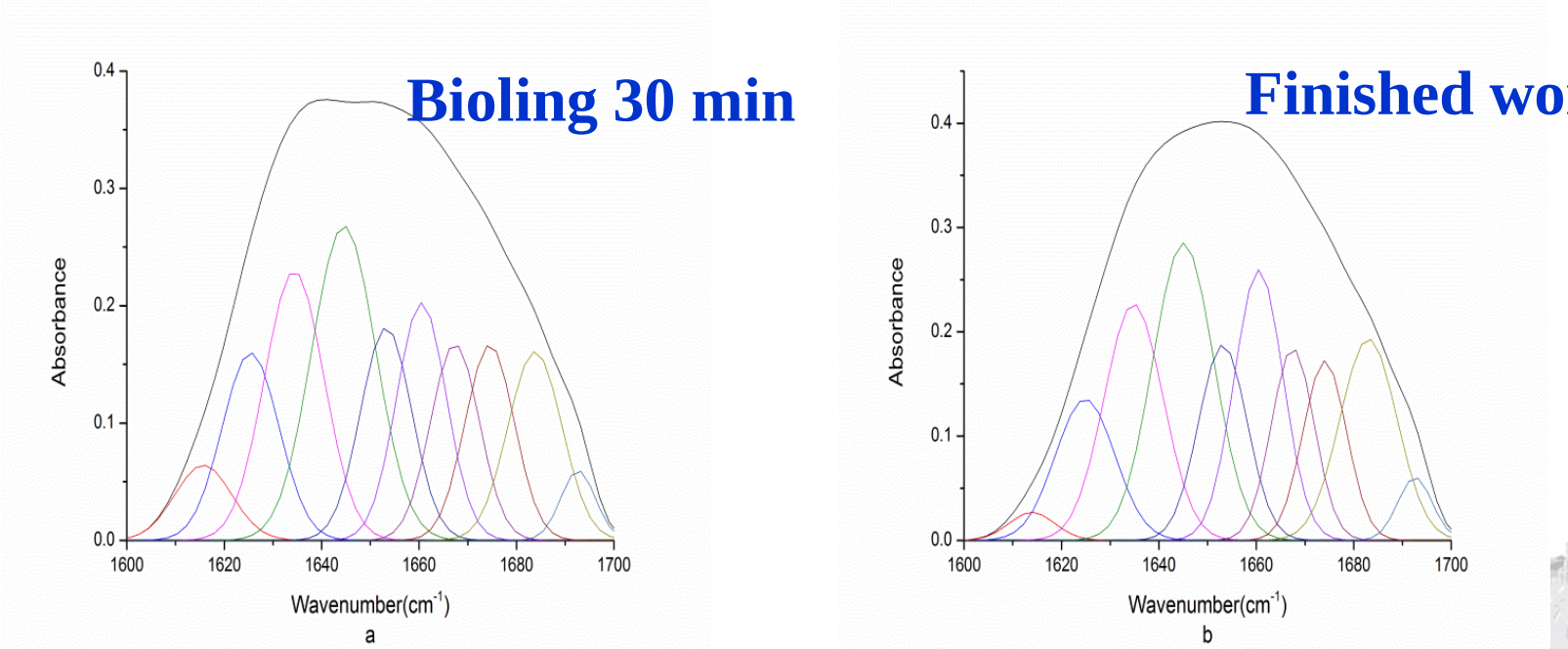
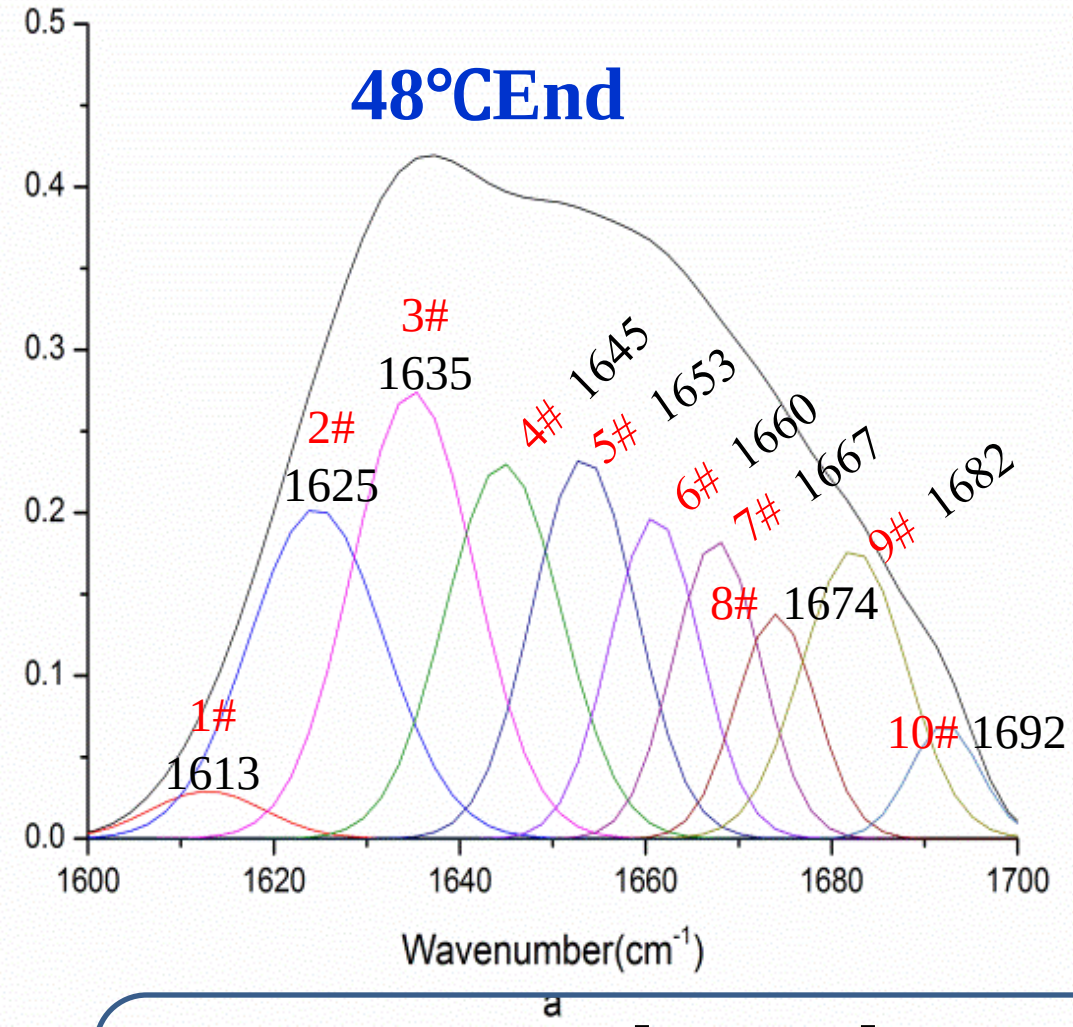
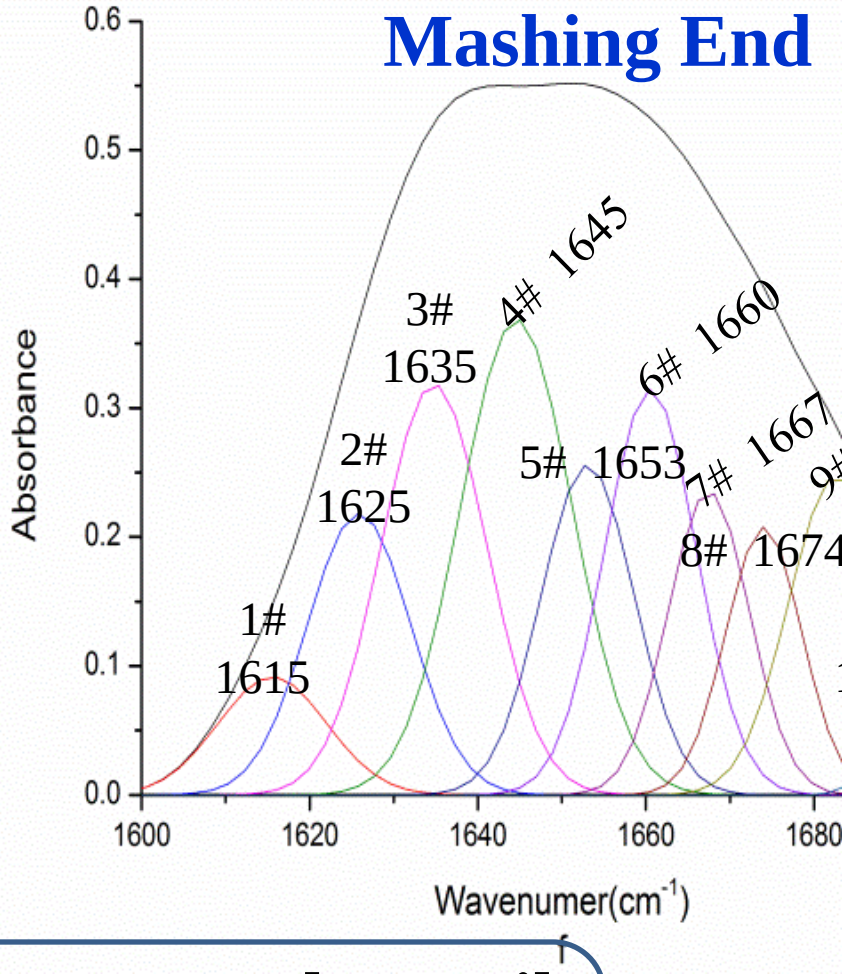


Fig. 13 Original (top) and curve-fitting (bottom) spectra of amide I region of protein Z during boiling process.





1#: Aggregated strands

2#: Intermolecular sheets

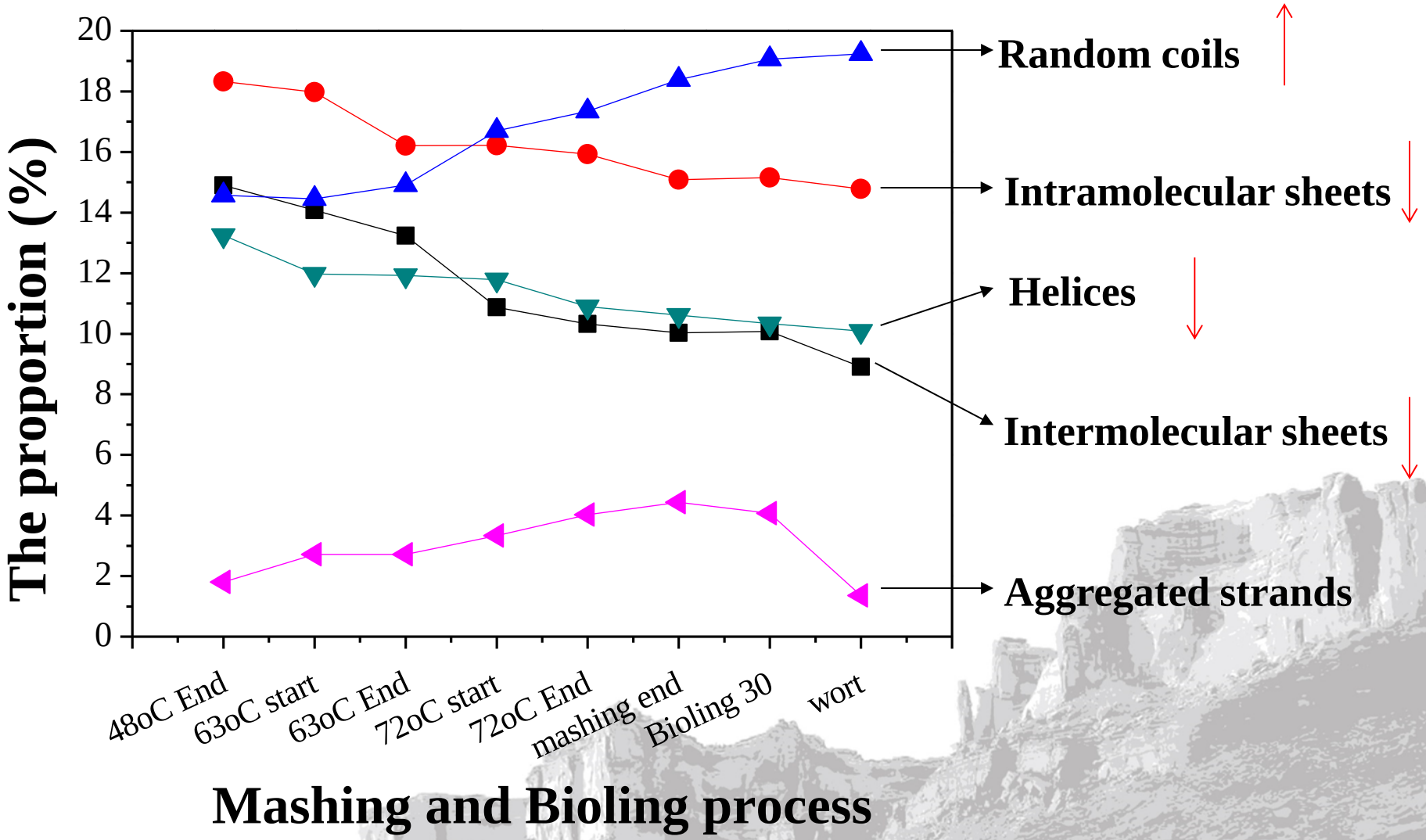
3#: Intramolecular sheets

4#: Random coils

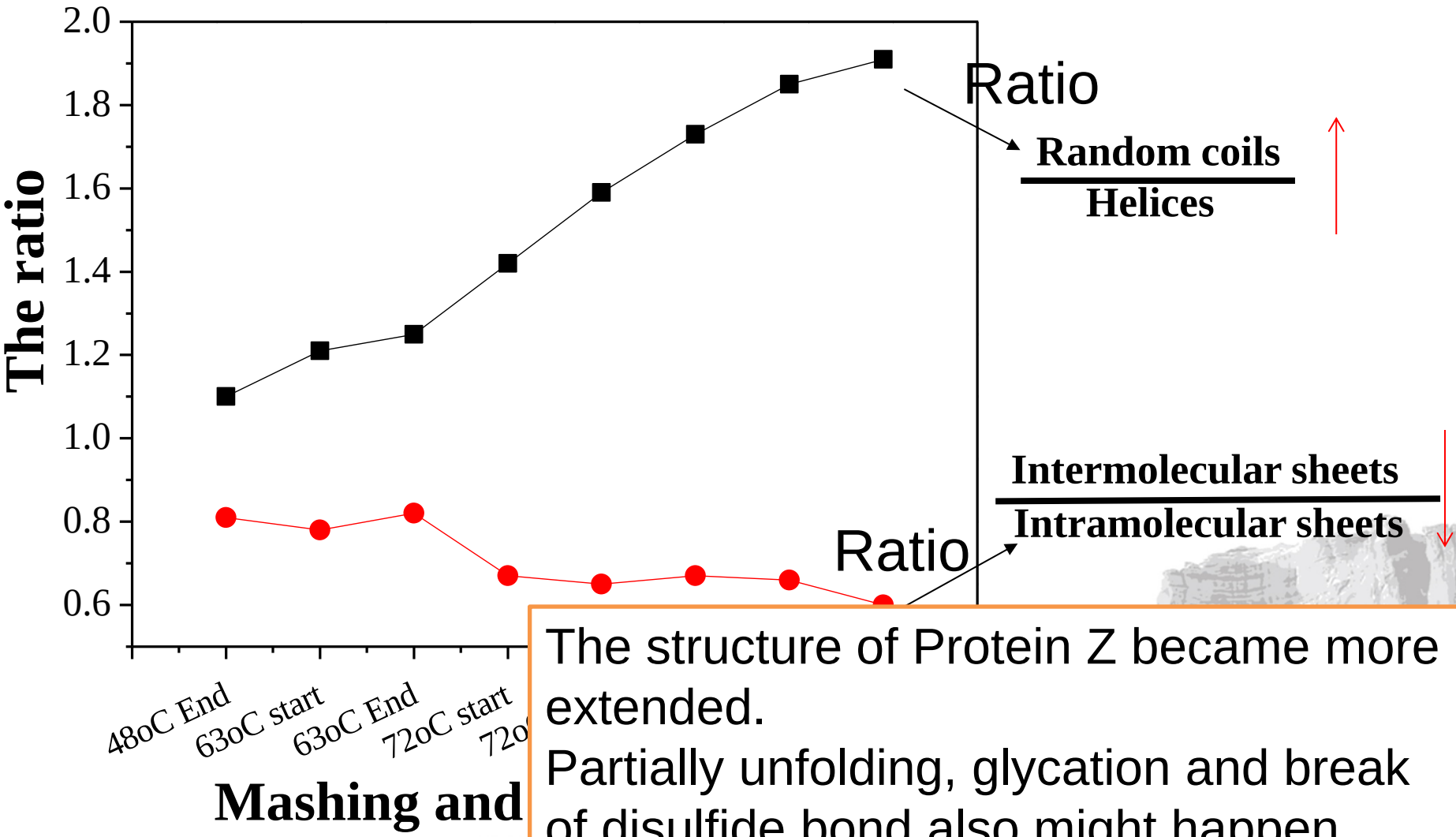
5#: Helices

6-10#: Turns

Internal structure changes in protein Z



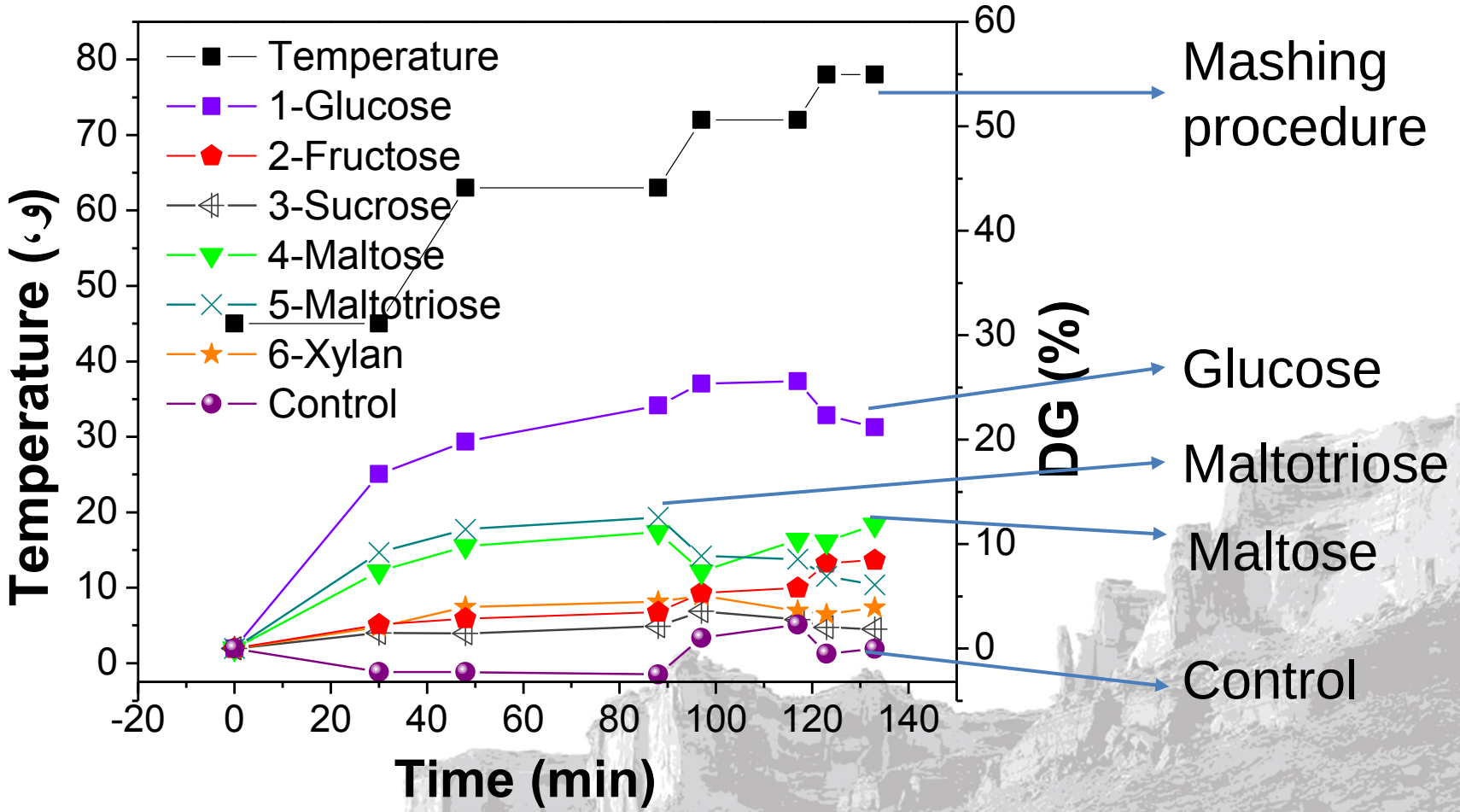
Internal structure changes in protein Z



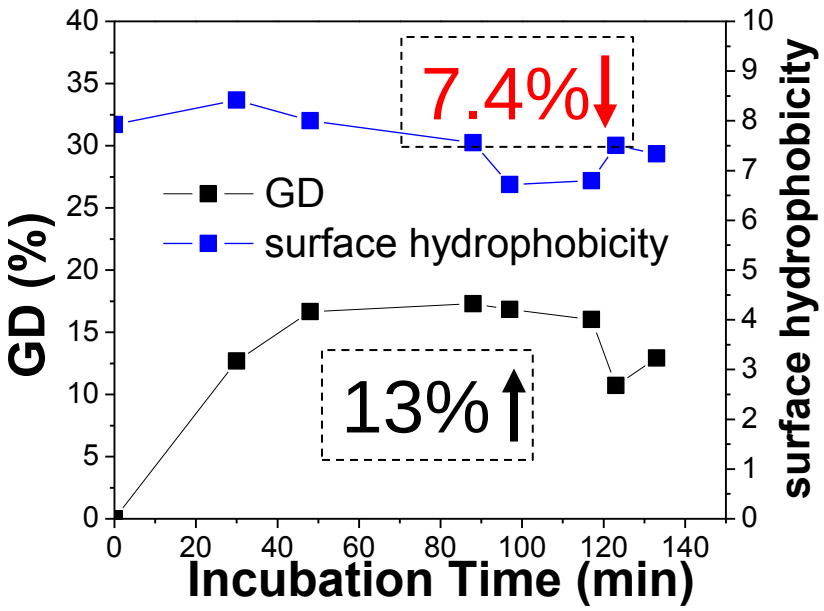
The structure of Protein Z became more extended.
Partially unfolding, glycation and break of disulfide bond also might happen.

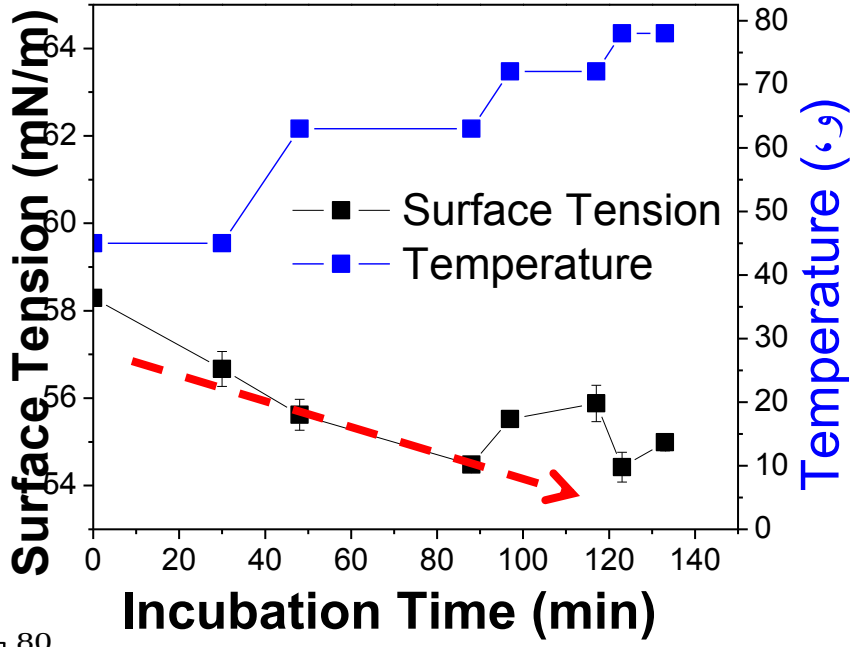
The grafting degree analysis

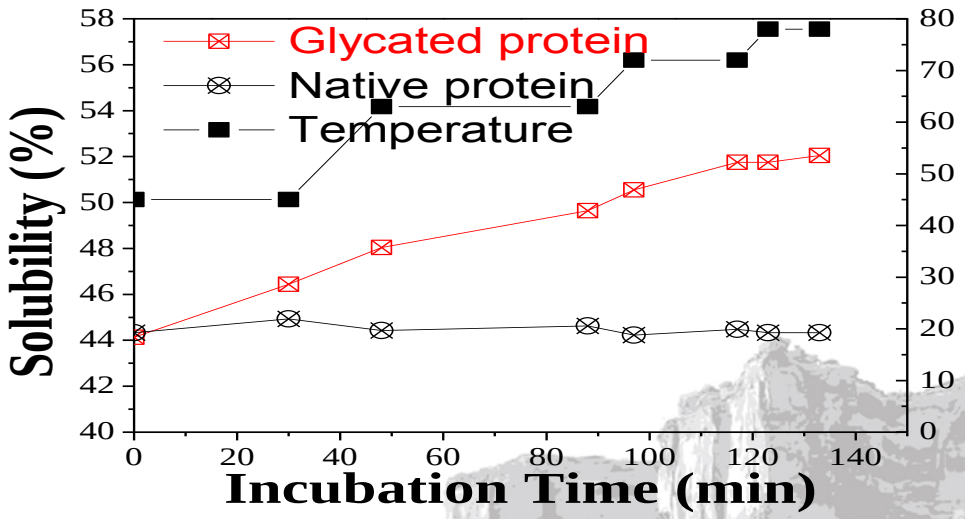
Protein Z extracted from malt + Saccharide → Mashing



The properties of glycated protein Z during mashing process







During Mashing process:

The grafting degree ↑

Surface hydrophobicity ↓

Surface tension ↓

Solubility ↑

Conclusions

- **The contents of α-helices and β-sheets decreased and opposite changes to β-turns and random coils. The complex environment rich in polysaccharides might lead the conformational alterations and modifications occurred to protein Z.**

Conclusions

- **The extended structural features provided more amino acid residues for modifications and exposed intra-hydrophobic regions.**

- **Glycation of extended protein Z decreased its surface hydrophobicity and surface tension, increased the solubility, which should be beneficial to maintain beer foam.**

Thank you for your attention !