



WORLD BREWING CONGRESS

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#ElevateBeer



Influence of high-molecular-weight proteins and polypeptides on smoothness of beer

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Outline

- **Background**
- **Objectives**
- **Experiments**
 1. **Preparative size-exclusion chromatography and sensory evaluation**
 2. **Dose response of peptides and maltodextrins on beer taste**
 3. **Identification of a 10-20 kDa protein**
- **Conclusions**



Background

- **Maltodextrins contribute to palate fullness & mouthfeel of beer** (Gastle et al., 2013, EBC)
- **High molecular nitrogen influences the fullness & bitter quality of beer**
(Ishizuka et al., 2014, MBAA)
- **Beer foam proteins have been identified by proteomic analysis** (Jimure et al., 2015, EBC)



Objectives

- **Few studies have focused on causal relationships between beer taste and high molecular weight (HMW) proteins or polypeptides.**
- **We therefore examined the influence of HMW proteins and polypeptides on beer taste.**

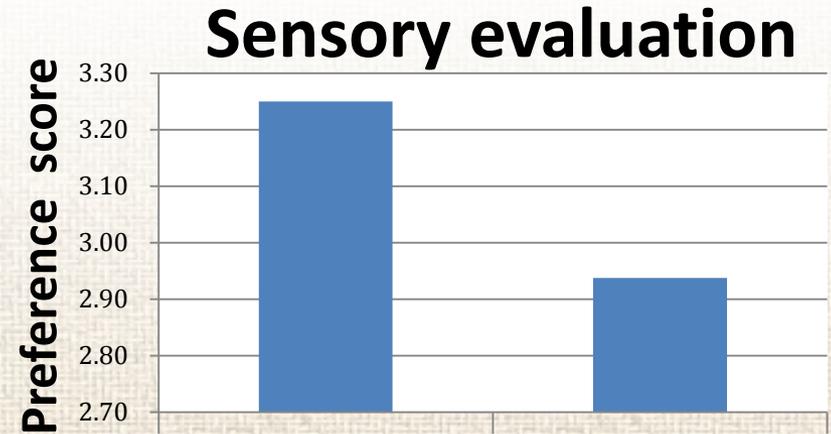
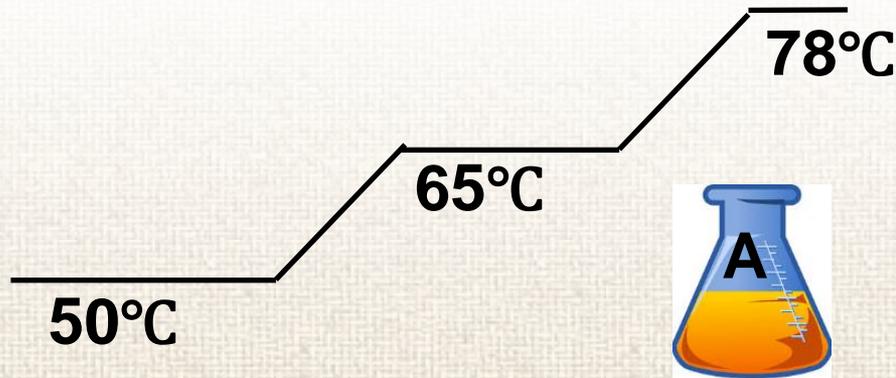
Experiment 1

Preparative size-exclusion chromatography and sensory evaluation



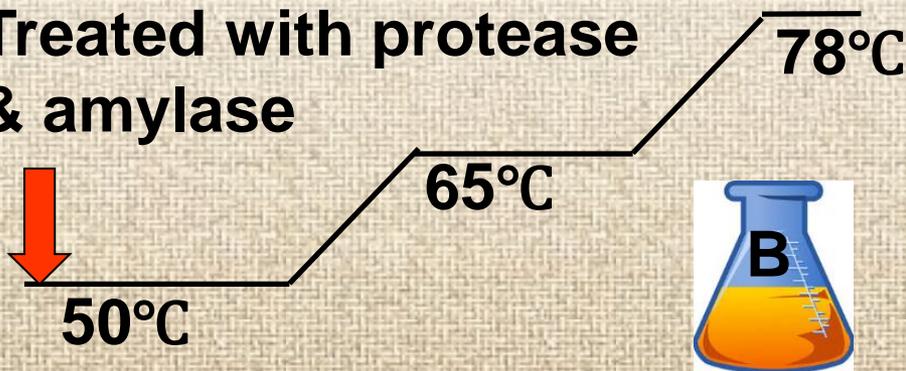
All-malt beer samples

Sample A



Sample B

Treated with protease & amylase



Sample A Sample B

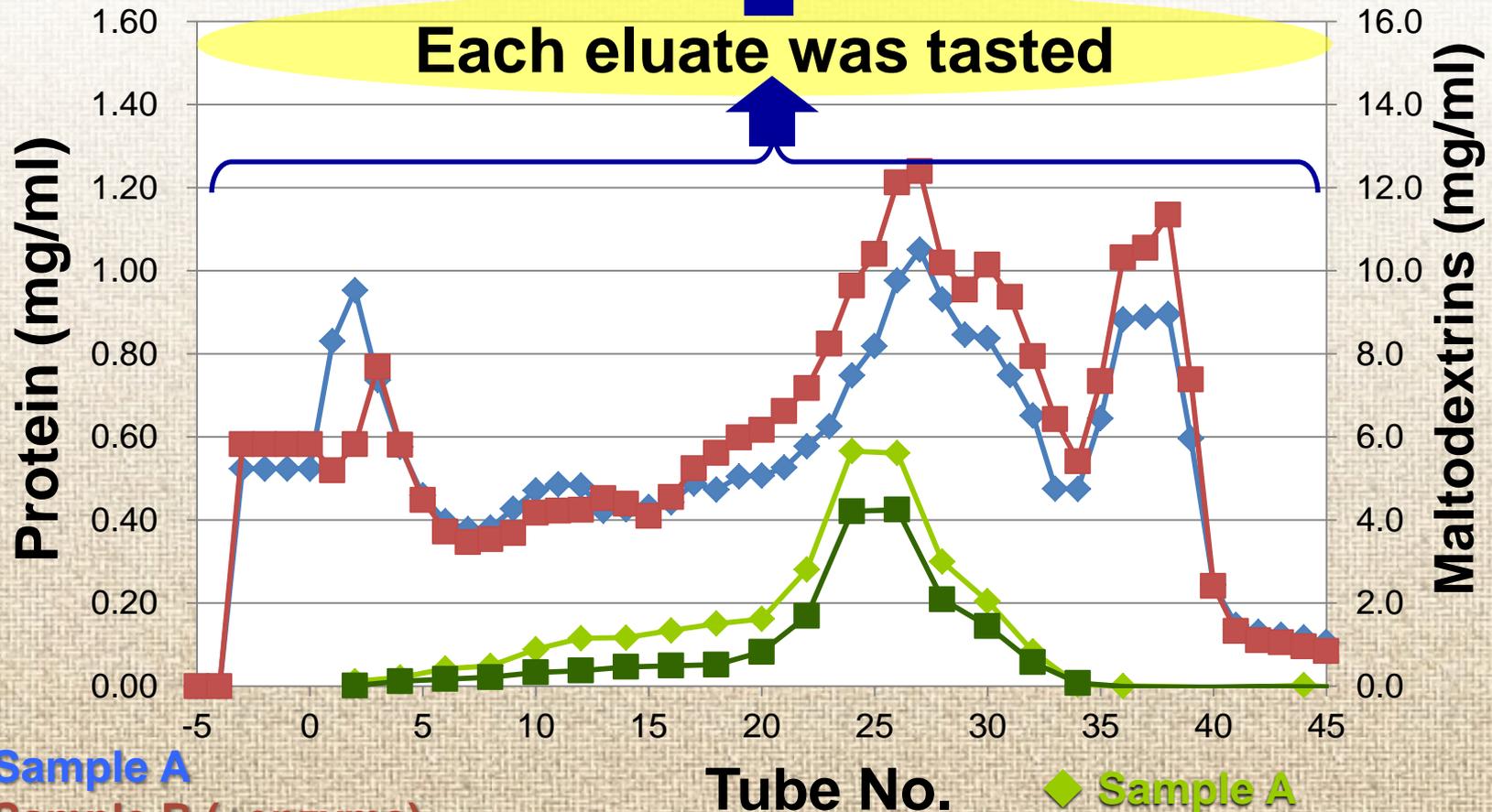
- ◆ Fermentation: 1 week at 12°C
- ◆ Maturation: 4 days at 10°C



Size-exclusion chromatography

Grouped according to taste profile

Each eluate was tasted



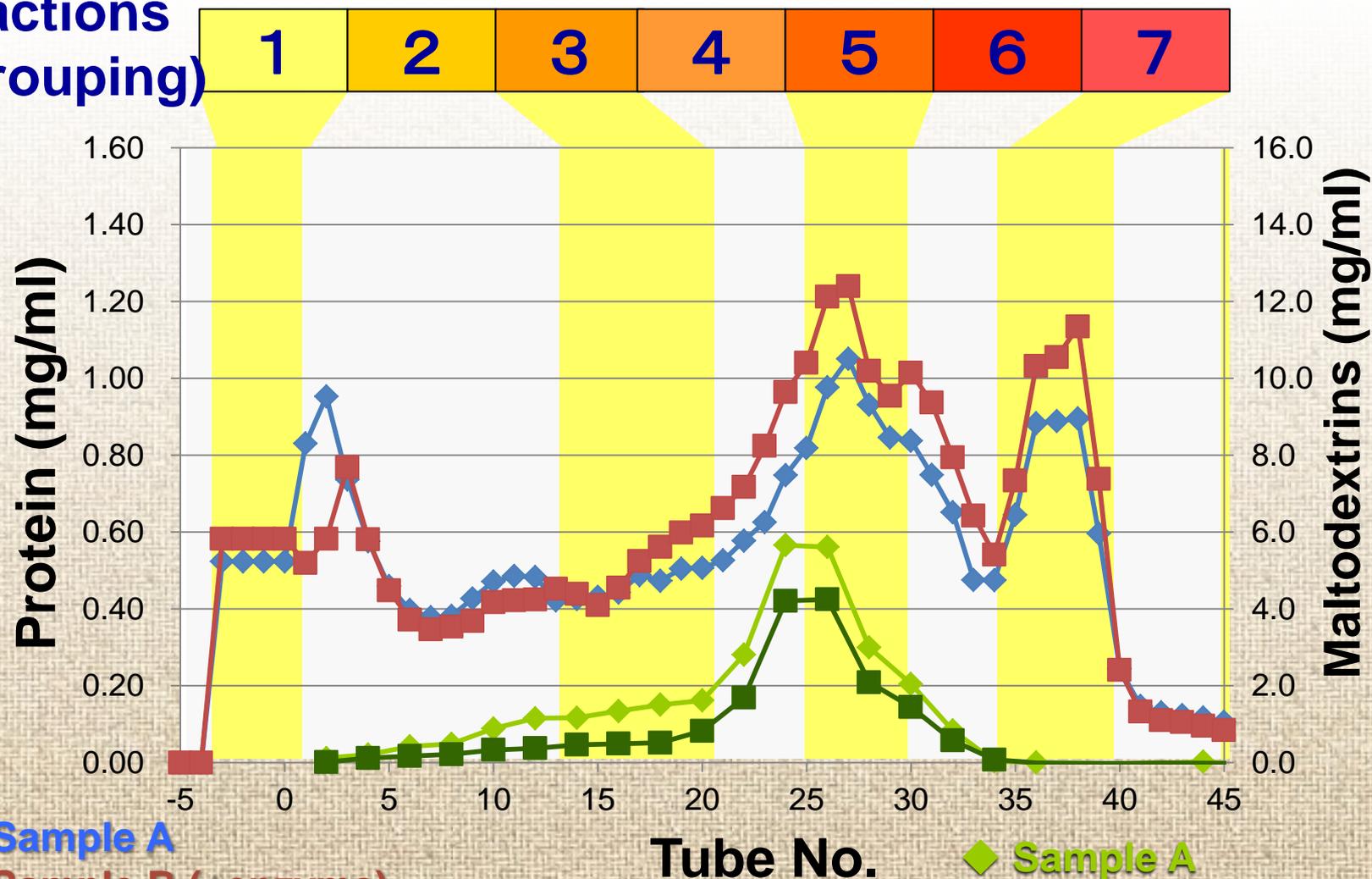
◆ Sample A
■ Sample B (+enzyme)

◆ Sample A
■ Sample B (+enzyme)



Size-exclusion chromatography

Fractions
(Grouping)



◆ Sample A

■ Sample B (+enzyme)

◆ Sample A

■ Sample B (+enzyme)

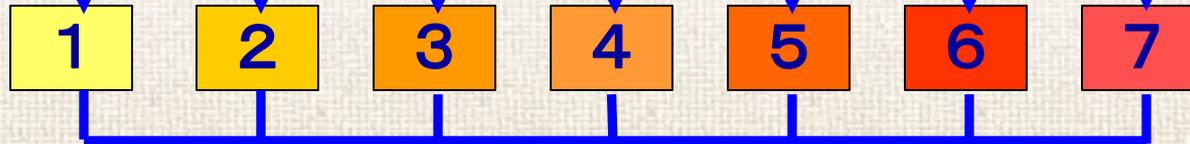


Purification scheme

Sample A or B

Size-exclusion chromatography

Fractions



C18 Solid phase extraction (SPE)

flow-through

← Ion-exchanger resin
← Activated charcoal

adsorbed



Maltodextrin fractions



Protein & peptide fractions

Sensory evaluation

M2 M3 M4 M5

P1 P2 P3 P4 P5 P6 P7

Maltodextrins/proteins & peptides from **Sample A or B**

+

50% increase from original sample

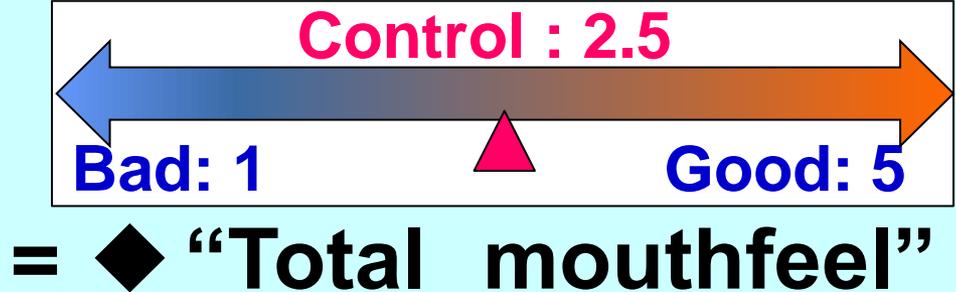


<49% malt

>51% barley "Happo-shu"

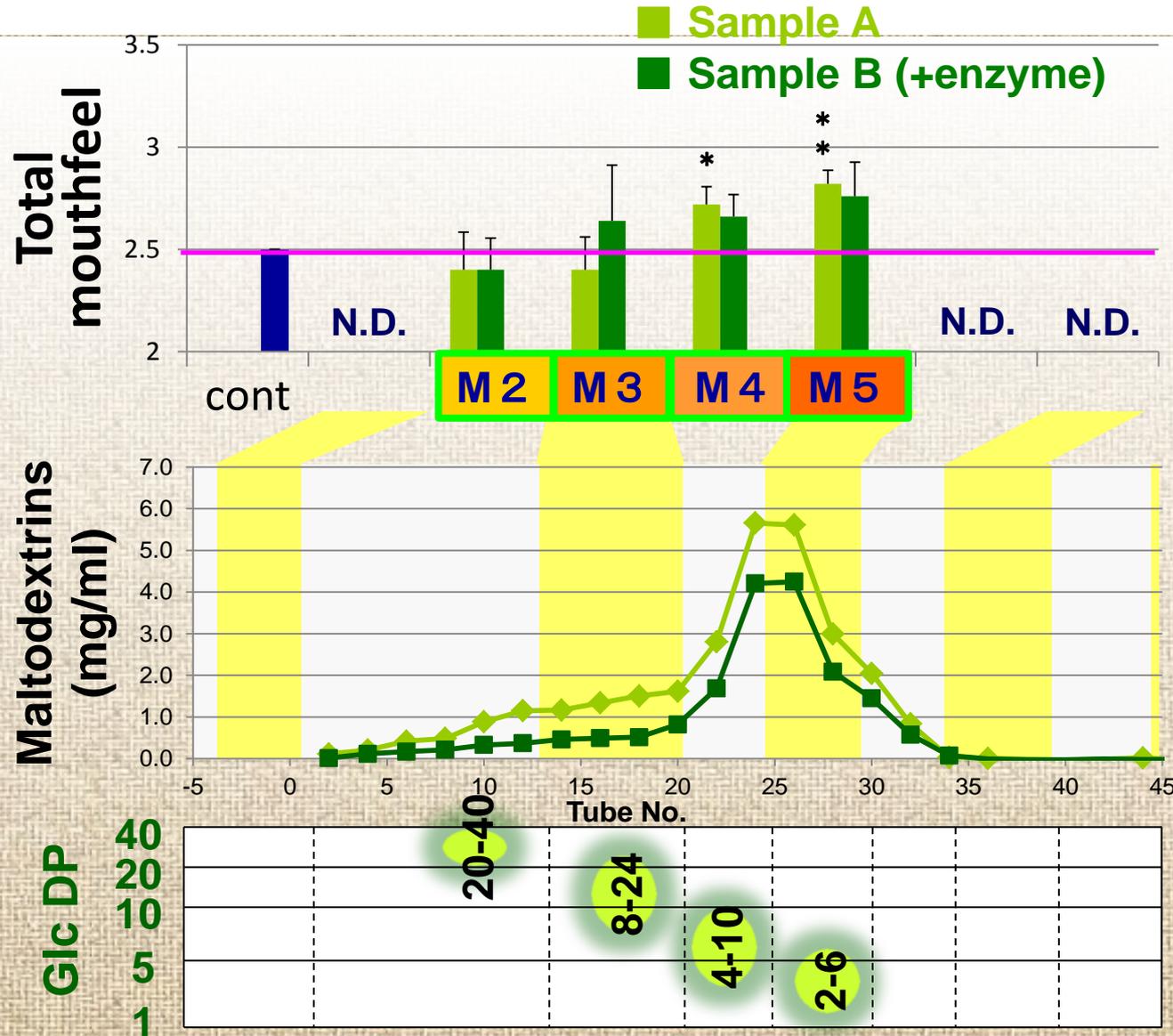
Sensory analysis

- Smoothness
- Softness
- Astringency



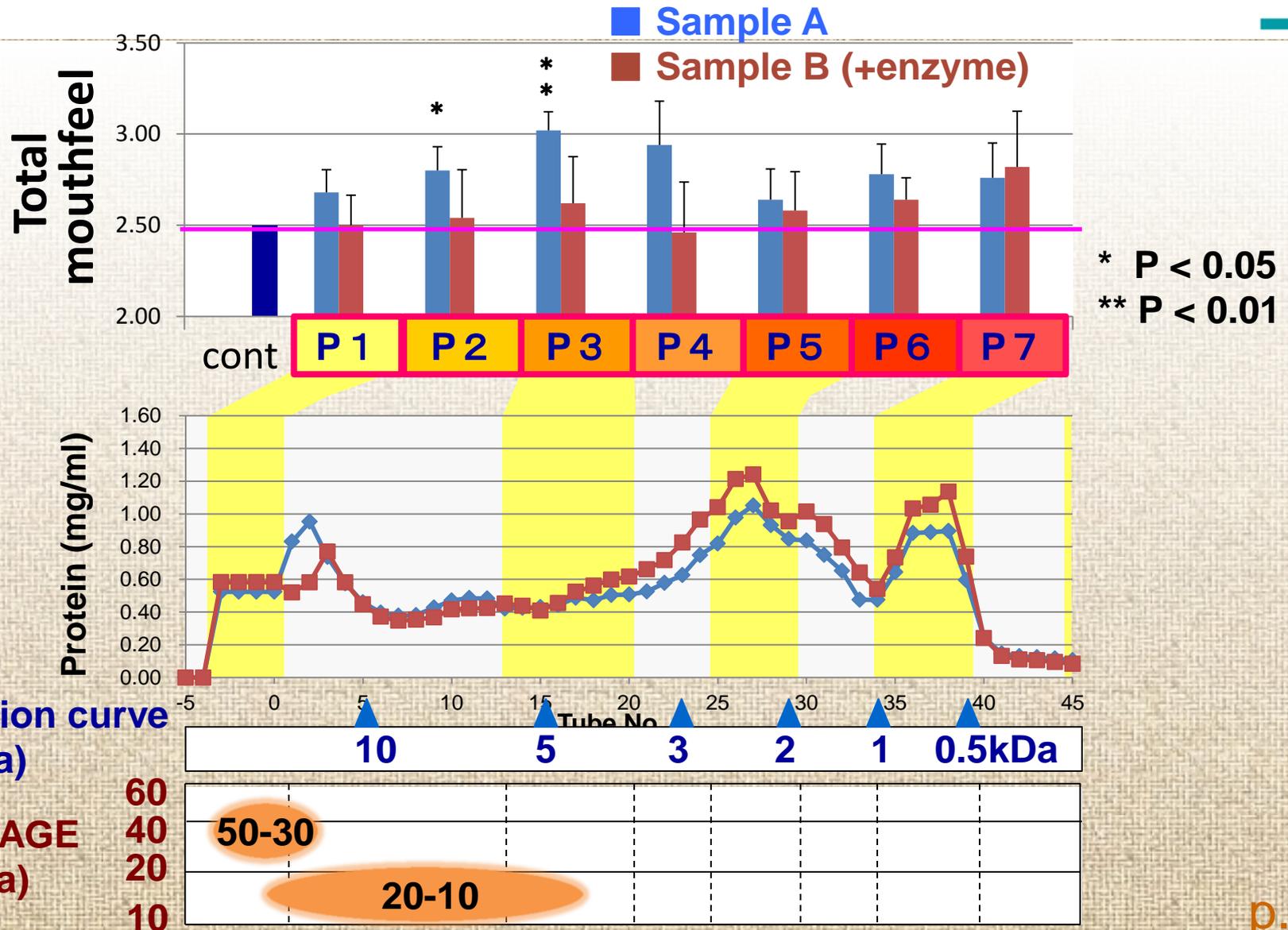


Maltodextrin fractions





Protein fractions





Short summary (Exp. 1)

- **Significant improvement in mouthfeel:**
 - Maltodextrin fractions **M4 & 5** (DP 2-10)
 - Protein fractions **P2 & 3** (10-20 kDa) from **Sample A**
- **Tendency for improvement:**
 - Polypeptide fractions **P4 to 7** (2-3, 0.5-2 kDa)
- **The effects appeared to be dose-dependent, but some fractions did not exhibit this relationship.**

Quality differences in the LMW polypeptides might be responsible for this lack of dose dependency.

Experiment 2

Dose response of peptides and maltodextrins on beer taste



Purification scheme

Sample C

All malt beer



Sample D

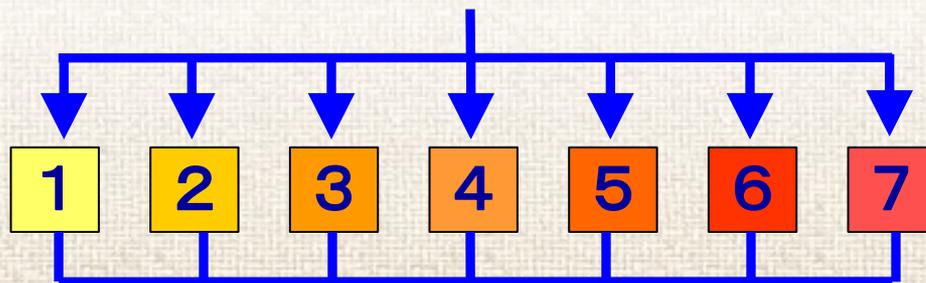
“Happo-shu”

<49% malt
>51% barley



Sample C or D

Size-exclusion chromatography



SPE (C18)

Flow-through

Adsorbed

M4 M5

P1 P2 P3 P4 P5

Maltodextrin fractions

Protein & peptide fractions

Sensory evaluation- maltodextrins

M4 + **M5**

Purified from **Sample D**

+

10% - 60% increase
from original sample



< 24% malt
& others
(barley, sugar syrup)
low-carb “Happo-shu”

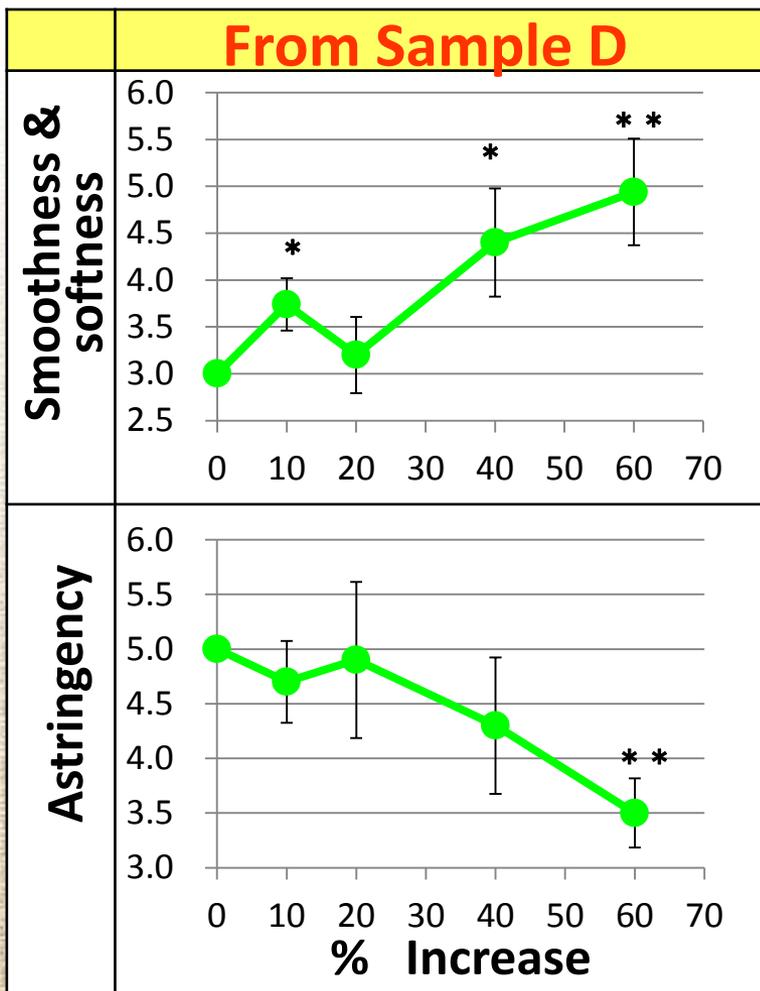


Sensory analysis

- ◆ Smoothness & softness
- ◆ Astringency

Weak: 1 Strong: 9

Maltodextrins: body & mild taste

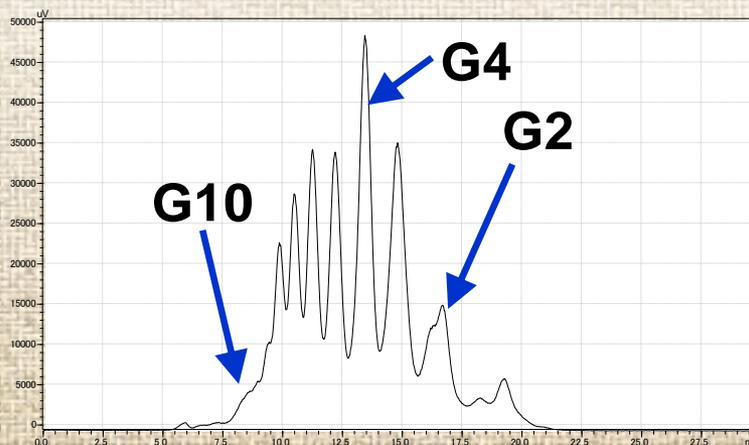


* P < 0.05

** P < 0.01

Panelist comments

	<div style="border: 1px solid red; padding: 2px; display: inline-block;">M4</div> <div style="border: 1px solid blue; padding: 2px; display: inline-block; margin-left: 5px;">M5</div>	<p>Body, weak sweetness, mild taste</p>
<p>G2-10 malto-dextrins</p>		



Sensory evaluation- proteins & peptides

P 1

P 2 + **P 3**

P 4 + **P 5**

Purified from **Sample C or D**

+

20% - 50% increase
from original sample



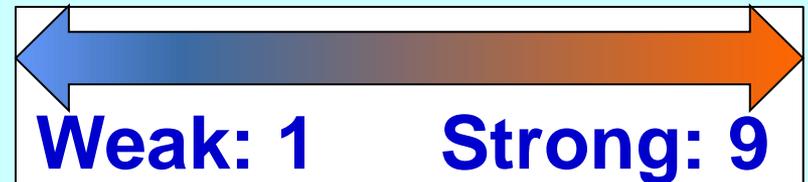
<49% malt

>51% barley "Happo-shu"



Sensory analysis

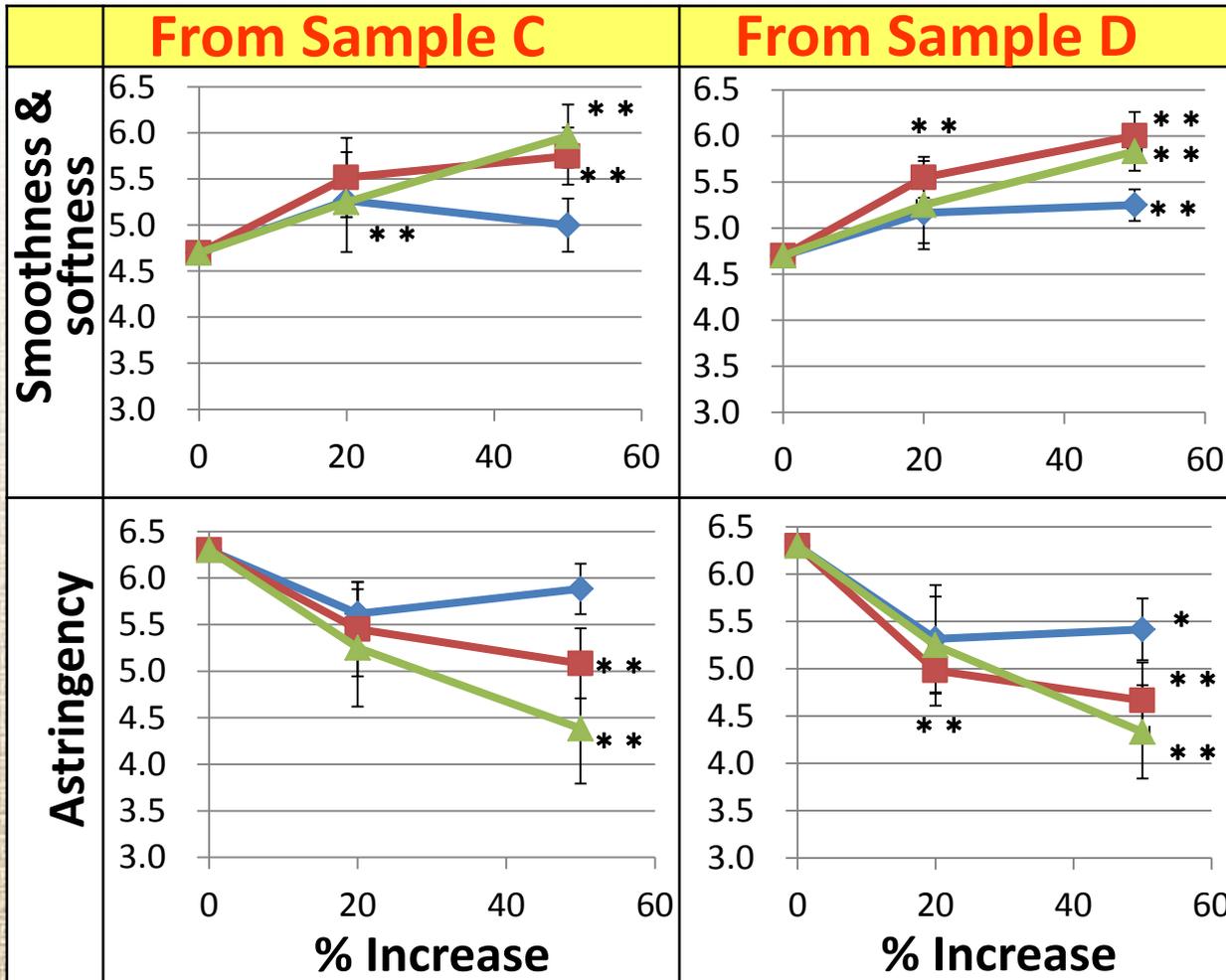
- ◆ Smoothness & softness
- ◆ Astringency





HMW proteins- improved mouthfeel LMW peptides- body & “umami” taste

Panelist
comments



◆	P 1	Roundness
	30-50 kDa HMW proteins	

■	P 2 P 3	Smooth texture
	10-20 kDa HMW proteins	

▲	P 4 P 5	Body, “umami” taste
	2-3 kDa LMW polypeptide	

* P < 0.05

** P < 0.01



Short summary (Exp. 2)

- **Palate fullness (body) was increased by the DP 2-10 maltodextrin fraction.**
- **Mouthfeel (smoothness & softness) was improved by the 10-20 kDa HMW protein fraction, and astringency was also reduced.**
- **Body and “umami” taste were increased by the 2-3 kDa LMW polypeptide fraction.**

Experiment 3

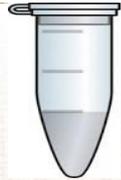
Identification of 10-20 kDa protein



Protein identification scheme

Fractions

P2 P3



TCA acetone purification

2D PAGE

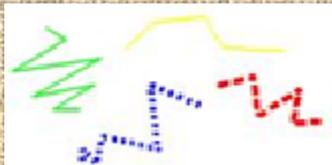


Sample A

Sample B
(+enzyme)

In-gel Digest

Peptides mix



Mass spectrometry

ESI LC-MS/MS



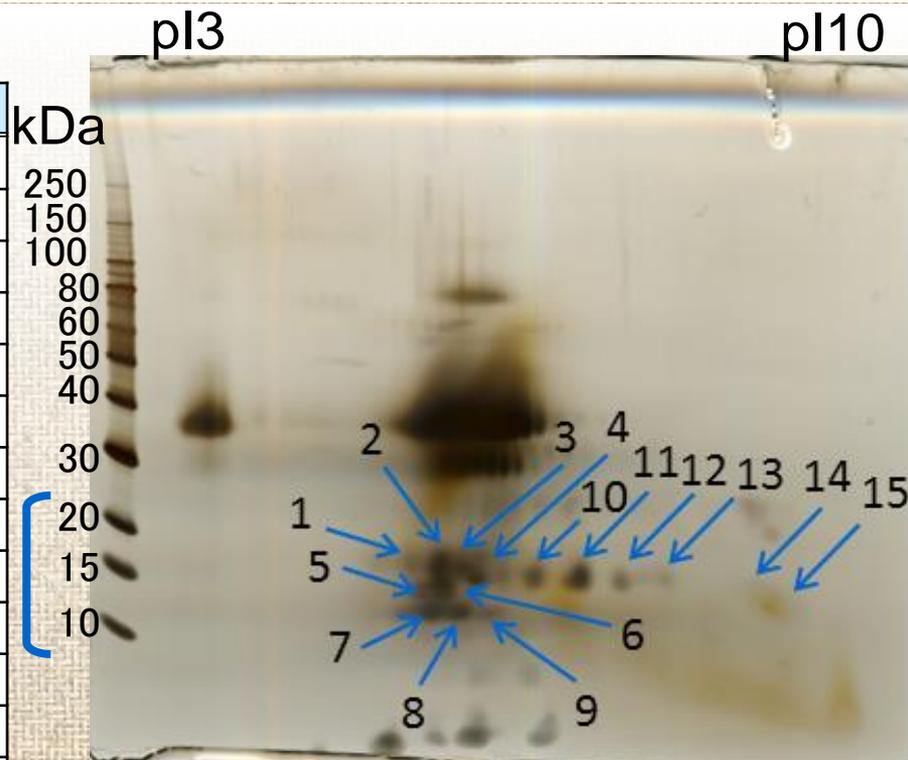
Database Search

Mascot Search Engine, Swiss-Prot Database

Protein identification

Protein identification results

Spot No.	Name of protein
1	Alpha-amylase/trypsin inhibitor CMb
2	Alpha-amylase/trypsin inhibitor CMb
3	Alpha-amylase/trypsin inhibitor CMd
4	Alpha-amylase/trypsin inhibitor CMb
5	Alpha-amylase inhibitor BDAI-1
6	Alpha-amylase inhibitor BDAI-1
7	Alpha-amylase inhibitor BDAI-1
8	Alpha-amylase inhibitor BDAI-1
9	Alpha-amylase inhibitor BDAI-1
10	Trypsin inhibitor Cme
11	Trypsin inhibitor Cme
12	Trypsin inhibitor Cme
13	Trypsin inhibitor Cme
14	Non-specific lipid-transfer protein 1
15	Non-specific lipid-transfer protein 1



◆ **10-20 kDa spots :**
Sample A > B (+enzyme)

◆ **Sensory analysis score :**
Sample A > B (+enzyme)



Conclusions

- **Mouthfeel (smoothness & softness) was improved by 10-20 kDa HMW proteins, and astringency was also reduced.**
- **Body and “umami” taste were increased by 2-3 kDa LMW polypeptide.**
- **In future studies, effects of HMW proteins and LMW polypeptides on beer taste profile will be examined.**

Thank you for your attention.