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Angiotensin I converting enzyme-inhibitory peptides in Asiago d'Alleva cheese

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Introduction

- ✓ **Milk proteins** are precursors of many different biologically **active peptides** called **BIOPEPTIDES**
 - inactive within the sequence of the precursor proteins
- ✓ They can be generated by: enzymes in milk, bacterial enzymes and gastro-intestinal enzymes
- ✓ They have some bio-activities:
 - antithrombotic (Sollier *et al.*, 1996)
 - antihypertensive** (Gobbetti *et al.*, 2004)
 - immunomodulating (Kayser & Meisel, 1996; LeBlanc *et al.*, 2002)
 - antibacterial (Zucht *et al.*, 1995; Gobbetti *et al.*, 2003)

Angiotensin-I converting enzyme (ACE)

ACE (E.C. 3.4.15.1): enzyme involved in the renin- angiotensin system regulating peripheral blood pressure.

Angiotensin-I $\xrightarrow{\text{ACE}}$ Angiotensin II
 Bradykinin $\xrightarrow{\text{ACE}}$ 

Characteristics of ACE-inhibitory peptides in dairy products

- 2-20 amino acid residues
- C-terminal position: basic, hydrophobic (aromatic or branched side-chains) residues
- Proline-containing peptides are generally resistant to degradation by digestive enzymes

(Vermeirssen *et al.*, 2004)

ACE and proteolysis in dairy products

- ✓ Usually inhibitory activity increased as proteolysis developed but only to a certain level after which ACE-inhibition index decreases;
- ✓ ACE-inhibitory peptides have been isolated from several italian cheeses characterized by short and medium ripening period;
- ✓ Higher ACE-inhibitory activity in 2- and 8-months old Manchego cheeses than in cheeses with 4 and 12 months: not followed any common pattern!!!

(Smacchi & Gobbetti, 1998; Saito *et al.*, 2000; Gómez-Ruiz *et al.*, 2006)

Aim of the research

Evaluate the ACE-inhibitory activity
of
peptides extracted from
Asiago d'Allevo cheese

Materials and Methods



Asiago cheese



- ✓ Asiago is an Italian PDO cheese (Reg. 1107 /96) produced with 2 distinct flavours: fresh and ripened varieties;
- ✓ *Asiago d'Allevo* variety is manufactured by processing evening skimmed and morning raw milk mixture and ripened from 6 to 18 (even more) months.

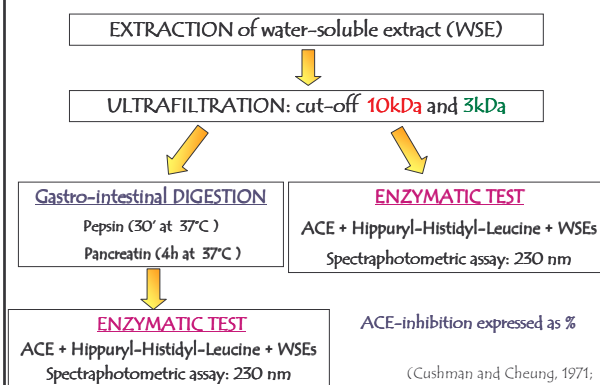


Materials and Methods

Wheels of *Asiago d'Allevo* cheese analysed

Age of ripening (months)	Milk production system / cheese making condition
6,12 and 18	May: Total mixed ration based on hay and concentrate (maize and soybean); cheese manufactured in a commercial dairy plant
6,12 and 18	July: Alpine grazing + concentrate (maize and other cereals grains) as supplement (5.0 kg DM /d); cheese manufactured in the farmhouse
6,12 and 18	Sept.: Alpine grazing + concentrate (maize and other cereals grains) as supplement (4.5 kg DM/d); cheese manufactured in the farmhouse

ASSAY



Statistical analysis

LINEAR MIXED MODEL (SAS 9.1)

$$Y_{ijklm} = \mu + R_i + P_j + MW_k + D_l + MW \times R + R \times D + MW \times D + \varepsilon_{ijklm}$$

where **fixed effects** are:

R: ripening (3 levels: 6, 12 and 18)

P: milk production system (3 levels: May-commercial dairy plant; July/alpine grazing-farm house; September/alpine grazing-farm house)

MW: molecular weight of peptides (2 levels: < 3 kDa and < 10 kDa)

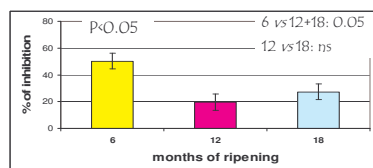
D: gastro-intestinal digestion (2 levels: no digestion, yes digestion)

random effect: wheel (R x P)

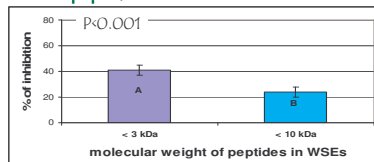
RESULTS

Lsmeans – % of ACE-inhibitory activity

Ripening effect

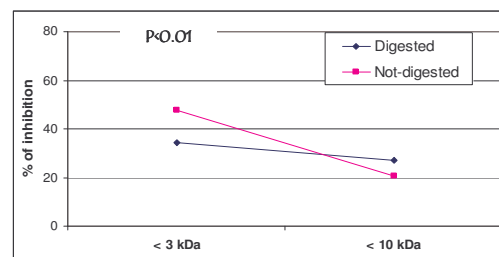


Molecular weight (MW) of peptides effect



WSE: water-soluble extract; R: ripening; MWxR: ns

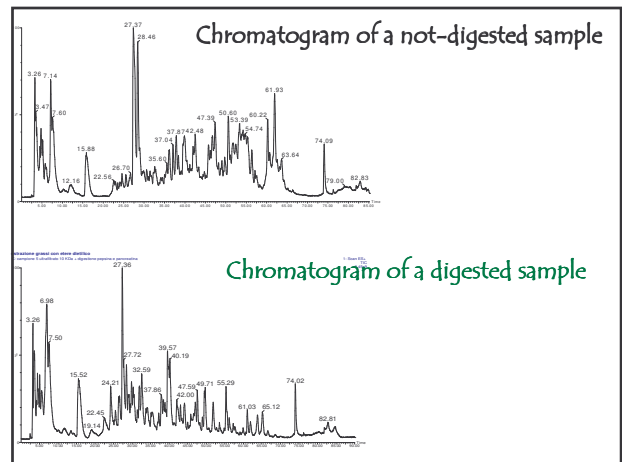
Gastro-intestinal digestion and Molecular weight of peptides effect



EFFECTS – milk production system: ns

Conclusions

- ✓ 6 months-old cheeses had higher ACE-inhibitory activity than cheeses ripened to 12 and 18 months ;
- ✓ The ACE-inhibitory activity was mainly associated with the low-MW peptide fraction;
- ✓ Milk production system did not influence ACE-inhibition index;
- ✓ Further gastro-intestinal digestion experiments will be performed using different intestinal enzymes (trypsin, α -chymotrypsin, elastase or carboxypeptidases A and B);
- ✓ Identification of peptides using HPLC-MS.



Thank you
for your attention

