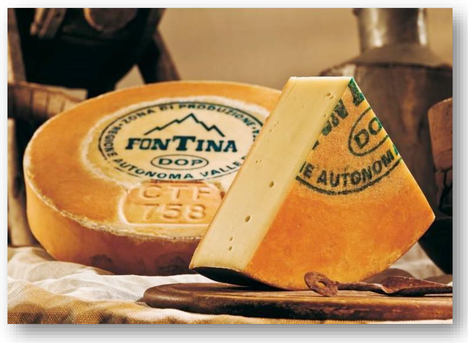
# CHARACTERIZATION OF BIOACTIVE PEPTIDES DERIVED FROM FONTINA CHEESE WHEY PROTEINS BY A PROTEOMIC APPROACH.

S. Valentini[1,2*], T. Flutto[1,2], A. Sado[,1,2], M. Chasseur[2], L. Vernetti-Prot[1,2], A. Barmaz[1,2]
*[1]Institut Agricole Régional, Aosta, Italy; [2]Research Unit HEART VdA, Aosta, Italy*
s.valentini@iaraosta.it

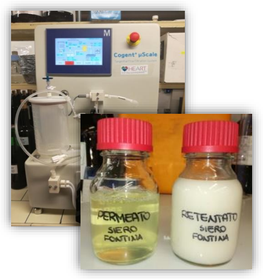
## INTRODUCTION

Whey is the by-product of cheese production. The concentration of whey proteins depends on the type of whey (acid or sweet), the source of milk, the time of the year, the type of feed, the stage of lactation and the quality of processing. Cheese whey represents a rich mixture of proteins with wide ranging chemical, physical and functional properties. Moreover, these proteins, once partially digested, serve as a source of bioactive peptides with further physiological activities. Aim of this study was to characterize potential bioactive peptides from Fontina cheese whey, liberated by enzymatic digestion simulating gastrointestinal condition, and to study which kind of bioactivity they potentially exert.



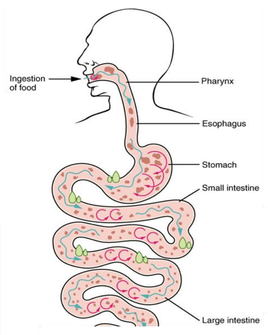

## MATERIALS & METHODS

**Clarification by thermocalcic precipitation**
Removal of non-centrifugeable residual lipids




**Ultrafiltration**
Cogent µScale
tangential flow filtration system
(10 kDa MW cut-off)

**In vitro GI hydrolysis of retentate**

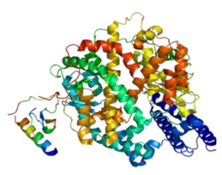



**GASTRIC PHASE**
Pepsin
pH2 – 37 °C – 2H

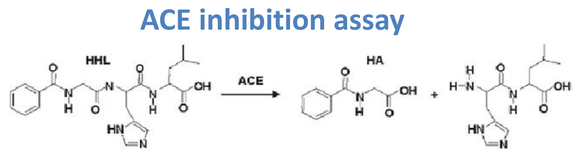
**INTESTINAL PHASE**
Trypsin and chymotrypsin
pH6.5 – 37 °C – 2.5H

**Degree of proteolysis**
ratio of the non protein Kjeldahl nitrogen (NPN) to the total Kjeldahl nitrogen (TN), according to the AOAC official methods

**Biochemical characterization**
HPLC: PLRP column 1x150mm, 300 A, 3 µm
LC-MS: Thermofisher LTQ XL ESI$^+$ -



**ACE inhibition assay**




## RESULTS

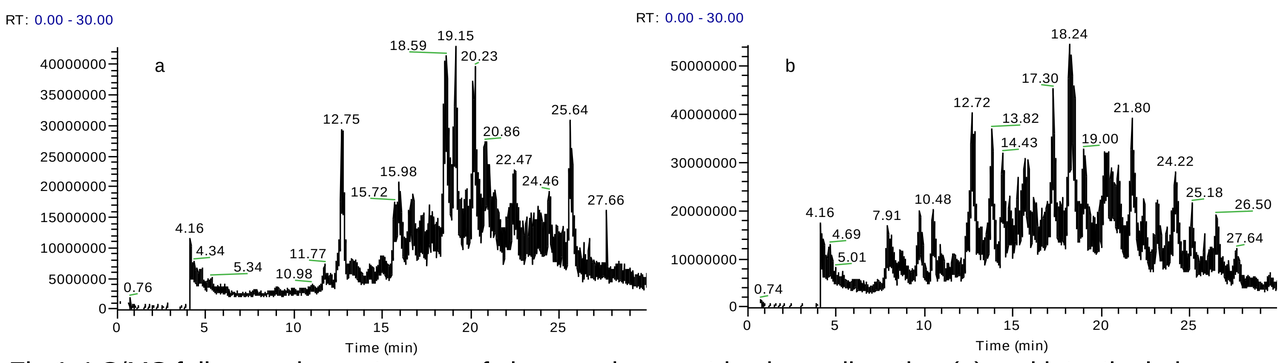



Fig.1: LC/MS full scan chromatogram of cheese whey gastric phase digestion (a) and intestinal phase digestion (b).

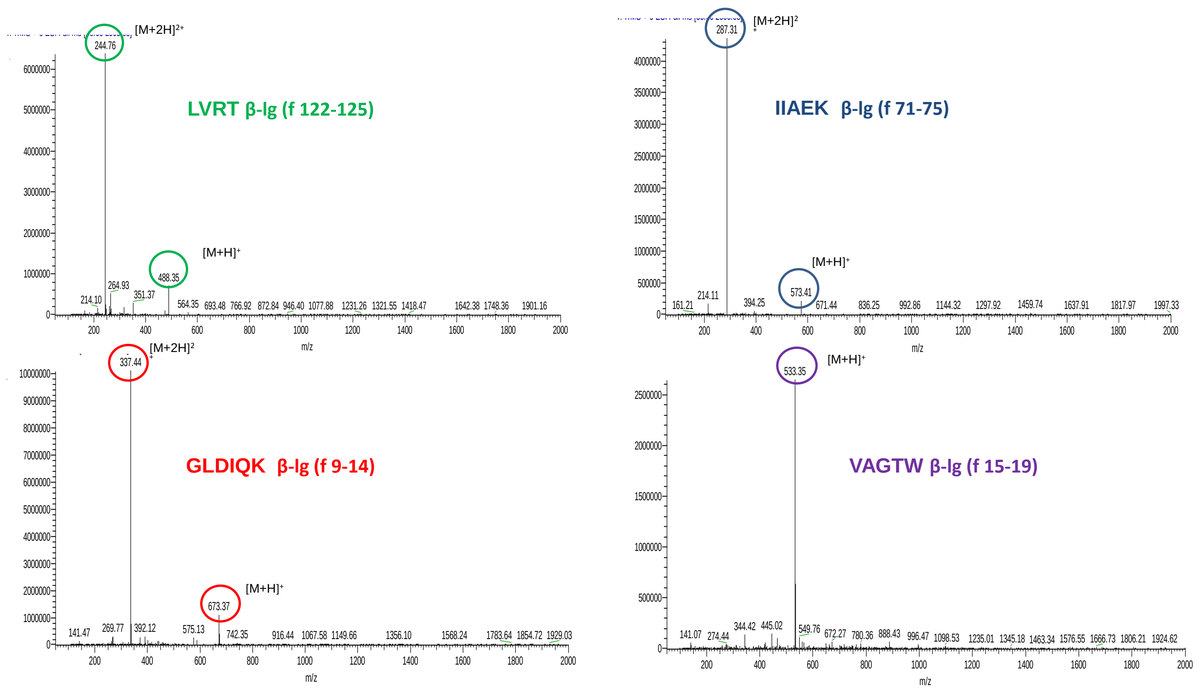



Fig.2: MS spectrum of identified β-lg fragments produced during intestinal phase with bioactive activity.

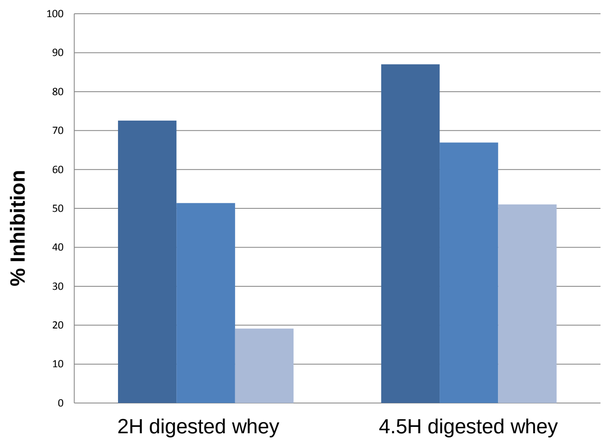



Fig.3: %ACE inhibition in the presence of digested cheese whey in 3 different dilutions (1, 1:2, 1:3 v:v).

## DISCUSSION & CONCLUSION

From the comparison of MS spectra obtained an important increase in the number of peaks has been recorded, corresponding to the formation of a greater number of digestion products (Fig.1).

In order to quantify this augmentation, the degree of proteolysis reached after the simulation of the gastrointestinal digestion has been determined by estimating the ratio of the nonprotein Kjeldahl nitrogen (NPN) to the total Kjeldahl nitrogen (TN) of the two types of whey protein concentrate and has been estimated in percentage 27.7 ± 3.4 and 57.4 ± 2.9 respectively.

A bioinformatic approach has been employed, using BIOPEP database, to assign a biological activity to protein fragments present in our samples. From this analysis we identified 4 peaks potentially corresponding to peptides ascribed with hypocholesterolemic and ACE inhibitory activity (Fig.2).

The determination of ACE inhibition by whey derived peptides in vitro, performed using a method developed by Guan Hong Li et al., starting from Cushman and Cheung procedure, confirmed that our samples have the potential to inhibit the enzyme at the extent of, in percentage, 72.55 ± 0.46 for the simulated stomach digestion and 87.03 ± 0.51 for the simulated complete gastointestinal digestion (Fig.3). Our preliminary results need to be further validated, in order to confirm the effective presence of these bioactive peptides and their unambiguous sequence.

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