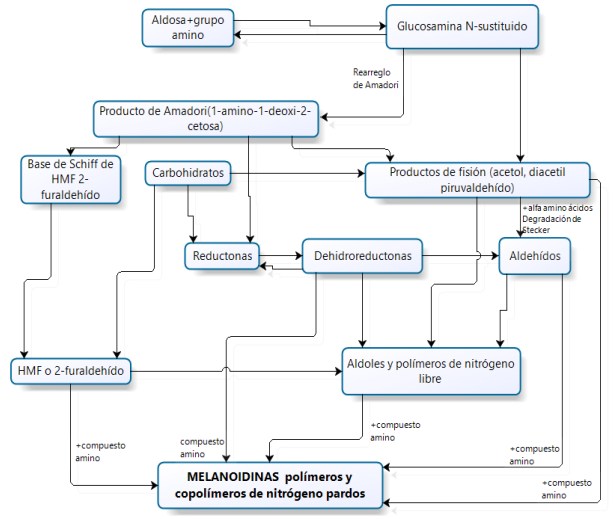
Study of melanoidins of the Maillard reaction in Dulce de Leche

Micaela Escobal1, Analía Rodríguez2, Luis Panizzolo2, Cristina Olivaro1, Fernando Ferreira1.
1-Espacio de Ciencia y Tecnología Química, Centro Universitario de Tacuarembó, Universidad de la República. 2- Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República

Introduction

The "Dulce de leche" (DL) is a dairy product elaborated by milk and sucrose concentration in favorable conditions for develop organoleptic properties, as aroma and color in the Maillard Reaction (MR). It is a non-enzymatic browning reaction of carbonyl compounds, especially reducing sugars with compounds which possess a free amino group such as amino acids, amines and proteins. The final stage is typified by the production of melanoidins, brown and nitrogen-containing polymers and co-polymers

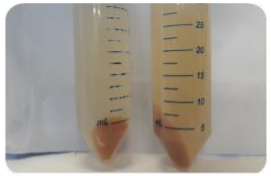
Recently research relate this products with antioxidant, antimicrobial, anti-inflammatory, antihypertensive or prebiotic activity. In foods melanoidins are linked to proteins in a complex structure as melanoproteins which is more difficult to analyze so is relevant the melanoidins separation and isolation from a complex matrix.



Materials and Methods

DL laboratory product

pH 7,2 70°Brix

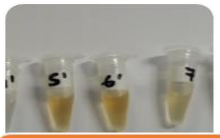


Melanoproteins: Dialysis and centrifugation

membrane 8 kDa 24h 4°C

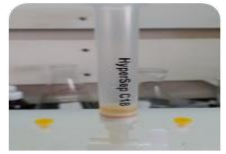
Enzimatic digestion and centrifugation

Enzyme pronase pH 8 T=37°C 24h



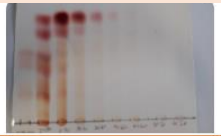
Gel filtration

Biogel P2 (1 a 1,8 kDa) 420nm

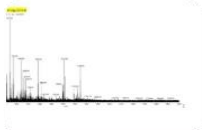


Solid phase extraction (SPE)

C18 100mg MeOH: H2O



Thin-layer cromatography (TLC)

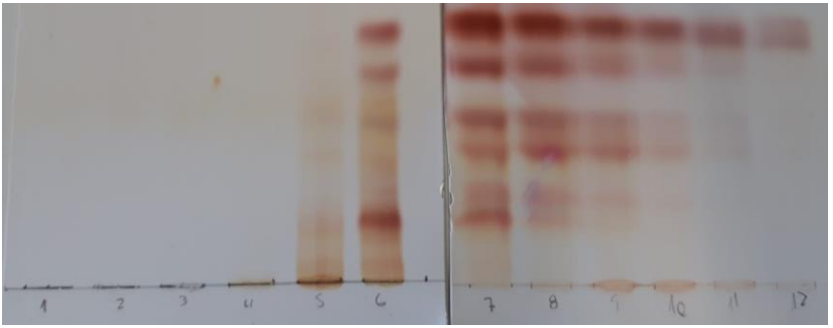


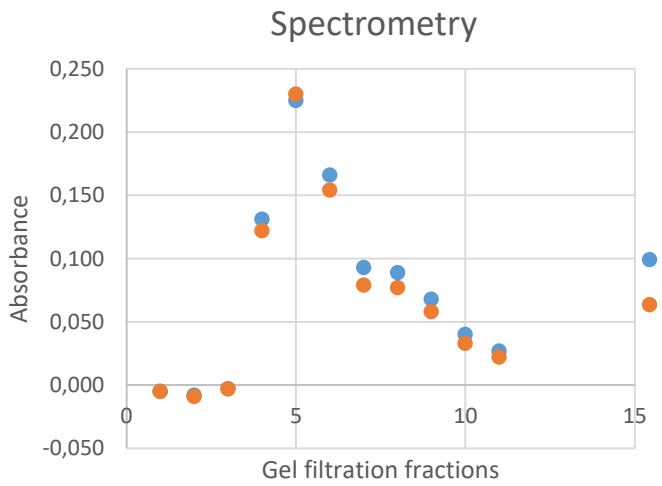
IC-ESI-IT MS^n
LC-ESI-IT MS^2

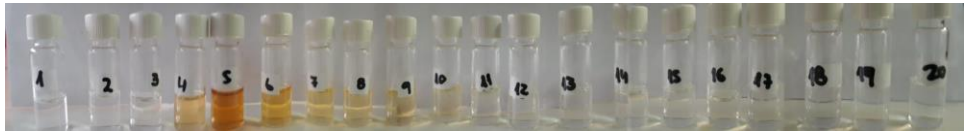
Monitoring to select fractions:
- TLC (spray reagent ninhydrin)
Mobile phase:
3:1:1 Buthanol:acetic acid: water
- Spectrometry (405nm and 420 nm)

Gel filtration: fraction 5 was selected for further analysis

TLC reveal ninhydrin

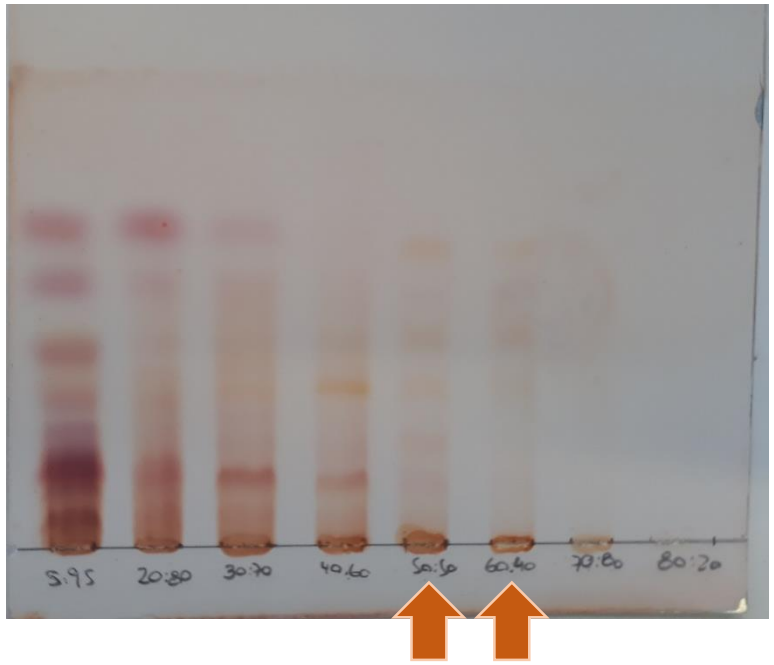




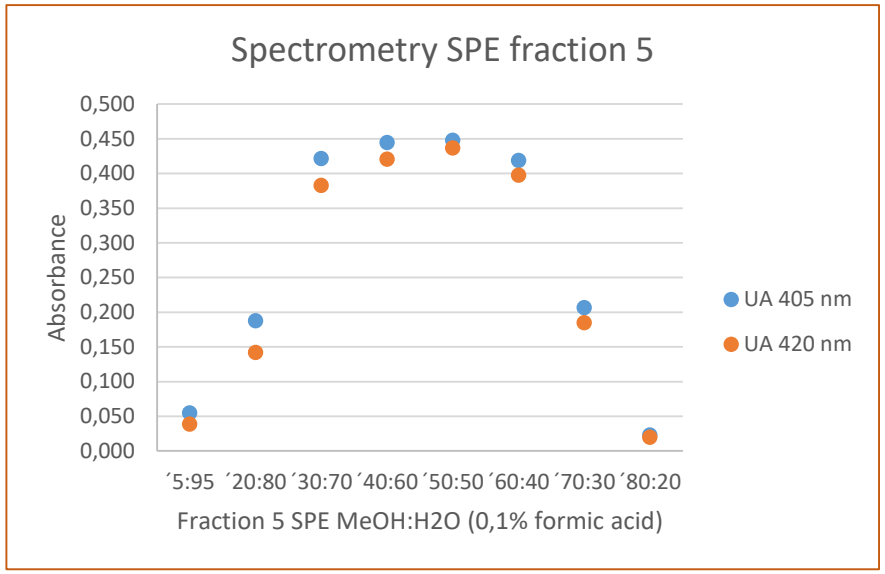


The fraction with the lower content of amino acids was selected

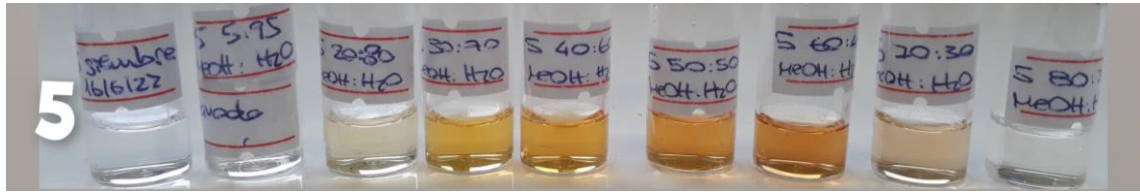
SPE:



Fraction 5 SPE MeOH:H2O (0,1% formic acid)



The fractions with the lower content of amino acids and more colored were selected for further analysis



Results and conclusions

Glucose and phenylalanine were used as references for sugars and aminoacids, respectively. The TLC plates were treated with orcinol and ninhydrin reagents and heated to detect sugars or amino acids, respectively. Fluorescence under irradiation at 365 nm was used as an indication of possible melanoidin compounds.

The mass spectrometric data produced is presently under analysis. A complex mixture of products with m/z < 1200 was found.