EVALUATING THE POTENTIAL EFFECT OF ETHANOL TREATMENT ON WHEY PROTEINS DIGESTIBILITY

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Introduction

Whey proteins (WP), although exhibiting high nutritional and functional attributes, demonstrate significant resistance to hydrolysis phenomena due to their globular structure [1; 2]. Studies have indicated that, in comparison with the traditional heat treatment, ethanol can irreversibly and more effectively denature proteins and change their secondary structures [3; 4]. However, to the best of our knowledge, data regarding the effect of ethanol on WP digestibility is scarce.

Objectives

This study focuses on the comparison of the degradation pattern and evaluation of differences in digestibility of WP under four different conditions, namely: in native WP isolates aqueous solution **(N-WPI)**, in water-ethanol WP solutions with different ethanol concentrations (i.e., 10% and 50% w/v, **E-10 WPI**, and **E-50 WPI,** respectively) and in WP aqueous solution obtained after heating at 90°C for 10 min **(H-WPI)**.

Method/Design

The protein content was measured with the Kjeldahl method according to ISO 8968-3:2007/IDF 20-3: 2007 [5]. To simulate the physiological digestion process (i.e., the oral **(OP),** gastric **(GP)**, and intestinal phases **(IP)**), all substrates were subjected to the INFOGEST static *in vitro* digestion protocol [6]. The enzyme activities (pepsin and trypsin) were quantified prior to the implementation of the INFOGEST protocol as proposed by *Minekus et al. (2014)* [7]. A discontinuous polyacrylamide gel electrophoresis (disc-PAGE) was initially applied on the samples obtained from the *in vitro* digestion (4 treatments x 3 phases) [8]. Furthermore, Sodium Dodecyl Sulphate PAGE (SDS–PAGE) was carried out following the method of *Laemmli (1970)* [9] with some modifications. A Molecular Weight Marker (MwM) composed of a mixture of standard proteins of known molecular weight (from 14.2 kDa to 66.3 kDa) was also run in parallel on the same gel. All experiments were conducted in triplicates and on a protein-equivalent basis.

Results

The mean protein content of undigested substrates, as well as OP, GP, and IP samples was 3.57% (±0.01), 1.84% (±0.07), 1.00 (±0.04) and, 0.55% (±0.02), respectively.

Results of disc-PAGE showed that:

- The OP samples gave, in all 4 substrates, bands with the same electrophoretic mobility (EM) as the protein zones corresponding to a-lactalbumin (a-la) and β-lactoglobulin (β-LG), ran on the same gel.
- At the end of the GP, the band with EM similar to a-la was not visible indicating that this component was quite susceptible to proteolysis by pepsin in all four matrices [10]. However, clear differences in the gastric proteolysis of the band with EM similar to β-LG were observed among the four treatments. Namely, this protein remained pronounced, i.e., more pepsin resistant throughout the whole GP phase, in N-WPI compared to E-10 WPI, E-50 WPI, and H-WPI *(data not shown)*.

A quite comparable proteolysis pattern was recorded by SDS-PAGE analysis **(Figure)**. Based on the data provided by the current literature [3; 4; 11], the following observations are of interest:

- In GP, a fainter band corresponding to intact β-LG was evident in E-10 WPI, E-50 WPI, and H-WPI, compared to the native system (N-WPI).
- The greater degradation of β-LG in the above three denatured protein substrates (E-10 WPI, E-50 WPI, and H-WPI) was accompanied by the appearance of a set of lower molecular weight peptides (< 14.4 kDa). It should be highlighted that, under the conditions of this study, no such peptides were visualized in N-WPI.
- Regarding the IP phase, the degradation patterns of E-10 WPI and H-WPI exhibited many similarities as opposed to the degradation profile of E-50 WPI and N-WPI. At this point it should be mentioned, that according to *Mao et al.* (2019) [12], the presence of ethanol influenced not only the hydrolysis of intact β-LG but also the hydrolysate profiles, resulting from the action of trypsin.

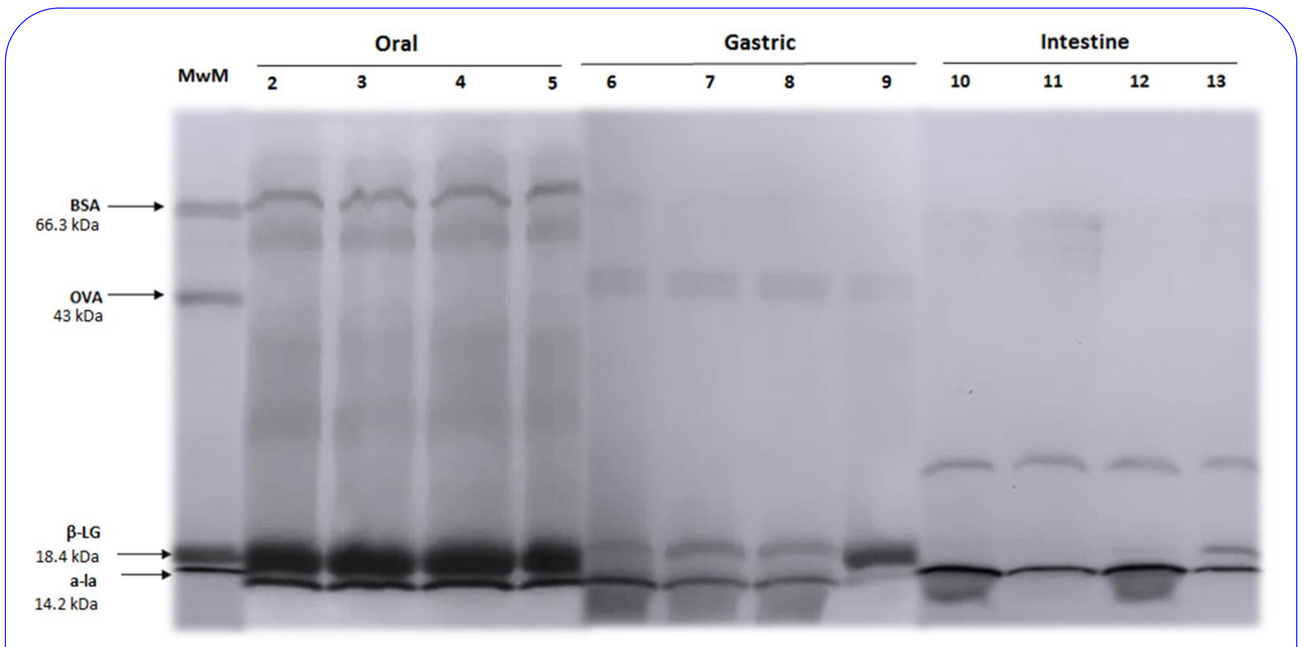

Figure: SDS-PAGE electrophoretograms. **(1)** MwM, **(2)** OP: E-10 WPI, **(3)** OP: E-50 WPI, **(4)** OP: H-WPI, **(5)** OP: N-WPI, **(6)** GP: E-10 WPI, **(7)** GP: E-50 WPI, **(8)** GP: H-WPI, **(9)** GP: N-WPI, **(10)** IP: E-10 WPI, **(11)** IP: E-50 WPI, **(12)** IP: H-WPI, **(13)** IP: N-WPI.

OP: Oral Phase, GP: Gastric Phase, IP: Intestine Phase, E-10 WPI: Water-ethanol Whey Protein Isolates solution with 10% w/v ethanol, E-50 WPI: Water-ethanol WPI solution with 50% w/v ethanol, H-WPI: Heated WPI aqueous solution, N-WPI: Native WPI aqueous solution, MwM: Molecular weight marker, BSA: Bovine Serum Albumin, OVA: Ovalbumin, β-LG: β-lactoglobulin, a-la: a-lactalbumin.

** Molecular weights as recorded by Andrews, A.T. (1986). Electrophoresis: theory, techniques and biochemical and clinical applications (No. 04; QD79. E44, A5.)*

Conclusions

Based on the preliminary data provided in this study, the structural modifications, induced by ethanol treatment, were shown to affect the release of the peptides generated during hydrolysis and consequently the digestibility of WP. Nevertheless, further investigation is required to confirm the aforementioned results and elucidate the underlying mechanisms of enzymic hydrolysis of ethanol-treated WP preparations.

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