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Development of a Potential Functional Yogurt Using Bioactive Compounds Obtained from the by-Product of the Production of Tannat Red Wine †

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Abstract: Tannat (*Vitis vinifera* cv. Tannat) grape pomace is an abundant by-product of the Uruguayan wine industry, which is mainly composed of peels and seeds. Tannat skin from grape pomace is a sustainable source of bioactive compounds and dietary fiber. In previous studies we have seen that it has antioxidant, antidiabetic, antiobesity and anti-inflammatory activity, with the potential to prevent the development of chronic diseases. In this work, the encapsulation of bioactive compounds of an ethanolic extract derived from Tannat grape skin by microparticles of whey protein isolate (without and with enzymatic hydrolysis) and inulin (3:1) is proposed, for its application in yogurt as a potential functional food. In addition, it is proposed to evaluate the bioaccessibility of the bioactive compounds for which an in vitro digestive simulation study is carried out simulating the conditions of the gastrointestinal tract. Among the most relevant results, it was found that the encapsulation efficiency was higher for the encapsulant without hydrolysis (29.7%). The incorporation of the encapsulated bioactive compounds in yogurt resulted in a significant increase ($p < 0.05$) of antioxidant capacity by ORAC-FL compared to the controls. Also, after in vitro digestion the extract did not lose antioxidant capacity (determined by ABTS and ORAC-FL) and the different yogurt formulations presented antioxidant capacity. In conclusion, spray drying is a suitable methodology for encapsulating Tannat grape skin extract for its application in yogurts as a natural colorant and antioxidant ingredient.

Keywords: anthocyanins; bioaccessibility; bioactivity; encapsulation; functional yogurt; Tannat grape peel; spray drying

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1. Introduction

Grape pomace, composed by peel and seeds, is the main by-products of the wine industry [1]. Uruguay is one of the main producers of Tannat red wine [2], consequently generates significant amounts of grape pomace. This by-product is a rich source of dietary fiber and phenolic compounds [3]. Among the phenolic compounds, anthocyanins are the main ones in the peel [4], which are associated with promoting good health [5]. Due to their high antioxidant capacity, investigations have been carried out regarding their use as functional ingredients to develop functional foods that reduce the risk of suffering non-communicable chronic diseases [6]. In the development of a functional food it is extremely

important to evaluate the bioaccessibility of the bioactive compounds, since only the compounds released from the food and stable in gastrointestinal conditions may be potentially available to exert their beneficial effects on the gastrointestinal tract [7]. Regarding Tannat grape skin, it has been reported the remaining bioactivity in the bioaccessible fraction, possessing great potential as a functional ingredient [8].

In this sense, Tannat grape skin could be incorporated into foods of high global consumption, such as yogurt, being possible to exert an effect on the health of the population. It should be taken into account that phenolic compounds are unstable to pH or temperature changes, so their addition to different foods represents challenges [9]. Micro-encapsulation technology could solve this problem by improving their stability [10].

In the present work, it is proposed to encapsulate the compounds present in an ethanol extract derived from the skin of Tannat grape pomace by spray drying, for the development of a functional yogurt. In addition, it is proposed to evaluate the bioaccessibility of said compounds to determine the remaining bioaccessible antioxidant compounds.

2. Methods

2.1. Tannat Grape Skin Treatment and Extract Preparation

The separation of grape pomace (provided by Bouza wine cellar) into peel and seeds was carried out manually. The skin was dried at 40 °C in a conventional oven until constant weight (24 h) and milled using a domestic coffee mill. To extract the bioactive compounds from grape skin powder an ethanol extraction was carried out [3].

2.2. Systems Preparation

The encapsulating materials used for the development of the nano-microparticles were whey protein isolate (WPI, Arla Foods Ingredients, Denmark) or whey protein isolate hydrolysate with alcalase (WPIH), together with inulin (I, BENEO-Orafti S.A., Belgium) in a 3:1 ratio.

Five systems were prepared as described below, which were subjected to spray drying (air inlet temperature: 180 °C, air flow: 600L/h, air atomization pressure: 0.14 MPa, feed temperature: 60 °C) [11].

- System 1—whey protein isolate and inulin (WPI + I) (control system)
- System 2—whey protein isolate hydrolysate and inulin (WPIH + I) (control system)
- System 3—encapsulant: extract (WPI + I + E) (1:1)
- System 4—encapsulant: extract (WPIH + I + E) (1:1)
- System 5—extract subjected to the spray drying temperature (E c/t) (control system)

After spray drying, a powder corresponding to each of the systems was obtained. In addition, a control system was prepared that was not subjected to spray drying:

- System 6—extract without temperature treatment (E s/t) (control system)

2.3. Evaluation of Encapsulation Efficiency

To evaluate the encapsulation efficiency the content of phenolic compounds on the surface of the nano-microparticles and the content of phenolic compounds after destabilization and rupture of the nano-microparticles were determined [12].

2.4. Yogurt Preparation

Seven formulations of yogurt (Y) were prepared:

- Yogurt 1—Encapsulant WPI + I (Y WPI + I)
- Yogurt 2—Encapsulant WPIH + I (Y WPIH + I)
- Yogurt 3—Extract with encapsulant WPI + I (Y WPI + I + E)
- Yogurt 4—Extract with encapsulant WPIH + I (Y WPIH + I + E)
- Yogurt 5—Extract at spray drying temperature (Y E c/t)
- Yogurt 6—Extract without drying temperature (Y E s/t)

- Yogurt 7—Base Formulation (Y B)

To the mixtures corresponding to the yogurts only with extract and only with encapsulating agent (Yogurt 1, 2, 5 and 6), 0.5 g of powder was added, while the other yogurts 1 g was added, to maintain the extract addition ratio in the yogurt formulation (encapsulant: extract 1:1). No system was added to the base formulation.

2.5. Bioaccessibility and Bioactivity Assays

The bioaccessibility studies in both the systems as well as incorporated in the yogurt were carried out by means of an in vitro digestive simulation. The simulation was carried out using the protocol described in INFOGEST [13].

To determine the antioxidant capacity in the systems, yogurts and bioaccessible fractions of both samples, Folin-Ciocalteu, ABTS and ORAC-FL were carried out according to Fernández-Fernández et al. [3].

2.6. Statistical Analysis

Results were expressed as means ± standard deviation (n = 3). The analysis of the results was performed by analysis of variance (ANOVA) and significant differences were determined by the Tukey test ($p < 0.05$) using Infostat v. 2015 program.

3. Results and Discussion

3.1. Evaluation of Encapsulation Efficiency

The efficiency to encapsulate the compounds present in the extract was significantly higher ($p < 0.05$) when the whey protein was in its native state (WPI) (29.65 ± 0.92%) compared to hydrolyzed whey protein (WPIH) (12.81 ± 1.39%). This means that the compounds present in the extract (mostly anthocyanins) interact in a stronger way with the protein in its native state. This is in agreement with the results obtained by Yin et al. [14].

3.2. Bioactivity Assays

3.2.1. Phenolic Compounds

Total polyphenol content (TPC) results before and after in vitro digestion can be observed in Figure 1. TPC values were unchanged for most systems after in vitro digestion (Figure 1a), meaning phenolic compounds were stable under gastrointestinal conditions. Also, after digestion the TPC value of the system containing the extract encapsulated with non-hydrolyzed whey protein (WPI) increased significantly with respect to the system with hydrolyzed protein (WPIH) (Figure 1a). The latter would imply that the extract might be more protected by the non-hydrolyzed encapsulating agent (WPI + I) than by the system with hydrolyzed whey protein (WPIH + I), which is in line with the encapsulation efficiency results.

Regarding yogurt formulations (Figure 1b), all formulations showed a significant increase in TPC values after digestion, reaching similar levels. This increase could be due to the release of peptides from milk proteins during the simulation of gastrointestinal digestion, interfering with the assay [15].

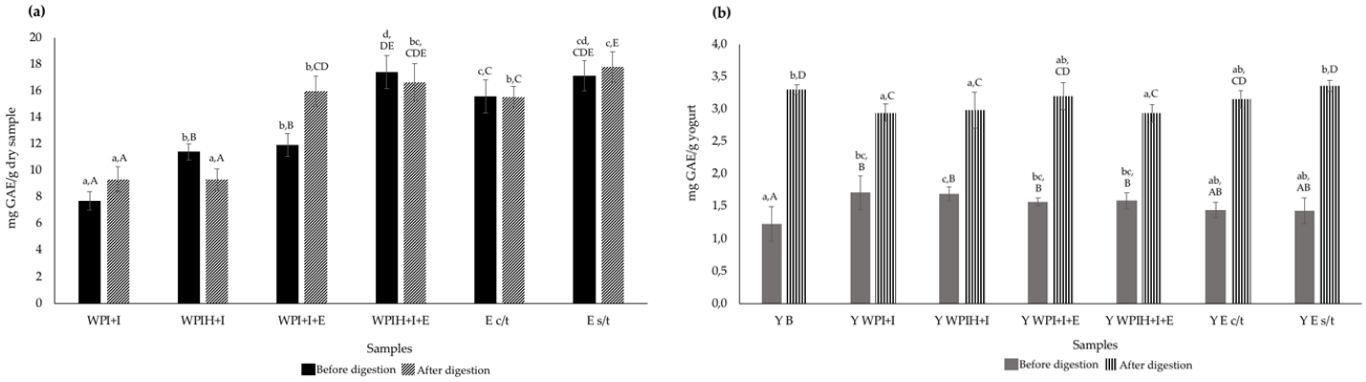

Figure 1. Total polyphenol content before and after in vitro digestion of powder systems on a dry basis (**a**) and of yogurts (**b**). The bars denote the mean values and the error bars the standard deviation. Different lowercase letters represent significant differences between the samples without digestion according to Tukey test ($p < 0.05$). Different capital letters represent significant differences between all samples (before digestion and after digestion) according to Tukey test ($p < 0.05$).

3.2.2. Antioxidant Compounds of the Systems and Yogurts

The systems showed increased antioxidant capacity (ABTS and ORAC-FL) after in vitro digestion (Figure 2). The increase in the antioxidant capacity of the systems WPI+I and WPIH+I (only encapsulating agent) may be due to the release of bioactive peptides by the proteolytic digestive enzymes. The increase in the antioxidant capacity of the encapsulated extracts after in vitro digestion measured by ORAC-FL (Figure 2b) showed encouraging results to use them as functional ingredients, shake formulations or as a natural colorant [16].

Also, the antioxidant capacity of the non-encapsulated samples (E s/t; E c/t) increased after in vitro digestion, probably because of changes in phenolic compounds chemical structure, leading to an increase in their antioxidant capacity [17].

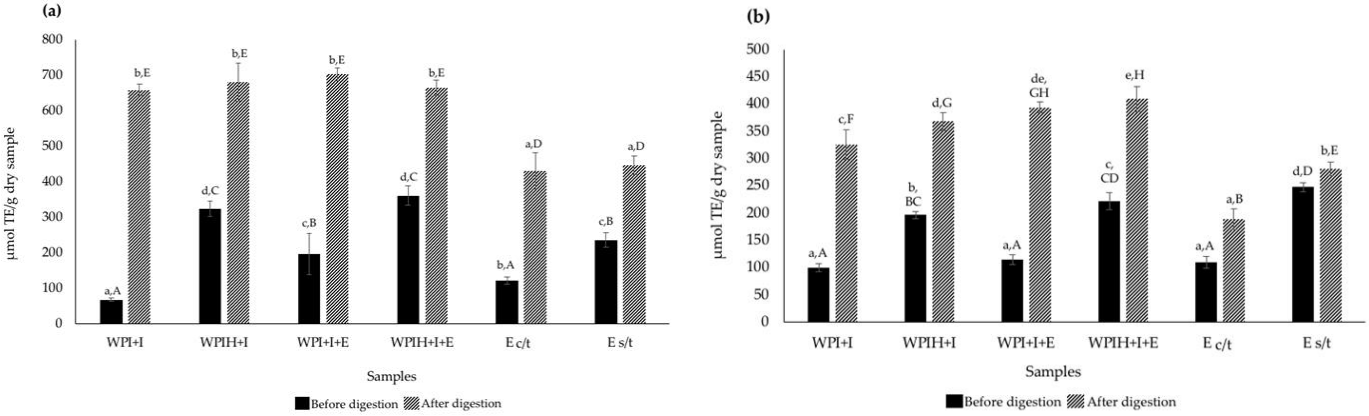

Figure 2. Antioxidant capacity before and after in vitro digestion of systems on a dry basis measured by ABTS (**a**) and ORAC-FL (**b**). The bars denote the mean values and the error bars the standard deviation. Different lower case letters represent significant differences between the samples without digestion according to Tukey ($p < 0.05$). Different capital letters represent significant differences between all samples (before digestion and after digestion) according to Tukey ($p < 0.05$).

Regarding the yogurts the incorporation of the encapsulated bioactive compounds resulted in a significant increase of antioxidant capacity by ORAC-FL before in vitro digestion compared to the base formulation (Figure 3b). However, this increase was not observed after in vitro digestion, probably because of the low amount of encapsulated extract added to the yogurt formulations. In future studies, it is proposed to add a higher amount of encapsulated extract in order to observe a significant increase in the antioxidant capacity. Moreover, the yogurt formulations with encapsulated extract may have other bioactive properties, different from antioxidant capacity.

The fact that the antioxidant capacity increased dramatically after in vitro digestion for all formulations except for the system containing hydrolyzed whey protein (Y WPIH + I) may be due to the release of bioactive peptides from yogurt proteins during digestion. These results are in agreement with the reported by Fernández-Fernández et al. [18] that showed increased antioxidant capacity of a milk protein after its in vitro digestion.

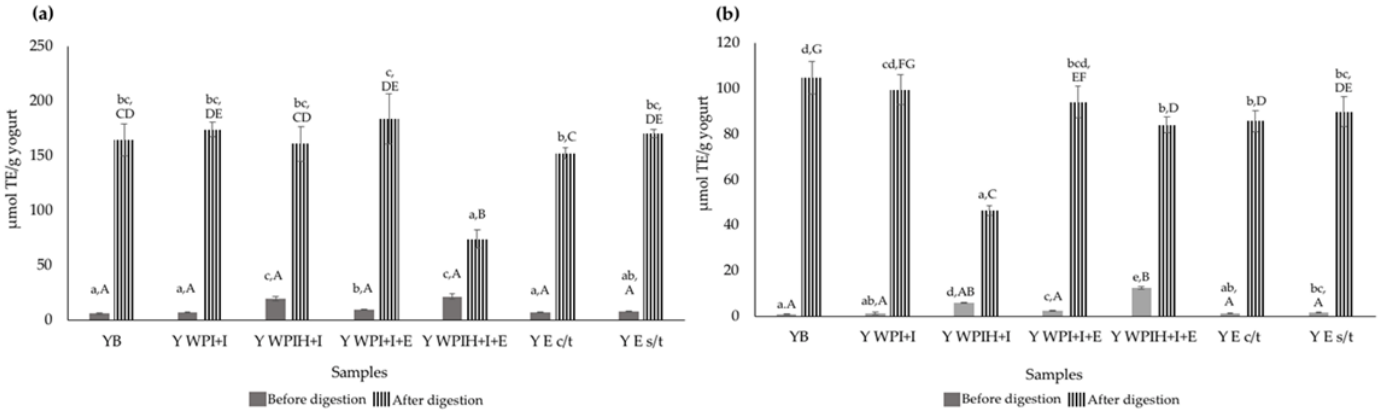

Figure 3. Antioxidant capacity before and after in vitro digestion of yogurts measure by ABTS (**a**) and ORAC-FL (**b**). The bars denote the mean values and the error bars the standard deviation. Different lower case letters represent significant differences between the samples without digestion according to Tukey ($p < 0.05$). Different capital letters represent significant differences between all samples (before digestion and after digestion) according to Tukey ($p < 0.05$).

4. Conclusions

In the present work, different encapsulates of natural antioxidants were developed from an ethanol extract of Tannat grape skin by spray drying obtaining an adequate encapsulation efficiency. The encapsulates were incorporated into a widely consumed food (yogurt). Bioaccessible fractions of the systems, showed increased ($p < 0.05$) antioxidant capacity due to the release of bioactive peptides during the in vitro simulation of gastrointestinal digestion and due to a possible change in the chemical structure of the phenolic compounds present in the extract. Regarding the bioaccessible fractions of yogurts, all the formulations showed increased ($p < 0.05$) antioxidant capacity due to the release of bioactive peptides from milk proteins that are part of the yogurt.

In conclusion, the antioxidant capacity determined in the developed yogurts with the encapsulated extract by spray drying, represent encouraging results to continue with the valorization of the by-product of the Uruguayan wine industry. Further studies regarding other bioactive properties as well as sensory analysis should be addressed on the different yogurt formulations.

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