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Proceeding Paper

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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† Presented at the 2nd International Electronic Conference on Foods—Future Foods and Food Technologies for a Sustainable World, 15–30 October 2021; Available online: https://foods2021.sciforum.net/.

Abstract: Tannat (*Vitis vinifera* cv. Tannat) grape pomace, which is mainly composed of peels and seeds, is an abundant by-product of the Uruguayan wine industry. Tannat skin from grape pomace is a sustainable source of bioactive compounds and dietary fiber. In previous studies, Tannat skin has shown antioxidant, antidiabetic, anti-obesity 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 studied for its application in yogurt as a potential functional food. Thus, it is proposed to evaluate the release of the bioactive compounds after digestion, for which an in vitro digestive simulation study was carried out simulating the conditions of the gastrointestinal tract. Among the most relevant results, the encapsulants showed increased total polyphenol content (TPC) and antioxidant capacity ($p < 0.05$) after in vitro digestive simulation. In addition, the different yogurt formulations showed increased ($p < 0.05$) TPC and antioxidant capacity after in vitro digestion, probably due to the release of bioactive peptides from milk proteins that are part of the yogurt. In conclusion, the antioxidant capacity shown after in vitro digestive simulation by the yogurts formulated with the spray drying encapsulated Tannat grape skin extract represent potential for its application in functional yogurts.

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

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Citation: Olt, V.; Báez, J.; Jorcin, S.; López, T.; Fernández, A.; Medrano, A. Development of a Potential Functional Yogurt Using Bioactive Compounds Obtained from the By-Product of the Production of Tannat Red Wine. *Biol. Life Sci. Forum* **2021**, *6*, 93. https://doi.org/10.3390/Foods2021-10998

Academic Editor: Domenico Cautela

Published: 14 October 2021

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1. Introduction

Grape pomace, composed of peel and seeds, is the main by-product generated by the wine industry [1]. Uruguay is one of the main producers of Tannat red wine [2], consequently generating 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 that are released from the food matrix and are

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 that the remaining bioactive properties in the bioaccessible fraction possess great potential as a functional ingredient [8].

In this sense, Tannat grape skin could be incorporated into foods with high global consumption, such as yogurt, making it 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 evaluate the release after in vitro digestive simulation of encapsulated compounds from an ethanol extract derived from the skin of Tannat grape pomace by spray drying, as well as the release of these compounds when incorporated into a yogurt formulation.

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 the 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., Tienen, 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 w/t)
- Yogurt 6—Extract without drying temperature (Y E wo/t)
- Yogurt 7—Base formulation (Y B)

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

2.5. Bioactive Compounds' Release after In Vitro Digestive Simulation

The release of bioactive compounds during digestion in both the systems and the systems incorporated in the yogurt were carried out by means of an in vitro digestive simulation. Digestion control (without any sample) was simultaneously carried out. 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, total polyphenol content by Folin-Ciocalteu, ABTS and ORAC-FL were carried out according to Fernández-Fernández et al. [3]. Digestion control was also determined in order to subtract the values of the digestion control from the values of the samples. The values were normalized taking into consideration the dilution factor that results from the sequential addition of the digestive fluids provided by the INFOGEST protocol.

2.6. Statistical Analysis

The 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 the Infostat v. 2015 program.

3. Results and Discussion

3.1. Evaluation of Encapsulation Efficiency

The efficiency of encapsulation of 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 with 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. Bioactive Compounds' Release

3.2.1. Phenolic Compounds

The total polyphenol content (TPC) results after in vitro digestion can be observed in Figure 1. After digestion, the TPC value of the system containing the extract encapsulated with both encapsulants (WPI + I + E and WPIH + I + E) increased significantly with respect to the systems without extract (WPI + I and WPIH + I) (Figure 1a). This would imply that the extract improves the content of phenolic compounds. Additionally, after digestion, the powder system WPI + I + E showed increased TPC values compared with the values before digestion (13.91 and 11.93 mg GAE/g dry sample, respectively) ($p < 0.05$) (data not shown) [15], which might be due to more protection of the extract bioactive compounds by the non-hydrolyzed encapsulating agent (WPI + I) than by the system with hydrolyzed whey protein (WPIH + I), which is consistent with the encapsulation efficiency results.

Regarding the yogurt formulations (Figure 1b), all the formulations showed a significant decrease in TPC values after digestion and showed the same tendency of the yogurts before digestion (data not shown), so that similar levels were reached for yogurts with and without extract ($p > 0.05$). Base yogurt (YB) showed TPC values due to the presence of proteins and peptides that may interfere with the assay [16] as a consequence of aromatic amino acids composing the proteins that may reduce the Folin-Ciocalteau reagent. Thus, the TPC values shown by all the yogurt formulations correspond to those components and not to the bioactive compounds composing the extract. Higher addition of the extract in the yogurt should be assessed to improve TPC values of the yogurt with extract.

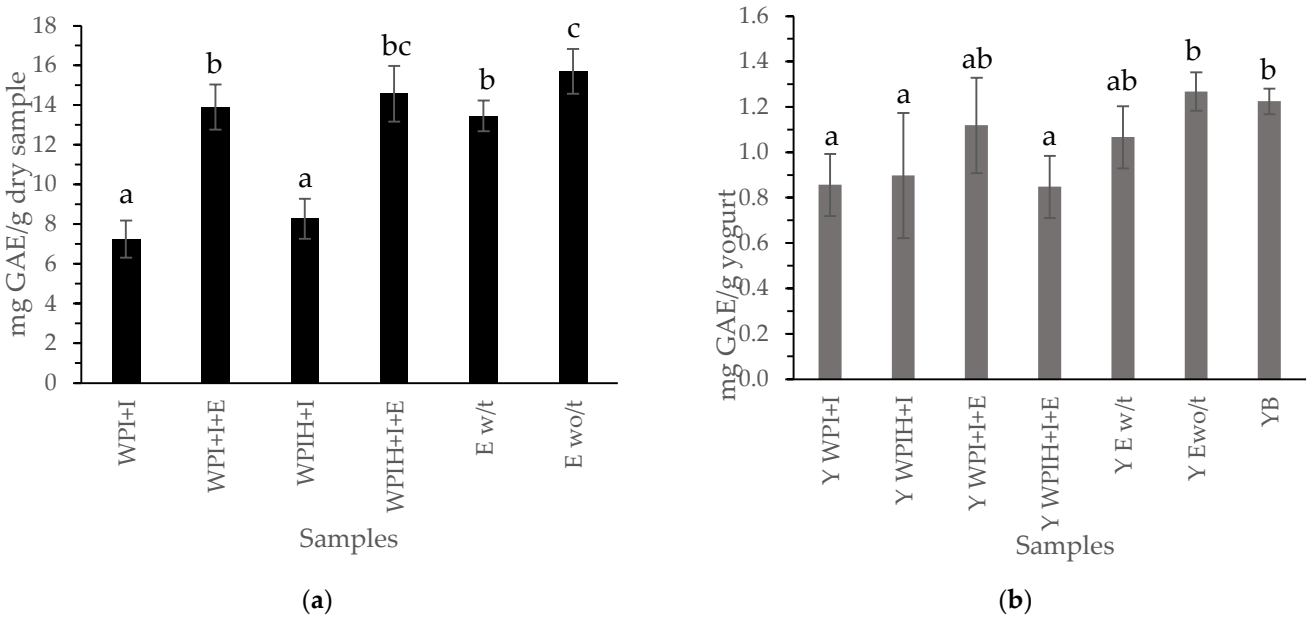


Figure 1. Total polyphenol content after in vitro digestion of powder systems on a dry basis (**a**) and of yogurts (Y) (**b**). The bars denote the mean values and the error bars the standard deviation. Different letters represent significant differences between the samples after digestion according to Tukey test ($p < 0.05$). WPI + I: whey protein isolate and inulin; WPIH + I: whey protein isolate hydrolysate and inulin; WPI + I + E: extract with encapsulant WPI + I; WPIH + I + E: extract with encapsulant WPIH + I; E w/t: Extract at spray drying temperature; E wo/t: extract without spray drying temperature.

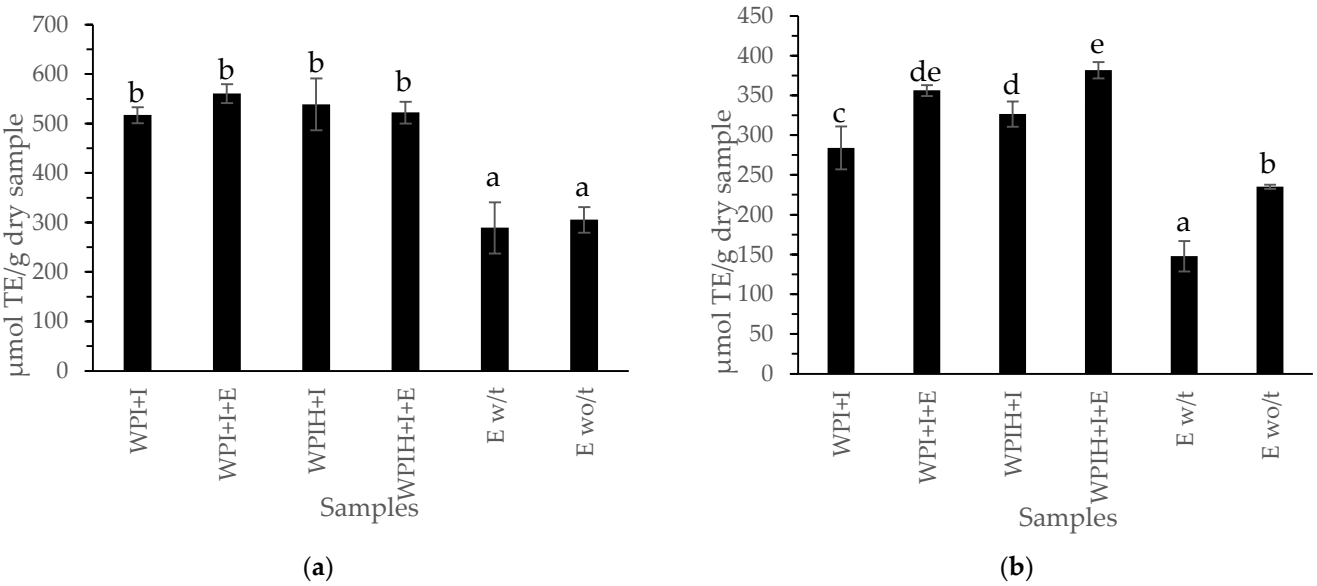
3.2.2. Antioxidant Compounds of the Systems and Yogurts

The systems showed increased antioxidant capacity (ABTS and ORAC-FL) after in vitro digestion (Figure 2) when compared with the antioxidant capacity before digestion ($p < 0.05$) [15]. 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 for their use as functional ingredients, shake formulations or as a natural colorant [17].

In addition, the antioxidant capacity of the non-encapsulated samples (E wo/t; E w/t) increased after in vitro digestion, probably because of changes in the phenolic compounds' chemical structures, leading to an increase in their antioxidant capacity [18].

Regarding the yogurts, the incorporation of the encapsulated bioactive compounds resulted in no significant increase ($p > 0.05$) of antioxidant capacity by ORAC-FL after in vitro digestion compared with the base formulation (Figure 3), or the antioxidant capacity assessed by ABTS (data not shown). This was 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 present other bioactive properties in addition to antioxidant capacity [19].

In addition, after digestion, the yogurts increased their antioxidant capacity ($p < 0.05$) when compared with the yogurts before digestion [15]. 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 those reported by Fernández-Fernández et al. [20] that showed increased antioxidant capacity of a milk protein after its in vitro digestion.



(**a**) (**b**)

Figure 2. Antioxidant capacity after in vitro digestion of powder 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 letters represent significant differences between the samples after digestion according to Tukey ($p < 0.05$). WPI + I: whey protein isolate and inulin; WPIH + I: whey protein isolate hydrolysate and inulin; WPI + I + E: extract with encapsulant WPI + I; WPIH + I + E: extract with encapsulant WPIH + I; E w/t: Extract at spray drying temperature; E wo/t: extract without spray drying temperature.

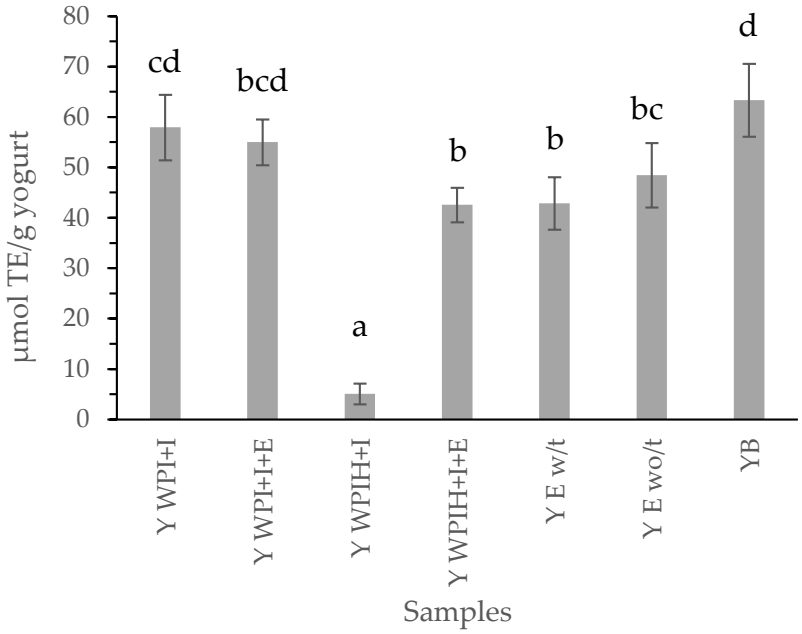


Figure 3. Antioxidant capacity after in vitro digestion of yogurts (Y) measured by ORAC-FL. The bars denote the mean values and the error bars the standard deviation. Different letters represent significant differences between the samples after digestion according to Tukey ($p < 0.05$). WPI + I: whey protein isolate and inulin; WPIH + I: whey protein isolate hydrolysate and inulin; WPI + I + E: extract with encapsulant WPI + I; WPIH + I + E: extract with encapsulant WPIH + I; E w/t: Extract at spray drying temperature; E wo/t: extract without spray drying temperature.

4. Conclusions

In the present work, different encapsulants of natural antioxidants were developed from an ethanol extract of Tannat grape skin by spray drying, and an adequate encapsulation efficiency was achieved. The encapsulants were incorporated into a widely consumed food (yogurt). After digestion, the powder systems with encapsulated extract presented higher antioxidant capacity ($p < 0.05$) than the non-encapsulated extract. Moreover, the powder systems showed increased ($p < 0.05$) total polyphenol content and antioxidant capacity after in vitro simulation of digestion compared with the undigested samples 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 yogurts, all the formulations showed increased ($p < 0.05$) total polyphenol content and antioxidant capacity after in vitro simulation of digestion due to the release of bioactive peptides from milk proteins that are part of the yogurt, with similar values between all the yogurt formulations ($p > 0.05$). Addition of higher contents of extract to the base formulation should be assessed in order to improve yogurt bioactive properties.

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 from the Uruguayan wine industry. Further studies regarding higher contents of extract and other bioactive properties as well as sensory analysis should be addressed on the different yogurt formulations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/Foods2021-10998/s1.

Author Contributions: Conceptualization, A.F. and A.M.; methodology, A.F.; validation, A.F. and A.M.; formal analysis, V.O., J.B., S.J. and A.F.; investigation, V.O., A.F. and A.M.; resources, A.F. and A.M.; data curation, V.O. and A.F.; writing—original draft preparation, V.O. and A.F.; writing—review and editing, V.O., A.F. and A.M; supervision, A.F. and A.M.; project administration, T.L., A.F. and A.M.; funding acquisition, T.L. and A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Comisión Sectorial de Investigación Científica (CSIC-UdelaR) project entitled "Recently developed bioactive compounds in the prevention of non-communicable chronic diseases incorporated in high-consumption functional dairy foods" (CSIC I+D-2018, Project ID: 186).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: We have Supplementary Data to show the results presented in Section 3.

Acknowledgments: The authors would like to thank Bouza wine cellar for providing Tannat grape by-product.

Conflicts of Interest: The authors declare no conflict of interest.

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