biology and life sciences forum

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Proceeding Paper

Development of Potential Functional Biscuits with the Incorporation of Tannat Grape Pomace and Sweetener †

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† Presented at the 3rd International Electronic Conference on Foods: Food, Microbiome, and Health—A Celebration of the 10th Anniversary of Foods' Impact on Our Wellbeing, Online, 1–15 October 2022; Available online: https://sciforum.net/event/Foods2022/.

Abstract: Nowadays, consumers are becoming more aware of their diet. In order to improve the nutritional aspects of people's diet and health, research on the development of functional foods is growing. Tannat grape pomace (TGP) is an abundant byproduct of the Uruguayan wine industry, with the potential to be used in the development of functional foods, mainly because of its composition of bioactive compounds and dietary fiber, which may improve the consumer's health and reduce environmental impact by reusing it in the formulation of a widely consumed product such as biscuits. The aim of the present study was to determine the bioactive properties (antioxidant, antidiabetic, and antiobesity) of different biscuit formulations (with variation in content of TGP and sweetener sucralose) to develop potential functional biscuits. A factorial design with central points was assessed varying the content of TGP and sucralose: 10 and 20% of TGP, 2 and 4% of sucralose, and the central point in triplicate with 15% TGP and 3% sucralose. Among the most relevant results, the addition of 20% of TGP and 4% of sucralose (20%; 4%) increased the total phenols content (TPC) (1.86 ± 0.04 mg GAE/g biscuit) and the antioxidant capacity measured by 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, 50.49 ± 1.86 µmol TE/g biscuit) and ORAC-FL (50.91 ± 3.66 µmol TE/g biscuit) compared to the control biscuit (without TGP, with 4% of sucralose) (TPC: 1.06 ± 0.05 mg GAE/g biscuit; ABTS: 14.43 ± 1.10 and ORAC-FL: 7.99 ± 1.15 µmol TE/g biscuit) ($p < 0.05$). Moreover, biscuits added with the byproduct showed a greater α-glucosidase and pancreatic lipase inhibition capacity compared to the biscuits without the addition of TGP ($p < 0.05$). In brief, biscuit formulations with the incorporation of TGP and sucralose showed potential as functional foods with the possibility to improve consumers' health.

Keywords: bioactivity; dietary fiber; functional biscuits; polyphenols; Tannat grape pomace

Citation: Olt, V.; Báez, J.; Curbelo, R.; Boido, E.; Dellacassa, E.; Fernández-Fernández, A.M.; Medrano, A. Development of Potential Functional Biscuits with the Incorporation of Tannat Grape Pomace and Sweetener. *Biol. Life Sci. Forum* **2022**, *18*, 50. https://doi.org/10.3390/Foods2022-13067

Academic Editor: Joana S. Amaral

Published: 17 October 2022

1. Introduction

A healthy lifestyle, including a good diet, is essential to prevent chronic diseases. Functional foods have the potential to decrease the risk of suffering chronic non-communicable diseases, consequently resulting in the promotion of healthy effects in consumers [1]. Functionality is almost exclusively associated with the action of bioactive compounds that have a beneficial impact on health by managing cell metabolism and its functions under certain conditions [2].

Grape pomace (GP) is a promising example of a bioactive compounds' source which can be used as an ingredient in the development of functional foods. As wine is one of the most consumed alcoholic beverages worldwide, the wine industry produces large amounts of GP as the main byproduct. This waste is a huge problem from the environmental point of view mainly due to their high organic load that hinders their rapid degradation [3]. Its potential use as a functional ingredient can be justified by its components, which represent a natural source of dietary fiber and polyphenols (phenolic acids, anthocyanins, resveratrol, and procyanidins) possessing important health benefits such as antioxidant, anti-inflammatory, antiproliferative, and antimicrobial properties [4,5]. The incorporation of this residue into food products, or its use as a nutritional supplement, is being studied as it might be useful for health promotion and the prevention of chronic diseases [6]. Previous studies showed associations between polyphenols and plant food intake with gut health promotion, amelioration of inflammation, and disease prevention. Specifically, recently it was reported that Tannat grape skin presents antioxidant, antidiabetic, antiobesity, and anti-inflammatory capacities [4].

The application of GP In popular bakery products could significantly improve nutritional value and bioactive potential to enhance consumers' health. Thus, biscuits seem to be the consumers' ideal bakery product, due to their rich nutrition content, different shapes and flavors, ready-to-eat form, and long shelf life [7].

In the present work, the development of potential functional biscuits with the addition of Tannat grape pomace (TGP) and "no added sugar" was proposed. In addition, it was proposed to evaluate the bioactive properties (antioxidant, antidiabetic, and antiobesity) of different biscuit formulations by varying TGP and sucralose contents.

2. Methods

2.1. Biscuits' Formulation

Five formulations of biscuits were prepared according to a factorial design with central points. Two levels of wheat flour substitution by TGP (10 and 20% *w*/*w* in the total wet biscuit mass), two levels of sucralose sweetener (2 and 4% *w*/*w* in the total wet biscuit mass) and a central point in triplicate with 15% TGP and 3% sucralose were used in the development of the biscuits' formulations. Additionally, control biscuits without substitution by TGP and with different percentages of sucralose (2, 3 and 4% *w*/*w* proportion in the total wet biscuit mass) were formulated for comparison. Biscuits' formulations were formulated using wheat flour, sucralose as sweetener, sunflower oil, egg, salt, and baking powder. The formulations were designed to comply with the nutrition claim "source of dietary fiber" and "no added sugar" according to MERCOSUR regulations [8]. The TGP (provided by Bouza wine cellar) used in the biscuit formulation was previously dried and milled using a coffee mill in order to obtain a particle size similar to that of commercial flour.

2.2. Determination of Bioactive Properties

2.2.1. Total Polyphenol Content

The total polyphenol content (TPC) was determined by the Folin-Ciocalteu method [3]. Briefly, 10 µL of sample or standard solution (gallic acid), 200 µL of 20% sodium carbonate solution and 50 µL of Folin-Ciocalteau reagent (1/5) were added to the 96-well plate. After 30 min in the dark, absorbance was measured at 750 nm in a microplate reader Multiskan™ FC Microplate Photometer (SkanIt 1.00.94 visual Software, Thermo Scientific, Waltham, MA, USA).

2.2.2. Antioxidant Capacity

To determine the ABTS radical scavenging capacity, each well of translucent flat-bottom 96-well plates contained 10 µL of sample or standard solution (Trolox) and 190 µL of the ABTS radical [3]. After 10 min incubation in the dark, absorbance was measured at 750 nm in a microplate reader Multiskan™ FC Microplate Photometer (SkanIt 1.00.94 visual Software, Thermo Scientific, Waltham, MA, USA).

To determine the peroxyl radical inhibition (ORAC-FL assay), each well of black flat-bottom 96-well plate contained 20 µL of sample or standard solution (Trolox) with 120 µL of fluorescein working solution and 60 µL of AAPH [3]. Fluorescence (λexcitation = 485 nm, λemission = 520 nm) was measured at 37 °C every 1 min for 104 min in a Varioskan^{TM} Lux (SkanIt RE 5.0 software, Thermo Scientific, Waltham, MA, USA) fluorimeter microplate reader.

2.2.3. Antidiabetic Capacity

To determine the potential antidiabetic capacity, the enzyme activity inhibition of α-glucosidase was evaluated [4]. The assay consisted in measuring the fluorescence (λexcitation = 360 nm, λemission = 460 nm) of the substrate (4-MUF-α-D-glucopyranoside) during 30 min in a Varioskan^{TM} Lux (SkanIt RE 5.0 software, Thermo Scientific, Waltham, MA, USA) fluorimeter microplate reader. Results were expressed as the concentration of biscuit causing 50% inhibition (IC_{50}, mg/mL) of α-glucosidase.

2.2.4. Antiobesity Capacity

To determine the potential antiobesity capacity, the enzyme activity inhibition of pancreatic lipase assay was performed [4]. The fluorescence (λexcitation = 360 nm and λemission = 460 nm) of the substrate (4-methylumbelliferyl-oleate) was measured for 30 min in a Varioskan^{TM} Lux (SkanIt RE 5.0 software, Thermo Scientific, Waltham, MA, USA) fluorimeter microplate reader. From the dose-response curves, the concentration necessary to inhibit 50% of pancreatic lipase was calculated (IC_{50}, mg/mL).

2.2.5. Identification of TGP Phenolic Compounds

LC-MS analyses were performed using a Shimadzu Triple Quadrupole MS detector (Shimadzu, Tokyo, Japan) using an electrospray ionization (ESI) interface as described by Boido et al. [9] with some modifications. Briefly, the separation of the flavanols was performed using an HPLC Kinetex C18-EVO reverse phase C18 column (5 µm particle size, 150 × 4.6 mm i.d.)(Phenomenex, Torrance, CA, USA) thermostated at 35 °C. The solvents used were: (A) 0.1% trifluoroacetic acid, (B) HPLC-grade acetonitrile, establishing the following gradient: from 0 to 100% A for 3 min, from 4 to 30% B for 50 min, from 30 to 98% C for 5 min, and isocratic 98% B for 2 min, at a flow rate of 1.3 mL min^{-1}. Detection was carried out at 280 nm as the preferred wavelength. LC-MS analyses were performed using a Shimadzu Triple Quadrupole MS detector (Shimadzu, Tokyo, Japan) using an electrospray ionization (ESI) interface. The source voltage used was 2.50 kV, and the capillary temperature was 250 °C. Spectra were recorded in positive ion mode between m/z 100 and 2000.

2.3. Statistical Analysis

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

3. Results and Discussion

3.1. Total Polyphenol Content and Antioxidant Capacity

The results for TPC and the antioxidant capacity are shown in Figure 1, where results indicated the substitution of wheat flour by TGP in the biscuits increased the content of total polyphenols and the antioxidant capacity compared with their controls (biscuits 2, 3

and 2%). The formulation presenting the highest antioxidant capacity was the one with the highest TGP (20% *w*/*w*) and sucralose (4% *w*/*w*) content. Instead, the formulations with the lowest antioxidant capacity were the control biscuits as they did not have the incorporation of TGP and thus were without the byproduct bioactive compounds with high antioxidant capacity [10]. The results of the current work are in agreement with the ones reported by Theagrajan et al. [11], in which they incorporated 6% of grape pomace in biscuits.

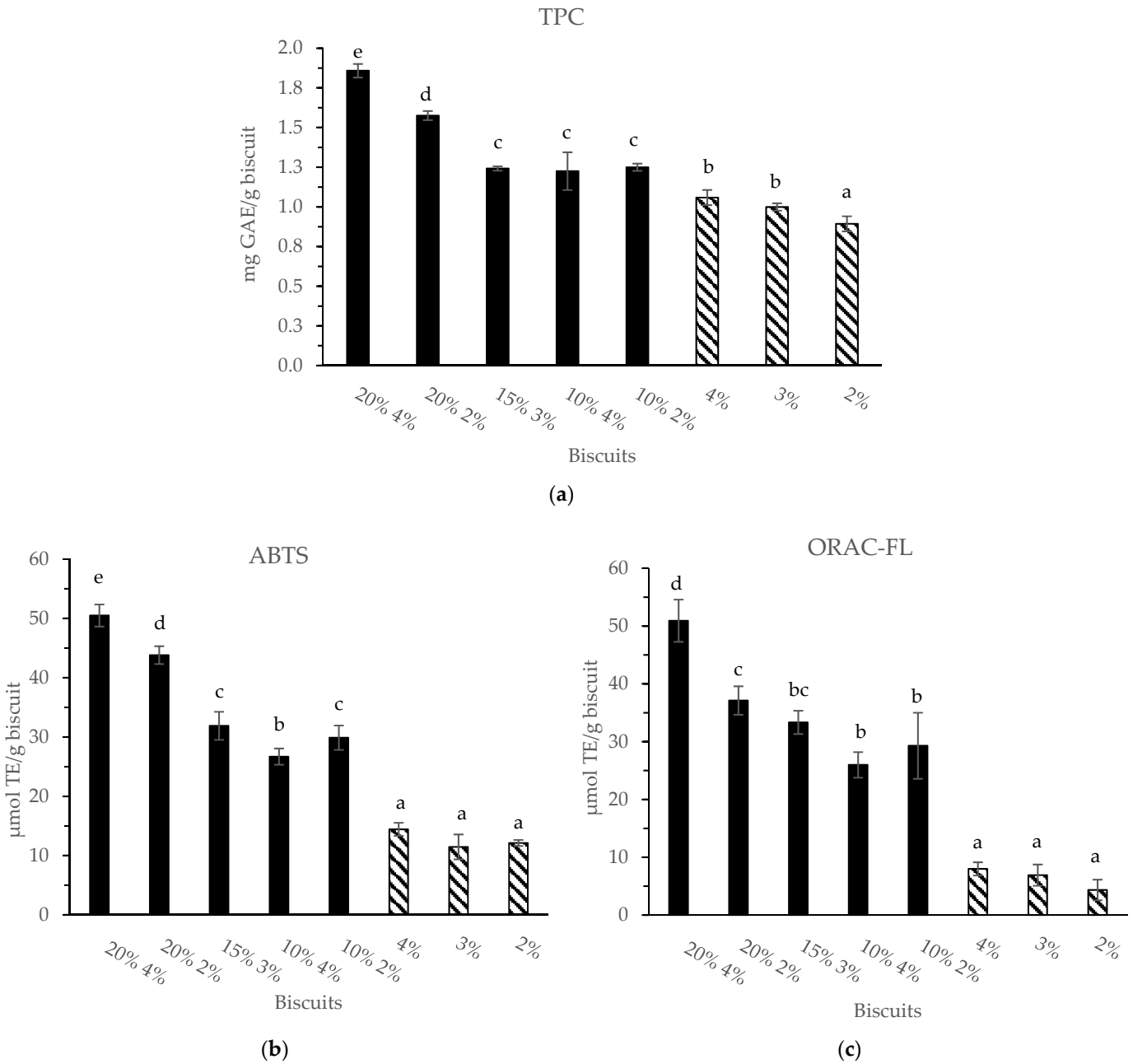
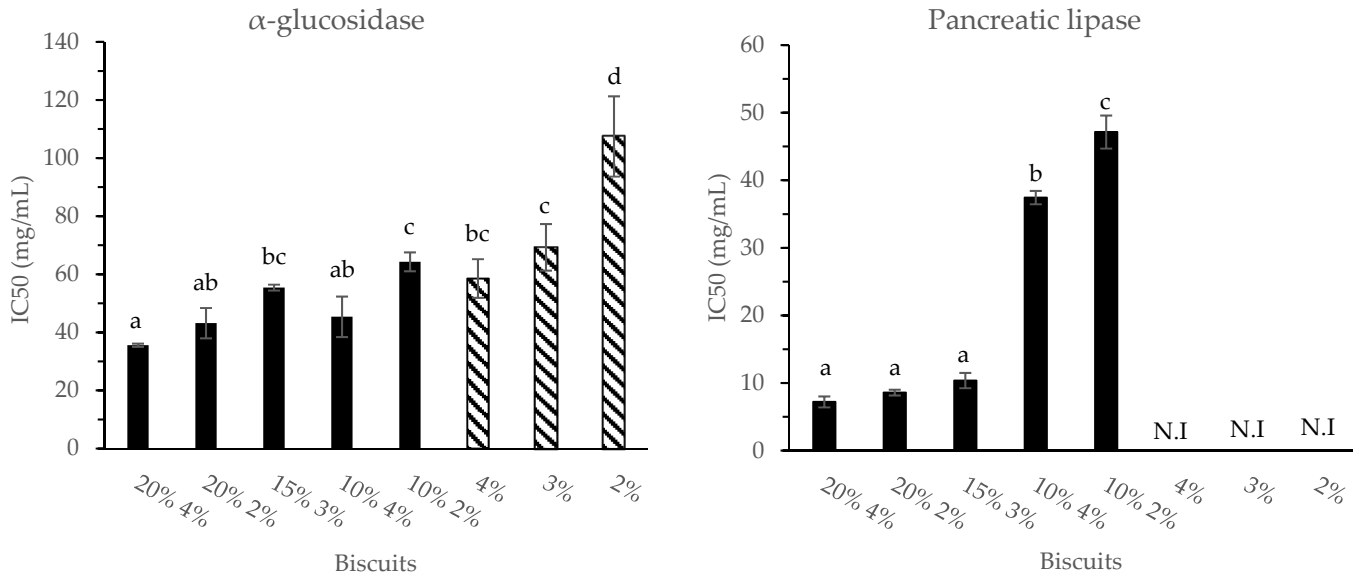


Figure 1. Total polyphenol content (**a**) and antioxidant capacity measured by ABTS (**b**) and ORAC-FL (**c**). Bars denote the mean values and error bars the standard deviation. Different letters represent significant differences according to the Tukey test ($p < 0.05$). TGP biscuits: 20% 4% (20% TGP and 4% sucralose), 20% 2% (20% TGP and 2% sucralose), 15% 3% (15% TGP and 3% sucralose), 10% 4% (10% TGP and 4% sucralose), and 10% 2% (10% TGP and 2% sucralose). Control biscuits (without TGP): 4% (4% sucralose), 3% (3% sucralose), and 2% (2% sucralose).

3.2. Antidiabetic and Antiobesity Capacity

As previously reported, the phenolic compounds are able to inhibit α-glucosidase and lipase enzymes through nonspecific binding [12]. Our results showed that the formulations with the highest substitution of wheat flour by TGP (20%) presented greater capacity to inhibit α-glucosidase and pancreatic lipase compared with the control (Figure 2). Although there is research demonstrating the inhibitory capacity of α-glucosidase and pancreatic lipase in red grape pomace (non-Tannat grape varieties) [12], extracts of Tannat grape skin [4], and biscuits with Tannat grape peel from pomace [13], to the best of our knowledge this is the first time that biscuits with the substitution of wheat flour by grape pomace have been reported to have pancreatic lipase and α-glucosidase inhibition capacities, making them a potential functional food for regulating post-prandial fat and glucose levels.



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

Figure 2. Antidiabetic and antiobesity capacities measured by α-glucosidase (**a**) and pancreatic lipase (**b**) inhibition. Bars denote the mean values and error bars the standard deviation. Different letters represent significant differences according to Tukey's test ($p < 0.05$). TGP biscuits: 20% 4% (20% TGP and 4% sucralose), 20% 2% (20% TGP and 2% sucralose), 15% 3% (15% TGP and 3% sucralose), 10% 4% (10% TGP and 4% sucralose), and 10% 2% (10% TGP and 2% sucralose). Control biscuits (without TGP): 4% (4% sucralose), 3% (3% sucralose), and 2% (2% sucralose). N.I: no inhibition.

3.3. Phenolic Compounds of TGP

Phenolic acids, flavan-3-ols, flavonols, and anthocyanins were identified by HPLC-DAD-MS (Table 1), being mainly p-coumaroyl hexose, procyanidin trimers, quercetin-3-O-glucoside and quercetin-7-O-neohesperidoside, and malvidin-3-O-(6′-p-coumaroyl)glucoside, respectively. These results agree with previous reports for Tannat grape skin [13].

Table 1. TGP phenolic compounds by HPLC-DAD-MS.

	Rt (min)	Area	Compound	
Chromatogram 280 nm	6.496	34,450	cis-caftaric acid	phenolic acids
	6.893	90,891	trans-caftaric acid	
	8.730	225,597	protocatechuic acid	
	12.313	265,757	trans-cutaric acid	
	12.457	**573,977**	**p-coumaroyl hexose**	
	4.628	39,983	procyanidin trimer C2	flavan-3-ols
	7.453	181,179	procyanidin dimer B1	
	8.022	303,632	procyanidin dimer B3	
	8.306	436,692	(+)-catechin	
	9.104	199,322	procyanidin trimer	
	10.608	**1,127,900**	**procyanidin trimer**	
	11.459	328,747	procyanidin dimer B4	
	11.832	204,148	procyanidin dimer B6	
	12.457	839,734	(−)-epicatechin	
	15.757	107,028	procyanidin dimer galloylated	
	16.024	89,969	procyanidin trimer	
	16.324	341,893	procyanidin trimer	
	17.158	161,716	procyanidin dimer B2	
	17.579	302,107	procyanidin dimer galloylated	
	22.827	200,876	procyanidin dimer B7	
	19.665	79,965	myricetin-3-O-galactoside	flavonols
	21.814	31,333	myricetin-3-O-glucoside	
	22.689	65,749	quercetin-3-O-galactoside	
	23.466	**162,693**	**quercetin-3-O-glucoside**	
	26.790	71,502	siringetin-3-O-glucoside	
	28.236	**160,861**	**quercetin-7-O-neohesperidoside**	
	35.226	57,127	quercetin aglycone	
Chromatogram 520 nm		0	delphinidin-3-O-glucoside	anthocyanins
		0	cyanidin-3-O-glucoside	
	17.573	52,866	petunidin-3-O-glucoside	
	19.744	74,672	peonidin-3-O-glucoside	
	20.804	882,877	malvidin-3-O-glucoside	
		0	delphinidin-3-O-(6′-acetyl)glucoside	
	26.547	34,547	petunidin-3-O-(6′-acetyl)glucoside	
	28.848	13,166	peonidin-3-O-(6′-acetyl)glucoside	
	29.545	211,121	malvidin-3-O-(6′-acetyl)glucoside	
	30.385	72,350	delphinidin-3-O-(6′-p-coumaroyl)glucoside	
	32.285	192,783	malvidin-3-O-(6′-caffeoyl)glucoside	
	32.677	28,817	cianidin-3-O-(6′-p-coumaroyl)glucoside	
	33.362	308,975	petunidin-3-O-(6′-p-coumaroyl)glucoside	
	35.785	195,123	peonidin-3-O-(6′-p-coumaroyl)glucoside	
	36.079	**2,268,418**	**malvidin-3-O-(6′-p-coumaroyl)glucoside**	

4. Conclusions

In this work, five biscuit formulations containing Tannat grape pomace (TGP) and sucralose were developed. Promising results were obtained, since they showed antioxidant, antidiabetic, and antiobesity properties due to their incorporation of the byproduct. The formulation with the highest content of TGP (20% *w*/*w* total wet biscuit mass) and sucralose (4% *w*/*w* total wet biscuit mass) presented the highest bioactive properties. This work shows the added value of an agro-food industry byproduct, highlighting what could be a powerful tool to increase the nutritional value of food with the potential to prevent the development of chronic diseases and also reduce negative environmental impact. Further studies regarding sensory analyses need to be carried out on the different biscuit formulations.

Author Contributions: Conceptualization, A.M.F.-F.; methodology, A.M.F.-F.; validation, A.M.F.-F.; formal analysis, V.O., R.C. (HPLC-MS), and A.M.F.-F.; investigation, V.O., J.B., A.M.F.-F., and A.M.; resources, A.M.F.-F. and A.M.; data curation, V.O., R.C. (HPLC-MS), E.B. (HPLC-MS), E.D. (HPLC-MS), and A.M.F.-F.; writing—original draft preparation, V.O. and A.M.F.-F.; writing—review and editing, V.O., J.B., E.D., A.M.F.-F., and A.M.; supervision, A.M.F.-F. and A.M.; project administration, A.M.F.-F. and A.M.; funding acquisition, A.M.F.-F. and A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Agencia Nacional de Investigación e Innovación (ANII), Project grant number FMV_3_2020_1_162341 and V.O. scholarship POS_NAC_M_2020_1_164532; and V.O. student scholarship from Programa de Desarrollo de las Ciencias Básicas (PEDECIBA).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: We do not have supplementary data to show other than the results presented in Section 3.

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

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

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