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Inhibition of Trypsin by Condensed Tannins and Wine

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Phenolic compounds are abundant vegetable secondary metabolites in the human diet. The ability of procyanidin oligomers and wine polyphenols to inhibit trypsin activity was studied using a versatile and reliable *in vitro* method. The hydrolysis of the chromogenic substrate *N*-benzoyl-D,L-arginine-*p*-nitroanilide (BApNA) by trypsin was followed by spectrophotometry in the presence and absence of condensed tannins and wine. A clear relationship between the degree of polymerization of procyanidins and enzymatic inhibition was observed. Trypsin activity inhibition was also detected in several types of wine. In general, the inhibition increased with the concentration of phenolic compounds in wines. These results may be relevant when considering these compounds as antinutritional factors, thereby contributing to a reduced absorption of nutrients.

KEYWORDS: Trypsin; condensed tannins; procyanidin; BApNA; wine; grape seeds

INTRODUCTION

Phenolic compounds are one of the most important groups of secondary metabolites in plants. This group of compounds is present in a great variety of foods and is particularly abundant in some beverages, such as wine, tea, and berry juices (*1*−*3*). Since the discovery of these compounds, great work has been done concerning their biological effects. Presently, the issues of bioavailability (*3*), bioefficacy (*4*), and stability (*5*, *6*) of phenolic compounds in several matrices are being studied.

Tannins are a group of phenolic compounds that generally have the ability to act as protein-complexing agents (*7*). Tannins are classically divided into hydrolyzable and condensed tannins; the first are composed of gallic acid and its dimer ellagic acid esterified with sugar molecules, and the latter are composed of flavan-3-ol units with various degrees of substitution and polymerization, constituting the largest group of proanthocyanidins (*1*). In the present work, procyanidins (a group of proanthocyanidins derived from catechin and epicatechin) are studied because of their large relevance in wine.

Work carried out during the past decade points to a high intestinal absorption of simple phenolic compounds, sugar conjugated molecules, and a reduced passage of large polymeric structures, such as condensed tannins (*4*, *8*). This data allows for the assumption that phenolic compounds that are poorly absorbed in the intestine remain in the lumen for long periods of time and therefore may have some degree of biological activity in this portion of the digestive tract (*9*).

The biological activity of phenolic compounds can be summarized in three main mechanisms; metal quelation, antioxidant activity, and enzyme inhibition (*10*). There have been several epidemiologic studies concerning the ability of these compounds to cure or prevent illness, especially the modern illnesses of hypertension, hypercholesterolemia (*11*, *12*), and cancer (*13*).

Condensed tannins are supposed to remain in the digestive tract for extended periods of time and act as protein-complexing agents (*14*). Thus, the study of the inhibition of digestive enzymes by them has been arousing interest in the last few years. Polyphenolic compounds have been found to inhibit the three main classes of digestive enzymes: lipases (*9*, *15*, *16*), glycosidases (*17*−*19*), and proteases (*9*, *20*, *21*).

There has been, for some time, the knowledge that some polyphenolic compounds may inhibit the activity of proteolytic enzymes (*21*). This ability is of particular relevance in disease processes, such as bacterial colonization, tumor survival, and metastasis (*12*). In digestive processes, there is evidence of reduced amino acid absorption in chicks upon feeding with hulls of field beans (*Vicia faba* L.) (*22*), and in ruminants, the amino acids methionine and lysine are poorly absorbed in procyanidin-rich diets (*23*). Extracts of cocoa, pears, and lentils have been found to inhibit trypsin; however, the composition of these extracts and the structure of the phenolic inhibitors involved was not clarified (*20*).

This work aims to study the role of proanthocyanins from grape seed in the inhibition of tryptic activity using an *in vitro* model with *N*-benzoyl-D,L-arginine-*p*-nitroanilide (BApNA) as a chromogenic substrate. BApNA was first developed in 1961 (*24*) and has been used as a substrate to follow reactions involving the rupture of ester bonds; trypsin is one of the enzymes that possesses this ability. This substrate was used in many types of assays to search for tryptic activity (*25*−*27*), to study the isoforms of this enzyme (*28*, *29*), and to test for inhibitors (*26*, *30*, *31*). Phenolic compounds have never been tested as inhibitors of trypsin activity using BApNA. A quick and versatile method to assess the trypsin activity inhibition by

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Table 1. Chromatographic Elution Times and Solvent Using a TSK Toyopearl Gel Column for Fractionating Proanthocyanins from the Grape Seed Extract[a]

	time (h)	eluent	average MW	average DP
fraction 1	0.50–0.75	methanol	513	1.7
fraction 2	0.75–1	methanol	823	2.8
fraction 3	1–5	methanol	949	3.2
fraction 4	5–19	5% CH_3COOH/methanol	1513	5.0
fraction 5	19–27	10% CH_3COOH/methanol	2052	6.8

[a] Average molecular weights (MWs) and average degree of polymerization (DP) were calculated using the masses of individual MS peaks and their relative abundance (*37*).

condensed tannins, using pig trypsin as a model for mammals, is described herein. The influence of the degree of polymerization of procyanidins to their ability to inhibit trypsin activity was studied. Furthermore, the inhibitory effect of white table wine, red table wine, and red Port wine (enriched with procyanidins) was also evaluated. Hopefully, this work will contribute to clarify the role of phenolic compounds as antinutritional agents (*23*, *32*, *33*).

EXPERIMENTAL PROCEDURES

Materials. Trypsin (type IX-S from porcine pancreas) and BApNA were obtained from Sigma Aldrich and stored at −20 °C. Trypsin was dissolved at room temperature in phosphate buffer (50 mM) and frozen, and BApNA was dissolved in dimethylsulfoxide (DMSO, 44 g L^{-1}) to prepare a stock solution that was stored at 0 °C. BApNA working solutions (2.5 g L^{-1}) were prepared immediately before use by dilution of the stock solution using deionized and distilled water.

Extraction and Fractioning of Grape Seed Proanthocyanin Oligomers (OPCs). Condensed tannins were extracted from *Vitis vinifera* grape seed tissues with a ethanol/water/chloroform solution (1:1:2, v/v/v) using a blender (Ultra-Turrax), according to the method described in the literature (*34*). The 50% aqueous ethanol upper layer, containing phenolic compounds, was separated from the chloroform layer, containing chlorophylls, lipids, and other undesirable compounds.

Ethanol was removed using a rotary evaporator (30 °C), and the resulting aqueous solution, containing the polyphenolic compounds, was extracted with ethyl acetate, followed by precipitation with hexane, to obtain the oligomeric procyanidins (OPCs) (*35*).

The OPCs were fractionated through a TSK Toyopearl HW-40(s) gel column (100 × 16 mm i.d.). Fractions where eluted according to **Table 1** following the procedure described in the literature with some modifications (*36*). The first fraction corresponding to the first 30 min was rejected.

All fractions were diluted with deionized water, and the organic solvent was eliminated using a rotary evaporator under reduced pressure at 30 °C. Fractions were frozen and freeze-dried.

Analysis of Procyanidin Fractions. The composition of proanthocyanidins in the fractions was determined with electrospray ionization mass spectrometry (ESI−MS) by direct injection using a Finnigan Surveyor series liquid chromatograph (**Figure 1**). The mass detection was carried out by a Finnigan LCQ DECA XP MAX (Finnigan Corp., San José, CA) mass detector with an atmospheric pressure ionization (API) source of ionization and an electrospray ionization (ESI) interface. The capillary voltage was 28 V; the capillary temperature was 270 °C; and the auxiliary and carrier gases were nitrogen, respectively, at 6 and 1.2 mL min^{-1}. Spectra were recorded in negative-ion mode between *m*/*z* 150 and 2200. Fraction 1 contains catechin, galloyl monomer, and procyanidin dimer; fraction 2 contains procyanidin dimer, galloyl derivatives, and procyanidin trimer; fraction 3 contains procyanidin trimer and tetramers and galloyl derivatives; fraction 4 contains procyanidin pentamer and the galloyl derivative; and fraction 5 contains procyanidin pentamer digalloyl, procyanidin tetramer tetragalloyl, procyanidin hexamer galloyl, procyanidin heptamer, and the galloyl derivative. The mean molecular weight and degree of polymerization in **Table 1** corresponds to the average molecular weights and degrees of polymerization of peaks, taking into account their relative abundance in the respective mass spectra.

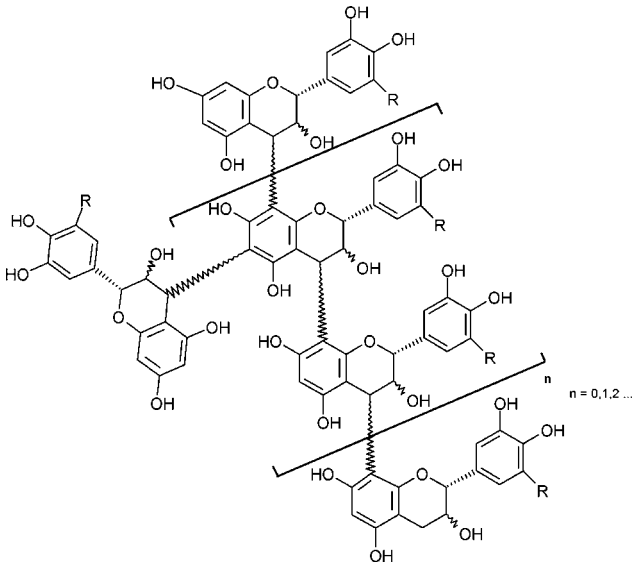

Figure 1. Structural representation of polymeric proanthocyanidins. R = H, procyanidin; R = OH, prodelphidin (*14*).

Port Wine Enriched with Proanthocyanidins and Commercial Wines. The Port wine (*Vitis vinifera*, Touriga Nacional cv.) was provided by Adriano Ramos Pinto, Vinhos S. A., alcoholic degree of 20% (v/v). Different quantities (0.5, 1.0, and 2.0 g L^{-1}) of oligomeric procyanidins extracted from grape seed were added directly to the bottled Port wine. After addition, the wine was homogenized and stored in the dark for 9 years. Other studied wines were commercially purchased. The red table wine was from Adriano Ramos Pinto, Vinho S. A., from the vintage of 1993 and had an alcoholic degree of 12% (v/v). The white wine was from Sogrape Vinhos S. A., from the 2005 crop and had a 9% (v/v) alcohol concentration. The commercially purchased red Port wine was from the 2003 vintage of the Symington Group and had an alcoholic degree of 21% (v/v).

Determination of Total Phenolic Compounds. The total phenolic compounds were determined using the Folin−Ciocalteu reagent according to the method described in the literature, with some modifications (*37*). The wine samples were diluted 10 times with deionized water. A total of 250 μL of diluted wine was mixed with 12.5 mL of deionized water, 1.250 mL of Folin−Ciocalteu reagent, and 5 mL of sodium carbonate (20%). After 30 min at room temperature without agitation, the absorbance at 750 nm was measured. A standard curve was obtained using (+)-catechin as a standard.

Enzymatic Activity Assays. Trypsin activity was assayed using BApNA as a substrate (*24*). From tryptic action, this substrate is degraded, releasing *p*-nitroaniline, detectable by spectrophotometry at 410 nm. Several concentrations of BApNA, from 0.05 to 0.40 g L^{-1}, were tested at 0.072 g L^{-1} of trypsin. The range of concentrations of BApNA from 0.15 to 0.30 g L^{-1} was shown to be appropriate to calculate the initial rate from the graphics of $\Delta Abs = f(time)$ in the experimental conditions used. In each well of a 96-well (300 μL) microreader plate, 20 μL of DL−BApNA working solution (final concentration of 0.18 g L^{-1}) was mixed with different volumes of samples (procyanidin fractions, dissolved in distilled water, or wine samples); the total volume prior to trypsin addition was 260 μL, adjusted with phosphate buffer. The reaction was followed for 3 min before the addition of trypsin. During this period, the absorbance remained constant, indicating no reaction between the substrate and the added procyanidins. After this, 20 μL of trypsin were added (1 g L^{-1} in phosphate buffer, pH 7.0, 50 mM), and the absorbance started to increase as a result of BApNA hydrolysis. The release of *p*-nitroaniline

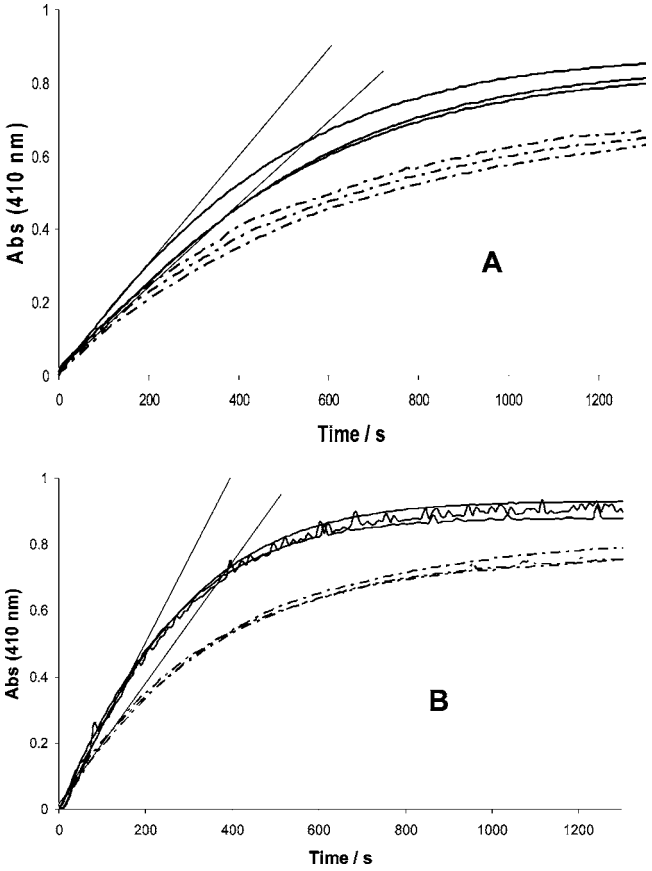

Figure 2. Comparison between the kinetics of trypsin activity (in triplicate) at two different enzyme concentrations: (**A**) 0.036 g L^{-1} and (**B**) 0.072 g L^{-1} in the absence (—) and presence (•••) of oligomers of grape seed (OPC) (0.18 g L^{-1}). The BApNA concentration was 0.18 g L^{-1}.

from BApNA was measured in a UV−vis microplate reader (Biotek Powerwave XS) and appropriate data processing software (KC4).The temperature was physiological (37 °C), and the pH (7.0) corresponds to the one occurring after neutralization of gastric fluid in the duodenum (*38*).

Statistical Analysis. All assays were performed in $n \geq 3$ repetitions. The mean values, standard deviations, and statistical differences were evaluated using analysis of variance (ANOVA), and the mean values were compared using a Tukey test; all statistical data were processed using the Origin Pro software (Origin Laboratory Corporation, MA).

RESULTS AND DISCUSSION

To determine the appropriate amount of trypsin to use in the inhibition assays, two different enzyme concentrations were tested (0.036 and 0.072 g L^{-1}) with and without condensed tannins (**Figure 2**). A concentration of OPC of 0.18 g L^{-1} was used to evaluate their inhibitory ability toward trypsin. A clear difference between both samples with different trypsin concentrations was obtained. For control assays (—), the one with the higher concentration of trypsin (B, 0.072 g L^{-1}) reaches its maximum absorbance (0.89 ± 0.03) at around 600 s, while for lower concentrations (A, 0.036 g L^{-1}), this maximum is not reached in the measuring time. **Figure 2** also shows the influence of procyanidin oligomers on the decrease of enzymatic activity (•••). For both trypsin concentrations, the initial rate and maximum absorbance corresponding to the BApNA hydrolysis are lower in the presence of procyanidins.

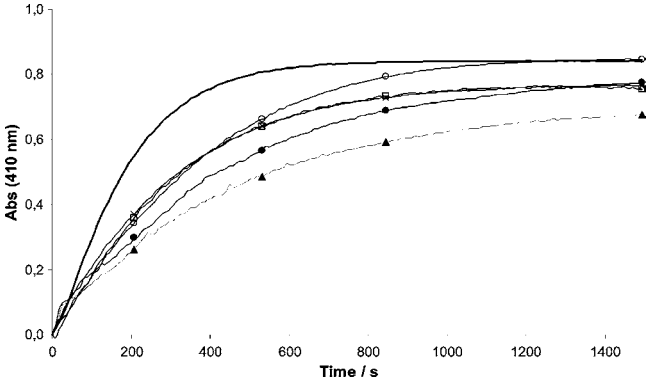

Figure 3. Kinetics of trypsin (0.072 g L^{-1}) activity for several concentrations of procyanidin oligomers fraction 4. Control (black line), (○) 89 mg L^{-1}, (×) 134 mg L^{-1}, (□) 179 mg L^{-1}, (●) 223 mg L^{-1}, and (▲) 312 mg L^{-1}. The BApNA concentration was 0.18 g L^{-1}. To simplify the figure, each curve is a representative of triplicate analysis.

The relative rate of the trypsin inhibition assays can be calculated as the ratio between the initial rate in the presence and that in the absence of phenolic compounds. This parameter can be used as a measure of trypsin inhibition by phenolic compounds. Some authors alternatively used the final absorbance as a measure of residual trypsin activity (*25*, *31*). When the relative rate (**Table 2**) at the two concentration studies are compared, the inhibitory effect is slightly more pronounced (higher sensitivity) and more reproductible (decrease in standard deviation) at a higher enzyme concentration (0.072 g L^{-1}).

The influence of the procyanidin concentration on the inhibitory effect was studied. For several oligomeric fractions with different molecular weights and degrees of polymerization (**Table 1**), a plot of $\Delta Abs = f$(reaction time) was made using different concentrations from 0 (control assay) to 313 mg L^{-1}. **Figure 3** and **Table 3** show the influence of the concentration of procyanidin fraction 4 (mean MW = 1513; mean DP = 5.0) on the inhibition of trypsin activity. In general, there is a decrease in the initial rate with the increase of the concentration of procyanidin. This behavior was observed for the other procyanidin fractions with different MW (data not shown). For the range of concentrations, 89−179 mg L^{-1}, the initial rate was similar and lower than for the control. However, the final absorbances for control and 89 mg L^{-1} are the same (0.84) and superior to the one of the intermediate concentrations (0.76). The most concentrated assay had the lower final absorbance value (0.67), showing the highest inhibition of the hydrolysis of BApNA by trypsin.

Table 2. Initial and Relative Rates (R_i/R_c) of Hydrolysis of BApNA at Two Trypsin Concentrations in the Absence and Presence of OPCs[a]

	initial rate ($s^{-1} \times 10^2$)		final absorbance	
trypsin concentration	0.036 g L^{-1}	0.072 g L^{-1}	0.036 g L^{-1}	0.072 g L^{-1}
without OPC (R_c)	0.127 ± 0.008	0.193 ± 0.03	0.84 ± 0.03	0.89 ± 0.03
with OPC (R_i)	0.097 ± 0.007	0.142 ± 0.01	0.65 ± 0.02	0.77 ± 0.02
relative rate (R_i/R_c)	0.764 ± 0.014 a	0.735 ± 0.004 b		

[a] Maximum absorbance values reached at 1300 s. Values with different letters are significantly different ($p < 0.05$), and values with the same letter are not significantly different ($p > 0.05$).

Table 3. Initial Rate and Relative Rate (R_i/R_c) of Hydrolysis of BApNA with Different Concentrations of OPC (Fraction 4)[a]

procyanidin concentration (g L^{-1})	initial rate ($s^{-1} \times 10^3$)	relative rate
0	2.37 ± 0.03 a	1.00 ± 0 a
89	1.66 ± 0.06 b	0.70 ± 0.02 b
134	1.05 ± 0.22 c	0.45 ± 0.08 c
179	1.43 ± 0.14 b	0.61 ± 0.07 b
223	1.26 ± 0.04 c	0.53 ± 0.03 c
313	0.83 ± 0.02 d	0.35 ± 0.03 d

[a] Values, in each variable, with different letters are significantly different ($p < 0.05$), and values with the same letter are not significantly different ($p > 0.05$).

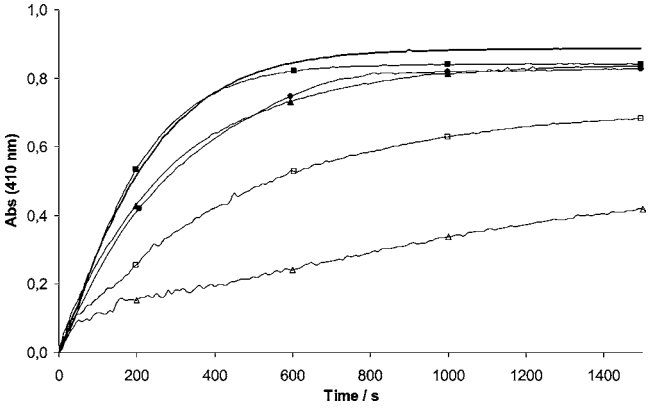

Figure 4. Kinetics of trypsin (0.072 g L^{-1}) activity in the presence of procyanidin fractions from the grape seed extract (223 mg L^{-1} of each fraction). Control (black line), (■) fraction 1, (▲) fraction 2, (●) fraction 3, (□) fraction 4, and (△) fraction 5. The BApNA concentration was 0.18 g L^{-1}. To simplify the graphics, each curve is a representative of triplicate analysis.

The decrease of trypsin activity with an increasing MW and degree of polymerization of the added procyanidin oligomers was also evaluated. **Figure 4** shows the increase of absorbance with time for procyanidin fractions 1−5 at the same concentration (223 mg L^{-1}). There is clearly a decrease in both the initial rate and the final absorbance with the size of procyanidin oligomers, especially for the two more polymerized fractions 4 and 5.

Assuming a relationship between trypsin inhibition and the concentration of procyanidins (shown in **Figure 3**), the representation of the relative reaction rate versus the concentration of phenolic compound [$R_i/R_c = f(|\text{polyphenol}|)$] relates to the ability of procyanidins to inhibit trypsin (**Figure 5**). The slope of these curves, defined herein as the inhibitory factor (Fi), is a measure of the ability of phenolic compounds to inhibit trypsin and can therefore be used to compare the inhibitory effect of different compounds tested under these conditions. **Table 4** shows the inhibitory effect of procyanidin fractions with different mean molecular weights.

Fractions 1−3 inhibit trypsin at the same extent, and their Fi values are lower than the one obtained for the more polymerized fractions. Indeed, fractions 4 and 5 presented an extensive inhibitory capacity toward tryptic degradation of BApNA.

For these fractions, it is possible to observe that the linearity was lost for high concentrations. This apparent saturation behavior probably relates to a point in which all of the trypsin molecules are inhibited to some extent but still retain some residual activity. Thus, the inhibitory factor (Fi) was calculated using the linear part of the curves (low concentrations).

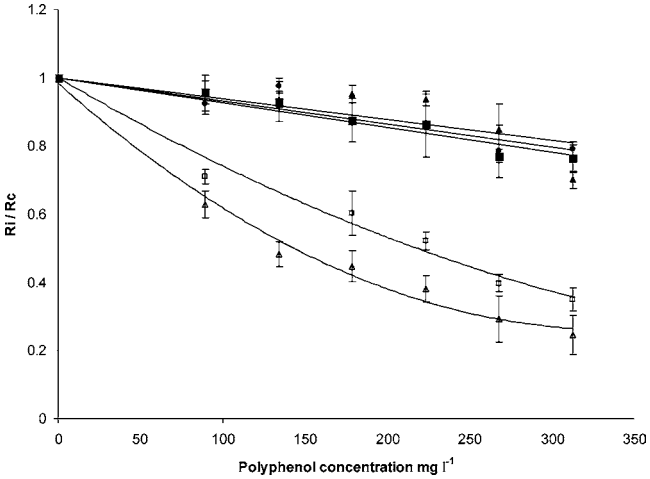

Figure 5. Relative rate of trypsin activity (R_i/R_c) as a function of the inhibitor concentration for procyanidin oligomers. (■) Fraction 1, (▲) fraction 2, (●) fraction 3, (□) fraction 4, and (△) fraction 5.

Table 4. Inhibition Factor (Fi) of the Trypsin Activity and Mean Molecular Weight for the Oligomeric Fractions Tested[a]

fractions	mean MW (*36*)	inhibition factor (Fi) × 10^3
1	513	0.82 ± 0.11 a
2	823	0.77 ± 0.09 a
3	949	0.74 ± 0.12 a
4	1513	2.00 ± 0.09 b
5	2052	2.34 ± 0.19 c

[a] Means with different letters are significantly different ($p < 0.05$), and means with the same letter are not significantly different ($p > 0.05$).

The inhibition factor remains unchanged for a MW below 1000 (**Table 4**), after which they rise probably as a result of the increase in the number of binding sites available to complex with trypsin. These results are in agreement with previous experimental observations on simple interactions between these procyanidin fractions and model proteins, such as bovine serum albumin, for which the ability to form protein−tannin aggregates increased with the degree of polymerization of procyanidins (*39*).

Another objective of the present work was to explore the antinutritional properties of beverages, in particular, wine with different concentrations of condensed tannins. The enological tannins (such as procyanidins) are used for the clarification of wines and to improve the gustatory properties. Their effect on the nutritional properties of wine is unclear, but the increased polyphenolic content might produce a high inhibition of trypsin. Thus, a commercial Port wine was enriched with an extract of grape seed procyanidin (OPC). Different concentrations of procyanidins were added to bottled Port wine to obtain the final concentrations of 0.5, 1.0, and 2.0 g L^{-1}. The Port wine without the addition of OPC corresponds to the reference. Plots of $\Delta\text{Abs} = f(\text{time})$ made at several wine volumes allowed for the calculation of the initial rates R_i and R_c and the relative rate (R_i/R_c). **Figure 6** shows the final plot of R_i/R_c as a function of the wine volume, and **Table 5** shows the Fi for the tested wines. The relative rate decreases with the increase of the wine volume, pointing to the inhibition of tryptic activity. However, this decrease is only linear for low wine volumes. At volumes superior to 70 μL, the R_i/R_c stays constant, which could mean that the trypsin sites available to complex with the polyphenols are saturated (as seen for procyanidin fractions 4 and 5).

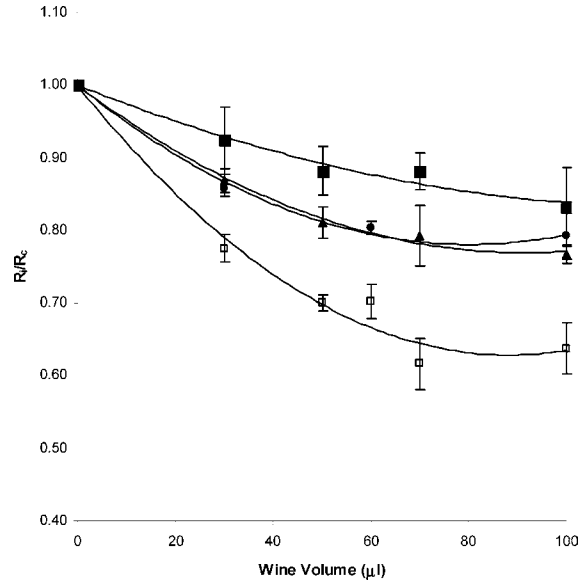

Figure 6. Relative rate of trypsin activity (R_i/R_c) as a function of the wine volume for the supplemented wines. (■) Reference (no added procyanidin), (▲) 0.5 g L^{-1} of OPC added, (●) 1.0 g L^{-1} of OPC added, and (□) 2.0 g L^{-1} of OPC added.

Table 5. Inhibition Factor (Fi) of the Trypsin Activity and Total Phenolic Content of Port Wines Enriched with Different Concentrations of Procyanidin Oligomers and Commercial Wines[a]

	wine	inhibition factor (Fi) × 10^3	total phenolics[b] (mg L^{-1})
enriched Port wines	reference (0 g L^{-1})	1.3 ± 0.2 a	0.26 ± 0.01 a
	0.5 g L^{-1}	2.3 ± 0.2 a	0.47 ± 0.01 b
	1.0 g L^{-1}	2.0 ± 0.3 a	0.49 ± 0.02 b
	2.0 g L^{-1}	5.1 ± 0.5 b	0.73 ± 0.01 c
commercial wines	white table wine	1.5 ± 0.4 a	0.015 ± 0.006 d
	red table wine	4.4 ± 0.7 b	1.80 ± 0.01 e
	red Port wine	13.7 ± 1.7 c	3.15 ± 0.02 f

[a] Values, for each variable, with different letters are significantly different ($p < 0.05$), and values with the same letter are not significantly different ($p > 0.05$). [b] Expressed as catechin equivalents.

The wine supplemented with 2.0 g L^{-1} of OPC presented the highest Fi value among the enriched wines, while the inhibitory factor of the 0.5 and 1.0 g L^{-1} supplemented wines is similar and higher than that of the reference; these results are in agreement with the total amount of phenolic content of each wine measured with the Folin−Ciocalteu method. It is advisable to refer that these wines are 9-year-old Port wines and that, during this period, several chemical reactions occurred in the wine, such as complexation and oxidation−reduction reactions involving tannins (both hydrolyzable and condensed), that lead to the formation of new compounds, including polymers that precipitate. In fact, when these bottled wines were opened, some deposit was observed probably corresponding to these compounds. Still, these wines may contain phenolic compounds other than condensed tannins that could also be effective in inhibiting enzyme activity.

This method was also applied to commercial wines: white table wine, red table wine (RTW), and red Port wine. **Figure 7** shows the plot of inhibitory R_i/R_c versus the wine volume. Once again, for high wine volumes, an apparent saturation of trypsin-complexing sites occurs, as R_i/R_c changes very little. The Fi results with table wines point to a clear difference between white (Fi = 1.5 ± 0.4) and red (Fi = 4.4 ± 0.7) wine, with the latter

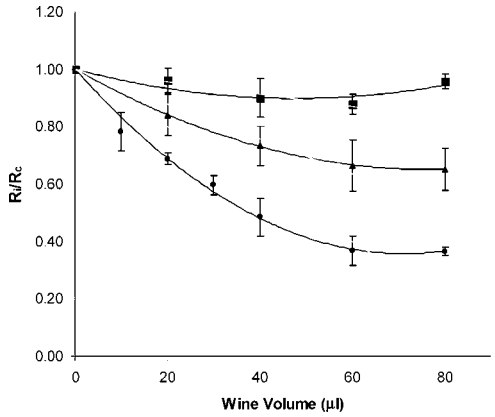

Figure 7. Relative rate of trypsin activity (R_i/R_c) as function of the wine volume for the commercial wines. (■) White table wine, (▲) red table wine, and (●) red Port wine.

presenting a much higher inhibition factor (**Table 5**). This could be explained by the higher total phenolic content of red table wine. Port wine is shown to reduce the activity of trypsin to a greater extent (Fi = 13.7 ± 1.7). The Fi for the commercial Port wine is 3.2 times larger than that of the RTW, and this wine also has a higher amount of potentially inhibitory phenolic compounds. When the RTW and the Port wine enriched with 2.0 g L^{-1} are compared, it is also possible to observe that they have similar Fi values, despite the total phenols in the red table wine being 2.5 times higher.

It is possible to conclude that procyanidin content is not the only factor affecting the activity of trypsin. A result that supports this is the one obtained with white table wine that, although having the lowest phenolic content, inhibits trypsin to the same extent as the Port wine reference in the enriched wine assays.

This method has proven to be versatile and reliable to evaluate trypsin inhibition by phenolic compounds, which has still been poorly studied. A direct relation between the mean degree of polymerization and enzymatic inhibition was observed. Furthermore, trypsin inhibition activity was observed in several types of wines with different phenolic content. This may be relevant in the consideration of condensed tannins as antinutritional factors, inhibiting the digestion of proteins and therefore contributing to a reduced absorption of nutrients. Bearing this, it seems to be a nutritional advantage for the ingestion of both tannin and protein-rich foods to prevent the inhibition of digestive enzymes in the intestine. Although extrapolations from *in vitro* assays to *in vivo* studies need to be taken into consideration with high caution, the evidence gathered here points to an ability of polyphenolic compounds, at least grape procyanidins, to inhibit trypsin activity.

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