



Relationships between antiplatelet activity, dry matter content and flavor in onion cultivars

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Abstract

A number of studies suggest that dietary intake of onion (*Allium cepa* L.) benefits cardiovascular health. The *in vitro* antiplatelet activity observed in Alliums is influenced by genotype and environmental factors. Few descriptions about Argentinean onion cultivars and their health benefits have been reported. To investigate the relationship between antiplatelet effect, pyruvic content, soluble solids content and total dry matter, eight Argentinean onion cultivars ('Angaco INTA', 'Antartica INTA', 'Ancasti INTA', 'Refinta 20', 'Valcatorce INTA', 'Valuno INTA', 'Cobriza INTA' and 'Navideña INTA') of different physiological requirements for bulbification and genetic background were grown during two years. Thirty days after harvest onion bulbs were sampled, and pungency, dry matter, soluble solids and *in vitro* antiplatelet activity were analyzed. Significant cultivar x year interactions were observed for all studied variables ($P < 0.05$). 'Refinta 20' and 'Antartica INTA' showed 100% inhibition of platelet aggregation, while 'Angaco INTA' presented the lowest inhibiting effect. Significant positive correlations were found between *in vitro* antiplatelet activity and pyruvic acid content, soluble solids and dry matter content in both years. There seems to be interesting differences among cultivars according to their origins. 'Angaco INTA' originated from 'Valencianita' populations shows low pungency and also low *in vitro* antiplatelet activity, whereas cultivars selected from 'Valenciana' type populations show intermediate pungency and antiplatelet activity. 'Refinta 20' selected from 'Southport White Globe' populations has the highest antiaggregatory effect, pungency and solids. Another interesting relationship seems to be related to bulb color; white-bulb cultivars ('Ancasti INTA', 'Refinta 20', and 'Antartica INTA') have more soluble solids content and *in vitro* antiplatelet activity than colored bulb cultivars.

Key words: Alliums, *Allium cepa* L., health benefits, antiaggregatory effect, platelets, pungency, solids, dry matter, flavor, organosulfur compounds.

Introduction

The onion crop is of great importance in Argentina, along with garlic, are the main fresh vegetables exported. About 600,000 tons of onions are produced yearly on 19,000 ha, for fresh market and the dehydration industry¹. For decades, onion-breeding programs in Argentina have been developing short, intermediate and long-day cultivars². However, no descriptions about their health benefits have been reported.

There is an increasing demand for foods with good organoleptic characteristics and also with beneficial attributes for health, nevertheless it is important to establish scientific bases to support the consumption of food with potential nutritional active ingredients.

Onion and garlic consumption have been associated with decreased cardiovascular events, because of their hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, antithrombotic and anti-hyperhomocysteinemia effects³, as well as with many other biological activities including antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory and prebiotic activity⁴.

Onions are primarily consumed for their distinctive flavor or their ability to enhance flavors in other foods⁵. The pungency⁶ and antiplatelet activity⁷ in onion and other Alliums are attributed

to the organosulfur products arising from the enzymatic decomposition of S-alk(en)yl-L-cysteine sulfoxides (ACSOs), following tissue disruption.

Platelet aggregation is an important cause of thrombosis leading to cardiovascular disease⁸. Inhibitors of aggregation can provide protection against these syndromes that affect millions of people worldwide⁹. Onion and garlic juice inhibits platelet aggregation in human blood *in vitro*^{10,11} and *in vivo*¹²⁻¹⁵. Several epidemiologic studies have reported that antiplatelet activity of onion is considered to be a property of organo-sulphur and flavonoid compounds. The possible mechanism of the antiplatelet effect of onion includes thromboxane A₂ synthase inhibition and TXA₂/PGH₂ receptor blockade¹⁶.

Among the first scientific investigations of onion antiplatelet activity, two separate studies were reported comparing bleeding time and fibrinolytic activity in blood^{17,18}. Both showed that heating onions increased coagulation time and fibrinolytic activity *ex vivo*. Furthermore, Bordia *et al.*¹⁹ showed that garlic oil administration to healthy subjects and patients with coronary artery disease (CAD) inhibited platelet aggregation *ex vivo*. Likewise, consumption of raw Welsh onion by rats has also been shown to inhibit aggregation and other platelet functions *ex vivo*²⁰.

In raw onions, significant correlations have been reported among *in vitro* antiplatelet activity, pungency and the solid content^{7, 21-23}, also common QTL for these properties have been detected^{11, 23}.

The Argentinean onion germplasm trace back mainly to European origins. 'Valenciana' populations were used to develop the cultivars 'Valcatorce INTA', 'Valuno INTA', 'Cobriza INTA' and 'Antartica INTA'. 'Valenciana' populations are related to 'Grano' type onions from the Valencia region of Spain; the long-storing cv. Pukekohe Long Keeper of New Zealand and its Australian derivative cv. Creamgold are believed to derive from the same Spanish origin²⁴. 'Torrentina' populations were used to develop 'Navideña INTA'. 'Torrentina' populations have been known for more than 100 years in Argentina and it may have been originated in the south of Spain. 'Angaco INTA' trace back to 'Valencianita' populations that were introduced to Argentina from southern Spain more than 100 years ago. 'Refinta 20' was originated from a selection of 'Southport White Globe' populations²⁵. S₁ family selection was the common strategy used for the development of 'Navideña INTA', 'Cobriza INTA' and 'Refinta 20'². Mass selection was used in the case of 'Valcatorce INTA', 'Valuno INTA' and 'Antártica INTA'. Currently available cultivars are described in Galmarini².

The beneficial health-effects attributed to onions are important for consumers and breeders. The characterization of germplasm according to antiplatelet activity would help the selection of onion cultivars with enhanced flavor and defined medicinal benefits. There are not published data about the variation of the *in vitro* antiplatelet activity of Argentinean onion cultivars. It is also relevant to investigate the relationship among antiplatelet effect, pungency, soluble solids content and total dry matter of cultivars from different origins.

Materials and Methods

Plant material and experimental design: Eight onion cultivars: 'Navideña INTA', 'Valuno INTA', 'Valcatorce INTA', 'Cobriza INTA', 'Ancasti INTA', 'Antartica INTA', 'Refinta 20' and 'Angaco

Table 1. Physical and chemical properties of La Consulta's soil.

Textural class	Loam
EC (dSm ⁻¹)	3.77
SAR ((meq/l) ^{1/2})	3.47
N (mg kg ⁻¹)	693.00
Pa (mg kg ⁻¹) *	3.15
Ki (mg kg ⁻¹)**	175.50
Ca (meq l ⁻¹)	31.80
Mg (meq l ⁻¹)	4.00
Na (meq l ⁻¹)	14.59
Cl ⁻ (meq l ⁻¹)	15.00
SO ₄ ²⁻ (meq l ⁻¹)	33.00
Organic carbon (g kg ⁻¹)	12.5

*Pa, available phosphorus, **Ki, exchangeable potassium.

Table 2. Average temperatures and rainfall during the period of grow in both studied years.

Average t (°C)*		Maximum t (°C)*		Minimum t (°C)*		Rainfall (mm)*	
2005	2006	2005	2006	2005	2006	2005	2006
18.1	18.2	26.3	27.1	9.9	9.8	44	23.5

*Values corresponding to the period September to February.

INTA', were chosen from the germplasm collection of INTA La Consulta. These cultivars were grown in la Consulta, Mendoza, Argentina (33°44'S, 69°07'W) during 2004-2005 and 2005-2006. A randomized complete block design with four replicates for each cultivar and year was used. The crop was implanted using seedlings in early September (09/08/04; 09/09/05), 72 plants per plot (2 m x 3 m) were implanted. The onions were grown in a loam soil. Physical and chemical properties of the soil are described in Table 1. The average temperatures and precipitations for both years are indicated in Table 2. Irrigation was applied every 8 days, using furrow irrigation. No fertilizers were applied. Onion bulbs were harvested when 50% of foliage leaves were collapsed. At harvest bulbs were collected and stored in sheds at room temperature. After harvest the bulbs were stored in a cool storage room.

Sample processing: Ten unsprouted bulbs per replication were sampled 30 days after harvest and evaluated for antiplatelet activity (IVAA), pyruvic acid content (PC), soluble solids content (SSC) and total dry matter (TDM). These ten chosen bulbs after removing the dry outer scales were quartered along the longitudinal axis to obtain a representative sample of outer and inner scales. One quarter of each bulb was combined and used to measure TDM and SSC. The other ten quarters were juiced using a mechanical juicer. The juice was collected, filtered and left on the bench for 15 minutes to allow enzymatic hydrolysis of the flavor precursors. For PC analysis a juice aliquot was added with an equal volume of 5% trichloroacetic acid and centrifuged for 10 minutes at 10,000 rpm. For IVAA determination, onion juice was centrifuged for 20 minutes at 10,000 rpm and the supernatant was frozen at -80°C⁷.

Measurement of *in vitro* antiplatelet activity: IVAA was measured using a whole blood electrical impedance aggregometer (Chrono-Log, Corp.) in whole blood²⁶. Blood was drawn from three healthy human donors who had abstained from eating onion; others related *Alliums*, or other known platelet-inhibitory foods (e.g., wine, beer, etc.) for at least 72 h. Also, the donors had not taken drugs known to affect platelet aggregation (e.g., aspirin) for at least two weeks prior to blood sampling. Venipuncture was performed by the Hematology Service of the 'Hospital Central' of Mendoza, following all the ethical and safety precautions required by Human Public Health Committee of Mendoza. These blood samples were anticoagulated with sodium citrate (3.8%, 1 vol anticoagulant:9 vol of blood) and were diluted 1:1 with TRIS-buffered saline (pH 7.4) and vortexed. The diluted blood was maintained at room temperature during the experiment and used within 2 hours of extracted.

For all experiments, 1 ml of blood/TBS was transferred to cuvettes containing a stir bar. After incubation at 37°C for 3 min., a dose of onion juice was added. The inhibitory dose of onion juice at which all cultivars showed antiplatelet activity was previously established (data not shown). Each dose was assayed twice. After juice addition (200 µl), the electrodes were inserted into the blood mixture and platelet aggregation was induced by the addition of 2.5 µl collagen (1 mg ml⁻¹). Change in the impedance was recorded over 6 minutes; this change is proportional to platelet aggregation.

IVAA was expressed as percent of inhibition of platelet aggregation, compared to control samples prepared in the same way but without adding onion juice.

Pungency determination: Pyruvate content was determined according to Schwimmer and Weston²⁷. For all experiments, 0.3 ml of juice/TCA previously centrifuged were taken and diluted with distilled water (1:20). One ml of 0.0125% 2,4-DNPH in 2 N HCl was added to 2 ml of juice/TCA diluted (1:20). The tubes were incubated at 37°C during 10 minutes in a thermostated bath, then 5 ml of NaOH 0.6 N were added. The color developed by the samples was measured in a spectrophotometer (Beckman DU Serie 500 UV/Visible) at 420 nm. The pyruvic acid concentration of the onion juice was determined using as a reference a standard curve developed with known concentrations of pyruvate. Values were expressed as $\mu\text{mol g}^{-1}$ of onion fresh weight.

Determination of total dry matter and soluble solids content: Fresh onion bulb tissues were crushed and dried in a stove at 50°C for 20 hours until constant weight. Values were expressed as g kg^{-1} .

Ten quarters were blended for a minute in a blender and the resulting juice was squeezed through cheesecloth. Several drops of juice were applied to a hand refractometer to measure soluble solids content (expressed as °Brix)²⁸.

Statistical analysis: The data were expressed as mean \pm SD. IVAA, PC, SSC and TDM were analyzed by ANOVA procedure using the software STATGRAPHICS Plus for Windows 4.0. Means of each group were compared by honestly significant difference (Tukey HSD) test. Correlation analysis among variables was performed using the same software.

Results

In vitro antiplatelet activity: Onion juice inhibited aggregation in a dose-dependent manner (data not shown). Based on these results a concentration of 200 μl onion juice/ml blood was chosen for all experiments. The ANOVA analysis revealed significant differences for antiplatelet activity among cultivars and years. The cultivar \times year interaction was significant at $P < 0.05$ (Fig. 1). Nevertheless, ANOVA analysis of cultivar, year and their interaction contributed 85.52, 0.53 and 10.58% of variation to the IVAA in this study, respectively (Table 3); indicating that genetic differences were the main factor determining IVAA, and that year and cultivar \times year interaction, played a minor role.

Averaged over all cultivars, antiplatelet activity was significantly higher in 2005 compared to 2006. 'Refinta 20' and 'Antartica INTA' showed 100% inhibition of platelet aggregation in both years, while 'Angaco INTA' presented the lowest inhibiting effect. 'Valcatorce INTA' showed greater IVAA during 2005 ($94.0 \pm 5.4\%$) than 2006 ($45.7 \pm 22.7\%$). However, 'Ancasti INTA' showed greater IVAA during 2006 ($89.9 \pm 12.4\%$) than 2005 ($58.0 \pm 4.5\%$), (Fig. 1, Tables 4 and 5).

There seems to be differences between Valencianita cultivars and Valenciana cultivars for this character. There are also differences between white colored bulb cultivars ('Ancasti INTA', 'Refinta 20') and colored ones for IVAA.

Pungency: Significant differences among cultivar and year \times

cultivar interactions for pungency were observed. The lowest levels of pyruvic acid were observed in 'Angaco INTA' (Tables 4 and 5). 'Refinta 20' exhibited the highest pyruvic acid content, 2.7-fold and 1.8-fold difference in 2005 and 2006, respectively, when compared to the 'Angaco INTA', the lowest pyruvic acid content cultivar. 'Valuno INTA' showed significant variability for pungency among years. The other cultivars did not show significant differences for pungency. The overall ranking was maintained. There seems to be differences between Valencianita, Torrentina and Valenciana cultivars and 'Refinta 20', originated from 'Southport White Globe', 'Refinta 20' being the most pungent one, and 'Angaco INTA', derived from Valencianita populations, sweeter.

Total dry matter and soluble solids content: 'Refinta 20' had the highest dry matter content whereas the cultivar 'Angaco INTA' showed the lowest among the studied materials, in both years. There are differences between white colored bulb cultivars ('Ancasti INTA', 'Refinta 20', and 'Antartica INTA') and colored ones for SSC.

Soluble solids content ranged from 21.6 °Brix and 22.3 °Brix for 'Refinta 20' in 2005 and 2006 respectively, to 7.7 °Brix for 'Valuno INTA' in 2005 and 6.9 °Brix for 'Angaco INTA' in 2006 (Tables 4 and 5). The cultivar \times year interaction was significant at $p < 0.05$ for SSC as well as for dry matter content.

'Refinta 20' had the highest dry matter content whereas the cultivar 'Angaco INTA' showed the lowest among the studied materials, in both years. There were differences between white colored bulb cultivars ('Ancasti INTA', 'Refinta 20', and 'Antartica INTA') and colored ones for SSC.

Relation between antiplatelet activity and other variables: Significant positive correlation were found between IVAA and PC ($r = 0.69^{***}$ in 2005 and $r = 0.80^{***}$ in 2006), SSC ($r = 0.38^*$ in 2005 and $r = 0.69^{***}$) and dry matter content ($r = 0.43^*$ in 2005 and $r = 0.71^{***}$ in 2006) in both studied years (Table 6). Soluble solids content and total dry matter correlated with pungency.

The principal components analysis indicated that the first principal component accounted for 79% of total trait variation and allows the discrimination of cultivars in four groups. In one the long-day cultivars 'Cobriza INTA', 'Valcatorce INTA' and 'Valuno INTA', all derived from selections of Valenciana populations, and the intermediate cultivar 'Navideña INTA',

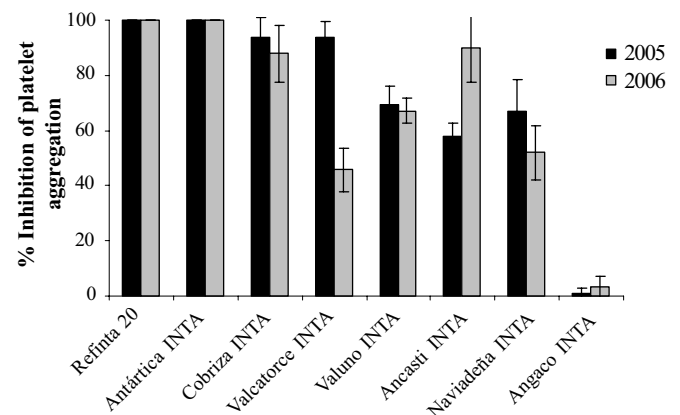


Figure 1. Antiplatelet activity of onion cultivars extracts during 2005 and 2006.

Table 3. ANOVA table of IVAA variation due to cultivar, year, and their interaction.

Source	d.f	Sum of square		F-value	P>F
Cultivar	7	55730.5	(85.52 %)	174.47	0.0000
Year	1	347.4	(0.53 %)	7.62	0.0082
Cultivar x year	7	6895.1	(10.58 %)	21.59	0.0000
Error	48	2190.26	(3.36 %)		
Total	63	65162	(100 %)		

Table 4. *In vitro* antiplatelet activity, pyruvic acid content, total dry matter and soluble solids content of onion cultivars in 2005.

Cultivar	PC ^a		TDM		SSC		IVAA	
Refinta 20	7.4 ± 1.2 ^b	a	220.8 ± 11.3	a	21.6 ± 1.2	a	100.0 ± 0.0	a
Antartica INTA	4.8 ± 0.3	b	163.8 ± 4.9	b	15.0 ± 0.7	b	100.0 ± 0.0	a
Cobriz INTA	5.0 ± 0.3	b	109.9 ± 5.6	c	9.2 ± 0.4	c	94.0 ± 7.0	a
Valcatorce INTA	4.9 ± 0.7	b	103.7 ± 1.4	cd	8.3 ± 0.4	cd	94.0 ± 5.4	a
Valuno INTA	3.7 ± 0.1	bc	96.0 ± 3.5	cde	7.7 ± 0.7	d	69.3 ± 6.7	b
Naviadeña INTA	3.9 ± 0.5	bc	89.3 ± 5.8	e	8.1 ± 0.5	cd	67.0 ± 11.5	b
Ancasti INTA	4.5 ± 1.0	b	173.2 ± 4.2	b	15.7 ± 0.3	b	58.0 ± 4.5	b
Angaco INTA	2.7 ± 0.1	c	89.9 ± 6.2	de	8.1 ± 0.2	cd	1.0 ± 2.0	c

^aPC, pyruvic acid content ($\mu\text{mol g}^{-1}$ of onion fresh weigh); TDM, total dry matter (g kg^{-1}); SSC, soluble solids content (^oBrix); IVAA, *in vitro* antiplatelet activity (%). ^bEach value is the mean ± SD of fresh material. Values followed by the same letter were not significantly different according to the Tukey test ($p \leq 0.05$).

Table 5. *In vitro* antiplatelet activity, pyruvic acid content, total dry matter and soluble solids content of onion cultivars in 2006.

Cultivar	PC ^a		TDM		SSC		IVAA	
Refinta 20	6.35 ± 0.2	a	242.0 ± 5.8	a	22.5 ± 0.3	a	100.0 ± 0.0	a
Antartica INTA	5.5 ± 0.1	b	160.8 ± 2.6	c	14.5 ± 0.5	c	100.0 ± 0.0	a
Cobriz INTA	5.3 ± 0.3	b	115.0 ± 2.5	d	9.0 ± 0.3	d	87.9 ± 10.3	a
Valcatorce INTA	4.6 ± 0.1	c	103.6 ± 4.6	de	8.7 ± 0.3	de	45.7 ± 22.7	c
Valuno INTA	4.6 ± 1.1	bc	91.7 ± 1.3	ef	7.9 ± 1.0	def	67.0 ± 30.2	b
Naviadeña INTA	3.6 ± 0.2	d	88.9 ± 6.6	f	7.5 ± 0.4	ef	52.1 ± 9.8	bc
Ancasti INTA	5.1 ± 0.4	bc	214.8 ± 9.9	b	20.0 ± 1.2	b	89.9 ± 12.4	a
Angaco	3.5 ± 0.2	d	80.5 ± 1.5	f	6.9 ± 0.2	f	3.3 ± 3.8	d

^aPC, pyruvic acid content ($\mu\text{mol g}^{-1}$ of onion fresh weigh); TDM, total dry matter (g kg^{-1}); SSC, soluble solids content (^oBrix); IVAA, *in vitro* antiplatelet activity (%). ^bEach value is the mean ± SD of fresh material. Values followed by the same letter were not significantly different according to the Tukey test ($p \leq 0.05$).

Table 6. Correlation values (r) among *in vitro* antiplatelet activity, pyruvic acid content, total dry matter and soluble solids content of onion cultivars in 2005 and 2006.

Variable	PC	TDM	SSC	IVAA
2005				
PC		0.76***	0.75***	0.69***
TDM			0.99***	0.43*
SSC				0.38*
IVAA				
2006				
PC		0.74***	0.71***	0.83***
TDM			0.99***	0.71***
SSC				0.69***
IVAA				

The symbol *, ***, denotes significant at $P < 0.05$, and 0.001 respectively.

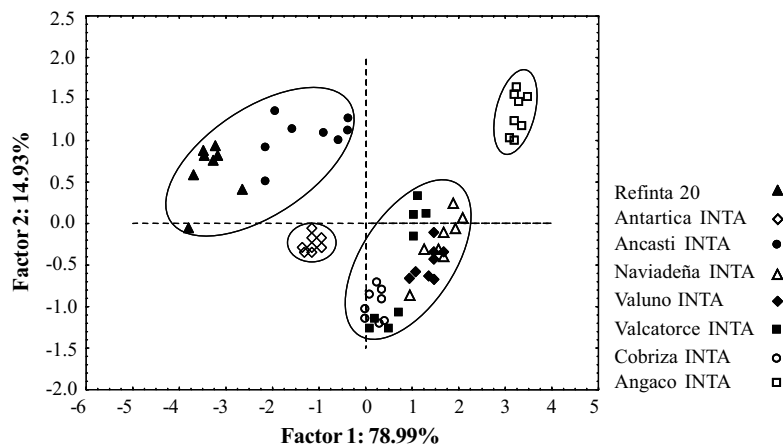


Figure 2. Principal components analysis of studied variables in onion cultivars.

selected from Torrentina populations are grouped. In other group 'Angaco INTA' a short-day Valenciana-type cultivar appears alone; in a third group a white Valenciana-type cultivar 'Antartica INTA' appears also alone and in the last group two white type-cultivars used for the dehydration industry, 'Refinta 20' and 'Ancasti INTA' are grouped together (Fig. 2).

Discussion

This is the first study on the relationship between antiplatelet activity, dry matter and flavor of Argentinean onion germplasm. The positive correlation observed between antiplatelet activity and pungency, soluble solids and dry matter content indicates that onion cultivars with higher solids and higher pyruvic content exhibit greater antiplatelet activity. These results agree with the phenotypic correlation previously found by Mann and Hoyle²⁸, Goldman *et al.*⁷ and Debaene *et al.*²⁹ and the phenotypic and genetic correlation reported by Galmarini *et al.*¹¹ using other genetic backgrounds.

Pungency and solids content are important attributes of onion bulb quality for processing and storage. Dry matter content is important for dehydration industry because it has a direct impact on the energy required for drying. Sinclair *et al.*³⁰ classified 49 varieties on the basis of their dry matter content. Varieties for fresh market showed dry matter content ranging from 74 to 147 g kg⁻¹, whereas the dry matter content of dehydrating varieties ranged from 159 to 215 g kg⁻¹. Thus, in agreement with Sinclair *et al.*³⁰, 'Refinta 20' and 'Ancasti INTA' are suitable for dehydration and other cultivars are classified as "fresh market" type; these two cultivars, as was mentioned, grouped together in our analysis. Previous studies have evaluated the relationship between soluble solids content and total dry matter with pungency²¹ indicating a positive correlation among these variables. Our results showed that cultivars with high solids content are more pungent. Onion cultivars are classified on the basis of pungency as low pungency/sweet (0-3 μmol pyruvic acid g⁻¹ fresh weight); medium pungency (3-7 μmol pyruvic acid g⁻¹ fresh weight) and high pungency (above 7 μmol pyruvic acid g⁻¹ fresh weight)³¹. According to this classification, 'Refinta 20' can be classified as highly pungent and the other cultivars as mild pungent.

The phenotypic correlation between pyruvic acid content and antiplatelet activity may be indicative of S-alk(en)yl-L-cysteine sulfoxides concentrations and alliinase activity³². ACSOs composition is important because it is responsible for nature and intensity of flavor. Once onion is crushed, these compounds are transformed into thiosulfates and disulfides³³ which have proven inhibitor effect of platelet aggregation *in vitro*⁹. The strong positive correlation observed in our study suggests that onion cultivars with greater antiplatelet activity have high levels of ACSOs. Pungency was a good indicator of IVAA; suggesting that this measure could be used as a predictor of the biological activity. In the near future we will analyze the ACSOs levels in Argentinean onion cultivars, to corroborate this hypothesis.

In this study all cultivars analyzed inhibited platelet aggregation in a dose-dependent manner exhibiting a broad range of variation (from 0 to 100%). Our results agree with those reported by Goldman *et al.*⁷, indicating that antiplatelet activity of onion extract is genotype dependent. Also cultivar x year interaction was observed, it indicates that the environmental factors in any given year influence onion-induced antiplatelet activity, observation

already made by Debaene *et al.*²⁹. Nevertheless, we found that most of the variation was due to genetic differences.

One way to express the difference among cultivars according to their capacity to prevent platelet aggregation is trying to establish the amount of fresh onion juice that can cause equal antiplatelet effects. As an example, in the case of 'Angaco INTA' it was necessary to triplicate the dose of juice to equal similar effects caused by 'Refinta 20', this is shown in Fig. 3. Assuming that *in vitro* evaluation is correlated with fresh onion consumption, there would be differences among cultivars in the quantity of fresh onion necessary to reach similar health-benefits effects. These results should be taken into consideration in onion breeding programs and also in the recommendations for onion consumers.

There seems to be interesting differences among cultivars according to their origins. 'Angaco INTA' originated from Valenciana populations shows low pungency and also low IVAA, cultivars selected from Valenciana type populations show intermediate pungency and also intermediate antiplatelet activity. 'Refinta 20' selected from SWG populations has the highest IVAA, PC and dry matter content.

Another interesting relationship seems to be related to bulb color. White-bulb cultivars ('Ancasti INTA', 'Refinta 20', and 'Antartica INTA') have more SSC and IVAA than colored bulb

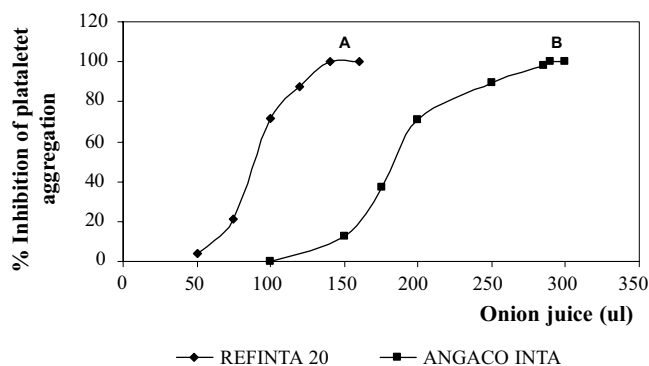


Figure 3. Dose-response curve of the highest (A) and lowest (B) antiplatelet activity onion cultivars.

cultivars. This relationship needs to be further explored.

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