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Evaluation of phenolic compounds, antioxidant activity and bioaccessibility in *Physalis peruviana* L.

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Evaluation of phenolic compounds, antioxidant activity and bioaccessibility in *Physalis peruviana* L.

This work evaluated phenolic compounds in Physalis peruviana as well as their antioxidant activity and bioaccessibility, using an in vitro model of the gastro intestinal system. Three combinations of solvents were evaluated for the extraction of phenolic compounds. All other chemical components, their antioxidant activity and bioaccessibility were evaluated using established methods. P. peruviana is rich in fibre (4.61 g/100g), vitamin C (26.70 mg/100g), carotenoids (5.95 µg/100g), total phenolic compounds (59.9 mgGAE/100g), flavonoids (0.340 mgQE/10 g) and ortho-diphenols (94.6 mgGAE/100g). The antioxidant activity varied from 7.7 to 13.7 µmolTE/g. The simulation of the digestive tract showed that only about 40-50% of the phenolic compounds remained available for intestinal absorption, and only 23-34% of the antioxidant activity was preserved after passing through the gastrointestinal system. Hence, these ratios have to be taken into consideration regarding the ingestion of phenolic compounds in order to expect desired health benefits for the human body, namely in terms of antioxidant activity.

Keywords: Physalis peruviana L.; extraction; phenolic compounds; bioaccessibility; antioxidant activity

Introduction

Physalis peruviana L. is a plant of the Solanaceae family, native of South America (region of the Andes). However, presently it is spread over a wide variety of countries, not only in south and central America, but also in Europe, in countries such as Portugal, for example (Oliveira et al., 2016; Bernal et al., 2016).

Besides being highly appreciated for their unique characteristics, like flavour, texture and colour, recent research has shown that *P. peruviana* fruits are pretty rich in many beneficial compounds. They are particularly rich in provitamin A and ascorbic acid, as well as some vitamins of the B complex (thiamine, niacin, and vitamin B_{12}). Moreover, they are also rich in essential fatty acids, crude protein (with contents exceptionally high for a fruit) and minerals like phosphorous or iron (Hassanien, 2011; Oliveira et al., 2016).

Some health benefits have been attributed to *P. peruviana*, such as purify blood, decrease the albumin in kidneys, reconstruct and fortify the optic nerve, alleviate throat infections, eliminate intestinal parasites or treat prostate problems. The fruit can also be used for the prevention and treatment of pterygia, or as an expectorant, diuretic, antibacterial, anti-inflammatory and anthelmintic agent. Furthermore, it is reported as beneficial for the treatment of diabetes, albuminuria and pertussis, as well as to strengthen teeth and prevent tooth decay. Besides, it has shown some cytotoxic and immunomodulatory effects (Rey et al., 2015; Sang-ngern et al., 2016, p.; Yang et al., 2016; Bernal et al., 2016).

Many of the health effects associated to foods are due to the presence of certain compounds that have bioactive properties when in the human body. These phytochemicals are responsible for antioxidant properties by eliminating reactive oxygen

species. Some of these families of compounds include phenolic compounds. Among these, phenolic acids, flavones, flavonols, flavanones, ortho-diphenols and anthocyanins constitute major bioactive substances with proven benefits for human health (Hellinger et al., 2014; Peixoto et al., 2018; Zheng et al., 2019). Thus, it is of prime importance to evaluate the presence of such compounds in foods, that act as their dietary sources, and in what way these substances are bioaccessible when going through the gastrointestinal system, since only through absorption they become bioavailable to act as health enhancers.

The ortho-diphenols have been reported as particularly active against free radicals, and can be found in fruits, such as for example olives (Soufi et al., 2014; Dossi et al., 2017), although there are no studies about their quantification in other products. Even though there are some studies about *P. peruviana*, the information on the scientific literature is still scarce, and therefore this study was undertaken to evaluate some physical-chemical characteristics of *P. periviana* produced in Portugal, as well as phenolic compounds, including ortho-diphenols, and their antioxidant activity. Furthermore, the bioaccessibility of those phenols was studied using an *in vitro* model of the gastrointestinal system. To our knowledge no information was published up to the present regarding the bioaccessibility of the phenolic compounds or antioxidant activity of *P. peruviana.*

Experimental procedure

Sampling

The fruits were harvested from one farm located in the North-Centre region of Portugal. Approximately 750 g of berries were collected, being selected randomly from several plants in different parts of the plantation. Then, the samples were transported to the

laboratory in appropriate plastic cuvettes, protected from light and under refrigeration. In the laboratory, the berries were kept is the refrigerator at a temperature of about 4 ºC and at 85 to 90% relative humidity, until the moment when they were analysed. Some fruits were also lyophilized for later analysis. For this, the samples were previously frozen in a conventional freezer (temperature of about -18 to -21 ºC) and then lyophilized in a Freeze Dryer TDF 5505 (Uniequip, Germany). The frozen samples were left in the freeze drier for 96 hours at a temperature of about -50 ºC and a pressure of 0.7 Pa. After lyophilization, the samples were kept in sealed containers and away from light until utilization.

Analysis of the physical-chemical properties

Moisture content was determined by drying until constant weight, using a Halogen Moisture Analyser HG53 (Mettler Toledo) at 120 ºC and medium drying rate (3 on a scale from 1 = very fast to 5 = very slow). Crude fibre was determined by adaptation of the method described by Patarra et a. (2010). The sample was submitted to acidic digestion with sulphuric acid 1.25%(v/v) followed by alkaline digestion with sodium hydroxide 1.25%(v/v), using a Dosi Fiber Selecta. Total and reducing sugars were evaluated according to the Luff-Schoorl technique, following the Portuguese standard NP-1420. Acidity was determined by titration with NaOH 0.025N following, the Portuguese standard NP-1421, with sample preparation according to Portuguese standard NP-783. Total soluble solids were determined in ºBrix by refractometry (refractometer ATAGO 3T) with temperature correction. Ascorbic acid (AA) was evaluated according to Ribeiro (2012) by titration with 2,6-dicloroindofenol, using a calibration curve obtained for concentrations between 0.2 and 1.0 mg AA. For determination of carotenoids was used the spectrophotometric method described by Carvalho et al. (2012), with absorbance read

at 450 nm in a spectrophotometer UV Mini-1240 (Shimadzu, Japan) (Oliveira, 2016). A minimum of three independent replicates was made for all determinations.

Extraction and quantification of phenolic compounds

To optimize the extraction of phenolic compounds, different methods were tested, so as to better compare procedures and select the most appropriate conditions that allowed the highest extraction of these compounds from the grounded solid matrix of physalis. The extraction procedure was adapted from Oliveira et al. (2011). The ratio mass:solution was 1:20 (g/mL) and three different methods were tested, with different combinations of extraction solvents:

- Method A: three sequential extractions made with acetone:methanol (70:30, v:v), methanol:water (70:30, v:v) and methanol:water:acetic acid (50:40:10, v:v:v).
- Method B: two sequential extractions made with methanol:water (70:30, v:v) and acetone:methanol (70:30, v:v).
- Method C: two sequential extractions made with methanol (100%).

In all methods the different solvent combinations were applied sequentially over one sample. Method A: in a 50 mL Erlenmeyer was placed 1 g of the freeze-dried sample, and then 20 mL of the first extracting solution (acetone:methanol) was added and the Erlenmeyer was left to rest for 45 minutes at 58 ° C. The solution was then filtered in a kitasate with a vacuum pump. The retained material was collected and transferred to another 50 mL Erlenmeyer flask where 20 mL of the second extraction solution (methanol:water) was also added and the Erlenmeyer was left again for 45 minutes at 58 ° C. The solution was again filtered into a kitasate using vacuum. This procedure was

finally repeated for the third extracting solution (methanol:water:acetic acid) as described for the first two.

Method B: this method was designed considering the results obtained with method A. Hence, one g of lyophilized sample was weighed and two sequential extractions were carried out: first extraction with methanol:water and a second with acetone:methanol. The experimental procedure was the same as described in Method A, only changing the extracting solutions and number of consecutive extractions performed over the same sample.

Method C: two sequential extractions were performed using methanol (100%), following the same procedure as described earlier for methods A and B.

For each method two assays were made with independent samples, following the exact same procedure and solvent combinations. The extracts obtained were used to quantify the total phenolic compounds, ortho-diphenols and flavonoids by spectrophotometric methods.

The total phenolic compounds (TPC) were determined by means of the Folin–Ciocalteu reagent, using gallic acid as standard (Guiné et al., 2015). To a tube were added 125 μL of diluted sample, 750 μL of distilled water and 125 μL Folin-Ciocalteu reagent, and the mixture was left to rest for 6 minutes. After that, were added 2 mL of 5 % sodium carbonate solution and it was left to rest for 60 minutes at room temperature in the dark (Santos et al., 2014). All measurements were performed in triplicate and the results of the readings, made in a spectrophotometer (model UV Mini-1240 from Shimadzu, Japan) at 760 nm, were expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of sample mass.

The quantification of ortho-diphenols (ODP) was based on the method described by Santos et al. (2014), whereby colorimetric evaluation was made though the

complexation of the ODP with molibdate ions, originating a coloured solution (orange). To a tube were added 0.5 mL of diluted sample and 1 mL of sodium molibdate solution (5 % (w/v): 5 grams to a 50% (v:v) methanol aqueous solution), being then agitated and left at room temperature for 15 min. The absorbance was measured at 370 nm. Gallic acid was used as standard to prepare a calibration curve and the ODP content was expressed as mg GAE/100 g.

For the determination of flavonoid (FLV) content was followed the method by Meda et al. (2005). To a tube was added 0.5 mL of extract diluted in a methanol solution of aluminium chloride at a concentration of 2%, which was then agitated and felt in the dark for 10 min before reading the absorbance at 330 nm. The calibration curve was obtained with quercetin at different concentrations up to 0.06 mg/L, and the results were expressed as mg quercetin equivalents (QE) per 100 g of sample.

Three replicates were made for the determination of TPC, ODP and FLV in each of the extracts analysed.

Evaluation of antioxidant activity

The antioxidant activity (AOA) was determined by two methods, using the free radicals DPPH* (2,2-Diphenyl-1-picrylhydrazyl) and $ABTS^{+}$ (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid). The results were based on the percentage of inhibition, compared with a standard antioxidant (Trolox) in a dose–response curve, being expressed as μmol Trolox equivalents (TE) per gram of sample.

The first method is based on the capture of the DPPH radical by the oxidants, reducing the absorbance due to transfer of electrons. The DPPH solution used had an absorbance of 0.700. To a tube were added 0.1 mL of sample and 2 mL of DPPH solution previously

prepared and then the flask was placed in a dark place at room temperature for 30 minutes. A blank was used by replacing the sample by 0.1 mL of distilled water. The absorbance was measured at a wavelength of 515 nm (Guiné et al., 2014).

The second method is based on the abilities of different substances to scavenge the $ABTS^+$ radical compared with the standard antioxidant Trolox. For the assay, $ABTS^+$ radical was prepared by mixing an $ABTS^+$ stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture was allowed to stand for 12–16 h at room temperature in the dark until a stable oxidative state. The $ABTS^+$ solution (1 mL) was diluted in 80 mL of ethanol or buffer solution prior to utilization. In a tube were placed 2 mL of $ABTS^+$ solution with 0.1 mL of sample and after agitation it was left to rest in the dark for 15 minutes. Then, the absorbance was measured at 734 nm to assess the percentage of inhibition, using the calibration curve obtained for concentrations up to 0.4 mg TE/L (Santos et al., 2014; Guiné et al., 2014).

The analyses for antioxidant activity were performed in triplicate with both methods for each of the extracts analysed.

In vitro simulation of the digestive tract for evaluation of bioaccessibility

To perform the *in vitro* simulation of the different stages of the digestive system was used the model proposed by McDougall et al. (2005). Several solutions were necessary for the experimental procedure, namely: sodium chloride 1% (w/v), saline solution α-amylase 1% (w/v) at pH 6.5, saline solution of pepsin 1% (w/v) at pH 2, brine pancreatin 0.3% (w/v) at pH 7 and a solution of bile salts 1% (w/v) at pH 7.

The *in vitro* model assumes the simulation of the conditions along the digestive tract system, starting in the mouth, then the stomach and finally in the intestine. Simulation of the mouth: in a tube were added 2 mL of sample and 2 mL of α-amylase

solution, which was then allowed to react for 2 minutes at 37 °C. Stomach: in a tube tube were added 2 mL of the above solution and 2 mL of pepsin solution and allowed to react for 2 hours at 37 °C. Intestine: to a tube was added 2 ml of the above solution and 1 ml of pancreatin solution and 1 mL of bile salts solution and allowed to react for 2 hours at 37 °C.

Two control assays were conducted with the same simulated conditions. In the first the enzymes were replaced by a sodium chloride solution as follows: NaCl pH 6.5 instead of alpha-amilase, NaCl pH 2 instead of pepsin and NaCl pH 7 instead of pancreatin and bile salts. In the second were used the same original conditions and only the sample was replaced by gallic acid. At the end of each step were evaluated the TPC content and the antioxidant activity by ABTS assay.

Statistical treatment

All values are presented as mean and corresponding standard deviation based on the different replicas made. Linear regression with the least squares method was used to fit the calibration curves.

The results for the phenolic composition, antioxidant activity and bioaccessibility were subjected to statistical analysis using the SPSS software, version 22, and comparisons between groups for all parameters were tested by Kruskal Wallis tet (KW) and U Mann Whitney (UMW) tests, still being used ANOVA with Tukey test for multiple comparisons, depending on the case and type of variables. In all tests, the significance level considered was 5% ($p < 0.05$).

Results and discussion

Chemical composition

Table 1 shows the results obtained for the physical-chemical analyses. The berries had a moisture content of about 83%, being similar to other values reported in literature for physalis fruits (Ramadan, 2011; Vega-Gálvez et al., 2014; Luchese et al., 2015). Crude fibre was found in a concentration of 4.5% (wet basis) while Vega-Gálvez et al.(2014) reported higher values, 6.3%, and Valdivia-Mares et al. (2016) reported substantially lower values also for *P. peruviana*, between 0.15 and 1.35 %. Total sugars and reducing sugars were 8.79 and 8.03%, indicating that the great majority of the sugars present were reducing sugars. Both the total and reducing sugars were slightly higher than the values encountered by and Oliveira et al. (2011) for *P. angulata*: 6.45% and 4.12%, respectively. Total soluble solids were found to be 13.60 ºBrix, corresponding to 13.6 g sucrose/100 g solution. Valdivia-Mares et al. (2016) reported TSS for *P. peruviana* varying from 11.60 to 15.30 g/100g, Licodiedoff et al. (2013) for *P. peruviana* varying from 14.28 to 14.50 g/100g and Oliveira et al. (2011) for *P. angulata* found values of TSS of 12.00 º Brix. Titrated acidity was 1.5%, expressed as citric acid, which was higher than the value reported by Oliveira et al. (2011) for *P. angulata*: 0.68% citric acid, but of the same order of that reported for *P. peruviana* by Licodiedoff et al. (2013), 1.54 – 1.83%. Regarding the carotenoids content, it was about 6 µg/g, which is very similar to the value reported by Oliveira et al. (2011), around 4 µg/g, but inferior to that reported by Ramadan (2011), 1.6 mg/100 g corresponding to 16 µg/g. The vitamin C content was about 27 mg ascorbic acid/100 g, being very similar to that reported by Oliveira et al. (2011), 25 mg/100 g, but lower than the value found by Ramadan (2011), 43 mg/100 g. On the other hand, the study by Licodiedoff et al. (2013) for ripe physalis fruits reported a much higher vitamin C content, of 151 – 163 mg/100 g. These results highlight the differences encountered in

the chemical composition of physalis, depending on the variety or even place of cultivation.

Table 1 – Chemical composition of *P. peruviana*.

Component	**Content** (Average ± standard deviation)
Moisture (g/100 g)	83.02 ± 0.94
Crude fibre (g/100 g)	4.61 ± 0.60
Total sugars (g/100 g)	8.79 ± 0.63
Reducing sugars (g/100 g)	8.03 ± 0.50
Total soluble solids, TSS (°Brix)	13.60 ± 0.34
Vitamin C (mg ascorbic acid /100 g)	26.70 ± 2.12
Carotenoids (µg/g)	5.95 ± 1.23
Titratable acidity (% citric acid)	1.25 ± 0.03

Phenolic compounds

Total phenolic compounds

To determine the content of phenolic compounds different extracts were used, being obtained through different methods/extracting solutions, as described earlier. Method A allowed obtaining three extracts: the 1st in a solution acetone:methanol (1-AcMe), the 2nd in methanol:water (2-MeWa) and the 3rd in methanol:water:acetic acid (3-MeWaAa); method B gave palce to two extracts: the 1st in methanol:water (1-MeWa) and the 2nd in acetone:methanol (2-AcMe); and method C originated also two extracts, both with

methanol (1-Me and 2-Me). This last method was applied to the fresh sample as well as the lyophilized one.

For validation of results, two independent replicates of all extraction processes were made for each method. Since there were no statistically significant differences (U Mann Whitney test, $p = 0.500$), the results of the three analyses based on each of the two samples were gathered, allowing to calculate a mean value and the corresponding standard deviation based on 6 observations, these values being presented in Table 2.

Table 2 shows the percentage of extraction of phenolic compounds obtained for each of the extracts considering the different methods. The results indicate that the extracting solution methanol:water allowed the highest recovery of phenolics in methods A and B, regardless of the order in which was used (2^{nd} for method A and 1rst for methods B). Nevertheless, when it was used as first, its extracting capacity was greatly increased, 86% against 53%. Furthermore, for method C, the first extraction was considerably more effective in extracting the phenolic compounds for both types of sample, fresh or lyophilized. Regarding method C, it was found that there were statistically significant differences (U Mann Whitney test, $p = 0.220$) with respect to the content of phenolic compounds when the method was used for the fresh or lyophilized samples. Nevertheless, in the case of the fresh sample it was observed a trend for increased extraction of these compounds, producing a total average of 53.4 mg GAE/100 g in comparison with the lyophilized sample, 42.7 mg GAE/100 g. This may result from the fact that in the lyophilized sample structural changes occurred, possibly not allowing such an efficient extraction of the phenolic compounds. Also some loss of phenolics could occur during the sublimation process underlying the lyophilization operation.

Table 2 – Total phenolic compounds content in different extracts of *P. peruviana*.

Sample	**Extraction method**	**Extract[1]**	**Percentage recovery** (%)	**Total phenolic compounds** (mg GAE/100 g)	**Global TPC** (mg GAE/100 g)
Lyophilized	A	1-AcMe	42.4	25.4 ± 2.9	59.9
		2-MeWa	52.7	31.6 ± 1.5	
		3-MeWaAa	4.9	2.9 ± 2.8	
	B	1-MeWa	86.0	46.3 ± 2.3	53.9
		2-AcMe	14.0	7.5 ± 6.5	
	C	1-Me	95.5	40.8 ± 1.7	42.7
		2-Me	4.5	1.9 ± 0.8	
Fresh	C	1-Me	87.5	46.8 ± 7.8	53.4
		2-Me	12.5	6.7 ± 3.3	

[1]Extract: order-solvents (Ac = acetone, Me = methanol, Wa = water, Aa = acetic acid)

Considering the different extracting solutions applied to the same sample, i.e., lyophilized, there were statistically significant differences (Kruskal-Wallis test, $p < 0.001$). The methanol:water solution extracted the greater amount of phenolic compounds (average of 46.3 mg GAE/100 g for method B and 31.6 mg GAE/100 g for method A). The second best extracting solution was found to be methanol (40.8 mg GAE/100 g) followed by the solution acetone:methanol (average of 25.4 mg GAE/100g and 7.5 mg GAE/100 g, for methods A and B, respectively).

Finally the solution methanol:water:acetic acid extracted the lowest amount of phenolic compounds, with an average of 2.9 mg GAE/100 g. However, it could be seen that not only the type of solution influenced the efficacy of the extraction but also the order in which the extracting solution was applied. In this way, the efficiency of extraction varied according to the order, having in mind that when successive extractions were performed, the sample had successively fewer compounds available to extract. In fact, it could be seen that there were statistically significant differences (Kruskal-Wallis test, p

<0.001) in the composition of the various extracts obtained in different orders for each of the applied methods. In this way, the extraction of phenolic compounds tended to decrease with increasing order, so that the last extraction consistently recovered a lower amount of phenolic compounds.

Comparing the different methods as to the overall efficiency of phenolic compounds' recovery (corresponding to the sum of the quantified amounts in the different extracts), method A was the best, allowing obtaining 59.9 mg GAE/100 g, followed by method B, with 53.9 mg GAE/100 g, and finally method C, 42.7 mg GAE/100 g, all these considering the same type of sample, i.e. lyophilyzed. However, it should be noted that these apparent differences were not statistically significant (Kruskal Wallis, $p = 0.381$).

The phenolic compounds' contents quantified in this study are within the range of those reported in literature for *P. peruviana*, namely: 40.5 mg GAE/100 g (Puente et al., 2011), 47.8 mg GAE/100 g for aqueous extract and 57.9 mg GAE/100 g for methanol extract (Rockenbach et al., 2008); and 49.8 mg GAE/100 g [212.84 mg GAE/100 g d.m., moisture = 78.61%] (Vega-Gálvez et al., 2014).

Ortho-diphenols

Table 3 shows the quantification of ortho-diphenols obtained for each of the extracts. It is possible to observe that for methods A and B 100% of ortho-diphenols were obtained using the extracting solution methanol:water, regardless of whether it was the 1st or the 2nd extraction. For method C, it can be seen that when the lyophilized sample was used the highest extraction of these compounds occurred in the 1st extraction, with about 82% of the total extracted. However, when the same method was used for the fresh sample, it was found that the 2nd extraction was the more efficient (77%). As described above, for each method were performed two independent replicates of all extraction processes, and

since there were no statistically significant differences between the levels of quantified ortho-diphenols in the two replications (Mann Whitney U test, $p = 0.323$), the results of the different analyses based on each of the two samples were used to calculate the mean and standard deviation based on 6 observations, values shown in Table 3.

The results presented in Table 3 show that for methods A and B the ortho-diphenols were quantified only when the solvent combination methanol:water was used, regardless of the order. In fact, these compounds could not be quantified with the other solutions (acetone: methanol and methanol:water:acetic acid) as precipitation occurred when they became in contact with the sodium molybdate, which may have occurred due to some side-reactions. The results further show that even though using the same extracting solution, the amount of recovered compounds could be different depending on the order, so that the 1st extraction had higher concentrations when compared to the 2nd (27.5 and 14.1 mg GAE/100 g, respectively).

With regards to method C, a higher extraction was obtained from the lyophilized sample, a total average of 94.6 mg GAE/100 g, as opposed to when the fresh sample was used (90.9 mg GAE/100 g). However, these differences were not statistically significant (Mann Whitney U test, $p = 0.280$).

Considering the extraction solutions used, statistically significant differences were found (Kruskal Wallis test, $p = 0.015$). Contrarily, there were no statistically significant differences (Kruskal-Wallis test, $p = 0.103$) with respect to the order in which the different extracting solutions are used. Comparing all methods used, there were significant differences (Kruskal-Wallis test, $p = 0.003$) in the total content of ortho-diphenols (obtained by summing the quantified levels in the various extracts). Method C allowed extracting a greater amount of compounds (94.6 mg GAE/100 g) while the method which extracted the smaller quantity (on average 14.1 mg GAE/100 g) was

method A. These results demonstrate that method C was the most efficient for the extraction of such compounds, and furthermore with only two extractions, thus saving time and reagents.

No reported values for the content of ortho-diphenols in physalis were found in the literature, and because they are reported as highly antioxidant (Gouveia et al., 2003), their presence in physalis is encouraging.

Table 3 – Ortho-diphenols content in different extracts of *P. peruviana*.

Sample	**Extraction method**	**Extract**[1]	**Percentage recovery** (%)	**Ortho-diphenols** (mg GAE/100 g)	**Global ODP** (mg GAE/100 g)
Lyophilized	A	1-AcMe	0	n.d.	14.1
		2-MeWa	100	14.1 ± 1.9	
		3-MeWaAa	0	n.d.	
	B	1-MeWa	100	27.5 ± 2.8	27.5
		2-AcMe	0	n.d	
	C	1-Me	82.3	77.9 ± 12.5	94.6
		2-Me	17.7	16.7 ± 0.9	
Fresh	C	1-Me	23.1	21.0 ± 8.1	90.9
		2-Me	76.9	69.8 ± 10.6	

[1]Extract: order-solvents (Ac = acetone, Me = methanol, Wa = water, Aa = acetic acid)
n.d. = not detected

Flavonoids

The results in Table 4 show that for method A there was a very similar extraction capacity when the 1st and 2nd solutions were used: 42% and 44% of the total extracted for 1-AcMe and 2-MeWa, respectively, and the third extraction (3-MeWaAa) represented a smaller

fraction of only 14%. In comparison, for method B the 1-MeWa solution yielded 55% of flavonoids, which suggests that the same solution used in different stages produces different results. For method C is appears that in the first extraction the flavonoid recovery was much greater, regardless of the physical state of the sample, 80% of the total extracted from lyophilized sample and 75% from the fresh sample.

Once again it no statistically significant differences were found with respect to flavonoid contents considering the independent extractions (Mann Whitney U test, $p = 0.052$), and therefore the results were joined as explained before for other compounds, being shown in Table 4.

There were no statistically significant differences (Mann Whitney U test, $p = 0.072$) in the values quantified by Method C when the lyophilized or fresh samples were used (0.184 and 0.186 mg QE/100 g, respectively). However, statistically significant differences were found (Kruskal-Wallis test, $p < 0.001$) with respect to the use of various extracting solutions, so that the 1-MeWa solution showed the highest value, 0.167 mg QE/100 g, contrarily to 2-Me which corresponded to the lowest concentrations (0.037 and 0.046, respectively for the lyophilized and fresh samples). There were statistically significant differences (Kruskal-Wallis test, $p < 0.001$) with respect to the order in which the extracting solutions were used, the first extraction corresponding to higher concentrations and so on. Comparing all methods, there were statistically significant differences (Kruskal-Wallis test, $p < 0.001$) in the total flavonoids content (obtained for each method by adding the quantified levels in the various extracts). Methods A and B allowed to extract a greater amount of these compounds (0.340 and 0.301 mg QE/100 g), as compared to method C (0.184 mg QE/100 g). Again, because no values of total flavonoids were found in the literature for physalis, our results couldn't be compared.

Table 4 – Flavonoids content in different extracts of *P. peruviana*.

Sample	**Extraction method**	**Extract**[1]	**Percentage recovery** (%)	**Flavonoids** (mg QE/100 g)	**Global FLV** (mg QE/100 g)
Lyophilized	A	1-AcMe	42.1	0.143 ± 0.008	0.340
		2-MeWa	43.5	0.148 ± 0.009	
		3-MeWaAa	14.4	0.049 ± 0.007	
	B	1-MeWa	55.5	0.167 ± 0.005	0.301
		2-AcMe	44.5	0.134 ± 0.060	
	C	1-Me	79.9	0.147 ± 0.006	0.184
		2-Me	20.1	0.037 ± 0.006	
Fresh	C	1-Me	75.3	0.140 ± 0.012	0.186
		2-Me	24.7	0.046 ± 0.016	

[1]Extract: order-solvents (Ac = acetone, Me = methanol, Wa = water, Aa = acetic acid)

n.d. = not detected

Antioxidant activity

DPPH antioxidant activity

The results in Table 5 show that for method A was obtained a higher antioxidant activity with 2-MeWa solution, 49%, and this solution also allowed the highest value for method B (89%), although the order of extraction was different. With respect to method C, it appears that, regardless of the state of the sample, a substantially greater antioxidant activity was quantified in the 1st extraction (88% and 82%, respectively for the lyophilized and fresh samples).

Once more there were no statistically significant differences between the independent extractions (Mann Whitney U test, $p = 0.749$), so the results were gathered and are presented in Table 5. The results obtained for method C showed that no

statistically significant differences existed in the antioxidant activity quantified by DPPH method (Mann Whitney U, p = 0.344) considering the use of fresh or freeze-dried samples (8.77 and 9.30 μmol TE/g, respectively).

Table 5 – DPPH antioxidant activity in different extracts of *P. peruviana*.

Sample	**Extraction method**	**Extract**[1]	**Percentage** (%)	**Antioxidant activity** (μmol TE/g)	**Global AOA** (μmol TE/g)
Lyophilized	A	1-AcMe	39.5	3.79 ± 0.40	9.61
		2-MeWa	48.5	4.66 ± 0.76	
		3-MeWaAa	12.0	1.15 ± 0.82	
	B	1-MeWa	89.1	6.89 ± 0.49	7.73
		2-AcMe	10.9	0.84 ± 0.23	
	C	1-Me	87.7	8.16 ± 0.53	9.30
		2-Me	12.3	1.14 ± 0.25	
Fresh	C	1-Me	82.5	7.24 ± 0.40	8.77
		2-Me	17.5	1.54 ± 0.48	

[1]Extract: order-solvents (Ac = acetone, Me = methanol, Wa = water, Aa = acetic acid)

Regarding the AOA quantified in the different extracts, there were statistically significant differences (Kruskal Wallis p <0.001). With regards to the order of the extractions, there were also statistically significant differences (Kruskal Wallis p <0.001), being again the first extract the one with greater AOA. When comparing the extraction methods, it was found that there were no statistically significant differences (Kruskal Wallis p = 0.151) in the total antioxidant activity obtained by summing the AOA quantified in the different extracts. Still, it was observed that method A seemed to result in higher values of AOA, with an average of 9.61 μmol TE/g.

The values obtained for AOA in this work prove to be considerably higher than the values reported in literature for antioxidant activity when determined by the same method in *P. peruviana* by Puente et al. (2011), who reported values in the range 1.92 – 2.11 µmol TE/g or by Vega-Gálvez et al. (2014), who presented a value of 94.07 µmol TE/g dry matter, corresponding to 0.22 µmol TE/g of fresh sample (with 78.61% moisture). The differences could be due to different extraction conditions, because as seen in this work the type of solvent and the extraction method influence the AOA, or even due to climatic and soil conditions where the bushes were grown, post-harvest practices or laboratory practices. In fact, according to Lima et al. (2012), the values of antioxidant capacity of plant products are largely influenced by genetic factors, environmental conditions, degree of ripeness of the berries at harvest, variety of the plant, the solvent and the extraction technique.

ABTS antioxidant activity

Equally to what was observed in previous cases regarding the repetitions performed, again no statistically significant differences were encountered (Mann Whitney U test, $p = 0.829$), for the antioxidant activity assessed by ABTS method, and therefore the results were treated considering the six observations and are presented in Table 6. Taking into account the results obtained with Method C, comparing the fresh with the lyophilized samples, it appeared that the fresh sample had a slightly higher AOA (13.71 µmol TE/g) when compared to the fresh sample (12.28 µmol TE/g), however, these differences were not statistically significant (Mann Whitney U test, $p = 0.077$).

Regarding the different extracts, statistically significant differences were observed (Kruskal Wallis $p < 0.001$), being the highest value for 1-MeWa (10.90 µmol TE/g). Comparing the order in which the solutions were used, there were statistically

significant differences (Kruskal-Wallis test, p <0.001), so that the first extract showed the most intense AOA. Comparing all methods, and looking at the lyophilized sample, method A yielded greater AOA (13.07 µmol TE/g). However, this was just a trend not confirmed statistically since there were no significant differences (Kruskal-Wallis test, p = 0.174) in the values obtained with different methods.

Table 6 – ABTS antioxidant activity in different extracts of *P. peruviana*.

Sample	**Extraction method**	**Extract**[1]	**Percentage** (%)	**Antioxidant activity** (µmol TE/g)	**Global AOA** (µmol TE/g)
Lyophilized	A	1-AcMe	28.0	3.65 ± 0.23	13.07
		2-MeWa	65.8	8.61 ± 1.35	
		3-MeWaAa	6.2	0.81 ± 0.09	
	B	1-MeWa	88.1	10.90 ± 1.35	12.38
		2-AcMe	11.9	1.48 ± 0.62	
	C	1-Me	84.6	10.39 ± 1.28	12.28
		2-Me	15.4	1.90 ± 0.43	
Fresh	C	1-Me	78.2	10.71 ± 0.98	13.71
		2-Me	21.8	2.99 ± 0.50	

[1]Extract: order-solvents (Ac = acetone, Me = methanol, Wa = water, Aa = acetic acid)

The values of AOA observed in this study are within the range of those reported in the literature for *P. peruviana* by the ABTS method, being higher than those reported by Licodiedoff (2012) and Licodiedoff et al. (2013), about 8 µmol TE/g, by Lima et al. (2012), 1.5 µmol TE/g or by Vasco et al. (2008), 9 µmol TE/g, but lower than those obtained by Rockenbach et al. (2008), 29-31 µmol TE/g.

Correlations

The data for the AOA obtained by the two experimental methods used (DPPH and ABTS) were well described by a linear function, since the correlation coefficient was high, indicating a strong association between the two variables considered ($R^2 = 0.9687$). Thus, the results obtained by both methods, although numerically different, exhibited a strong connection between them, as expected.

The antioxidant capacity of a food is due to the presence of different compounds with antioxidant properties, including phenolic compounds, carotenoids or ascorbic acid. Since this study evaluated the levels of phenolic compounds, carotenoids and ascorbic acid in the *P. peruviana*, it was interesting to investigate the extent in which these compounds contributed to the AOA. Hence, the relations between the AOA determined by the two methods (DPPH and ABTS) with the content of phenolic compounds, ascorbic acid and carotenoids were analysed, and the correlation coefficients are shown in Table 7. The results indicate that there was a strong positive correlation between phenolic content and DPPH ($R^2 = 0.9687$) and also ABTS ($R^2 = 0.9329$). Furthermore, it was found a strong correlation between ascorbic acid and AOA by both methods ($R^2 = 0.8642$ and $R^2 = 0.9832$, respectively for the DPPH and ABTS). As regards the carotenoids there was also a correlation with AOA ($R^2 = 0.7857$ and $R^2 = 0.7163$, for DPPH and ABTS methods, respectively). Thus, the results confirm that the antioxidant properties of physalis are largely due to the presence of phenolic compounds, ascorbic acid and carotenoids, as it would be expected.

Table 7 – Correlations between antioxidant activity and some types of compounds.

Compounds	**Antioxidant activity**	
	DPPH	**ABTS**

Total phenolic compounds	0,9374	0,9329
Ascorbic acid	0,8642	0,9832
Carotenoids	0,7857	0,7163

Bioaccessibility

Bioaccessibility refers to the proportion of a certain compound that is ingested and which is released from the food matrix and incorporated into micelles in the gastrointestinal tract, being thus available for intestinal absorption, whereas bioavailability refers to the portion of the compound which is in fact absorbed in the body, enters in systemic circulation and becomes available for utilization in normal physiological functions or for storage in the human body (Rodriguez-Amaya, 2015; Saini et al., 2015). In the present work the bioaccessibility of total phenolic compounds and antioxidant activity were evaluated by means of an *in vitro* model simulating the gastrointestinal system.

Bioaccessibility of total phenols

The evaluation of the alterations in the TPC content along the digestive tract was simulated considering an *in vitro* model as described earlier. The results were expressed as a percentage of the amount initially present that was found in the different extracts obtained for *P. peruviana* and are presented in Table 8. The results show that in all extracts analysed a decrease occurred in the phenolic compounds over the different phases of the gastrointestinal tract. This reduction was higher while passing the mouth (with losses between 24% and 34%), whereas passing through the stomach apparently produced a smaller decrease (losses between 1% and 15%).

Comparing the different extracts as to the overall effect (Table 8) it was observed that the 1-AcMe extract with the lyophilized sample allowed maintaining a higher

proportion of phenolic compound up to the end, being therefore potentially available for absorption in the intestine 50% of the total amount initially present. In all other extracts the percentage of phenolic compounds available in the intestine for absorption was smaller, only about 40% of the initial amount. Thus, the 1-AcMe extract, with solvents acetone and methanol, appears as more feasible to allow the preservation of phenolics along the digestive tract.

Table 8 – Total phenolic compounds along the digestive tract.

Sample	Extraction method	Extract[1]	Total phenolic compounds (%)			
			Initial	Mouth	Stomach	Intestine
Lyophilized	A	1-AcMe	100 ± 20	76 ± 15	75 ± 11	50 ± 10
		2-MeWa	100 ± 28	66 ± 20	64 ± 15	41 ± 9
		3-MeWaAa	n.e.	n.e.	n.e.	n.e.
	B	1-MeWa	100 ± 16	69 ± 7	58 ± 7	40 ± 6
		2-AcMe	n.e.	n.e.	n.e.	n.e.
	C	1-Me	100 ± 20	70 ± 12	55 ± 8	40 ± 13
		2-Me	n.e.	n.e.	n.e.	n.e.
Fresh	C	1-Me	100 ± 24	67 ± 14	61 ± 13	41 ± 10
		2-Me	n.e.	n.e.	n.e.	n.e.
Average among all extracts analysed[2]			**100[a]**	**70[b]**	**63[b]**	**43[c]**

[1]Extract: order-solvents (Ac = acetone, Me = methanol, Wa = water, Aa = acetic acid)

[2]Values with the same letter are not statistically different at the level of significance of 5%.

n.e. = not evaluated

To determine the significance of differences in the multiple stages along the gastrointestinal tract ANOVA was applied to the results, considering mean values for the various extracts evaluated. The results showed that significant differences existed between the different stages considered ($F = 92.543$; $p = 0.000$). It was found that the content of phenolic compounds decreased from 100% at the start to only 43%, across the whole digestive tract. The multiple comparisons test Tukey (post-hoc) showed significant differences from the beginning to the mouth and from the stomach into the intestine, however, the differences from the mouth to the stomach were not significant. According to Vega-Gálvez, et al. (2016), the bioavailability of phenolic compounds can be affected

by binding microstructure of these compounds into the matrix of the foods, which is also directly related to the processing of fruit.

Bioaccessibility of antioxidant activity

The change in antioxidant activity as evaluated by the ABTS method considering the *in vitro* simulation of the gastrointestinal tract, for the various extracts is presented in Table 9. It can be seen that there is a decrease in antioxidant activity throughout the digestive tract, and this is higher from the beginning to the mouth, similarly to what was previously observed for the concentration of total phenols. It can be confirmed also that the 1-AcMe extract using the lyophilized sample was that which maintained a higher percentage of antioxidant activity, 34% of the total, while the 1-MeWa extract was the one that originated a higher loss in the antioxidant activity (77% loss) but very close to the other extracts (varying between 76% and 75%). It was confirmed that the extract which allowed the best preservation of the phenolic compounds was the one that also preserved the antioxidant activity.

Table 9 – ABTS Antioxidant activity along the digestive tract.

Sample	Extraction method	Extract[1]	Antioxidant activity (%)			
			Initial	**Mouth**	**Stomach**	**Intestine**
Lyophilized	A	1-AcMe	100 ± 21	41 ± 17	40 ± 13	34 ± 20
		2-MeWa	100 ± 11	46 ± 14	39 ± 10	24 ± 4
		3-MeWaAa	n.e.	n.e.	n.e.	n.e.
	B	1-MeWa	100 ± 14	45 ± 8	31 ± 7	23 ± 3
		2-AcMe	n.e.	n.e.	n.e.	n.e.
	C	1-Me	100 ± 21	45 ± 8	29 ± 9	25 ± 4
		2-Me	n.e.	n.e.	n.e.	n.e.
Fresh	C	1-Me	100 ± 16	63 ± 20	39 ± 14	24 ± 8
		2-Me	n.e.	n.e.	n.e.	n.e.
Average among all extracts analysed[2]			**100[a]**	**48[b]**	**36[c]**	**26[d]**

[1]Extract: order-solvents (Ac = acetone, Me = methanol, Wa = water, Aa = acetic acid)

[2]Values with the same letter are not statistically different at the level of significance of 5%.

n.e. = not evaluated

An ANOVA was performed to ascertain the statistical differences in antioxidant activity at various stages throughout the gastrointestinal tract. The results showed significant differences ($F = 187.093$; $P = 0.000$), and the antioxidant activity decreased from 100% to only 26% when reaching the intestine, this corresponding to only about ¼ being available in the intestine. The multiple comparisons test Tukey (post-hoc) showed that there were significant differences in all stages along the digestive tract.

The obtained results are relevant because the potential functional value of physalis is strongly determined by its phenolic content. In fact, only a percentage of their phenolic compounds can be biologically active in the body, as they have to be absorbed through the gastrointestinal tract and reach the bloodstream (this refers to their bioavailability)

(Ribas-Agustí et al., 2019). Some studies have been performed about the bioavailability of phenolic compounds in different foods, such as for example grapes (Iglesias-Carres et al., 2019), cranberries (Barak et al., 2019), bread (Świeca et al., 2017), millet (Hithamani and Srinivasan, 2017) or tomato (Martínez-Huélamo et al., 2015), but not in physalis up to the present. In those studies, the bioavailability of phenolic compounds was assessed in other plant foods and the changes along the intestinal tract were not reported. Furthermore, by not using the same simulation model used in the present study, it was not possible to compare the percentage of losses of phenolic compounds and antioxidant activity along the digestive system, being these values reported for the first time in the present work.

Conclusions

The results obtained highlighted some important chemical components present in *P. peruviana*, namely fibre, sugar, vitamin C and carotenoids. Regarding the extraction of phenolic compounds, it was observed that the solution methanol:water was the most efficient to recover these compounds from the berries, regardless of the extracting order, and therefore it could be recommended for this kind of extraction. Besides, the extraction methods that used the combination of the solutions methanol:water and acetone:methanol showed a good capacity to extract the phenolic compounds, allowing quantifying phenolics in the range 53.9-59.9 mg GAE/100 g. Also the flavonoids were better extracted with these combinations of extracting solutions, originating concentrations of 0.301-0.340 mg QE/100 g. Contrarily, the recovery of ortho-diphenols was more efficient when methanol was used for the extraction, allowing obtaining 90.9-94.6 mg GAE/100 g. This highlights the relevance of using combined solvents to extract the phenolic compounds form physalis, in order to obtain maximum yield. By using combinations of solvents, the

affinity with the compounds is enlarged because some are of more polar nature while others are more apolar. Also the chemical structure of the functional groups linked to the benzene ring originates differences in the affinity towards the solvents. The results of the *in vitro* simulation of the digestive tract showed that only about 40-50% of the phenolic compounds present in the fruits remained available for intestinal absorption, being the highest reduction observed in the mouth. This indicates that, although physalis demonstrates a very high antioxidant capacity, the dose ingested has to be adapted so as to effectively contain the necessary amounts of bioactive compounds that are expected to bring benefits for the human body, after absorption in the intestine and release into the blood stream.

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