What makes apples that good?

Erasmus + APPLES International Teem[1], 12thB Class 2017[2]

Resumo

Como é do conhecimento geral, as maçãs possuem diversos benefícios para a saúde, devendo ser consumidas regularmente. Neste artigo pretendemos verificar outras propriedades desta fruta, especificamente o seu conteúdo fenólico e potencial antioxidante.

Após a preparação do extrato de uma maçã, homogeneizando a fruta em água e ácido ascórbico seguido do método de centrifugação, foi efetuada a avaliação da presença de compostos fenólicos. De seguida, foi analisado o potencial antioxidante da maçã.

Relativamente ao conteúdo fenólico, comparando os valores da absorvência do sumo de maçã com os valores de referência, foi possível calcular o conteúdo de composto fenólicos, expresso como equivalente do ácido pirogálico.

Concluímos que o sumo de maçã tem fortes propriedades antioxidantes, especialmente se for menos diluído e não sofrer aquecimento. As implicações práticas desta conclusão é que devemos escolher sumos o mais naturais possível.

No entanto, não podemos descurar a importância da pasteurização na conservação dos produtos alimentares. Nesta conformidade, será necessário realizar novos estudos que permitam desenvolver uma técnica que mantenha as propriedades antioxidantes assim como a conservação do produto.

Abstract

As is well known, apples have several health benefits and should be consumed regularly. In this article, we intend to verify other properties of this fruit, specifically its phenolic content and antioxidant potential.

After the preparation of apple extract, homogenization of the fruit in water and ascorbic acid followed by the centrifugation method, the presence of phenolic compounds was evaluated. Then, the antioxidant potential of the apple was analysed.

As far as the phenolic content is concerned, comparing the apple juice absorbance values with the reference values, it was possible to calculate the content of phenolic compounds, expressed as the equivalent of pyrogallic acid.

We conclude that apple juice has strong antioxidant properties, especially if it is less diluted and does not undergo heating. The practical implications of this conclusion are that we should choose juices as natural as possible.

However, we cannot overlook the importance of pasteurization in the preservation of food products. Accordingly, further studies will be required to develop a technique that maintains antioxidant properties as well as preservation of the product.

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Keywords: apple juice, apple pomace, antioxidant activity, phenolic content, health, health conditions, nutrition, essential nutrients, functional food.

Introdution

Apple juice has some unique properties for health, due to the high content of vitamins and polyphenols. These compounds (e.g. quercetin, catechin) have an important antioxidant capacity, which is essential for overall health [1]. Some studies highlight the biological properties of the apple as a functional food that may also help prevent the onset of Alzheimer's [2] and Parkinson's disease [3] as well as decrease the risk of cancer, specially lung cancer.

In this experiment, some biological properties of apples will be addressed with the quantification of phenolic content and evaluation of antioxidant potential of apple juice and apple pomace, solid remains generated after fruit processing, with potential to be an active ingredient in food [4, 5].

The objectives of the project were to identify the antioxidants present in apple juice and to understand its health implications.

In this project, we used, essentially, apple juice and apple extract. All the procedures were performed in the laboratory.

Materials and methods

Preparation of apple extract:

1. Weigh 1 washed apple
2. Add 35 mL of ultrapure water + 5 mL of ascorbic acid 5%
3. Homogenize in a blender for 1 minute
4. Separate into 2 falcon tubes
5. Heat the 1st tube at 90 ° C for 10 seconds and keep at room temperature
6. Centrifuge at 6000 g for 10 seconds at 4 ° C
7. Transfer the supernatant into new falcon tube
8. Centrifuge at 6000 g for 5 seconds at 4 ° C
9. Collect the supernatant and keep in an ice bath

Evaluation of Phenolic content

1. Identify 5 Eppendorf tubes (1-5) and add 200 µl of ethanol in each
2. Add 200 µl of standard solution of pyrogallic acid in Eppendorf 2 and homogenize
3. Transfer 200 µl of Eppendorf 2 to Eppendorf 3 and homogenize
4. Repeat this procedure to prepare the solutions of Eppendorf 4 and 5
5. Identify 5 new Eppendorf tubes (6-10) and add 200 µl of water in each
6. Prepare dilution 1/2 of apple extract as procedure above
7. In a 96-well microplate, add 235 µl of water and 5 µl of each solution (1-10) in triplicate
8. Add 15 µl of Folin reagent and wait 5 min.
9. Add 45 µl of sodium carbonate and incubate at 40 ° C for 30 minutes
10. Read the absorbance at 630 nm

Evaluation of Antioxidant Activity:

1. Identify 5 Eppendorf tubes (1-5) and add 200 µl of ethanol in each
2. Add 200 µl of quercetin standard solution to Eppendorf 2 and homogenize
3. Transfer 200 µl of Eppendorf 2 to Eppendorf 3 and homogenize
4. Repeat this procedure to prepare the solutions of Eppendorfs 4 and 5
5. Identify 5 new Eppendorf tubes (6-10) and add 200 µl of water in each
6. Prepare dilution 1/2 of apple extract as procedure above.
7. In a 96-well microplate, add 30 µl of each solution (1-10) in triplicate
8. Add 200 µl of DPPH solution
9. Incubate at 40 ° C for 30 minutes in the dark
10. Read the absorption at 517 nm

Results

Preparation of apple juice:

44 g of apple were blended with water and the final volume was 275 ml
Concentration: 160 g of apple / L of juice

Quantification of Total Phenols in apple juice

The quantification of total phenol content was accessed by the Folin-Ciocalteu method using pyrogallic acid (PGA) as standard.

Table 1 – Phenolic content of apple juice

Tube	[pyrogallic Acid] (mg/L)	Absorvance
1	0	0.053
5	62.5	0.091
4	125	0.186
3	250	0.475
2	500	0.921
	Concentration (g apple /L juice)	
6 (water)	0	
7 (pure apple juice)	160	0.793
8 (apple juice dil 1/2)	80	0.355
9 (apple juice dil 1/4)	40	0.176
10 (apple juice dil 1/8)	20	0.129

The calibration curve was determined as showed in Figure 1.

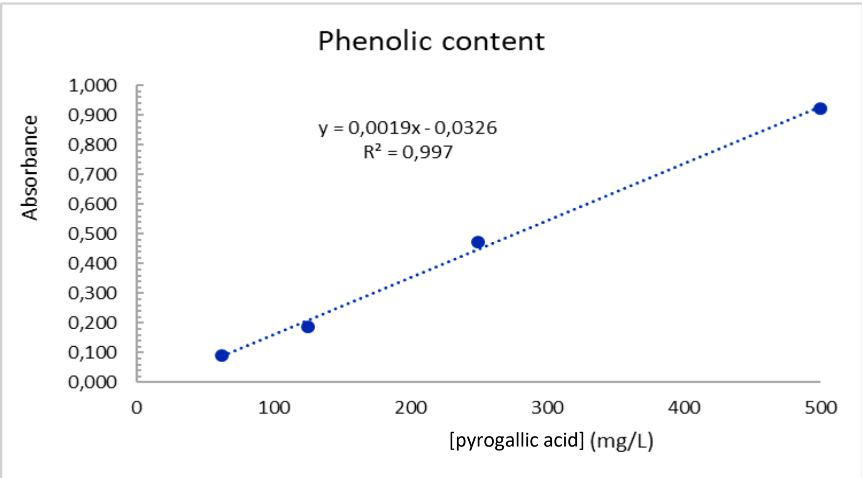

Figure 1 - Total phenolic content expressed in milligrams equivalents of pyrogallic acid (PGA) per gram of each fraction.

Comparing absorbance values of apple juice and standard we can calculate the content in phenol expressed as equivalent of pyrogallic acid (mg pyrogallic acid/g apple). Thus, the apple juice has a high content in phenol compounds equivalent to 2.7 ± 0.1 mg PGA /g apple.

Antioxidant Activity

The antioxidant activity was accessed with the free radical DPPH method using quercetin as standard. The calibration curve (figure 2) was determined according to table 2.

Table 2 – Antioxidant Activity of quercetin and apple juice

Tube	[Quercetin] (mg/L)	log [Quercetin]	Antioxidant Activity
1	0		
**	15.625	1.194	61.3 ± 0.7
**	31.25	1.495	69.2 ± 0.4
5	62.5	1.796	79.6 ± 1.9
4	125	2.097	83.1 ± 1.6
3	**250**	**2.398**	**85.6 ± 1.1**
2	500	2.699	87.1 ± 0.6
	Concentration (g apple /L juice)		
7	0		
8	**160**		**86.4 ± 3.9**
9	80		82.4 ± 2.5
10	40		65.0 ± 2.3
**	20		9.4 ± 1.1

** - these tubes were not used initially but were necessary to add to determine calibration curve.

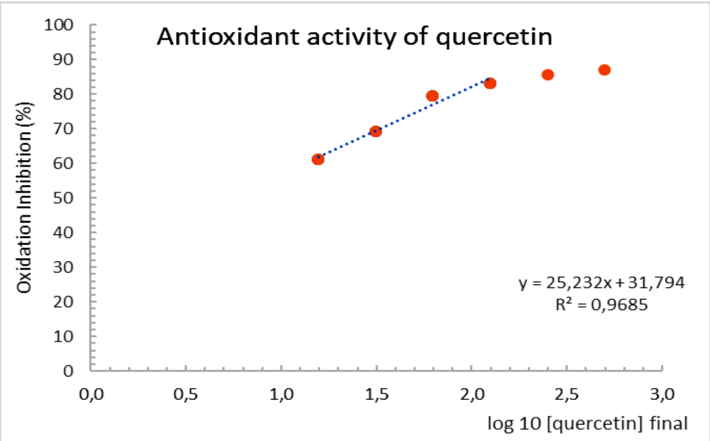

Figure 2 - Calibration curve with standard (quercetin)

The antioxidant activity of apple juice was determined without dilution (160 g/L), and with successive dilutions 1/2 (Table 1 and Figure 2).

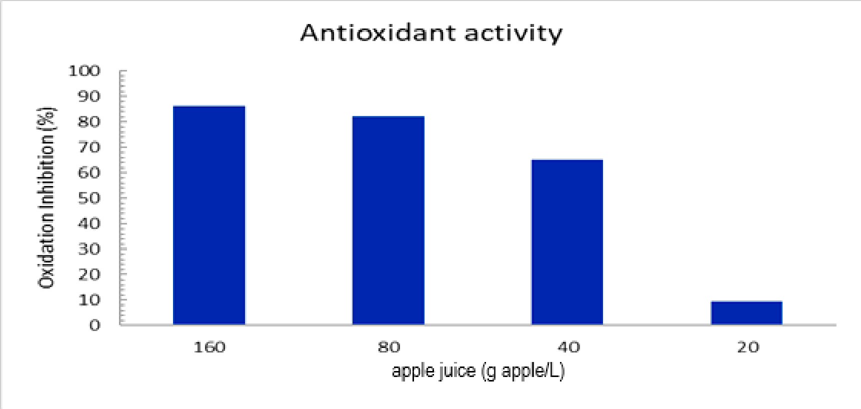

Figure 3 – Antioxidant activity of apple juice

When comparing the activity of juice, we observed that 89 g apple/L of juice were equivalent to 250 mg/L of quercetin (highlighted at table 1).

When using apple juice heated at 90ºC for 10 seconds, a decrease of the antioxidant activity comparing to the apple juice keep at room temperature was noticed (Figure 4).

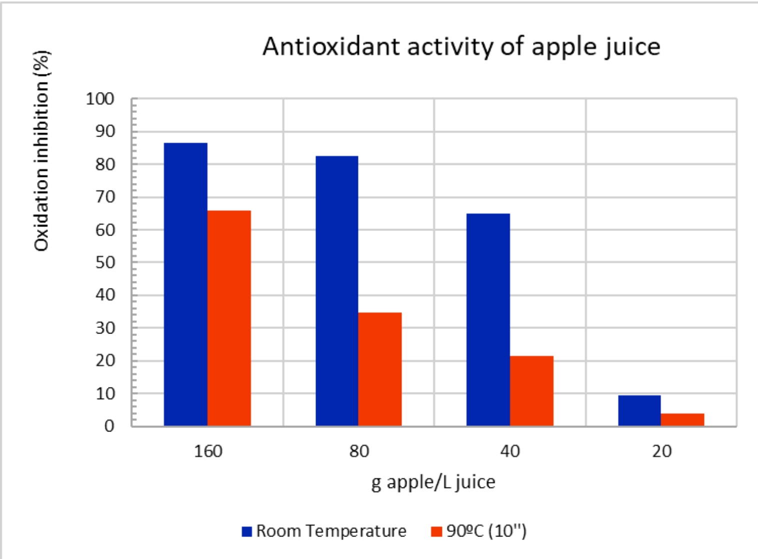

Figure 4 – Antioxidant activity of apple juice when prepared at room temperature or when heated at 90 ºC for 10 seconds.

Discussion

When comparing the absorbance values of apple juice and standard (pyrogallic acid), we can calculate the phenolic content expressed as equivalent in pyrogallic acid (mg pyrogallic acid / g apple). Thus, we confirm that apple juice has a high content of phenolic compounds, equivalent to 2.7 ± 0.1 mg PGA / g apple.

Since polyphenols are antioxidants naturally present in fruit and other foods, it is not surprising that the results have a high antioxidant capacity in apple juice, as it was rich in phenols.

It is also worth noting that the antioxidant concentration in apple juice decreases considerably when diluted. For this reason, if we choose juice with low concentration of apple extract, its antioxidant capacity may be compromised, not having the beneficial effects we would expect. However, natural juices can sometimes be more caloric due to the fruit sugars.

On the other hand, when comparing the results of the apple extract that was heated with those maintained at room temperature, we noticed that the antioxidant concentration in the juice that was previously heated was much lower. This comparison aimed to simulate the effects of pasteurization on apple juice composition. Although this technique is essential for the preservation of products, the results of our experience show that it degrades the quality of the juice, regarding antioxidants.

For these reasons, our study shows that, supporting common sense, the healthiest option is the consumption of juice as natural as possible, always integrated into a varied and balanced diet.

Conclusion

We can conclude that the higher the concentration of apple juice the greater the amount of polyphenols (antioxidants), therefore it is more advantageous to consume more concentrated juices.

The results obtained were as expected, though some minor errors at the procedures not relevant for the success of the experiment since it was a small-scale study and therefore not very rigorous.

Studies will be needed to prove that "An apple a day keeps the doctor away" proverb and that it is healthier to consume pasteurized juices than to consume juices with all the original antioxidants, since the polyphenols contained in apple juice are susceptible to the temperatures used in the pasteurizing process.

Studies are underway to develop pasteurization methods that preserve polyphenols (e.g. [6]).

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