Journal of Berry Research 8 (2018) 11–23
DOI:10.3233/JBR-170168
IOS Press

Phytochemical characterization, and evaluation of rheological and antioxidant properties of commercially available juices of berries

Ângelo Luís[a], Sónia Sousa[b], Ana Paula Duarte[a], Luísa Pereira[c] and Fernanda Domingues[a,*]
[a]*Centro de Investigação em Ciências da Saúde (CICS-UBI), Universidade da Beira Interior, Covilhã, Portugal*
[b]*Materiais Fibrosos e Tecnologias Ambientais (FibEnTech), Universidade da Beira Interior, Covilhã, Portugal*
[c]*Centro de Matemática e Aplicações (CMA-UBI), Universidade da Beira Interior, Covilhã, Portugal*

Received 8 August 2017; accepted 2 September 2017

Abstract. The consumption of berries and their contribution to improving the human health is a subject of considerable interest, have already resulted in several research projects and numerous clinical trials in humans. The main goal of this study was to evaluate the phytochemical composition (total reducing sugars, total phenolics, flavonoids, and anthocyanins) of some commercially-available juices of berries and associating it to their physicochemical (pH, density, and total solids), rheological and antioxidant properties. It was observed that in general, the juices analysed presented great concentrations of polyphenols and anthocyanins; and, in what concerns to their rheological properties, non-Newtonian pseudoplastic fluid characteristics ($n < 1$) were observed. The antioxidant properties of the juices were evaluated using both the DPPH free radical scavenging assay and the β-carotene bleaching test. Overall, the results of the % Inhibition of DPPH free radical by the tested juices indicate that they possess considerable capacity to scavenge free radicals, resulting in good antioxidant properties. This study demonstrated that the physicochemical properties of the juices influence their rheological behavior; and the phytochemical composition impacts their antioxidant properties. Due to their antioxidant capacities, juices of berries could be considered functional foods.

Keywords: Berries, juices, phytochemicals, rheology, antioxidants

1. Introduction

The consumption of berries and their contribution to improving the human health is a subject of considerable interest, have already resulted in several research projects and numerous clinical trials in humans [1–3]. Among all the varieties of berries, the most consumed ones are blackberries, raspberries, blueberries, cranberries, strawberries, and also acai, blackcurrants, chokeberries, elderberries, mulberries and goji berries [1]. In general, berries are considered to be powerful disease-fighting foods, making up the largest proportion of fruit consumed in the human diet [4].

*Corresponding author: Prof. Fernanda Domingues, Centro de Investigação em Ciências da Saúde (CICS-UBI), Universidade da Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal. Tel.: +351 275 319 700; Fax: +351 275 329 183; E-mail: fdomingues@ubi.pt.

1878-5093/18/$35.00 © 2018 – IOS Press and the authors. All rights reserved

Berries provide significant health benefits because of their high levels of bioactive compounds, namely polyphenols, antioxidants, and vitamins [4]. The polyphenols of berries represent a diverse group of compounds including phenolic acids, such as hydroxybenzoic and hydroxycinnamic acids conjugates; and flavonoids, such as flavonols, flavanols, and anthocyanins (fruit colorants) [5]. Many polyphenols, individually or combined, possess high antioxidant properties and are of great interest for nutritionists and food technologists due to the opportunity to use them as ingredients for functional foods [5].

Berries are popularly consumed, not only in their fresh and frozen forms, but also as processed and derived products, including dried and canned fruits, yogurts, beverages or juices, jams, and jellies [4]. Juices of berries have become very popular for people due to the consumer demand for healthy nutraceutical foods that could possibly reduce some health risks and improve various health conditions, in addition to their widespread market availability and low price [6]. Therefore, there is a huge opportunity to study the chemical composition of juices of berries, together with their antioxidant properties. Moreover, in the study of juices, their physicochemical and rheological properties need to be evaluated, since the flow behavior of fruit juices and their fluid derivatives are strongly affected by both juice and fruit characteristics [7].

Thus, the objectives of this study were to evaluate the phytochemical composition of some commercially-available juices of berries associating it with their physicochemical, rheological and antioxidant properties.

2. Material and methods

2.1. Juices

The juices of berries ($n = 25$) were acquired commercially in local supermarkets (Covilhã and Lisbon, Portugal). Table 1 details the characteristics of the juices considered in this study. The juices were maintained in the fridge (4°C) and aliquots were slowly frozen (–20°C). The juices were randomly numbered (from 1 to 25) to simplify their designation throughout the study.

2.2. Physicochemical properties – pH, density, and total solids

A digital pH meter (Metrohm 827 pH Lab, Switzerland) was used to measure the pH values of the juices of berries at 25°C. The density of the juices was measured by weighing samples of 5 mL of each juice at 25°C, and was expressed as g/mL. The total solids were estimated by total evaporation in an air ventilated oven (105°C, 48 h) of aliquots of 5 mL of each juice, being the results expressed in terms of percentage of total solids relatively to the total mass.

2.3. Rheological properties

The rheological behaviour of the juices of berries was studied with a Haake RS150 rheometer (Thermo Fisher Scientific, USA), using a cone/plate geometry of 2º/35 mm, at 25°C, by varying the shear rate from 0 to 1300 s^{-1} in 180 seconds. Apparent viscosity (η_{app}) was determined at 1300 s^{-1}. The shear rate versus shear stress was used to calculate the different rheological parameters (consistency coefficient – k, and flow behaviour index – n) using the Haake software, according to the Ostwald-de-Waele model, also known as the power law model, given by the equation [8, 9]:

$$\tau = k \times \dot{y}^n, \quad (1)$$

where τ is the shear stress (Pa); k is the consistency coefficient (Pa.s^n); $\dot{y}$ is the shear rate (s^{-1}), and n is the flow behaviour index.

Table 1
Characteristics of the juices of berries considered in this study

Juice number	Juice	Principal ingredients
1	Cem Porcento Forma^{+} – Raspberry ketone PLUS	2% of hydrophilic extract of raspberry (*Rubus idaeus*) – minimum ketone content: 17%
2	Cem Porcento Forma^{+} – Super Fruits Energy	3% of dried extract of pomegranate (*Punica granatum*), 2.5% of dried extract of blueberry (*Vaccinium myrtillus*), 1.5% of dried extract of raspberry (*Rubus idaeus*), 1% of liquid extract of grape seed (*Vitis vinifera*)
3	Optima super fruit juice Acai	Juice of acai berries (*Euterpe oleracea*), mixture of grapes, lycopene, and resveratrol
4	Vitalbio – Apple, Raspberry, Blueberry, 100% fruits	70% apple, 25% raspberry, 5% blueberry
5	Vitalbio Cool Fruits – Apple, Strawberry, Blueberry, + Acerola	69% apple, 20.7% strawberry, 3% blueberry, 3% acerola
6	Luso Fruta – Red Fruits	7.8% apple, 1.5% grape,1% purple-carrot, 1% cranberry, 0.1% strawberry, 0.1% raspberry
7	Luso Fruta – Pomegranate and Acai	10.2% apple, 0.9% pomegranate, 0.2% acai, purple-carrot
8	Compal Vital Equilíbrio – AOX Red Fruits	12% grape, 9% apple, 6% raspberry, 5% blackcurrant, 4% strawberry, 4% cranberry
9	Coldpress – Raspberry, Pear, Apple, Smoothie	49% apple, 24% pear puree, 20% apple puree, 7% raspberry puree, extract of purple-carrot
10	Compal Vital Equilíbrio – AOX Pomegranate-Goji	24% pomegranate juice, 1% goji juice
11	Solevita – Natural juice of grape, pomegranate, and blackcurrant	65% grape juice, 25% pomegranate juice, 10% blackcurrant juice
12	Sonatural – Fresh Super Juices – Pomegranate	50% pomegranate, 46% apple, 4% berries
13	Sonatural – Fresh Fruit Red Fruits – Apple, Banana, Raspberry, Blueberry	82% apple, 8% banana, 6% raspberry, 4% blueberry
14	Compal Essencial Fruta – Strawberries	62% strawberry pulp, 10% strawberry juice
15	Compal Essencial Fruta – Red Fruits & Acai Protection	22% apple pulp, 21% apple juice, 15% strawberry pulp, 15% plum pulp, 13% grape juice, 3% pulp and juice of raspberry, 1% acai pulp
16	Solevita – Apple juice with elderberries	97% apple juice, 3% of elderberries extract
17	Pleno Tisanas – Pomegranate and chamomile	0.53% pomegranate and berries juices, 0.17% of extracts of tea and chamomile

(*Continued*)

Table 1
(*Continued*)

Juice number	Juice	Principal ingredients
18	IKA – Berries	5% grape juice, 1.5% strawberry juice, 1% pomegranate juice, 1% elderberries juice, 0.5% blackberry juice, 0.5% sweet cherries juice, 0.5% raspberry juice
19	Santal Active Drink – Red fruits	apple juice, 3.6% grape juice, 3% carrot juice, 0.7% pulp and juice of raspberry, 0.2% pulp and juice of strawberry, pulp and juice of blackcurrants and cranberries
20	Paquito – Cranberry Light	75% mineral water, 25% cranberry juice with origin in North America
21	Paquito – Syrup Grenadine Red Fruits	8% red fruits juice (raspberry, elderberry, blackcurrant, and cassis)
22	Cem Porcento Forma^{+} – Cranberry Plus	2.6% of dried extract of cranberry (*Vaccinium macrocarpon*)
23	Cem Porcento Forma^{+} – Pomegranate	5.3% hibiscus extract (*Hibiscus sabdariffa*), 5% pomegranate juice (*Punica granatum*), 2% sugar-beet juice (*Beta vulgaris*), 1.5% berries juice (raspberry, blackberry, and blueberry), 0.7% of dried extract of grape seed
24	Cem Porcento Forma^{+} – Goji	5% of dried extract of goji berries (*Lycium barbarum*), 3.8% hibiscus extract (*Hibiscus sabdariffa*), 1.5% berries juice (raspberry, blackberry, and blueberry), 1% of dried extract of grape seed
25	Maison Prosain – Cranberry Pur Jus Origine: Québec (Canadá)	100% cranberry juice from organic culture (Agriculture Canadá FR-BIO-01)

2.4. Phytochemical characterization

2.4.1. Total reducing sugars

The total reducing sugars were determined by dinitrosalicylic acid (DNS) method, as described previously [10]. Initially, the DNS solution (1% w/v) was prepared by dissolving 1 g of DNS (Sigma-Aldrich, USA) in 20 mL of sodium hydroxide (2 M) (Sigma-Aldrich, USA). Then, 30 g of potassium sodium tartrate (Sigma-Aldrich, USA) were added, being this mixture diluted with distilled water (1 L). After this, 500 μL of the standard solutions or juices were mixed with 500 μL of the DNS solution. These reaction mixtures were vigorously shaken using a vortex and incubated in a water bath (100°C) for 5 minutes. After cooling the reaction mixtures in an ice bath, 5 mL of cooled distilled water were finally added [10]. The absorbance of these solutions was recorded at 540 nm against a blank, using a UV-Vis spectrophotometer (Helios–Omega, Thermo Scientific, USA). The linear regression equation ($y = 5 \times 10^{-5}x$; $R^2 = 0.9930$) was obtained using glucose (Sigma-Aldrich, USA) standard solutions (50, 100, 200, 400, 600, 800 and 1000 mg/L). The results were expressed in terms of mg of glucose equivalents (GE)/100 mL of juice.

2.4.2. Total phenolics

The phenolics were determined by Folin-Ciocalteu's colorimetric method [11]. Initially, 50 μL of each juice or standard solutions were diluted in 450 μL of distilled water. Then, 2.5 mL of 0.2 N Folin-Ciocalteu's reagent (Sigma-Aldrich, USA) were added, being these mixtures vortexed and allowed to stand for 5 minutes, before the addition of 2 mL of an aqueous solution of sodium carbonate (Na_2CO_3) (Sigma-Aldrich, USA) (75 g/L). The reaction mixtures were finally incubated at 30°C for 90 minutes. The total phenolics were determined by measuring the absorbances at 765 nm, using a UV-Vis spectrophotometer (Helios–Omega, Thermo Scientific, USA). The standard curve ($y = 0.0009x$; $R^2 = 0.9875$) was prepared using several solutions of gallic acid (Sigma-Aldrich, USA) in methanol (Scharlab, Spain) [11]. Total phenolic values were expressed as mg of gallic acid equivalents (GAE)/100 mL of juice.

2.4.3. Flavonoids

The aluminum chloride colorimetric method was used to determine the content in flavonoids according to the previously implemented method [11]. Firstly, 500 μL of each juice were mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride (Sigma-Aldrich, USA), 0.1 mL of 1 M potassium acetate (Sigma-Aldrich, USA) and 2.8 mL of distilled water. These solutions remained at room temperature for 30 minutes. Finally, the absorbances were measured at 415 nm using a UV-Vis spectrophotometer (Helios–Omega, Thermo Scientific, USA). The calibration curve ($y = 0.0074x$; $R^2 = 0.9980$) was constructed by preparing eight quercetin (Sigma-Aldrich, USA) solutions at concentrations ranging from 12.5 to 200 μg/mL in methanol [11]. Flavonoid values were expressed as mg of quercetin equivalents (QE)/100 mL of juice.

2.4.4. Anthocyanins

Anthocyanins were measured by the pH differential method [12]. Monomeric anthocyanin pigments reversibly change color with a change in pH; the colored oxonium form occurs at pH = 1.0, and the colorless hemiketal form predominates at pH = 4.5 [12].

Primarily, two buffer solutions were prepared: pH = 1.0 buffer – potassium chloride, 0.025 M (Sigma-Aldrich, USA); and, pH = 4.5 buffer – sodium acetate, 0.4 M (Sigma-Aldrich, USA). Then, each juice was separately diluted (1 : 100 v/v) in each buffer solution, and the absorbances of the resulting mixtures were measured at both 520 nm ($A_{520\,nm}$) and 700 nm ($A_{700\,nm}$), using a UV-Vis spectrophotometer (Helios–Omega, Thermo Scientific, USA), against a blank constituted by distilled water [12]. To calculate the concentration of anthocyanins, expressed as mg of cyanidin-3-glucoside equivalents (cy-3-glu Eq)/100 mL of juice, or as mg malvidin-3-glucoside equivalents (mal-3-glu Eq)/100 mL of juice, the following equation was used:

$$Anthocyanins = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1} \times \frac{1}{10}, \tag{2}$$

where

$$A = (A_{520nm} - A_{700nm})_{pH=1.0} - (A_{520nm} - A_{700nm})_{pH=4.5}\,; \tag{3}$$

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside, or 463.3 g/mol for malvidin-3-glucoside; DF = dilution factor = 100; 10^3 = factor for conversion from g to mg; ε = molar extinction coefficient = 26900 L × mol^{-1} × cm^{-1} for cyanidin-3-glucoside, or 28000 L × mol^{-1} × cm^{-1} for malvidin-3-glucoside; 1 = pathlenght in cm; and $\frac{1}{10}$ = factor for conversion from L to 100 mL [12,13].

2.5. Antioxidant properties

2.5.1. DPPH free radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to evaluate the antioxidant activity of the juices of berries. In this work the method previously described was used [14]. Briefly, 100 μL of each juice were added to 3.9 mL of a 0.1 mM DPPH (Sigma-Aldrich, USA) methanolic solution, which was prepared daily. These samples were vigorously shaken and kept in the dark at room temperature for 15 minutes. After this time, the absorbances were measured at 517 nm, using a UV-Vis spectrophotometer (Helios–Omega, Thermo Scientific, USA). The percentage of inhibition of DPPH free radical by the samples (% Inhibition) was determined using the following equation:

$$\%\ Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100, \tag{4}$$

where $A_{control}$ is the absorbance of the control (composed by 100 μL of methanol and 3.9 mL of DPPH solution), and A_{sample} is the absorbance of the samples as described above [14].

2.5.2. β-carotene bleaching test

The β-carotene bleaching test was also employed to evaluate the antioxidant properties of the juices. After the preparation of a β-carotene (Sigma-Aldrich, USA) solution (20 mg/mL in chloroform), 500 μL were added to 40 μL of linoleic acid (TCI Europe N.V., Belgium), 400 μL of Tween 40 (Riedel-de Häen, Germany) and 1 mL of chloroform (Scharlab, Spain). The chloroform was then removed under vacuum (45°C) and finally 100 mL of oxygenated distilled water were added to the mixture to form an emulsion. Then, 5 mL of the emulsion were mixed with 300 μL of each juice, being the control sample composed by 5 mL of the emulsion and 300 μL of methanol. These samples remained at 50°C for 60 minutes. The absorbances of the samples were measured at 470 nm, using a UV-Vis spectrophotometer (Helios–Omega, Thermo Scientific, USA), against a blank where the emulsion was prepared without the addition of the β-carotene solution. The absorbances were measured at the initial time (t = 0 h) and at the final time (t = 1 h) of the incubation. The antioxidant activity was expressed in terms of the percentage of inhibition of β-carotene's oxidation (% Inhibition) by the following equation:

$$\%\ Inhibition = \frac{A^{t=1h}_{sample} - A^{t=1h}_{control}}{A^{t=0h}_{control} - A^{t=1h}_{control}} \times 100, \tag{5}$$

where $A^{t=1h}$ is the absorbance of the sample or control at the final time of incubation, and $A^{t=0h}$ is the absorbance of the control at the initial time of incubation [15].

2.6. Statistical analysis

All the measurements were carried out in triplicate assays. The data were analyzed using the statistical program SPSS version 24. The mean values and standard deviation (SD) were calculated for all cases. One-way analysis

Table 2
Physicochemical properties (pH, density, and total solids) of the juices of berries studied

Juice number	pH	Density (g/mL)	Total solids (%)
1	4.09 ± 0.01 **q**	1.01 ± 0.02 **ab**	1.64 ± 0.01 **cd**
2	4.50 ± 0.01 **s**	1.02 ± 0.05 **ab**	2.61 ± 0.01 **e**
3	3.22 ± 0.01 **j**	1.12 ± 0.02 **bc**	22.08 ± 0.02 **m**
4	3.34 ± 0.01 **l**	1.02 ± 0.04 **ab**	11.32 ± 0.25 **j**
5	3.66 ± 0.01 **p**	0.89 ± 0.08 **a**	11.95 ± 0.06 **k**
6	3.05 ± 0.01 **f**	1.02 ± 0.01 **ab**	4.83 ± 0.01 **g**
7	3.06 ± 0.01 **fg**	1.03 ± 0.02 **ab**	5.06 ± 0.01 **g**
8	3.14 ± 0.01 **hj**	1.03 ± 0.02 **ab**	3.75 ± 0.14 **f**
9	3.10 ± 0.01 **gh**	1.04 ± 0.05 **ab**	9.30 ± 0.09 **i**
10	3.15 ± 0.01 **i**	1.02 ± 0.02 **ab**	2.06 ± 0.01 **d**
11	3.27 ± 0.01 **k**	1.07 ± 0.02 **ab**	13.41 ± 0.01 **l**
12	3.17 ± 0.01 **i**	1.05 ± 0.02 **ab**	9.21 ± 0.17 **i**
13	3.60 ± 0.01 **o**	1.08 ± 0.02 **ab**	11.66 ± 0.33 **jk**
14	3.52 ± 0.01 **n**	1.07 ± 0.03 **ab**	9.34 ± 0.01 **i**
15	3.42 ± 0.01 **m**	1.02 ± 0.07 **ab**	11.96 ± 0.30 **k**
16	3.53 ± 0.01 **n**	1.06 ± 0.02 **ab**	12.01 ± 0.05 **k**
17	2.99 ± 0.01 **e**	1.01 ± 0.02 **ab**	0.77 ± 0.01 **a**
18	2.82 ± 0.01 **c**	1.02 ± 0.02 **ab**	4.75 ± 0.06 **g**
19	2.98 ± 0.01 **e**	1.01 ± 0.02 **ab**	3.70 ± 0.04 **f**
20	2.88 ± 0.01 **d**	1.00 ± 0.01 **ab**	1.19 ± 0.02 **abc**
21	2.58 ± 0.01 **b**	1.34 ± 0.06 **c**	61.25 ± 0.02 **n**
22	4.44 ± 0.01 **r**	0.99 ± 0.03 **ab**	1.43 ± 0.01 **b**
23	2.95 ± 0.01 **e**	1.01 ± 0.01 **ab**	1.05 ± 0.01 **abc**
24	2.90 ± 0.01 **d**	1.02 ± 0.01 **ab**	1.23 ± 0.01 **abc**
25	2.46 ± 0.01 **a**	1.05 ± 0.01 **ab**	6.86 ± 0.05 **h**

Data expressed as means ± SD of triplicate assays; Mean values in a column with different letters are significantly different ($p < 0.05$).

of variance (ANOVA) was undertaken to test for significant differences among means ($p < 0.05$ was considered significant).

3. Results and discussion

3.1. Physicochemical properties

The physicochemical properties of the juices of berries considered in this study are reported in Table 2. In general, juices are acidic solutions, presenting pH values lower than 4.50. Juice 25, composed solely by cranberries, presented the lowest pH value (2.46), which is probably due to the chemical composition of cranberries. This low pH makes cranberries and their juice protective agents against recurrent urinary tract infections, acting by producing acidic urine due to the excretion of hippauric acid [16]. Regarding the density of the juices, it was near 1 for all the samples, which is consistent to the aqueous nature of the juices. Concerning total solids, juice 21 presented the highest value (61.25%) indicating the presence of high quantities of organic matter, which is also observed in its density (1.34 g/mL). This juice is a syrup of various berries which may explain the results obtained.

Table 3
Rheological properties of the juices of berries studied

Juice number	Apparent viscosity – η_{app} (Pa.s) × 10^{-3}	Consistency coefficient – k ($Pa.s^n$)	Flow behavior index – n	Regression coefficient – R^2
1	5.094 ± 0.202 **a**	0.244 ± 0.001 **a**	0.448 ± 0.005	0.994
2	5.418 ± 0.053 **a**	0.294 ± 0.017 **a**	0.434 ± 0.006	0.995
3	4.740 ± 0.664 **a**	0.074 ± 0.005 **a**	0.580 ± 0.006	0.942
4	50.260 ± 2.430 **e**	4.021 ± 0.148 **d**	0.386 ± 0.018	0.986
5	65.460 ± 2.660 **f**	8.028 ± 0.185 **e**	0.320 ± 0.013	0.992
6	2.060 ± 0.046 **a**	0.045 ± 0.003 **a**	0.533 ± 0.002	0.950
7	1.837 ± 0.180 **a**	0.047 ± 0.002 **a**	0.521 ± 0.005	0.955
8	2.608 ± 0.228 **a**	0.048 ± 0.014 **a**	0.590 ± 0.048	0.970
9	12.630 ± 0.976 **b**	0.164 ± 0.034 **a**	0.626 ± 0.041	0.989
10	1.895 ± 0.024 **a**	0.029 ± 0.001 **a**	0.592 ± 0.007	0.953
11	2.664 ± 0.121 **a**	0.045 ± 0.008 **a**	0.579 ± 0.020	0.956
12	2.214 ± 0.114 **a**	0.042 ± 0.008 **a**	0.581 ± 0.037	0.969
13	4.496 ± 0.032 **a**	0.077 ± 0.018 **a**	0.596 ± 0.031	0.973
14	22.210 ± 0.170 **c**	0.660 ± 0.027 **bc**	0.517 ± 0.005	0.992
15	22.220 ± 1.390 **c**	0.795 ± 0.040 **c**	0.501 ± 0.019	0.971
16	3.016 ± 0.099 **a**	0.035 ± 0.003 **a**	0.640 ± 0.010	0.975
17	1.957 ± 0.033 **a**	0.041 ± 0.007 **a**	0.547 ± 0.021	0.950
18	1.978 ± 0.005 **a**	0.043 ± 0.001 **a**	0.537 ± 0.002	0.953
19	2.051 ± 0.233 **a**	0.041 ± 0.002 **a**	0.561 ± 0.002	0.962
20	2.050 ± 0.020 **a**	0.063 ± 0.013 **a**	0.494 ± 0.022	0.952
21	42.950 ± 0.396 **d**	0.045 ± 0.001 **a**	0.987 ± 0.006	0.999
22	5.804 ± 0.360 **a**	0.350 ± 0.002 **ab**	0.415 ± 0.005	0.989
23	1.936 ± 0.151 **a**	0.039 ± 0.010 **a**	0.555 ± 0.044	0.957
24	1.740 ± 0.207 **a**	0.049 ± 0.004 **a**	0.510 ± 0.010	0.958
25	2.130 ± 0.309 **a**	0.048 ± 0.003 **a**	0.543 ± 0.020	0.974

Data expressed as means ± SD of triplicate assays; Mean values in a column with different letters are significantly different ($p < 0.05$).

3.2. Rheological properties

The rheological properties of the juices of berries were also evaluated, being the results presented in Table 3. Concerning the values of η_{app}, it was found that most of the juices presented low viscosity, with six of them (juices 4, 5, 9, 14, 15 and 21) presenting significantly higher ($p < 0.05$) viscosity than the remaining. In general, the juices that exhibited higher viscosity presented simultaneously higher content in total solids and total reducing sugars (Tables 3 and 4), except for juice 21. The values of k and n are adequately ascertained employing the Ostwald-de-Waele model, as showed by the regression coefficients (R^2) (Table 3). Overall, the values of k follow the same trend than the ones of η_{app}, being observed that most juices have low k, with juices 4 and 5 presenting the highest consistency. The juices analysed presented non-Newtonian pseudoplastic fluid characteristics ($n < 1$), with time-dependent (thixotropic) behaviour. Only the juice 21 had a Newtonian behaviour, since their n is equal to 1. This juice is the one with the highest total solids content (Table 2), which may explain its Newtonian behaviour, caused by the low molar mass of the solutes which constitute it [17]. Different fluid characteristics were detailed in literature for juices and nectars of berries; other researchers had previously verified that raspberry

Table 4
Phytochemical characterization of the juices of berries considered in this study

Juice number	Total reducing sugars (mg GE/100 mL)	Total phenolics (mg GAE/100 mL)	Flavonoids (mg QE/100 mL)	Anthocyanins	
				mg cy-3-glu Eq/100 mL	mg mal-3-glu Eq/100 mL
1	199.00 ± 1.41 **bc**	276.96 ± 8.89 **f**	5.28 ± 0.64 **c**	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
2	427.00 ± 4.24 **de**	310.59 ± 12.77 **g**	9.94 ± 0.41 **e**	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
3	1487.00 ± 12.73 **i**	327.17 ± 17.36 **g**	20.59 ± 0.59 **hi**	0.22 ± 0.09 **a**	0.22 ± 0.09 **a**
4	2049.00 ± 72.12 **l**	110.52 ± 11.26 **c**	22.07 ± 0.27 **i**	6.00 ± 0.69 **e**	5.95 ± 0.68 **f**
5	2249.00 ± 18.38 **m**	114.45 ± 13.20 **c**	19.10 ± 0.07 **gh**	6.59 ± 0.35 **ef**	6.53 ± 0.34 **f**
6	94.00 ± 2.83 **a**	11.04 ± 0.64 **a**	0.68 ± 0.04 **a**	0.88 ± 0.06 **a**	0.87 ± 0.06 **ab**
7	137.00 ± 4.24 **abc**	28.67 ± 2.08 **a**	1.84 ± 0.07 **ab**	1.35 ± 0.05 **ab**	1.33 ± 0.05 **ab**
8	450.00 ± 5.66 **de**	81.11 ± 0.16 **b**	6.46 ± 0.14 **c**	3.93 ± 0.44 **d**	4.14 ± 0.07 **e**
9	1388.00 ± 19.80 **h**	118.95 ± 9.82 **c**	15.86 ± 0.96 **f**	1.44 ± 0.18 **ab**	1.43 ± 0.18 **abc**
10	59.00 ± 1.41 **a**	74.44 ± 3.69 **b**	7.93 ± 0.35 **d**	0.83 ± 0.08 **a**	0.83 ± 0.08 **ab**
11	455.00 ± 1.41 **e**	174.44 ± 6.76 **d**	18.15 ± 0.27 **g**	10.48 ± 0.64 **g**	10.73 ± 0.28 **h**
12	563.00 ± 1.41 **f**	387.52 ± 9.49 **h**	28.22 ± 0.77 **i**	10.56 ± 0.45 **g**	10.46 ± 0.45 **h**
13	1745.00 ± 7.07 **j**	225.89 ± 8.01 **e**	19.67 ± 0.43 **gh**	4.09 ± 0.06 **d**	4.05 ± 0.06 **e**
14	2125.00 ± 26.87 **l**	217.89 ± 0.31 **e**	14.65 ± 0.98 **f**	8.30 ± 0.60 **f**	8.22 ± 0.59 **g**
15	1880.00 ± 48.08 **k**	132.94 ± 5.58 **c**	22.78 ± 0.54 **i**	3.72 ± 0.61 **cd**	3.69 ± 0.61 **e**
16	653.00 ± 7.07 **g**	81.99 ± 0.64 **b**	9.97 ± 0.09 **e**	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
17	89.00 ± 1.41 **a**	15.09 ± 0.47 **a**	1.84 ± 0.06 **ab**	1.54 ± 0.00 **ab**	1.52 ± 0.00 **abc**
18	71.00 ± 1.41 **a**	22.84 ± 0.60 **a**	2.78 ± 0.08 **ab**	0.70 ± 0.02 **a**	0.69 ± 0.02 **ab**
19	228.00 ± 14.14 **c**	30.37 ± 1.00 **a**	3.72 ± 0.08 **b**	1.52 ± 0.08 **ab**	1.51 ± 0.08 **abc**
20	77.00 ± 1.41 **a**	43.97 ± 0.69 **a**	5.35 ± 0.09 **c**	1.98 ± 0.09 **bc**	1.97 ± 0.09 **bcd**
21	183.00 ± 7.07 **bc**	32.82 ± 1.61 **a**	3.81 ± 0.34 **b**	2.99 ± 0.05 **bcd**	2.96 ± 0.05 **cde**
22	197.00 ± 4.24 **bc**	20.40 ± 0.99 **a**	2.48 ± 0.12 **ab**	0.14 ± 0.03 **a**	0.14 ± 0.03 **a**
23	180.00 ± 5.66 **bc**	16.70 ± 0.53 **a**	2.03 ± 0.06 **ab**	0.87 ± 0.02 **a**	0.87 ± 0.02 **ab**
24	182.00 ± 2.83 **bc**	16.59 ± 1.46 **a**	2.02 ± 0.18 **ab**	0.50 ± 0.06 **a**	0.50 ± 0.06 **ab**
25	370.00 ± 5.66 **d**	92.33 ± 1.90 **bc**	10.99 ± 0.45 **e**	3.50 ± 0.05 **cd**	3.47 ± 0.05 **de**

GE – glucose equivalents; GAE – gallic acid equivalents; QE – quercetin equivalents; cy-3-glu Eq – cyanidin-3-glucoside equivalents; mal-3-glu Eq – malvidin-3-glucoside equivalents; Data expressed as means ± SD of triplicate assays; Mean values in a column with different letters are significantly different ($p < 0.05$).

and blueberry juices had predominantly Newtonian behaviour over the studied range of temperature and total solids content [18]. Similarly to the results found in the present study, the pseudoplastic behaviour was observed for redcurrant juices [7]. Several variables greatly affect the rheological behaviour of juices, such as solute type, size, shape, flexibility, and solute-solvent interactions. Since each juice of berries is composed by soluble and insoluble solids in an aqueous phase, together with particles in suspension, their rheological behaviour results from the complex interactions among the aforementioned variables.

3.3. Phytochemical characterization

The results of the phytochemical characterization of the juices of berries are summarized in Table 4. The total reducing sugars, total phenolics, flavonoids and anthocyanins were determined for all the samples being tested.

Sugar is the general term for a group of sweet-flavored compounds used as food, and a relevant ingredient in many foods and beverages; it also occurs naturally in fruits, vegetables, milk, and honey [10]. Juice 10 presented the lowest concentration of total reducing sugars (59.00 mg GE/100 mL), in contrast with juice 5, which presented the highest concentration (2249.00 mg GE/100 mL). However, juice 5 is only made of fresh fruit, indicating that the sugars present are only the naturally-occurring ones. Generally, the sugars present in natural juices are glucose, fructose and sucrose [19].

Berries are characterized by their high concentration of antioxidant molecules, namely phenolic compounds [20]. Juice 12 presented simultaneously the highest concentration of total phenolics, flavonoids and anthocyanins, in strong contrast with juice 6 that had the lowest concentrations. Juice 12 is mainly composed by pomegranate (*Punica granatum*), which is also a polyphenol-rich food and often consumed in combination with berries [21], whereas juice 6 is mostly constituted by water (88.2%), explaining the lowest values obtained for bioactive phytochemicals. In terms of phytochemicals, the results obtained in the present study are similar to what was observed with citrus juices [22]. It is difficult to establish a correlation between the phytochemical characterization and the composition of the juices, namely in what concerns to the type of berry, since the juices are a complex mixture of compounds, and in many cases a mixture of several types of berries with other fruits, namely apple/pear, which are used as basis for the juices.

3.4. Antioxidant properties

The antioxidant properties of the juices under study were evaluated using both the DPPH free radical scavenging assay, and the β-carotene bleaching test; the results are presented in terms of % Inhibition and are listed on Table 5. These two methods were chosen since they measure distinct antioxidant properties, and because the juices are complex mixtures of compounds that may possess similar or diverse antioxidant mechanisms. Other methods to test the antioxidant activity of the juices could be employed, like the Ferric Reducing Ability of Plasma (FRAP), which was previously applied in strawberry extract [23] and Saskatoon berry [24]. However, it was also verified that the results of DPPH method follow the same trend that the ones obtained with the FRAP method [23, 24]. Therefore, in the present work, it was decided to employ the DPPH assay and another method that is less commonly used, like β-carotene bleaching test, which evaluates the capacity of the samples to inhibit the lipid peroxidation. Moreover, recently, the DPPH method was updated, being proposed the use of the diphenyl-1-pyrenylphosphine (DPPP) oxide which is formed by the stoichiometric reaction of lipid hydroperoxide and DPPP, to follow plasma lipid oxidation induced by different oxidants [25]. This method was applied to blueberry extracts [25], however, it needs optimization and implementation by other researchers in order to be widely used, as it was happened with the DPPH method.

Overall, the results of the % Inhibition of DPPH free radical by the tested juices indicate that they possess considerable capacity to scavenge free radicals, resulting in good antioxidant properties. Since berries are claimed to be functional foods, mainly due to their antioxidant bioactive compounds, and being widely used to minimize the side effects of oxidative-stress related pathologies [26], it is now demonstrated that juices of berries maintain the antioxidant properties attributed to berries. Juice 3, supplemented with resveratrol, presented the highest % Inhibition of DPPH radicals (99.54%). Resveratrol is a well-known polyphenolic antioxidant compound also naturally present in some types of berries [4, 27], which explains the results obtained for this juice. In contrast, juice 6 showed the poorest antioxidant activity as measured by the DPPH method (% Inhibition = 23.53%), but this juice is mostly made of water, presenting low quantities of bioactive phytochemicals (Tables 4 and 5). Another relevant result was obtained for juice 22 (% Inhibition = 96.05%), which is composed by cranberries, indicating that this type of berries possesses remarkable antioxidant properties, which is also verified in juice 25 (100% cranberries), presenting % Inhibition = 89.47%.

Besides the DPPH method, the β-carotene bleaching test was also employed to evaluate the antioxidant activity of the juices. This activity is variable and depends on the method used, since an antioxidant mechanism in various biological matrices is very complex and several factors may intervene [28]. Regarding the results obtained with this method (Table 5), it was possible to verify that in general the antioxidant activity is weaker than that measured by the DPPH free radical scavenging assay. Juice 23 possesses no capacity to inhibit the oxidation of β-carotene,

Table 5
Antioxidant properties of the juices of berries considered

Juice number	DPPH free radical scavenging assay % Inhibition	β-carotene bleaching test % Inhibition
1	90.69 ± 0.17 **ghi**	81.65 ± 1.85 **j**
2	61.51 ± 0.37 **c**	89.60 ± 0.39 **l**
3	99.54 ± 0.14 **kj**	25.48 ± 1.00 **e**
4	53.72 ± 1.04 **b**	84.39 ± 0.33 **j**
5	84.08 ± 0.13 **f**	89.01 ± 1.22 **l**
6	23.53 ± 0.43 **a**	24.99 ± 0.31 **e**
7	66.16 ± 0.08 **d**	47.40 ± 0.36 **h**
8	86.35 ± 0.57 **f**	37.44 ± 0.66 **g**
9	49.38 ± 1.67 **b**	46.61 ± 0.19 **h**
10	94.26 ± 0.07 **gij**	8.82 ± 1.15 **b**
11	87.82 ± 0.25 **f**	25.45 ± 0.33 **e**
12	89.54 ± 0.69 **fg**	14.99 ± 0.65 **c**
13	76.74 ± 0.41 **e**	19.47 ± 0.57 **d**
14	86.66 ± 1.98 **f**	29.78 ± 1.11 **f**
15	53.66 ± 1.75 **b**	30.13 ± 0.76 **f**
16	86.23 ± 1.42 **f**	0.57 ± 0.05 **a**
17	27.45 ± 0.89 **a**	5.91 ± 0.55 **b**
18	90.16 ± 0.49 **fgh**	1.76 ± 0.22 **a**
19	61.91 ± 1.19 **c**	0.37 ± 0.09 **a**
20	70.63 ± 1.80 **d**	39.04 ± 1.60 **g**
21	54.43 ± 0.11 **b**	3.38 ± 0.52 **b**
22	96.05 ± 1.40 **ij**	56.06 ± 1.70 **i**
23	85.50 ± 1.37 **f**	0.00 ± 0.00 **a**
24	78.49 ± 0.76 **e**	20.61 ± 1.34 **d**
25	89.47 ± 0.87 **fgh**	15.14 ± 0.86 **c**

Data expressed as means ± SD of triplicate assays; Mean values in a column with different letters are significantly different ($p < 0.05$).

indicating that it is not able to inhibit the lipid peroxidation cascade reactions. In contrast, juice 2 presented 89.60% of % Inhibition, being this the highest measured value of inhibition. In addition to several berries, this juice is also made with pomegranate and grape seeds, a mixture of known antioxidant foods. In general, the juices of berries considered in this study had higher antioxidant properties than those obtained by other researchers dealing with the characterization of citrus and tamarind juices [22, 29].

4. Conclusions

This study demonstrated that the physicochemical properties of the juices influence their rheological behavior; and the phytochemical composition impacts their antioxidant properties. This study also confirmed the potential health benefits of berries and berries-derived products, namely juices, that maintain the bioactive phytochemicals and the antioxidant properties of the original berries. Due to their phytochemical composition and antioxidant capacities, juices of berries could be considered functional foods or a potential source of nutraceuticals.

Acknowledgments

Ângelo Luís acknowledges the Post-Doc research fellowship within the scope of the protocol signed between Universidade da Beira Interior and bank Santander/Totta with the reference BIPD/ICI-FC-BST-UBI 2016. This work was supported by FEDER funds through the POCI-COMPETE 2020-Operational Program Competitiveness and Internationalization in Axis I-Strengthening research, technological development, and innovation (Project POCI-01-0145- FEDER-007491) and National Funds by FCT-Foundation for Science and Technology (Project UID/Multi/00709/2013).

Conflict of interest

The authors have no conflict of interest to report.

References

[1] Basu A, Rhone M, Lyons TJ. Berries: Emerging impact on cardiovascular health. Nutr Rev. 2010;68(3):168-77.

[2] Zhu Y, Miao Y, Meng Z, Zhong Y. Effects of *Vaccinium* berries on serum lipids: A meta-analysis of randomized controlled trials. Evidence-based Complement Altern Med. 2015. doi: 10.1155/2015/790329

[3] Huang H, Chen G, Liao D, Zhu Y, Xue X. Effects of Berries Consumption on Cardiovascular Risk Factors: A Meta-analysis with Trial Sequential Analysis of Randomized Controlled Trials. Sci Rep. 2016. doi: 10.1038/srep23625

[4] Nile SH, Park SW. Edible berries: Bioactive components and their effect on human health. Nutrition. 2014;30(2):134-44.

[5] Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J. Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci. 2015;16(10):24673-706.

[6] Pinto P, Cardoso S, Pimpão RC, Tavares L, Ferreira RB, Santos CN. Daily polyphenol intake from fresh fruits in Portugal: Contribution from berry fruits. Int J Food Sci Nutr. 2013;64(8):1022-9.

[7] Ibarz A, Falguera V, Garza S. Rheology of redcurrant juices. Afinidad LXVII. 2010;335-41.

[8] Šimunek M, Jambrak AR, Dobrović S, Herceg Z, Vukušić T. Rheological properties of ultrasound treated apple, cranberry and blueberry juice and nectar. J Food Sci Technol. 2014;51(12):3577-93.

[9] Manjunatha SS, Raju PS, Bawa AS. Modelling the Rheological Behaviour of Enzyme Clarified Lime (*Citrus aurantifolia* L.) Juice Concentrate. Czech J Food Sci. 2005;30(5):456-66.

[10] Baskan KS, Tütem E, Akyüz E, Özen S, Apak R. Spectrophotometric total reducing sugars assay based on cupric reduction. Talanta. 2016;147:162-8.

[11] Luís Â, Neiva D, Pereira H, Gominho J, Domingues F, Duarte AP. Stumps of *Eucalyptus globulus* as a Source of Antioxidant and Antimicrobial Polyphenols. Molecules. 2014;19:16428-46.

[12] Lee J, Durst R, Wrolstad R, Barnes K, Eisele T, Giusti M, et al. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative Study. J AOAC Int. 2005;88(5):1269-78.

[13] Lee J, Rennaker C, Wrolstad RE. Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods. Food Chem. 2008;110:782-6.

[14] Colon M, Nerín C. Synergistic, antagonistic and additive interactions of green tea polyphenols. Eur Food Res Technol. 2016;242(2):211-20.

[15] Luís Â, Duarte A, Domingues F. Bioactive compounds, RP-HPLC analysis of phenolics, and antioxidant activity of some portuguese shrub species extracts. Nat Prod Commun. 2011;6(12):1863-72.

[16] Micali S, Isgro G, Bianchi G, Miceli N, Calapai G, Navarra M. Cranberry and recurrent cystitis: More than marketing? Crit Rev Food Sci Nutr Sci Nutr. 2014;54(8):1063-75.

[17] Astolfi-Filho Z, Telis VRN, de Oliveira EB, Coimbra JS dos R, Telis-Romero J. Rheology and fluid dynamics properties of sugarcane juice. Biochem Eng J. 2011;53(3):260-5.

[18] Nindo CI, Tang J, Powers JR, Singh P. Viscosity of blueberry and raspberry juices for processing applications. J Food Eng. 2005;69(3):343-50.

[19] Leopold LF, Leopold N, Diehl H-A, Socaciu C. Quantification of carbohydrates in fruit juices using FTIR spectroscopy and multivariate analysis. Spectroscopy. 2011;26(2):93-104.

[20] Hidalgo G-I, Almajano MP. Red fruits: Extraction of antioxidants, phenolic content, and radical scavenging determination: A review. Antioxidants. 2017. doi: 10.3390/antiox6010007

[21] Duarte AP, Luís Â, Domingues FC. Pomegranate (*Punica granatum*): A Natural Approach to Combat Oxidative Stress-Related Diseases. In: Rodrigues da Silva L, Silva B, editors. Natural Bioactive Compounds from Fruits and Vegetables as Health Promoters. BENTHAM SCIENCE PUBLISHERS LTD.; 2016. pp. 143-79.

[22] Sicari V, Pellicanò TM, Giuffrè AM, Zappia C, Capocasale M. Bioactive compounds and antioxidant activity of citrus juices produced from varieties cultivated in Calabria. J Food Meas Charact. 2016;10(4):773-80.

[23] Gasparrini M, Forbes-Hernandez TY, Giampieri F, Afrin S, Alvarez-Suarez JM, Mazzoni L, et al. Anti-inflammatory effect of strawberry extract against LPS-induced stress in RAW 264.7 macrophages. Food Chem Toxicol. 2017;102:1-10.

[24] Lachowicz S, Oszmiański J, Seliga L, Pluta S. Phytochemical composition and antioxidant capacity of seven saskatoon berry (*Amelanchier alnifolia* Nutt.) Genotypes Grown in Poland. Molecules. 2017. doi: 10.3390/molecules22050853

[25] Morita M, Naito Y, Yoshikawa T, Niki E. Antioxidant capacity of blueberry extracts: Peroxyl radical scavenging and inhibition of plasma lipid oxidation induced by multiple oxidants. J Berry Res. 2017. doi: 10.3233/JBR-170152

[26] Céspedes CL, Valdez-Morales M, Avila JG, El-Hafidi M, Alarcón J, Paredes-López O. Phytochemical profile and the antioxidant activity of Chilean wild black-berry fruits, *Aristotelia chilensis* (Mol) Stuntz (*Elaeocarpaceae*). Food Chem. 2010;119(3):886-95.

[27] Duarte A, Martinho A, Luís Â, Figueiras A, Oleastro M, Domingues FC, et al. Resveratrol encapsulation with methyl-β-cyclodextrin for antibacterial and antioxidant delivery applications. LWT - Food Sci Technol. 2015;63:1254-60.

[28] Çam M, Hişil Y, Durmaz G. Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. Food Chem. 2009;112(3):721-6.

[29] Muzaffar K, Dar BN, Kumar P. Assessment of nutritional, physicochemical, antioxidant, structural and rheological properties of spray dried tamarind pulp powder. J Food Meas Charact. 2016;1-12.