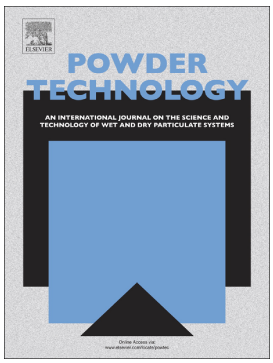


Spray-dried powders from berries extracts obtained upon several processing steps to improve the bioactive components content

Maite Gagneten, Rocío Corfield, Milagros Gómez Mattson, Agustina Sozzi, Graciela Leiva, Daniela Salvatori, Carolina Schebor

PII:	S0032-5910(18)30775-7
DOI:	doi:10.1016/j.powtec.2018.09.048
Reference:	PTEC 13722
To appear in:	*Powder Technology*
Received date:	14 December 2017
Revised date:	23 July 2018
Accepted date:	19 September 2018

Please cite this article as: Maite Gagneten, Rocío Corfield, Milagros Gómez Mattson, Agustina Sozzi, Graciela Leiva, Daniela Salvatori, Carolina Schebor , Spray-dried powders from berries extracts obtained upon several processing steps to improve the bioactive components content. Ptec (2018), doi:10.1016/j.powtec.2018.09.048

Spray-dried powders from berries extracts obtained upon several processing steps to improve the bioactive components content

Maite Gagneten[1], Rocío Corfield[1,4], Milagros Gómez Mattson[2,4], Agustina Sozzi[2,4], Graciela Leiva[3,*], Daniela Salvatori[2,4], Carolina Schebor[1,4]

(1) Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Industrias, Buenos Aires, Argentina.

(2) PROBIEN (CONICET-Universidad Nacional del Comahue), Argentina.

(3) Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento deQuímica Orgánica, Buenos Aires, Argentina.

(4) CONICET, Argentina.

* Corresponding author: leiva@qo.fcen.uba.ar

Abstract

This work aimed at developing powders rich in antioxidant compounds and pigments from three berries grown in argentine Patagonia: blackcurrant, raspberry and elderberry. Ultrasound-assisted extraction (UAE) was applied on the precipitates generated after juice production in order to improve bioactive compounds recovery. The best extracts (UAE 10 min for raspberry and blackcurrant, and 5 min for elderberry), obtained after 2-3 extraction cycles were mixed and spray-dried. The glass transition temperatures (43-50°C), and the low water content (0.92-1.44 %) and water activity values (<0.06) of the powders suggest that they could be stored at room temperature without risk of physical deterioration. Elderberry and blackcurrant powders showed good flowability and low cohesiveness while raspberry powders presented an intermediate cohesiveness and fair flowability. This was consistent with the smooth surface and the less shrinkage of the raspberry powder particles observed by microscopy. Elderberry powders presented higher concentration of bioactive compounds (polyphenols content 4.57±0.23 mg gallic acid (GA)/g d.m. and monomeric anthocyanins content 2.39±0.12 mg cyd-3-glu/g d.m.), and higher antioxidant capacity (3.33±0.57mg GA/ g d.m.) when compared to raspberry and blackcurrant. The obtained powders result possible ingredients to be used as natural colorants or antioxidants.

Keywords

Berries; Ultrasound-assisted extraction; Bioactive compounds; Spray-drying

1. Introduction

Berries are a rich source of bioactive compounds such as anthocyanins, flavonols and polyphenols, which contribute to their antioxidant activity and different biological functions [1-3]. Anthocyanins have demonstrated promising health-promoting properties like anti-inflammatory, immunomodulatory, antimicrobial, improvement of vision and neuroprotective effects [4-8]. Because of these properties and, given their good taste and appearance, consumer demand of this type of fruits has been growing. Due to their high respiration rate, berries exhibit a very short postharvest life [9]. For this reason, there is increasing interest in developing processed berry products that can be consumed as is or incorporated as ingredients in different kinds of food. Additionally, the high level of anthocyanins found in these fruits offers interesting applications as natural food colorants [10,11]. However, the use of anthocyanins as colorants may face some problems due to the stability loss during storage caused by temperature, oxygen, and light [12]. In order to overcome these difficulties and preserve the bioactive compounds, encapsulation technologies, such as spray drying can be applied [13].

Fruit powders are glassy materials, therefore, they are prone to suffer changes related to glass transition, like stickiness, caking, and collapse [14,15], as wellas color changes [16]. For that reason, the addition of an appropriate carrier matrix is relevant to get physically stable and non-sticky powders. Many researchers published studies about encapsulation byspray-drying of anthocyanins from different sources like fruit juices or extracts from pomegranate [17], blackmulberry [18], and extracts of *Garcinia indica* Choisy [19], and black carrot [13]. Some studies were conducted to obtain spray-dried

powders from other berry juices (blackberry, blackcurrant, raspberry) and using maltodextrin as carrier matrix [20,21].

Upon fruit juice extraction, particularly from berries, a solid residue rich in phenolic compounds can be obtained. Therefore, by applying an appropriate extraction method it is possible to take advantage of the valuable components present in this residue. Conventional solvent extraction procedures take long times, and can deteriorate anthocyanins [22,23]. Currently, there is a growing demand for extraction procedures that reduce the extraction time, the energy consumption, and the use of unsafe solvents. An alternative technique is ultrasound-assisted extraction (UAE), which has shown a positive effect on phenolic compounds extraction from several fruit sources [24,25]. In the case of berries, several authors applied UAE to obtain extracts:raspberry [26] and black chokeberry [27]. Also, Galván d'Alessandro et al. [28] applied UAE to the wastes of black chokeberry juice extraction and recovered extracts rich in anthocyanins and polyphenols.

The objectives of the present work were: 1) Improve the extraction of bioactive compounds by UAE from blackcurrant, raspberry and elderberry fruits; and 2) Use the spray-drying process to dehydrate the optimized berry extracts and characterize the physical and functional properties of the powders with the potential use as healthy food colorants or functional ingredients. This study will also support the development of products with more added value for the growing industry of berry fruits.

2. Materials and methods

2.1. Fruits

Three types of berries grown in Patagonia Argentina where used: blackcurrant (*Ribes nigrum*) of the Titania cultivar, raspberry (*Rubus idaeus* L.) of the Autumn Bliss cultivar and elderberry (*Sambucus nigra*). The fruit was purchased frozen by individual quick-freezing (IQF) technology at a local market and stored at -18°C until use.

2.1.1. Fruits characterization

A characterization according to AOAC methods [29] was done: soluble solids (932.12), acidity (945.26), pH (945.27). Moisture was determined according to Código Alimentario Argentino (CAA) [30], method 13.21 (1989).

2.1.2. Preparation of fruit extracts

The following steps were taken: thawing of the frozen fruit under controlled conditions (bath at 40–45°C), steam blanching (100°C) for 60 seconds, addition of water in the ratio 2:1 (water:fruit), milling for 60 seconds and 10 minutes rest in ice bath. The resulting product was divided in three fractions. Two of the fractions were subjected to ultrasound treatment in an ice bath for 5 min (U5) and 10 min (U10) using an UP100H ultrasonic processor (Teltow, Germany) at a frequency of 30 kHz and 100% amplitude. The third fraction did not receive ultrasound treatment (C).The samples were then centrifuged (4°C, 9000 rpm) for 20 minutes. The remaining solid was separated from the supernatant and it was weighed. Water was added to the precipitate in a ratio 2:1 (water:fruit), and the corresponding treatment (U5, U10 or C) was applied. This procedure was done once for raspberry and twice for blackcurrant and elderberry. Functional properties (total phenolic content, antioxidant capacity and monomeric

anthocyanin content) were analyzed after each extraction cycle to select the best pretreatment (U5, U10 or C) for each fruit. Once the best treatment for each berry was selected, the supernatants of the consecutive treatments were collected to obtain one pool of extracts for each fruit. The total soluble solids content of each pooled extract was determined. The obtained values were: 2.9 ± 0.1; 3.10 ± 0.05 and 2.23 ± 0.06 per 100g of extract for raspberry, blackcurrant and elderberry, respectively.

2.2. Drying process

Some selected extracts were spray-dried using as carrier matrix (20% w/w) maltodextrin (MD) DE 12 from Givaudan S.A. (Buenos Aires, Argentina). The MD:fruit solids proportion was 8.6, 8.06 and 11.2 for raspberry, blackcurrant and elderberry, respectively. A laboratory scale device, Mini Spray Dryer BüchiB290 (Flawil, Switzerland) was used.

The operational conditions of the drying process were: inlet air temperature of 170 ± 3 °C, flow rate 8mL min^{-1}, air pressure 3.2 bar, and nozzle diameter 1.5 mm. Once obtained, the powders were collected into sealed polyvinylidene chloride bags or in individual vials and then stored at -18°C.

2.3. Physical Properties

All measurements were made in triplicate and the average values were informed.

2.3.1. Water content determination

Karl Fisher titration was carried out at 25 °C with a Karl Fisher TIM 980 titration manager (Radiometer Analytical, France), applying the one component technique with Hydranal Titrant Composite 5 from Riedel-de Haën (Seelze, Germany). Pure methanol was used as solvent and they were purchased from Biopack (Buenos Aires, Argentina). Approximately 20mg samples were analyzed.

2.3.2. Water sorption isotherms

The water sorption isotherms were determined by the static isopiestic method (n=3). The humidification of samples was performed at 20 °C. Approximately 1g of fruit powders were put into vacuum desiccators over saturated salt solutions from 11% to 52% relative humidity (Greenspan, 1977). The “equilibrium” was determined when samples achieved constant weight.

2.3.3. Water Activity

Water activity was measured using an electronic dew point water activity meter Aqualab Series 3TE (Decagon Devices, Pullman, Washington, USA).

2.3.4. Thermal Transitions

Glass transitions were determined by differential scanning calorimetry (DSC; onset values) using a DSC 822e Mettler Toledo calorimeter (Schwerzenbach, Switzerland). The instrument was calibrated with indium (156.6 °C), lead (327.5 °C), and zinc (419.6 °C). All measurements were performed at a heating rate of 10°C/min. Hermetically sealed 40 mL medium pressure pans were used (an

empty pan served as reference). Thermograms were evaluated using Mettler Star^{e} program.

2.3.5. Superficial Color

Superficial color was determined by photocolorimetry using a handheld colorimeter (Minolta Co, model CR400, Japan). Color functions were calculated for illuminant C at 2° standard observer and in the CIELAB uniform color space. L* (brightness/darkness), a* (redness/greenness), and b* (yellowness/ blueness) values were obtained. Measurements were performed using glass vials containing enough powder to complete 1 cm height. A white cylindrical cup was used to cover the vial and standardize the measurements.

2.3.6. Solubility

Solubility was determined according to Franceschinis et al. [20] with some modifications. 0.5g of juice powder was hydrated in 50 mL distilled water and centrifuged at 3000 g during 5 min. Supernatant (10 mL) was transferred to a glass capsule and oven-dried at 105°C up to constant weight. Solubility (%) was calculated by weight difference.

2.3.7. Bulk and Compacted Density, flowability (Carr index) and cohesiveness (Hausner ratio).

Bulk density (g/mL) was determined according to Jinapong et al. [31] with some modifications by measuring mass with an analytical balance and volume using a graduated cylinder. The volume occupied by 0.6 g of berry powders in a 10 mL cylinder was determined. The volume occupied by the same sample of powder

after holding the cylinder on a vortex vibrator for 1 minute was determined to inform the compacted density (g/mL).

The Hausner ratio (H) was related to the powder cohesiveness (Eq. 1) where levels below 1.2 are considered low, between 1.2-1.4 are intermediate and above 1.5 are high [31]. The classification of powder flowability was based on Carr´s compressibility index (CI) (Eq. 2). The following scale was used: a) CI = 10 is excellent, b) 11 <CI< 15is good; c) 16 <CI< 20is fair; d) 21 <CI< 25isacceptable; and e) 26 <CI< 31ispoor [32].

$$H = (compacted\ density)/(bulk\ density) \quad (1)$$

$$CI = (compacted\ density - bulk\ density)/(compacted\ density) \quad (2)$$

2.3.8. Hygroscopicity

Hygroscopicity was determined using Swaminathan et al. [33] method with slight modifications. 1 g of powder was placed in a closed desiccator at 25ºC containing a saturated solution of NaCl (75% RH). Duplicate samples were weighed periodically during nine days, and hygroscopicity was expressed as the average of grams of adsorbed water per 100 grams of dry matter (g a.w./100 g d.m.).

2.3.9. Particle Morphology and Size Distribution

The microstructural characteristics of powders were analyzed by scanning electron microscopy (SEM) using a Zeiss microscope Supra 40 (Oberkochen, Germany). The samples were placed in an aluminum support using a double-sided adhesive tape conductive carbon and then coated with gold nanoparticles

using a sputter coater (Cressington Scientific Instruments 108). The images were taken with the detector within the lens, using an acceleration voltage of 3.00 kV.

Particle size distribution was measured using a laser light diffraction instrument under the dry powder method (LA 950 V2, Horiba, Kyoto, Japan). Average particle size was expressed as volume mean diameter D[4,3], and the distribution width was characterized in terms of span (Eq. 3). The span index was calculated as follows:

$$Span=((D90-D10))/D50 \quad (3)$$

where D_{90}, $D_{50}$and $D_{10}$are the diameters for which 90%, 50% and 10% of the population is below each value, respectively. The closer the span value is to 1, the narrower the particle size distribution [34].

2.4. Chemical and Functional Properties

All measurements were made in triplicate and the average values were informed. In the case of powders, all determinations were done on the reconstituted extract, which was prepared as follows: approximately 0.5 g of each powder was dissolved in 2.5 mL distilled water. A spectrophotometer ultraviolet-visible Jasco V-630 (Tokyo, Japan) was used in all cases.

2.4.1. Soluble Solids, Total Acidity and pH

The soluble solids content was analyzed by measuring the refraction index in a digital refractometer model AR200 (Reichert, USA) at 25 °C. Total acidity was

expressed as percent of citric acid (%, wet basis). A pH meter model 340 (Mettler Toledo, UK) was used for pH and total acidity measurements.

2.4.2. Berry extracts for measurement

Methanolic extracts of samples (frozen fruits, extracts and reconstituted powders) were prepared and used for total phenolic content (TPC) and antioxidant capacity (AC) measurements. For extract preparation, 1 g of sample was homogenized in 5 mL of absolute methanol, shaken for 5min using a magnetic stirrer and filtered under vacuum using a Büchner funnel. The pellet was extracted again with 5 mL of absolute methanol. The extracts were combined, and methanol was added to constitute a total volume of 10 mL.

Ethanolic extracts of samples (frozen fruits, extracts and reconstituted powders) were prepared and used for monomeric anthocyanin content (ACY). For extract preparation, 1 g of the sample was homogenized in 5 mL of ethanol acidified with hydrochloric acid, shaken for 15min using a magnetic stirrer and filtered under vacuum using a Büchner funnel. The pellet was extracted again with 5 mL of ethanol acidified with hydrochloric acid. The extracts were combined, and ethanol was added to constitute a total volume of 10 mL.

In the case of frozen fruits prior to weighing, they were thawed (water bath at 40–45°C) and milled for 60 seconds.

2.4.3. Total phenolic content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu reagent according to Singleton and Rossi [35] with some modifications. 100 µL of extract was mixed with 900 µL water, 100 µL Folin–Ciocalteu reagent and 600

µL 20% sodium carbonate in 1.5N NaOH. After incubation during 25 min at40 °C and centrifugation (10000 rpm, 5 min), the supernatant absorbance was measured at 765 nm.

2.4.4. Antioxidant capacity (AA)

Antioxidant capacity was measured using the bleaching method of 2,20-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid] radical cation ($ABTS^{+\cdot}$), according to Coria Cayupán et al. [36]. ABTS was dissolved in distilled water to yield a 7mM solution. Radical cation solution was prepared by incubating the ABTS solution with a 2.45mMpotassium persulfate solution for 16 h in the dark at room temperature and subsequently diluted with phosphate buffer to a final absorbance of 1.00±0.01 at 734 nm. For antioxidant capacity determination, 0.1 mL of sample extract was added with 1.9 mL of the $ABTS^{+\cdot}$ solution and incubated at 25°C for 30 min. The decrease in absorbance at 734nm was monitored. A calibration curve was done with gallic acid as standard. The results were expressed as gallic acid equivalents in milligrams per 1 g of dry matter.

2.4.5. Monomeric anthocyanin content (ACY)

ACY was determined using the pH differential method [37]. A 1:3 (extract:buffer) dilution was performed using buffer solution of pH 1.0 or 4.5.The samples were left in the dark for 15 min and the absorbances at 510 and 700 nm were then measured. Monomeric anthocyanins content was expressed as cyanidin-3-glucoside (MW: 445.2 and a molar extinction coefficient ε=26 900 L cm^{-1} mol^{-1}) per 1g of dry matter using eq. (4) and (5).

$$A = (A_{510} - A_{700})_{Ph_{1,0}} - (A_{510} - A_{700})_{Ph_{4,5}} \quad (4)$$

$$ACY = ((A \times MW \times DF \times 1000))/((\varepsilon \times l)) \quad (5)$$

2.4.6. Polymeric color percentage (%PC)

The percent of polymeric color was expressed as a % of total color density (%PC = PC/CD x 100). Color density (CD) and polymeric color (PC) parameters were determined using the bisulfite bleaching method, according to [38]. Total color density is a measure of the total color strength of the sample solution, it was calculated as follows:

$$CD = [(Abs_{420nm} - Abs_{700nm}) + (Abs_{\lambda \text{vis}-\max} - Abs_{700nm})] \times Dilution\ factor \quad (6)$$

Polymeric color, an indicator of polymerized pigments, including tannins, and brown compounds was calculated by using the same equation as for CD but applied to bleached samples, assuming that only monomeric anthocyanins get bleached.

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (California, USA, 2014) and InfoStat statistic Software (Córdoba, Argentina). An ANOVA analysis and the Tukey's test were carried out to detect differences ($p<0.05$) between treatments.

3. Results and discussion

Table 1 shows the characterization of the three studied fruits: raspberry, blackcurrant and elderberry. There is a vast bibliography that evidences the strong variability of ACY and TPC values and AA in berries due to numerous factors including: species and cultivar, harvest season, field management practices, ripeness grade and environmental conditions, among others [39-43].

Ultrasound assisted extraction (UAS) was used to improve bioactive compounds extraction from the fruits. The used treatments were: control, application of ultrasound for 5 min (U5) and 10 min (U10); after one, two or three extraction cycles. Bioactive compounds concentration (ACY and TPC), as well as antioxidant capacity were determined in order to evaluate the performance of the different treatments (**Figure 1**). Blackcurrant (**Figure 1b**) and elderberry (**Figure 1c**) received three extraction cycles, while for raspberry (**Figure 1a**) only two extraction cycles were performed as there was no improvement on bioactive compounds after the third stage. The observed trends for ACY were similar to those presented for TPC and AA. Main effects of both factors ($p < 0.05$) “ultrasound treatment” and “extraction cycle” were observed for all response variables. Nevertheless, it could be highlighted that in the case of raspberries, the AA variable presented significant interaction between the factors (**Figure 1(3)a)**. Elderberry showed the highest bioactive values, followed by blackcurrant and raspberry. In spite of the first extraction cycle showed significantly higher values of ACY (**Figure 1(1)**), TPC (**Figure 1(2)**) and AA (**Figure 1(3)**) than the following ones, all the obtained extracts were considered relevant for a fruit powder development, taking into account that the aim of this work was to recover as much as possible the bioactive

compounds from the fruits. Globally analyzing the bioactive content obtained after each ultrasound treatment, an extraction method was selected for each fruit: U10 was the most appropriate for raspberry and blackcurrant, and U5 for elderberry. After applying the optimum treatment, one pool was obtained from each fruit by collecting the supernatant of the consecutive treatments. Then, maltodextrin was added to the extracts (20% w/w) to produce the fluid feeds for spray-drying. After spray-drying the three fruit powders were analyzed, taking into account both bioactive compounds and physical properties. **Figure 2** shows the total phenolic content (a), anthocyanins content (b) and antioxidant activity (c) of the three fruit powders. The ACY loss upon spray drying was lower than 8% in all cases. Bakowska-Barczak and Kolodziejczyk [44] observed that at inlet temperatures lower than180 °C no significant changes in anthocyanins content was detected in blackcurrant spray-dried powders. Silva et al. [45] reported that 180 °C was the optimum inlet temperature to obtain high retention of anthocyanins in jaboticaba peel extract containing 30% MD. On the other hand, an increase in TPC and AA was detected. These results are in accordance with those reported by Poonam Mishra et al. [46], who attributed the TPC increase to polymerization and synthesis of polyphenols due to high temperatures.

When analyzing the ACY content of the fruit powders (**Figure 2**) in comparison with those of the fruits (**Table 1**), it can be observed that the proportion of ACY decrease observed for blackcurrant powder was much higher than that observed for elderberry powder. Therefore, we analyzed the percentage of polymeric color (%PC) in order to evaluate the formation of polymeric compounds which are not detected by the method used to measure ACY. %PC

values were 3.59 ± 0.05, and 2.1 ± 0.6, for blackcurrant and elderberry, respectively. Blackcurrant powder presented higher %PC values than elderberry ones. This fact together with a differential behavior of each fruit due to divergence in composition could account for the observed behavior.

The superficial color variables for the three studied powders are shown in **Figure 3**. Positive a* values were obtained in all cases, having blackcurrant the highest a* value, consistent with the intense reddish tone of this fruit. Regarding b* parameter, elderberry and blackcurrant showed negative values, related to the purple shade of these fruits in comparison to raspberry, that showed a positive b* value and a red/orange hue. Regarding luminosity, elderberry powder was the darkest one, followed by blackcurrant and raspberry. Murugesan and Orsat [47] studied the behavior of spray-dried elderberry juices using different matrices (maltodextrin, soy milk powder, soy protein powder, isolated soy protein and gum acacia). They concluded that the use of maltodextrin minimizes the variation of chromatic values in comparison with others carriers.

Table 2 shows the results of the measurement of several physical properties to the berry powders. Water activity and water content values were relatively low, and glass transition temperature (T_g) values were well above 25°C, suggesting that these glassy powders could be stored at room temperature without risk of physical deterioration. Bakowska-Barczak and Kolodziejczyk [44] reported 5.5% water content for blackcurrant powders containing maltodextrin added to reach 35°Brix.

The three studied powders showed high solubility values (above 90%). Also, Hausner ratio values indicate that elderberry and blackcurrant powders have

low cohesiveness, while raspberry powder has an intermediate cohesiveness according to Jinapong et al. [31].On the other hand, elderberry powder showed very low Carr index, indicating an excellent flowability, while blackcurrant powders showed a good flowability and raspberry powders presented higher CI values related to a fair flowability. Swaminathan et al. [33] when studying spray-drying of jamun fruit juice with 15 to 30% MD (20 DE) as carrier agent obtained powders with high cohesiveness, with Hausner ratio values ranging from 1.57 to 1.72,and poor flowability, with CI values from36 to 42. Caliskan and Dirim [48] reported results similar to Swaminathan et al. [33] in their study of spray-dried powder of sumac extract with 20% MD (DE10–12) as matrix. They reported a Hausner ratio value of 1.5 and a CI value of 34.

Figure 4 shows the external morphology of raspberry (**Fig. 4a**), blackcurrant (**Fig. 4b**), and elderberry (**Fig. 4c**) spray-dried powders. Usually, upon spray-drying spherical particles are produced, as this is the more stable droplet shape [49]. For all the analyzed fruit powders, spherical particles with some degree of shrinkage were obtained. Similar results were observed for blackberry powders spray dried with maltodextrin as carrier [20].

In general, powders flowability depends on the particle shape and size, showing better flow properties the more spherical particles. In the case of small particles (<50 μm) interparticle adhesive forces are significant, and roughness on the surface can obstruct the ability of particles to approach each other. Then, rough particles may show better flowability properties than smooth ones [50]. In the case of the fruit powders, although the spray-dried particles were smaller than 50μm (**Table 3**), adequate flow properties were obtained. This fact could be related to the shrinkage of the spherical particles, which was more evident for

elderberry and blackcurrant powders (**Figure 4**). Raspberry powders showed a smooth surface and less shrinkage than the other studied powders, and this could be related with the lower flowability detected in this powder.

Bulk density values were in the range of those reported by Franceschinis et al. [20] for blackberry powders and Fazaeli et al. [18] for black mulberry powders.

Regarding hygroscopicity, all the analyzed powders showed similar values. Muzaffar et al. [51] optimized the spray drying conditions for production of quality pomegranate juice powder, and the hygroscopicity range proposed to meet the optimum characteristics was between 10.84 and 15.42. Our results fall within the suggested hygroscopicity range. Similar results were reported by Tonon et al. [52] for açai spray dried powder.

Figure 5 shows the water content and the glass transition temperature as a function of water activity for the three fruit powders. A similar water sorption (filled symbols) behavior was observed among the analyzed powders, being blackcurrant the one that adsorbed less water in the range of a_w studied. The glass transition temperature curves (open symbols) indicate that all the powders maintained the glassy state at room temperature (20ºC) up to 52 %RH. This behavior is promising as the three fruit powders show a broad RH range for storage in the glassy state.

All the analyzed powders showed unimodal particle size distribution (**Figure 6**), as previously reported for other spray dried products Gallo et al. [53]. The mean volume particle diameter ($D_{4,3}$) was between 6 and 9 μm, showing elderberry the smaller particle size and blackcurrant the bigger particle size (**Table 3**). This size difference could be attributed, at least in part, to the higher MD proportion present in elderberry powders. Also, span was calculated considering the D_{10},

D_{50} and D_{90} values (**Table 3**); the observed span value for elderberry powder was close to 1, indicating that the size distribution was narrow. In the case of raspberry and blackcurrant powders, the span value was higher indicating a broader size distribution, as it can also be seen in **Figure 6**. Therefore, the type of fruit extract influenced the particle size.

Syamaladevi et al. [54] found similar values of particle diameter for encapsulated red raspberry powder with pre-treatment of high-pressure homogenization. Tonon et al. [52] reported comparable range of particle sizes in spray dried açai juice.

4. Conclusions

Three powders of different physical and bioactive properties were obtained from berries grown in argentine Patagonian. We could increase the recovery of phytochemicals of each fruit improving the extraction process by the application of UAE and several extraction steps. UAE proved to be a satisfactory procedure to improve bioactive compounds extraction. The three berry powders showed low values of a_w and water content and high values of glass transition temperature and solubility. In addition, relatively good flow properties were detected. Altogether the observed physical properties suggest that these powders could be used as stable and versatile ingredients for the food, cosmetic and pharmaceutical industries. Moreover, important differences in color were observed, suggesting their possible applications as natural colorants in several foodstuffs and cosmetics. Also, the powders presented high concentration of phenolic compounds and high antioxidant capacity, suggesting

that they could result in an attractive contribution if used as ingredients in functional foods.

Acknowledgments

This work was supported by University of Buenos Aires (UBACYT 20020150100188BA), the National Council for Scientific and Technical Research (PIP 00224 and PUE0067), and the National Agency for the Promotion of Scientific and Technical Research (PICT-2014-2250).

References

[1] O. Paredes-López, M.L. Cervantes-Ceja, M. Vigna-Pérez, T. Hernández-Pérez, Berries: Improving Human Health and Healthy Aging, and Promoting Quality Life—A Review, Plant Foods Hum. Nutr., 65(2010) 299-308.

[2] J.J. Vulić, A.S. Velićanski, D.D. Četojević-Simin, V.T. Tumbas-Šaponjac, S.M. Dilas, D.D. Cvetković, S.L. Markov, Antioxidant, antiproliferative and antimicrobial activity of freeze-dried raspberry, Acta Period. Technol., 45 (2014) 99–116.

[3] A. Gopalan, S.C. Reuben, S. Ahmed, A.S. Darvesh, J. Hohmann, A. Bishayee, The health benefits of blackcurrants, Food Funct. 3(8)(2012)795-809.

[4] T. C Wallace, Anthocyanins in cardiovascular disease, Adv. Nutr. 2(1) (2011) 1-7.

[5] X. Han, T. Shen, H. Lou, Dietary Polyphenols and Their Biological Significance, Int. J. Mol. Sci. 8(9) (2007) 950-988.

[6] S. Ramos, Cancer chemoprevention and chemotherapy: Dietary polyphenols and signalling pathways, Mol. Nutr. Food Res. 52 (2008) 507–526.

[7] M.A. Soobrattee, T. Bahorun, O. Aruoma, Chemopreventive actions of polyphenolic compounds in cancer, BioFactors 27 (2006) 19–35.

[8] C.C. Neto, Cranberry and blueberry: Evidence for protective effects against cancer and vascular diseases, Mol. Nutr. Food Res. 51 (2007) 652 – 664.

[9] P. Gómez Riera, I. Bruzone, D. Kirschbaum, Visión prospectiva de la cadena de frutas finas al 2030. 1a ed. - Buenos Aires: Ministerio de Ciencia, Tecnología e Innovación Productiva, E-Book. ISBN 978-987-1632-38-1.2014.

[10] D.B. Rodriguez-Amaya, Natural food pigments and colorants, Curr. Opin. Food Sci., 7 (2016) 20–26.

[11] L. Szalóki-Dorkó, M. Stéger-Máté, L. Abrankó, Evaluation of colouring ability of main European elderberry (*Sambucus nigra* L.) varieties as potential resources of natural food colourants, Int. J. Food Sci. Technol., 50 (2015) 1317-1323.

[12] F.J. Francis, P.C. Markakis Food colorants: Anthocyanins, Crit. Rev. Food Sci. Nutr., 28(4) (1989) 273-314.

[13] S. Ersus, U.Yurdagel, Microencapsulation of anthocyanin pigments of black carrot (*Daucuscarota* L.) by spray drier, J. Food Eng., 80(3) (2007) 805-812.

[14] B.R. Bhandari, N. Datta, T. Howes, Problems associated with spray drying of sugar-rich foods, Drying Technol., 15(2) (1997) 671-684.

[15] J. Aguilera, J.del Valle, M. Karel, Caking phenomena in amorphous food powders, Trends Food Sci. Technol., 6(5)(1995) 149-155.

[16] N. Acevedo, C. Schebor, M.P. Buera, Water–solids interactions, matrix structural properties and the rate of non-enzymatic browning, J Food Eng., 77(2006)1108-1115.

[17] S. Youssefi, Z. Emam-Djomeh, S.M. Mousavi, Comparison of artificial neural network (ANN) and response surface methodology (RSM) in the prediction of quality parameters of spray-dried pomegranate juice, Drying Technol., 27(7-8)(2009) 910-917.

[18] M. Fazaeli, Z. Emam-Djomeh, A. Ashtari, M. Omid, Effect of spray drying conditions and feed composition on the physical properties of black mulberry juice powder, Food Bioprod. Process., 90 (2012) 667–675.

[19] C.A. Nayak, N.K. Rastogi, Effect of selected additives on microencapsulation of anthocyanin by spray drying, Drying Technol., 28(12) (2010) 1396-1404.

[20] L. Franceschinis, D.M. Salvatori, N. Sosa, C. Schebor, Physical and functional properties of blackberry freeze- and spray-dried powders, Drying Technol., 32 (2014) 197–207.

[21] B.R. Bhandari, A. Senoussi, E.D. Dumoulin, A. Lebert, Spray drying of concentrated fruit juices, Drying Technol., 11(5) (1993) 1081-1092.

[22] V. Camel, Microwave-assisted solvent extraction of environmental samples, Trends Anal. Chem, 19(4) (2000) 229-248.

[23] B. Lapornik, M. Prošek, A.G. Wondra, Comparison of extracts prepared from plant by-products using different solvents and extraction time. J Food Eng., 71(2) (2005) 214-222.

[24] F. Chemat, M.K. Khan, Applications of ultrasound in food technology: processing, preservation and extraction, Ultrason. Sonochem., 18(4) (2011) 813-835.

[25] M.K. Khan, M. Abert-Vian, A.S. Fabiano-Tixier, O. Dangles, F. Chemat, Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel. Food Chem., 119(2) (2010) 851-858.

[26] F. Chen, Y. Sun, G. Zhao, X. Liao, X. Hu, J. Wu, Z. Wang, Z., Optimization of ultrasound-assisted extraction of anthocyanins in red raspberries and identification of anthocyanins in extract using high-performance liquid chromatography–mass spectrometry, Ultrason. Sonochem., 14(6) (2007) 767-778.

[27] L. Galván d'Alessandro, K. Kriaa, I. Nikov, K. Dimitrov, Ultrasound assisted extraction of polyphenols from black chokeberry, Sep. Purif. Technol., 93 (2012) 42-47.

[28] L.Galván d'Alessandro, K. Dimitrov, P. Vauchel, I. Nikov, Kinetics of ultrasound assisted extraction of anthocyanins from *Aroniamelanocarpa* (black chokeberry) wastes, Chem. Eng. Res. Des., 92(10) (2014) 1818-1826.

[29] W. Horwitz, W. Horwitz, W., Official methods of analysis, 17th ed., AOAC (Association of Official Analytical Chemists), Virginia, USA, 2000.

[30] Código Alimentario Argentino http://www.anmat.gov.ar/alimentos/normativas_alimentos_caa.asp (accesed November 2017).

[31] N. Jinapong, M. Suphantharika, P. Jamnong, Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration, J. Food Eng. 84 (2008)194–205.

[32] United States Pharmacopeia and National Formulary (USP 30-NF25), The United States Pharmacopeial Convention. United States Pharmacopeia and National Formulary, Rockville, MD, 2007.

[33] S. Swaminathan, J.D.B. Sowriappan. F. Sneha, S. Mallela, Effect of inlet temperature on physicochemical properties of spray-dried jamun fruit juice powder, Powder Technol., 274 (2015) 37–43.

[34] R. Sarrate, J.R. Ticó, M. Miñarro, C. Carrillo, A. Fàbregas, E. García-Montoya, J.M. Suñé-Negre, Modification of the morphology and particle size of pharmaceutical excipients by spray drying technique, Powder Technol., 270 (2015) 244-255.

[35] V. Singleton, J. Rossi J. Colorimetry of total phenolics with phosphomolybdic-phosphotungsticacidre agents, Am. J. Enol. Vitic, 16 (1965) 144–158.

[36] Y.S. Coria Cayupán, M.J. Ochoa, M.A. Nazareno, Health-promoting substances and antioxidant properties of Opuntia sp. fruits. Changes in bioactive-compound contents during ripening process, Food Chem., 126 (2011) 514–519.

[37] M. Giusti, R. Wrolstad R., Anthocyanins: Characterization and measurement with UV–visible spectroscopy, Curr. Protoc. Food Analyt. Chem.,2 (2001) 1–13.

[38] M.M. Giusti, R.E. Wrolstad, Characterization and measurement of anthocyanins by UV-visible spectroscopy, in: Wrolstad, R.R., Schwartz, S.J. (Eds.), Food Analytical Chemistry, Vol. 2: Pigments, colorants, flavors, texture, and bioactive food components. John Wiley & Sons Inc., New York, pp. 19-3, 2005.

[39] M.J. Anttonen, R.O. Karjalainen, Environmental and genetic variation of phenolic compounds in red raspberry, J. Food Comp. Anal., 18 (2005) 759–769.

[40] S. Benvenuti, F. Pellati, M. Melegari, D. Bertelli, Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of Rubus, Ribes, and Aronia. J. Food Sci., 69 (3) (2004).

[41]J. Lee, C.E. Finn, Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. J. Sci. Food Agric., 87 (2007) 2665–2675.

[42] R.A. Moyer, K.E. Hummer, C.E. Finn, B. Frei, R.E. Wrolstad, Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes, J. Agric. Food Chem., 50 (2002) 519–525.

[43] S. Silva, E. Costa, C. Calhau, R. Morais, M. Pintado, Anthocyanin extraction from plant tissues: A review, Crit. Rev. Food Sci. Nutr., 57 (14) (2015) 3072-3083.

[44] A.M. Bakowska-Barczak, P.P. Kolodziejczyk, Black currant polyphenols: Their storage stability and microencapsulation, Ind Crops Prod., 34(2)(2011) 1301-1309.

[45] P.I. Silva, P.C. Stringheta, R.F. Teófilo, I.R. de Oliveira, Parameter optimization for spray-drying microencapsulation of jaboticaba (*Myrciaria jaboticaba*) peel extracts using simultaneous analysis of responses, J Food Eng., 117(4) (2013) 538-544.

[46] P. Mishra, S. Mishra, C.L. Mahanta, Effect of maltodextrin concentration and inlet temperature during spray drying on physicochemical and antioxidant properties of amla (*Emblica officinalis*) juice powder, Food Bioprod. Process., 92(3) (2014) 252-258.

[47] R. Murugesan, V. Orsat, Spray drying of Elderberry (*Sambucus nigra* L.) juice to maintain its phenolic content, Drying Technol., 29 (2011) 1729–1740.

[48] G. Caliskan, S.N. Dirim, The effect of different drying processes and the amounts of maltodextrin addition on the powder properties of sumac extract powders, Powder Technol., 287 (2016) 308–314.

[49] A.B.D Nandiyanto, K. Okuyama, Progress in developing spray-drying methods for the production of controlled morphology particles: from the nanometer to submicrometer size ranges. Adv. Powder Technol. 22 (2011) 1–19.

[50] D. Schulze, Powders and bulk solids: Behavior, characterization, storage and flow, Springer-Verlag, Berlin, Heidelberg Germany, 2008.

[51] K. Muzaffar, B.V Dinkarrao, P. Kumar, Optimization of spray drying conditions for production of quality pomegranate juice powder, Cogent Food Agric., 2(1) (2016) 1127583.

[52] R.V. Tonon, C. Brabet, M.D. Hubinger, Anthocyanin stability and antioxidant activity of spray-dried açai (*Euterpeoleracea Mart*.) juice produced with different carrier agents, Food Res Int., 43(3) (2010) 907-914.

[53] L. Gallo, M.V. Ramírez-Rigo, J. Piña, J., V. Bucalá, A comparative study of spray-dried medicinal plant aqueous extracts. Drying performance and product quality, Chem. Eng. Res. Des., 104 (2015) 681-694.

[54] R.M. Syamaladevi, S.K. Insan, S. Dhawan, P. Andrews, S.S. Sablani, Physicochemical properties of encapsulated red raspberry (*Rubus idaeus*) powder: Influence of high-pressure homogenization, Drying Technol., 30 (2012) 484–493.

Table 1 - Characterization of raspberry, blackcurrant and elderberry frozen fruits. Total monomeric anthocyanins (ACY), total phenolic content (TPC), antioxidant activity (AA)

Property	Raspberry	Blackcurrant	Elderberry
Water content (g H_2O/100 g fruit)	85 ± 3	78 ± 2	82 ±2
Total soluble solid (°Brix at 25 °C)	16.75 ± 0.07	16.62 ± 0.36	10.4 ± 0.2
Total acidity (mg citric acid/100 g fruit)	2.08 ± 0.03	2.83 ± 0.04	1.02 ± 0.04
pH	2.82 ± 0.01	3.11 ± 0.04	4.20 ± 0.02
ACY(mg cyn-3-glu/1 g fruit)	0.34 ± 0.02	2.42 ± 0.03	2.30 ± 0.05
TPC (mg gallic acid/1g fruit)	1.74 ± 0,14	4.32 ± 0.18	7.31± 0.34
AA (mg gallic acid /1 g fruit)	0.93 ± 0.03	2.47 ± 0.05	5.48 ± 0.20

Table 2: Physical properties of spray dried raspberry, blackcurrant and elderberry powders.

	Raspberry	Blackcurrant	Elderberry
Water content (% drybasis)	1.40 ± 0.12	1.44 ± 0.10	0.92 ± 0.17
a_w	<0.058	<0.058	<0.059
T_g (°C)	50 ± 1	50± 1	43± 1
Bulk density (g/mL)	0.42 ± 0.02	0.38 ± 0.04	0.38 ± 0.02
Compacted density (g/mL)	0.52 ± 0.03	0.43 ± 0.04	0.41 ± 0.02
Hausner ratio	1.23 ± 0.02	1.15 ± 0.05	1.08 ± 0.02
Carr Index	18.45 ± 1.35	15.35 ± 0.91	5.89 ± 0.24
Solubility (%)	95.4 ± 1,1	92.5 ± 2.2	93.2 ± 0.1
Higroscopicity (%)	13.14 ± 0.95	11.8 ± 0.3	12.74 ± 1.50

Table 3:Particle sizes and span index of the spray –dried fruit powders.

Powder	D[4,3] (μm)	D_{10}(μm)	D_{50}(μm)	D_{90}(μm)	span
Raspberry	8.48±0.30	4.22±0.19	7.48±0.26	13.41±0.49	1.23

Blackcurrant	9.10±0.10	4.64±0.15	8.33±0.02	14.42±0.42	1.17
Elderberry	6.06±0.59	3.16±0.55	5.69±0.61	9.53±0.80	1.12

Figure 1: Monomeric anthocyanin content (ACY) (1), Total phenolic content (TPC) (2) and Antioxidant activity (AA) (3) after first (white bar), second (gray bar), and third (black bar) extraction cycles for raspberry (**a**); blackcurrant (**b**); and elderberry (**c**). Means with the same letter were not significant different ($p < 0.05$). Lowercase letters and uppercase letters were used for main effect of factors “ultrasound treatment” and “extraction cycle”, respectively. In Figure 1 (3)a, interaction between the studied factors was significant ($p < 0.05$), in this case the Tukey test was run for the interaction.

Figure 2: (**a**) Total phenolic content (TPC); (**b**) monomeric anthocyanin content (ACY); and (**c**) antioxidant activity (AA).Raspberry (white bar), blackcurrant (gray bar), and elderberry (black bar) powders. Means with a different letter are significantly different ($p < 0.05$).

Figure 3: b* vs a* (**a**), and L* (**b**) color coordinates for the three studied powders.

Figure 4: Scanning electron micrographs of the surface of spray-dried berries powders. Raspberry (**a**); blackcurrant (**b**); and elderberry (**c**). The magnification was 15000 X.

Figure 5: Water content (filled symbols) and glass transition temperature (T_g, open symbols) as a function of water activity at 20°C, for raspberry (▲,△), elderberry (■,□), and blackcurrant (●,○).

Figure 6: Particle size distributions for the spray-dried fruit powders.

Highlights

- Berry extracts rich in bioactive compounds obtained by several-steps extraction.
- Ultrasound-assisted extraction is suitable to rapidly extract bioactive compounds.
- Berry powders have good physicochemical and functional properties.
- Spray-dried powders can be used as natural colorant or functional ingredient.

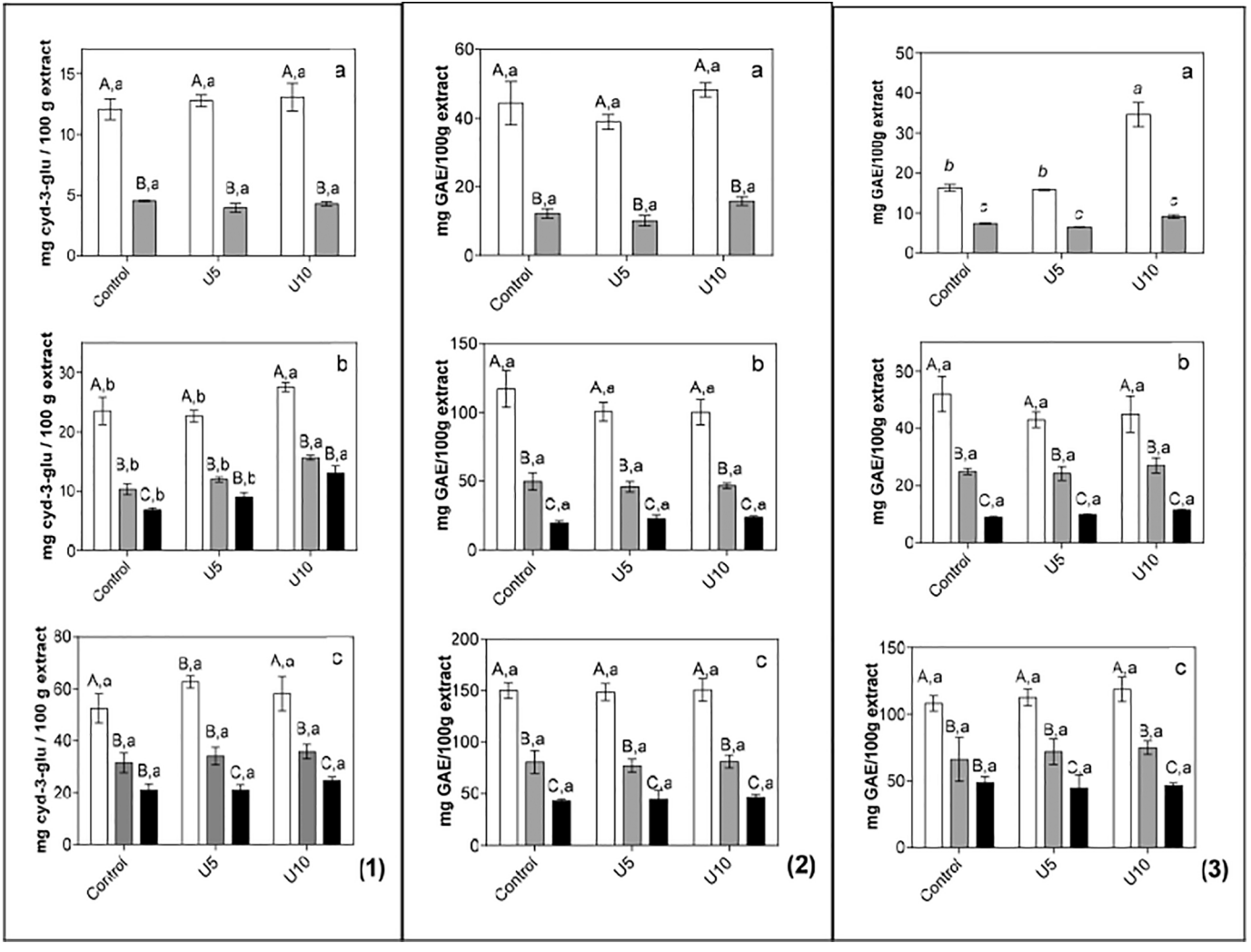

mg cyd-3-glu / 100 g extract
mg GAE/100g extract
Control
U5
U10
(1)
(2)
(3)

Figure 1

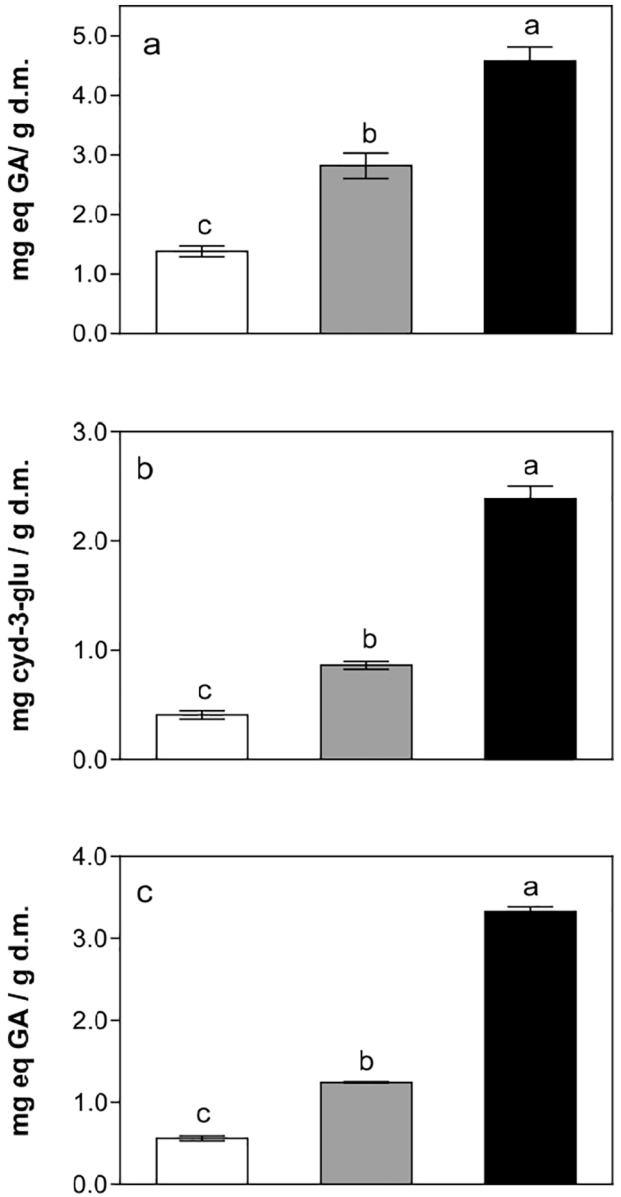

a
mg eq GA/ g d.m.
5.0
4.0
3.0
2.0
1.0
0.0
a
b
c
b
mg cyd-3-glu / g d.m.
3.0
2.0
1.0
0.0
a
b
c
c
mg eq GA / g d.m.
4.0
3.0
2.0
1.0
0.0
a
b
c

Figure 2

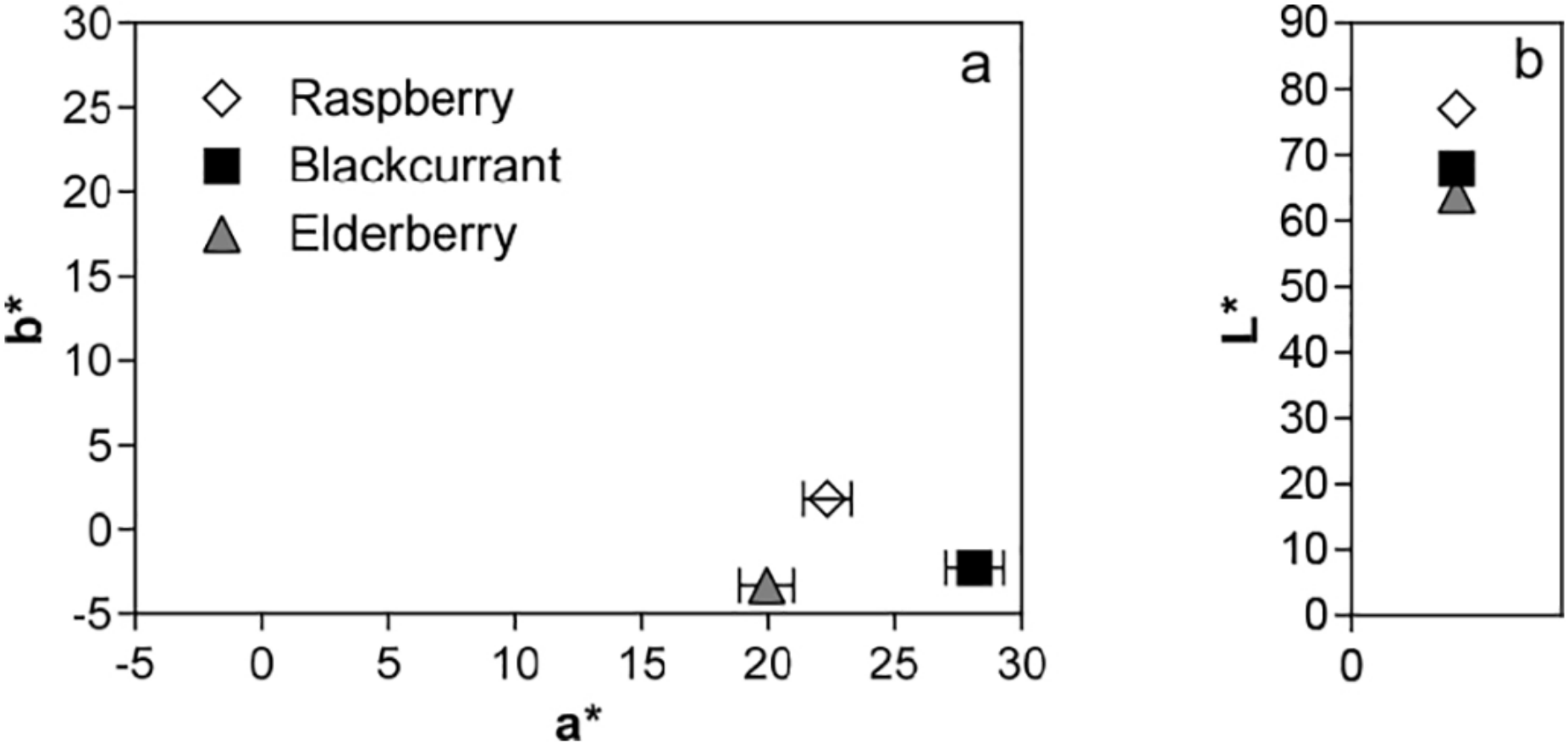
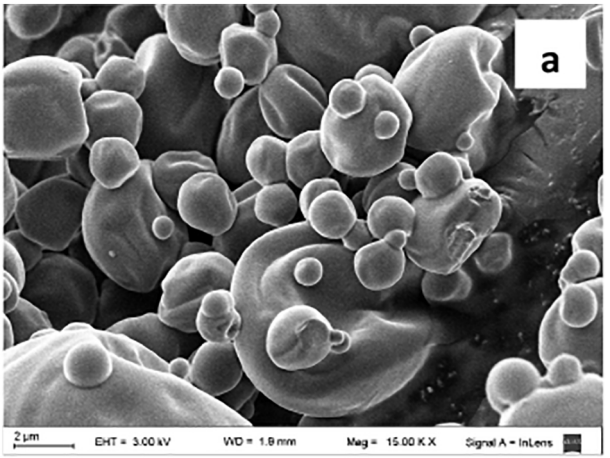
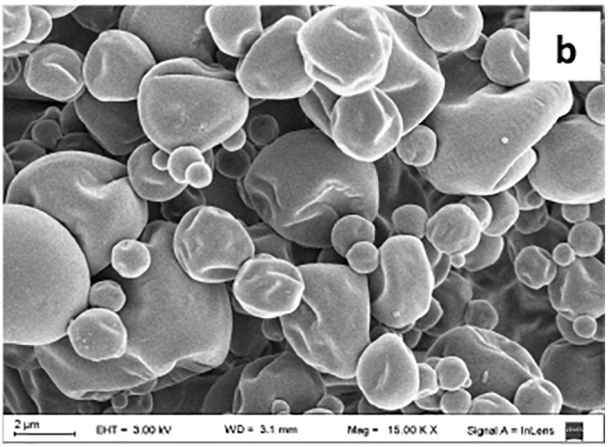

a
Raspberry
Blackcurrant
Elderberry
b*
a*
30
25
20
15
10
5
0
-5
-5
0
5
10
15
20
25
30
b
L*
90
80
70
60
50
40
30
20
10
0
0

Figure 3


a
2 µm
EHT = 3.00 kV
WD = 1.9 mm
Mag = 15.00 K X
Signal A = InLens


b
2 µm
EHT = 3.00 kV
WD = 3.1 mm
Mag = 15.00 K X
Signal A = InLens

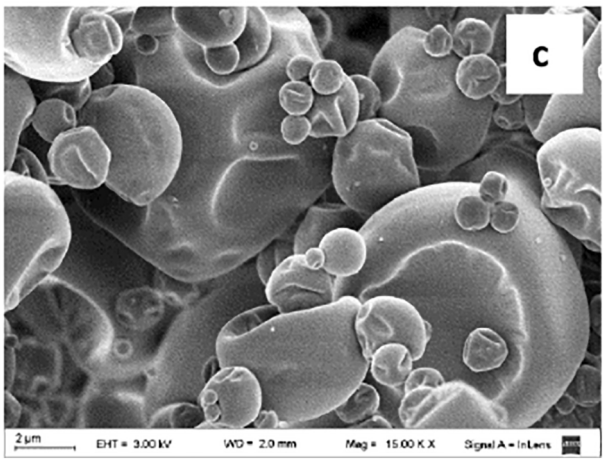

c
2 µm
EHT = 3.00 kV
WD = 2.0 mm
Mag = 15.00 K X
Signal A = InLens

Figure 4

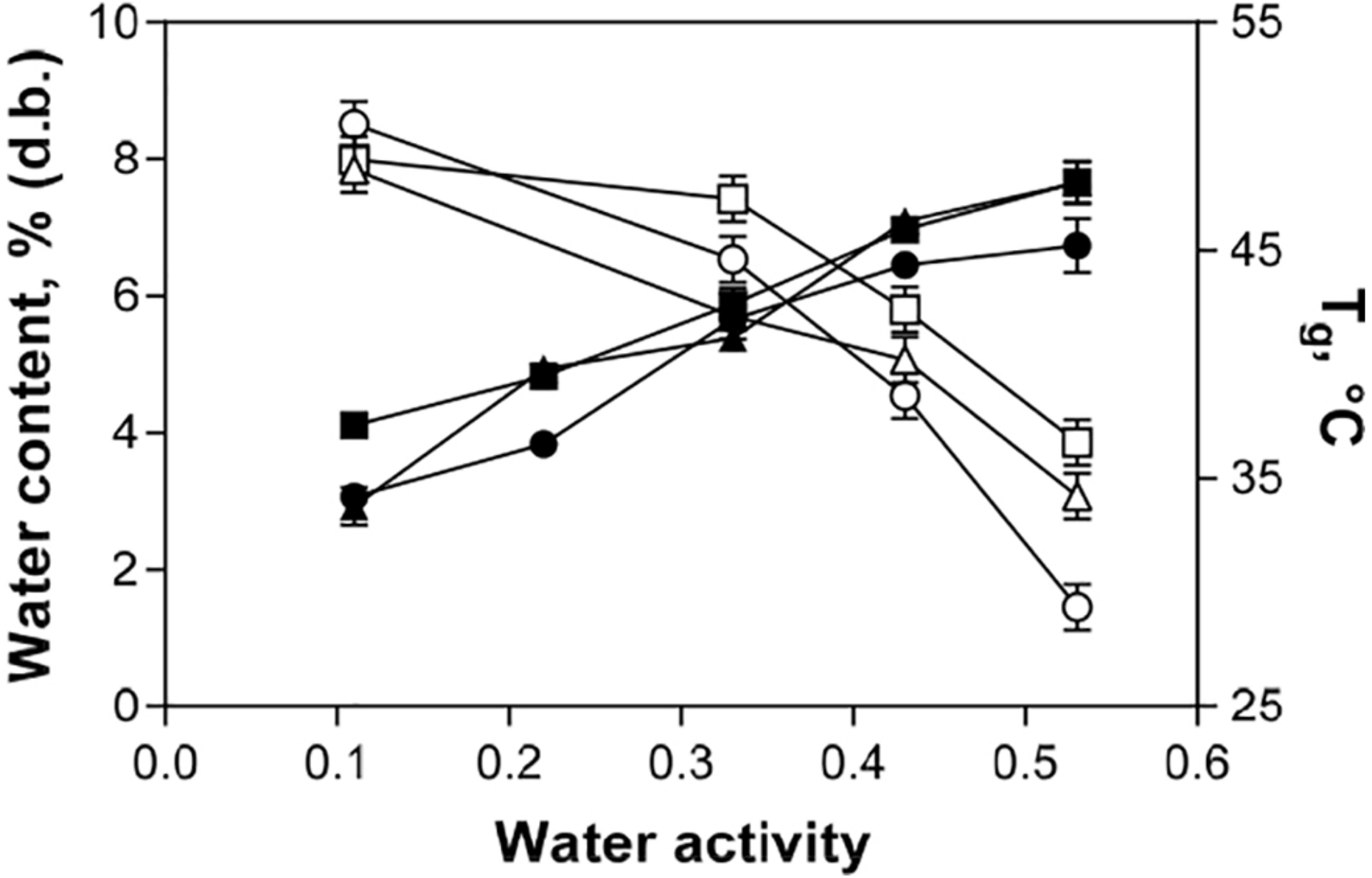

Water content, % (d.b.)
10
8
6
4
2
0
55
45
35
25
T_g, °C
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Water activity

Figure 5

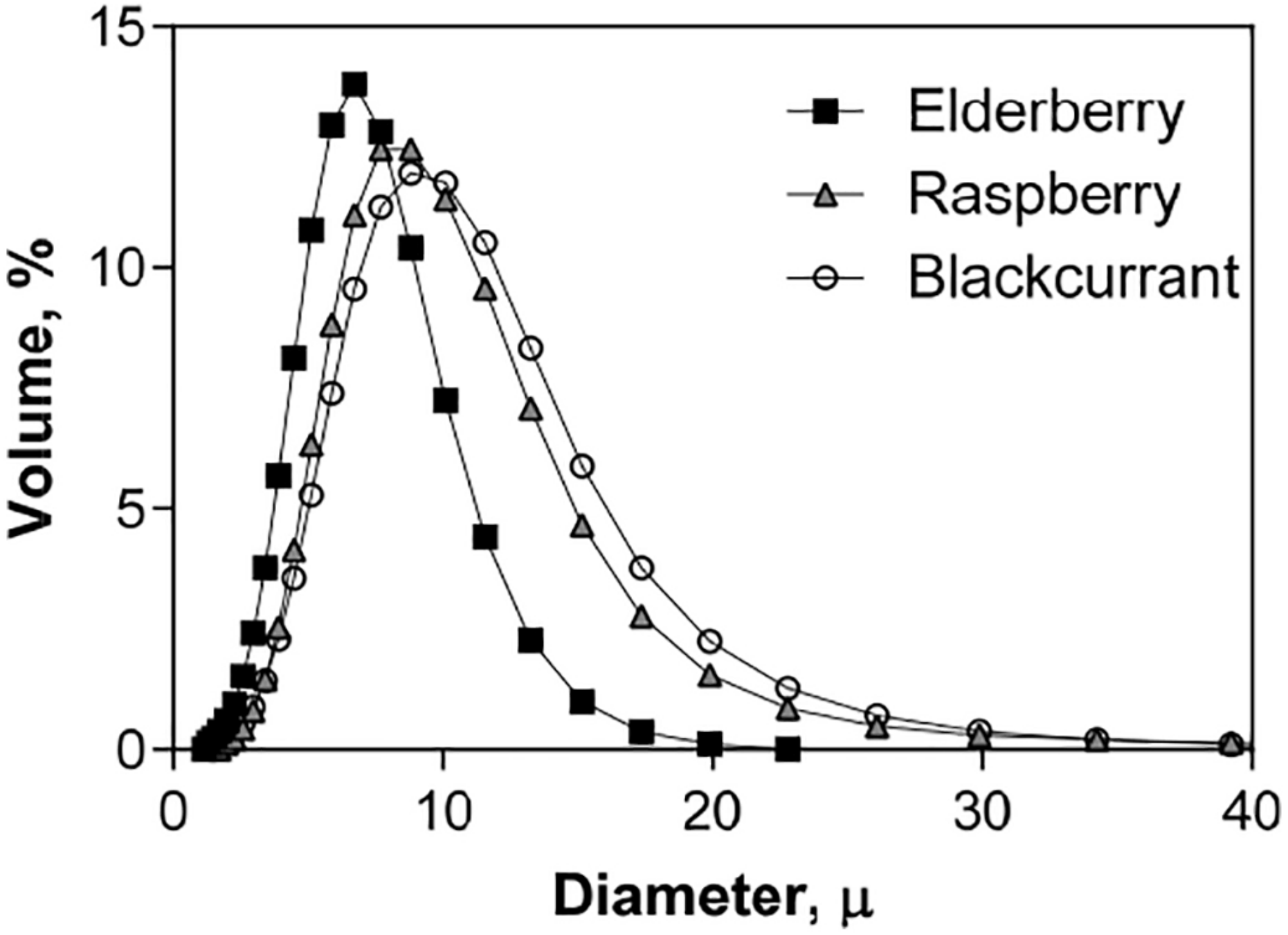

15
10
5
0
Volume, %
0
10
20
30
40
Diameter, μ
Elderberry
Raspberry
Blackcurrant

Figure 6