VACUUM IMPREGNATION AND AIR DRYING TEMPERATURE EFFECT ON INDIVIDUAL ANTHOCYANINS AND ANTIRADICAL CAPACITY OF BLUEBERRY JUICE INCLUDED INTO AN APPLE MATRIX

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Vacuum impregnation and air drying temperature effect on individual anthocyanins and antiradical capacity of blueberry juice included into an apple matrix

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Abstract

Blueberries are an important source of bioactive compounds that can be consumed as fresh or processed fruit. The aim of this work was to study the addition of blueberry juice into apple discs by vacuum impregnation and further stabilization of the impregnated apple by air-drying and freeze-drying in order to produce a low-humidity fruit-like natural snack. The effect of processing operations was studied in terms of functional properties. Results indicated that it was possible to add blueberry juice into the structure of fresh apple slices without a negative effect on bioactive compounds. While air-drying operation implied a significant loss of the initial anthocyanin content, the final product stabilization by freeze-drying did not cause any loss of individual anthocyanin content. Results of the different analyses showed the best final product was obtained by freeze-drying or air-drying stabilization at 40ºC.

Keywords: `Vacuum impregnation, Blueberry juice, Antioxidant capacity, Functional foods, Fruit-like snacks`

1. Introduction

In the last fifteen years, the functional food market has grown significantly as a consequence of increased promotion and consumers awareness of healthy eating and lifestyle (Day et al., 2009). This trend is also promoted and supported by governments and consumers around the world (Arai, 2002; Lajolo, 2002; Milner, 2002; Roberfroid, 2002).

Nowadays, blueberry consumption *(Vaccinium corymbosum)* is of high interest due to its high content of antioxidant compounds (flavonoids and anthocyanins), vitamin C, B, E and A. Blueberries are well known for their anticancer, anti-inflammatory and anti-diabetic properties. In addition, they have important functions in weight loss, macular degeneration and Alzheimer prevention, as well as aging signs reversion, circulation increase and protection and cholesterol reduction (Faria et al., 2005; Yi et al., 2005; Seeram, 2008; Krikorian et al., 2010). Finally, the effectiveness of blueberries in the treatment of urinary tract disorders has been demonstrated (Kalt and Dufour, 1997; Howell et al., 2005; Jepson and Craig, 2007).

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Vacuum impregnation technology is a mass transfer operation between a liquid phase and a porous solid structure, like a fruit or vegetable. Creating pressure gradients in the system constituted by the solid structure immersed into the liquid phase causes a gas outlet and a liquid inlet into the interior of the porous structure (Fito, 1994).

Previous studies have demonstrated vacuum impregnation technology is an effective tool for designing nutritionally balanced, healthy and/or functional new products (Fito et al., 2001; Gras et al., 2003; Sanzana et al., 2011; Betoret et al., 2012b,a).

Moreover, if vacuum impregnation is combined with a stabilization operation like air-drying, it is possible to make a wide range of natural functional products (Fito et al., 2001; Betoret et al., 2003; Alzamora et al., 2005; Betoret et al., 2007, 2011; Hironaka et al., 2011; Betoret et al., 2012b,a; Schulze et al., 2012). The addition of compounds into a food matrix could protect them against deteriorating reactions (Betoret et al., 2011) and aid to modulate the release of these compounds being able to influence on their assimilation (Ribeiro et al., 2006).

The aim of this work was to assess vacuum impregnation with blueberry juice, as well as study the effect of vacuum impregnation and stabilization processes on the content of the most important anthocyanins (delphinidin, cyanidin and malvidin) of blueberry juice included in an apple matrix. The antiradical capacity of the final product and its physicochemical characteristics were also evaluated.

2. Materials and methods

2.1. Reagent and standards

For the HPLC analysis methanol and acetonitrile multisolvent HPLC grade (Scharlab S.L.) and formic acid 98-100% (Scharlab S.L.) were utilized. As standards for HPLC method *Cyanidin-3-O-glucoside* (Kuromanin chloride), *Delphinidin-3-O-glucoside* (Myrtillin chloride) and *Malvidin-3-O-glucoside* (Oenin Chloride) (Extrasynthese S. A.) were utilized. To determine the antiradical capacity the 2,2-diphenyil-picrylhydrazyl (DPPH) (Aldrich, Sigma-Aldrich Co. LLC.) and the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Fluka, Sigma-Aldrich Co. LLC.) were used. For total phenolic determination the Folin-Ciocalteu reagent and monohydrated galic acid $C_6H_2(OH)_3COOH \cdot H_2O$ (Scharlab S.L.) were used. The depectinization was carried out with Viscozyme ® L (Novozymes Corp.). This enzymatic pack is a multi-enzymatic complex of *Aspergillus aculeatus* that degrades the cell walls. Contains β-glucanases (endo-1,3(4)-), cellulases, hemicellulases and xilanases activities.

2.2. Sample preparation

Blueberry juice was obtained from frozen blueberries *(Vaccinium corymbosum)* from a cooperative of Asturias (Spain). Firstly, blueberries were thawed and crushed. Then a depectinization was performed by adding 0.1g of Viscozyme® L per 100g of crushed buleberries (see reagents and standards). The enzymatic treatment was carried out at 50ºC during 2.5h in a reciprocal shaking bath (Unitronic Reciprocal, JP Selecta, Spain). Following enzymatic treatment, the juice was filtered throw a sieve of 0.5mm. This fresh blueberry juice depectinized and filtered will be referred as blueberry juice. Vacuum impregnation was carried out in a vacuum chamber with pressure control (Heraeus vacuum oven, Thermo Fisher Scientific Inc.) using the methodology described by Betoret et al. (2007). Apples *(Granny Smith)* were sliced into discs of 5mm thick, 65mm outer diameter and 20mm inner diameter. Apples discs were immersed in the blueberry juice and a vacuum pressure of 5000Pa was applied for 10min. Then, atmosphere pressure was restored, maintaining apple discs immersed for 10min more. Impregnated apples were air-dried at 30, 40 and 50ºC until a water activity of 0.3 in a tray drier CLW 750 TOP+ (Pol-Eko-Aparatura SP.J., Poland) with transverse air flow, air velocity 2m/s, and an air renewal fee of 50%. Impregnated apples were freeze-dried during 48h in a freeze-drier Lioalfa-6 (Telstar, Spain). In Figure 1 the process carried out and the samples obtained are shown.

2.3. Physicochemical analysis

Moisture and a_w. Water activity was measured using a dewpoint hygrometer (DECAGÓN Aqualab CX-2, ± 0.003) and the water content was quantified by vacuum drying at 60ºC until a constant weight was achieved (method 20.013 (AOAC International, 1980)). The values stated are the average of triplicates.

Suspended pulp and cloudiness. Suspended pulp was measured weighing the precipitate obtained by centrifuging (Medifriger BL-S, J.P. Selecta S.A., Barce-lona, Spain) 10 mL of sample at 365 x g for 10min at 27ºC. The transmittance of the supernatant was measured at 650nm with a UV/visible spectrophotometer (Helios Zeta UV-VIS, Thermo Scientific, Germany) to analyze cloudiness at 365 x g. Then, 5mL of the supernatant obtained at 365 x g was centrifuged at 3000 x g, the precipitate was weighed and the transmittance of the supernatant was measured for cloudiness. The suspended pulp was expressed as grams of suspended pulp per 100g of sample, respect to the initial weighed of the sample and the cloudiness was expressed as a transmittance percent (%T). The results stated are the average of triplicates.

Particle size. Particle size was determined by a laser diffraction instrument, Mastersizer 2000 (Malvern Instruments Limited, Worcestershire, U.K.). The dispersant medium was water. The refraction index used for the samples was 1.350 and the absorption index was 0.1. The results stated are the average of quintuplicates.

Rheology. Rheological measurements were carried out with a rheometer (Haake RheoStress 1, Thermo Electron Corporation, Karlsruhe, Germany) using a concentric cylinder geometry (Z34 DIN 53019). The control rate experiments were done for 300s with an increasing shear rate from 0 to $250 s^{-1}$. The temperature was maintained constant at 20ºC. Product flow behaviour was modeled using the Ostwald-de-Waele model. The parameters k (consistency index ($Pa * s^n$)) and n (flow behaviour index (dimensionless)) for the model were obtained by regression using the software HAAKE RheoWin Data Manager v.3.61.0004. The results stated are the average of triplicates.

Color. Instrumental color was measured using a spectrocolorimeter (CM-3600d, Minolta Co. Ltd., Japan). A white tile and a black chamber supplied by the manufacturer were used for calibration. For each sample three determinations at 120º were done. The results were expressed in CIE L*a*b* color space for illuminant D65 and 10º observer. Total color difference were calculated as $((L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2)^{(1/2)}$. Croma difference and hue difference were calculated as $\Delta C = (C_2 - C_1)$ and $\Delta h = (h_2 - h_1)$. The results stated are the average of triplicates.

Textural analysis. Texture analyses were carried out in a Universal Texture Analyzer (TA. XT2, Stable Micro Systems) by a puncture test. The test was done with a cylinder probe 2mm diameter (P/2) and with a probe advancing speed 2.0mm/s, a trigger force of 0.04903N and a 10mm of distance. The results stated are the average of triplicates.

2.4. Functional components and antiradical capacity

Monomeric anthocyanins. Total monomeric anthocyanins were measured by the pH differential spectrophotometric method developed by Giusti and Wrolstad (2001). Anthocyanin content was quantified using the molar extinction coefficient for *Cyanidin-3-O-glucoside* (2690 m^2/mol). Results were expressed as milligrams of *Cyanidin-3-O-glucoside* equivalents per liter of juice. Monomeric anthocyanins determinations were carried out by triplicate. The total anthocyanins on the air-dried apple at 40ºC were determined by a solid-liquid extraction with methanol acidified with hydrochloric acid (HCl) (1mL/100mL).

Anthocyanins by HPLC. The content of *Cyanidin-3-O-glucoside*, *Delphinidin-3-O-glucoside* and *Malvidin -3-O-glucoside* was determined on the blueberry juice, fresh apple (the fresh apple sample is referred to the natural fresh fruit before vacuum impregnation), impregnated apple and air-dried apple at 30, 40 and 50^{o}C. To determine the anthocyanin content in solid samples each slice was milled and a solid-liquid extraction was performed with methanol acidified with hydrochloric acid (HCl) (0.1mL/100mL). The anthocyanin content was determined by an Alliance® HPLC system (2695 Separation Module, Waters Co. Milford, Mass., USA) coupled to a diode array detector (DAD 2996, Waters Co. Milford, Mass., USA). The chromatographic separation was performed by a Luna II C18 column 250mm x 4.6mm and 5μm (Phenomenex , Torrance, USA). The chromatographic method proposed by Cassano et al. (2011) was used. The injection volume was 10μL. Anthocyanins were identified by their retention times and characteristic spectra compared with standards (*Cyanidin-3-O-glucoside, Delphinidin-3-O-glucoside and Malvidin-3-O-glucoside*). Quantification was performed using calibration curves from 5 - 300mg/kg. Results were expressed as milligrams of anthocyanin per 100g of product or dry matter.

Total phenolic. Total phenolic content was measured using the Folin-Ciocalteu method developed by Waterhouse (2001) and monohydrate gallic acid was utilized as standard ($C_6H_2(OH)_3COOH \cdot H_2O$). Values were measured by triplicate. Results were expressed as milligrams of gallic acid equivalents per liter of juice.

Antiradical capacity. Antiradical capacity was determined by the free radical method proposed by Brand-Williams et al. (1995). The measure was done at 515nm for the radical 2,2-Diphenyil- picrylhydrazyl (DPPH). The antiradical capacity also was determined against the 2,2'-azino-bis(3- ethylbenzothiazoline -6-sulphonic acid) (ABTS) (Re et al., 1999). The absorbance was determined at 734nm. For solid samples, a solid-liquid extraction was performed in the same way as total anthocyanins determination. For the solid samples, the results were expressed as milligrams of citric acid equivalents and milligrams of trolox equivalents per 100g of product or 100g of dry matter, for the DPPH and ABTS method respectively. In the case of antiradical capacity of blueberry juice, the results were expressed in milligram equivalents per liter of juice.

2.5. Statistical analysis

An analysis of variance (ANOVA) test (post hoc analysis) was carried out to determine the significant differences with a confidence level of 95% ($p<0.05$). The software used was Statgraphics Centurion v.16.1.11.

3. Results and discussion

3.1. Vacuum impregnation operation and its effect on functional properties

By means of vacuum impregnation, a cellular structure may be used as a vehicle of the antioxidant compounds of a liquid media, protecting them from oxygen and other deteriorative agents. The amount of liquid media impregnated in a porous structure depends on operation conditions and some physicochemical properties of liquid and solid phases. The characteristics of liquid media, in this case blueberry juice, required to a quantitative impregnation are: a particle size smaller than the porous channels in the cellular structure, a cloud stability assuring suspended particles will not precipitate or block the flow of juice inside the porous matrix and rheological properties that assure the blueberry juice flows inside porous structures. In Table 1 particle size, suspended pulp, cloudiness and viscosity of blueberry juice are shown.

A great difference can be observed between the Sauter Mean Diameter and De Brouckere Mean Diameter which may be attributed to the shape of most of the suspended particles. According to the volume weighted size monomodal distribution represented in Figure 2 and the d_{10}, d_{50} and d_{90} values being near to De Brouckere Mean Diameter, the latter is the most suitable to compare with apple average pore size. Apple pore has a wide range of sizes and varies between cultivars. Near the surface of the skin, the pores appear roughly spherical and do not show much orientation. Inwards, close to the core, the pores appear elongated and show some orientation (Mendoza et al., 2010). Some authors determined different characteristic dimensions of the pores. Rahman et al. (2005) reported a mean pore diameter of 12.07±2.69μm, a linear weighted

average diameter of 109.0±82.1μm and a Sauter mean diameter of 142.6±95.6μm. In our case, considering apple pore size between $12 - 100\mu$m near the 50% of particles can be incorporated into apple structure by vacuum impregnation.

Suspended pulp and cloudiness were determined at two centrifugal forces allowing the evaluation of different kinds of pulp in the cloud of the juice. Centrifuging at 365 x g only removes the less stable pulp while centrifuging at 3000 x g values the more stable cloud because only the smaller particles remain in the supernatant. Although the suspended pulp obtained for blueberry juice after centrifuging at 3000 x g is around the value for commercial pulped fruit juices (Kale and Adsule, 1995; Carbonell et al., 2011; Cerdán-Calero et al., 2013), the great differences between the values determined at two centrifugal forces, make us consider a great amount of not stable pulp suspended in the cloud. In agreement with the available knowledge on cloudiness and turbidity in fruit juices, it can be assumed that the turbid substances mainly consist of suspended proteinacious pectin particles (Grassin and Fauquembergue, 1996; Hilz et al., 2005). However, fractions of other type of cell wall material might also have contributed to part of the turbidity. While the precise locations of anthocyanins and other phenolics compounds and their type of bonding or possible physical entrapment in the cell wall networks of blueberry skin are largely unknown at present, may be a fraction is located in suspended particles and consequently it could not be incorporated by vacuum impregnation. Related with rheological properties, consistency index and flow behaviour index allow the juice to flow inside a porous structure. Due to the characteristics of the blueberry juice does not ensure an homogeneous impregnation of apple slices, the uniformity in the bioactive compounds inclusion was analyzed by quantifying the total phenolic content, total anthocyanin content and the antiradical capacity of the blueberry juice and residual juice after three successive impregnations. Results are shown in Table 2.

As it can be observed in Table 2, there are no statistical differences among blueberry juice and residual juices so it can be assumed that the anthocyanins, phenolics and the other compounds with antiradical capacity are homogeneously incorporated. This result is only possible if the amount of bioactive compounds present in the suspended particles, of a juice whose cloudiness is rather unstable, is negligible. This is coherent with the results of Landbo and Meyer (2004). Taking into account that bioactive compounds have been incorporated into the apple matrix homogeneously, it is possible to predict by the hydrodynamic model (HDM) proposed by Fito et al. (1996), the quantity of anthocyanins included into the apple matrix.

By the experimental procedure described by Fito et al. (1996), it is possible to determine the amount of liquid incorporated (X (m^3 of liquid incorporated/ m^3 of sample) and the volumetric deformation γ (m^3 of sample deformation/ m^3 of sample)). When the vacuum impregnation occurs homogeneously, it is possible to estimate, through the equation proposed by Fito et al. (2001), the content of functional compounds that are going to be included in the porous food matrix. The impregnation of apple discs with blueberry juice has a value of X = 0.179 ± 0.005 m^3 juice incorporated/m^3 fresh apple and γ = - 0.048 ± 0.013 m^3 apple deformation/m^3 apple (Castagnini et al., 2012). The content of cyanidin, delphinidin and malvidin predicted theoretically beside the experimental values determined by HPLC are presented in Table 3.

3.2. Freeze-drying and air-drying temperature effect on functional properties of blueberry juice incorporated into an apple structure

Dried fruits generally have a higher total energy content, increased nutritional density, fiber content and a significantly higher antioxidant capacity compared to fresh, as a result of the concentration that occurs during drying (Bennett et al., 2011). However, the drying process can also result in losses of antioxidant compounds mainly depending on dehydration conditions (temperature, air flow velocity, drying time) (Vinson et al., 2005).

Lots of research works suggest that freeze-drying is the process that produce less losses of bioactive compounds (Genin and René, 1995; Irzyniec et al., 1995; Ratti, 2001; Schulze et al., 2014), making this operation the ideal stabilization method for products with a high content of antioxidant compounds. However, the high costs associated with the operations makes it more difficult their implementation. In this work, the anthocyanin content of vacuum impregnated and freeze-dried samples was analyzed in order to serve as reference. Table 3 shows a strong similarity between the values experimentally obtained and estimated theoretically. Only in the case of cyanidin, uncontrolled factors cause a higher experimental value compared to the theoretical one. A possible explanation could be that the interchange of the apple native intercellular

liquid phase by blueberry juice during vacuum impregnation could induce conversion reactions from procyanidins presents in apple disc to cyanidins being then detected by HPLC analysis. In fact, Guyot et al. (2002) inform that a *Granny smith* apple has a total procyanidin content of 753 ± 131 mg/kg (fresh weight), also Wellmann et al. (2006) reveal that reactions leading to anthocyanidins and, in particular, to proanthocyanidins have remained under debate. By the way, they proposed different type of reactions, enzymatic and non-enzymatic pathways that explain the synthesis of cyanidin and *cyanidin-3-glucoside* from (+)-catechin. In terms of drying methods, as expected, freeze-drying maintained the higher content of anthocyanins in the final product while the air-drying process caused important losses.

Table 3 shows a decrease in anthocyanin content due to the effect of hot air. At 40ºC the content of anthocyanins is less affected than at 30 and 50ºC. It could be related with the combination of temperature and time of treatment. In fact impregnated apples remain 28h at 30ºC, 20h at 40ºC and 12h at 50ºC. The kinetic parameters of anthocyanins degradation in solid and semi-solid foods may depend upon the individual anthocyanin stability, in addition to the influence of composition, structure, physicochemical properties and the presence of other compounds such as flavones and/or organic acid in the food (Patras et al., 2010). The thermic degradation of anthocyanins has been extensively studied for various fruits and their processed products (Jaiswal et al., 2010; Marquez et al., 2012; Ouchemoukh et al., 2012; Rababah et al., 2012). The increments of individual anthocyanins can be explained by mean of the concentration effect due to the loss of water by evaporation and due to the cell wall disruption of the grape skin. The decrement in the content of anthocyanins can be related with the different chemical reactions in which anthocyanins can take part such as polymerization, copigmentation, tannin reactions, and formation of adducts (Marquez et al., 2012). Additionally, it has been reported that losses in the order of 21-25% of total phenolics, 30-43% total anthocyanins and 50-60% of the antioxidant activity in blueberries osmodehydrated with solutions of sucrose, glucose and fructose and dried by hot air at 70ºC (Giovanelli et al., 2013). If it is considered the sumatoria of the content of this three anthocyanins determined by HPLC, the air-drying process induce a decrease of 70%, 56% and 75% for the temperatures of 30, 40 and 50ºC respectively. Bueno et al. (2012) has estimated the anthocyanins intake in the range $12.5 - 250$ mg/day depending on nutritional, social, and cultural differences of the populations investigated, as well as on the methodological approaches applied. Taking into account that the total monomeric anthocyanins content (spectrophotometrically determined) is 54.1 ± 6.5 mg *cyanidin-3-glucoside*/100g dried apple (40ºC), the consumption of 40g of the product provides 21.6mg of anthocyanins, so it can be assumed that this portion of blueberry based food contributes with an interesting content of anthocyanins and phenols to the daily recommended intake.

From the antiradical capacity point of view, even though in Figure 3 there can be observed a decrease in the antiradical capacity, the combination of blueberry juice included into the apple matrix and dehydrated preserve a high antiradical capacity. This is related with the concentration that occurs during the air-drying process and that this "losses" in anthocyanins and phenolics, could be masked by chemical reconversion (Bennett et al., 2011). In this sense, while the dried fruit polyphenols have shown to be bioavailable (Vinson et al., 2005), the relative bioavailability of the non-oxidized forms versus oxidized has not been systematically compared (Bennett et al., 2011).

In Table 4 the results related to the water activity and moisture content of the apple discs after each operation are presented. Water activities of air-dried samples are around 0.3 that assure the stabilization of the product (Labuza et al., 1970; Fennema, 1996; Slade and Levine, 2004). Also, from the functional point of view, it was observed that anthocyanins are more stable with decreasing water activity (Khachik et al., 1986). The freeze-dried sample reach lower water activity values than samples air-dried.

HPLC analysis allow to verify, identify and quantify the incorporation of anthocyanins into the apple matrix. Figure 4 shows the chromatograms for the blueberry juice, fresh apple, freeze-dried sample and vacuum impregnated and air-dried at 30, 40 and 50ºC samples. It can be seen that all the peaks present in the juice are reproduced in the impregnated samples.

3.3. Physical characteristics of the final product

In order to quantify the macroestructural effect of the different stabilization conditions on the final product a textural analysis and instrumental color determinations in the CIE L*a*b* color space were carried out.

The color characteristics are presented in Table 5, it can be seen that practically there is no difference in terms of luminosity for the different stabilization treatments. As a consequence of the air-drying process occurs a shift toggle more saturated colors in the case of AD40 and AD50 samples. Also, in the three conditions of air-drying process, a change in the hue angle towards purple color takes place.

Total colorimetric differences were calculated for the different stabilization process and conditions taking as reference the impregnated apple with blueberry juice. We were unable to find any published studies correlating the total color differences (ΔE) with the visual perception of color changes in blueberry products. Stojanovic and Silva (2007) analyzed the color changes in fruits with different treatments. They measured the color in the Hunter Lab space. They found differences in ΔE of 7 points, but these color changes were subtle to the eye (visual observation), indicating no major changes in the appearance of the blueberries. In this sense although there are differences between the three colorimetric parameters calculated, it could not be assumed that these differences could be significant from the point of view of the consumer. Differences were found between samples freeze-dried and air-dried. The ΔE value for freeze-dried samples were 50% higher than values obtained for air-dried samples.

From the point of view of textural properties, the results presented in Table 7 indicate that air-drying at 40 and 50^{o}C increases the maximum force and slope. A higher slope indicates a higher rigidity of the sample (Vargas et al., 2000) and a higher maximum force indicates a higher resistance of the product (Prothon et al., 2001). In combination these two parameters are related with a crispier behavior. In terms of area, that is an index of energy required to break the structure, there are no significant differences between air-dried samples. In the case of freeze-dried sample, the three values resulted lower than that obtained for air-dried samples. The breaks in the apple cellular structure produced by the freeze-drying process explain this values.

4. Conclusion

It is possible to incorporate 22 milliliters of blueberry juice into the structure of 100 grams of fresh apple discs by vacuum impregnation without negative effect on bioactive compounds present in blueberry juice. Physicochemical properties of blueberry juice makes possible a homogenous impregnation, consequently it is possible to quantify the composition of the impregnated apple by hydrodynamic model equations. Air-drying operation involves losses of 70 and 75% for temperatures of 30 and 50^{o}C respectively, nevertheless, at 40^{o}C it is possible to maintain around 50% of the initial anthocyanin content determined by HPLC. The final product stabilization by freeze-drying does not produce losses in the content of individual anthocyanins. From the point of view of antiradical capacity, air-drying at 40^{o}C allows to maintain a high antiradical power in the final product.

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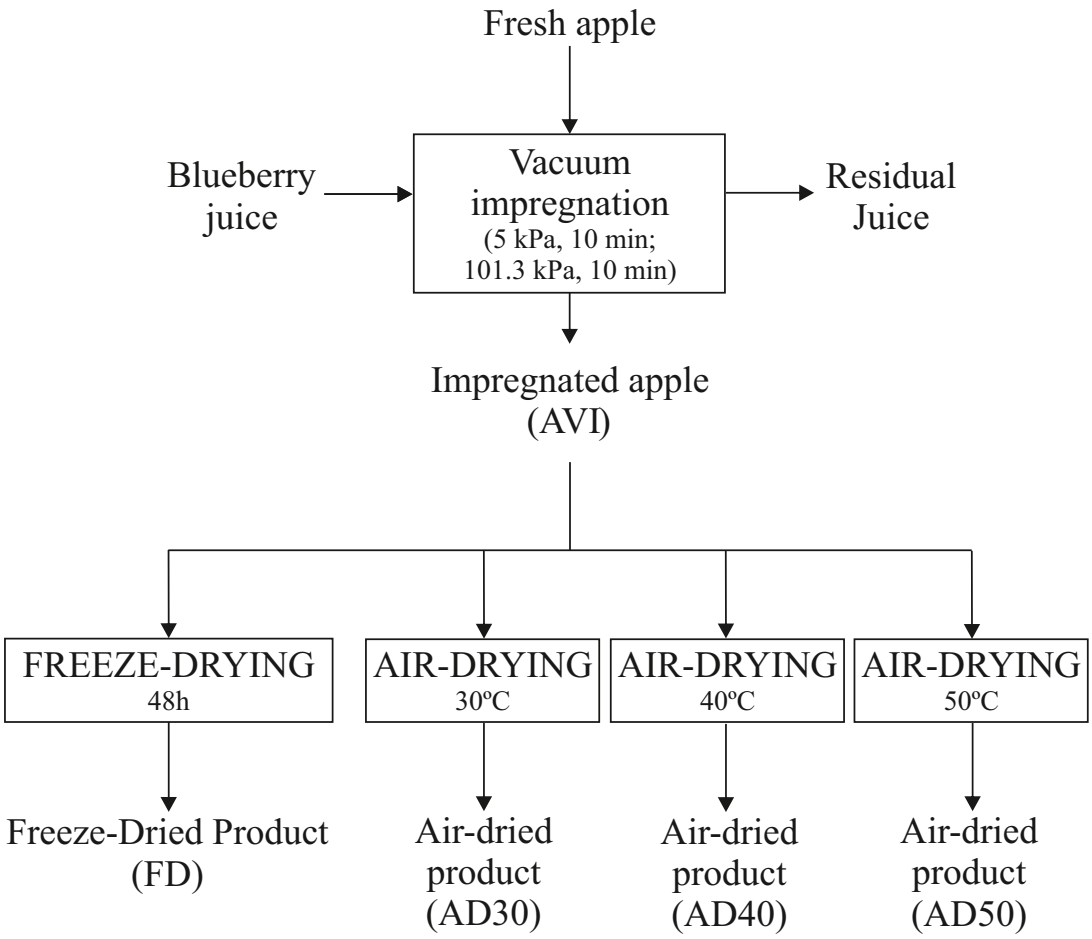

Figure 1: Experimental procedure diagram

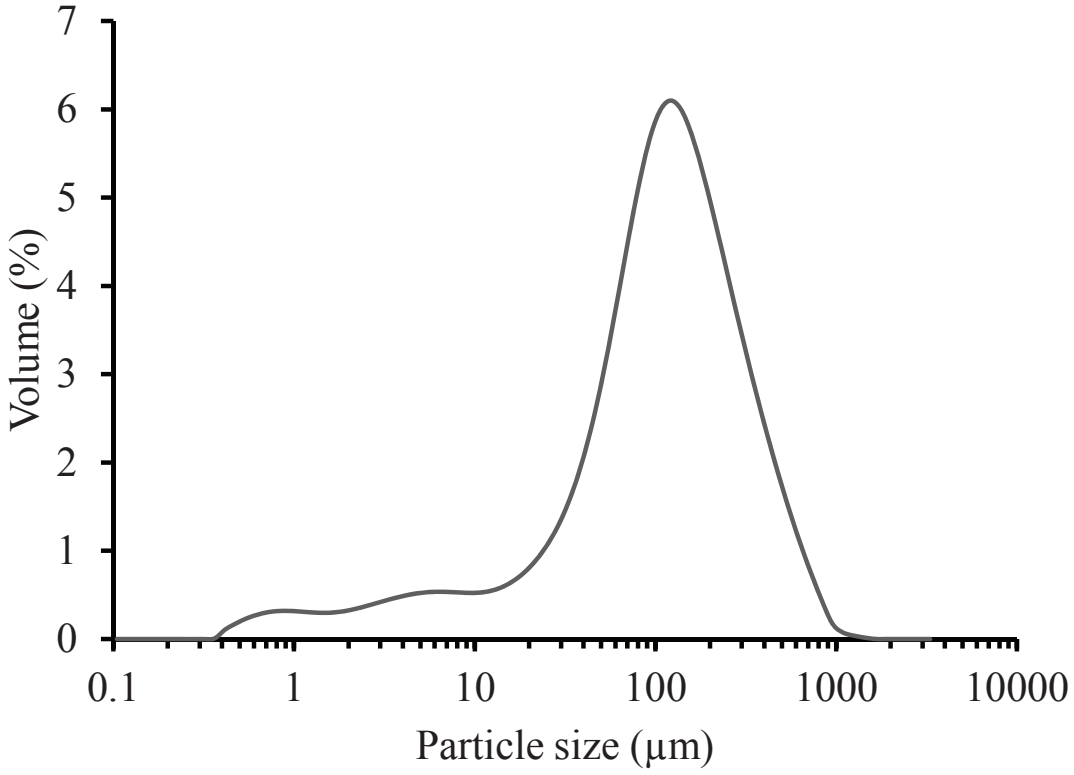

Figure 2: Blueberry juice suspended particle size distribution

Table 1: Particle size, suspended pulp, cloudiness and rheological properties of blueberry juice (mean ± standard deviation)

	Blueberry juice
$D_{[4,3]}$ (μm)	147.21 ± 6.04
$D_{[3,2]}$ (μm)	15.23 ± 0.37
d_{10} (μm)	12.93 ± 1.00
d_{50} (μm)	105.28 ± 2.43
d_{90} (μm)	331.25 ± 14.57
Suspended Pulp 365 x g(g/100g)	36.12 ± 2.19
Suspended Pulp 3000 x g (g/100g)	7.78 ± 0.84
Cloudiness 365 x g (%T)	0.12 ± 0.03
Cloudiness 3000 x g (%T)	14.23 ± 1.46
k	0.57 ± 0.03
n	0.33 ± 0.02

$D_{[3,2]}$ Surface area mean diameter (Sauter Mean Diameter): is the weighted average surface diameter, assuming spherical particles of the same surface area as the actual particles. $D_{[4,3]}$ Volume mean diameter (De Brouckere Mean Diameter): is the weighted average volume diameter, assuming spherical particles of the same volume as the actual particles. d_{10}, d_{50} and d_{90}: a particle size value indicating that, respectively, 10%, 50% and 90% of the distribution is below this value.
k and n: consistency index and flow behaviour index according with the Ostwald-de-Waele model.

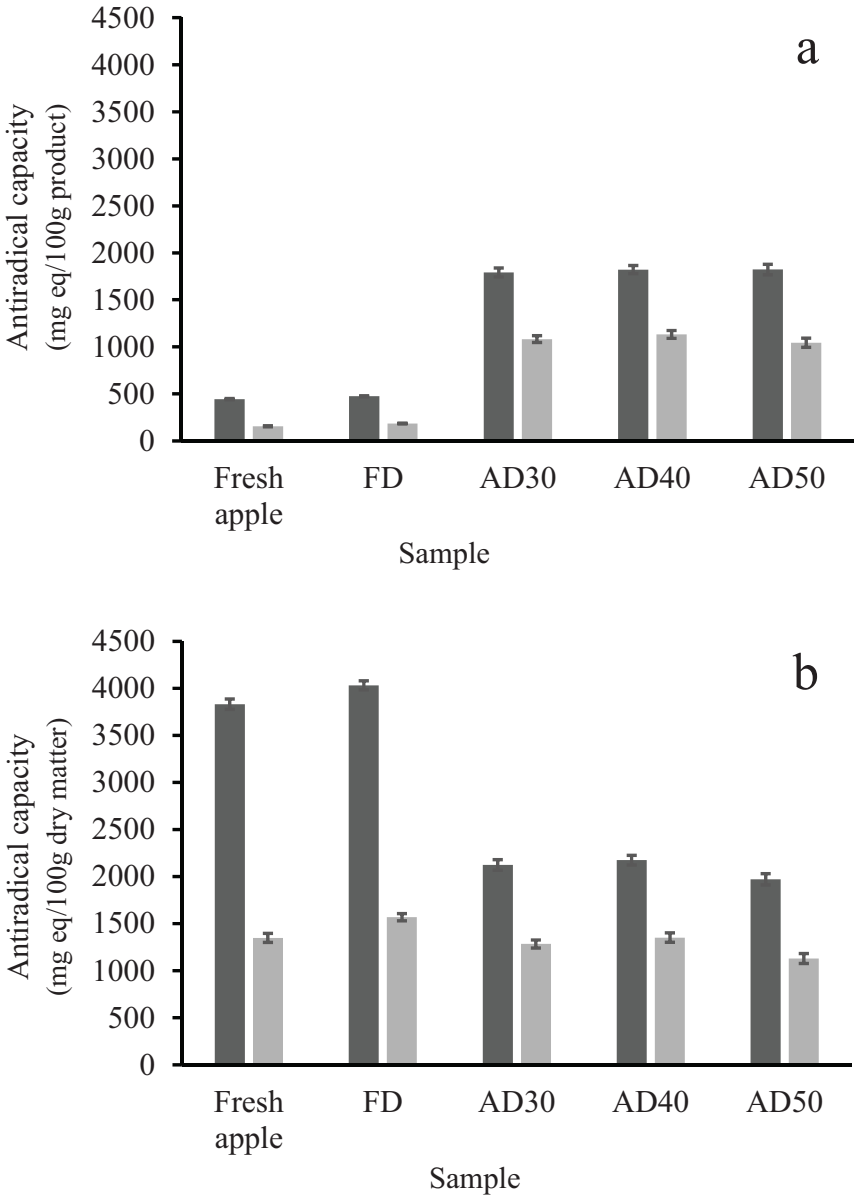

Figure 3: Antiradical capacity of different samples per 100g of product (a) and 100g of dry matter (b). Concentration expressed in milligrams of ascorbic acid equivalents for DPPH method and milligrams equivalent of trolox equivalent for ABTS method

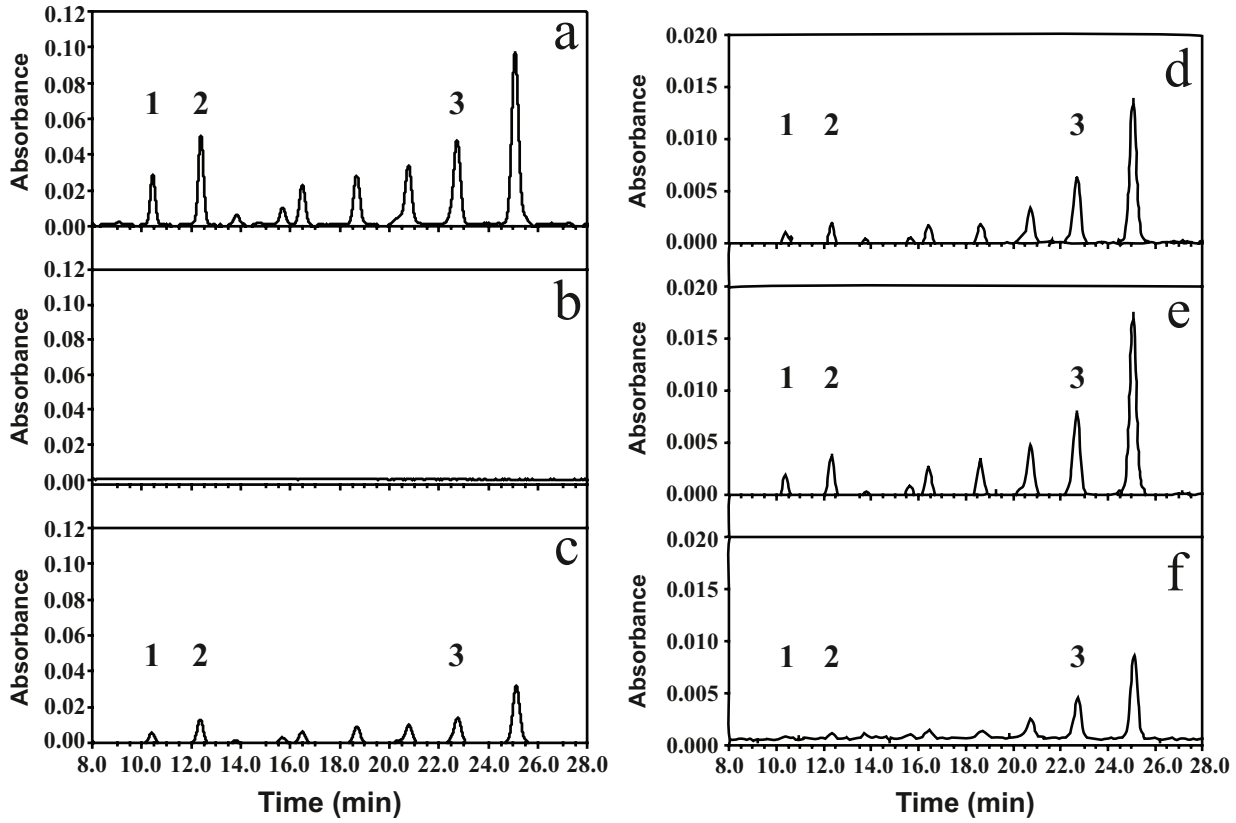

Figure 4: Chromatograms of the samples analyzed by HPLC. a. Blueberry juice, b. Fresh apple, c. Freeze-dried product, d. Air-dried product at 30°C, e. Air-dried product at 40°C, f. Air-dried product at 50°C. Peak N° 1: delphinidin, 2: cyanidin and 3: malvidin

Table 2: Total phenolics content, total anthocyanins content and the antiradical capacity of the blueberry juice and residual juice after three successive impregnations

		Anthocyanins	Total phenolics	Antiradical capacity	
				DPPH	ABTS
Blueberry juice	(mg/L)	447.03 ± 43.03^{a}	1979.76 ± 307.67^{b}	1603.88 ± 154.45^{c}	3499.24 ± 181.14^{d}
JVI_1	(mg/L)	443.69 ± 28.46^{a}	1874.45 ± 253.57^{b}	1604.05 ± 165.56^{c}	3357.45 ± 190.71^{d}
JVI_2	(mg/L)	421.81 ± 35.43^{a}	1690.84 ± 211.16^{b}	1552.82 ± 113.90^{c}	3317.65 ± 80.79^{d}
JVI_3	(mg/L)	404.38 ± 23.85^{a}	1817.05 ± 257.78^{b}	1522.20 ± 135.07^{c}	3420.32 ± 58.27^{d}

Anthocyanins content expressed in mg *cyanidin-3-O-glucoside*/L, total phenolics in mg gallic acid equivalent/L, antioxidant capacity by DPPH method in mg ascorbic acid equivalent/L, antioxidant capacity by ABTS method in mg trolox equivalent/L. Different superscript in the same column means significant differences. JVI: Juice Vacuum Impregnated after 1, 2 or 3 impregnations.

Table 3: Individual anthocyanin content in the juice and impregnated apple

		Delphinidin	Cyanidin	Malvidin	Total
Blueberry juice	mg/L	21.35 ± 2.09	3.07 ± 1.34	43.23 ± 4.67	67.62
AVI*	mg/100g d.m.	3.25	0.47	6.59	10.31
FD	mg/100g d.m.	3.19 ± 0.16	3.46 ± 0.11	6.04 ± 0.17	12.69
AD30	mg/100g d.m.	1.42 ± 0.15	0.03 ± 0.01	2.18 ± 0.16	3.63
AD40	mg/100g d.m.	1.94 ± 0.11	0.39 ± 0.08	3.10 ± 0.35	5.43
AD50	mg/100g d.m.	n.d.	n.d.	3.06 ± 0.11	3.06
Blueberry juice	mg/100g	2.01 ± 0.20	0.29 ± 0.13	4.08 ± 0.44	6.39
AVI*	mg/100g	0.38	0.05	0.77	1.20
FD	mg/100g	0.37 ± 0.02	0.41 ± 0.01	0.71 ± 0.02	1.49
AD30	mg/100g	1.20 ± 0.13	0.02 ± 0.01	1.84 ± 0.14	3.06
AD40	mg/100g	1.72 ± 0.09	0.35 ± 0.08	2.75 ± 0.31	4.77
AD50	mg/100g	n.d.	n.d.	2.83 ± 0.11	2.83

*calculated theoretically.
n.d.: not detected. d.m.: dry matter.

Table 4: Moisture and water activity of fresh, vacuum impregnated, freeze-dried and air-dried samples.

	a_w	Moisture (dry basis) (kg water/kg dry matter)	Moisture (wet basis) (kg water/kg product)
Fresh apple	0.989 ± 0.002^{d}	7.631 ± 0.072^{c}	0.884 ± 0.001^{e}
AVI	0.988 ± 0.001^{d}	7.498 ± 0.421^{c}	0.883 ± 0.002^{e}
FD	0.215 ± 0.003^{a}	0.014 ± 0.003^{a}	0.014 ± 0.002^{a}
AD30	0.348 ± 0.007^{c}	0.186 ± 0.019^{b}	0.156 ± 0.014^{d}
AD40	0.301 ± 0.007^{b}	0.129 ± 0.026^{b}	0.114 ± 0.019^{c}
AD50	0.304 ± 0.004^{b}	0.082 ± 0.003^{b}	0.076 ± 0.003^{b}

Table 5: Instrumental color in the CIE L*a*b* space

Sample	L*	a*	b*	C*	h
Fresh apple	66.2 ± 4.5^{d}	0.3 ± 1.7	18.6 ± 3.6	18.7 ± 3.7^{c}	89.6 ± 4.6^{d}
AVI	27.0 ± 4.1^{ab}	8.0 ± 1.5	4.8 ± 2.1	9.5 ± 1.7^{a}	30.1 ± 11.6^{c}
FD	37.2 ± 2.1^{c}	24.7 ± 1.5	0.6 ± 0.5	24.7 ± 1.5^{d}	1.3 ± 1.1^{a}
AD30	26.9 ± 4.1^{a}	13.5 ± 3.5	2.0 ± 1.0	13.7 ± 3.4^{b}	8.7 ± 4.5^{b}
AD40	31.0 ± 2.0^{b}	20.7 ± 2.5	4.1 ± 1.8	21.1 ± 2.7^{c}	11.0 ± 3.9^{b}
AD50	29.7 ± 4.0^{ab}	18.8 ± 2.0	4.2 ± 1.1	19.3 ± 1.8^{c}	12.9 ± 4.3^{b}

L*: luminosity index; a* green-red color coordinate; b* blue-yellow color coordinate; C*: croma; h: hue.

Table 6: Colorimetric differences

Sample	ΔE	ΔC	Δh
FD	20.1 ± 1.6	15.2 ± 1.5	-28.8 ± 1.1
AD30	7.6 ± 2.8	4.2 ± 3.4	-21.4 ± 4.5
AD40	13.5 ± 2.6	11.6 ± 2.7	-19.0 ± 3.9
AD50	11.8 ± 2.4	9.8 ± 1.8	-17.2 ± 4.3

Colorimetric differences calculated taking as reference the impregnated apple. ΔE: Total color difference. ΔC: Croma difference. Δh: Hue difference.

Table 7: Textural characteristics

Sample	Maximum force (N)	Slope (N/mm)	Area (N.mm)
FD	7.9 ± 2.3^{a}	3.7 ± 1.1^{a}	8.1 ± 4.4^{a}
AD30	11.8 ± 1.1^{b}	2.9 ± 0.6^{a}	17.5 ± 4.0^{bc}
AD40	15.8 ± 1.7^{d}	5.2 ± 0.6^{b}	19.8 ± 2.7^{c}
AD50	14.0 ± 2.4^{c}	5.1 ± 2.0^{b}	14.8 ± 3.4^{b}