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ORIGINAL ARTICLE

Blueberry balsamic vinegar: bioactive compounds and antioxidant activity during processing and assessment of diverse evaporation techniques for juice

Vinagre balsâmico de mirtilo: compostos bioativos e atividade antioxidante durante o processamento e a avaliação de diferentes técnicas de evaporação de suco

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Abstract

Blueberries are widely recognized for their beneficial health effects due to their bioactive compounds content. In addition, balsamic vinegars trade developed quickly because of their wide acceptance in gourmet food. A novel product made with second quality berries, being suitable for human consumption, i.e., blueberry balsamic vinegar, was evaluated. This work aimed to assess changes in Total Anthocyanins (TA), Total Phenolics (TP), and antioxidant activity during production process of blueberry balsamic vinegar, at the following stages: raw material, blueberries juice after enzyme treatment, blueberries alcoholic substrate, blueberries vinegar, concentrated blueberry juice and blueberries balsamic vinegar. Additionally, three alternative evaporation systems, rotary vacuum evaporator, microwave and vacuum microwave, were evaluated in order to determine the concentration method that best retains TA and TP in blueberry juice for its further use in this process. The highest TA and TP retention was achieved by blueberry juice concentration with a rotary vacuum evaporator. On the other hand, both alcoholic fermentation and acetification negatively affected those compounds and antioxidant activity during vinegar production. However, mixing with concentrated juice to obtain blueberry balsamic vinegar allowed balancing nutrient concentration reductions due to processing. The present study showed that production of blueberry balsamic vinegar gives rise to an interesting possibility to reduce losses due to fruit waste while getting added value products with healthy qualities.

Keywords: total anthocyanins; total phenolics; acetification; alcoholic fermentation; juice concentration; *Vaccinium corymbosum* L; berries; gourmet products.

Resumo

Os mirtilos são amplamente reconhecidos por seus efeitos benéficos à saúde pelo conteúdo em compostos bioativos. Além disso, o mercado de vinagre balsâmico se desenvolveu muito rapidamente, em razão da ampla aceitação de alimentos *gourmet*. Neste trabalho, um novo produto foi avaliado, o vinagre balsâmico de mirtilo, produzido com bagas de segunda qualidade, mas adequado ao consumo humano. O objetivo foi analisar as variações de antocianinas totais, compostos fenólicos totais e atividade antioxidante durante o processo de obtenção de vinagre balsâmico de mirtilos, nas seguintes etapas: matéria-prima, suco de mirtilo após tratamento enzimático, substrato alcoólico de mirtilo, vinagre de mirtilo, suco concentrado de mirtilo e vinagre balsâmico de mirtilo. Além disso, para determinar o método de concentração que retém a maior concentração de antocianinas e fenóis totais no suco de mirtilo, três sistemas de evaporação alternativos foram analisados: evaporador rotativo a vácuo, forno de micro-ondas e forno de micro-ondas a vácuo. As maiores concentrações foram obtidas por evaporação em evaporador rotativo a vácuo. Por outro lado, a fermentação alcoólica e a acetificação afetaram negativamente as antocianinas totais, os fenóis totais e a atividade antioxidante durante a produção de vinagre. No entanto, misturar com suco concentrado para obter vinagre balsâmico de mirtilo permitiu equilibrar essas perdas devidas ao processamento. Este trabalho mostra que a produção de vinagre balsâmico de mirtilo abre uma possibilidade interessante de reduzir perdas por descarte de frutos e, ao mesmo tempo, obter produtos de valor agregado com características saudáveis.

Palavras-chave: antocianinas totais; fenóis totais; acetificação; fermentação alcoólica; concentração de suco; *Vaccinium corymbosum* L.; bagas; produtos *gourmet*.

1 Introduction

Blueberries are widely recognized for their bioactive properties as they contribute to protect against coronary and neuronal diseases, cancer, diabetes, among others (Folmer et al., 2014; Beaulieu et al., 2016). Particularly, these berries contain high levels of phenolics, carotenoids, vitamins A, C and E, folic acid and minerals that have shown to prevent oxidative processes by neutralizing free radicals in the human body. The phenolic profile is mainly composed by flavonoids, mostly anthocyanins and some flavonols such as quercetin, and phenolic acids. These compounds are affected not only by plants genetic differences, environmental conditions prior to harvest, fruit maturity at harvest, but also by post-harvest physiological changes, especially during processing and storage (Eichholz et al., 2015; Hornedo-Ortega et al., 2017).

At the beginning of the 2000s, Argentina became an important blueberry supplier to the United States of America (USA), Canada and European Union (EU). However, the increasing volume of discarded fruits (berries), not suitable for export, has caused negative social, economic and environmental impacts (Zapata, 2014). Their high sugars content (10% to 15%), the increasing trends to consume natural products and the fast development of balsamic vinegars trade due to their wide acceptance in gourmet food, open up a possibility of making a novel product, i.e., balsamic type blueberry vinegar. Previous studies regarding its production were not found.

Generic balsamic vinegars are defined as dark products with sweet and sour flavor which must comply with general vinegar regulations (5 to 12 g of acetic acid per 100 mL of product) regardless of their composition and maturing method. They are flavored vinegars and may have low or high viscosity and density (Giudici et al., 2015). Although balsamic vinegars are not regulated in Argentina, national legislation specifies that "[...] vinegars not specifically contemplated by this Code […] must contain a minimum acidity of 4.0% [...]" (Código Alimentario Argentino, 2021, art 1336, p.79).

This research work aimed to evaluate changes in Total Anthocyanins (TA), Total Phenolics (TP), and antioxidant activity in each step of blueberry balsamic vinegar processing. In addition, traditional fruit juice concentration processes result in large quality losses and they are time consuming. Thus, in order to select

the method that best retains TA and TP for blueberry juice concentration destined for subsequent production of blueberry balsamic vinegar, different concentration alternatives (rotary evaporator, conventional microwave, and microwave under vacuum) were assessed.

2 Material and methods

2.1 Raw material

Blueberries (*Vaccinium corymbosum* L.) of different cultivars from *Concordia*, *Entre Ríos* (Argentina) were collected in their optimal stage of maturity during the harvest period (from October to December 2018). Berries discarded by size, deformations, or overproduction but suitable for human consumption were frozen at -18 °C until use in these assays. Prior to beginning the process, 1000 g sample were crushed and used for physicochemical characterization. Total Soluble Solids (TSS), Total Reducing Sugars (TRS), Direct Reducing Sugars (DRS), pH, Total Acidity (TAc), TA, TP, and Antioxidant Activity (AA) were determined.

2.2 Production of blueberry balsamic vinegar

Blueberry balsamic vinegar was obtained according to the flow sheet shown in Figure 1. In order to analyze the influence of each step in the production of blueberry balsamic vinegar, samples were taken in the following stages: blueberries juice, blueberries alcoholic substrate, blueberries vinegar, concentrated blueberry juice and blueberries balsamic vinegar. Total content in main classes of antioxidant compounds that are associated to TA and TP, AA as well as to other quality parameters were assessed.

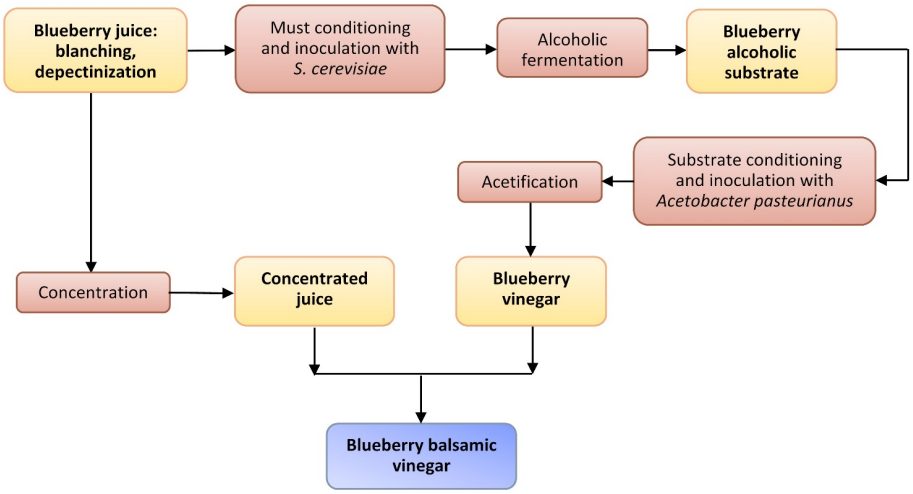

Figure 1. Flow sheet to produce blueberry balsamic vinegar.

2.2.1 Blueberry juice processing

Frozen blueberries were thawed up to 5 °C to prevent microbial, sensorial, and nutritional decay and washed with chlorinated water. Then, they were blanched in a water bath at 95 °C for 2 min and immediately cooled in ice water until reaching 38 °C. This treatment inactivates the endogenous polyphenoloxidases, thus allowing the preservation of anthocyanins and other polyphenolic components with nutritional value (Sablani et al., 2010). The fruits were crushed in a domestic blender until obtaining a homogeneous mixture. In order to avoid must gelling due to high pectin concentration a commercial pectolytic enzyme (Rohapect 10L) was added. Once enzymatic treatment was ended, juice was extracted by filtering through a fine mesh cloth. The volume was divided into two fractions: one was assigned to the preparation of blueberry alcoholic

substrate and the other was maintained at -18 °C until its concentration for must preparation. The TSS, TRS, TAc, pH, TA, TP, and AA were analyzed.

2.2.2 Alcoholic fermentation of blueberry juice

In order to obtain a 12% v/v alcoholic substrate, an additional source of sugars was used to get a fermentation must with 24 ± 1 °Brix. Considering eucalyptus honey is an important regional raw material, required amount to reach that soluble solids content was added to the blueberry juice. It was also supplemented with diammonium phosphate solution (0.5 g/L) as nitrogen and phosphorus source for wine yeasts and sulfite (0.2 g/L potassium bisulfite) to prevent chemical oxidation and growth of spoilage bacteria. The must was inoculated with *Saccharomyces cerevisiae* (IOC 18-2007, Institut Œnologique de Champagne) active dried yeast, and transferred into 10 L glass containers. The alcoholic fermentation was carried out at 25 °C to 28 °C to allow fast ethanol production and the highest phenolic extraction, until no sugar reduction was registered. Alcoholic substrate was racked once the vinification process finished and yeast cells were separated by centrifugation at 2000 × g prior to the acetification stage. Then, the sample was analyzed for ethanol concentration (E), SST, TRS, pH, TAc, TA, TP, and AA.

2.2.3 Acetification of blueberry alcoholic substrate

Alcoholic must was then inoculated with an *Acetobacter pasteurianus* culture isolated in laboratory from regional blueberries (Gerard, 2015), previously revitalized in a reinforced acetic acid and ethanol (RAE) broth. In order to avoid ethanol inhibition over acetic acid bacteria at values up to 6% v/v, the alcoholic substrate was diluted to 4% v/v. A solution with KH_2PO_4 (0.8 g/L), $K_2HPO_4.3H_2O$ (0.35 g/L) and $(NH_4)_2SO_4$ (1.5 g/L) (Davies, 2015) was added to balance nutrient concentrations.

Acetic oxidation was developed by repeated fed-batch fermentation since this operation mode reduces the risk of ethanol inhibition and acetic acid repression, contributes to improve yield and acetification rate over surface fermentations and allows for the reuse of the acetifying culture in the subsequent cycle (Gullo et al., 2014). The laboratory acetification equipment (New Brünswick Cientific Co., Bioflo 2000, New Jersey, USA) consisted mainly of a glass vessel with a maximum working volume of 2 L, PID agitation and temperature sensors, air flowmeter and a sparger located on the bottom of the vessel. The fermenter reload was carried out with a silicon hose adapted to the dispenser of fresh alcoholic substrate. The feeding rate was regulated by a drip valve at 14 mL/min (Davies, 2015).

The *A. pasteurianus* were cultivated in 0.7 L of alcoholic substrate (Ethanol: 4% v/v, Acetic acid: 0.9% to 1.0% w/v) at constant agitation, 677 rpm, and aeration rate, 0.38 vvm (Davies, 2015). Temperature was controlled at 30ºC. Foam produced during the process was suppressed with silicone antifoam (AETN 22, Silicon Argentina SRL).

The assay consisted of the development of four-step feeding. The first one was ended when total acidity reached 4.0% to 5.0% w/v, as acetic acid, in order to avoid bacterial inhibition (Nanba et al., 1984). The second cycle consisted of slowly and progressively addition of 500 mL of fresh substrate to avoid sudden modifications of the biomass environmental conditions that would generate a new lag phase of bacterial growth (de Ory et al., 2004). During the successive feeds, 12% v/v alcoholic substrate was incorporated into the must in order to gradually increase the alcoholic concentration and consequently the acetic acid produced. The acetification process was finished once total acidity stabilized its value (around 8.0% w/v, as acetic acid).

Blueberry vinegar was analyzed for TAc, pH, E, TSS, TA, TP, and AA.

2.2.4 Blueberry concentrated juice

Previous experiments in order to determine the concentration method that best retains antioxidant compounds in blueberry juice were developed. For that purpose, alternative evaporation systems such as rotary vacuum evaporator, conventional microwave, and vacuum microwave were evaluated.

Blueberry juice was concentrated up to 47 ± 2 ºBrix, in the following conditions:

- Rotary vacuum evaporator (RV): a YAMATO RE-46 (Japan) rotary vacuum evaporator was employed to concentrate the juice, in a water bath at 60 ± 2 ºC (Zapata, 2014);
- Microwave (M): a TCL microwave, model 20P10M (Argentine), with 700 W of maximum irradiation power was used. The samples were placed in a 15 cm diameter plastic container suitable for microwaves and concentrated at 250 W (Elik et al., 2016);
- Vacuum microwave (VM): a TOP HOUSE microwave, model MM820CTM (China) was modified in order to manually regulate the continuous power and with an added vacuum system. The samples were placed in a 15 cm diameter plastic container.

In all cases, a 180 mL sample was used because volume was limited by the evaporating flask of the rotary evaporator. All tests were performed in duplicate.

Blueberry concentrated juice was analyzed for TSS, TAc, pH, TA, TP, and AA.

2.2.5 Preparation of blueberry balsamic vinegar

Blueberry balsamic vinegar was obtained by mixing blueberry vinegar with concentrated blueberry juice (Figure 1). Considering the reference legislation, a mass balance was formulated to determine necessary amounts of each component to obtain a product with 6.0% w/v, expressed as acetic acid, and 25 ºBrix. In order to present a complete physicochemical characterization of this new product, TAc, volatile acidity, fixed acidity, pH, dry extract, total ashes, density, TSS, TRS, E, TA, TP, and AA were determined.

2.3 Analytical methods

The following analytical methods were developed: content of TSS (refractometric method), TRS and TDS (Association of Official Analytical Chemists, 2005a), TAc, fixed and volatile acidity (Association of Official Analytical Chemists, 2005b), pH (potentiometric method), E (enzymatic UV method, Boehringer Mannheim/R-Biopharm, Cat. N° 10176290035), dry extract (Association of Official Analytical Chemists, 2005b), total ashes (Association of Official Analytical Chemists, 2005b), and density (digital Mettler Toledo Densito 30PX).

The TA were measured following the pH-differential method (Zapata, 2014) and TP, by the Folin-Ciocalteu reactive reduction (Zapata, 2014). Since some researchers reported differences between testing systems for AA determination, caused by synergistic interactions among the components, the use of at least two assays is recommended (Sakanaka & Ishihara, 2008). Thus, AA was determined by two procedures: 2,2′-azino-bis-(3ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) radical-scavenging assay and 2, 2-diphenyl-1-picrylhydrazyl-hydrate ($DPPH^{\bullet}$) radicals absorbance reduction method (Zapata, 2014). L-ascorbic acid was used as reference antioxidant compound. Assays were performed in triplicate.

2.4 Statistical analysis

Statistical analysis was performed using Statgraphics Centurion XV Corporate software. Data was submitted to Analysis of Variance (ANOVA) and significant differences ($p < 0.05$) between means were determined by Fisher´s Least Significant Difference (LSD) method. Correlation coefficients (r) were

calculated to assess the relationship between antioxidant compounds and AA. When $p < 0.05$, both variables were considered significantly correlated.

3. Results and discussion

3.1 Physicochemical characterization of raw material

Main physicochemical parameters of employed blueberry fruits are shown in Table 1. In order to simplify the analysis, bioactive parameters are depicted in Table 2. The TSS content was 12.5 °Brix which are within the average values (8.9 to 14.0 Brix) reported by Zapata et al. (2013) for blueberries grown in this region. The TRS resulted slightly higher than DRS, indicating that sucrose is present in a minor concentration. Results for pH were similar to that (3.24) registered in previous works (Davies, 2015). Likewise, the TAc agreed with values reported by Zapata et al. (2013) who determined a range from 0.3% to 1.4% w/v as citric acid, for regional blueberries.

Table 1. Physicochemical parameters of blueberry fruits (mean ± standard deviations).

Physicochemical parameters	Determined values
Total soluble solids (°Brix)	12.5 ± 0.2
Total acidity (% w/v, as citric acid)	0.8 ± 0.0
pH	3.34 ± 0.04
Total reducing sugars (% w/v)	10.7 ± 0.2
Direct reducing sugars (% w/v)	9.2 ± 0.3

3.2 Production of blueberry balsamic vinegar

3.2.1 Blueberry juice processing

After fruit enzymatic treatment, an increase in TSS (13.7 ± 0.1 °Brix), TRS (11.9 ± 0.2% w/v) and TAc (0.9 ± 0.0% w/v) was determined while pH (3.14 ± 0.02) was lower than raw material. In addition, an increment in TP was recorded (Table 2). This behavior was already observed in other berries (Klopotek et al., 2005; Ubeda et al., 2013) and grapes (Romero Cascales, 2008) and is probably due to pectolytic degradation of fruit cell wall that allows a relevant diffusion of vacuole components (phenolics, sugars, acids) and thus favoring a better must extraction during pressing stage (Gómez-Plaza et al., 2010). However, the TA did not show significant differences. This behavior was previously reported by Cvejić & Atanacković (2015) who established that in most cases, addition of pectolytic enzymes did not increase TA, though an increase in TP was observed. The AA did not show statistically significant differences in both analyzed methods, probably owing to AA in *Vaccinium* species which is mainly provided by anthocyanins (Cásedas et al., 2017) or released phenolics contributed AA to a lesser extent (Dávalos et al., 2005).

3.2.2 Alcoholic fermentation of blueberry juice

Prior to the inoculation with *S. cerevisiae,* the addition of honey to the blueberry juice increased TSS up to 24.2 ± 0.1 °Brix and TRS to 22.3 ± 0.2% w/v. After 15 days of alcoholic fermentation no TSS reduction was registered, indicating the end of this biological stage. At this point, the TSS values were 9.5 ± 0.1 °Brix, TRS, 0.9 ± 0.0% w/v, TAc, 0.9 ± 0.1% w/v expressed as citric acid, and E, 12.017% v/v. The reduction in TSS and TRS demonstrated sugar assimilation by yeasts with the consequent ethanol production. According to these values, typical fermentative parameters were calculated. It was verified 95.9% of substrate consumption, an ethanol yield ($Y_{P/S}$) of 0.44 g ethanol/g total sugars consumed, and a process efficiency (η, ratio considering theoretical yield of ethanol: 0.511 g ethanol/g sugar) of 83.2%. Although the substrate

showed a high assimilation percentage, difference with efficiency value might indicate an inhibition of metabolic activity associated to the accumulation of ethanol or a possible exhaustion of nutrients along the fermentation. Similar results were reported by Fonseca et al. (2018) who determined 96.5% of substrate consumption, an ethanol yield of 0.43 g/g, and a process efficiency of 84% when performing a blueberry alcoholic fermentation.

A reduction in all bioactive components was observed after alcoholic fermentation, however, the highest loss was registered in TA content (85%). This agrees with previous investigations where TA decreased 87% during blueberries alcoholic fermentation (Davies, 2015) using the same *S. cerevisiae* yeasts strain as inoculum. Similar results were found by Ubeda et al. (2013) who determined a 63% to 85% decrease during strawberry alcoholic fermentation and Klopotek et al. (2005), with a reduction of 69% to 79% when strawberry vinegar was produced. Indeed, the TA degrade could be related to their adsorption to the cell walls of yeast strains (Ubeda et al., 2011, 2013; Morata et al., 2019; Echeverrigaray et al., 2020), and condensation reactions with acetaldehyde formed during metabolism of yeasts (Bosso & Guaita, 2008). In addition, the disappearance of monomeric anthocyanins has been reported during vinification due to polymerization reactions and formation of new pigments of low and high molecular weight (Pérez-Trujillo et al., 2011).

The TP decreased 63.5% after alcoholic fermentation. Other researchers also found losses in these compounds as following: 49% for strawberry wine (Hornedo Ortega et al., 2017), and 43% for persimmon wine (Ubeda et al., 2011). According to Vasantha Rupasinghe et al. (2017), most phenolic acids were increased by 27% to 188%, while others decreased by 5% to 30% during fermentation of Korean black raspberry juice. They concluded fermentation induced changes in phenolic composition and the proportion of the variation is related to the metabolism of the yeast strains.

After alcoholic fermentation, both DPPH• and $ABTS^{+}$ scavenging activity was lower than AA of the juice used as substrate by almost 40.7% and 14.4%, respectively. Other authors have also informed decay by 16% to 39% in the AA of pomegranate juice after winemaking (Mena et al., 2012; Ordoudi et al., 2014) and they suggested that it was due to TP reduction.

Table 2. Total Anthocyanins (TA) and Total Phenolics (TP) content along with DPPH and ABTS radical scavenging activity at different stages during blueberry balsamic vinegar processing (mean ± standard deviations).

	Total Anthocyanins (TA) (mg cyanidin-3-glucoside/L)	Total Phenolics (TP) (mg GAE/100 mL)	DPPH (mg AAE/100 mL)	ABTS (mg AAE/100 mL)
Blueberries (fruit)	$1419.39 \pm 1.49^{(1)d}$	$381.73 \pm 0.03^{(2)d}$	$383.11 \pm 1.77^{(3)d}$	$249.02 \pm 3.31^{(3)d}$
Blueberry juice with enzymatic treatment	1430.51 ± 16.49^{d}	523.45 ± 3.30^{e}	396.71 ± 3.14^{d}	261.61 ± 2.41^{d}
Blueberry alcoholic substrate	212.21 ± 11.80^{b}	191.12 ± 1.02^{b}	235.10 ± 5.12^{c}	223.91 ± 8.78^{c}
Blueberry vinegar	6.45 ± 1.02^{a}	138.35 ± 5.82^{a}	103.75 ± 3.31^{a}	69.05 ± 11.92^{a}
Blueberry juice concentrate	2044.37 ± 78.23^{e}	623.50 ± 18.56^{f}	651.79 ± 41.95^{e}	258.26 ± 6.48^{e}
Blueberry balsamic vinegar	284.25 ± 19.12^{c}	288.72 ± 2.68^{c}	187.95 ± 4.99^{b}	88.14 ± 7.73^{b}

Different letters in each column indicate statistically significant differences ($p < 0.05$) among components means in each process stage. GAE: Gallic Acid Equivalent. AAE: Ascorbic Acid Equivalent. [(1)]TA values were expressed as mg cyanidine-3-glucoside/kg of fresh fruit; [(2)]TP values were expressed as mg GAE/100 mg of fresh fruit; [(3)]Antioxidant activity (DPPH and ABTS) was expressed as mg AAE/100 mg of fresh fruit.

3.2.3 Acetification of blueberry alcoholic substrate

The assay consisted of the development of four acetification cycles and was finished when the acetic acid production stabilized. The first cycle took 46 hours and was developed until TAc reached 4.7% w/v expressed as acetic acid. An addition of alcoholic substrate was done and the second cycle reached 5.4% w/v at 80 hours processing. After the next substrate incorporation, maximum acidity was reached (8.2% w/v). However,

when the last addition of substrate was carried on, TAc decreased due to the dilution effect, but did not continue the increasing trend and only reached the previous maximum value. Finally, the TAc was 8.2% w/v and was obtained after 120 hours processing. This value remained constant for the next 36 hours so the process was finished. The pH was 2.51 ± 0.03, similar to other vinegars (2.47 ± 0.02) previously produced with regional berries (Davies, 2015). Regarding the E concentration, it resulted in 0.577 ± 0.034% v/v which indicated the presence of ethanol not metabolized possibly owing to acetic acid inhibition. The TSS showed a small decrease (8.7 ± 0.1 ºBrix) with respect to the alcoholic substrate (9.5 ºBrix). Since *Acetobacter* species show diauxic growth on media containing both ethanol and glucose, this reduction may be due to ethanol repression on glucose assimilation (Gullo et al., 2014).

Both TA and TP showed a reduction in their contents, 97% and 28% respectively, with respect to final values determined after alcoholic fermentation. Decrease in these bioactive compounds has been recorded by several authors (Su & Chien, 2007; Cerezo et al., 2010; Ubeda et al., 2013; Davies, 2015; Cunha et al., 2016; Hornedo-Ortega et al., 2017) although ratio varies according to acetification system, contact of the fruit with its skin during alcoholic fermentation, and phenolic composition of the raw material.

The significant reduction in the TA content was attributed to their great susceptibility to oxidation (Cerezo et al., 2010; Ubeda et al., 2013) considering the strong aeration and high processing time, although other researchers attributed this reduction to polymerization reactions with other phenolics (Andlauer et al., 2000; Cerezo et al., 2010). Although anthocyanins are more stable at low pH, the reduction does not prevent the effects of acetic biooxidation (Hornedo-Ortega et al., 2017). Studies in strawberry vinegars recorded reductions between 97.2% (Klopotek et al., 2005) and 99% (Ubeda et al., 2013) after acetification stage, while Su & Silva (2006) reported a 79% reduction during blueberry vinegar processing and this was attributed to the aforementioned factors. A 44% decrease was recorded in previous experiences with blueberry vinegars in batch system, however the duration of this stage was 40 h approximately (Davies, 2015), while in the present experience, the exposure to oxygen was higher (144 h).

The TP decrease was also corroborated during the acetification of blueberry wines with losses of approximately 35% (Ubeda et al., 2013), whereas Cunha et al. (2016) reported reductions among 17% and 30% during blackberry vinegar processing. Different TP final values depend on substrate individual composition since polymeric phenolics are oxidized more slowly than monomeric ones (Andlauer et al., 2000). In addition, slow acetification systems (Orleans method) contribute to minor losses of such compounds in relation to processes that use air injection (Ubeda et al., 2013).

Both AA assays showed a loss from alcoholic substrate to vinegar, i.e., a 56% and 69% reduction was registered when using DPPH and ABTS methods, respectively. Many research studies (Dávalos et al., 2005; Cerezo et al., 2010; Bakir et al, 2016) have reported lower AA in vinegars than in wines, indicating acetification could decrease phenols content with high AA, and/or generate new ones with weaker AA than those present in the alcoholic substrate. Bakir et al. (2016) reported an antiradical potential almost 56% lower than that of the fresh juice when acetifying pomegranate and it was attributed to degradation of strong radical scavengers during fermentation processes.

3.2.4 Blueberry concentrated juice

Evolution of TSS against time of process for three concentration methods is indicated in Figure 2.

Time required to reach 47 ± 2 ºBrix was 60 min for M, 65 min for RV and 90 min for VM. Several investigations (Yousefi et al., 2013; Elik et al., 2016) reported microwave energy decreases concentration times because the heat is generated within the food by water molecular friction. As previously mentioned, TP and specifically TA are highly sensitive to degradation during traditional fruit juices concentration (Barba et al., 2012). Since temperature is one of the factors affecting TA stability, final product temperature

was registered in each concentration process. While 49 ± 1 ºC and 48 ± 1 ºC were the final juice concentrated temperatures in both vacuum methods, RV and VM, 75 ± 1 ºC was reached in conventional M.

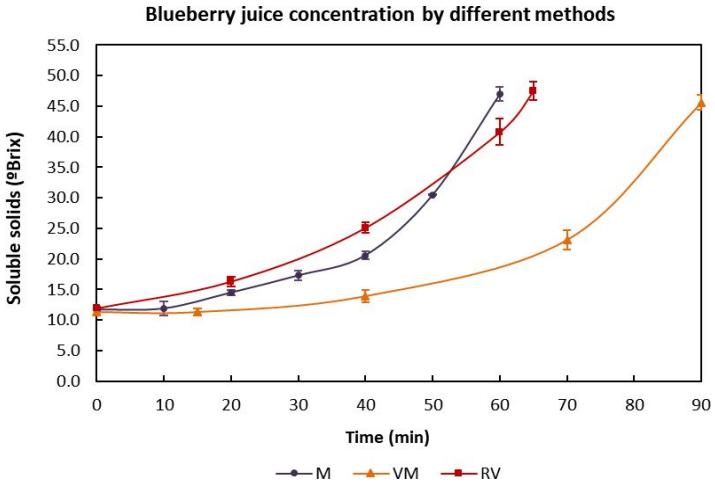

Figure 2. Total soluble solids (means ± SD) in blueberry juice during concentration with different methods. M: microwave, RV: rotary vacuum evaporator, VM: vacuum microwave.

The TA in blueberry juice was 1414.45 ± 9.66 mg cyanidin-3-glucoside/L, whereas TP value was 662.92±7.03 mg GAE/100 mL. Statistical analysis after concentration showed significant differences ($p < 0.05$) according to the evaporation method (Table 3). The highest concentration of both TA and TP was determined in samples treated with RV, probably by maintaining the temperature at levels (48 ± 1 °C) in which bioactive compounds stability is favored or their degradation is diminished. Although similar temperatures were recorded in VM samples (49 ± 1 °C), the lower values of TA and TP may be due to longer processing time (90 min) with respect to M and RV (60 min and 65 min, respectively). Similar results were reported by Kirca & Cemeroğlu (2003).

Elik et al. (2016) reported higher TP content in microwave treated samples (200 and 250 W) than in those concentrated with rotary evaporator. They attributed this result to the shorter process time required in the first method. However, the sample volume was 75 mL, significantly lower than the one used in the present research study (180 mL), which may be affecting the concentration times.

Table 3. Total Anthocyanins (TA) and Total Phenolics (TP) in blueberry concentrated juice after different concentration methods.

Sample	TA (cyanidin-3-glucoside/L)	TP (mg GAE/100 mL)
RV	2164.30 ± 49.60^{a}	813.42 ± 64.57^{a}
M	1299.14 ± 81.42^{c}	724.40 ± 14.40^{b}
VM	1561.80 ± 79.34^{b}	545.94 ± 14.43^{c}

Different letters indicate significant statistical differences ($p < 0.05$). GAE: Gallic Acid Equivalent. RV: rotary vacuum evaporator, M: conventional microwave, VM: vacuum microwave.

Based on these results, blueberry juice was concentrated in a vacuum rotary evaporator at 60±2 ºC in order to be used in blueberry balsamic vinegar preparation. As expected, both TA and TP increased their concentrations after evaporation treatment in RV (Table 1). The AA determined with DPPH assay also increased (64%), whereas with ABTS technique no significant differences were recorded. This could be explained by the fact that each method measures a different aspect of antioxidant activity and the capacity of free radical scavenging does not always correlate well with the capacity to inhibit oxidation (Niki, 2011; Ubeda et al., 2011).

3.2.5 Preparation of blueberry balsamic vinegar

According to the mass balance, blueberry vinegar was blended with concentrated juice. Both TAc and TSS were determined in the mixed product: 5.9 ± 0.1% w/v (expressed as acetic acid) and 26.1 ± 0.1°Brix, respectively. Furthermore, an increment in all bioactive compounds was recorded. Specifically, a 44-fold increase in TA and 2-fold increase in TP was determined with respect to those in vinegar samples (Table 2). Therefore, vinegar mixed with concentrated juice allowed balancing concentration reductions of bioactive compounds due to processing.

As stated previously, blueberry balsamic vinegar is a novel product so, a physicochemical characterization was performed (Table 4). As can be seen, the TAc is in the range (5% to 12% w/v) indicated by Giudici et al. (2015). In order to have more reference sources, *Código Alimentario Español* (España, 2012) was consulted and according to it, balsamic vinegars must contain a minimum total sugar content of 150 g/L, and a maximum residual alcohol of 0.5% v/v. Therefore, it can be said blueberry balsamic vinegar fulfills those few established parameters.

Table 4. Physicochemical characterization parameters of blueberry balsamic vinegar (mean ± standard deviations).

Physicochemical parameters	Determined values
Total acidity (% w/v)	5.9 ± 0.1
Fixed acidity (% w/v)	1.1 ± 0.0
Volatile acidity (% w/v)	4.8 ± 0.0
pH	2.74 ± 0.02
Total dried extract (% w/v)	19.90 ± 0.02
Total ashes (% w/v)	0.47 ± 0.01
Density (at 15 °C, g/mL)	1.0947 ± 0.0021
Total soluble solids (°Brix)	26.1 ± 0.1
Total reducing sugars (% w/v)	25.0 ± 0.0
Ethanol (% v/v)	0.030 ± 0.002

3.2.6 Correlations between bioactive compounds and antioxidant activity

Correlations analyses of AA against TP and TA were performed during the whole process. Significantly high correlations were observed for TP and TA when AA was determined by DPPH assay, and slightly positive correlations were obtained when ABTS technique was performed (Table 5). These results indicate that TA and TP greatly contribute to AA in blueberry balsamic vinegar. Other studies have reported good correlation between them in blueberry wines (Ortiz et al., 2013) and vinegars (Su & Chien, 2007), revealing that TP and tannins were clearly responsible for the antioxidant activity (Zhang et al., 2016). In addition, the high correlation coefficient between both AA methods (r = 0.7929) indicate either of them have validity for determining antioxidant activity without distinction in blueberry balsamic vinegar.

Table 5. Correlation coefficients (r) among antioxidant components and antioxidant activity (ABTS and DPPH methods) in blueberry balsamic vinegar ($p < 0.05$).

	ABTS	DPPH
Total Anthocyanins (TA)	0.7743	0.9606
Total Phenolics (TP)	0.6942	0.9257
ABTS	---	0.7929

4 Conclusions

The evaluation of the juice concentration method that best retains Total Anthocyanins (TA), Total Phenolics (TP) indicated that the best method was associated with a rotary vacuum evaporator.

Both alcoholic fermentation and acetification negatively affected TA, TP and antioxidant activity during blueberry vinegar production. However, mixing with concentrated juice to obtain blueberry balsamic vinegar allowed balancing bioactive compounds decrease due to processing.

The present study showed that production of blueberry balsamic vinegar opens an interesting possibility to reduce losses due to fruit waste while getting added value products with healthy qualities. Therefore, it might be considered a competitive product that increases the variety of seasonings with regional characteristics in the commercial market.

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References

Andlauer, W., Stumpf, C., & Fürst, P. (2000). Influence of the acetification process on phenolic compounds. *Journal of Agricultural and Food Chemistry*, *48*(8), 3533-3536. http://dx.doi.org/10.1021/jf000010j

Association of Official Analytical Chemists – AOAC. (2005a). *Official methods of analysis of the Association of Official Analytical Chemists* (18th ed., 923.09). Gaithersburg: AOAC.

Association of Official Analytical Chemists – AOAC. (2005b). *Official methods of analysis of the Association of Official Analytical Chemists* (18th ed., 930.35). Gaithersburg: AOAC.

Bakir, S., Toydemir, G., Boyacioglu, D., Beekwilder, J., & Capanoglu, E. (2016). Fruit antioxidants during vinegar processing: changes in content and in vitro bio-accessibility. *International Journal of Molecular Sciences*, *17*(10), 1-12. http://dx.doi.org/10.3390/ijms17101658

Barba, F. J., Jäger, H., Meneses, N., Esteve, M. J., Frígola, A., & Knorr, D. (2012). Evaluation of quality changes of blueberry juice during refrigerated storage after high-pressure and pulsed electric fields processing. *Innovative Food Science & Emerging Technologies*, *14*, 18-24. http://dx.doi.org/10.1016/j.ifset.2011.12.004

Beaulieu, J. C., Stein-Chisholm, R. E., Lloyd, S. W., Bett-Garber, K. L., Grimm, C. C., Watson, M. A., & Lea, J. M. (2016). Volatile, anthocyanidin, quality and sensory changes in rabbiteye blueberry from whole fruit through pilot plant juice processing. *Journal of the Science of Food and Agriculture*, *97*(2), 469-478. http://dx.doi.org/10.1002/jsfa.7748

Bosso, A., & Guaita, M. (2008). Study of some factors involved in ethanal production during alcoholic fermentation. *European Food Research and Technology*, *227*(3), 911-917. http://dx.doi.org/10.1007/s00217-007-0805-7

Cásedas, G., Les, F., Gómez-Serranillos, M. P., Smith, C., & López, V. (2017). Anthocyanin profile, antioxidant activity and enzyme inhibiting properties of blueberry and cranberry juices: a comparative study. *Food & Function*, *8*(11), 4187-4193. http://dx.doi.org/10.1039/C7FO01205E

Cerezo, A. B., Cuevas, E., Winterhalter, P., Carmen Garcia-Parrilla, M., & Troncoso, A. M. (2010). Anthocyanin composition in Cabernet Sauvignon red wine vinegar obtained by submerged acetification. *Food Research International*, *43*(6), 1577-1584. http://dx.doi.org/10.1016/j.foodres.2010.03.006

Código Alimentario Argentino. (2021). *Ley 18284/69 y Decreto Reglamentario 2126/1971. Capítulo XVI: Correctivos y Coadyuvantes, Art. 1336, p. 79. Actualizado 01/2021*. Retrieved in 2021, March 26, from https://www.argentina.gob.ar/sites/default/files/caa_cap_xvi_feb2021.pdf.

Cunha, M. A., Lima, K. P., Santos, V. A. Q., Heinz, O. L., & Schmidt, C. A. P. (2016). Blackberry vinegar produced by successive acetification cycles: production, characterization and bioactivity parameters. *Brazilian Archives of Biology and Technology*, *59*(1), 1-10. http://dx.doi.org/10.1590/1678-4324-2016150136

Cvejić, J., & Atanacković, M. (2015) Effect of wine production techniques on wine resveratrol and total phenolics. In: V.R. Preedy (Ed.), *Processing and impact on active components in food* (pp. 501-508). Cambridge: Academic Press. http://dx.doi.org/10.1016/B978-0-12-404699-3.00060-3.

Dávalos, A., Bartolomé, B., & Gómez-Cordovés, C. (2005). Antioxidant properties of commercial grape juices and vinegars. *Food Chemistry*, *93*(2), 325-330. http://dx.doi.org/10.1016/j.foodchem.2004.09.030

Davies, C. V. (2015). *Estudio de los procesos biotecnológicos de acetificación para la producción de vinagre de naranja y vinagre de arándanos* (Doctoral dissertation). Universidad Politécnica de Valencia, España. http://dx.doi.org/10.4995/Thesis/10251/59399

de Ory, I., Romero, L. E., & Cantero, D. (2004). Operation in semi-continuous with a closed pilot plant scale acetifier for vinegar production. *Journal of Food Engineering*, *63*(1), 39-45. http://dx.doi.org/10.1016/S0260-8774(03)00280-2

Echeverrigaray, S., Scariot, F. J., Menegotto, M., & Delamare, A. P. L. (2020). Anthocyanin adsorption by *Saccharomyces cerevisiae* during wine fermentation is associated to the loss of yeast cell wall/membrane integrity. *International Journal of Food Microbiology*, *314*, 108383. http://dx.doi.org/10.1016/j.ijfoodmicro.2019.108383

Eichholz, I., Huyskens-Keil, S., & Rohn, S. (2015) Blueberry phenolic compounds: fruit maturation, ripening and post-harvest effects. In V. R. Preedy (Ed.), *Processing and impact on active components in food* (pp. 173-180). Cambridge: Academic Press. http://dx.doi.org/10.1016/B978-0-12-404699-3.00021-4

Elik, A., Yanik, D. K., Maskan, M., & Göğüş, F. (2016). Influence of three different concentration techniques on evaporation rate, color and phenolics content of blueberry juice. *Journal of Food Science and Technology*, *53*(5), 2389-2395. http://dx.doi.org/10.1007/s13197-016-2213-0

España. (2012). *Boletín Oficial del Estado (BOE-A-2012-5529), Real Decreto 661/2012, de 13 de abril, por el que se establece la norma de calidad para la elaboración y la comercialización de los vinagres, n° 100, sección I, 32031-32036*. Retrieved in 2020, November 20, fom https://www.boe.es/eli/es/rd/2012/04/13/661/con.

Folmer, F., Basavaraju, U., Jaspars, M., Hold, G., El-Omar, E., Dicato, M., & Diederich, M. (2014). Anticancer effects of bioactive berry compounds. *Phytochemistry Reviews*, *13*(1), 295-322. http://dx.doi.org/10.1007/s11101-013-9319-z

Fonseca, M., Santos, V., Calegari, G., Dekker, R., Barbosa-Dekker, A., & Cunha, M. (2018). Blueberry and honey vinegar: successive batch production, antioxidant potential and antimicrobial ability. *Brazilian Journal of Food Technology*, *21*, e2017101. http://dx.doi.org/10.1590/1981-6723.10117

Gerard, L. M. (2015). *Caracterización de bacterias del ácido acético destinadas a la producción de vinagres de frutas* (Doctoral dissertation). Universidad Politécnica de Valencia, España. http://dx.doi.org/10.4995/Thesis/10251/59401

Giudici, P., Lemmetti, F., & Mazza, S. (2015) *Balsamic vinegars. Tradition, technology, trade.* USA: Springer International Publishing Switzerland. https://doi.org/10.1007/978-3-319-13758-2

Gómez-Plaza, E., Romero Cascales, I., & Bautista-Ortín, A. B. (2010). Use of enzymes for wine production. In: A. Bayindirli (Ed.), *Enzymes in fruits and vegetables processing chemistry and engineering applications* (pp. 215-244). Boca Raton: CRC Press. http://dx.doi.org/10.1201/9781420094343-c9

Gullo, M., Verzelloni, E., & Canonico, M. (2014). Aerobic submerged fermentation by acetic acid bacteria for vinegar production: process and biotechnological aspects. *Process Biochemistry*, *49*(10), 1571-1579. http://dx.doi.org/10.1016/j.procbio.2014.07.003

Hornedo-Ortega, R., Álvarez-Fernández, M. A., Cerezo, A. B., Garcia-Garcia, I., Troncoso, A. M., & García-Parrilla, M. C. (2017). Influence of fermentation process on the anthocyanin composition of wine and vinegar elaborated from strawberry. *Journal of Food Science*, *82*(2), 364-372. http://dx.doi.org/10.1111/1750-3841.13624

Kirca, A., & Cemeroğlu, B. (2003). Degradation kinetics of anthocyanins in blood orange juice and concentrate. *Food Chemistry*, *81*(4), 583-587. http://dx.doi.org/10.1016/S0308-8146(02)00500-9

Klopotek, Y., Otto, K., & Böhm, V. (2005). Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, *53*(14), 5640-5646. http://dx.doi.org/10.1021/jf047947v

Mena, P., Gironés-Vilaplana, A., Martí, N., & García-Viguera, C. (2012). Pomegranate varietal wines: phytochemical composition and quality parameters. *Food Chemistry*, *133*(1), 108-115. http://dx.doi.org/10.1016/j.foodchem.2011.12.079

Morata, A., Escott, C., Loira, I., Del Fresno, J. M., González, C., & Suárez-Lepe, J. A. (2019). Influence of *Saccharomyces* and non-*Saccharomyces* yeasts in the formation of pyranoanthocyanins and polymeric pigments during red wine making. *Molecules (Basel, Switzerland)*, *24*(4490), 1-18. http://dx.doi.org/10.3390/molecules24244490

Nanba, A., Tamura, A., & Nagai, S. (1984). Synergistic effects of acetic acid and ethanol on the growth of *Acetobacter* sp. *Journal of Fermentation Technology*, *62*, 501-505.

Niki, E. (2011). Antioxidant capacity: which capacity and how to assess it? *Journal of Berry Research*, *1*(4), 169-176. http://dx.doi.org/10.3233/JBR-2011-018

Ordoudi, S. A., Mantzouridou, F., Daftsiou, E., Malo, C., Hatzidimitriou, E., Nenadis, N., & Tsimidou, M. Z. (2014). Pomegranate juice functional constituents after alcoholic and acetic acid fermentation. *Journal of Functional Foods*, *8*(1), 161-168. http://dx.doi.org/10.1016/j.jff.2014.03.015

Ortiz, J., Marín-Arroyo, M. R., Noriega-Domínguez, M. J., Navarro, M., & Arozarena, I. (2013). Color, phenolics, and antioxidant activity of blackberry *(Rubus glaucus* Benth.), blueberry (*Vaccinium floribundum* Kunth.), and apple wines from Ecuador. *Journal of Food Science*, *78*(7), 985-993. http://dx.doi.org/10.1111/1750-3841.12148

Pérez-Trujillo, J. P., Hernández, Z., López-Bellido, F. J., & Hermosín-Gutiérrez, I. (2011). Characteristic phenolic composition of single-cultivar red wines of the Canary Islands (Spain). *Journal of Agricultural and Food Chemistry*, *59*(11), 6150-6164. http://dx.doi.org/10.1021/jf200881s

Romero Cascales, I. (2008). *Extracción de compuestos fenólicos de la uva al vino. Papel de los enzimas de maceración* (Doctoral dissertation). Universidad de Murcia, Murcia. Retrieved in 2020, August 3, from http://hdl.handle.net/10803/11064

Sablani, S. S., Andrews, P. K., Davies, N. M., Walters, T., Saez, H., Syamaladevi, R. M., & Mohekar, P. R. (2010). Effect of thermal treatments on phytochemicals in conventionally and organically grown berries. *Journal of the Science of Food and Agriculture*, *90*(5), 769-778. http://dx.doi.org/10.1002/jsfa.3882

Sakanaka, S., & Ishihara, Y. (2008). Comparison of antioxidant properties of persimmon vinegar and some other commercial vinegars in radical-scavenging assays and on lipid oxidation in tuna homogenates. *Food Chemistry*, *107*(2), 739-744. http://dx.doi.org/10.1016/j.foodchem.2007.08.080

Su, M. S., & Chien, P. J. (2007). Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinium ashei*) fluid products as affected by fermentation. *Food Chemistry*, *104*(1), 182-187. http://dx.doi.org/10.1016/j.foodchem.2006.11.021

Su, M. S., & Silva, J. L. (2006). Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinium ashei*) by-products as affected by fermentation. *Food Chemistry*, *97*(3), 447-451. http://dx.doi.org/10.1016/j.foodchem.2005.05.023

Ubeda, C., Callejón, R. M., Hidalgo, C., Torija, M. J., Troncoso, A. M., & Morales, M. L. (2013). Employment of different processes for the production of strawberry vinegars: effects on antioxidant activity, total phenols and monomeric anthocyanins. *Lebensmittel-Wissenschaft + Technologie*, *52*(2), 139-145. http://dx.doi.org/10.1016/j.lwt.2012.04.021

Ubeda, C., Hidalgo, C., Torija, M. J., Mas, A., Troncoso, A. M., & Morales, M. L. (2011). Evaluation of antioxidant activity and total phenols index in persimmon vinegars produced by different processes. *Lebensmittel-Wissenschaft + Technologie*, *44*(7), 1591-1596. http://dx.doi.org/10.1016/j.lwt.2011.03.001

Vasantha Rupasinghe, H. P., Joshi, V. K., Smith, A., & Parmar, I. (2017). Chemistry of fruit wines. In M. R. Kosseva, V. K. Joshi, & P. S. Panesar (Eds.), *Science and technology of fruit wine production* (pp. 105-176). Cambridge: Academic Press. http://dx.doi.org/10.1016/B978-0-12-800850-8.00003-X

Yousefi, G., Yousefi, S., & Emam-Djomeh, Z. (2013). A comparative study on different concentration methods of extracts obtained from two raspberries (*Rubus idaeus* L.) cultivars: evaluation of anthocyanins and phenolics contents and antioxidant activity. *International Journal of Food Science & Technology*, *48*(6), 1179-1186. http://dx.doi.org/10.1111/ijfs.12073

Zapata, L. M. (2014). *Obtención de extracto de antocianinas a partir de arándanos para ser utilizado como antioxidante y colorante en la industria alimentaria* (Doctoral dissertation). Universitat Politécnica de Valéncia, España. http://dx.doi.org/10.4995/Thesis/10251/39105

Zapata, L., Heredia, A., Malleret, A., Quinteros, F., Cives, H., & Carlazara, G. (2013). Evaluación de parámetros de calidad que ayuden a definir la frecuencia de recolección de bayas de arándanos. *Revista Iberoamericana de Tecnología Postcosecha*, *14*(2), 186-194. Retrieved in 2020, August 3, from http://www.redalyc.org/articulo.oa?id=81329290013

Zhang, L., Li, N., & Gao, X. (2016). Phenolic compounds and antioxidant activity of wines fermented using ten blueberry varieties. *American Journal of Food Technology*, *11*(6), 291-297. http://dx.doi.org/10.3923/ajft.2016.291.297

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