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## Study of the influence of berry-blanching on syneresis in blueberry purées

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### Abstract

A wider range of fruit-based products, preserving high nutritional and sensory attributes, aid towards achieving a healthy diet. Pigmented fruit, specially blueberries, are a natural source of phenolic phytochemicals which could be exploited through proper technologies, limiting by-product loss and detrimental impact of processing on fruit bioactive compounds. The influence of the mild thermal pretreatment of steam blanching on the syneresis of frozen blueberry purées was studied. Syneresis kinetic parameters, colour and phenolic profile of the serum from syneresis were analyzed, highlighting the relationship between quality data and pigment localization in the berry by a correlative microscopy study. Blanching induced a marked anthocyanin diffusion from the vacuoles of pigmented epidermal cells down to the core of berries. Monomeric anthocyanin pigments (MAP) decreased in blanched (BL) berries (−17.6%), but no further decrease was observed in BL purées, while puréeing of not blanched (NB) berries caused a significant loss in MAP (−28.0%) and total phenolic compounds (TPC) (−35.8%) and an increase in percent polymeric color. As a result BL purées had higher MAP and TPC compared to NB ones. A different syneresis behaviour was evidenced between BL and NB purées and a model fitting data was proposed; the retention of syneresis serum was higher in BL purée during the first 180 min after thawing, then it was higher in NB ones till 340 min, and thereafter was similar. BL serum samples showed higher TPC (+69%), MAP (+215%), lightness ( $L^*$ ) and red colour component ( $a^*$ ) compared to NB ones. The introduction of a berry-blanching step improves physical and chemical stability of ready-to-eat frozen blueberry purées. Correlative microscopy provides a useful integrated approach to fulfil quality requirements.

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*Keywords:* Blueberry purée; blanching; syneresis; phenolics; microscopy

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## 1. Introduction

Consumer interest for ready to eat fruit-based products is growing, especially with regards to pigmented fruit, rich in phenolic antioxidant compounds. The need to supply convenience food preserving health-related compounds, moves research towards the application of mild technologies and the introduction of innovative solutions for the consumer. Frozen blueberry purée could be a versatile product, processed without pomace loss and potentially rich in all the bioactive compounds which are characteristic of the whole berry. By-product waste is especially detrimental to blueberry phytochemical background; anthocyanin and several phenolic antioxidant compounds are in fact localized in the berry epidermal layers which are also the main by-product constituents [1, 2]. Phytochemicals abundance, colour and physical stability of the serum/pulp system are main quality parameters in fruit and vegetables purées, all parameters deeply affected by the impact of thermal and mechanical treatments on cells environment [3, 4, 5]. A complex relationship has been assessed between thermal processing and fruit anthocyanin stability, directly influenced by the designed thermal unit operation and time/temperature combination [6]. Blanching is a mild thermal treatment, often applied to raw plant tissues as a preliminary step to freezing, canning or dehydrating, to inactivate oxidative enzymes thus extending product shelf life and enhancing colour. The impact of blanching on plant cell membranes and wall pectins may result in softening or improved texture, with crucial implications on rheological properties of the final product [7]. The ability of a steam blanching pretreatment to improve anthocyanin yield and recovery from pomace has been assessed in blueberry juice and it has been related to thermal inactivation of fruit phenolic-oxidative enzymes [8, 9, 10]. Important structural and ultrastructural alterations induced by blanching in pigmented epidermal cells have been documented [11]. Furthermore, an improving effect of blanching on quality aspects and kinetics of mass transfer was evidenced in osmo-air dehydrated blueberries [12]. Potential health benefits of blueberry fruits together with their peculiar microstructure features need proper processing technologies to be developed.

This work aimed at studying the influence of blueberry blanching pretreatment on the kinetic of purée syneresis and on colour parameters and phenolic composition of the separated serum. Correlation between thermal treatment and tissue localization of pigments in whole berries was highlighted by macro- and micrographs and implications on purée quality attributes discussed.

## 2. Materials and Methods

Three kilograms of highbush blueberries (*Vaccinium corymbosum* L., cv Brigitta), harvested in a commercial orchard in Trentino region (Italy) at market maturity, on arrival were immediately individually quick frozen (IQF) in a tunnel at  $-40^{\circ}\text{C}$  and then stored at  $-20^{\circ}\text{C}$  for 1 month. Afterwards they were divided into two lots: one lot of berries was processed into purée after thawing at  $20^{\circ}\text{C}$  for 3h (NB), while the other was processed after steam-blanching for 3min and tap water-cooled in a pilot steam blanching tunnel (BL) [9]. A small sub-sample of BL lot was frozen at  $-20^{\circ}\text{C}$  for subsequent analyses. For purée processing, 1.5 kg of NB and BL berry samples were homogenized for 1 min using a commercial food processor, then 300g aliquots of purées were packed in 400-mL plastic vessels, sealed under partial vacuum using a 25  $\mu\text{m}$  thick polypropylene film and frozen and stored at  $-20^{\circ}\text{C}$  till analysis. Frozen NB and BL berries were analysed for monomeric anthocyanin pigments (MAP), total phenolic compounds (TPC), percent polymeric colour (%PC), index of browning (IB) and for macro- and micrographs on half berries using microscope. Frozen NB and BL purées were analysed for the syneresis kinetic following thawing, and for MAP, TPC, %PC and IB. The syneresis liquid was analysed for colour and for MAP, TPC, %PC and IB.

For microscopy, frozen NB and BL berries were cut in half using a razor blade and placed on ice in a polystyrene bowl; macrographs of the longitudinal section of berries were taken using a Sony T100 Digital camera (Sony Corporation, Tokyo, Japan). Epicarp samples (1 mm thick) were excised from

frozen berries with a razor blade, embedded in 6% agar at room temperature and cut into sections (30–40  $\mu\text{m}$ ) with a manual tissue slicer Vibroslice NVSL (World Precision Instruments Inc, Sarasota, USA). Samples were immediately examined with an Olympus BX50 (Olympus, Japan) light microscope, equipped with a differential interference contrast.

The purée syneresis kinetic was studied at 20°C by placing frozen purées (3 replicates) in a cheesecloth-lined funnel. The amount of liquid separated from the thawing pulp was collected in a flask underneath the funnel and weighed at 30 min intervals up to 6 hours, and then after 24h. On weight data of syneresis liquids a kinetic fitting model was studied by Simple Regression procedure (Statgraphics v7, Manugistic Inc., Rockville, MD).

Colour coordinates  $L^*$ ,  $a^*$  and  $b^*$  were measured on the 24 h syneresis liquid samples using a Spectrophotometer CM-2600 (Minolta Co, Ltd, Osaka, Japan). Values (2 replicates) were recorded at four different points on the bottom of a Petri dish (80 mm diameter) filled with 25 g syneresis liquid and covered with a black cap to shade external light.

Polyphenols analysis on just thawed NB and BL berries and on purées thawed in a fridge at 4°C for 24 h (2 replicates) were carried out after extraction (two replicates) in 5% aqueous formic acid media [8]. Anthocyanins were determined by differential method (MAP) and subtractive method (%PC and IB) according to Giusti & Wrolstad [13], TPC were determined by Folin-Ciocalteu according to Singleton & Rossi [14].

Data were submitted to one way analysis of variance (ANOVA procedure, Statgraphics v7) and means were compared by Tukey's test at  $P < 0.05\%$ .

### 3. Results and Discussion

Blueberries after blanching got rounder in shape and softened, but the integrity of the fruit epidermal barrier was largely maintained. Steam blanching induced a marked anthocyanin diffusion from pigmented epidermal and sub epidermal layers down to the core of berries, as evidenced in Figure 1(A1, B1).

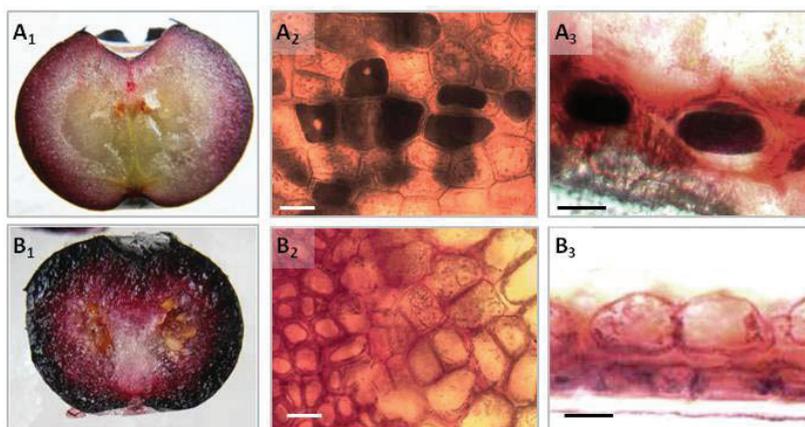


Fig. 1. Effect of steam blanching on pigments diffusion in *V. corymbosum* L. (A, not blanching; B, blanching). 1- Berries longitudinal sections. 2- Epidermis panoramic views. 3- Epidermal tissue cross-sections (Bars=30  $\mu\text{m}$ ).

Pigments, stored in the vacuoles of epicarp cells (Figure 1, A2 and A3), leak out through tissues, due to thermal induced plasmolysis and cell wall softening. Figure 1, B2 and B3, show blanched depigmented epidermal cells, with altered membranes and thickened intercellular spaces.

Relaxation of cell wall structures with reduced adhesion between cells, also previously observed by other authors in blanched plant tissues, have been explained by heat-induced pectin solubilization phenomena and alteration of cellulosic cell wall network [11, 15, 16].

Blanching-induced flow of pigments throughout berry tissues was related to a decrease in MAP (−17.6%), but no further decrease was observed in corresponding BL purées. In contrast, puréeing of NB berries caused a significant loss in MAP (−28.0%) and TPC (−35.8%). There was an higher %PC in NB samples, which further increased with puréeing (Table 1). As a consequence, BL purées achieved a higher content in MAP (+11.3%) and TPC (+51.6%) and a lower %PC compared to NB purées. The same data trend for TPC and MAP, further amplified by a final pasteurization step, was found in NB and BL purées from organic and conventional blueberries [4].

Table 1. Total Phenolic Compounds (TPC), Monomeric Anthocyanin Pigments (MAP), Percent Polymeric Color (%PC) and Index of Browning (IB) in BL and NB blueberry fruits, purées and syneresis liquid from purées.

	Berries		Purées		Syneresis liquid	
	NB	BL	NB	BL	NB	BL
TPC (mg/100g)	284.40 a,A	279.70 a,A	182.71 b,B	276.97 a,A	104.01 b	175.89 a
MAP (mg/100g)	114.41 a,A	94.23 b,A	82.38 b,B	91.69 a,A	16.67 b	52.59 a
PC (%)	3.84 a,B	1.55 b,A	7.81 a,A	1.91 b,A	6.79 a	4.35 a
BI ( $A_{420nm}$ )	0.05 b,A	0.25 a,B	0.1 b,A	0.30 a,A	0.25 a	0.14 a

Means followed by different letters are statistically different at  $P < 0.05\%$  (Tukey's test); small letters refer to the effect of blanching within the products, capital letters refer to the effect of puréeing within the heat treatment.

These results would suggest the coexistence of a direct detrimental impact of heat treatments on anthocyanin compounds, evidenced in berries, and an indirect protective effect mediated by heat inactivation of oxidative enzymes, evidenced after purée processing. Heat application on intact berries, before cell membranes disruption due to puréeing, would enhance the protective effects against the detrimental ones.

Overall appearance of BL and NB purées differed in terms of colour and liquid-holding capacity. BL purées had a more jelly-like structure, while syneresis phenomena clearly occurred only in NB samples after thawing in plastic vessels. The different liquid-holding behaviour was measured by removing frozen purées from the vessel and placing them in a cheesecloth-lined funnel at 20°C; the retention of liquid portion in these conditions was higher in BL purées during the first 180 min after thawing, then it was higher in NB ones till 340 min (Figure 2). After this time, the liquid retention did not change and the syneresis serum was on average about 60% of the initial weight for both purées. Hence, BL and NB syneresis kinetics were modelled using the Simple Regression procedure (Statgraphics) considering the weight data of syneresis liquid till 400 minutes. The best model fitting both BL and NB data is reported in Eq. 1.

$$W = a + b\sqrt{t} \quad (1)$$

where  $W$  is the weight of serum,  $t$  is the syneresis time, and  $a$  and  $b$  are the estimated parameters of the model (Table 2). The square-root X model better fitted the BL syneresis kinetic, having a higher  $R^2_{adj}$ .

Table 2. Estimate and standard error of model parameters for BL and NB syneresis kinetics

Sample	N obs	Estimate		Standard error		R <sup>2</sup> <sub>adj</sub>
		<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	
BL	42	-16.205	10.646	3.425	0.261	97.6
NB	42	-26.749	11.516	5.255	0.400	95.7

By the comparison of the regression models, it was evidenced that intercept (*a*) is not significantly different, while the slope (*b*) has a P-value near the significance ( $P = 0.07\%$ ). Therefore, even if at selected syneresis times there was statistically difference for serum weight between the samples, the kinetic models were not statistically different.

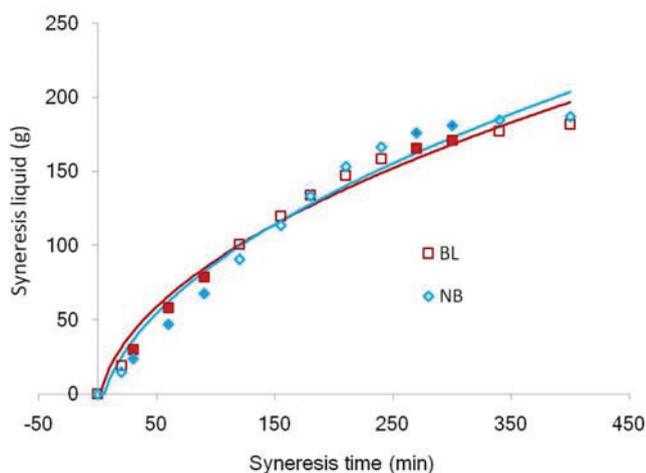


Fig. 2. Weights (g) of syneresis liquid from blached (BL) and not-blached (NB) purées in function of time at 20°C. Symbols refer to experimental data mean and lines to fitted model; filled symbols refer to BL and NB means statistically different within the same syneresis time (Tukey's test;  $P < 0.05\%$ ).

Although the NB and BL serum weights were the same after 24 h at 20°C, their chemical composition was different. Serum from BL purées had a higher content in TPC (+69%) and triple the amount in MAP (+215.5%) compared to NB serum sample (Table 1); in addition BL syneresis liquid had higher lightness ( $L^*$ ) and red colour component ( $a^*$ ) (Table 3).

These data, also supported by microscopy observations, highlighted a marked phenolic-enriching effect of blanching on the liquid portion of blueberry purées, especially concerning anthocyanin compounds.

Hence, both polyphenols content and extractability from the food matrix are enhanced in frozen blueberry purées due to the blanching pretreatment. These phenomena, together with heat solubilisation of tissue pectins, could account for the different liquid-holding capacity of BL and NB purées. Furthermore, by affecting food matrix and dietary fiber, a blanching-mediated influence on polyphenols bioavailability could be expected [17].

Table 3. Colour values of syneresis liquids from NB and BL blueberry purées.

Colour coordinates	Syneresis liquid	
	NB	BL
$L^*$	26.02 b	26.27 a
$a^*$	3.07 b	4.71 a
$b^*$	0.74 a	1.03 a

Different letters in the same row indicate a statistical difference between means at  $P < 0.05\%$  (Tukey's test).

#### 4. Conclusion

The introduction of a berry-blanching step in the processing of ready-to-eat frozen blueberry purées would give products with high nutritional and sensory qualities. In frozen blanched purées, the phenolic bioactive compounds are well preserved, the syneresis phenomena are limited and the colour properties are improved. The increased release of phytochemical components from food matrix in blanched purées could have further nutritional and technological implications to be better investigated. Our results stress that correlative microscopy provides a useful integrated approach in the understanding of the phenomena occurring with processing.

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