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ARTICLE

Effect of enzymes and clarifying agents on quality parameters of elderberry juice

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ABSTRACT Natural food colorants are usually used in concentrated form. Production of concentrate including enzymes, temperature, clarifying agents and mechanical operation may have significant influence on valuable components of the end product. In our study, we investigated the effects of two different pectolytic enzyme preparations (Fructozym P and Pectinex BE XXL) and five clarifying agents (three bentonites and two silica sols) on total polyphenol, total anthocyanin content and colour parameters of Haschberg and Samocco elderberry juices. Our results showed that enzymes and clarifying agents applied during juice production influenced the investigated quality parameters of both varieties. Generally, in case of Samocco variety Fructozym P with bentonites, while in case of Haschberg Pectinex BE XXL with silica sols proved the most effective combinations to reach high quality values. This fact should be considered in case of industrial application.
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Introduction

In the food industry, natural or synthetic food colorants are used to improve, restore or establish the colour of the final product. The regulation of the European Commission 1333/2008/EC states that food containing certain artificial colouring material, must be labelled with the following phrase: „may have an adverse effect on activity and attention in children" (OJEU 2008). For this reason, food colorants from natural sources are becoming preferred as alternatives of synthetic food agents to adjust food colour.

Natural food colorants are usually used in concentrated form in the food industry. However, various physical and biological factors such as temperature, enzymes, heat and mechanical stress may have influence on the quality parameters of the end product (Stéger-Máté et al. 2006; Szalóki-Dorkó et al. 2014).

Many studies investigated the effect of processing technology on valuable compounds in fruit juice. Heat treatment caused degradation of anthocyanin content, (Bakker and Pildre 1992; Wesche-Ebeling and Argaiz-Jamet 2002; Bakowska-Barczak et al. 2003; Garzon and Wrolstad 2003; Talcott et al. 2003; Kirca et al. 2007; Patras et al. 2010; Sui 2014; Szalóki-Dorkó et al. 2015) pH reduction and storage temperature influenced also the colour stability of strawberry nectar (Gössinger et al. 2009). In case of red cabbage anthocyanin composition and content were changed during fermentation, storage and stewing (Wiczkowski et al. 2015). Total polyphenol and total pigment content of *Rubus coreanus* Miquel fruit were degraded during jam processing (Lee et al. 2013). Effects of clarification and pasteurisation on anthocyanins and the colour parameters of pomegranate juice were also investigated (Turfan et al. 2011; Vegara et al. 2013; Valero et al. 2014) and found that clarifying agents, such as bentonites or gelatines improve the colour, flavour and physical stability of the treated juice (Turfan et al. 2011).

Anthocyanin colorants are suitable for food colouration with a low pH level such as soft drinks, water ice-cream, dairy products, table jellies, because of the molecules are stable in acidic medium (Cooper-Driver 2001; Bakowska-Barczak et al. 2003; Valero et al. 2014). Elderberry (*Sambucus nigra* L.) may be suitable colouring material in food industry, because of the high pigment content (Seruga et al. 2007; Lee and Finn 2007; Veberic et al. 2009). Elderberry anthocyanins are almost exclusively cyanidin-glycosides, from which cyanidin-3-glucoside and cyanidin-3-sambubioside were detected as the most abundant compounds (Veberic et al. 2009). According to a previous study, cyanidin-3-sambubioside was the most stable pigment molecule against processing technology (Drdak and Daucik 1990). However, the effect of enzyme and clarifying agents on valuable compounds of elderberry during processing is a less researched area.

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Table 1. Parameters of applied enzyme preparations.

	Pectinex BE XXL	Fructozym P
Origin	*Aspergillus niger*	*Aspergillus niger*
Activity	pectin-lyase	pectin-esterase, pectin-lyase, endo-polygalacturonase
pH optimum	pH 4.5	<pH 3.0
Temperature optimum	50-55 °C	20-55 °C

The aim of this research was to investigate the effects of enzyme preparations and clarifying agents on some quality parameters of elderberry juices. Total polyphenol, total anthocyanin content and colour parameters were investigated in elderberry juices made from two varieties. Further aim was to determine the suitable combination of applied enzymes and clarification agents to reach higher quality values.

Materials and Methods

Two different elderberry (*Sambucus nigra* L. sbsp. *nigra*) varieties, namely Haschberg (Austrian) and Samocco (Danish) were harvested from the growing area of Vál of the Hungarian Elderberry Grower Cooperative, Hungary in 2013.

During the experiment, two type of enzyme preparations were used (Table 1): Pectinex BE XXL and Fructozym P (Kerttrade Ltd., Dunavarsány, Hungary).

Furthermore, three type of bentonite clarifying agents were applied (Erbslöh, Germany): SodiBent Supra is a natural finely milled sodium powder to clarify wine, fruit juice and vinegar. GranuBent PORE-TEC is a natural sodium bentonite granulated by PORE-TEC technology, using in clarification of wine, fruit juice and vinegar. NaCalit PORE-TEC containing premium quality granulated sodium-calcium bentonite to clarify wine. In additional, two types of silica sols (Erbslöh, Germany), namely Klar-Sol 30 (alkaline) and Klar-Sol Super (acid) were investigated.

Preparation and measurements

Elderberry juices were prepared according to industrial protocols under laboratory conditions (Fig. 1).

Total anthocyanin concentration was determined by the method of Lee et al. (2005). Results were calculated by the following equation and were expressed in mg cyanidin-3-glucoside equivalents (CGE) per L of elderberry samples:

$(A \times MW \times D \times 10^3)/(\varepsilon \times l)$

where $A = (A_{520nm}-A_{700nm})$ pH1-($A_{520nm}-A_{700nm}$) pH 4.5; MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol); D = dilution; 10^3 = factor for conversion from g to mg; ε = molecular extinction coefficient for cyanidin-3-glucoside (26 900 L/(mol x cm) and l is the path length (cm).

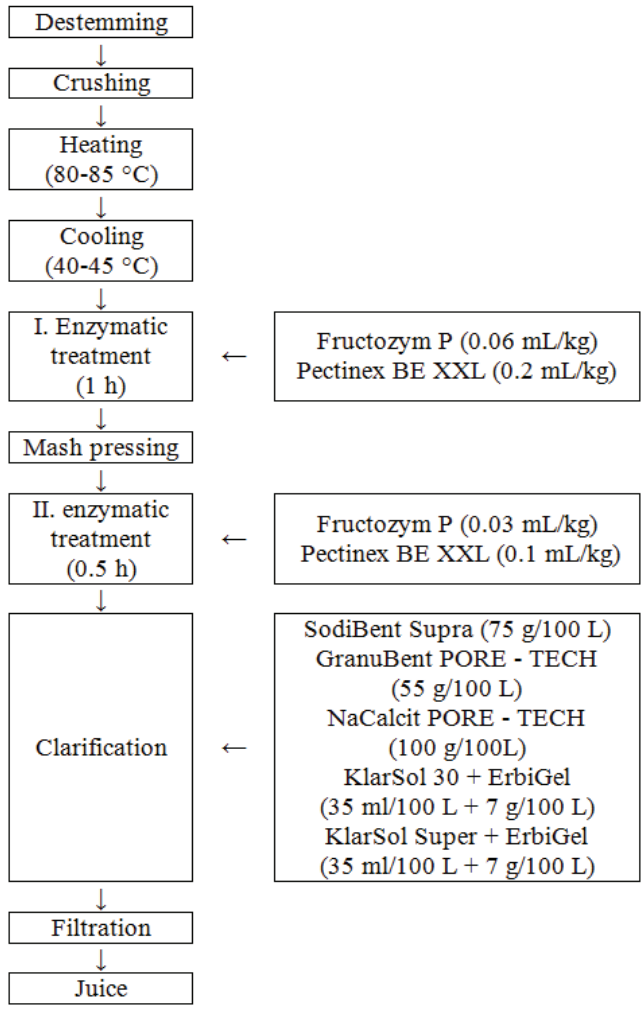

Figure 1. Production scheme of elderberry juice processing.

Total polyphenol content was determined according to the method of Singleton and Rossi (1965) and values were expressed in mg gallic acid equivalent (GAE) per L.

Colour parameters were measured by digital colorimeter (Konica Minolta CR 410) and L* (lightness factor), a* (green-red) and b* (yellow-blue) values were recorded. Colour difference (ΔE*) was calculated from a*, b* and L* values according to the following equation and data were evaluated compared to the control samples according to Table 2.

$\Delta E^* = (\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{1/2}$

Statistical analysis

T-test was used (SPSS 13.0 version) to analyze the signifi-

Table 2. Evaluating of colour difference (ΔE*).

ΔE*	Visible difference
ΔE*≤0.5	non-visible
0.5<ΔE*≤1.5	less visible
1,5<ΔE*≤3.0	visible
3.0<ΔE*≤6.0	obvious
6.0<ΔE*	very obvious

Table 3. Identification numbers of the applied clarifying agents.

Identification number	Applied clarifying agents
1	SodiBent Supra
2	GranuBent PORE-TEC
3	NaCalit PORE-TEC
4	Klar-Sol 30 + ErbiGel
5	Klar-Sol Super + ErbiGel

cance of anthocyanin and polyphenol contents changes compared to the control sample. The difference was considered significant when P<0.05.

Results and Discussion

The sample No. 0 was considered as control sample, which was treated by enzyme, but not by clarifying agent. For simplicity, treatments with clarifying agents were indicated as No. 1-5, as shown in Table 3.

Total polyphenol content

Figure 2 illustrates total polyphenol content of the Haschberg juices. The Fructozym P with SodiBent Supra (1) and GranuBent PORE-TEC (2) combination resulted the highest polyphenol content (10 083 ± 473 mg/L and 10 080 ± 287 mg/L), while Klar-Sol 30 + Erbigel (4) combination the lowest (7805 ± 226 mg/L).

In case of Pectinex BE XXL enzyme-treated juices, NaCalit PORE-TEC (3) clarifying agent caused the highest polyphenol concentration (10 139 ± 83 mg/L). The lowest polyphenol content was measured in case of Pectinex BE XXL enzyme and Klar-Sol Super - ErbiGel (5) combination (7944 ± 212 mg/L). This result is reduced by 20% compared to sample No. 0. Considering the two applied enzyme preparations, values of bentonite-treated juices were higher compared to the silica sol-treated juices.

Figure 3 shows total polyphenol content of Samocco variety juices. Fructozym P and SodiBent Supra (1) combination resulted the highest polyhenolic value in Samocco juice (21 833 ± 629 mg/L), which is 17% higher than the control sample. The lowest polyphenol content (19 722 ± 1052 mg/L) was measured in case of Fructozym P enzyme treated control sample.

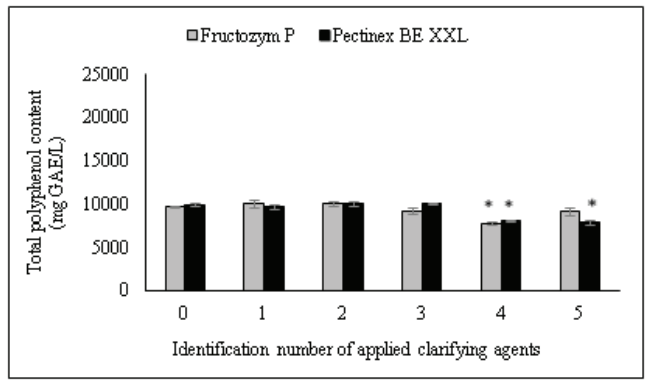

Figure 2. Total polyphenol content of Haschberg juice treated by several enzymes and clarifying agents. * represents significant differences (P<0.05) in total polyphenol content compared to the untreated control sample.

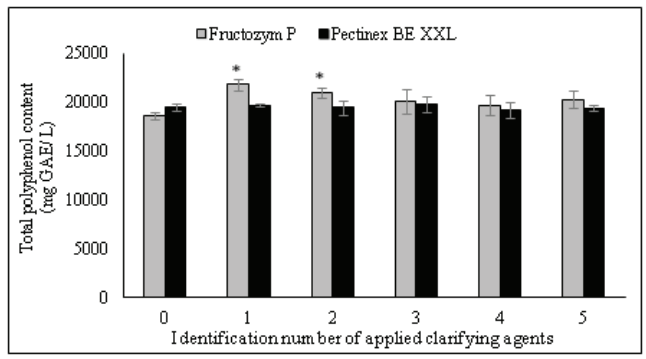

Figure 3. Total polyphenol content of Samocco elderberry juice treated by different enzymes and clarifying agents. * represents significant differences (P<0.05) in total polyphenol content compared to the untreated control sample.

Among the Pectinex BE XXL treated elderberry juices, SodiBent Supra (1) and NaCalit PORE-TEC (3) proved to be the most effective clarifying agents. These clarifying agents resulted the highest total polyphenol concentration (19 750 ± 173 mg/L and 19 778 ± 798 mg/L). The applied Klar-Sol 30 + ErbiGel (4) caused the lowest value (19 250 ± 822 mg/L), although the difference was not considerable (3%).

Generally, the applied clarifying agents combined with Fructozym P enzyme resulted higher total polyphenol content compared to the Pectinex BE XXL-treated and initial samples in case of Samocco juices.

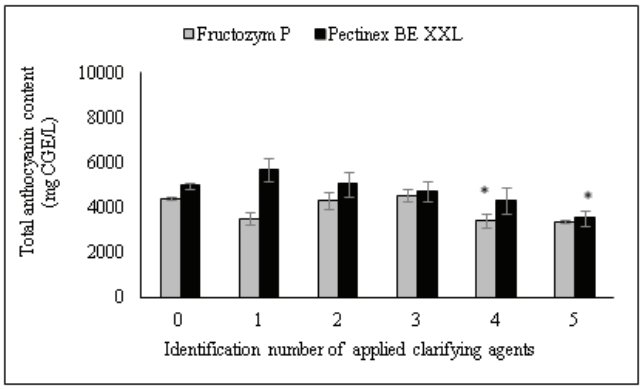

Figure 4. Total anthocyanin content of Haschberg elderberry juice treated by different enzymes and clarifying agents. *represents significant differences (P<0.05) in total anthocyanin content compared to the untreated control sample.

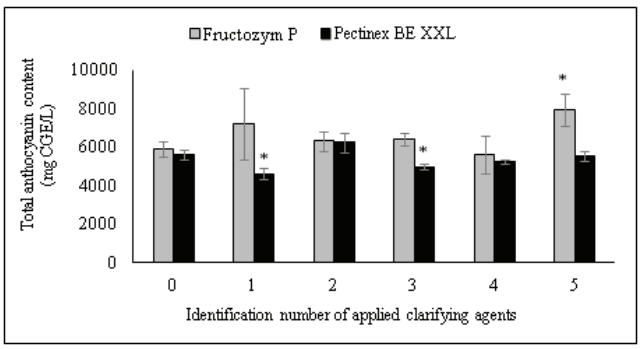

Figure 5. Total anthocyanin content of Samocco elderberry juice treated by several enzymes and clarifying agents.*represents significant differences (P<0.05) in total anthocyanin content compared to the untreated control sample.

Total anthocyanin content

Total anthocyanin content of Haschberg samples is illustrated in Figure 4. Pectinex BE XXL-treated juices had higher total pigment concentration than Fructozym P-treated samples. Among the clarifying agents, SodiBent Supra (1) provided the highest anthocyanin yield (5728 ± 511 mg/L), which is 15% higher; while the lowest anthocyanin content was measured in sample (5) (Klar-Sol Super + ErbiGel), which is 29% lower compared to the initial Pectinex BE XXL sample.

In case of Fructozym P-treated elderberry juice, the lowest anthocyanin content was measured (3384 ± 54 mg/L) in Klar-Sol Super + ErbiGel-treated sample (5), which is 31% lower than the control juice (4798 ± 685 mg/L). As regard the enzyme and clarifying agent combination, total anthocyanin content increased in case of bentonite treated juices, while silica sols combining with gelatine, resulted reduction in pigments content compared to the untreated samples. This result is consistent with a Turkish study, which was described about black carrot juice (Valero et al. 2014). This fact should be considered in case of industrial application.

The results of Samocco juices (Fig. 5) showed that Fructozym P enzyme was more efficient to reach higher pigment content than Pectinex BE XXL enzyme preparation. This observation is controversial to the results obtained by Haschberg juices, which may explained by the different enzyme composition and variety characteristic. Among the applied Fructozym P enzyme-treated juices, Klar-Sol Super + ErbiGel combination (5) proved the highest total anthocyanin value (7971 ± 848 mg/L), in this case pigment yield increased by 35% relative to the control sample. Klar-Sol 30 + ErbiGel combination (4) resulted in the lowest pigment content (5611 ± 998 mg/L) of Samocco juices.

Among the Pectinex BE XXL enzyme-treated juices, sample 2 (GranuBent PORE-TEC) had the highest total anthocyanin content (6245 ± 489 mg/L), which is 12% higher value than the control sample. The juice that contained the lowest anthocyanin content was treated by Pectinex BE XXL enzyme and SodiBent Supra (1) clarifying agent combination (4614 ± 285 mg/L).

Table 4. Colour difference (ΔE*) values of the treated elderberry juices.

Clarifying agent	1	2	3	4	5
Haschberg	-	-	-	-	-
ΔE* Fructozym P	1.32	2.71	0.90	2.49	3.78
ΔE* Pectinex BE XXL	0.40	0.20	0.81	0.65	3.06
Samocco	-	-	-	-	-
ΔE* Fructozym P	3.64	0.73	2.02	0.52	2.52
ΔE* Pectinex BE XXL	1.76	4.10	0.53	0.53	3.69

Colour parameters

Colour differences (ΔE*) varied during the experiment (Table 4). ΔE* values were under the level of 0.5, so no visible difference was observed after SodiBent Supra (1) and GranuBent PORE-TEC (2) treatments in case of Pectinex BE XXL in Haschberg elderberry juices. In most cases NaCalit PORE-TEC (3) and Klar-Sol 30 + ErbiGel (4) clarifying agents caused less visible difference, while obvious visible colour difference was detected after using Klar-Sol Super + ErbiGel (5) in both varieties. Generally, colour difference can be observed in enzyme-treated elderberry juices, if they are combined by bentonite and silica sol clarifying agents.

Conclusion

This study showed that applied enzyme preparations and clarifying agents influenced the investigated quality parameters of both varieties during juice production. Generally, in case of Samocco variety Fructozym P with bentonites, while in case of Haschberg juices Pectinex BE XXL with silica sols proved to be the most effective combinations to reach high quality values. This phenomenon is probably variety dependent characteristic due to different phenolic composition, which should take into account in case of industrial application. Therefore, it would be worthwhile to investigate the structure of anthocyanidin-saccharides of elderberry juice that may influence the effectiveness of enzymatic treatment and clarification. Summary, the application of enzymes and clarification agents during technology can influence the colour parameters of the final elderberry product.

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