EFFECT OF ENZYMES FOR INCREASING AMOUNT OF ANTHOCYANIN IN BLACK CARROT JUICE

Şükrü KARATAŞ[1], Dilek DÜLGER ALTINER[1] , Eda TARİN[1]

Abstract

In this study, the effect of two different enzyme, Natuzym DP+ (Weiss BioTech:10-A10166) and Rapidase Pac (DSM Pac: 414 294 401) on the black carrot juice were investigated for increasing amount of the anthocyanin content.

The black carrot juice were previcely heated at 85°C and cooled to 50°C than were added three different enzyme concentrations (25, 50, 75 µg/L) for one hour period at 50°C. At end of each experimental work the amount of anthocyanin content were determined by UV spectrophotometric method and compared with initial value.

The amount of anthocyanin content was increased from 831 mg/L up to 1337 mg/L and also It was found that the Natuzym DP+ was more effective than Rapidase Pac at 50°C for one hour period.

Keywords: *Black carrot juice, anthocyanin content, enzyme*

1. Introduction

Anthocyanins are members of the flavonoid group of phytochemicals, which is a well-known natural colorants and provide bright red colour in foods group such as teas, honey, wines, fruits, vegetables, nuts. (Kırca et al. 2007, Lila 2004). The sources include red cabbage, blueberries, cherries, raspberries, strawberries, black carrots, purple grapes and red wine (Mazza 2007). Because of their strong red to blue coloring,

[1]*Istanbul Aydın University, Faculty of Engineering, Department of Food Engineering*

anthocyanins are the most recognized, visible members of the bioflavonoid phytochemicals (Ray et al. 2009).

Anthocyanins have been shown to be strong antioxidants and may be responsible for some biological activities including the prevention the risk of cardiovascular disease, diabetes, arthritis and cancer (Kahkonen 2003, Wang 1997). Anthocyanins have a good effects and interest to the artificial colorants The stability of anthocyanins is changed by different factors such as pH, temperature, light, pigment, metallic ions, the presenc of enzymes etc (Devi et al. 2012).

Black carrot is one of the most important root vegetable of *Apiaceae (Umbelliferae)* family (*Daucus carota* L.) originated from Central Asia, grown and consumed in Turkey, Afghanistan, Egypt, Pakistan and India ((Kaur et al. 2013, Elham et al. 2006). It is often used in juice, concentrate, shalgam i.e., food colorant in food sector (Türkyılmaz et al. 2012). It is among the fruits that contain high amounts of anthocyanins (Kırca et al. 2006) and other polyphenolics. Mazza and Miniati (1993) have reported a range of 1750 mg/100 g fresh weight and 45. 5 g/kg to 17.4 g/kg dry matter total anthocyanin amounts (Kammerer et al. 2004).

According to the investigations of scientists related to the black carrots, the results indicated that it contains high amounts of acylated anthocyanins such as sinapic, ferulic and p-cumaric acid (Dougall et al. 1998, Kırca et al. 2006). Because of the fact that related to the black carrots these starts to gain in the importance of further research activities of many scientists.

Black carrot is much more important in many cases due to its using mainly in food industries such as natural food coloring, drinks, beverage, confectionary, dairy, candies, yoghurt and although other like cosmetics, pharmaceutical starts to use the benefits of the black carrots.

Enzymes such as pectinase enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples and sapota. Pectinases have also been used in wine production (Anonim 2008). Enzymatic treatment alone or in combination with others, is one of the

potential pretreatment, which results in increased yield with better juice quality, colour and acceptability (Kaur and Sharma 2013). Khandare et al. (2011) indicated that enzyme-assisted black carrot juice processing significantly improved the antioxidant and total phenolics composition of black carrot juice. It was reported that overall increase of 33% in juice yield, 27% in total phenolics and 46% in total flavonoids. Khandare et al. (2011) reported that pre-press maceration treatment effected antioxidant composition of black carrot juice with different doses of cell wall degrading enzyme pectinase. Sharma et al. (2005) optimized the enzymatic process parameters for increased juice yield from carrot. In this study, it was reported that enzymatic treatment resulted in increase in juice yield by 13.95%. Kaur and Sharma (2013) reported that some factors affecting colour of carrot juice during storage are pH, acidity, processing temperature, duration and fruit cultivar.

Several researchers have recently reported that enzyme treatments significantly increase the juice yield, enhance the recovery of anthocyanins and phenolics and enhance the total anthocyanin content in black currant juice (Landbo and Meyer, 2004; Wang et al. 2009)

The aim of this research was to increase amount of pigments from black carrot juice by using a Natuzym DP+ and Rapidase enzymes at 50° C at an hour period time and also the effect of two different enzymes were investigated for increasing amount of the anthocyanin content

2. Materials and methods

2.1. Materials

Black carrots used in the study were obtained from the Büyükçekmece bazaar district of the city of Istanbul were processed immediately. A flow diagram of juice enzimatization and concentrate processing was shown in Fig. 1.

2.2. Juice Concentrate Processing

Carrots were washed and damaged parts were separated. Than grinding process of black carrot juice was made with juice extraction machine. Then, at 85 ºC for 10 minutes fruit juice is not boiled which is called blanching in order to inactivate enzymes. The purpose of this step was to provide the inactivation of enzyme naturally present in vegetables. If don't apply this

temperature enzymes can be effect on the depocosition of the phonelic compounds. The pure black carrot juice was processed by seperation of variable active substances. Black carrot juices was centrifuged at 40*100 rpm/rcf for 3 minutes. After centrifugation, carrot juice was hold 85-87 °C for 2-3 minutes in flash pasteurization, then cooling to 50-55 °C. Microorganisms in vegetable juice and other enzymes were inactivated and prevented quality loss in color and quality of mash.

The vegetable water that heated to 50 °C, enzyme was added to do experiment, the enzymatic fermentation was performed 60 minutes with Natuzym DP+ (Weiss BioTech:10-A10166) and Rapidase enzyme (DSM Pac:414294401) dosage. Thus, this process increased efficiency, vegetable juice color quality and color intensity. Undesirable particles in liquid was seperated with clarification. Coarse filtration with filter paper was preferred in carrot juices processing. They were analyzed for anthocyanins content

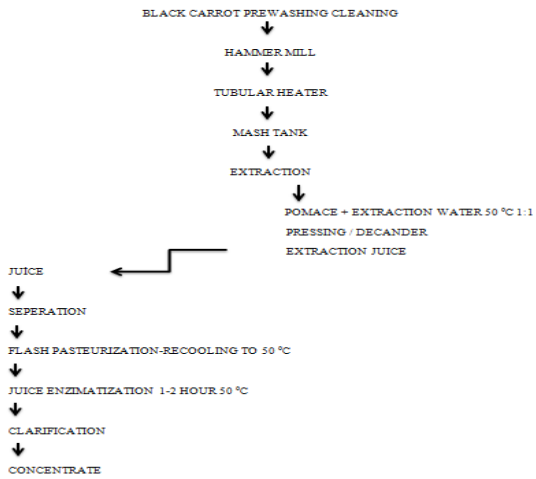

Fig. 1. Flow diagram for the processing of black carrot juice concentrate.

2.3. Methods

2.3.1. Other analyses

Brix was measured at 20°C using an Abbe's Refractometer and pH with pH meter (İnolab pH 720)

2.3.2. Enzymatic fermentation

In this research, Natuzym DP+ enzyme (25-50-75 µl enzyme solution/ L black carrot juice) and Rapidase Pac enzyme (25-50-75 µl enzyme solution/1 L black carrot juice) were added and kept at 50°C for 1 h which were filtrated and analyzed for total anthocyanins and other analyses. The effect of two different enzymes were investigated for increasing amount of the anthocyanin content.

2.3.2. Anthocyanin analysis

Total monomeric anthocyanins of samples were determined in duplicate using the pH-differential method as described by Giusti and Wrolstad (2001). For this reason, the aliquots of black carrot concentrate were first brought to pH 1.0 and 4.5 and were allowed to equilibrate 1 h at room temperature. The absorbance of equilibrated solutions was then measured at 560 (λ max) by using a UV-VIS spectrophotometer (JENWAY 6315). Pigment content was calculated based on cyanidin-3-glucoside (Alasalvar et al. 2005, Kirca 2007) with molecular weight of 449.2 and with extinction coefficient of 26,900 (Giusti and Wrolstad 2001). The difference in absorbance values at pH 1.0 and 4.5 was directly converted proportional to anthocyanin concentration. Quartz cuvettes of 1 cm pathlength were used and all measurements were carried out at room temperature. Absorbance readings were made against distilled water as a blank.

3. Results and discussion

While each application were processing, Brix and pH measurements were made. This measurements were made in raw material, after boiling, centrifuge, enzymation, pasteurization and after filtration. Table 1 and Table 2 shows that Brix and pH values of black carrots juices..

Respectively, stability of anthocyanins from black carrots was studied at various solid contents, °Brix and pH values in Natuzym DP+ and Rapidase Pac enzyme application as shown in Table 1 and Table 2.

Table 1. Brix and pH values of black carrots juices in Natuzym DP+ enzyme application.

Samples	Brix	pH
İn raw materials	10.9	6.15
After boiling	10.9	6.16
After centrifuge	9.5	6.22
After enzymation	9.5	6.08
After pasteurization	9.5	6.10
After filtration	9.5	6.11

Table 2. Brix and pH values of black carrots juices in Rapidase Pac enzyme application.

Samples	Brix	pH
İn raw materials	10.2	5.81
After boiling	10.2	5.86
After centrifuge	9.5	5.80
After enzymation	9.5	5.81
After pasteurization	9.5	5.80
After filtration	9.5	5.80

According to analysis; all the application and enzyme added processes didn't observed to exchange pH, but brix of the samples is changed by centrifugation.

It was observed, the implementation process did not changed the pH in carrot juice, but the centrifugation process was caused a decrease in the amount of dry matter. Enzyme application didn't change the pH at all. According to the readings, increasing concentrations of the Natuzym DP+ enzyme were found to be increased values of absorbance as shown in in Table 3.

Table 3. Total anthocyanins contents

Enzyme content	NATUZYM DP +	RAPIDASE PAC
Pre-enzyme treatment	831 mg/ L	1122 mg/ L
25 µl enzyme / 1 L black carrot juice	1177 mg/ L	1322 mg/ L
50 µl enzyme / 1 L black carrot juice	1235 mg/ L	1340 mg/ L
75 µl enzyme / 1 L black carrot juice	1337 mg/ L	1385 mg/ L

Initial anthocyanins contents were 831 mg/ L, 1122 mg/ L for Natuzym DP+ and Rapidase Pac respectively as shown in Table 3. These reference values were different each other due to several composition of carrot juices. The enzyme were used in this research was not affecton the pH of juice but the filtration and centrifugation were caused a reduction in degre of Brix content.

In this investigation was observed that the yield of anthocyanin depended on the additional amount of available enzyme as shown in Table 3. Effect of Natuzym DP+ enzyme related with concentration treatment for increasing anthocyanin concentration was shown in Fig 2 and also effect of Rapidase Pac enzyme treatment on increasing of anthocyanins concentration was shown in Fig 3.

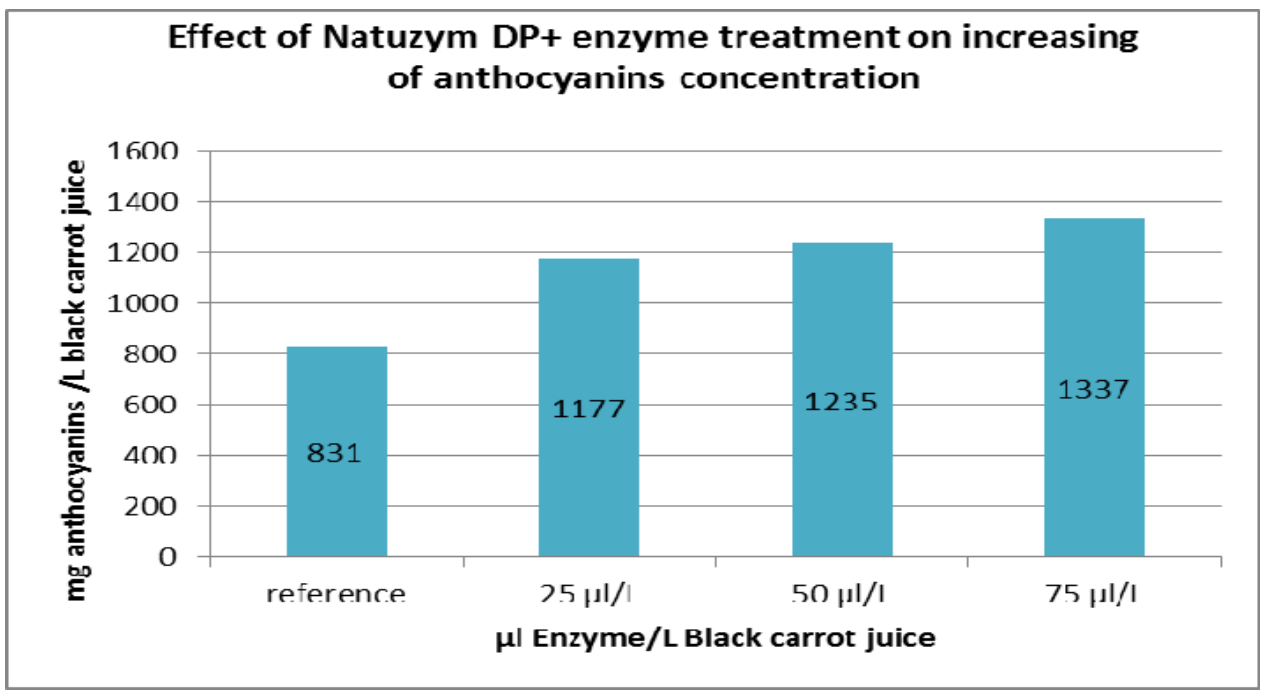

Figure 2. Effect of Natuzym DP+ enzyme treatment on the anthocyanins concentration

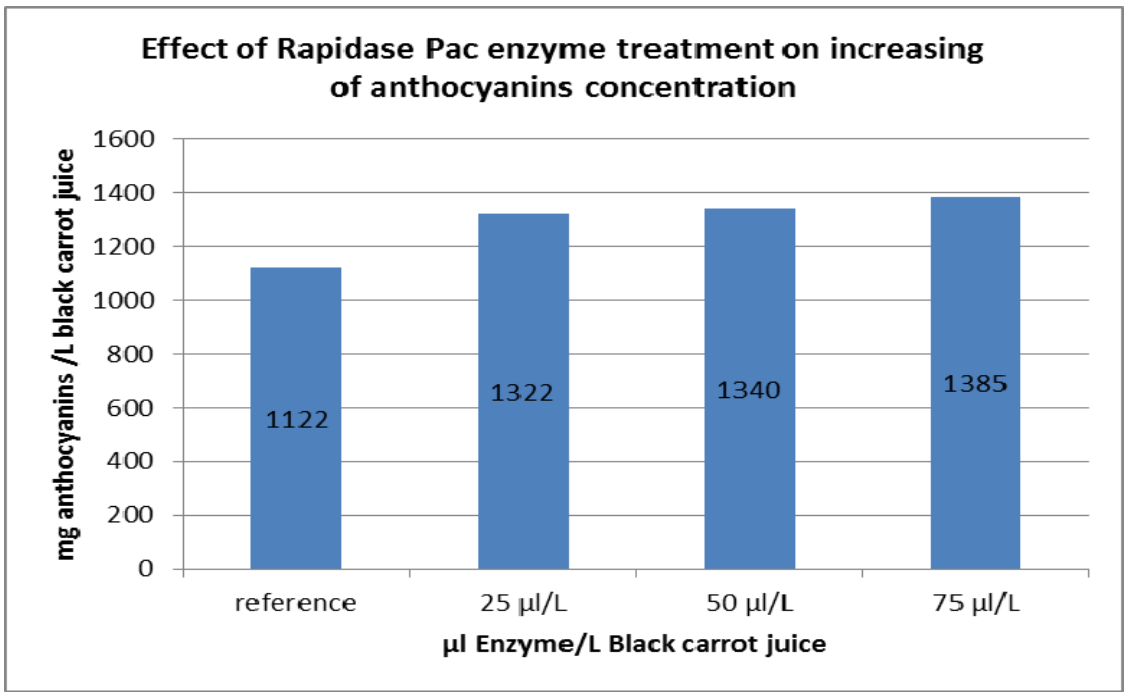

Figure 3. Effect of Rapidase Pac enzyme treatment on the anthocyanins concentration

Table 2. Enzyme yield depending on anthocyanins contents of black carrot juice

Enzyme used	Enzyme dosage (µl/l black carrot juice)	Yield of enzyme (%)
Natuzym DP+	25 µl	41.63
	50 µl	48.61
	75 µl	60.89
Rapidase Pac	25 µl	17.82
	50 µl	19.43
	75 µl	23.45

In Table 2 as can be seen that the yield of anthocyanins contents in black carrot juice depending on the enzyme concentration. According to Table 2, when enzyme dosage was increased, total amount of anthocyanins in black carrot juice were also increased. Thus, the result indicates that enzyme yield of Natuzym DP+ was more effective than Rapidase Pac enzyme were shown in Table 2. The highest enzyme yield of anthocyanin were obtained by additional of 75 µl Natuzym DP+ (60.89%), but the yield estimated with Rapidase Pac was 17.82%.

Compared with literature, Pectinex Ultra SP-L, Pectinex Smash, Pectinex BE 3-L and Biopectinase CCM were increased the total content of anthocyanins between 13–41% in the bilberry juices and 18–29% in the blackcurrant juices according to Buchert et al. 2005. In this study, It was reported that the most efficient enzyme preparation to increase the anthocyanin extraction was Pectinex BE-3L, increasing the yield by 41%. This result is in agreement with the results of our study.

The yield of enzyme found in black carrot juice in our study was higher than the values reported by author, such as Karadeniz and Ekşi (1999), who reported the effect of mash enzymation (Pectinex BE 3-L, Novo Nordisk Ferment) on juice yield and chemical composition of sourcherry juice, the increase in juice yield due to mash enzymation was found values of 6.25-9.0%. The contents of anthocyanins in the sourcherry juices ranged from 94 to 140 mg/l. It was caused differences so the structure of the fruit and enzymes used in this study than in black carrot juice. In our study, the total anthocyanins contents of black carrot juice were determined values of 831 -1337 mg/l. in Natuzym DP+ enzyme treatment, while the contents of total anthocyanins was 1122-1385 mg/l in Rapidase Pac enzyme treatment. Contents of total anthocyanins in juices of bilberry ranged from 1823 to 20174 mg/kg reported by Buchert et al. 2005. The differences may be due to the structure of juices.

Türkyılmaz et al. (2012) were found that depectinisation and bentonite treatments resulted in 7% and 20% were increased in monomeric anthocyanins content of black carrot juice respectively, but gelatine-kieselsol treatment and pasteurisation were caused to 10% and 3-16% reduction. Contents of total anthocyanins of two different the black carrot extracts value of 93.8 and 126.4 mg/ 100 g fw, were reported by Algarra et al. 2014. As comparing with our enzyme treatments were increased anthocyanin content than other researchers.

The authors Junker (1987) and Yücel (1993) were found that in apple juices by increased additional mash enzymes which were increased yield of anthocyanin between 5 to 30% . It also lower than our results. It was reported that a process for the increased juice extraction form carrot, enzyme

concentration changed from 50-650 mg/kg of grated carrot (Sowbhagya 2010).

Landbo and Meyer (2004) reported that juice yields ranged from 66.4% to 78.9% by wet weight of black currant mash in pre-press maceration treatments with 10 different pectinolytic enzyme preparations in experimental black currant juice production. The yields of anthocyanins in the juices were increased from 900 to 2200 mg/kg wet weight black currant mash. Similar results were also reported in black carrot juice. The differences may be due to cell structure of black carrot and black currant

Mieszcczakowska (2012) investigated that impact of enzyme on quality of balackcurrant and juices. Results were reported that the best pressing-yield for blackcurrant was achieved with polygalacturonase and pectin lyase, 65 g/100 g after 1 h and 74 g/100 g after 4 h of pectinolysis. The macerating mixture gave about 58-59 g/ 100 g yield, pressing-yield of plum juices was in the range of 94-97 g/ 100 g . This results associated and comparable with our results.

Tochi et al. (2009) reported that two commercial enzyme preparations, a pectinase and a liquid pectinase/hemicellulases were used singly or in combination at a rate 0.03% (w/w) in a two step extraction of pineapple juice. The concentrated juices were extracted twice treated with (Fluka) Rapidase enzymes. According the results of sensory evaluation, juice extracted using Rapidase scored better than that extracted with either Fluka. These observations were in agreement with our results.

4. Conclusions

The most efficient enzyme preparation to increase the anthocyanin extraction was 75 µl Natuzym DP+ enzyme dosage in black carrot juice, was increased the yield up to 60.89%. The enzyme treatments had quite significant effects on the total anthocyanins content in black carrot juice. This study indicates that the enzyme treatment were used in this processing strongly releated additional enzyme concentration, time and temperature on the anthocyanin extraction. It can be conculed that black carrot is a rich source of anthocyanins and an important colorant foods such as beverages, fruit juice

processing, confectionary, dairy products with good functional and nutritional value.

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