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EFFECTS OF PECTINASE AND CELLULASE ENZYMES ON THE BLACKCURRANT JUICE BY REVERSE OSMOSIS

UTICAJ PEKTINSKIH I CELULOZNIH ENZIMA NA SOK RIBIZLE U REVERZNOJ OSMOZI

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SUMMARY

The blackcurrant is an important and popular fruit which contains a number of constituents, recognized for their biological activity. The aim of the present study was to prepare a concentrated blackcurrent juice by reverse osmosis and preserve the valuable components of the berry. A Paterson Candy International (PCI) apparatus was used for juice concentration, equipped with a tubular B1 RO membrane module; the active membrane area was 0,9 m^2. The concentration of the juice was carried out at 20°C and 60 bar pressure. The effects of pretreatments with two pectinolytic enzyme preparations on the permeate flux were examined to explore the applicability of the reverse osmosis for concentration of the blackcurrant juice. For the control sample, the permeate flux was the lowest and the maximum TSS of the concentrate reached was the lowest at 22,5 °Brix. The highest permeate flux was achieved during the concentration of juice that has been previously pretreated by Cellobiase from Aspergillius niger.

***Key words**: blackcurrant, reverse osmosis, pectinase and cellulase enzymes.*

INTRODUCTION

The blackcurrant (*Ribes nigrum* L.) is a species of currant that is native to central and northern Europe as well as northern Asia that produces a very dark purple (almost black) berry fruit. Blackcurrants are a very popular fruit in Europe [1]. It contains in great amounts mineral salts and vitamins, which have beneficial effects to human health. The intense colour is due to the high concentrations of anthocyanins, and the berries are a rich source of vitamin C; their C-vitamin concentration is 4–5 times higher than that of lemon. The fruit is also rich in P -, B1 -, B2 - vitamin, in provitamin A, in pigment and anthocyanins [2]. Although some blackcurrants are consumed as fresh fruit, the majority are processed to produce juices, syrups, cordials, purees, and concentrates which are often incorporated into jams/conserves, jellies, pie fillings, and various ready-to-drink beverages including smoothies. Blackcurrant extracts are sold as a commodity and are widely used in the food industry, particularly for their colour but also their flavour which can be rather astringent. Blackcurrant (*Rubus fruticosus* sp.) are used in various food products such as juice, ice cream, jams and for nutraceutical applications. Juices produced from blackcurrant are rich in various antioxidants including anthocyanins [3].

In recent years, membrane processes such as nanofiltration (NF), reverse osmosis (RO) and alternative membrane-based separation technologies, e.g. membrane distillation (MD) and osmotic distillation (OD), have been evaluated as fruit juice concentration processes [4]. RO has achieved some commercial interests in the fruit juice concentration since it maintains the advantages of a low temperature product processing (better preservation of heat-labile ingredients), reduced energy consumption and lower capital equipment costs. However, the final concentration of juices is limited to about 25–30 °Brix, due to the high osmotic pressure of the retentate at those levels [5].

The aim of the present study was to concentrate black currant juice by using reverse osmosis. A further objective was to compare the results throughout the concentration beween the pectinolytic enyme pretreated juice to the juice without any pretreatment.

MATERIALS AND METHODS

Blackcurrant juice

The blackcurrant juice was purchased from Tolcsva (Hungary). This is a typical hungarien berry growing area. The initial total soluble solids content of the feed was 15-17 °Brix. This blackcurrant juice was used in the concentration studies using reverse osmosis.

Enzymatic pretreatment

To minimise fouling and reduce juice viscosity, the blackcurrant juices were pretreated by two enzyme: Pectinase from *Aspergillius aculeatus*, and cellobiase from *Aspergillius niger* (Novozyme). Such treatment is known to facilitate the filtration process during juice concentration. A small amount of the liquid enzyme preparations (8 ml / 20 l) were used and the hydrolysis treatment lasted 24 hours at 25°C.

(a) The treatment time was 24 hours at 25°C. We add 8ml Novozyme enzyme (Sigma Aldrich enzyme complex, 200 U/ml) to the 20 l blackcurrant juice.

(b) The treatment time was 24 hours at 25°C. We add 8ml Pectinase enzyme (Sigma Aldrich, 9500 U/ml) to the 20 l blackcurrant juice.

(c) The treatment time was 24 hours at 25°C. We add 2*8ml from both (Novozyme and Pectinase) enzyme prepeparations to the 20 l blackcurrant juice.

Membrane processes

A Paterson Candy International (PCI) apparatus was used for juice concentration, equipped with a tubular B1 module(RO membrane); the salt retention of the membrane was 99%, and the active membrane area was 0,9 m^2. The module actually contained an AFC 80 polyamide tubular membrane [6]. The temperature was controlled (~ 25 ^{o}C), using cold water circulating through the tubular heat exchanger. The applied low temperature (26-30 °C) preserves the valuable berry components (anthocyanins, phenols, C-vitamin) and the maintains the antioxidant

capacity of the juice throughout the process. The transmembrane pressure was fixed at 60 bar, and 60 liter of the juices were concentrated in each batch. Before and after each experiment, the water flux was measured with destilled water at 25 °C. Membrane regeneration was achieved by washing in a 0.1 w/w% NaOH solution and rinsing with distilled water. Finally, a 0.5% citric acid solution has been used and circulated for 30 min, followed by rinsing of the membrane with distilled water. A typical industrial cleaning procedure is carried out and followed by another determination of the clean membrane resistance:

$$J_W = \frac{\Delta p_{TM}}{\eta_W \cdot R_M} \quad (1)$$

where J is the permeate flux rate (m s^{-1}) and Δp_{TM} is the transmembrane pressure (Pa). The resistance of the membrane (R_M) was calculated from the flux using:

$$R_M = \frac{\Delta p_{TM}}{\eta_W \cdot J_W} \quad (2)$$

where η_W is the dynamic viscosity of water (Pa s).

The fouling resistance (RF) calculated from the flux of the clear water measured after juice concentration and following gel layer removal:

$$R_F = \frac{\Delta p_{TM}}{J_F * \eta_W} \quad (3)$$

The gel-layer resistance (RG) calculated from the measured flux at the end of the juice concentration process:

$$R_G = \frac{\Delta p_{TM}}{J_F * \eta_m} - R_M - R_F \quad (4)$$

where η_m is the viscisity of the blackcurrant juice at the end of the concentration process [7].

The total resistance (R_T) is calculated as:

$$R_T = R_M + R_F + R_G \quad (5)$$

where R_T is the total resistance, R_M is the membrane resistance, R_F is the fouling resistance, and R_G is the gel-layer resistance[8].

Total soluble solids (TSS) content was measured using an Atago PR-101α digital refractometer; the TSS data were expressed as °Brix.

RESULTS AND DISCUSSION

The effect of the transmembrane pressure (TMP) and the applied pretreatment on the permeate flux was investigated before the concentration tests. The initial fluxes and the slope of these changes were different and depended on the applied pretreatment (Fig. 1).

With increasing TMP the permeate flux increased substantially. The highest permeate flux was achieved during concentration of juice previously treated by cellobiase from *Aspergillius niger* (Novozyme). A lower permeate flux was achieved with the control sample to which no enzymatic pretreatment was applied. However, the lowest permeate flux was achieved with the sample to which both enzymes have been applied. At 30 bar pressure, only the cellobiase treated blackcurrant juice provided measurable flux data, because the viscosity values of the cellobiase treated samples decreased better than all other samples. Apparently, the largest viscosity value was noticed with the juice sample treated with both enzymes (Table 1).

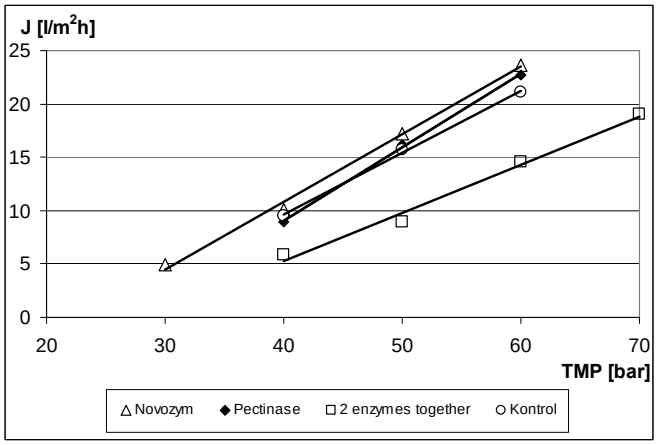

Fig. 1. The fluxes of the different pretreated blackcurrant on different pressure

Sl. 1. Fluks različitih predtretmana crne ribizle na različitim pritiscima

Table 1.Viscosity values of blackcurrant juice

Tabela 1. Vrednosti viskoziteta soka od crne ribizle

Temp. (25 °C)	Novozyme	Pectinase	The 2 enzymes together	Control
Viscosity(mPas)	1.61	1.84	2.20	1.82

The initial total soluble solids content of the feed varied between 15-17 and reached 26,5 °Brix for Pectinase, 29,5 °Brix for Novozyme enzyme after, 28,5 °Brix for the dual enzyme treatment and 22°Brix for the control juice. The concentration of the control sample ended after 130 minutes, whereas the contentration of the enzyme treated samples ended after 200 minutes for Novosyme, 160 minutes for Pectinase and 270 minutes for the dual enzyme treatment. The concentration time of together with two enzymes treated juices was longer than that of the Novozyme. It have a shrewd idea that the two enzymes entered into an interaction with each other. The TSS increase was the sharpest for the Novozyme-treated sample. The latter treatment also yielded the highest TSS level (29.5 °Brix).

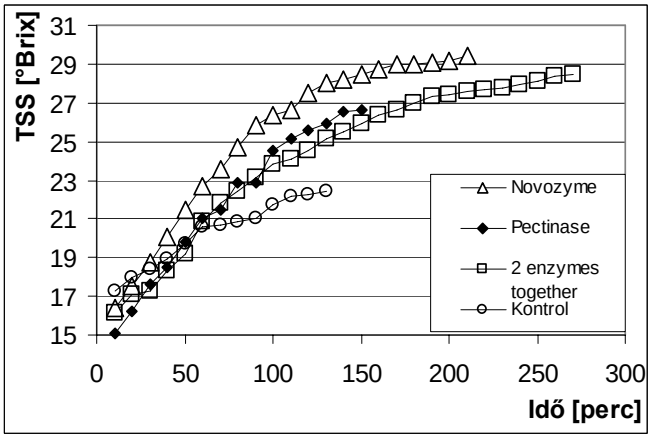

Fig. 2. Comparison of TSS values during concentration of blackcurrant juice

Sl. 2. Poređenje TSS vrednosti tokom ugušćavanja soka od crne ribizle

The resistance values (RM, RT, RF, and RG), of the filtration proecess are presented in Figure 3. The fouling resistance is elevated for the Novozyme-treated sample, while the gel-layer resistance is raised in the case of the other two enzymatically-treated samples, since the cellobiase preparation degrades more effectively the cell wall materials and reduces the viscosity of the blackcurrant juices better than the pectinase preparation.

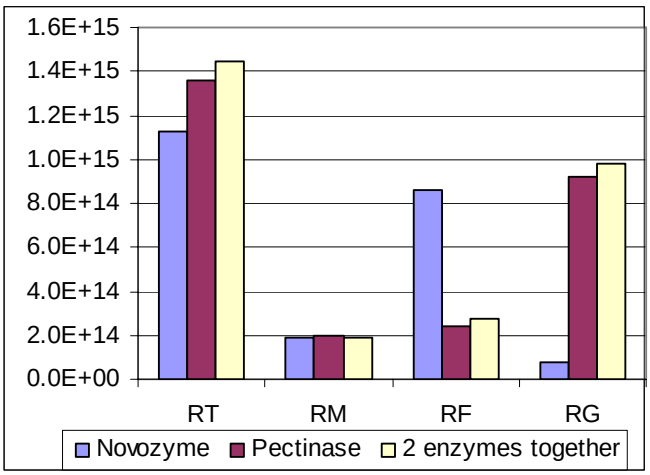

Fig. 3. A comparison of the resistance values among enzymztically-pretreated blackcurrant juices
Sl. 3. Poređenje rezistentne vrednosti između enzimski predtretiranog soka od crne ribizle

CONCLUSIONS

The aim of this study was to examine the applicability of reverse osmosis for the concentration of blackcurrant juice using an AFC 80 polyamide tubular membrane. The effects of pretreatment with different hydrolytic enzymes have been also explored. Two commercially available enzyme preparations (Pectinase from Aspergillius Aculeatus, Cellobiase from Aspergillius niger) were used in this respect, and the results were compared to the control sample (untreated juice).

For the control sample, the permeate flux was the lowest, the flux decline rate the highest and the maximum TSS of the concentrate reached was at 22 °Brix. The highest permeate flux was achieved during the concentration of juice that has been previously pretreated by Novozyme, and the flux decline rate was the smallest for this sample as well. The highest concentration ratio was observed in the case of the Novozyme-treated juice.

It can be concluded that reverse osmosis is a viable method for concentration of blackcurrant juices with the applied transmembrane pressure at 60 bar and 25°C operating temperature, when an enzymatic pretreatment is used before the processing. It was also found that the juice treated with Novozyme was the most effectively concnetrated by reverse osmosis.

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