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Continuous Clarification of Barberry Juice with Pectinase Immobilized by Oxidized Polysaccharides

Running head: Continuous clarification of the barberry juice

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SUMMARY

Research background. Barberry juice is a rich source of bioactive compounds and shows different health properties such as antioxidant and anticancer activities. Clarification, as the removal process of suspended material, is an important step in production process of fruit juice due to its significant effect on the appearance, flavor and commercialization of juice. Pectinase is the most important enzyme applied in juice clarification that breaks down the pectin polymer structure and leads to reduce the undesirable turbidity. Pectinase immobilization is a way to overcome free enzyme drawbacks such as instability, high cost, the difficulty of recovery and recyclability. Also, continuous clarification process which is highly preferred in fruit juice industry is not possible without enzyme immobilization.

Experimental approach. Pectinase enzymes were immobilized on the functionalized glass beads (glass bead+3-aminopropyl triethoxysilane) by glutaraldehyde, polyaldehyde pullulan and

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polyaldehyde kefiran and the barberry juice clarification was accomplished in the batch and continuous process in a packed bed reactor (PBR). Also, the effect of clarification process on the physicochemical and antioxidant properties of the barberry juice samples was evaluated.

Results and conclusions. The clarification optimum conditions in the PBR were obtained at a flow rate of 0.5 mL/min, the temperature of 50 °C and treatment time of 63 min. The clarification process led to a decrease in turbidity, pH, total soluble solids, viscosity, total phenol content and antioxidant activity of the juice samples. Also, this process increased the clarity, acidity, reducing sugar content and the lightness parameter of the barberry juice. It was found that the greatest effect on the studied properties was related to polyaldehyde kefiran-immobilized pectinase treatment in the continuous process and both new cross-linkers had better enzyme immobilization performance than common cross-linker (glutaraldehyde).

Novelty and scientific contribution. For the first time, barberry juice was clarified with pectinase immobilized by polyaldehydes of pullulan and kefiran and the obtained results showed that the pectinase immobilization by these new cross-linkers was much more efficient than by the glutaraldehyde as common cross-linker. Therefore, it seems that these findings can be of use towards an industrialised production of fruit juices.

Key words: barberry juice, pectinase, polyaldehyde polysaccharide, immobilization, packed bed reactor

INTRODUCTION

Barberry (*Berberis vulgaris* L.) fruit, as the largest genus of *Berberidaceae* family, is a rich source of bioactive compounds such as carotenoids, flavonoids, anthocyanins and polyphenols and therefore shows different health properties such as antioxidant and anticancer activities (*1*). Besides, in traditional medicine, this fruit especially its juice has wide application in the treatment of colitis, chronic inflammation, high blood pressure and also, the nervous, liver and heart problems (*2*). Considering these health benefits, it is highly recommended to consume barberry juice as one of the most important nutraceutical juices. An overview of the production process of barberry juice reveals that its main stages include separation of fruit and washing, raw juice production, clarification, heat treatment and packaging. Among these steps, the clarification, which is theoretically defined as the removal process of suspended material causing turbidity and sediment in the product, is particularly important due to its significant effect on the appearance,

flavor, customer attention and commercialization of juice. The main cause of this turbidity and sediment is a hydrocolloid named pectin as a polymer of α-1,4- galacturonic acid that makes up approx. 30% of the primary cell wall of plants and enters the product during the juicing process (*3,4*) and therefore it is necessary to remove it in the mentioned step. In conventional industrial methods, this process is done with the help of gelatine, bentonite and different types of membranes (*5*). However, with the advancement of science and the increasing demand for more selective and clean processes in recent years, the enzymes have gained a special place in this process because they have high efficiency, selectivity, low toxicity and also operate under mild reaction conditions (*6-8*).

Pectinase is the most important enzyme employed in juice clarification process that breaks down the pectin polymer structure and leads to reduce the undesirable turbidity, cloudiness and sediment (*9*). Although the use of free pectinase in the juice production process can be considered as a potential process, it has some drawbacks such as instability, high cost, the difficulty of recovery and recyclability that limit its industrial application (*10*). The way to solve these problems is carrier-bound and carrier-free immobilization. Carrier-bound immobilization refers to the process of connecting an enzyme on a suitable support surface by the different methods including adsorption, entrapment and covalent binding (*11-13*), while carrier-free immobilization is prepared by directly cross-linking different enzyme preparations (*14,15*). Wahab *et al*. (*16*) stated that the covalent immobilization of pectinase on the alginate-agar gel by glutaraldehyde increased significantly the stability of enzymes. Hassan *et al*. (*17*) immobilized pectinase and xylanase enzymes on the alginate beads and reported that the used method had a high efficiency for clarification of apple juice. Benucci *et al*. (*18*) reported that the immobilization of pectinase and protease enzymes on the chitosan beads by polydialdehyde starch can be considered as an efficient method for clarifying pomegranate juice. As can be seen, there are two key parts of the support and immobilization method in this definition that determine the capabilities and performance of the system. In the case of support, it would be wise to choose glass beads because the enzymatic system undergoes stress during the juice clarification process and so it is important to choose resistant support. In addition to excellent resistance to the stress condition, the glass bead is insoluble support with high capacity to bind enzyme which has led to considerable attention to it (*19*). In the case of the immobilization method, it seems that covalent binding, as one of the strongest chemical bonds used to immobilize enzymes, is the best method in the industrial applications due to the lower probability of the release of enzymes from support

and therefore higher stability and reusability (*20-22*). For the binding of the enzyme to support, the existence of a cross-linker is essential. To our best knowledge, in all studies in which glass bead is used as an enzyme carrier, glutaraldehyde is applied as cross-linker (*19,23*), that is a toxic material in all doses and therefore its use is associated with concerns and challenges. In previous studies, for the first time, we applied poly-aldehyde kefiran and pullulan (produced by partial oxidation with sodium periodate) as new cross-linkers to immobilize pectinase on the functionalized glass bead (glass bead+3-aminopropyl triethoxysilane) and the obtained results showed that the used polyaldehyde polysaccharides were comparable with glutaraldehyde and also could bind more amount of enzyme on the support (*24*).

One of the most important advantages of immobilized enzymes is the capability to perform a continuous enzyme process after immobilization. The first step in performing a continuous enzymatic process is the preparation or manufacture of a bioreactor. Packed bed reactor (PBR) is a simple and inexpensive reactor with a double-walled cylinder packed with support-immobilized enzymes (Fig. 1) which so far has been used successfully in many studies (*11,25*). For example, Dal Magro *et al*. (*26*) reported that pectinase immobilized on chitosan particles and used in packed bed reactor seems to be a good alternative for large scale application on juice clarification. In another study, Benucci *et al*. (*27*), who used from packed bed reactor for white wine protein stabilization by the immobilized cysteine proteases, reported that the enzymatic treatment in packed bed reactor can be used as an alternative to bentonite fining for white wine protein stabilization.

As mentioned above, the feasibility of using polyaldehyde polysaccharides (polyaldehyde kefiran and pullulan) as new cross-linkers for the immobilization of pectinase on the glass beads was investigated in previous studies (*24,28*). However, the performance of this immobilization system under the actual conditions remains unclear while without checking the obtained results from the juice clarification, the efficiency of this system cannot be judged. Therefore, keeping in mind the broad applications in the juice industry, in this study, a continuous clarification of barberry juice using PBR charged with polyaldehyde polysaccharides-immobilized pectinase on the glass beads was aimed. Also, the physicochemical and antioxidant properties of the barberry juice samples clarified by polyaldehyde polysaccharides-immobilized pectinase were compared with the samples clarified by free and glutaraldehyde-immobilized enzymes.

MATERIALS AND METHODS

Materials

The pectinase enzyme (Pectinex® Ultra Color) with a protein content of 80 mg/mL was obtained from Novozymes (Bagsvaerd, Denmark). Also, the preliminary experiments showed that the specific activity of the applied pectinase was (2.93±0.13) U/mg at the temperature of 50 °C and pH of 5.0 (obtained optimum conditions for free pectinase activity).

Apple pectin with DE of 72-76 % was bought from Herbstreith & Fox KG (Neuenbuerg, Germany). Kefiran and pullulan exopolysaccharides ware supplied by Microbiology Lab., Department of Food Science and Engineering, University of Tehran (Karaj, Iran) and Herbstreith & Fox KG (Neuenbuerg, Germany), respectively. Glass beads with 6 mm diameter were provided by Iran Beads Co. (Esfahan, Iran). The 3-aminopropyl triethoxysilane (3-APTES), 3,5-dinitrosalicylic acid (DNS), glutaraldehyde, hydroxylamine hydrochloride, Sodium periodate and D-(+)-galacturonic acid monohydrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

Preparation of polyaldehyde polysaccharides

The partial oxidation reaction with sodium periodate ($NaIO_4$) was applied to produce the polyaldehyde kefiran and pullulan (*29*). To produce any polyaldehyde polysaccharide, 5 g of sodium periodate powder was dissolved in 100 mL distilled water and the obtained solution was slowly added to 250 mL polysaccharide aqueous solution (4 % *m*/*V*) under stirring. After adjusting pH to 5 by adding 1 M sulfuric acid solution, the reaction was continued in the dark for 3 h at (40±2) °C. To precipitate polysaccharides, 2:1 (*V*/*V*) of 96 % ethanol was added and the resulting mixture was stored at refrigerator (7 °C for 3 days). Afterward, the oxidized polysaccharides were separated by centrifuging at 10 000×*g* for 20 min, were washed three times with distilled water and subsequently were freeze-dried for 1 day. The aldehyde content of polyaldehyde kefiran and pullulan, respectively, was obtained (23.6±0.9) and (19.9±1.3) % by the reported method by Kholiya *et al*. (*30*) using hydroxylamine hydrochloride.

Immobilization of pectinase on the glass bead

In the current study, the cross-linkers of glutaraldehyde, polyaldehyde kefiran and polyaldehyde pullulan were applied to immobilize pectinase enzymes on the glass beads based to Gomez *et al*. (*19*) method. 23 cm^3 glass beads (approx.110 beads) were washed with 5 % HNO_3 at approx. 85 °C for 120 min and then were dried at 110 °C for 24 h. In the next step, the

functionalized glass beads were prepared by mixing glass beads with 10 % (*V*/*V*) 3-APTES solution for 3 h at 60 °C and a constant pH of 4 (pH adjusted by 6.0 and 0.1 M HCl). After washing with distilled water and drying at 80 °C for 24 h, the glass beads were slowly stirred in glutaraldehyde solution (2.5 % *V*/*V*) or polyaldehde kefiran (2.5 % *m*/*V*) or polyaldehyde pullulan (2.5 % *m*/*V*) for 2.5 h at room temperature (approx. 23 °C). The glass beads were then immersed in pectinase solution diluted by sodium acetate buffer (pH=4 and ratio of 1:4 *V*/*V* (5 mL enzyme solution with protein concentration of 80 mg/mL and 20 mL buffer)) for 5 h at room temperature (approx. 23 °C), were washed with the same buffer and were stored at 4 °C, respectively.

The protein content of the glass beads after pectinase immobilization by glutaraldehyde, polyaldehyde kefiran and polyaldehyde pullulan, respectively, was (4.51±0.68), (57.75±1.12) and (20.13±0.98) mg in 23 cm^3 glass beads according to Lowry method (*31*) by formula below:

$$\text{Protein content (mg in 20 cm}^3\text{ glass beads)} = m_{\mathrm{I}} - (m_{\mathrm{R}} + m_{\mathrm{W}}) \qquad /1/$$

where m_I, m_R and m_W were the protein content of initial, remained and washing solutions, respectively.

Also, the specific activity of pectinase immobilized by glutaraldehyde, polyaldehyde kefiran and pullulan, respectively, was 2.56±0.12 (activity recovery of 87.4 %), 1.87±0.24 (activity recovery of 63.8 %) and 2.21±0.18 (activity recovery of 75.4 %) U/mg protein according to the described method in the previous study (DNS method) (*24*). It should be stated that one unit was defined as the enzyme content required to form 1 μmol/min of galacturonic acid under the experimental conditions.

Kinetic parameters

The Michaelis–Menten constant (K_m) and the maximum reaction rate (v_{max}) were determined as following equation:

$$\frac{1}{v} = \frac{K_{\mathrm{m}}}{v_{\max}} \cdot \frac{1}{[\mathrm{S}]} + \frac{1}{v_{\max}} \qquad /2/$$

where [S] was pectin concentration (2-20 mg/mL).

It should also be noted that the enzymatic reaction conditions were based on DNS method (*24*). For this purpose, 20 cm^3 of the glass beads immobilized enzyme were added to 10 mL of 1 % (*m*/*V*) pectin solution prepared by 0.1 M sodium acetate buffer (pH=5.5) and the hydrolysis

reaction was done for 15 min at 50 ºC. After that, 1 mL of the reaction medium was poured in a test tube and 1 mL of DNS reagent was added to it. The resulting solution was immediately placed in Ben Marie (100 ºC, 5 min) and then was cooled in an ice-water bath. Afterward, 3 mL of distilled water was added to the test tube and its absorbance was determined at 540 nm. D-galacturonic acid (10-1000 µg/mL) was used to draw standard curve.

Preparation of barberry juice

The barberry fruits were obtained from a local store in Karaj, Alborz, Iran. The fruits were first separated from the branches and then were thoroughly washed. In the next step, a household juicer (BRAUN, SJ 3000 W, China) was applied to produce barberry juice. In final, the obtained juice was filtered on a Whatman No. 1 paper to separate insoluble materials and then was stored at refrigerator until next use.

Packed bed reactor (PBR) system

To carry out a continuous process, a jacketed glass reactor with an inner diameter of 14 mm and a length of 150 mm was applied. This bioreactor was well packed with 23 cm^3 of pectinase-immobilized glass beads (approx. 110 beads). The PBR was fed from the bottom with 1 % (*m*/*V*) of buffered pectin solution (pH of 3) or barberry juice (pH of 3.01) while the water was flowed to adjust the temperature in the outer layer (Fig. 1). As can be calculated, the total volume of PBR was 23.08 cm^3 while the actual volume of 23 cm^3 of glass beads (110 beads) was 12.43 cm^3 and thereby the void volume was 10.65 cm^3 according to the formula below:

$$V_V = V_T - V_A \qquad /3/$$

where V_V is the void volume of the PBR, V_T is the total volume of the PBR and V_A is the actual volume of 23 cm^3 of glass beads.

Effect of flow rate, temperature and treatment time on the PBR performance

The effect of flow rate, temperature and treatment time on the activity of the immobilized pectinase under a continuous process in PBR was evaluated using one factor at a time design.

For flow rate, this factor was variable (0.1-10 mL/min) while temperature and pH of the 1 % (*m*/*V*) pectin solution was constant in 50 °C and 3.0 (pH of the tested juice), respectively. For each flow rate, the residence time can also be calculated as follows:

$$\text{Residence } t \text{ (min)} = \frac{V_\text{V}}{F} \qquad /4/$$

where V_V is the void volume of the reactor (mL) and F is the flow rate (mL/min).

In the case of temperature, the mentioned factor was varied from 30 to 90 °C and the flow rate and pH of 1 % (*m*/*V*) pectin solution was considered constant in 0.5 mL/min and 3.0, respectively. For flow rate and temperature, the substrate was pectin solution (1 % *m*/*V*) and the response was reducing sugar (mg/mL) that was determined by the DNS method as stated earlier (*32*).

To determine the best treatment time, this factor was varied from 0 to 105 min while the flow rate (0.5 mL/min) and temperature (50 °C) was fixed. In this section, based on the study aim, the barberry juice was substrate and the turbidity was the response. For this purpose, after the clarification process in different treatment times, the turbidity of the obtained juice was determined by a portable turbidimeter (WTW, 350 IR, Weilheim, Germany) and the results were reported as Nephelometric Turbidity Unit (NTU).

Volumetric productivity of free and immobilized pectinases in batch and continuous systems

In batch condition, the volumetric productivity of free pectinase and the enzymes immobilized by different cross linkers was determined by adding 56 µL of free enzyme or 23 cm^3 of the glass bead-immobilized enzymes to 10.65 mL of the pectin solution and performing the hydrolysis reaction under optimal conditions as mentioned earlier. The volumetric productivity was reported based on mg of the produced reducing sugar per mL of medium per *min* (mg/(mL·min)). In the continuous process, this parameter was also calculated as follows (*33*):

$$P_\text{V} \text{ (mg/mL} \cdot \text{min)} = \gamma \times \frac{1}{\text{Residence } t} \qquad /5/$$

where γ is mg of the produced reducing sugar per mL.

Enzymatic clarification of barberry juice

For this purpose, the free and immobilized pectinase enzymes under batch and continuous operations were used. In the batch process, 56 µL of free enzyme or 23 cm^3 of enzyme-immobilized glass beads were immersed into 10.65 mL barberry juice and the clarification treatment was done at 50 °C for 63 min. In the case of the continuous operation, the fruit juice was pumped into the PBR with a flow rate of 0.5 mL/min at the same conditions (50 °C, 63 min). Generally, in the current study, seven clarification treatments were considered and the obtained results were compared with untreated juice. The treatments were included: raw barberry juice (RBJ); barberry juice clarified by the free pectinase (BFP); barberry juice clarified using the pectinase immobilized on the glass beads by glutaraldehyde in the batch operation (BGB); barberry juice clarified using the pectinase immobilized on the glass beads by glutaraldehyde in the continuous operation (BGC); barberry juice clarified using the pectinase immobilized on the glass beads by polyaldehyde pullulan in the batch operation (BPB); barberry juice clarified using the pectinase immobilized on the glass beads by polyaldehyde pullulan in the continuous operation (BPC); barberry juice clarified using the pectinase immobilized on the glass beads by polyaldehyde kefiran in the batch operation (BKB); barberry juice clarified using the pectinase immobilized on the glass beads by polyaldehyde kefiran in the continuous operation (BKC).

The physicochemical characterization of barberry juice

In this section, the physicochemical properties of raw and treated barberry juice were determined.

The turbidity of the juice samples was determined by a portable turbidimeter (WTW, 350 IR, Weilheim, Germany) and was reported based on NTU.

The clarity of juice samples diluted with distilled water (five-fold) was obtained by recording the absorbance at 660 nm using a spectrophotometer (CE 2502, CECIL, Cambridge, UK) and calculating the transmittance percentage (% T) according to the equation below:

$$\mathrm{T} = 10^{(2-\mathrm{A})} \qquad /6/$$

pH was obtained using a pH meter (GLP 22, Crison, Spain). Total titratable acidity (TTA) was determined by the titration with 0.1 M NaOH in the presence of phenolphthalein reagent and was reported based on g malic acid/100 mL juice.

Total soluble solids (TSS) were obtained by a refractometer (Abbe, Bellingham-Stanley, London, England) at room temperature and was reported based on ºBrix.

The reducing sugar mass concentration of the samples was measured using the DNS method as mentioned earlier and was reported based on mg/mL.

The viscosity of the barberry juice samples was determined by a programmable viscometer (Brookfield Ametek, DV3TLVTJ0, USA) at a shear rate of 50 rpm and 23 °C.

The pectin presence test was performed by adding two-volume of ethanol to juice samples and then storing the obtained mixtures at 4 ºC for 24 h. In this test, the lack of supernatant formation shows the complete elimination of pectin.

The color parameters of different samples were obtained by a color reader (CHROMA METER, CR-400, Tokyo, Japan).

Total phenol content (TPC) was determined using the Folin-Ciocalteu method (*34*,*35*). 100 µL of barberry juice was added to 500 µL of 10 % (*V*/*V*) Folin-Ciocalteu reagent solution and then 400 µL of 10 % (*m*/*V*) sodium carbonate solution was poured into it. After 1 h in darkness, the absorbance was read at 517 nm using a spectrophotometer (CE 2502, CECIL, Cambridge, UK) and the TPC was calculated by the comparison of the determined absorbance with the standard curve obtained from gallic acid (10-100 µg/mL). The TPC was reported as mg gallic acid equivalent per mL barberry juice (mg GAE/mL).

Determination of antioxidant activity of barberry juice

Antioxidant activity was evaluated by the DPPH radical scavenging activity of barberry juice (*34*,*36*). For this purpose, 100 µL of the barberry juice diluted with distilled water (200-fold) was added to 1.9 mL of DPPH ethanolic solution (0.1 mM) and the obtained mixture was vortexed. The absorbance of samples was recorded at 517 nm after storing in darkness for 30 min and the DPPH radical scavenging activity (DPPH-RSA/%) was calculated as follows:

$$\mathrm{DPPH-RSA}\ (\%) = \frac{A_{\mathrm{Control}} - A_{\mathrm{Sample}}}{A_{\mathrm{Control}}} \cdot 100 \qquad /7/$$

ABTS radical scavenging activity (ABTS-RSA/%) was determined in a completely similar way to DPPH-RSA. It should only be noted that to produce ABTS radical solution, 7.4 mM ABTS solution was mixed with 2.6 mM potassium persulfate and after storing in darkness for 18 h, its

absorbance was adjusted to 0.7±0.02 at 734 nm by adding distilled water (*34*). Also, in this experiment, the absorbance of the obtained samples was read at 734 nm.

Statistical analysis

All the experiments were performed in triplicate and their results were expressed as mean±SD. The obtained data were subjected to one-way analysis of variance (ANOVA) using SPSS software version 16 (*24*). It must also be stated that a significance level of α=0.05 was applied.

RESULTS AND DISCUSSION

Kinetic parameters

The kinetic parameters of the immobilized pectinases including Michaelis–Menten constant (K_m) and the maximum reaction rate (v_{max}) were calculated at temperature of 50 °C and pH of 5.5. The obtained results showed K_m for the pectinase enzymes immobilized by glutaraldehyde, polyaldehyde pullulan and polyaldehyde pullulan was 10.07±0.37, 11.21±0.24 and 11.58±0.31 mg/mL, respectively. Also, v_{max} value for the pectinase enzymes immobilized by the mentioned cross-linkers was 2.93±0.29, 2.24±0.18 and 2.17±0.14 μmol/(min·mg of protein), respectively. As can be seen, K_m and v_{max} of the pectinase immobilized by polyaldehydes of pullulan and kefiran were higher and lower than the pectinase immobilized by glutaraldehyde, which can be due to the diffusion limitation effects created by the polymeric network and three-dimensional structure of the polyaldehydes of pullulan and kefiran (*28*).

Effect of the variables on the PBR performance

To determine the effect of flow rate on the immobilized pectinase activity in the PBR, the reducing sugar production by pectinase immobilized using different cross-linkers was studied (substrate was 1 % *m*/*V* buffered pectin solution at pH 3 and temperature was constant at 50 °C). Fig. 2a showed that the reducing sugar production in bioreactor packed with pectinase immobilized by polyaldehyde kefiran was higher than that for the enzymes immobilized by two other cross-linkers (glutaraldehyde and polyaldehyde pullulan) at all flow rates. This observation was probably due to more protein immobilization by polyaldehyde kefiran (57.75±1.12 mg/(23 cm^3 glass beads)) than polyaldehyde pullulan (20.13±0.98 mg/(23 cm^3 glass beads)) and glutaraldehyde (4.51±0.68 mg/(23 cm^3 glass beads)) on the glass beads. Also, as can be seen,

the reducing sugar production was decreased when the flow rate was increased from 0.1 (residence time of 106.5 min) to 10 (residence time of 1.065 min) mL/min which can be related to the decrease in contact duration between immobilized enzymes and substrate with increasing the flow rate (*37*). However, as can be seen, there is no significant difference between reducing sugar productions in flow rates of 0.1 to 0.5 mL/min. Therefore, to save time and cost, other experiments in this study were performed at a flow rate of 0.5 mL/min (residence time of 21 min).

Fig. 2b showed the effect of the treatment temperature (30-90 °C) on the reducing sugar production by pectinase immobilized using different cross-linkers while other parameters were constant (pH of 3 and flow rate 0.5 mL/min). In this section, like what was seen for the flow rate, the reducing sugar production in the bioreactor packed with pectinase immobilized by polyaldehyde kefiran was higher than that with the enzymes immobilized by glutaraldehyde and/or polyaldehyde pullulan at all temperatures. Besides, the obtained results indicated that the maximum reducing sugar production was obtained at a temperature of 50 °C for pectinase immobilized by all three cross-linkers. These results were probably attributed to the optimum temperature of the immobilized pectinase by the mentioned cross-linkers as shown in previous studies (*24*). However, it seems that the type of immobilization method and used cross-linker are the key factors in this analysis. For instance, Dal Magro *et al*. (*38*) reported that the optimum temperature of the pectinase enzyme immobilized on the glutaraldehyde-activated magnetite was 60 °C, while de Oliveira *et al*. (*11*) stated that this parameter for pectinase immobilized on the alginate beads was at 50 °C.

To evaluate the effect of treatment time on the fruit juice clarification by the bioreactor packed with pectinase enzymes immobilized on the glass beads, the turbidity of the barberry juice was measured in different treatment time (0-105 min) while the flow rate and temperature were constant in 0.5 mL/min and 50 °C, respectively. As can be seen in Fig. 2c, the turbidity of the samples reached a minimum after 63 min and a further increase in time had no significant effect on this factor. However, the slope of the turbidity reduction in the bioreactor packed with pectinase immobilized by polyaldehyde kefiran was greater than the other two applied cross-linkers which was possibly due to the higher amount of the enzyme immobilized by this cross-linker. Therefore, all tests on the treated barberry juice were performed after clarification at a flow rate of 0.5 mL/min, the temperature of 50 °C and treatment time of 63 min for continuous operation and temperature of 50 °C and treatment time of 63 min for the batch operation.

The volumetric productivity of the free and immobilized pectinase in the batch and continuous operations

In this section, the volumetric productivity of the free pectinase and the pectinase immobilized by different cross-linkers in the batch and continuous operations was determined and the obtained results were shown in Fig. 3. As can be seen, the maximum volumetric productivity was due to the pectinase immobilized by polyaldehyde kefiran in continuous operation and then the enzymes immobilized by polyaldehyde kefiran in batch operation was in second place. The higher volumetric productivity by polyaldehyde kefiran-immobilized pectinase than the other applied cross-linkers was due to the more capable of this cross-linker in enzyme immobilization as stated earlier. Also, the higher volumetric productivity in continuous operation than the batch type was possibly due to better and more contact between the immobilized pectinase and the substrate in the continuous process. Besides, the volumetric productivity of polyaldehyde pullulan-immobilized pectinase was between polyaldehyde kefiran-immobilized and glutaraldehyde-immobilized enzymes. This observation is also due to the amount of the immobilized enzyme by polyaldehyde pullulan that is between two other cross-linkers. As mentioned earlier, the protein content of the free pectinase and the glutaraldehyde-immobilized enzymes was equal and according to Fig. 3, the volumetric productivity of free enzymes was more that glutaraldehyde-immobilized ones which was possibly related to the decrease in enzyme activity due to the immobilization process as shown in previous studies (*20,24*).

Effect of clarification on the barberry juice properties

After clarification process under optimum conditions (flow rate of 0.5 mL/min, the temperature of 50 °C and treatment time of 63 min for continuous operation and temperature of 50 °C and treatment time of 63 min for the batch operation) using the free pectinase and the pectinase enzymes immobilized by different cross-linkers in the batch and continuous operations, the physicochemical, antioxidant and color properties of the treated barberry juice were evaluated and the obtained results were compared with the untreated sample.

Physicochemical properties

Table 1 showed the physicochemical properties of the untreated and clarified juice samples. The most important goal in the fruit juice clarification is to reduce the turbidity and increase clarity. The main cause of this turbidity is the pectin present in fruit juice and therefore its hydrolysis is

necessary (*39*). Table 1 indicated that after clarification, the turbidity of barberry juice samples was significantly decreased from approx. 2068.8 to approx. 69.3 NTU and thereby the clarity was increased from approx. 28.38 to approx. 73.63 %. Also, the obtained results in Table 1 indicate that the used immobilization method (the use of polyaldehyde kefiran and pullulan as cross-linker) for the clarification of the barberry juice was much more efficient than the common method (the use of glutaraldehyde as cross-linker). Besides, the mentioned results were in accordance with the reported data for the clarification of different fruit juice samples by Bilal *et al*. (*40*) and the clarification of the apple juice by Deng *et al*. (*41*).

As can be seen in Table 1, the pH of barberry juice samples was decreased after the clarification process which was in line with the obtained data for the apple juice clarified by the pectinase immobilized into calcium alginate microspheres (*41*). Also, the TTA of the juice samples was significantly increased by clarification process which can be related to the liberation of organic acids especially galacturonic acid due to the pectin hydrolysis by pectinase enzymes (*42*).

The obtained results from the TSS determination indicated that this parameter was decreased after the clarification process. This change was probably related to the deposition of the suspended solid compounds after pectin hydrolysis and the destruction of the network formed by it. Also, the findings showed that the clarification process led to an increase in the reducing sugar content of the treated fruit juice which can be due to the liberation of these sugars by pectin hydrolysis.

The pectin has a high water-holding capacity and creates a cohesive network structure. Therefore, the pectin hydrolysis by the free and/or the immobilized pectinase can reduce this water holding capacity, release the water into the fruit juice and thereby reduce the viscosity (*11*). As can be seen in Table 1, the viscosity of the fruit juice samples clarified by all treatments was significantly lower than the untreated sample. Similar results were also reported by other researchers (*25,43*).

The results of the pectin presence test showed that no pectin was observed in the fruit juice samples after clarification by the free pectinase and the enzymes immobilized by different cross-linkers in the batch and continuous operations indicating that pectin was completely hydrolyzed.

Three color parameters including *L* (lightness), *a* (green-red value) and *b* (blue-yellow value) of the untreated barberry juice and the samples clarified by free and immobilized enzymes were evaluated and the obtained data were shown in Table 2. As can be seen, the lightness factor was increased by the enzymatic clarification process. This result was predictable because a decrease

in the turbidity and an increase in the clarity lead to an increase in *L* factor which was in agreement with the reported data by de Oliveira *et al*. (*11*) and Sin *et al*. (*43*) for the clarified apple juice and sapodilla juice, respectively. Also, the obtained results indicated that the clarification process led to a decrease in the green-red value and an increase in the blue-yellow value (Table 2). The decrease in red color after the pectin hydrolysis can be due to the deposition of some antioxidant compounds that are red in the acidic medium of fruit juice.

Table 1 showed that the TPC was significantly reduced by the clarification process which was in agreement with the reported data by Landbo *et al*. (*42*) and Diano *et al*. (*25*) for the clarified black currant juice and apple juice, respectively. The mentioned observations were possibly attributed to the deposition of these compounds after pectin hydrolysis and also the change of the phenolic compounds profile due to the presence of secondary enzymatic activities (*11*).

By looking closely at Table 1, it is well understood that the bioreactor packed with polyaldehyde kefiran-immobilized pectinase had the greatest effect on the measured parameters which could be attributed to the more enzyme immobilization by the mentioned cross-linker. Also, for this reason, the effect of polyaldehyde pullulan-immobilized pectinase treatment was more than glutaraldehyde-immobilized enzyme treatment in most characteristics. Besides, it can be seen that the continuous process changes the fruit juice properties more than the batch type which is probably due to the better and more contact between the immobilized pectinase and the substrate in the continuous operation as stated earlier.

Antioxidant properties

In this study, to evaluate the antioxidant properties, DPPH and ABTS radical scavenging activity of the untreated and treated barberry juice samples were determined and the obtained results were shown in Fig. 4. As can be seen, both the parameters were significantly reduced after the clarification process. These observations were probably related to the decrease in TPC that has a direct relationship with antioxidant properties (*11,44*) and also, it can be due to the deposition of some other antioxidant compounds after pectin hydrolysis. Fig. 4 showed that the polyaldehyde kefiran-immobilized pectinase treatment led to the greatest reduction in antioxidant properties that is due to the ability of the cross-linker to more immobilization of the enzymes.

CONCLUSIONS

In the current study, different cross-linkers (glutaraldehyde, polyaldehyde pullulan and polyaldehyde kefiran) were applied to immobilize pectinase on the functionalized glass beads and the clarification of the barberry juice was done by the batch and continuous operations using the mentioned immobilized enzymes. The observations showed that the best PBR performance was obtained at a flow rate of 0.5 mL/min, the temperature of 50 °C and treatment time of 63 min. Also, the physicochemical, color and antioxidant properties of the barberry juice were significantly changed after the clarification process. Under optimum conditions, the turbidity of the fruit juice samples clarified by polyaldehyde pullulan-immobilized pectinase treatment and polyaldehyde kefiran-immobilized pectinase treatment was significantly lower than the samples clarified by glutaraldehyde-immobilized pectinase treatment which was related to the greater ability of these new cross-linkers to immobilize the pectinase enzymes.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

AUTHORS' CONTRIBUTION

S.S. Hosseini contributed in data collection, data analysis and interpretation, drafting the article and critical revision. F. Khodaiyan participated in design of the work, performing the analysis, critical reviews and supervision. S.M. Mousavi assisted in design of the work, performing the analysis, final approval and supervision. S.Z. Azimi contributed in planning data collection and performing the analysis.

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REFERENCES

1. Alizadeh HR, Mortezapour H, Akhavan HR, Balvardi M. Performance of a liquid desiccant-assisted solar juice concentration system for barberry juice. Sol Energy. 2019;184:1–10. https://doi.org/10.1016/j.solener.2019.03.085
2. Sarraf M, Beig Babaei A, Naji-Tabasi S. Investigating functional properties of barberry species: an overview. J Sci Food Agr. 2019;99:5255–69. https://doi.org/10.1002/jsfa.9804
3. Kazemi M, Khodaiyan F, Labbafi M, Hosseini SS. Ultrasonic and heating extraction of pistachio by-product pectin: physicochemical, structural characterization and functional measurement. J Food Meas Charact. 2020;14:679–93. https://doi.org/10.1007/s11694-019-00315-0
4. Asgari K, Labbafi M, Khodaiyan F, Kazemi M, Hosseini SS. High-methylated pectin from walnut processing wastes as a potential resource: Ultrasound assisted extraction and physicochemical, structural and functional analysis. Int J Biol Macromol. 2020;152:1274–82. https://doi.org/10.1016/j.ijbiomac.2019.10.224
5. Shahrestani H, Taheri-Kafrani A, Soozanipour A, Tavakoli O. Enzymatic clarification of fruit juices using xylanase immobilized on 1, 3, 5-triazine-functionalized silica-encapsulated magnetic nanoparticles. Biochem Eng J. 2016;109:51–8. https://doi.org/10.1016/j.bej.2015.12.013
6. Goh WJ, Makam VS, Hu J, Kang L, Zheng M, Yoong SL, et al. Iron oxide filled magnetic carbon nanotube-enzyme conjugates for recycling of amyloglucosidase: toward useful applications in biofuel production process. Langmuir. 2012;28:16864–73. https://doi.org/10.1021/la303046m
7. Dal Magro L, de Moura KS, Backes BE, de Menezes EW, Benvenutti EV, Nicolodi S, et al. Immobilization of pectinase on chitosan-magnetic particles: Influence of particle preparation protocol on enzyme properties for fruit juice clarification. Biotechnol Rep. 2019;24:e00373. https://doi.org/10.1016/j.btre.2019.e00373
8. Cerreti M, Liburdi K, Benucci I, Esti M. The effect of pectinase and protease treatment on turbidity and on haze active molecules in pomegranate juice. LWT-Food Sci Technol. 2016;73:326-33.

https://doi.org/10.1016/j.lwt.2016.06.030

9. Sojitra UV, Nadar SS, Rathod VK. A magnetic tri-enzyme nanobiocatalyst for fruit juice clarification. Food Chem. 2016;213:296–305. https://doi.org/10.1016/j.foodchem.2016.06.074
10. Azar RISL, da Luz Morales M, Maitan-Alfenas GP, Falkoski DL, Alfenas RF, Guimaraes VM. Apple juice clarification by a purified polygalacturonase from *Calonectria pteridis*. Food Bioprod Process. 2020;119:238–45. https://doi.org/10.1016/j.fbp.2019.11.013
11. de Oliveira RL, Dias JL, da Silva OS, Porto TS. Immobilization of pectinase from *Aspergillus aculeatus* in alginate beads and clarification of apple and umbu juices in a packed bed reactor. Food Bioprod Process. 2018;109:9–18. https://doi.org/10.1016/j.fbp.2018.02.005
12. Rong J, Zhou Z, Wang Y, Han J, Li C, Zhang W, et al. Immobilization of horseradish peroxidase on multi-armed magnetic graphene oxide composite: improvement of loading amount and catalytic activity. Food Technol Biotech. 2019;57:260–71. https://doi.org/10.17113/ftb.57.02.19.5832
13. Cui J, Jia S. Organic–inorganic hybrid nanoflowers: A novel host platform for immobilizing biomolecules. Coordin Chem Rev. 2017;352:249-63. https://doi.org/10.1016/j.ccr.2017.09.008
14. Cui JD, Jia SR. Optimization protocols and improved strategies of cross-linked enzyme aggregates technology: current development and future challenges. Cr Rev Biotechn. 2015;35:15-28. https://doi.org/10.3109/07388551.2013.795516
15. Bilal M, Cui J, Iqbal HM. Tailoring enzyme microenvironment: State-of-the-art strategy to fulfill the quest for efficient bio-catalysis. Int J Biol Macromol. 2019;130:186-96. https://doi.org/10.1016/j.ijbiomac.2019.02.141
16. Wahab WA, Karam EA, Hassan ME, Kansoh AL, Esawy MA, Awad GE. Optimization of pectinase immobilization on grafted alginate-agar gel beads by 2^4 full factorial CCD and thermodynamic profiling for evaluating of operational covalent immobilization. Int J Biol Macromol. 2018;113:159-70. https://doi.org/10.1016/j.ijbiomac.2018.02.086

17. Hassan SS, Williams GA, Jaiswal AK. Computational modelling approach for the optimization of apple juice clarification using immobilized pectinase and xylanase enzymes. Cur Res Food Sci. 2020;3:243-55. https://doi.org/10.1016/j.crfs.2020.09.003

18. Benucci I, Mazzocchi C, Lombardelli C, Cacciotti I, Esti M. Multi-enzymatic systems immobilized on chitosan beads for pomegranate juice treatment in fluidized bed reactor: effect on haze-active molecules and chromatic properties. Food Bioprocess Tech. 2019;12:1559-72. https://doi.org/10.1007/s11947-019-02315-w

19. Gomez JL, Bodalo A, Gomez E, Bastida J, Hidalgo AM, Gomez M. Immobilization of peroxidases on glass beads: an improved alternative for phenol removal. Enzyme Microb Tech. 2006;39:1016–22. https://doi.org/10.1016/j.enzmictec.2006.02.008

20. Lei Z, Jiang Q. Synthesis and properties of immobilized pectinase onto the macroporous polyacrylamide microspheres. J Agr Food Chem. 2011;59:2592–9. https://doi.org/10.1021/jf103719t

21. Dahiya P, Chand S, Dilbaghi N. Immobilization of organic solvent-tolerant lipase from *Pseudomonas mendocina* M-37 with potential synthetic activities. Food Technol Biotech. 2014;52:368-75.

22. Cui J, Ren S, Sun B, Jia S. Optimization protocols and improved strategies for metal-organic frameworks for immobilizing enzymes: Current development and future challenges. Coordin Chem Rev. 2018;370:22-41. https://doi.org/10.1016/j.ccr.2018.05.004

23. Bodalo A, Gomez E, Gomez JL, Bastida J, Maximo MF, Diaz F. A comparison of different methods of β-galactosidase immobilization. Process Biochem. 1991;26:349–53. https://doi.org/10.1016/0032-9592(91)85025-J

24. Hosseini SS, Khodaiyan F, Mousavi SME, Azimi SZ, Gharaghani M. Immobilization of pectinase on the glass bead using polyaldehyde kefiran as a new safe cross-linker and its effect on the activity and kinetic parameters. Food Chem. 2020;309:125777. https://doi.org/10.1016/j.foodchem.2019.125777

25. Diano N, Grimaldi T, Bianco M, Rossi S, Gabrovska K, Yordanova G, et al. Apple juice clarification by immobilized pectolytic enzymes in packed or fluidized bed reactors. J Agr Food Chem. 2008;56:11471–7. https://doi.org/10.1021/jf8019437

26. Dal Magro L, Pessoa JP, Klein MP, Fernandez-Lafuente R, Rodrigues RC. Enzymatic clarification of orange juice in continuous bed reactors: Fluidized-bed versus packed-bed reactor. Catal Today. 2021;362:184-91. https://doi.org/10.1016/j.cattod.2020.02.003

27. Benucci I, Lombardelli C, Liburdi K, Acciaro G, Zappino M, Esti M. Immobilised native plant cysteine proteases: packed-bed reactor for white wine protein stabilisation. J Food Sci Technol. 2016;53:1130-9. https://doi.org/10.1007/s13197-015-2125-4

28. Hosseini SS, Khodaiyan F, Mousavi SM, Kennedy JF, Azimi SZ. A health-friendly strategy for covalent-bonded immobilization of pectinase on the functionalized glass beads. Food Bioprocess Technol. 2021;14:177-86. https://doi.org/10.1007/s11947-020-02524-8

29. Guo J, Ge L, Li X, Mu C, Li D. Periodate oxidation of xanthan gum and its crosslinking effects on gelatin-based edible films. Food Hydrocolloid. 2014;39:243–50. https://doi.org/10.1016/j.foodhyd.2014.01.026

30. Kholiya F, Chaudhary JP, Vadodariya N, Meena R. Synthesis of bio-based aldehyde from seaweed polysaccharide and its interaction with bovine serum albumin. Carbohyd Polym. 2016;150:278–85. https://doi.org/10.1016/j.carbpol.2016.05.022

31. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265–75.

32. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31:426–8. https://doi.org/10.1021/ac60147a030

33. Singh RS, Saini GK, Kennedy JF. Continuous hydrolysis of pullulan using covalently immobilized pullulanase in a packed bed reactor. Carbohyd Polym. 2011;83:672–5. https://doi.org/10.1016/j.carbpol.2010.08.037

34. Kazemi M, Khodaiyan F, Hosseini SS, Najari Z. An integrated valorization of industrial waste of eggplant: Simultaneous recovery of pectin, phenolics and sequential production of pullulan. Waste Manage. 2019;100:101–11. https://doi.org/10.1016/j.wasman.2019.09.013

35. Feizy J, Jahani M, Ahmadi S. Antioxidant activity and mineral content of watermelon peel. J Food Bioprocess Eng. 2020;3:35-40. https://doi.org/10.22059/jfabe.2020.75811

36. Karimi S, Sharifzadeh S, Abbasi H. Sequential ultrasound-microwave assisted extraction as a green method to extract essential oil from *Zataria multiflora*. J Food Bioprocess Eng. 2020;3:101-9. https://doi.org/10.22059/jfabe.2020.308833.1064

37. Ansari SA, Husain Q. Lactose hydrolysis by β galactosidase immobilized on concanavalin A-cellulose in batch and continuous mode. J Mol Catal B-Enzym. 2010;63:68–74. https://doi.org/10.1016/j.molcatb.2009.12.010

38. Dal Magro L, Silveira VCC, de Menezes EW, Benvenutti EV, Nicolodi S, Hertz PF, et al. Magnetic biocatalysts of pectinase and cellulase: synthesis and characterization of two preparations for application in grape juice clarification. Int J Biol Macromol. 2018;115:35–44. https://doi.org/10.1016/j.ijbiomac.2018.04.028

39. Sharma HP, Patel H, Sharma S. Enzymatic extraction and clarification of juice from various fruits–a review. Trends Post Harvest Technol. 2014;2:01-14.

40. Bilal M, Asgher M, Iqbal HMN, Hu H, Zhang X. Delignification and fruit juice clarification properties of alginate-chitosan-immobilized ligninolytic cocktail. LWT-Food Sci Technol. 2017;80:348–54. https://doi.org/10.1016/j.lwt.2017.02.040

41. Deng Z, Wang F, Zhou B, Li J, Li B, Liang H. Immobilization of pectinases into calcium alginate microspheres for fruit juice application. Food Hydrocolloid. 2019;89:691–9. https://doi.org/10.1016/j.foodhyd.2018.11.031

42. Landbo AKR, Pinelo M, Vikbjerg AF, Let MB, Meyer AS. Protease-assisted clarification of black currant juice: synergy with other clarifying agents and effects on the phenol content. J Agr Food Chem. 2006;54:6554–63. https://doi.org/10.1021/jf060008d

43. Sin HN, Yusof S, Hamid NSA, Rahman RA. Optimization of enzymatic clarification of sapodilla juice using response surface methodology. J Food Eng. 2006;73:313–19. https://doi.org/10.1016/j.jfoodeng.2005.01.031

44. Su MS, Silva JL. Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinium ashei*) by-products as affected by fermentation. Food Chem. 2006;97:447–51. https://doi.org/10.1016/j.foodchem.2005.05.023

Table 1. Physicochemical characterization of barberry juice including turbidity, Clarity, pH, total titratable acidity (TTA), total soluble solids (TSS), reducing sugar content, viscosity, pectin presence test and total phenolic content

Parameters	RBJ	BFP	BGB	BGC	BPB	BPC	BKB	BKC
Turbidity/NTU	(2068.8±0.00)^{a}	(72.6±0.4)^{d}	(75.1±0.8)^{b}	(73.8±0.3)^{c}	(73.0±0.4)^{d}	(72.7±0.3)^{d}	(71.7±0.4)^{e}	(69.3±0.6)^{f}
Clarity/%	(28.38±0.16)^{h}	(61.72±0.18)^{e}	(54.13±0.10)^{g}	(60.32±0.14)^{f}	(63.00±0.11)^{d}	(65.13±0.09)^{c}	(68.21±0.10)^{b}	(73.63±0.13)^{a}
pH	(3.21±0.02)^{a}	(2.99±0.02)^{e}	(3.18±0.01)^{b}	(3.14±0.02)^{c}	(3.09±0.02)^{d}	(2.98±0.04)^{e}	(2.77±0.01)^{f}	(2.70±0.03)^{g}
TTA/(g/100 mL)	(0.75±0.03)^{g}	(1.04±0.02)^{d}	(0.91±0.03)^{f}	(0.97±0.01)^{e}	(1.13±0.01)^{c}	(1.14±0.02)^{c}	(1.21±0.02)^{b}	(1.29±0.03)^{a}
TSS/ ºBrix	(30.5±0.0)^{a}	(25.5±0.0)^{d}	(29.0±0.0)^{b}	(29.0±0.0)^{b}	(26.0±0.0)^{c}	(25.5±0.0)^{d}	(21.5±0.0)^{e}	(21.0±0.0)^{f}
γ(reducing sugars)/(mg/mL)	(53.17±0.16)^{h}	(63.36±0.21)^{e}	(60.32±0.16)^{g}	(62.80±0.13)^{f}	(64.12±0.10)^{d}	(66.16±0.12)^{c}	(69.10±0.13)^{b}	(69.57±0.09)^{a}
η/(mPa.s)	(4.59±0.18)^{a}	(4.07±0.04)^{b}	(4.21±0.17)^{b}	(4.18±0.12)^{b}	(4.01±0.13)^{bc}	(3.91±0.07)^{c}	(3.57±0.11)^{d}	(3.31±0.12)^{e}
Pectin test	Have	None	None	None	None	None	None	None
γ(total phenolics)/(mg/mL)	(20.31±0.08)^{a}	(19.31±0.14)^{b}	(19.60±0.17)^{b}	(19.47±0.11)^{b}	(19.40±0.09)^{b}	(18.91±0.10)^{c}	(18.81±0.15)^{c}	(18.54±0.04)^{d}

RBJ=raw barberry juice; BFP=barberry juice treated by the free pectinase; BGB=barberry juice treated using the pectinase immobilized by glutaraldehyde in batch operation; BGC=barberry juice treated using the pectinase immobilized by glutaraldehyde in continuous operation; BPB=barberry juice treated using the pectinase immobilized by polyaldehyde pullulan in batch operation; BPC=barberry juice treated using the pectinase immobilized by polyaldehyde pullulan in continuous operation; BKB=barberry juice treated using the pectinase immobilized by polyaldehyde kefiran in batch operation; BKC=barberry juice treated using the pectinase immobilized by polyaldehyde kefiran in continuous operation. Different letters in the same row indicate significant differences ($p<0.05$)

Table 2. The color parameters of the untreated and treated barberry juice

Parameters	RBJ	BFP	BGB	BGC	BPB	BPC	BKB	BKC
L	(10.21±0.03)^{e}	(18.10±0.15)^{c}	(17.14±0.18)^{d}	(17.20±0.13)^{d}	(17.46±0.16)^{d}	(18.32±0.09)^{c}	(20.41±0.16)^{b}	(21.14±0.12)^{a}
a	(22.21±0.11)^{a}	(19.18±0.13)^{c}	(20.33±0.11)^{b}	(20.21±0.08)^{b}	(18.44±0.11)^{d}	(18.26±0.17)^{d}	(17.21±0.10)^{e}	(17.14±0.07)^{e}
b	(4.13±0.13)^{c}	(5.03±0.16)^{b}	(5.00±0.16)^{b}	(5.09±0.1)^{b}	(5.13±0.1)^{b}	(5.17±0.14)^{b}	(5.51±0.08)^{a}	(5.66±0.10)^{a}

RBJ=raw barberry juice; BFP=barberry juice treated by the free pectinase; BGB=barberry juice treated using the pectinase immobilized by glutaraldehyde in batch operation; BGC=barberry juice treated using the pectinase immobilized by glutaraldehyde in continuous operation; BPB=barberry juice treated using the pectinase immobilized by polyaldehyde pullulan in batch operation; BPC=barberry juice treated using the pectinase immobilized by polyaldehyde pullulan in continuous operation; BKB=barberry juice treated using the pectinase immobilized by polyaldehyde kefiran in batch operation; BKC=barberry juice treated using the pectinase immobilized by polyaldehyde kefiran in continuous operation. Different letters in the same row indicate significant differences ($p<0.05$).

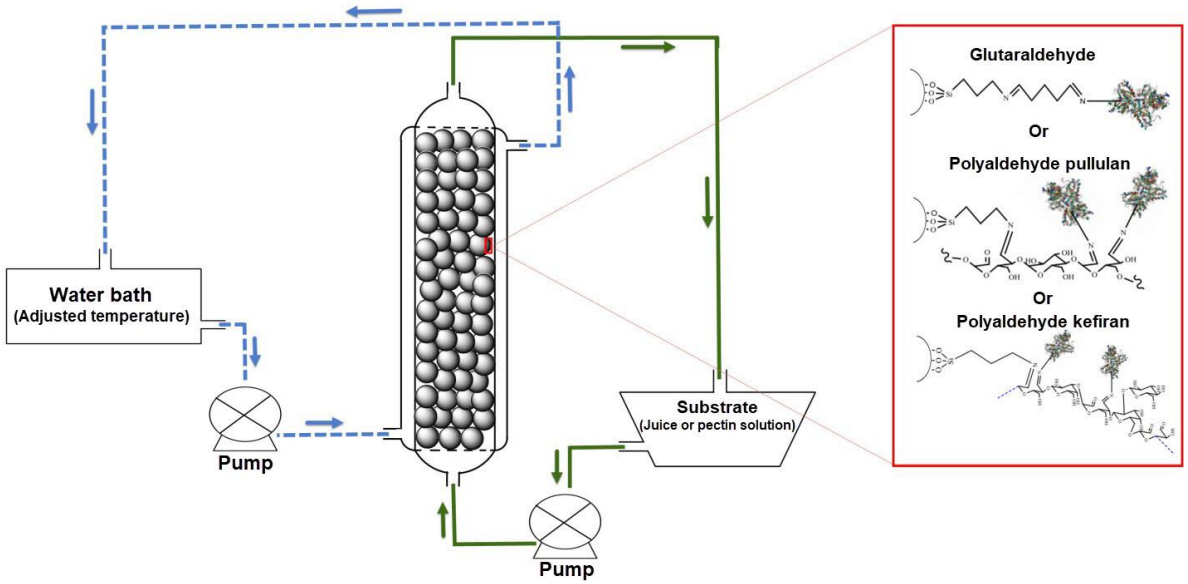

Fig. 1. Schematic illustration of the configuration of the continuous packed bed reactor used in this study

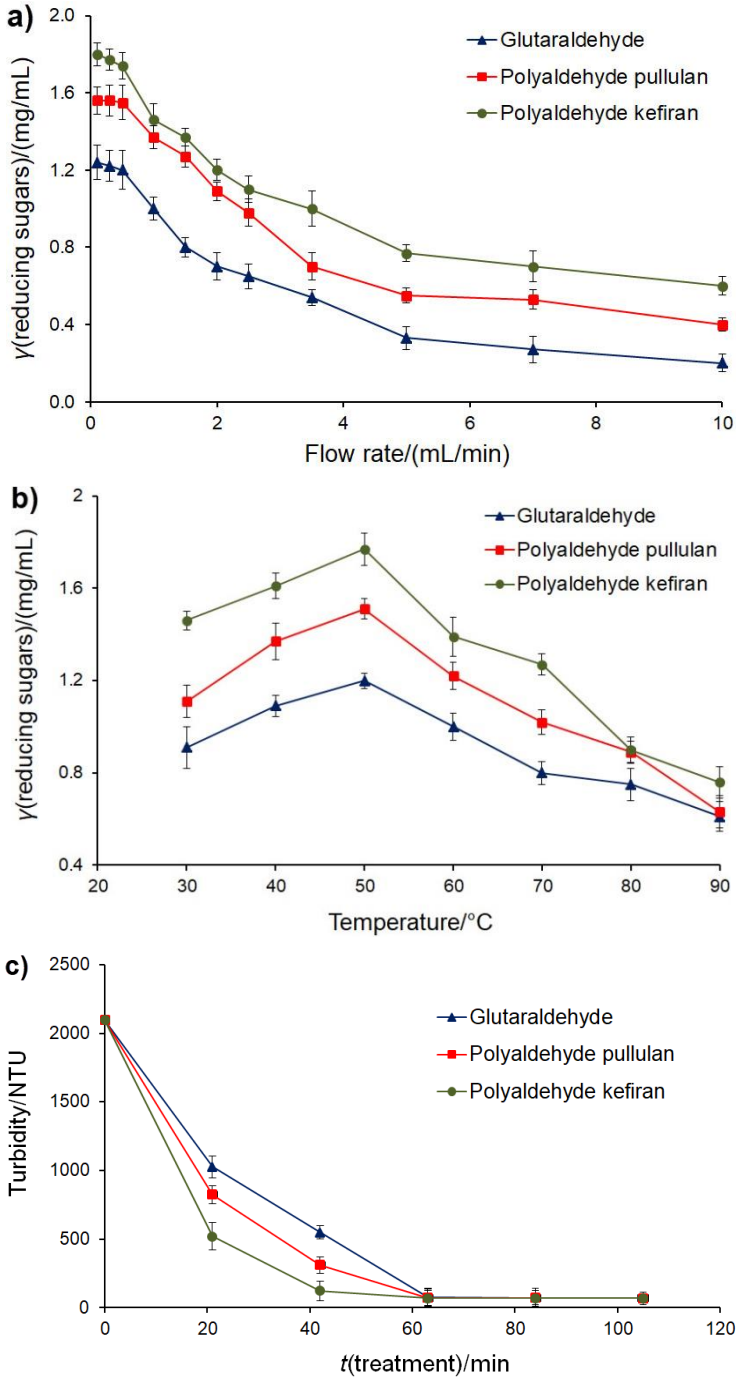

Fig. 2. (a) The effect of flow rate on the packed bed reactor performance at temperature of 50 °C. (b) The effect of temperature on the packed bed reactor performance at flow rate of 0.5 mL/min. (c) The effect of treatment time on the packed bed reactor performance at temperature of 50 °C and flow rate of 0.5 mL/min. Glutaraldehyde=the bioreactor pecked by the pectinase enzymes immobilized on the glass beads by glytaraldehyde; polyaldehyde pullulan=the bioreactor pecked by the pectinase enzymes immobilized on the glass beads by polyaldehyde pullulan; polyaldehyde kefiran=the bioreactor pecked by the pectinase

enzymes immobilized on the glass beads by polyaldehyde kefiran. Data are presented as mean value±S.D. of three replications.

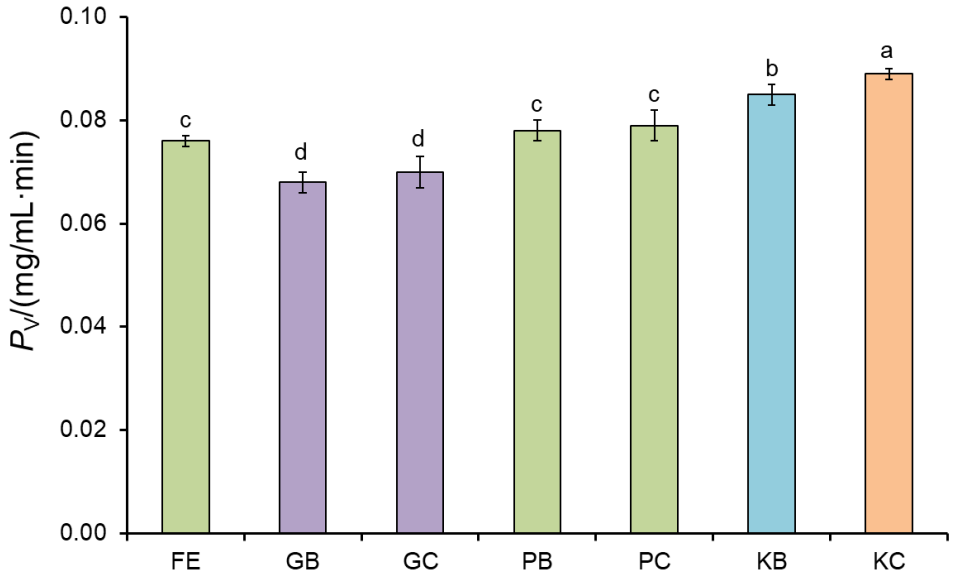

Fig. 3. Volumetric productivity of free and immobilized pectinase in the batch and continuous operations. FE=free pectinase; GB=pectinase immobilized by glutaraldehyde in the batch operation; GC=pectinase immobilized by glutaraldehyde in the continuous operation; PB=pectinase immobilized by polyaldehyde pullulan in the batch operation; PC=pectinase immobilized by polyaldehyde pullulan in the continuous operation; KB=pectinase immobilized by polyaldehyde kefiran in the batch operation; KC=pectinase immobilized by polyaldehyde kefiran in the continuous operation. Data are presented as mean value±S.D. of three replications. Different letters indicate significant differences ($p<0.05$)

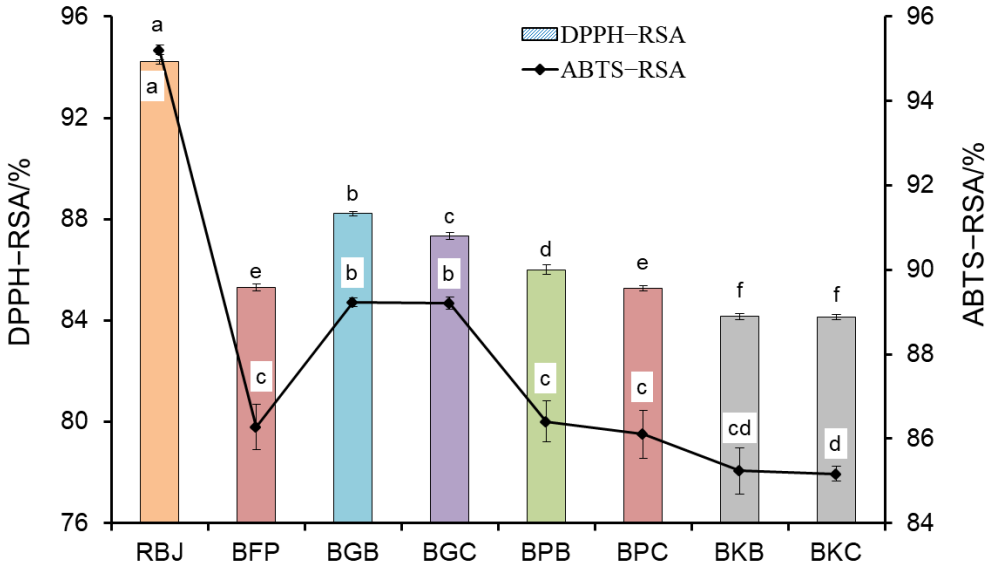

Fig. 4. DPPH radical scavenging activity (DPPH-RSA/%) and ABTS radical scavenging activity (ABTS-RSA/%) of the untreated and treated barberry juice. RBJ=raw barberry juice; BFP=barberry juice treated by the free pectinase; BGB=barberry juice treated using the pectinase immobilized by glutaraldehyde in batch operation; BGC=barberry juice treated using the pectinase immobilized by glutaraldehyde in continuous operation; BPB=barberry juice treated using the pectinase immobilized by polyaldehyde pullulan in batch operation; BPC=barberry juice treated using the pectinase immobilized by polyaldehyde pullulan in continuous operation; BKB=barberry juice treated using the pectinase immobilized by polyaldehyde kefiran in batch operation; BKC=barberry juice treated using the pectinase immobilized by polyaldehyde kefiran in continuous operation. Data are presented as mean value±S.D. of three replications. Different letters indicate significant differences ($p<0.05$)