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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(SI-2): 45-57. **OPEN ACCESS**

Effect of Incubation time and temperature on the physical properties of Beetroot juice clarification and Its quality as affected by enzyme treatments

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The fresh beetroot is perishable, led to have a relatively short shelf life. Transforming of fresh beetroot into juice through thermal processing with the aid of enzymes is one of the preservation methods to increase the yield and preserve their quality. This research was performed to evaluate the effect of different concentrations of commercial enzymes on the extraction yield. Besides, the effect of incubation time and temperature on physical properties of beetroot juice clarification and the quality of beetroot juice as affected by enzymes treatments were also evaluated. For juice extraction, the beetroot pulp was blended with distilled water (3:1) and treated with two commercial enzymes (pectinase or cellulase) and the combination (pectinase + cellulase) at different concentrations from 0.5 to 2 U/mL for 4 hrs at 40 °C. For juice clarification, the extracted juice was treated with the aforementioned enzymes at concentration of 1.0 U/mL for different incubation temperatures from 40 to 50 ^{0}C and time from 15 to 30 min. Determination of juice yield, colour, cloud value, total soluble solids (TSS), ascorbic acid, pH, total titratable acidity, and total sugar content of juice were conducted. Overall, the juice treated with pectinase at 1.5 U/mL had significantly ($p < 0.05$) highest extraction yield. For clarification, a prolongation of incubation temperature from 40 °C to 50 °C and incubation period from 15 min to 30 min resulted higher lightness, redness, and yellowness values for the juice. Juice incubated with enzymes at 50 °C for 15 min showed the optimum clarity value. Enzymes treatments significantly increased the total titratable acidity, total sugar content, and Vitamin C content in juice, but the pH of extracted juice was deceased. The total soluble solids content remained unaffected with the enzymatic treatments. In conclusion, the enzymatic treatments had resulted in desirable characteristics and improved the quality of beetroot juice.

Keywords: pectinase, cellulase, clarification, physical properties, beetroot juice.

INTRODUCTION

Beetroot (*Beta vulgaris* L.), which belongs to the Chenopodiaceae family, is a root vegetable also known as “bit” in Malaysia, commonly known as “beet” in North America and also known as “red beet”, golden beet”, sugar beet”, garden beet” or table beet” (Masih et al. 2019). Recently, there has been an increasing interest in the biological effect of beetroot. Earlier studies have reported that there are significantly high amounts of biologically active compounds such as nitrate, phenolic (flavonoids, phenolic acids, and phenolic amides), ascorbic acid, carotenoids, and betalains (betacyanins and betaxanthins) in beetroot (Clifford et al. 2015; Masih et al. 2019) that possess positive health effects as anti-inflammation, anti-carcinogenic, anti-allergic, anti-mutagenic agent (Ceclu and Nistor, 2020; Mirmiran et al. 2020). Beetroot also contain several nutritive compounds such as sugars,

vitamins B, folate, minerals, and fibres (Ceclu and Nistor, 2020). As a result, beetroot has potential to be utilized as functional food for health promoting and disease prevention. Considering the fact that beetroot has a glycemic index (GI) of 61, it is considered as moderate and may be included in the diabetic friendly diet for stable blood glucose levels. Anwar et al. (2018), reported that food with low GI has essential role in dietary management of diabetes, peak sport performance, weight reduction, reduce risks of disease associated with hypertension and heart disease. However, fresh vegetables and fruits are subjected to rapid microbial and enzymatic deterioration during storage due to their high moisture content (90% moisture) and water activity (Sharma et al. 2014). Thus, transforming the fresh fruit into food products after harvesting is important in order to extend their shelf life (Yusof et al. 2020). High water level of fruits and vegetables is suitable for juice production, but the quality of the juice after processing is a major challenge.

Fruit juices or drinks have become important as the consumer market is becoming health and convenience conscious. The demand for fruit juices with fresh, natural taste, convenient, nutritious, and safe food products is rising (Abid et al. 2013). According to Adhikari et al. (2004), fruit juices contain more than 90% of solids; low molecular weight sugars (glucose, sucrose, and fructose) and organic acids (citric, malic, and tartaric acids). Thus, fruit juices are one of the important food sources supplying energy (Norjana and Noor Aziah, 2011). Raw fruit juices are naturally cloudy and colloidal suspensions due to the presence of proteins, metals, tannins, and polysaccharides such as starch, cellulose, hemicelluloses, pectin, and lignin (Vaillant et al. 2011). According to Norjana and Noor Aziah (2011), juices that have turbidity or unstable cloud are considered 'cloudy' and unpleasant to be marketed as clear juices due to unacceptable by the customers (Tribess and Tadini, 2006). As a result, clarification of juice is very important in juice processing. During clarification, clusters are formed when juice is clarified with enzymes, this helps in facilitating filtration and results in higher clarity and colour improvement (Padma et al. 2017).

In juice processing, enzymes are a catalyst in improving the quality of the end-product and hence is commercially cost effective. Juice treated with enzymes gives a significant increase in juice yield compared to any other extraction process (Sharma et al. 2014). The enzymatic treatment of fruit pulp not only facilitates extraction by softening the plant tissue but also results to the release of cell contents that leads to high recovery (Nguyen and Nguyen, 2018) and also ensures highest possible quality of the end products. Pectinase and cellulase had been an essential part of the modern fruit processing technology. Pectinase is an enzyme that breaks down pectin which is a polysaccharide found in plant cell walls, while cellulase is an enzyme that breaks down the cellulose plant fibers into beta-glucose and short–chain polysaccharides. Cellulose breakdown is of considerable economic importance because it makes a major plants constituent available for consumption (David and Roy, 2019). Several researchers have reported that pectinase and cellulase enzymatic treatments can significantly improve the juice yield. For example, Joshi et al. (2012) applied pectinase for the extraction of tamarind juice and has a yield of 92.4%. Ajayi et al. (2017) used cellulase for extraction of apple, soursop, pear, and orange juices. Similarly, Srivastava and Tyagi (2013) applied cellulase for extraction of apple juice with increased juice yield.

External features such as visual appearance/clarity and colour are the major attributes influencing the customer's initial decision to purchase the juice. However, the decision for subsequent purchases is depended upon consumer satisfaction based on internal quality (Shewfelt, 2009; Chen and Opera, 2013). In present study, different commercial enzymes such as pectinase and cellulase and their combination were used in extraction and clarification of beetroot juice. The effect of different concentrations of commercial enzymes on the extraction yield were determined. Besides, parameters like incubation time and temperature during clarification were studied to obtain optimum clarified juice. The biochemical quality of beetroot juice as affected by enzymes treatments were also evaluated.

MATERIALS AND METHODS

Sample preparation

The ripened fresh beetroot fruits (*Beta vulgaris* L.) was purchased from the local wet market, located at the district of Besut, Terengganu, Malaysia. All fruits were carefully inspected for free from insects and damages before procurement. The two commercial enzymes, namely pectinase (DIS-1030) from *Aspergillu niger*, with the following specifications: claimed activity (60,000 U/ mL), density (1.08 g/

mL), optimum temperature (50^0C - 60^0C), and pH (2.5-5.5); and cellulase (DIS-1017) from *Trichoderma reesei*, with the following specifications: claimed activity (≥100,000 U/ g), optimum temperature (40^0C - 50^0C), and pH (4.5 - 7.5) were bought from creative enzymes (New York, USA). All of the chemicals used were analytical reagent (AR) grade.

Fruit juice preparation

The fresh beetroot was thoroughly washed and cleaned with running tap water before peeling to remove foreign materials. The pulp was then chopped into small pieces and blended with addition of distilled water at 1:3 ratios to obtain homogenous pulp. The homogenous pulp obtained was separated into two groups: one was used for enzymatic treatments and another one served as control (without enzymatic treatment). These homogenous samples are then used for juice extraction and clarification studies.

Enzyme preparation

Commercial enzymes such as pectinase, cellulase, and the combination of pectinase + cellulase at concentrations of 0.5, 1.0, 1.5, and 2.0 U/ mL were freshly prepared in sodium acetate buffer (pH 5) prior treatment (Padma et al. 2017). All the prepared enzymes were maintained under cold conditions.

Juice extraction

The enzymatic extraction of juice was carried out as described by Joshi et al. (2011) with mild modifications. Pectinase, cellulase, and the combination of pectinase + cellulase were separately added into the beakers containing homogenous fruit pulp with the ratio of 1:30 (v/w) and incubated at 40 °C for 4 hrs. Further this, all the treated pulp was filtered through a muslin cloth to separate the dispersed solid particles and juices and was calculated for juice extraction yield.

Juice clarification

Juice clarification was done according to the procedures as described by Padma et al. (2017), with minor modification. Each of the extracted juice (50 mL) was subjected to enzyme treatments (pectinase, cellulase, and the combination of pectinase + cellulase) (1 mL) at concentration of 1 U/ mL under the desired incubation temperature (40 °C and 50 °C) and time (15 min and 30 min) in a water bath. Then, all of the mixtures were held at 90 °C for 5 min to inactivate the enzyme activity before being filtered using No. 1 Whatman filter paper to remove fine solid particles and collected filtrate was used for further analysis.

Determination of juice yield

The yield of the extracted beetroot juice after treating with or without enzymes was calculated using the formula as shown below:

$$Juice\ extraction\ yield\ (\%) = (\text{Amount of fruit juice collected (mL) after treatment}) / Amount\ of\ homogenous\ fruit\ pulp\ used\ (mL)\ x\ 100\%$$

Measurement of colour

The colour of the samples was measured using Chroma Meter CR-400 (Konica Minolta, Tokyo, Japan). The colour was measured in terms of *L**, *a**, and *b** scale, whereby, *L** indicates degree of lightness (L*=100, white and L*=0, black), *a** shows the red chromaticity (+60) to green chromaticity (-60), and *b** denotes the yellow chromaticity (+60) to blue chromaticity (-60) of space values. The equipment was calibrated with the reference, white calibration plate before the analysis was conducted.

Measurement of cloud value

Approximately, 15 mL of juice samples was centrifuged at 760 x g for 10 min, then the collected supernatant was used for analysis. The absorbance of the supernatant was measured using a UV-Visible Spectrophotometer (Shimadzu UV mini-1240**,** Shanghai, China) at 660 nm (Zhu et al. 2019).

Determination of pH value

The pH value of beetroot juice samples was determined using pH meter (Thermo Scientific Orion 2 -Star Benchtop, MA, USA). It was calibrated using pH 4 and pH 5 buffer solutions at ambient temperature before analysis.

Measurement of total soluble solids (TSS) content

The total soluble solid of the juice samples were determined using a hand-held refractometer (Kern ORA 80BB Series Analogue Refractometer, Kuala Lumpur, Malaysia), scale ranging from 0 to 20°Bx and the value was expressed in degree Brix (Ho et al. 2020).

Determination of total titratable acidity

The total titratable acidity of the beetroot juice samples were determined according to the standard procedure as described by Antony and Chanrda (1997). Five millilitre of beetroot juice

samples was mixed in 20 mL of distilled water. Two to three drops of phenolphthalein indicator solution were added into the mixture and titrated against 0.05 M sodium hydroxide (NaOH) to determine the end point of phenolphthalein. Total titratable acidity was expressed as oxalic acid (%) and calculated using the formula as shown below:

$$\text{Total titrable acidity } (\%) = \frac{\text{Nx V2 x 63}}{\text{V1 x 1000}} \text{ x } 100\%$$

Where:
N= Normality of NaOH, 0.05 M
V1 = Volume of juice sample (mL)
V2 = Volume of NaOH used (mL)
Equivalent weight of oxalic acid = 63 g

Determination of total sugar content

The total sugar content of beetroot juice was determined according to the method as described by Sewwandi et al. (2020). Approximately, 10 mL of the juice sample was centrifuged at 4,000 rpm for 20 minutes to obtain the supernatant. Approximately, 1 mL of each sample or standard solution (reference) was respectively mixed with 1 mL of 5% aqueous solution of phenol and 5 mL of concentrated sulphuric acid. The mixture is then placed in a shaking water bath (100 °C) for 5 min for reaction occurred. Then, vortexed for 30 secs before scanning for the absorbance value. The calibration curve was prepared by using a mixture of glucose, fructose, and galactose (5, 10, 15, 20, 25, 30 mg/L) as a standard solution. The absorbance was measured at 490 nm using UV-Vis spectrophotometer and distilled water was used as blank. The amount of total sugar content was expressed as g/ 100 mL of juice.

Analysis of vitamin C content

The vitamin C content of the samples was determined using iodine titration method (AOAC, 2005). 10 mL of juice samples was added into 10 mL of distilled water. Then, 0.5 mL of starch indicator was added into the mixture and titrated with 0.1 M iodine solution. The end point of titration was identified as the first permanent trace of a dark blue-black colour due to starch-iodine complex. The amount of ascorbic acid was expressed as mg/ 100 mL of juice. The vitamin C content was calculated using the formula as shown below:

$$\text{Vitamin C content } \left(\frac{\text{mg}}{\text{100mL}}\right) = \frac{\text{Volume of titre (mL)x 0.88}}{\text{Volume of aliquot taken (mL)}}$$

Where:
0.88 = equivalent weight of ascorbic acid

Statistical analysis

Experimental data were analysed using the IBM SPSS Statistics for windows, version 25.0 (IBM Corp, Armonk, N.Y., USA). The reported results from this study were represented as the mean values of three individual replicates ± the standard deviation. A one-way analysis of variance (ANOVA) procedure followed by Tukey's HSD test was applied to analyse the significant differences among the mean values of the samples at a significance level of $p<0.05$.

RESULTS AND PRODUCTION

Juice yield

Figure 1 shows that both types of enzyme and enzyme concentrations significantly influenced on the yield of beetroot juice. There was an increase in the extraction yield when using enzymes in beetroot juice extraction as compared with control juice (without enzyme treatment). The increase in juice yield ranged from 72.22 to 82.22% after treated with pectinases, cellulase, and the combination of enzyme. Beetroot juice extraction using combination of enzymes did not show good synergistic effect on yield. However, applying pectinase alone at 1.5 U/mL proved to give a significant ($p < 0.05$) highest juice yield (82.22%) after extraction. When enzyme concentration increased to 2.0 U/mL, the yield decreased significantly. Therefore, it is recommended that beetroot juice could be extracted with 1.5 U/mL pectinase for optimum yield of extraction.

Pectin, a sugar-acid derivative polymer that was situated in the cell wall and inter-linked with other structural polysaccharides and proteins to form water-insoluble pectic substances (protopectin) (Tapre and Jain, 2014). Hence, this pectin form as cementing material and trap the juice inside the vacuole and cell wall of pulp of the fruit. Upon enzyme treatment, breakdown of the pectin into methanol and galactouronic acid leading to a decrease in the water holding capacity of the pectin that might have loosened the cell wall and released more juice in the mixture, thus, juice yield increased (Joshi et al. 2011; Nguyen and Nguyen, 2018; Reddy et al. 2018).

On the other hand, cellulose is a structural carbohydrate (crystalline polymer) consisting of complex sugar. The strong chain of cellulose is linked by inter- and intra- chain hydrogen bonds with the adjacent sheet over-lined one another via

weak Van-der Waals forces (Sharma et al. 2016). Cellulose is either presents in the nature form; pure state or present as cellulose fibre that embedded in a matrix of other structural biopolymers (i.e., hemicelluloses and lignin) (Sharma et al. 2016; Danalache et al. 2018). Cellulase is a common enzyme applied to degrade the cellulose into glucose by hydrolyzing the polysaccharides cell wall and substituted celluloses (Sharma et al. 2016). Moreover, according to Kumar (2015), cellullase is used as part of extraction enzymes acting on soluble pectin hydrolysis and on cell wall components.

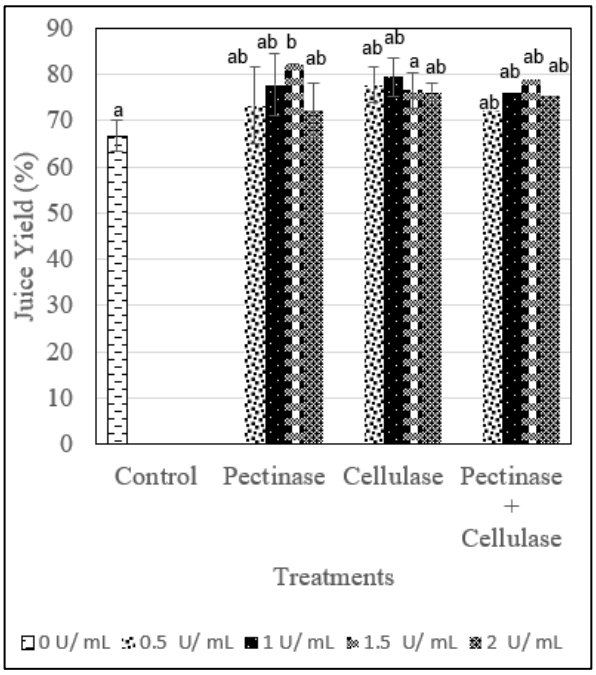

Figure 1. Effect of different concentrations of enzymes treatment on yield[1] of extraction of juice.

[1]Data are presented as mean ± standard deviation (n = 3).

Mean values with different letters within the same group of treatment of each enzyme are statistically significant from each other at P<0.05.

This result was in conjunction with those observed from the previous studies performed by Abbes et al. (2011) on date (*phoenix dactylifera* L) (variety deglet nour) juice treated with pectinase and cellulase combinations with juice recovery of 72.37%. Also, Joshi et al. (2012) had a juice yield of 92.4% after treating tamarind (variety Ajanta) with pectinase at enzyme concentration of 5mg/100g. Singh et al. (2012) had an optimum juice yield of 86.6% after treating bael fruit with pectinase. However, there is variation in the juice yield of different fruit because the amount and activity of the indigenous pectinase enzyme in each fruit varies.

Effect of incubation temperature and time on the physical properties of juice during clarification

Due to the presence of polysaccharides like cellulose, starch, pectin, hemicellulose, proteins, lignin and other substances like tanins and metals, fruit juices are naturally cloudy (Sharma et al. 2016). Pectin is suspended in the juice as the endocarp cells during extraction and left a small portion of the cloud material (Aghajanzadeh et al. 2017) in the juice. Cloud positively influences visual appearance for both colour and organoleptic properties of the fruit juice. The juice clarification process is used to reduce the colloidal particles in suspension after extraction. The effect of incubation temperature (40-50 °C) and incubation time (15-30 min) on colour (lightness: L^*, redness: a^*, and yellowness: b^*) and cloud values of beetroot juice after clarification process are depicted in Figure 2. In general, the results revealed that the higher the incubation time and incubation temperature, the higher the colour values (L^*, a^*, and b^*) of the enzymatically-clarified juice. All the juices treated with pectinase alone, cellulase alone, and pectinase in combination with cellulase showed lightness, redness, and yellowness values increased with prolonging incubation temperature and time, which reflected that the juice became lighter. Fruit juice colour is mainly attributed to various pigments such as anthocyanins, carotenoids, and betalains. Colour degradation is common for natural pigments due to their pH, heat, and light sensitive.

Heating may cause degradation of pigments during processing and produces considerable loss in the quality of foods (Vendruscolo et al. 2013). The study performed by Aghajanzadeh et al. (2017) showed that thermally pasteurised sour orange juice with pectin methylesterase enzyme exhibited lower lightness than fresh juice. Also, the lightness of the grapefruit juice decreased during thermal treatment (Igual et al. 2014). Interestingly to note that the enzymatically-clarified juice with different incubation temperature and time had resulted in more attractive colour (brighter, intense of red and yellow) than the control juices (Juice extracted with pectinase without clarification and fresh juice) (Figure 2).

This was due to the increase in cluster formation during clarification, which ease segregation through filtration and centrifugation. Therefore, improve the clarity and colour of the juice (Santana et al. 2020). Moreover, upon heating and enzyme treatment, the colour (redness and yellowness) of the beetroot juice increased apparently due to the release of pigments such as anthocyanins, carotenoids, and betalains from plant cells. These results in intense red and yellow colour of the end product. Joshi et al. (2011) reported that the anthocyanin pigment of fruit generally increased in juice treated with pectinases because the enzyme assists in the release of pigments from plant cells into the juice.

For the cloud value (Figure 3), juice treated with a combination of enzymes (pectinase + cellulase) revealed that the higher the incubation temperature and time, except for the juice treated with cellulase, the higher the clarity value of the beet juice (Figure 3). Overall, enzymatically-treated juices incubated at 50 °C for either 15 or 30 minutes had significance ($p<0.05$) higher clarity value (47.1-58.3%) than the control juices (juice clarified without enzyme (41.20%) and fresh juice (2.5%)). The raw juice is naturally cloudy or have colloidal suspensions due to individual compositions (i.e., polysaccharides, proteins, and metals) (Vaillant et al. 2011; Sharma et al. 2016).

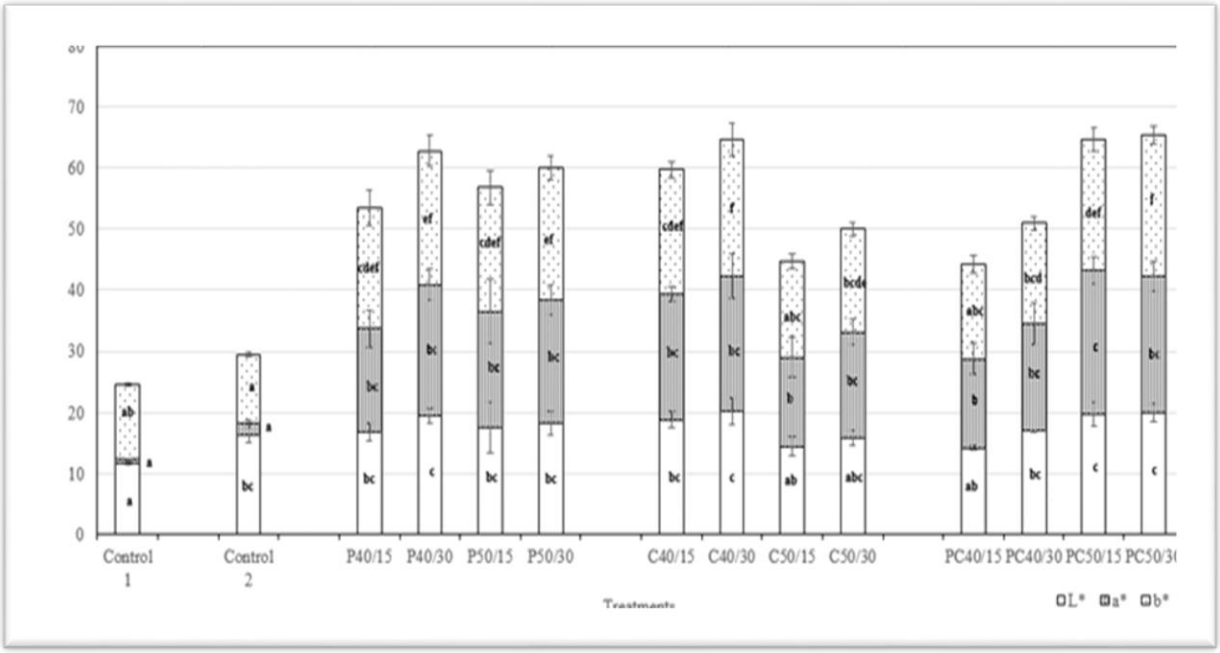

Figure 2: Effect of incubation temperature and time on the colour1 of juice2 during clarification.
Data are presented as mean ± standard deviation (n = 3).

1Mean values with different letters within the same group of treatment of each colour are statistically significant from each other at $P<0.05$

2P50/30: Juice treated with pectinase and incubated at temperature 50 °C for 30 minutes; P50/15: Juice treated with pectinase and incubated at temperature 50 °C for 15 minutes; P40/30: Juice treated with pectinase and incubated at temperature 40 °C for 30 minutes; P40/15: Juice treated with pectinase and incubated at temperature 40 °C for 15 minutes; C50/30: Juice treated with cellulase and incubated at temperature 50 °C for 30 minutes; C50/15: Juice treated with cellulase and incubated at temperature 50 °C for 15 minutes; C40/30: Juice treated with cellulase and incubated at temperature 40 °C for 30 minutes; C40/15: Juice treated with cellulase and incubated at temperature 40 °C for 15 minutes; PC50/30: Juice treated with pectinase + cellulase and incubated at temperature 50 °C for 30 minutes; PC50/15: Juice treated with pectinase + cellulase and incubated at temperature 50 °C for 15 minutes; PC40/30: Juice treated with pectinase + cellulase and incubated at temperature 40 °C for 30 minutes; PC40/15: Juice treated with pectinase + cellulase and incubated at temperature 40 °C for 15 minutes; Control 1: Fresh juice without extraction nor clarification; Control 2: Juice extracted with pectinase without clarification.

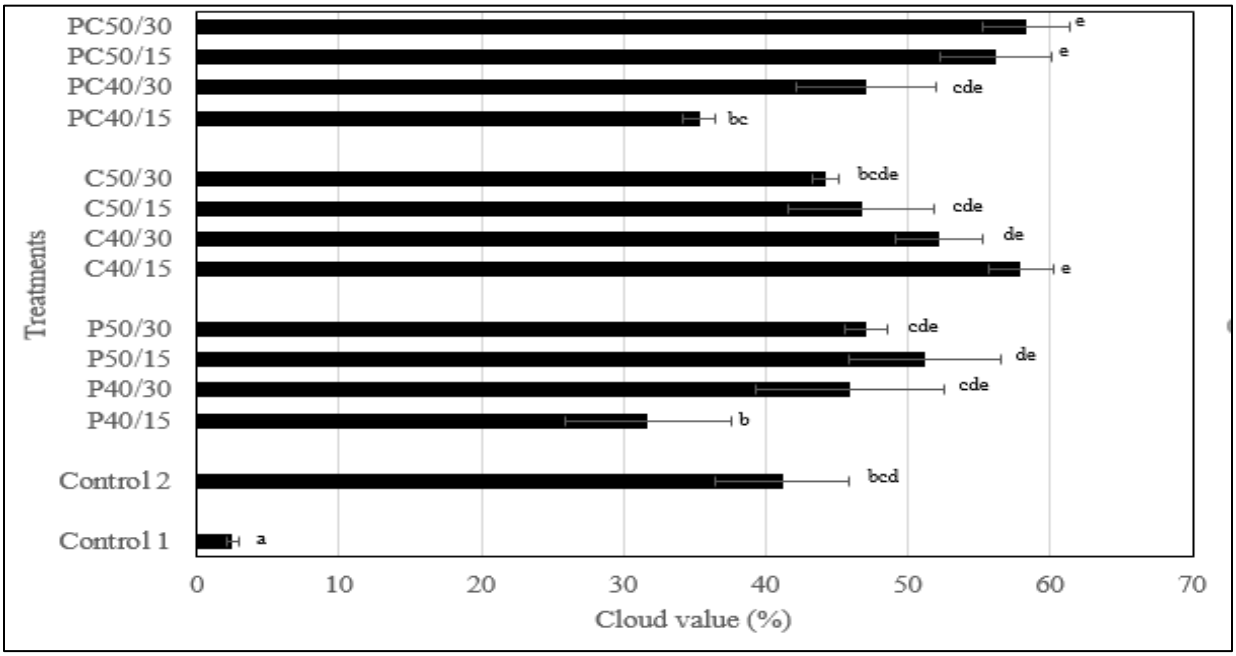

Figure 3: Effect of incubation temperature and time on the cloud value1 of juice2 during clarification

1Data are presented as mean ± standard deviation (n = 3).

Mean values with different letters within the same group of treatment of each cloud value are statistically significant from each other at P<0.05.

2P50/30: Juice treated with pectinase and incubated at temperature 50 °C for 30 minutes; P50/15: Juice treated with pectinase and incubated at temperature 50 °C for 15 minutes; P40/30: Juice treated with pectinase and incubated at temperature 40 °C for 30 minutes; P40/15: Juice treated with pectinase and incubated at temperature 40 °C for 15 minutes; C50/30: Juice treated with cellulase and incubated at temperature 50 °C for 30 minutes; C50/15: Juice treated with cellulase and incubated at temperature 50 °C for 15 minutes; C40/30: Juice treated with cellulase and incubated at temperature 40 °C for 30 minutes; C40/15: Juice treated with cellulase and incubated at temperature 40 °C for 15 minutes; PC50/30: Juice treated with pectinase + cellulase and incubated at temperature 50 °C for 30 minutes; PC50/15: Juice treated with pectinase + cellulase and incubated at temperature 50 °C for 15 minutes; PC40/30: Juice treated with pectinase + cellulase and incubated at temperature 40 °C for 30 minutes; PC40/15: Juice treated with pectinase + cellulase and incubated at temperature 40 °C for 15 minutes; Control 1: Fresh juice without extraction nor clarification; Control 2: Juice extracted with pectinase without clarification.

The pectinase and cellulase used in this study was able to breakdown pectin and cellulose present in the beetroot juice through aggregation of colloid particles after pectin de-esterification by adding enzymes, whereby, the de-esterified colloid particle diffuses until it meets another particle to form a 'fractile cluster' or an 'island structure' or joins an existing island to extend it (Aghajanzadeh et al. 2017), which facilitates separation through filtration and centrifugation. Thus, resulted in the increase in clarity of the beet juice samples treated with enzymes in compared to the control juice samples. In addition, a research conducted by Nadaroğlu et al. (2010) applied commercial pectinase (Pectinex 100L plus) enzyme in the clarification of apple, banana, carrot and orange juices. After clarification, Nadaroğlu et al. (2010) found that the juices had highest clarity rate when incubated at an optimum temperature of 50 ˚C.

A similar report by Mahdu et al. (2015), when pectinase, cellulase, and xylanase were used in orange and apple juice clarification, an optimum temperature was observed at 48 ˚C with clarity rate of 48.6% and 42.16% respectively. Temperature increases rate of enzymatic reactions as far as the temperature is below the optimum temperature required for the enzyme to denature (Lee et al. 2006). According to Amulu et al. (2017), the increase in the kinetic energies (heat) of the molecules which increases the dissolution of the soluble solid thus increased the

clarity. Besides, Amulu et al. (2017) also reported that the increase in temperature during the enzyme treatment contributed to pectin cells destruction. Furthermore, enzymatic reactions with application of heat accelerate hydrolysing the residual pectin which leads to juice clarification through neutralization of electrostatic charges between uronic acid, tannins and proteins (Danalache et al. 2018). Thus, increase rate of clarification.

Biochemical characteristics of beetroot juice clarification

The results of pH, total titratable acidity (%), total soluble solids (°Brix) (brix), ratio of total soluble solids to titratable acidity, total sugar content (g/ 100 mL), and Vitamin C content (mg/ 100 mL) of beetroot juice treated with or without enzymes are summarized in Table 1. The analyses of biochemical specifications of beetroot juice after clarification showed that the enzyme extract improved the process responsible for the clarification of beetroot juice.

Results of pH showed that the addition of enzyme (pectinase and cellulase) decreased the pH value (6.06-6.07) of beetroot juice. The fresh pressed juice (without enzymatic treatment) had the highest pH value of 6.32, therefore, less acidic. The lower pH of the enzymatic-treated juice than the freshly pressed juice (control) might be due to release of carboxyl groups and galacturonic acid from polysaccharides and pectin after treatment (Reddy et al. 2018). In addition, the pH is very crucial intrinsic characteristics influencing the survival and growth of microorganisms in food (Food Safety Authority of Ireland, 2019). For high acid food, at a pH < 4.5, the risk of growth and toxin production by *Clostridium botulinum* is extremely low and for products with pH values between 4.0 and 4.5, processes by mild heat treatment are aimed at controlling the survival and growth of spore forming organisms such as *Bacillus macerans*, *Bacillus polymyxa*, and *Bacillus coagulans* (Kathiravan et al. 2015). However, food with pH value of pH>4.6 is consider low acid food and required severe heat treatment to achieved 'botulinum cook' (Anderson et al. 2011). Therefore, the beetroot juices produced in this study fall under category of low acid food and thus, further processing, such as acidification and canning is necessary to prolong the shelf life of the juice.

Similar to research by Abbes et al. (2011), there was significant decrease in pH in the case of carrot and date (variety Deglet nour, alligandkentichi) syrup treated with enzyme in compared to the untreated sample as reported In another study, Yusof and Ibrahim (1994) also reported a similar result when pectinase was applied in the treatment of soursop juice (*Annona muricata* L), they found that decrease in pH was not significant for the first hour of incubation for each level of enzyme used, but as the incubation time increased for 2-3 hours, decrease in pH values of the fruit juice was significantly different from the initial value.

Total soluble solids, total titratable acidity, and the ratio of brix-acidity are the common quality indicators used to assess sweetness, tartness of a fruit juice, and degree of maturity of the fruits from which the juice was extracted, respectively (Seow et al. 2015). For total soluble solids, the beetroot juice produced in this study ranged from 4.80 to 4.93 °Brix. However, the total soluble solids of the clarified beetroot juice remained unaffected with the enzymatic treatment (Table 1). The current obtained results showed slightly lower than the results observed by Thakur and Gupta (2006) on brix value (6.0-7.2%) of beetroot juice clarified with enzyme Pectinex Ultra SPL at concentration of 0.15% at 45 °C for 2.5 h. On the other hand, the beetroot juice investigated in this study has similar degree of brix with minimally processed beet (4.5-degree brix) investigated by Vitti et al. (2005). According to Seow et al. (2015), organic acids, amino acids, minerals, and other water-soluble components are the major components contributed to high sugar content or brix value, whereas high titratable acidity indicates high organic acid contents.

Beetroots contain significant quantities of oxalic acid (Wruss et al. 2015). The total titratable acidity of the beetroot juice was expressed as oxalic acid (% w/v). The total titratable acidity varies from 0.15-0.42% in the different enzymatic and non-enzymatic treatment (Table 1). In general, higher titratable acidity in the form of oxalic acid was found in enzymatically treated beetroot juice (0.20-0.42%) as compared to the freshly pressed or non-treated juice (0.15%). After 15 min of incubation at 50 °C, the beetroot juice that used alone (PC50/15) for clarification had the highest value of total titratable acidity (0.42%). The possible reason to the increase in the total titratable acidity that the enzyme, particularly pectinase assists in pectin hydrolysis by de-esterification and degradation of polysaccharides, then release of carboxylic acids and galacturonic acids (Joshi et al. 2011; Akesowan and

Choonhahirun, 2013; Reddy et al. 2018). Thereby, the increasing of oxalate acid released during enzymatic activity (cell wall degradation) and the liberation of galacturonic acid inducted by pectinase adjunction. This obtained results is in agreement with previous studies carried by Nguyen and Nguyen (2018), they also had a similar result with mulberry juice obtained through extraction using enzyme Pectinex Ultra SP-L and Viscozyme L, which significantly had higher total titratable acidity than the pressed juice.

The brix/acid ratio of beetroot juice decreased by the addition of enzymes (pectinase and cellulase), application of pectinase alone (P50/15) in clarification apparently increase in titratable acidity than total soluble solids of the treated juice. Joshi et al. (2011) recorded a related result of a decrease in brix/acid ratio of a pectinase treated apple pomace. According to Seow et al. (2015), high titratable acidity indicates high organic acid contents. This brix/acid ratio result indicated that beetroot juice produced in this study has higher organic acid content (high total titratable acidity) than total soluble solid (low brix value).

The total sugar content of the beetroot juice was found to be 7.83-14.40% (Table 1).

In general, clarification of beetroot juice with enzymes had resulted an increase of total sugar content (9.80-14.40 g/100 mL). After 15 min of incubating at 50 °C, the beetroot juice that used pectinase + cellulase (PC50/15) for clarification had the highest value of total sugar content (14.40 g/100 mL), increasing by approximately 84% as compared to that of fresh juice without treatment. This indicates that beetroot juice clarification using combination of enzymes showed good synergistic effect on total sugar content. The increase in total soluble solids is associated to a greater degree of tissue breakdown which lead to the releasing more sugar compounds to the juice (Nguyen and Nguyen, 2018). According to Tapre and Jain (2014), pectic substances are complex colloidal acid polysaccharides, with a backbone of galacturonic acid residues linked by α (1-4) linkage and bounded with side chains of the pectin molecule that consists of neural sugars. The application of enzymes such as pectinase and cellulase in juice processing causes the degradation of cell wall polysaccharides by hydrolysis, therefore, soluble compounds are released, principally the neutral sugars and the D-galacturonic acid. Influence of enzymes on the cell wall release neutral sugars such as D-galactose, D- xylose, D- arabinose and L-rhamnose that are bonded to the pectic substances and therefore become soluble (Kumar, 2015). Wruss et al. (2015) reported that sucrose is the main sugar available in beetroot with little amount of glucose and fructose. A high sucrose and low fructose content of the fruit juice is preferable for development into sports drinks because fructose can reduce exercise capacity of human (Wruss et al. 2015).

Table 1: Biochemical composition[1] of juice[2] with or without enzymes treatment

Composition	Control 1	Control 2	P50/15	C50/15	PC50/15
pH	6.32±0.01^{c}	6.14±0.01^{b}	6.07±0.02^{a}	6.06±0.01^{a}	6.06±0.00^{a}
Total soluble solid (^{0}brix)	4.83±0.06^{a}	4.80±0.00^{a}	4.93±0.06^{a}	4.93±0.06^{a}	4.83±0.07^{a}
Total titrable acidity (%)	0.15±0.01^{a}	0.20±0.01^{b}	0.40±0.01^{c}	0.42±0.01^{c}	0.41±0.01^{c}
Brix/Acid	33:1	24:1	12:1	12:1	12:1
Total sugar content (g/100mL)	7.83±2.40^{a}	9.5±1.47^{ab}	13.53±2.10^{bc}	9.8±0.66^{ab}	14.40±1.47^{c}
Vitamin C content (mg/100mL)	42.83±2.03^{a}	38.13±2.69^{ab}	42.83±2.03^{b}	46.35±1.02^{c}	45.76±3.52^{b}

[1]Data are presented as mean ± standard deviation (*n* = 3).
Mean values with different superscripts within the same row are statistically significant from each other at P<0.05.
[2]P50/15: Juice treated with pectinase and incubated at temperature 50 °C for 15 minutes; C50/15: Juice treated with cellulase and incubated at temperature 50 °C for 15 minutes; PC50/15: Juice treated with pectinase + cellulase and incubated at temperature 50 °C for 15 minutes; Control 1: Fresh juice without extraction nor clarification; Control 2: Juice extracted with pectinase without clarification.

Similarly, an increase in total sugar of the juices added with enzymes was found to be due to the increase of fructose, galactose and sorbitol as observed by Tung et al. (1995) when plum was treated with pectinase. Joshi et al. (2011) and Trappey et al. (2008) reported that plum, apricot, pear peach, and mayhaw juice pectinase-treated had a large total soluble solid level as compared to the untreated juices.

The vitamin C content of the beetroot juice treated with enzyme and incubated at 50 °C for 15 min (Table 1) were significantly similar as compared with that of the control. Juice treated with cellulase (C50/15) had the highest vitamin C (46.35 mg/ 100 mL) content among the treated juices. This may be due to the enzymes such as pectinase and cellulase hydrolysis on the beetroot and vitamin C released into the juice. This is similar to the report by Nguyen and Nguyen (2018), they conducted a research on mulberry juice, and it shows that there were significant increases in vitamin C content in Mulberry juice treated with enzymes (Pectinex Ultra SP-L and Viscozyme L) compared to non-enzymatic treated juice.

CONCLUSION

In this study, enzymatic extraction with pectinase alone at concentration of 1.5 U/ mL at 40 °C for 4 hrs increased the juice yield as much as 23.3%, in comparison with the enzyme untreated-juice. For juice clarification, the longer the incubation temperature (from 40 °C to 50 °C) and time (from 15 min to 30 mins) had resulted in lighter (high L* value), intense red (high a* value), and yellow (high b* value) colours of the enzymatically-clarified juice than the control juices (Juice extracted with pectinase without clarification and fresh juice). Enzymatically-clarified juices incubated at 50 °C for either 15 or 30 minutes had significance ($p<0.05$) higher clarity value than the control juices. Moreover, enzymatic treatments showed improving quality in terms of higher value of total titratable acidity, total sugar, and Vitamin C, but slightly reduction in pH value of the beetroot juice. Therefore, it is recommended that beetroot juice could be produced through extraction using 1.5 U/ mL of pectinase at 40 °C for 4hrs for optimum juice yield, then followed with clarification with 1 U/ mL of the enzymes (pectinase, cellulase, and pectinase + cellulase) at 50 °C for 15 min for a clear juice production.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

All the authors would like to thank Universiti Sultan Zainal Abidin (UniSZA), Malaysia for providing facilities to conduct this research.

AUTHOR CONTRIBUTIONS

Abdussalam Muhyideen Zainab Funmilayo performed the Laboratory work. Both Abdussalam Muhyideen Zainab Funmilayo and Lee-Hoon Ho wrote the manuscript. All authors read and approved the final version.

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