

Effects of Enzymatic Treatment on Physicochemical Properties of Sugar Palm Fruit Juice

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Abstract— The interest in fruit and vegetable juices production has increased significantly all over the world due to their benefit value, quality of production and increasing of consumer awareness and preference for healthy food. The production of sugar palm (*Arenga pinnata*) fruit juice with exotic characteristics has the potential to be explored based on the new technologies and methods in juices industry. In beverage industry, enzyme is an essential tool for quality improvement and cost saving by increasing the yield of the fruit juice. The purpose of this study is to determine the effects of different enzymatic treatment on sugar palm fruit juice processing. Sugar palm fruit purees were treated individually and in combination using two types of commercial enzymes: Novozymes Cellulase and Pectinex Ultra SP-L at a concentration of 0.05% (w/w) and incubated at 45°C for 60 min. The results showed that there are significant ($p < 0.05$) reduction on proximate content such as crude fibre, crude protein and carbohydrate for the enzymes treated juices as compared to the untreated juice. The enzyme treatment also significantly ($p < 0.05$) reduced the juice viscosity and ascorbic acid content, promoted juice clarification and increased L value, yield, TSS and sugar content. In conclusion, the quality of sugar palm fruit juice can be improved by using the combination treatment of pectinase and cellulase enzymes.

Keywords— Sugar palm fruit; juice; enzyme treatment; pectinase; cellulose.

I. INTRODUCTION

The fruit juice industry has become one of the world's biggest agribusinesses [1]. In recent years, the interest in fruit and vegetable juices production has increased significantly all over the world due to their benefit values and quality of production. The production of fruit juices can be classified into several methods and purposes and depending on the physical and physiochemical of the fruit.

Sugar palm (*Arenga pinnata*) fruit also known as *Kabong*, *Kolang Kaling* or *Kolak*, is commonly used for cocktail, salad ingredient and local refreshment [2]. These palm tree is an economically important feather palm native to tropical Asia, from eastern India east to Malaysia, Indonesia, and the Philippines [3]. Although considered as a minor forest species in Malaysia, it provides two important food products which are the exotic sweet kabong fruit and sap juice.

Enzyme technology has led to a significant improvement in the consistency and quality of cloudy juices and concentrates [4], [5]. According to Lee *et al.*, [6], extraction of juice using the commercial pectinolytic enzymes and amylolytic enzyme will produce clearer fruit juice without

cloudy appearance. In order to develop and produce new fruit juice in market, enzymatic hydrolysis can be used to promote high yield, better viscosity and quality fruit juice [7]. Processing of fruit juice with enzyme treatment not only provide many advantages but sometimes lead to several outcomes depending on physicochemical factors such as incubation time, temperature, environment exposed and enzyme concentration.

The production of sugar palm fruit juice with exotic characteristics has the potential to be explored based on the new technologies and methods in juices industry. Thus, the aims of this study were to examine the effects of different enzyme treatments on the sugar palm fruit juice properties.

II. MATERIALS AND METHODS

A. Sample

Ready to eat sugar palm fruit was obtained from local fresh fruit market. The flesh of sugar palm fruit was stored at 4°C before use.

B. Chemicals

Cellulase from *Aspergillus niger* (Sigma-Aldrich Company, USA) and Pectinex Ultra SP-L (Modernist Pantry, USA) were used for the enzymatic treatment. The optimum activity conditions were at pH 3.5 to 6.0 and temperatures below 50°C. The enzymes were stored at 4°C before use.

C. Preparation of Sugar Palm Fruit Juice

Sugar palm fruit was washed with tap water and sliced into 3 pieces. Distilled water was added at 1:3 (v/w) ratios and the mixture was blended for 3 min into fruit puree. The sugar palm fruit puree was subjected to different enzymatic treatment and filtered through a cheese cloth to obtain the juice. The juices were bottled and pasteurized at 85 °C for 5 min before storage.

D. Enzymatic Treatment of Sugar Palm Fruit Puree

Four different enzymatic treatments were used [I: Fruit puree without enzyme treatment (control), II: Fruit puree with Cellulase, III: Fruit puree with pectinase, IV: Fruit puree with Cellulase and Pectinase]. 500 g sugar palm fruit puree was added with 0.05% enzymes (w/w), accordingly. The mixtures were incubated at 45°C and the exposure were stopped after 60 min for each treatments by heating the treated fruit puree at 90°C for 5 min in a water bath. The incubation temperature was controlled using a water bath (Blue Pard, Yiheng Technical Co. Ltd, P.R. China). Then, the treated fruit puree were pressed using a basket screw-press and the juices were strained through cheese cloth.

E. Physico-Chemical Analysis

1) *Determination of pH*: pH meter (Model 320, Mettler-Toledo Ltd., Essex, UK) was used to measure pH of each sample. pH meter was calibrated prior to use.

2) *Total Soluble Solids*: The total soluble solids content was determined using a digital refractometer (Atago, Tokyo, Japan) with a scale of 0–10°Brix.

3) *Yield*: The juice yield was estimated as a percentage of weight of the juice obtained to the initial puree.

$$\text{Yield} = [(w_j - w_w) / w_f] \times 100 \quad (1)$$

w_j = weight of juice
w_w = weight of added water
w_f = weight of fruit

4) *Determination of Juice Clarity*: The juice was shaken and 10 mL was centrifuged at 3000 rpm for 10 min to remove pulp and cloud particles. The clarity of the juice obtained was measured by measuring the transmittance at a wavelength of 570 nm using UV-VIS spectrophotometer (UV 5704SS, Electronics Corporation of India Ltd.). Distilled water was used as reference. The percent transmittance was considered as a measure of juice clarity.

5) *Colour Measurement*: Colour was evaluated using the CR-400 Chroma Meter-Konica Minolta and de-ionized water as blank. Two millilitres of sample were pipetted into a round case and the reflectance was

measured directly from the juice sample. The mean of three values was considered to evaluate the colour in the -L, a, b space system. The system provides the values of three colour components: the higher L (black-white component, luminosity) indicates higher lightness.

6) *Determination of Viscosity*: The viscosity was measured by a Brookfield viscometer (Model RVDV-II, Brookfield Engineering Laboratory, Stoughton, MA, USA) equipped with a spindle no.02 at 100 rpm. Each 20 ml sample was prepared in a 50 mL-beaker and the measurement was made at room temperature.

7) *Proximate Composition*: Moisture content, protein content, crude fat, crude fibre and ash content were determined using standard method [8]. The carbohydrate content were calculated using the difference energy method.

$$\text{Carbohydrate \%} = 100\% - \% \text{ protein} - \% \text{ fat} - \% \text{ crude fiber} - \% \text{ ash} - \% \text{ moisture} \quad (2)$$

8) *Determination of Natural Sugar Content*: The amount of sugar content was determined using the HPLC machine using (Shimadu LC- 6A, Japan). The percentage of Fructose, Sucrose and Glucose was prepared in four concentrations which were 0.5%, 1.0%, 1.5% and 2.0%. Mobile phase used was 80% Acetonitrile with 20% water. The column used was Ammonium. The samples was injected into Alpha Analytical machine with time setup 18 min per injected. The reading of each sample was display in computer connected with different peak of sugar content.

9) *Determination of Vitamin C*: The ascorbic acid content depends on its ability to reduce the redox indicator (colouring) 2, 6-dichlorophenolindophenol. Ascorbic acid were extracted from sample and titrated with that indicator in the presence of oxalic acid. 20 mL of sample were homogenized in 100 mL of oxalic acid for 3x10 seconds. Then the homogenized sample were filtrated and titrated with dye solution.

$$\text{Ascorbic acid content (mg/100g)} = [\text{titre (mL)} \times \text{dye factor (mg/mL)} \times V_f \times 100] / [\text{Aliquot for extraction (g)} \times V_s] \quad (3)$$

F. Statistical Analysis

Statistical significant differences of mean were calculated by using Minitab 16 Version, by aid of one-way ANOVA; results are stated as means ± SEM. A probability value of p<0.05 is regarded to indicate the statistically significant differences.

III. RESULTS AND DISCUSSION

A. Effect on pH and Total Soluble Solid

Table 1 shows the pH and total soluble solid (TSS) of untreated and enzymes treated sugar palm fruit juice. The pH values of the juices produced without enzyme treatment was

significantly higher ($p < 0.05$) as compared to the juices with enzymes treatment but there were no significant difference ($p > 0.05$) among the treated samples. According to Acar et al. [9], the juice treated with enzyme became more acidic, which might be due to the formation of galacturonic acid by the enzymatic breakdown of pectin.

The TSS was significantly different ($p < 0.05$) for both untreated and enzyme-treated samples with juice treated with the combination of both enzymes had the highest content in total solid solid (4.93 ± 0.02 °Brix). As reported by Schobinger et al. [10], the rise in TSS could be partially due to the increment of soluble sugars, which may result from the conversion of insoluble pectin by pectinolytic enzymes and the action of cellulase on cellulose to produce soluble sugars.

TABLE I
EFFECT OF ENZYMATIC TREATMENT ON PH AND TOTAL SOLUBLE SOLID OF SUGAR PALM FRUIT JUICE

Samples	pH	TSS (°Brix)
Untreated	4.28 ± 0.01^a	4.27 ± 0.03^d
Cellulase treated	4.19 ± 0.01^b	4.60 ± 0.00^c
Pectinase treated	4.16 ± 0.01^b	4.73 ± 0.02^b
Treated with both Cellulase and Pectinase	4.15 ± 0.01^b	4.93 ± 0.02^a

Data are mean \pm SE (n=3). Means with the same superscript within a column are significantly difference ($p < 0.05$)

B. Effect on Juice Yield (%) and Viscosity

The percentage of juice yield recovery is as shows in Fig 1. There are significantly different ($p < 0.05$) among the treatments in terms of juice yield. After enzymatic treatments, yields over 60% were obtained. The sugar palm fruit puree treated with combination of both enzymes gave the highest yield of 62.64%. This is in agreement with the suggestion of Pilnik and Vorange [11] which stated that the use of pectic enzymes in fruit processing is essential to get better juice yields, improve filtration rate and produce clear juices of high quality for the concentration process. According to Ramadan and Moersel [12], an increase in juice yield is mostly associated with an increase in sugars soluble in juice. For the enzymation of fruit juices, the middle lamella and cell wall pectin of the product are degraded with exogene enzymes and transformed to soluble materials such as acid and neutral sugar.

The used of enzymes in fruit juice production also supported by Joshi et al. [13] which mentioned that pectolytic enzymes have been used for increasing the yield of juice from stone fruits like peaches, plums and apricots. Imungi et al. [14] also reported that yield of cloudy juice is significantly affected by the enzymatic treatments. Enzymatic hydrolysis of the cell wall constituents is claimed to offer a number of advantages in producing carrot juice such as high yield, better colour and cloud stability [7].

The impact of the enzymes on juice characteristics may also be detected visually as the juices prepared with enzymes were more fluid and less viscous than juices prepared without enzymatic treatments. The viscosity of sugar palm fruit juice treated with the combination of pectinase and cellulase (8.05 Pa.s) had a significant reduction at $p < 0.05$ (refer to Fig 2). Then followed by juices treated by pectinase

and cellulase with the values of viscosity (8.18 Pa.s) and (9.15 Pa.s), respectively. The pectinase enzymatic treatment lowers the viscosity of juice effective when compared with the cellulase enzymatic treatment. This is because the pectinaceous substances possess a high water holding capacity and developed a cohesive network structure. Degradation of pectin by enzyme led to a reduction of water holding capacity and therefore, free water was released to the system to further reduce the viscosity been reported by Urlaub [15].

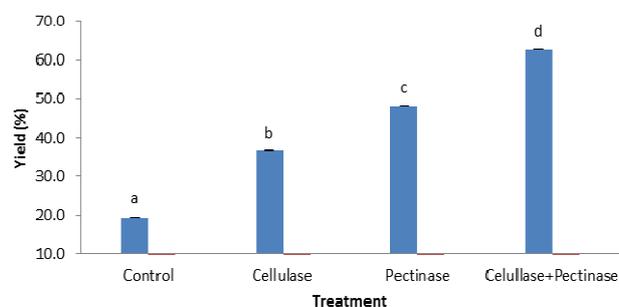


Fig. 1 Yield of sugar palm fruit juice

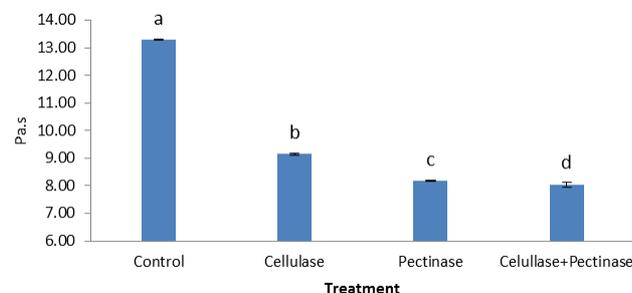


Fig. 2 Viscosity of sugar palm fruit juice

Finding by Cheryan and Alvarez [16] stated that in orders to enhance filtration performance, fruit juices are usually treated before filtrated with enzyme preparation aimed at hydrolyzing mainly soluble polysaccharides responsible for high viscosity. In the previous researches ([17], [18], [19]), fruit juices with high viscosity may lead to problems during the filtration process. The formation of a highly swollen fouling layer on the membrane surface will greatly reduce the performance.

C. Effect on Juice Clarity and Color

The clarity of the juice depended on the enzymatic treatment used and it shows a significant increase in the clarity of the juice ($p < 0.05$) (refer to Table 2). The juice treated with combination of cellulase and pectinase produced the highest clarity, and then followed by Pectinase and Cellulase. According to Kilara [20], temperature may aid in the rate of enzymatic clarification process as the temperature is below denaturation temperature (40–60°C). Using a combination of enzymes may increase the rate of clarification by exposing part of the positively charged protein beneath, thus reducing electrostatic repulsion between cloud particles which cause these particles to aggregate to larger particles and eventually settle out.

Upon enzyme treatment, pectolytic enzymes break down the pectin molecules, which facilitate the formation of pectin-protein flocs, leaving a clear supernatant and significantly removing the colloidal aspect of the juices [17], [21]. According to Rombouts and Pilnik [22], Pectinase hydrolyzes pectin and causes pectin protein complexes to flocculate, while cellulase degrades the cell wall inside.

Color is an important sensory attribute [23]. The L value is a measure of lightness and so this should be as high as possible for clarified juices. A dark color of product is usually less appealing to the consumers as it may indicate deterioration and L value generally showed similar trends as clarity of juice samples where more clear juice gave more light color for juice. The enzymatic treatments are significantly ($p < 0.05$) increased the lightness of sugar palm fruit juice. Increasing in L values, could probably be due to the absence enzymatic browning.

According to Mackinney and Chichester [24], color deterioration in fruit was due to the formation of brown pigments. Heating can create an opportunity for oxidative reactions, which cause a degradation of the pigments [25]. Other factors that also contribute to L value were temperature and time for incubation. Temperature and time used in incubation process was 60°C and 45 min showed some interaction had occurred on the browning enzyme in fruit juice. This may be due to increased agglomeration of floc as more pectin was degraded by the enzyme used.

TABLE II
EFFECT OF ENZYMATIC TREATMENT ON CLARITY AND COLOR OF SUGAR PALM FRUIT JUICE

Samples	Clarity (OD)	Color (L value)
Untreated	0.55 ± 0.00 ^a	51.49 ± 0.57 ^a
Cellulase treated	0.49 ± 0.00 ^b	52.65 ± 0.11 ^{ab}
Pectinase treated	0.48 ± 0.00 ^b	52.68 ± 0.19 ^{ab}
Treated with both Cellulase and Pectinase	0.39 ± 0.00 ^c	53.32 ± 0.43 ^b

Data are mean ± SE (n=3). Means with the same superscript within a column are significantly difference ($p < 0.05$)

D. Effect on Proximate Composition

There are no significant changes on ash and crude fat content between untreated and treated sugar palm fruit juice samples (refer Table 3). Crude fibre content was reduced significantly ($p < 0.05$) in juices treated with combination of cellulase and pectinase and also in juice treated with pectinase alone. There is no significant difference for the amount of carbohydrate reduction in samples treated with single enzyme. According to the Meloan et al. [26] crude fibre is defined as an organic food residue which has been hydrolyzed by acid and aqueous alkaline. Crude fibre method measures cellulose and lignin and therefore there is no specific component being determined. Ben-shalom [27] has proved that a commercial enzyme contains a combination of cellulase and pectinase was hydrolysed the cell walls when applying for fruit juice processing. Degradation of cell wall depends on sequential hydrolysis of the structural polysaccharides and addition of pectinases and the consequent degradation of pectic polymers allow greater

exposure of cellulose and hemicellulose for the enzymatic action of cellulases and hemicellulases [28].

The crude protein content was decreased significantly after the fruit puree was treated with the enzymes. In the previous study by Torres et al. [29], degradation of the crude protein content may be due to breakdown of pectin by action of pectinase enzyme.

TABLE III
EFFECT OF ENZYMES TREATMENT ON PROXIMATE COMPOSITION AND VITAMIN C CONTENT OF SUGAR PALM FRUIT JUICE

Samples	Ash	Crude Fat	Crude Fiber	Crude Protein	Carbohydrate
Untreated	0.10 ± 0.02 ^a	0.52 ± 0.13 ^a	2.72 ± 0.78 ^a	2.83 ± 0.09 ^a	0.88 ± 0.02 ^a
Cellulase treated	0.08 ± 0.01 ^a	0.48 ± 0.23 ^a	2.21 ± 0.77 ^a	1.85 ± 0.01 ^b	0.85 ± 0.01 ^a
Pectinase treated	0.09 ± 0.01 ^a	0.33 ± 0.06 ^a	0.95 ± 0.22 ^b	1.85 ± 0.01 ^b	0.87 ± 0.01 ^a
Treated with both Cellulase and Pectinase	0.07 ± 0.02 ^a	0.28 ± 0.03 ^a	0.64 ± 0.14 ^b	1.41 ± 0.01 ^c	0.82 ± 0.01 ^b

Data are mean ± SE (n=3). Means with the same superscript within a column are significantly difference ($p < 0.05$)

E. Effect On Reducing Sugar

Glucose and fructose were the most abundant sugars in all treated samples but lowest in untreated sample (refer Table 4). Enzymatic treatments increased the amounts of natural sugars in the juices by partly degrading the existing sucrose. These results were similar to those found by Fang et al. [30], where the concentration of reducing sugars (fructose and glucose) increased over time while that of sucrose decreased. The increase in the reducing sugars concentration was higher in the juices pasteurized at higher temperatures on the processing of sugar palm fruit juice.

TABLE IV
NATURAL SUGARS CONTENT IN ENZYMES TREATED SUGAR PALM FRUIT JUICE

Samples	Fructose (%)	Sucrose (%)	Glucose (%)	Ascorbic acid (mg/100g)
Untreated	0.13 ± 0.01 ^b	0.12 ± 0.00 ^b	0.27 ± 0.00 ^a	199.20 ± 0.00 ^a
Cellulase treated	0.14 ± 0.01 ^{ab}	0.11 ± 0.01 ^b	0.34 ± 0.02 ^a	190.90 ± 1.66 ^b
Pectinase treated	0.16 ± 0.03 ^{ab}	0.22 ± 0.03 ^a	0.02 ± 0.01 ^a	189.24 ± 0.00 ^b
Treated with both Cellulase and Pectinase	0.18 ± 0.00 ^a	0.20 ± 0.02 ^a	0.09 ± 0.06 ^a	172.64 ± 1.66 ^c

Data are mean ± SE (n=3). Means with the same superscript within a column are significantly difference ($p < 0.05$)

During enzyme treatment the number of reduction groups increased according to the increase in galacturonic acid and oligosaccharides [31], [32]. These substances are determined as sugars, so the sugar content of the final product was found to be higher. Besides that, the reducing sugar content in the enzymatically treated samples was slightly more than that of the control samples due to action of cellulolytic enzyme which causes conversion of cellulose to glucose [33].

F. Effect on Ascorbic Acid

Interestingly, ascorbic acid is present in high amounts in all treatments with the values in the range of 199-172 mg 100 g⁻¹. The untreated juices had slightly higher ascorbic acid content than enzyme-treated juices. The amounts of ascorbic acid degraded when undergo the enzymatic treatment. From the research by Moser and Bendich [34], the degradation of ascorbic acid may due to the heat treatment in processing steps and exposed to the oxygen too long which is the common cause in the loss of vitamin C inside a fruit juice.

IV. CONCLUSIONS

The effect of different enzymatic treatment on the sugar palm fruit juice extraction gave a significant ($p < 0.05$) different on the physical characteristics of sugar palm fruit juice while on the proximate composition no significant affect was observed. The result for viscosity and ascorbic acid were significantly decrease and result for % yield, TSS, pH, color, clarity and sugar content were significantly increase when compare from untreated and all treated sugar palm fruit juices. In conclusion, the using of combination pectinase and cellulase enzymatic treatment is the most effective for the production of sugar palm fruit juice. For the future research, other factors such as different concentration of enzymes in the production of sugar palm fruit juice could be considered in order to save the cost of production. The effect of temperature and time also can be study due to the sugar palm fruit juice enzymatic treatment stability and physicochemical properties.

REFERENCES

- [1] D.S. Retnowati, A.C. Kumoro, and C.S. Budiati, "Modeling o Rheological Properties of Tropical Fruit Juices", RSCE-SOMCHE, pp. 851-856, 2008.
- [2] C. Orwa, A. Mutua, R. Kindt, R. Jamnadass, and S. Anthony, "Agroforestry Database: a tree reference and selection guide version 4.0" World Agroforestry Centre, Kenya, 2009.
- [3] N.W. Uhl, and J. Dransfield, "Genera Palmarum - A classification of palms based on the work of Harold E. Moore". Lawrence, Kansas: Allen Press. ISBN 0-935868-30-5 / ISBN 978-0-935868-30-2, 1987.
- [4] R.A. Beker, and J.H. Bruemmer, "Pectinase stabilization of orange juice cloud", Journal of Agriculture and Food Chemistry, vol. 20 pp. 1169-1173, 1972.
- [5] B. Korner, G. Zimmermann and Z. Berk, "Orange pectinesterase: Purification, properties and effect on cloud stability" Journal of Food Science, vol. 45, pp. 1203-1206, 1980.
- [6] W.C. Lee, S. Yusof, N.S.A. Hamid, and B.S. Baharin, "Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM)", Journal of Food Engineering, vol.73, pp.55-63, 2006.
- [7] L. Qin, S.Y. Xu, and W.B. Zhang, "Effect of enzymatic hydrolysis on the yield of cloudy carrot juice and the effects of hydrocolloids on color and cloud stability during ambient storage", Journal of Science Food Agriculture, vol. 85, pp. 505-512, 2005.
- [8] AOAC International, "Official Methods of Analysis. 19th edition", USA, 2012.
- [9] J. Acar, N. Alper, and O. Esturko, "The production of cloudy apple nectar using total liquefaction enzymes", Fruit Process, vol. 8, pp. 314-317, 1999.
- [10] U. Schobinger, P. Durr, and A. Akesson, "Technologische und analytische Daten zur enzymatischen Verflüssigung von Äpfeln und Birnen", Lebensmittel Technologie, vol. 20, pp. 37-42, 1981.
- [11] W. Pilnik, and A.G. J. Vorange, "Effect of enzyme treatment on the quality of processed fruit and vegetable", ACS Symposium Series vol. 405, pp. 205-69, 1989.
- [12] M.F. Ramadan, and J.T. Moersel, "Impact of enzymatic treatment on chemical composition, physicochemical properties and radical scavenging activity of golden berry (*Physalis peruviana* L.) juice", Journal of the Science of Food and Agriculture, vol. 87, pp. 452-460, 2007.
- [13] V.K. Joshi, S.K. Chauhan, and B.B. Lal, "Extraction of juices from peaches, plums and apricots by pectinolytic treatment", Journal of Food Science and Technology, vol. 28, pp. 64-65, 1991.
- [14] J.K. Imungi, P. Scheffeldt, and P. SaintHilaire, "Physicochemical changes during extraction of clear guava juice" *Leben smittel-Wissen chaft und Technologie*, vol. 13, pp. 248-251, 1980.
- [15] R. Urlaub, "Advantages of enzymatic apple mash treatment and pomace liquefaction", Fruit Processing, vol. 6, pp. 399-406, 1996.
- [16] M. Cheryan, and J.R. Alvarez, "Food and beverage industry application", in R.D. Noble, S.A. Stern (Eds.), *Membrane separations technology principles and application*, Elsevier, Amsterdam. pp. 415-445, 1995.
- [17] S. Alvarez, R. Alvarez, F.A. Riera, and J. Coca, "Influence of depectinization on apple juice ultra-filtration. Colloids and Surfaces", *Physicochemical and Engineering Aspects*, vol. 138, pp. 377-382, 1998.
- [18] D.R. Kashyap, "Applications of pectinases in the commercial sector: a review", *Bioresource Technology*, vol. 77, pp. 215-227, 2001.
- [19] F. Vaillant, A. Millan, M. Dornier, M. Decloux, and M. Reynes, "Strategy for economical optimization of the clarification of pulpy fruit juices using crossflow microfiltration", *Food Engineering*, vol. 48, pp. 83-90, 2001.
- [20] A. Kilara, "Enzymes and their uses in the processed apple industry: a review", *Process Biochemistry*, vol. 23, pp. 35-41, 1982.
- [21] S. Yusof, and I. Nurzarina, "Quality of soursop juice after pectinase enzyme treatment", *Food Chemistry*, vol. 51, pp. 83-88, 1994.
- [22] F.M. Rombouts, and W. Pilnik, "Enzymes in fruit and vegetable juice technology", *Journal of Process Biochemistry*, vol. 13, pp. 9-13, 1978.
- [23] C.J.B. Brimelow, and C.A. Groesbeck, "Colour measurement of foods by colour reflectance instrumentation" in E. Kress-Rogers, Editor. *Instrumentation and sensors for the food industry*, Oxford: Butterworth Heinemann, pp. 63-96, 1993.
- [24] G. Mackinney, and C.O. Chichester, "Color deterioration in strawberry preserves", *Canner*, vol. 114, pp. 13, 1952.
- [25] P. Markakis, G.E. Livingstone, and C.R. Fellers, "Quantative aspects of strawberry pigment degradation", *Food Research* vol. 22, pp. 117-130, 1957.
- [26] C.E. Meloan, and Y. Pomeranz, "Crude Fiber" in *Food Analysis Laboratory Experiments*. 2nd Edition. AVI Publishing Company. Connecticut, pp. 94-96, 1980.
- [27] N. Ben-shalom, "Hindrance of hemicelluloses and cellulose hydrolysis by pectic substances", *Journal of Food Science*, vol. 51, pp. 720-725, 1986.
- [28] P. Massiot, J.F. Thibault, and X. Rouau, "Degradation of carrot (*Daucus carota*) fibres with cell-wall polysaccharide-degrading enzymes", *Journal of the Science of Food and Agriculture*, vol. 49, pp. 45-58, 1989.
- [29] E.F. Torres, C. Aguilar, J.C.C. Esquivel, and G.V. Gonzalez, "Pectinases", in A. Pandey, C. Webb, C.R. Soccol, C. Larroche, (Eds.), *Enzyme Technology*, pp. 273-296, 2005.
- [30] T. Fang, H.E. Chen, and L.M.J. Chiou, "Effects of heat treatment and subsequent storage on the quality of passion fruit (*Passiflora edulis*) juice", Paper presented on 19th Symposium of International Federation of Fruit Juice Producers, Den Haag, Netherlands, 1986.
- [31] N. Demir, J. Acar, K. Saroglu, and M. Mutlu, "The use of commercial pectinase in fruit juice industry, Part 3: Immobilized Pectinase for mash treatment", *Journal Food Engineering*, vol. 47, pp. 275-80, 2001.
- [32] A.K. Landbo, K. Kaack, and A.S. Meyer, "Statistically designed two step response surface optimization of enzymatic prepress treatment to increase juice yield and lower turbidity of elderberry juice", *Innovative Food Science Emerging Technology*, vol. 8, pp. 135-142, 2007.
- [33] M. Mandels, R. Andreotti, and R. Charles, "Measurement of saccharifying cellulose", *Biotechnology and Bioengineering Symposium*, vol. 6, pp. 21-2, 1976.
- [34] U. Moser, and A. Bendich, "Vitamin C", in *Handbook of Vitamins*, Edited by L.J. Machlin. Marcel Dekker, New York; Chapter 5, 1990.