The effect of enzyme concentration on physical characteristics of pumpkin (*Cucurbita moschata*) puree and its dried extract

F Kormin¹ *, R A Sakinah¹, A C Iwansyah², A Hesen¹

¹ Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Pagoh Educational Hub, 84600 Pagoh, Johor, Malaysia

² Research Centre for Appropriate Technology, Indonesian Institute of Sciences, Jl. KS. Tubun No. 5 Subang West Java, Indonesia, 41213

*Corresponding author: faridahk@uthm.edu.my

Abstract. Pumpkin (*Cucurbita moschata*) contain many nutritional and biologically active components such as carotenoid content, polysaccharides, sterols, vitamins and protein. Since pumpkin has perishable characteristics, pumpkin must be preserved to increase the shelf life. The present study describe the possibility of producing spraydried pumpkin extract to increase the shelf life. Since pumpkin have high viscosity, it tend to cause deposit in the wall of spray drying chamber. With the help of enzyme liquefaction technique, the viscosity of the pumpkin can be reduced. In this research, pumpkin puree were individually treated with Pectinex Ultra-SP (0-2.5% w/v). Then, concentration Pectinex Ultra SP that produce the best viscosity reduction of pumpkin puree were chosen and treated with Celluclast 1.5 L (0-2.5% w/v). The results showed that 2.5% w/v of both Pectinex and Celluclast 1.5 L produced high viscosity reduction, clarity, total soluble solid and low pH. Then this pumpkin extract are subjected to spray drying condition and physical properties are measured. The spray drying of pumpkin extract with 2.5% w/v Celluclast 1.5 L and 2.5% w/v of Pectinex Ultra SP showed the higher yield of pumpkin powder, while no differences in moisture content, water activity and colour.

Key words: Celluclast, *C. moschata*, dried extract, liquefaction, pumpkin

1. Introduction

Pumpkin, from genus Curcutibita from family Curcutitaceae contained many nutrient that are beneficial for health such as pectin, vitamins, carotene, mineral salts and other substances. Various product can be produced from the pumpkin due to its high nutritional and protective value such as instant noodles, spices, bakery product and colouring agent in food. Since pumpkin is a fresh seasonal crop, it is very sensitive to microbial spoilage and have shorter shelf life which cause problem to the storage. Moreover, many tropical fruit like pumpkin tend to deteriorate and cause wastage during peak harvesting season due to insufficient storage space and processing facilities [1]. Thus, pumpkin need to be preserved through drying or refrigerated condition to increase its shelf life and to maintain its nutritional qualities.

There are various method to preserve pumpkin. One of the method to preserve pumpkin are by using drying method. By using drying method, shelf life of the food product can be extended with lower requirements for packaging and reducing the weights of shipping. Tonon et al. [2] stated that spray
drying method is one of the methods that are suitable for heat sensitive food products and results in powder with low water activity, good qualities and facilitate the storage and transportation.

In preservation of pumpkin by using spray drying method, there are several problems that can affect the product yield in the drying process. The major degradation of the pumpkin powder produced by spray drying is mainly due to the stickiness and high viscosity in the nature of fruit material. According to Tonon et al. [3] this will lead to the operational problems and low product yield. Thus, the optimum enzyme concentration need to be considered to produce high qualities of powder product. Moreover, there were also lack of published results on liquefaction of combined enzyme treatment on spray dried pumpkin powder. Thus, this study aimed to evaluated the effect of enzyme in combination on physical characteristics of pumpkin puree and its dried extract.

2. Materials and Methods

2.1. Materials
Pumpkin fruits (Cucurbita moschata) were used in this study. Pumpkins were purchased from the fresh market in area Pagoh and Muar, Johor. Maltodextrin (DE 10–12) were obtained from Personel Formula Resources. The enzymes, Pectinex Ultra SP-L and Celluclast 1.5 L (for liquefaction) were purchased from Tricell Bioscience Resources and stored at 4°C.

2.2. Preparation of puree pumpkins and dried extract
The pumpkin fruits were peeled, deseeded and fleshed cut into small pieces [4]. Then, the slice pumpkin were blended by using Waring blender (WARING; model 700G) with water in ratio of 1:2 for approximately 30-60 s until a homogenous puree obtained [5]. After that, the homogenous pumpkin puree were filtered using muslin cloth to obtained pumpkin extract.

For enzymatic liquefaction, 500 g of homogenised pumpkin puree was individually treated with different concentrations (0–2.5% w/v) of Pectinex Ultra SP-L (Novozymes, Denmark). The enzyme-added purees were incubated at 50°C using a water bath (Memmert Lab Companion, Jeio Tech, Selangor, Malaysia). The viscosity and colour were measured at 1.5 h in 50°C. The enzyme in the treated puree was deactivated at 90°C for 5 min. Combined enzymes treatment was then performed by adding the optimised Pectinex Ultra SP-L concentration with Celluclast 1.5 L at different concentrations (0–2.5% v/w). After that, viscosity was chosen as the key evaluation to determine the best parameter for enzymatic liquefaction [6].

The dried pumpkins extract was performed by using a laboratory mini spray dryer (AGRIDON; model AG-1-06) and was operated concurrently with a 0.7 mm diameter spray nozzle. The inlet temperature was 180°C and the outlet temperature were between 75 - 85 °C. Then, the solution was feed into the drying chamber through peristaltic pump with pressure and feed flow rate of 2.0 kgcm⁻² and 0.5 Lh⁻¹, respectively. During spray drying, the feed containing maltodextrin, 50% w/v in order to encapsulated high sugar content of pumpkin extract, was stirred continuously to ensure its homogeneity. The resulting powder obtained was immediately removed and collect in glass collection vessel and stored in an air tight zip-lock bag and was kept in the desiccator containing silica gel before determining its characteristics [4].

2.3. Experimental design
This research uses a completely randomized design (CRD), with Celluclast 1.5 L at different concentration, namely, A0 (0%), A1 (0.5%), A2 (1.0%), A3 (1.5%), A4 (2.0%) and A5 (2.5%), with three replications, 18 trial units were obtained. The parameters tested were: physical properties, such as: viscosity, clarity, colour, pH and total soluble solid. The dried extract of pumpkins was measured moisture content, water activity, and yield.
2.4. Procedure Analysis

2.4.1 Viscosity Viscosity measurement were carried out using Rheometer (Discovery Hybrid Rheometer; model DHR-3) using concentric cylinder (28.03 mm diameter and 41.90 mm length) under controlled temperature. The shear rate measurement were performed using in the range 2.5-950 s⁻¹. The measured shear stress was within 0.5 to 50 Pa. Viscosity characterization was carried out in triplicate at room temperature.

2.4.2 Clarity Clarity was determined by measuring the absorbance at 660 nm using a Shimadzu UV– VIS spectrophotometer (Model UV-1201, Shimadzu corporation, Japan). Distilled water was used as the reference [7].

2.4.3 Colour The colour of the clarified juice was measured using a Hunter Laboratory Calorimeter (model SN 7877, Ultrascan, Hunter Associates Laboratory, Inc., Virginia), where +L represents lightness and –L represents darkness. L a and b where L denotes lightness and darkness, a redness and greenness and b yellowness and blueness [8].

2.4.4 Total solid The total soluble solids content was determined using a digital refractometer (ATAGO; model Pal-1) with a scale of 0–10°Brix [8].

2.4.5 pH value pH meter (ATAGO; model Pal-1) was used to measure pH value of each sample. pH meter was calibrated prior to use [8].

2.4.6 Moisture content The pumpkin’s powder moisture content was determined by using Moisture Analyzer (TOVATECH; model DSC 71P). 5 g of sample was weighed and placed on an aluminium pan. Based on the standard in the manual, the heating temperature was standardize into 160°C. Moisture content of the samples was expressed in percentage (%) and the experiment was done in triplicate at room temperature of 25 °C.

2.4.7 Water activity (a_w) Water activity (a_w) of samples were measured by water activity meter (DECAGON; model Aqua Lab). The samples were prepared in triplicated with cassava fermented flour (1 g) was placed in the sample holder. The sample was placed in the water activity meter and the measurement was recorded.

2.4.8 The yield cyclone and sweep recoveries were added together by total up the weight of powder by sweeping of the wall of spray-dryer glass chamber (sweep recovery) and the weight that will be collect in cyclone chamber (cyclone recovery). Process yield is expressed as the relationship between the total solid content in the feed mixture and the total solid content in resulting powder [3].

2.5 Statistical Analysis The data are presented in mean ± standard deviation (sd) (n=3). Data were analyzed by analysis of variance (ANOVA), with a confidence interval of 95% and tested for normality of the data first. Duncan's multiple comparison test was used to determine the average difference between each treatment. The data processing used Microsoft Excel 2017 and statistic programme.

3. Results and Discussion

3.1 Viscosity based on different concentration enzyme The viscosity of the pumpkin puree after treatment by enzyme Pectinex Ultra SP ranged from 0.08 Pa.s to 0.24 Pa.s (Figure 1). In order to determine the optimum enzyme liquefaction parameter, viscosity
measurement can be used and it was helpful to prevent the clogging of spray dryer and maximize the amount of juice yield [9]. As seen, increasing concentration of Pectinex Ultra SP causing decreasing in viscosity from initial of after enzymatic treatment. The highest viscosity reduction was when added 2.5% w/v Pectinex Ultra SP-L individually to the pumpkin that produced the enzyme range between 0.08 and 0.11 (P<0.05). From Figure 1, 0.08 and 0.11 are the viscosity amounts of the pumpkin puree (P<0.05). Therefore, 2.5% w/v Pectinex Ultra SP-L was chosen to treat pumpkin puree in combination with different concentration Celluclast 1.5L (0-2.5% w/v).

This study was similar to Gabas [10] reported that milk often exhibits simple Newtonian behavior, concentrated milk shows pseudoplastic behavior. Moreover, there are several factors that influence the rheological behaviour of liquid, which were particle size, total dry matter material, temperature and total dissolved solids [11]. The concentration of soluble and insoluble solids has a significant non-linear effect on Newtonian fluid viscosity, consistency index and apparent viscosity of non-Newtonian fluid [12].

![Figure 1. The effect of enzyme Pectinex Ultra SP 1.5 h concentration on viscosity reduction of pumpkin puree. a>b>c>d. Values followed by different alphabetic in bar shows significant differences (P<0.05).](image)

3.2 Physical properties of pumpkin puree
In contrast, Figure 2 showed that the absorbance quality increased with the increase in enzyme concentration. This showed that the rate of clarification increase by exposing part of the positively charged protein beneath, thus increasing the electrostatic repulsion between cloud particles which causes these particles to accumulate into larger particles and eventually settle out. There are also several factors that can effect clarity which is temperature, enzyme concentration and incubation time. Clarification are important in as to reduce the stickiness in the wall of spray drying chamber.
Figure 2. The effect of enzyme concentration on clarification of pumpkin puree. Data presented mean±standard errors (n=3). a>b>c>d>e. Values followed by different alphabetic in bar shows significant differences (P<0.05).

The results for the Pectinex Ultra SP and Celluclast 1.5 L mixture were similar to the viscosity of the Pectinex Ultra SP alone (figure 3). This could be due to the low reaction temperature and incubation time resulting the Celluclast 1.5 L not in their optimum reaction. Moreover, the use of celluclast alone to treat the pulp viscosity failed to reduced the viscosity of the pulp to the extent of enzyme combination and pectinex alone while the combination of pectinase and cellulase able to synergistically degrade the polysaccharide that contain high molecular weight and serve as an index for the amount of juice liquified [13][14][15]. Therefore, the combined enzymes, 1.0% (w/v) Pectinex Ultra SP-L and 0.5% (w/v) Celluclast 1.5 L were chosen as the desired enzyme concentration.

Figure 3. The effect of enzyme concentration (Pectinex Ultra SP and Celluclast 1.5 L mixture) on viscosity of pumpkin puree. Data presented mean±standard errors (n=3). a>b>c>d>e values followed by different alphabetic in bar shows significant differences (P<0.05).

Pumpkin fruit are classified as medium acid foods with pH range of 4.99-5.50. The effect of enzyme concentration of Celluclast 1.5L (0-2.5 w/v%) on total soluble solids (TSS) and pH was described on table 1. Table 1 shows that the effect of different concentration of Celluclast 1.5L with
2.5% Pectinex Ultra SP on pH. The pH decreased from 5.19 when the enzyme Celluclast 1.5 L increased from 0.5 to 2.5 ($P<0.05$). The pumpkin puree with celluclast 2.5% w/v had the lowest pH (5.02). This was due to the release of carboxylic acid from galacturonic acid upon demethylation, and of acetic acid as a result of the pectinesterase and acetylesterase activities [16][17] contained in the enzymatic preparation, and to liberation of galacturonic acid [18] from the pectin molecule during the enzymatic depolymerization [19].

| Samples | pH     | Total solid (°Brix) | L*          | a*          | b*          |
|---------|--------|---------------------|-------------|-------------|-------------|
| A0      | 6.51±0.00 | 2.80±0.55            | 47.04±0.10  | 15.22±0.22  | 69.56±0.26  |
| A1      | 5.19±0.00 | 4.83±2.20            | 50.45±0.07  | 1.90±0.25   | 7.06±0.42   |
| A2      | 5.14±0.01 | 5.97±2.44            | 50.64±0.21  | -1.83±0.11  | 7.34±0.05   |
| A3      | 5.09±0.00 | 5.97±2.44            | 51.19±0.31  | 1.47±0.30   | 30.88±0.88  |
| A4      | 5.08±0.00 | 6.10±2.47            | 52.73±0.21  | 2.69±0.52   | 32.23±1.90  |
| A5      | 5.02±0.02 | 6.57±2.56            | 53.41±0.15  | 0.21±0.23   | 23.26±1.52  |

Data were presented mean±standard deviation (SD) (n=3). A0: control, A1: celluclast 0.5%, w/v, A2: celluclast 1% w/v, A3: celluclast 1.5% w/v, A4: celluclast 2.0% w/v, A5: celluclast 2.5% w/v. a>b>c>d>e. Values followed by different letters in same columns showed significant differences ($P<0.05$).

Table 1 showed that the highest value of total solid obtained was at the concentration of Pectinase 2.5 w/v% and Celluclast 2.5% w/v at 1.5 hour ($P<0.05$). The amount of TSS increased as enzyme ratio was higher, due to an increase of volume of liquid phase released [18] in these conditions, caused by the cell walls water retention capacities loss which leads to the liberation of sugars that contribute to the °Brix. In other words, the tissue breakdown leads to the more components including nutritional components released by soluble solids as reported by the Norjana & Noor Aziah [20].

Color was represented by $L^*$, $a^*$, and $b^*$, where $L^*$ values range from black (0) to white (100), $a^*$ values range from green (−) to red (+), and $b^*$ values range from blue (−) to yellow (+). Hue is described as the color from the rainbow or spectrum of colors. The $L^*$ value indicated the lightness and the pumpkin is considered clarified if the value of $L^*$ is high. Based on the table 1, $L^*$ value range from 50.45 to 53.41. This may due to the high pectin degradation by higher enzyme concentration that resulting in agglomeration of particle. As time increased, the fine particles in the juice may also slowly settle down.

3.3 Physical properties of dried pumpkin extract

Process yield, colour, moisture content and water activity are important parameter to determine the good quality of dried pumpkin extract. The process yield of the pumpkin varies from 15.04 to 13.01% (figure 4). This was probably due to the high concentration of Celluclast that cause reduction in process yield of the pumpkin powder which lead to the production of low molecular weight sugar due to the degradation of cellulose. Study by Shavakhi et al. [4] on spray dry pumpkin powder also indicated that the Celluclast concentration also may reduce the quantity of maltodextrin needed to produce suitable feed for spray drying. Another factor that also contribute to the increase in spray drying is the maltodextrin concentration and inlet temperature.
Figure 4. The effect of enzyme concentration on the yield of dried pumpkin extract. Data presented mean±standard errors (n=3). a>b>c>d values followed by different alphabetic in bar shows significant differences (P<0.05).

The water activity is a measurement of availability of free water in a food system that is responsible for any biochemical reactions [21], while the moisture content is describes water composition in a food system. It was preferable to be low moisture content and water activity in order to increase the shelf life of the powder [22]. Table 2 shows that the moisture content was between 0.57±0.01 to 0.54±0.04, while the water activity was between 0.22 ± 0.07 to 0.21 ± 0.02. Moreover, the dried pumpkin extract produced were micorbiologically stable as the moisture content in this study were below 10% [23], while the water activity also considered as stable (<0.6) against microbial, browning, auto-oxidation, lipid oxidation, hydrolytic reactions and enzymatic activity [24].

Based on the Table 2, L* value of powder varied from 83.19±0.25 to 83.61±0.90 which showed variation of darkness/whiteness of dried pumpkin extract under the different designed conditions. The high *L value may due to the concentration of maltodextrin. The white colour of maltodextrin may cause increase in *L value. Similar results have been reported for spray dried pineapple juice powders [25], gac fruit powder [26] and sweet potato powders [27]. The lightness was also decreased slightly with increase in feed flow rate.

Table 2. Effects of enzyme concentrations to moisture content, water activity and color of dried pumpkin extract

| Samples | Moisture (%) | Water activity (a_d) | L* | a* | b* |
|---------|--------------|----------------------|----|----|----|
| A1      | 0.57±0.01    | 0.22±0.07            | 83.19±0.25 | 2.80±0.21 | 25.68±0.96 |
| A2      | 0.64±0.12    | 0.21±0.04            | 88.92±0.20 | 2.36±0.06 | 22.54±0.05 |
| A3      | 0.57±0.07    | 0.22±0.03            | 85.67±0.30 | 2.31±0.53 | 25.55±1.24 |
| A4      | 0.65±0.06    | 0.17±0.02            | 82.07±0.95 | 2.17±0.33 | 26.05±0.94 |
| A5      | 0.54±0.04    | 0.21±0.02            | 83.61±0.90 | 2.54±0.11 | 26.81±0.56 |

Data were presented mean±standard deviation (SD) (n=3). A1: cellulast 0.5%,w/v, A2: cellulast 1% w/v, A3: cellulast 1.5% w/v, A4: cellulast 2.0% w/v, A5: cellulast 2.5% w/v. a>b>c>d>e. Values followed by different letters in same columns showed significant differences (P<0.05).

4. Conclusion
The effect of enzyme liquefaction on spray dried pumpkin extract was determined based on the physical analysis. The combination usage of 2.5% w/v Pectinex Ultra SP L and 2.5% w/v Celluclast 1.5L shows the highest viscosity reduction of pumpkin puree. The pumpkin extract also exhibit
Newtonian behaviour under room temperature. Viscosity measurement showed 2.5% w/v of Pectinex Ultra SP with different concentration Celluclast 1.5L (0-2.5 w/v%) subjected to different measurement which is colour, clarity, brix and pH. The pumpkin extract with enzym 2.5% w/v of Celluclast with combination of Pectinex Ultra SP (A5) shows highest *L value, clarity, brix and pH. Moreover, the effect of different concentration of Celluclast 1.5L with 2.5 w/v Pectinex Ultra-SP on the spray-dried pumpkin extract shows effect on the product yield while no differences on colour, water activity and moisture content.

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