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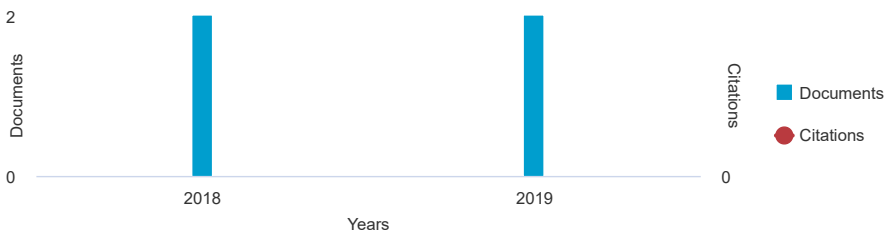

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The effect of fermentation process on physical and chemical characteristics of pitaya (*Hylocereus polyrhiuzus* [F.A.C. Weber] Britton & Rose) stem flour

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The effect of fermentation process on physical and chemical characteristics of pitaya (*Hylocereus polyrhiuzus* [F.A.C. Weber] Britton & Rose) stem flour

H Soedjatmiko[1], R Chrisnasari[2,*] and P H Hardjo[1]

[1]Magister of Biotechnology, Faculty of Biotechnology, University of Surabaya, Jalan Raya Kalirungkut, Surabaya 60293, East Java, Indonesia.
[2]Department of Biology, Faculty of Biotechnology, University of Surabaya, Jalan Raya Kalirungkut, Surabaya 60293, East Java, Indonesia

*Corresponding author: ruth_c@staff.ubaya.ac.id

Abstract. Research interest on fiber has increased rapidly in the last decade due to its complexity and functional properties towards the human body. In this research group, pitaya (*Hylocereus polyrhiuzus* [F.A.C. Weber] Britton & Rose) stem flour is a promising candidate as new food resources since it contains a high amount of fiber and vitamin C as well as demonstrates both antioxidant and antibacterial activities. However, this pitaya stem flour has very low solubility in water that makes its application in food product very limited. In this study, pitaya stem flour has been fermented by *Trichoderma reesei* in a variation of inoculum concentration (3 % and 5 %) and fermentation time (24 to 60 h). The fermentation processes reduce the ash and water content of the flour, increase the soluble fiber content as well as its solubility. During the fermentation, the colour of the flour has changed. It becomes brighter and brownish. The best fermentation condition marked by its highest soluble fiber content is achieved by 3 % (w/v) of *T. reesei* inoculum with 60 h incubation time.
Keywords: Fermented flour, incubation time, insoluble fiber, pitaya stem, soluble fiber.

1. Introduction

Dietary fiber is non-starch polysaccharides, i.e., cellulose, lignins, chitins, pectins, beta-glucans, resistant starch, resistant dextrins, inulin, and oligosaccharides that cannot be digested by human small intestine [1]. A large number of researches have been conducted to gather as much information about its resources, molecular and physical characteristics, bioavailability after entering human body as well as its biological effects and functions [2, 3]. This great interest in dietary fiber has shown that dietary fiber becomes one of the most essential and promising food resources in this era.

Dietary fiber is divided into two types that are soluble fiber and insoluble fiber. Soluble fiber viscously dissolves in water and postpones gastric emptying which results in an extended feeling of fullness. It is usually called as prebiotic fibers because it is easily fermented by colon microflora produces gases, bioactive by-products, such as short-chain fatty acids, vitamin, etc. and confers health benefits [4]. In other hand, insoluble fiber is an undissolved molecule which inert to upper gastrointestinal digestive enzymes. This fiber provides bulking by absorbs water as they move through the digestive system and easing defecation [1, 4].

Currently, research on the exploration of agricultural biomass as a fiber resource has gained rapidly. One of the agricultural biomass is pitaya (*Hylocereus polyrhiuzus* [F.A.C. Weber] Britton &

Rose) stem that can be utilized as new fiber resources. Generally, pitaya enters harvesting season when they are approximately 32 cm to 33 cm wide and 13 cm to 15 cm m long. They can reach a weight of 300 g to 600 g each [5]. After the fruits are harvested, the stems of the fruits are usually thrown away. Each hectare of pitaya farm land can produce around 60 t of wasted stems. This huge amount of waste will be a problem. People use those wasted stems as animal feed by directly chopping those stems after their thorns are removed. However, the stems reported having high ascorbic acid content more than the fruit flesh [6]. The previous research on flour made out of pitaya stem shows that this flour contains almost 50 % dietary fiber but has very low solubility. This low solubility is probably correlated with the existence of insoluble fiber in the flour. To deal with this issue, fermentation of pitaya stem is carried out aiming to change the structure of insoluble fiber into the soluble fiber.

There are some microorganisms that are capable of fermenting fiber such as *Aspergillus niger* and *T. reesei* [7, 8]. *Aspergillus niger* is a fungi conventionally used to hydrolyze fiber. This microorganism produces high β-glucosidases but relatively low cellobiohydrolases and endoglucanase [9]. While on the contrary, *T. reesei* produces cellobiohydrolases and endogluconase that reach 80 % but lower β-glucosidases. This causes the main hydrolysis product by *T. reesei* are cellodextrin and cellobiose, where both are more soluble in water, and a small amount of glucose [10, 11]. In this study, *T. reesei* was selected to ferment pitaya stem flour because it can generate high soluble fiber but produce a low level of glucose, which is important to chemical and functional characteristic of the flour. High level of glucose will cause browning and change the taste of the flour.

In the fermentation process using *T. reesei,* incubation time is the main factor. The precise incubation time will produce cellodextrin and cellobiose or the other short oligomer. However, if the fermentation process lasts longer, the fiber will be degraded into simple sugar. Furthermore, inoculum concentration is also a determining factor in the efficiency of the fermentation process. *T. reesei* can produce cellulase by solid-state fermentation with the highest activity at a temperature of 30 °C incubation [12]. In addition, cellulase production using 1 % (w/v) of *T. reseei* on kinnow pulp shows the highest activity in 96 h of incubation [13]. While cellulose production from rice straw using 2 % (w/v) of *T. reseei* results in the highest of glucose on 120 h incubation [14]. This research was conducted to observe the effect of inoculum concentration of *T. reesei* and incubation time during fermentation of pitaya stem to a physical and chemical characteristic of the resulting pitaya stem flour.

2. Materials and methods

2.1. Materials

Pitaya (*Hylocereus polyrhiuzus* [F.A.C. Weber] Britton & Rose) stems were obtained from Malang, East Java, Indonesia and *T. reesei* were obtained from Faculty of Biotechnology, University of Surabaya, Indonesia.

2.2. Making fermented starters

As much as one ose of the initial solid inoculum *T. reesei* was put into PDA medium (Merck) and incubated for 96 h to 120 h until green spores appeared. Then into this solid medium, physiological natrium chloride solution was added until the surface of the media was completely submerged and the green spores released from the media. The spores suspension then added by physiological natrium chloride solution. The spore density was measured by spectrophotometry at a wavelength of 600 nm and maintained at OD ~0.5 when it used as inoculum.

2.3. Preparation of pitaya stem and fermentation procedure

Mature pitaya stem is removed from its sharp spines and then washed with running water. The stem then sliced in a rectangular shape (2 × 6) cm^{-2}. The sliced pitaya stem was then added by 3 % (w/v) and 5 % (w/v) inoculum starter of *T. reesei* and incubated for 24 h, 36 h, and 60 h at 35 °C with initial pH maintained at 5.5. The fermented stems are then inactivated by heating at a temperature of 75 °C for 20 min. These inactivated fermented stems were then tempered in an oven at 60 °C until the

moisture content reaches 10 % (w/w). The dried stem then grinded into flour and sieved using 70 mesh sieve.

2.4. Analysis of pitaya stems flour

2.4.1. Protein analysis (Kjeldahl). As much as 1 g of the sample is placed inside a Kjeldahl flask. The addition of 15 g of K_2SO_4, 1 mg of $CuSO_4$ catalyst solution, 1 g of catalyst selen, boiling stone and 25 mg of concentrated H_2SO_4 are then given. Hereafter, the mixture is heated until it is boiled and experiences color alteration into a clear green. The mixture is then cooled off and diluted using as much distilled water as needed. Afterwards, an addition of 75 mg of 30 % NaOH solution is given before it is distilled for 5 min to 10 min or rather until the solution reaches 150 mL, with 50 mL of 4 % H_3BO_3 solution posing as the container. The solution is then titrated using 0.1 M HCl. The difference in the total value of the titrated sample and the blank poses as the total value of nitrogen. The protein's content is obtained through the process of multiplying N % with 6.25 convection factor [15].

2.4.2. Ash content analysis. As much as 3 g to 5 g of powder is charcoaled on a Bunsen burner by using a cup that has been previously spawned. Afterwards, the powder is passed through the ashing process at 550 °C until it turns white or grey within 5 h to 8 h. It is then cooled off inside an exicator for 30 min and weighed, before being put inside the oven with the same temperature once again for an hour. The cooling off process is done for another hour inside the exicator before it is weighed for the second time. The process is repeated continuously until a constant weight is obtained [16].

2.4.3. Fat content analysis. The flour sample is extracted using petroleum ether as a solvent and Soxhlet for 4 h. The existence of residue on the bottom part of the round-bottom flask after the solvent has been evaporated indicates the fat that the sample contained. The residue is repeatedly weighed until a constant heft is successfully obtained [17].

2.4.4. Vitamin C analysis. The sample is weighed as much as 100 mg before addition of 75 mL of aquades is given, then stirred for 2 min to 3 min. Amylum indicator for 5 mL is then added into the solution and titrated using 0.1 N of Iodine solvent that has been standardized with $Na_2S_2O_3$, which has also gone through standardization itself using the primary standard solution of 0.1 N KIO_3. Afterwards, titration is ceased when the solution has experienced color alteration into a dark blue that lasts for estimation of 1 min [18].

2.4.5. Starch analysis. As much as 5 g of the sample is added into 50 mL of distilled water inside a 250 mL beaker glass and is stirred for 1 h. The suspension is then filtered and rinsed using distilled water until the filtrate volume reaches 250 mL before that filtrate is then disposed of. Afterwards, the residue (a sample that contains fat) is washed five times by using filter paper with 10 mL ether until it evaporates. It is then rinsed, with the intention of releasing the dissolved carbohydrate, by applying 150 mL of 10 % alcohol. Hereafter, the residue is washed with 200 mL of distilled water and 20 mL of 25 % HCl inside an Erlenmeyer that is then covered using a condenser and is heated using a water bath until it boils, estimatedly for 2.5 h. The solution is cooled off afterwards, followed by neutralization using 45 % NaOH and dilution until its volume reaches 500 mL. Filtration is then conducted, and the glucose heft is calculated. Lastly, the glucose heft is multiplied by 0.9, the result posing as the starch heft [19].

2.4.6. Dietary fiber analysis. With petroleum ether posing as the solvent, an extraction of fat is conducted towards the dry sample for 15 min in room temperature. As much as 1 g of the fat-free sample is put into an Erlenmeyer, before additions of 25 mL of 0.1 M Natrium phosphate and pH 6 phosphate buffer are added, and the suspension is made. After 0.1 mL termamyl has been added, the Erlenmeyer is covered with aluminum foil and is put through an incubation process in 100 °C for 15 min. It is then taken away and cooled off. Hereafter, 20 mL of distilled water is added, and the pH value is adjusted into 1.5 by pouring in 4 M HCl. Then, 100 mg of pepsin is also added before the

Erlenmeyer is closed and put through the incubation and agitation process once again at 40 °C for 60 min. Afterwards, 20 Ml of distilled water is added, and the pH value is adjusted to 6.8. An addition of 100 mg pancreatin is then given, the Erlenmeyer is covered once again, and the same incubation and agitation process are repeated with the same temperature and duration. The pH is adjusted by using HCl until it becomes 4.5, then filtered using dried crucible with two porosities that contain dried celite and has been weighed. Lastly, it is washed twice by using distillate water afterwards [20].

2.4.7. Insoluble Fiber (IDF). The sample is first washed with 10 mL acetone twice and dried under 105 °C temperature until the heft becomes constant (approximately 12 h). The sample is then weighed after going through the cooling off process inside a desiccator. Afterwards, the sample is put through the ashing process inside a 500 °C furnace for a minimum duration of 5 h long and is then weighed after being cooled off inside a desiccator once again.

2.4.8. Soluble Fiber (SDF). The filtrate volume is managed by using distilled water until it reaches 100 mL before addition of 4 mL of 95 % warm ethanol and the sample is precipitated for 1 h. Afterwards, the sample is filtered by using dry crucibles with two porosities that contain 0.5 g dry celite and is washed with 10 mL of 78 % ethanol and 10 mL of acetone twice. The sample is then dried under the temperature of 105 °C until the weight becomes constant, before going through the cooling process using desiccator and weighed. Hereafter, the sample is put through the ashing process inside a 500 °C furnace for a minimum duration of 5 h. Then, the same cooling and weighing process is repeated.

2.4.9. Total Dietary Fiber/TDF. The total of dietary fiber can be determined by summing up the SDF value with IDF. The blank value for IDF and SDF can be obtained through the same method but without the use of the samples.

2.4.10. Total sugar analysis. Each sample solution is added with 1 mL of 5 % phenol and homogenized. Afterwards, the addition of 5 mL H_2SO_4 is given immediately by inserting it perpendicularly to the surface of the solution, then left still for 10 min before homogenization is conducted. Afterwards, the samples are put above the water bath for 15 min. The process is then continued by an absorbance measurement on 490 nm of wavelength. Sugar content was calculated using glucose as a standard [21].

2.4.11. Moisture content analysis. An empty and dry cup is weighed. As much as 5 g of the sample is put inside the cup, and its total weight is measured. The cup and the sample are put into an oven, and the temperature is set to 110 °C. The drying process is conducted until a constant, final weight is obtained. The water content is stated in percent (%), where the difference between the addition of the initial and final heft total and the final heft total divided by the final heft of the sample (that has been dried) is calculated [22].

2.4.12. Water solubility. The water solubility index and swelling power analysis are conducted by weighing 0.1 g (W1) of the sample and dissolving in 10 mL of distilled water inside a 15 mL centrifuge tube that has been weighed prior. The sample is stirred using a vortex for 15 sec before it is put inside the water bath for 30 min on 85 °C. The process is followed by continued stirring for 10 sec after 5 min, 15 min, and 25 min. The cooling process on room temperature is then conducted, followed by 2 000 rpm centrifugation for 30 min (1 rpm = 1/60 Hz). The supernatant is then taken, and its residue is weighed. Afterwards, the supernatant is put inside a petri dish that has been weighed prior. The drying process of the petri dish uses an oven at 105 °C temperature. The process is ceased when a constant weight is finally obtained; then the petri dish is weighed (W2). The water solubility is stated in percent (%), where the difference between initial weight (W1) and final weight (W2) [23].

2.5. Statistical Analysis

Data analysis has carried out using SPSS 20 software (IBM Corporation, New York, United States). The experiment was done with a factorial design and repeated three times. The resulting data were tested first with the Normality Test and Homogeneity test and followed by a two-way ANOVA with multiple comparisons using the Duncan test.

3. Result and discussion

Fermentation is a stage where the complex structure of polysaccharide can be degraded into a much simpler structure through the action of enzymes produced by the microorganism. In this research, pitaya stem is fermented for 24 h, 36 h, and 60 h using the variation of 3 % (w/v) and 5 % (w/v) inoculum.

Figure 1. Fermented pitaya stem. (a) Pre-Fermentation, (b) 3 % (w/v) Inoculum. 24 h, (c) 3 % (w/v) Inoculum. 36 h, (d) 3 % (w/v) Inoculum. 60 h, (e) 5 % (w/v) Inoculum. 24 h, (f) 5 % (w/v) Inoculum. 36 h, (g) 5 % (w/v) Inoculum. 60 h.

In figure 1, it is shown that when the fermentation time increased, the colour of the stem turned brown. This is also followed by a decrease in pH fermentation (Figure 2). This is most likely to have been caused by the ability of *T. reesei* degrade a complex cellulose fiber into a simpler structure and enters the TCA cycle. The side products of the TCA cycle are citric acid, malic acid, and also other acids. These acids create a decrease in the pH value. In an acidic condition, this substitute reaction will occur between two hydrogen atoms with magnesium that is found within the chlorophyll, thus producing a brownish green fefofitin-a [24]. Chlorophyll is known as a natural dye because chlorophyll is very widely used in the food industry to give the appearance of food color to make it more attractive and appetizing [25]. Besides, that chlorophyll can also act as an anticancer agent [26]. Therefore, the degradation of chlorophyll due to a decrease in pH during the fermentation is unexpected. After the fermentation process, the pitaya stem is dried, grinded and sieved to produce pitaya stem flour as shown in figure 3.

From table 1, it can be seen that there is an increasement in the brightness (L) level of the flour. Furthermore, the longer fermentation time, the red colour level has increased for each inoculum, this can be seen from the larger Δa. The level of yellow colour (Δb) in the 3 % (w/v) inoculum is higher than the 5 % (w/v) inoculum. Overall, there are differences in colour changes in each fermentation, with a positive ΔE value. The longer fermentation duration, the increase in the redness level escalates even more in every inoculum, as seen from the higher value of Δa. A greater increase was also found in the 3 % (w/v) inoculum rather than in the 5 % (w/v) inoculum for the yellowness level. There are differences in the colour alteration in each fermentation, followed with a positive ΔE value. This red and yellow colour escalation creates more brownish flour due to the mixture of its red and green

colour. While this yellow colour escalation creates a brighter brown colour. In consequence, the increased fermentation time changes the pitaya stem flour to be brownish green and slightly brown.

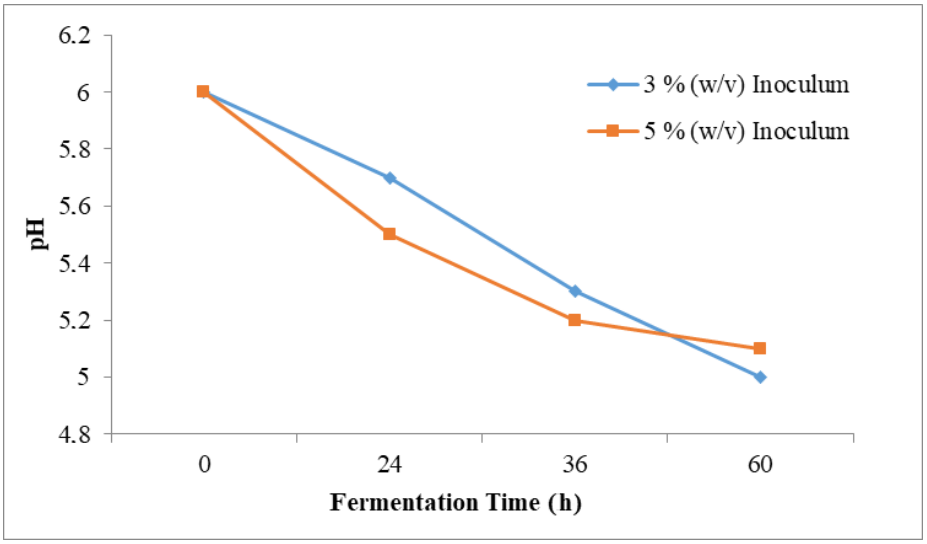

Figure 2. The pH profile during pitaya stem fermentation by *T. reesei*.

Figure 3. Flour of fermented pitaya's stem. (a) Pre-Fermentation, (b) 3 % (w/v) Inoculum. 24 h, (c) 3 % (w/v) Inoculum. 36 h, (d) 3% (w/v) Inoculum. 60 h, (e) 5 % (w/v) Inoculum. 24 h, (f) 5 % (w/v) Inoculum. 36 h, (g) 5 % (w/v) Inoculum. 60 h.

Yield is the comparison between the final flour product (dry weight) and the main raw material (wet weight). Inoculum and time turn out to be factors that give significantly different yield result, as well as the interaction between both. The results of the yield analysis show that the longer the fermentation time, the yield will decrease (table 2). The smallest amount of yield was obtained in 3 % (w/v) of inoculum fermentation for 60 h. This yield decrease is caused by the existence of fiber that hydrolyzed into sugar. Thus it is able to dissolve in water and escape during the process. *T. reesei* possesses a β-glucosidase enzyme that has the ability to alter cellobiose into glucose [27, 28].

The ash content also decreases following the increase of fermentation time and inoculum concentration. The decrease in the ash content is because being utilized by *T. reesei* its growth. Moisture content also decreases along with the increase of fermentation time. The decomposition of cellulose into simpler molecules (such as glucose or cellobiose) causes the lower binding capacity of water [29]. Furthermore, the water activity (Aw) value of the fermented flour that sits within the range of 0.42 to 0.57, decreases around 0.05 to 0.2 compared to the pre-fermented one. This low Aw implies that this fermented flour is relatively safe from microbiological spoilage. The minimum Aw value needed for microorganism to grow i.e. yeast > 0.75, mold > 0.7 and bacteria > 0.8 [30].

Table 1. Colour Index of fermented pitaya stem flour.

Inoculum% (w/v)	Time (h)	Colour Index						
		L	a	B	ΔL	Δa	Δb	ΔE
3	24	66.91^{c}	3.3^{e}	29.21^{d}	3.81^{c}	0.8^{e}	0.71^{e}	3.96
3	36	67.22^{b}	4.2^{d}	29.55^{c}	4.12^{b}	1.5^{d}	1.05^{d}	4.51
3	60	67.2^{b}	8.7^{a}	31.63^{a}	4.1^{b}	6.2^{a}	3.13^{a}	8.06
5	24	67.88^{a}	4.5^{d}	29.6^{c}	4.78^{a}	2.1^{c}	1.1^{c}	5.34
5	36	66.22^{d}	5^{c}	30.22^{b}	3.12^{d}	2.5^{c}	1.72^{b}	4.35
5	60	65.78^{e}	6.5^{b}	30.2^{b}	2.68^{e}	4^{b}	1.7^{b}	5.16
Pre-Fermentation		63.1^{f}	2.5^{f}	28.5^{e}	-	-	-	-

L: brightness index, a: the green/red coordinate, b: the yellow/green coordinate, ΔL: the darkness and brightness difference (A positive value of ΔL indicates a brighter index increase, while a negative value indicates a darker index), Δa: the red and green difference (A positive value of Δa indicates an increase in the red coordinates, while a negative value indicates an increase in the green coordinates), Δb: the yellow and blue difference (A positive value of Δb indicates an increase in the yellow coordinates, while a negative value indicates an increase in the blue coordinates), ΔE: complete colour alteration. Different letters in the same column indicate a significant difference based on the Duncan test (*P* value < 0.05).

Table 2. Yield, ash content, water content, water activity of fermented pitaya's stem flour.

Inoculum% (w/v)	Time (h)	Yield% (w/w)	Ash Content% (w/w)	Moisture Content% (w/w)	Water Activity (Aw)
3	24	$11.07^{b} \pm 0.0794$	$10.84^{b} \pm 0.0153$	$2.71^{b} \pm 0.0208$	$0.57^{b} \pm 0.0218$
3	36	$10.52^{d} \pm 0.0306$	$8.85^{d} \pm 0.0300$	$1.83^{f} \pm 0.0289$	$0.51^{c} \pm 0.0198$
3	60	$10.03^{e} \pm 0.0252$	$7.22^{g} \pm 0.0208$	$1.23^{g} \pm 0.0300$	$0.42^{e} \pm 0.0508$
5	24	$11.08^{b} \pm 0.0306$	$10.14^{c} \pm 0.0361$	$2.41^{c} \pm 0.0321$	$0.51^{c} \pm 0.0318$
5	36	$10.91^{b} \pm 0.0300$	$8.32^{e} \pm 0.0252$	$2.31^{d} \pm 0.0115$	$0.48^{d} \pm 0.0350$
5	60	$10.82^{c} \pm 0.0208$	$8.22^{f} \pm 0.0153$	$2.22^{e} \pm 0.0252$	$0.47^{d} \pm 0.0281$
Pre-fermentation		$11.50^{a} \pm 0.0107$	$13.21^{a} \pm 0.0120$	$4.22^{a} \pm 0.0102$	$0.61^{a} \pm 0.0108$

Different letters in the same column indicate a significant difference based on the Duncan test (*P* value < 0.05)

Table 3. Vitamin C, Protein, Starch and Fat of fermented pitaya stem flour.

Inoculum% (w/v)	Time (h)	Vitamin C% (w/w)	Protein% (w/w)	Starch% (w/w)	Fat% (w/w)
3	24	$3.57^{a} \pm 0.0351$	$10.99^{b} \pm 0.0153$	$3.41^{a} \pm 0.1026$	$3.15^{a} \pm 0.0551$
3	36	$3.54^{a} \pm 0.0503$	$10.60^{c} \pm 0.0173$	$3.41^{a} \pm 0.0917$	$3.15^{a} \pm 0.0416$
3	60	$3.53^{a} \pm 0.0520$	$10.10^{e} \pm 0.0153$	$3.42^{a} \pm 0.1249$	$3.15^{a} \pm 0.0611$
5	24	$3.53^{a} \pm 0.0751$	$10.97^{b} \pm 0.0200$	$3.42^{a} \pm 0.1050$	$3.15^{a} \pm 0.0651$
5	36	$3.54^{a} \pm 0.0231$	$10.51^{d} \pm 0.0100$	$3.41^{a} \pm 0.0917$	$3.15^{a} \pm 0.0503$
5	60	$3.54^{a} \pm 0.0361$	$9.87^{d} \pm 0.0153$	$3.42^{a} \pm 0.1249$	$3.16^{a} \pm 0.0361$
Pre-fermentation		$3.53^{a} \pm 0.0184$	$11.52^{a} \pm 0.0107$	$3.42^{a} \pm 0.0102$	3.15^{a}

Different letters in the same column indicate a significant difference based on the Duncan test (*P* value < 0.05)

As seen in table 3, inoculum concentration, fermentation duration, and the interaction between these two factors give a significant difference towards the protein level. The protein level decreases along with the increase in the fermentation duration and the amount of inoculum given. The lowest protein level (N-total) was found in 5 % (w/v) inoculum for 60 h. During the fermentation process, the protein degrades into amino acids and peptides by *T. reesei* [30]. The amino acids will be metabolized

to volatile compounds and ammonia which will be released in high amounts during the fermentation process thus allowing a decrease in N-Total levels [31].

Vitamin C, fat and starch level do not show any change during the fermentation. Inoculum concentration, fermentation duration, and the interaction between these two factors do not give any significant difference towards the vitamin C, fat and starch level. *T. reesei* does not possess the ability to metabolize vitamin C and fat found in the pitaya stem. The vitamin C content in this flour is higher than the reported highest ascorbic acid fruit, acerola cherries, which contains 2.75 % [32], proving that fermented pitaya stem flour can possibly be a new source of food products that are highly rich in vitamin C.

Table 4. Solubility, Total Sugar, Soluble & Insoluble Fiber of fermented pitaya stem flour

Inoculum% (w/v)	Time (h)	Solubility% (w/w)	Total Sugar% (w/w)	Soluble Fiber% (w/w)	Insoluble Fiber% (w/w)	Total Fiber% (w/w)
3	24	$54.75^{f} \pm 0.0321$	$7.23^{f} \pm 0.0208$	$22.94^{f} \pm 0.0404$	$30.82^{b} \pm 0.0321$	$53.77^{a} \pm 0.0513$
3	36	$69.60^{d} \pm 0.0153$	$8.31^{d} \pm 0.0200$	$27.64^{d} \pm 0.0721$	$26.13^{d} \pm 0.0577$	$53.77^{a} \pm 0.0231$
3	60	$73.05^{a} \pm 0.0503$	$10.46^{a} \pm 0.0173$	$36.25^{a} \pm 0.0451$	$17.53^{g} \pm 0.0289$	$53.79^{a} \pm 0.0231$
5	24	$56.73^{e} \pm 0.0252$	$7.92^{e} \pm 0.0208$	$24.01^{e} \pm 0.0231$	$29.78^{c} \pm 0.0681$	$53.78^{a} \pm 0.0651$
5	36	$69.83^{c} \pm 0.0252$	$8.42^{c} \pm 0.0306$	$30.65^{c} \pm 0.0603$	$23.15^{e} \pm 0.0709$	$53.81^{a} \pm 0.0115$
5	60	$69.99^{b} \pm 0.0208$	$8.50^{b} \pm 0.0153$	$31.27^{b} \pm 0.0321$	$22.54^{f} \pm 0.0473$	$53.80^{a} \pm 0.0153$
Pre-fermentation		$35.52^{g} \pm 0.0150$	$5.05^{g} \pm 0.0051$	$20.21^{g} \pm 0.0088$	$33.58^{a} \pm 0.0069$	$53.79^{a} \pm 0.0135$

Different letters in the same column indicate a significant difference based on the Duncan test (P value < 0.05)

From table 4 can be seen inoculum concentration, fermentation duration, and the interaction between these two factors give a significant difference towards the solubility level, total sugar, soluble fiber as well as insoluble fiber. Along with the increasing of fermentation duration, soluble fiber content and total sugar content are also increases. Increasing of total sugar and soluble fiber will affect the increasing of flour solubility. In the other hand, the decreasing of the insoluble fiber occurs as a consequence of fiber degradation during fermentation.

4. Conclusion

The research concluded that the best condition of pitaya stem fermentation using *T. reesei* is 3 % (w/v) inoculum for 60 h. This can be seen from the resulting flour that insoluble fiber content has decreased and followed increasing of soluble fiber which affects to increase of the flour solubility. However, the colour of the flour becomes lighter and brownish due to chlorophyll degradation. Further research to maintain the green color of the fermented pitaya stem flour is suggested to be done. This fermented pitaya stem flour can be a new source of a functional food with high soluble fiber.

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