

Conversion of food waste via two-stage fermentation to controllable chicken Feed Nutrients by local isolated microorganism

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Abstract

Purpose Food waste can be referred to as edible food materials that can create problems to the environment if it is not disposed properly. Therefore, the purpose of this study is to produce chicken feed from food waste via two-stage fermentation.

Methods Food waste was converted with combination of fungi A (isolated from degraded onion) and yeast B (isolated from local fermented fruit - Durian). Four batches of food waste were obtained from different sources. Food waste was fermented with fungi A and yeast B in bioreactor for 5 days with 80% moisture content. Fermented food waste was then dried in the oven.

Results Glucose and carbohydrate contents were investigated during fermentation process and it was found that carbohydrate content decreased from 0.1857 g/g before fermentation to 0.1305 g/g after fermentation. Glucose content was found to increase at the first 48 hours and then dropped from 48th hour until the fermentation was done. To get consistent with the results of the last product, the process control elements such as C:N ratio, pH, aeration, agitation, temperature, antiseptic technique and sterilization of food waste were controlled. The standard of the fermented food waste was compared with the standard of chicken feed used in Malaysia. The crude protein content, crude fat content, crude fiber content, total ash content and total energy content of chicken feed met the requirement set by Malaysia standard (MS 20:2008) of chicken feed.

Conclusion Thus, it is shown that food waste has a massive potential in the production of chicken feed.

Keywords Food waste, Fermentation, Probiotic, Chicken feed

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Abbreviation

ml	Milliliter
L	Liter
mg	Milligram
g	Gram
kg	Kilogram
kcal	Kilocalorie
SCP	Single Cell Protein
COD	Chemical Oxygen Demand
DNS	3, 5-dinitrosalicylic Acid
mm	Millimetre
PDA	Potato Dextrose Agar
NA	Nutrient Agar
rpm	Revolutions Per Minute
SSF	Solid State Fermentation
TKN	Total Kjeldahl Nitrogen
w/v	Weight Per Volume
v/v	Volume Per Volume
g/g	Gram/Gram
kcal/g	Kilocalorie/Gram

Introduction

Food waste can be referred to as edible food materials that are produced and made available to be consumed by human but left uneaten. Food waste can be any waste discharged from different sources such as kitchens, restaurants and cafeterias (Chen et al. 2017). In many parts of the world, this food waste will not be treated and discarded without being used properly for specific purposes. Food waste can be seen everywhere, as it is produced and left unused at every stage of the food system (Xue et al. 2017). In this era where food security is a major problem around the world, food waste can be considered as a component of the world's food system challenges. Each year, there is an approximate of 1.3 billion tonnes of food for humans which is lost and wasted globally (Gustavsson et al. 2011). This amount can feed more than a billion people worldwide. Food waste was known to create a lot of problems for many years. This is due to the rapid increase in the amount of food waste from drastic economic development together with urbanization and rising commercialization in the world (Abd Razak 2017). The world is now facing huge challenges in handling and treating food waste. Improper handling of food waste can lead to many environmental issues, for example, improper separation of municipal solid waste leads to the production of greenhouse gases in landfills (Lim et al. 2016). From an environmental viewpoint, there is a need for an appropriate management of waste especially food waste (Ma et al. 2009). Food waste is mainly composed of carbohydrate polymers such as starch, proteins, lipids, cellulose, and other inorganic parts. It can be very useful in some of the fermentation processes that produce products such as biofuel. However, it can also become remaining substrate that is considered as waste which requires well management and treatment. Compared to agro-industrial raw materials, food waste can be a better raw material for microbial fermentation without nutrients supplement. From this viewpoint, food waste has been used as the sole microbial feedstock for the development of various types of value-added bioproducts such as single-cell protein (SCP), enzymes, feed additives, biofuels, animal feeds as well as other useful chemicals or products (Buschke et al. 2013; Han and Shin 2004). Therefore, through the fermentation of food waste, it is possible to produce value added products such as chicken feed.

Recovering food waste for animal feeding (ReFeed) is an useful option that can be practiced and has the potential to solve the issues and problems faced in waste management such as food waste, landfilling, food security as well as other environmental challenges (Zheng et al. 2018). In the past, many countries practiced to use food waste and residuals from food production to feed animals (Westendorf 2000). Nevertheless, this old-time practice is no longer being implemented by most of the people, as the emergence of intensive animal feeding operations has already taken over the ancient practice. Another reason is the infection of diseases due to untreated food waste being feed directly to the animals. Nowadays, soybeans and maize are preferable as it is easier to control their nutrients (Banhazi et al. 2012). Today, in order to mitigate the environmental damages stemmed from food waste, the society attempts to sustainably feed the growing human population without producing a lot of waste. The society also creates a way to solve the food waste problem and hence, there is a renewed interest in reviving the practice (Stuart 2009). For instance, producing animal feed from food waste is a solution to reduce related issues effectively (ReFED 2016). Among all the approaches used in the bioconversion of food wastes into value added products, solid state fermentation (SSF) has been studied widely. There are many researches describing different types of bioreactor designs and microorganisms used for the production of various value added products like SCP and feeds (Couto and Sanroman 2006).

Poultry products are sources of protein that can be considered as affordable and cheap. This is because the poultry meat or white meat is more affordable as compared to cow meat or red meat (Wahyono and Utami 2017). The poultry industry plays an important role in Malaysia and also its economy as it provides cheap source of protein to the country. In 2012, the poultry industry accounted for 57.5% in the livestock sector (DVS 2012). In Malaysia, broiler meat is the primary protein source for majority of population. Statistics have shown that consumption of chicken meat per capita was about 37.7 kg. (Ministry of Agriculture 2005). In 2019, the poultry consumption per capita in Malaysia is expected to amount to about 46.9 kilograms per person annually (Hirschmann 2019). In the poultry industry, high cost of conventional feeds and drugs can account for up to 85% of the total production (Umeh and Udo 2002). Hence, any attempt to reduce the cost of the feed may lead to a significant reduction in the total cost of

production. Thus, further study on producing animal feed from fermented food waste is needed to reduce the production cost of chicken. Additionally, it can also reduce the problems associated to food waste nowadays.

The aim of this research is to produce consistent nutrient of chicken feed which complies with MS 20:2008. Based on results via two-stage fermentation, food waste has been successfully converted to chicken feed with controllable nutrients.

Material and methods

Fig. 1 shows the process flow to convert food waste into chicken feed. The size of working volume of

bioreactor was 50kg. The bioreactor and food waste were sterilized at temperature of 121°C. Then, all transferring inoculum and sampling were operated via the antiseptic technique. For fermentation process, Fungi A and Yeast B were used with temperature of 37°C, 50L/min aeration and 2 rpm agitation. The process of fermentation started from day 1 to day 3 with 50 kg food waste and 1000 ml (0.02%, w/v) of inoculum Fungi A. Then, the fermentation was continued using yeast B from day 3 to day 5 with 1000 ml (0.01%, w/v) of inoculum. Finally, fermented food was dried at 70°C temperature to protect protein and microorganism inside the food waste.

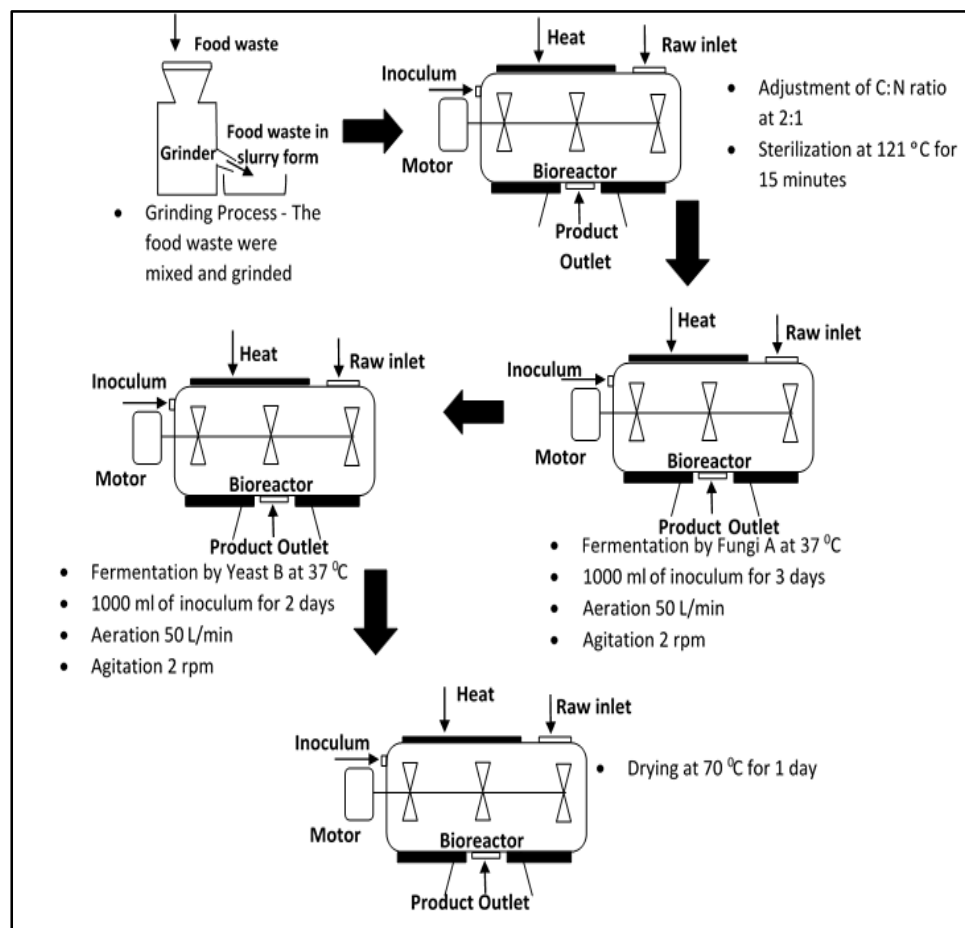


Fig. 1 The process flow to convert food waste into chicken feed via two-stage fermentation

Type and Source of Food Waste

Four batches of food waste were fermented individually in this study weighing 50 kg for each batch. The first batch of food waste was obtained from resident area while the second batch of food waste was from hospital. Third batch of food waste

was obtained from hotel and restaurant, followed by the fourth batch of food waste which was acquired from the wet market consisting of only vegetables. Table 1 shows the composition of raw food waste for all batches.

Table 1 Composition of raw food waste

Types of food	Average Percentage (%)			
	Batch 1	Batch 2	Batch 3	Batch 4
Rice, noodles	29	34	36	0
Eggs shell	2	4	7	0
Meats, bones	25	28	36	0
Vegetables, fruit peels	44	34	21	100

The characteristics of all batches of the waste before fermentation process are shown in Figure 4 to 8, such as crude protein, crude fat, crude fibre total ash and total energy. All those parameters' values were labelled before fermentation.

Preparation of Food Waste

The mixed food waste was grinded into slurry form. Then, the grinded food waste was then transferred into the solid state bioreactor to be fermented. The carbon-to-nitrogen (C:N) ratio of the food waste was determined before the fermentation process in order to standardize the C:N (2:1) ratio of every batch of food waste. The carbon (C) ratio was determined using similar method to determine the carbohydrate content. Nitrogen (N) ratio was determined using Nessler Method, Method 8038 (Hach Method), Nitrogen, Ammonia. The sample which is lack of carbon will be added with glucose powder and sample lack of nitrogen will be added with amino acid powder. For all batches, it only used between 0.1g-1g of glucose powder and 0-0.1g amino acid powder per 50 kg of sample.

Fermentation Process

The food waste was first inoculated with 1000 ml of Fungi A (0.02%, w/v) containing 2.5×10^6 spores/ml for 48 hours fermentation. Then, it was inoculated with 1000 ml (1% glucose) of Yeast B (0.01%, w/v) for another 72 hours (Chintagunta et al. 2016). The fermentation process was carried out at 37°C for a total of 120 hours in the 70 kg working capacity of solid state bioreactor (FERMSOSTAT). Periodical aeration of 50 L/min for 15 minutes per hour was implemented and agitation of 2 rpm was set to the bioreactor. The pH values of all food waste batches were between pH 4.0 to 6.0, therefore no adjustment was needed as optimum pH ranges were employed for fermentation. Sample was taken at an interval of 24 hours for 120 hours.

Chicken Feed Production

The fermented food waste was dried in the oven at 70°C for 24 hours until the food waste was completely dried and the moisture content was below 13%. The fermented food waste or chicken feed was grinded into smaller size of about 2 mm. The size was checked and confirmed using a sieve test (Model: Sieve Shaker Minor M200, Endecotts, UK). The grinded chicken feed was then transferred into a plastic bag with silica gel to prevent excessive moisture. Then, the chicken feed was stored at a cool and dry place.

Investigation of viability of Fungi A and Yeast B in chicken feed

The viability of Fungi A and Yeast B in chicken feed were investigated via plating method. Two different agars were prepared for the respective viability testing of Fungi A and Yeast in the chicken feed. Potato Dextrose Agar (PDA) was prepared for the viability testing of Fungi A, while nutrient agar (NA) was prepared for the viability testing of Yeast A in chicken feed. Viability of chicken feed was investigated at 3rd, 7th, 21st and 50th day after the fermentation when dried fermented food waste was produced.

Analysis

Determination of pH

The Method 9045D (EPA Method 2003) 16 hours was used to determine the pH of food waste before, during and after the fermentation. Samples weighing 20 g were taken and 100 mL deionized water was added. The suspension was continuously stirred for 5 minutes and then left for about 15 minutes. The suspension was centrifuged and the supernatant was used for pH measurement using pH meter (Model: pH 510, Eutech Instruments, Singapore).

Determination of Glucose Content

Determination of glucose content was carried out using 3, 5-dinitrosalicylic acid (DNS) method. DNS method was used to determine the glucose content of food waste although this method was mainly used for the determination of reducing sugar because the content of other reducing sugars is very low in food waste as reported by Thongdumyu et al. (2014). The DNS reagent was prepared by mixing 10 g of 3, 5-dinitrosalicylic acid, 16 g sodium hydroxide and 300 g sodium potassium tartrate in distilled water to achieve final volume of one liter. For 1.5 mL of sample, 2 mL of DNS reagent was added followed by boiling at 100°C for 5 minutes. After the mixture was cooled down, the absorbance was recorded at 540 nm. The glucose content was determined using glucose as the standard (Miller 1959) (Appendix 1).

Determination of Carbohydrate Content

The carbohydrate contents of dried food waste and fermented food waste were determined using Anthrone method (Sadasivam and Manickam 1996). An approximate amount of 0.1 g of the sample was hydrolyzed with 5 mL of 2.5 N hydrochloric acid in boiling water bath for three hours and cooled to room temperature. The sample was neutralized with sodium carbonate until the effervescence ceased and the sample was made up to 100 mL. Then, the suspended particles were removed by centrifugation. Next, 4 mL of Anthrone reagent was added to 1 mL of the supernatant and heated for 8 minutes in the boiling water bath. Then, it was rapidly cooled and the absorbance was recorded at 630 nm. Glucose solution was used as standard for the quantification. From the standard curve, the amount of carbohydrate present in the sample was calculated as depicted in Appendix 2.

Chemical Oxygen Demand (COD)

The COD analysis was carried out in accordance with EPA Method 8000 from Hach Company. Two milliliters of well blended and homogenized sample were added into Hach COD Digestion Reagent Vial. Blank sample was prepared using distilled water. The vial was inverted gently several times to mix and be heated for two hours using a reactor (Model: DRB200 reactor, Hach, USA). The vial was cooled, then the reading for COD was recorded (Model: DR 2800, Hach, USA), 435 COD HR.

Determination of Moisture Content

The moisture content of the food waste was determined using a moisture analyzer (Model: MX50, AND Weighing, USA). The moisture analyzer was pre-set with temperature of 200°C. An approximate amount of 0.5 g of food waste sample was weighed on the moisture analyzer. Then, the moisture content of the food waste was determined by starting the heating process of the moisture analyzer.

Total Kjeldahl Nitrogen (TKN) and Crude Protein Content

The total crude protein content in food waste was determined using Micro-Kjeldahl method (AOAC Method 2012). The percentage of crude protein was calculated by multiplying the percentage of nitrogen with conversion factor of 6.25. The formulae used to determine TKN and crude protein content are represented by Eq. (1) and Eq. (2), respectively.

$$\text{Percentage of nitrogen} = \frac{(\text{Volume of HCL used for sample}) \times \text{Molarities} \times 14 \times 100}{(\text{Volume of HCL used for blank}) \times \text{mass of sample}} \quad (1)$$

$$\text{Crude protein content (\%)} = \text{\% nitrogen} \times \text{conversion factor of 6.25} \quad (2)$$

Crude Fat Content

The total crude fat content in food waste was determined using Soxhlet method (AOAC Method 2012). The formula used to determine crude fat content is given by Eq. (3).

$$\text{Crude fat content (\%)} = \frac{(\text{Weight of flask after extraction} - \text{Weight of flask before extraction})}{\text{Weight of dried sample}} \times 100 \quad (3)$$

Crude Fiber Content

The crude fiber content in food waste was determined using Gravimetric Method (AOAC Method 2012). The formula used to determine crude fiber content is given by Eq. (4).

$$\text{Crude fiber content (\%)} = \frac{(A-C)-B}{S} \times 100 \quad (4)$$

Where, S = Weight of sample (dried and defatted)

C = Weight of filter paper

A = Weight of crucible + filter paper + dried precipitate

B = Weight of crucible + ash

Total Ash Content

The total ash content in food waste was determined using dry ashing method (AOAC 2012). The ash content was calculated based on dry and wet basis. The formula used to determine total ash content is as shown in Eq. (5).

$$\text{Total ash content (\%)} = \frac{\text{Weight of residue (g)}}{\text{Weight of sample (g)}} \times 100 \quad (5)$$

Total Energy Content

The total energy content of food waste was calculated by multiplying the weight of carbohydrate, protein, and fat by 4, 4, and 9, respectively. Then, the results were added together. The formula used to determine total energy content is represented by Eq. (6).

$$\begin{aligned} \text{Total energy content } \left(\frac{\text{kcal}}{\text{g}} \right) = & \\ \left(\text{Carbohydrate, } \frac{\text{g}}{\text{g}} \times 4 \frac{\text{kcal}}{\text{g}} \right) + & \left(\text{Protein, } \frac{\text{g}}{\text{g}} \times 4 \frac{\text{kcal}}{\text{g}} \right) + \\ \left(\text{Fat, } \frac{\text{g}}{\text{g}} \times 9 \frac{\text{kcal}}{\text{g}} \right) & \end{aligned} \quad (6)$$

Phosphorus Content

The method used to determine the phosphorus content in food waste was EPA Method 8048. Approximately 10 ml of food waste sample was prepared and transferred into the sample cell. One PhosVer 3 Phosphate Reagent Powder Pillow was added into the sample cell. The sample cell was closed immediately and shook vigorously for 20 to 30 seconds. The sample cell was left for a 2-minute reaction. Blank sample was prepared using distilled water. The phosphorus content can be determined using the set program 490 P React. PP in spectrophotometer DR 2800.

Chlorine Content

The chlorine content of the food waste was determined using titration Method IS (India Standard): 3025 (IS 1988). An approximate of 1 g of sample was added to the conical flask containing 200 mL distilled water. Five milliliters of acetic acid was added into the conical flask followed by 1 g of potassium iodide (KI) and 1 mL of starch solution. Then, titration was performed quickly using sodium thiosulphate until the blue colour of the solution faded off. The formula used to determine chloride content is Eq. (7).

$$\text{Chloride content} = \frac{(A-B) \times 0.01 \times 35.45}{200} \quad (7)$$

Where, A = Volume of sodium thiosulphate used for sample

B = Volume of sodium thiosulphate used for blank

Results and Discussion

Analysis of food waste before fermentation and after fermentation (dried food waste)

Glucose content

Based on Fig. 2, it was observed that the glucose content increased for the first 48th hours to 0.099 g/g as the highest and recorded as B2. IT was then decreased gradually until 144th hours. This indicated that during the first 48th hours of fermentation, Fungi A converted the carbon source in the mixed food waste into glucose by the metabolism of Fungi A. After the inoculation of Yeast B at 48th hour, the glucose content started to decrease. This indicated that the Yeast B utilized the glucose for metabolism and owned biomass production. Yeast species, especially Yeast B has been identified as one of the most important components of a matrix of protein production hosts (Freigassner et al. 2009).

Carbohydrate content

Based on the results obtained from Fig. 3, it can be observed that the carbohydrate content decreased gradually from 0th hour to 144th hours. The lowest carbohydrate content recorded was 0.12 g/g after the fermentation of B4. This indicated that the carbohydrate content of the mixed food waste was utilized and converted into glucose by Fungi A. Ghasem (2007) reported that Fungi A uses starch as substrates for fermentation as it is cheap and high product yield can be obtained. The carbohydrate content that was converted by Fungi A to glucose was utilized by Yeast B for its own biomass production from 48th hour to 144th hour. It was reported that the regulatory mechanism in Yeast B results in the preferential consumption of glucose over other carbon sources (Raamsdonk et al. 2001). Therefore, after the inoculation of Yeast B at 48th hour, both carbohydrate and glucose content continued to decrease until 144th hour.

Crude Protein Content

Fig. 4 shows that the average of crude protein content before fermentation was 14.07. It was reported that the crude protein content of household food waste ranged from 12% to 14% which was also very similar to the crude protein content of food waste in this

study (Bandeswaran 2016). After the fermentation process, it was found that crude protein content of food waste had increased to 30.82%. This was due to production of single-cell protein (SCP) from Yeast B. Yeast B could utilize a variety of substrates for the production of SCP. The production of single-cell protein (SCP) Yeast B using food waste as a substrate (Pond et al. 1995; Teresa et al. 2018) reported that the

crude protein content that is required by broilers in the diet ranges from 18% to 23%. The crude protein content of fermented food waste in this study was as high as 30.82%. It was higher than the requirement range set and Malaysia standard. Therefore, this fermentation food waste with high protein content is suitable to be used as animal feed for the consumption of broilers.

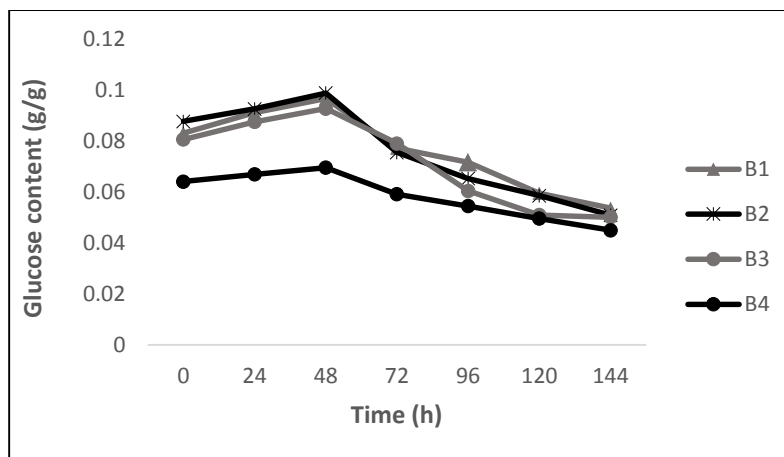


Fig. 2 Graph of glucose content (g/g) versus time (h) for Batch 1 of fermentation (B1), Batch 2 of fermentation (B2), Batch 3 of fermentation (B3) and Batch 4 of fermentation (B4)

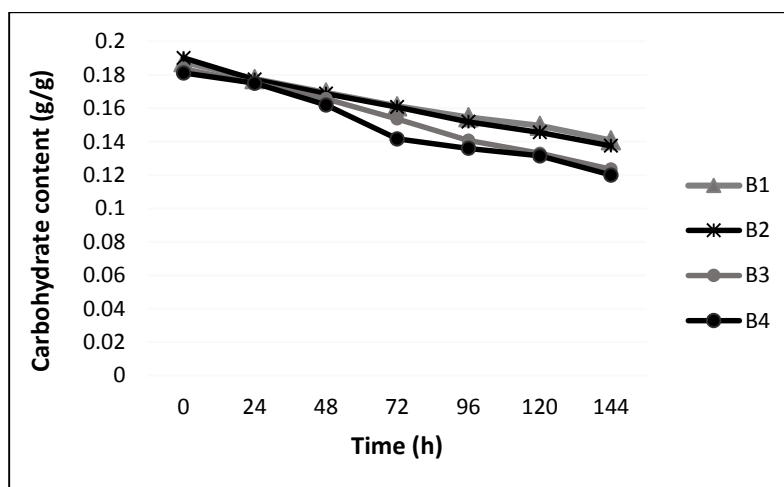


Fig. 3 Graph of carbohydrate content (g/g) versus time (h) for fermentations of Batch 1 (B1), Batch 2 (B2), Batch 3 (B3) and Batch 4 (B4)

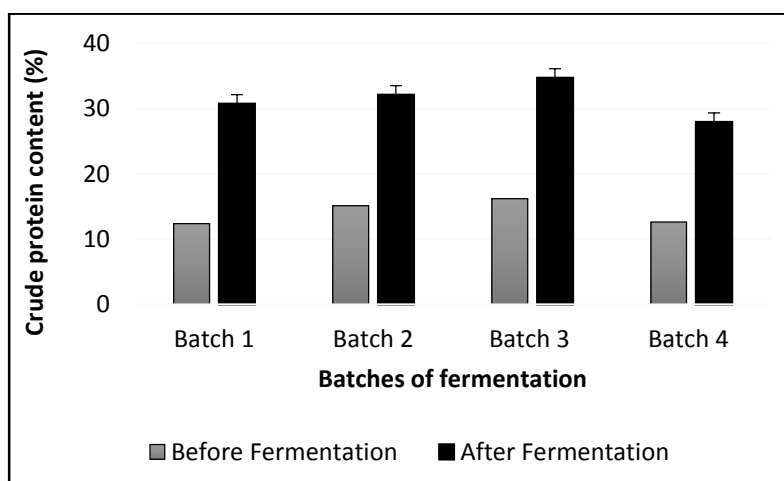


Fig. 4 Crude protein content (%) of food waste fermentation batches. Std = 1.21% (after fermentation)

Crude Fat Content

From Fig. 5, it can be observed that all 4 fermentation batches have lower crude fat contents after fermentation compared to before fermentation. This indicated that the fermentation process by Fungi A and Yeast B decreased the crude fat content of the food waste. The crude fat content of commercial chicken feed is approximately 4%. Standard deviation

of crude fat content after fermentation among the 4 batches was 1.30%. Khetarpaul and Chauhan (1989) reported that pure culture fermentation will either decrease or will not have any effect on the fat content. Fermentation with Yeast B will lead to less fat as compared to before fermentation. It was also reported that sequential culture fermentation will reduce the fat content (Khetarpaul and Chauhan 1989).

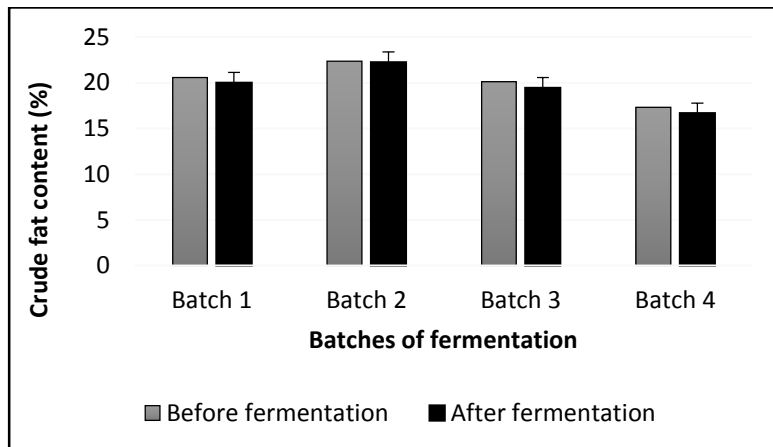


Fig. 5 Crude fat content (%) of food waste fermentation batches. Std = 1.30 % (after fermentation)

Crude Fiber Content

Based on Fig. 6, all 4 fermentation batches showed lower crude fiber contents after fermentation compared to before fermentation. This indicated that the fermentation process by Fungi A and Yeast B decreased the crude fiber content of the food waste. The crude fiber content of commercial chicken feed is approximately 6%. Standard deviation of crude fiber content after the fermentation among 4 batches was

0.17%. Liang et al. (2008) reported that some fermenting microorganisms have the ability to degrade the fiber content of the food which will also further loosen the food matrix. Fungi A and Yeast B could possibly exhibit similar ability. Degradation of fiber by microorganisms during fermentation could also increase the ease of digestion. Therefore, it could be a good way of increasing the digestibility of broilers which consume the feed.

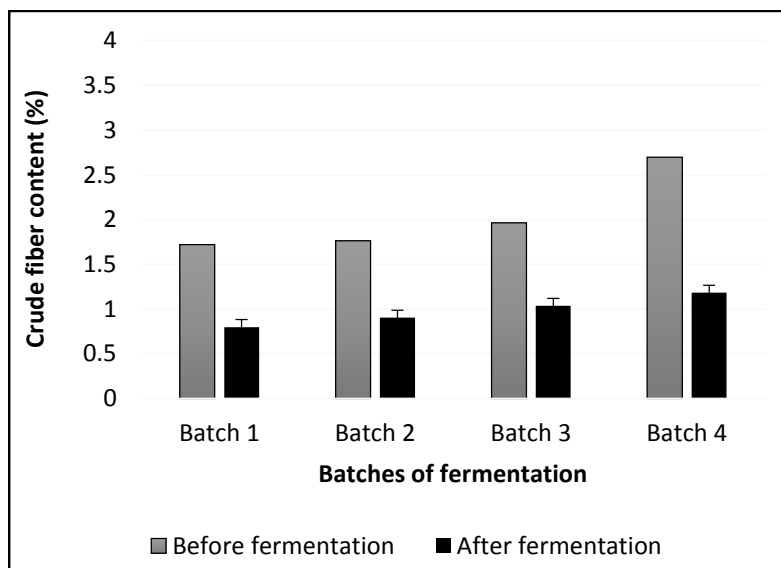


Fig. 6 Crude fiber content (%) of food waste fermentation batches. Std = 0.17 % (after fermentation)

Total Ash Content

Fig. 7 shows higher total ash contents after fermentation for all 4 batches compared to before fermentation. The highest total ash content recorded was 4.5441% after the fermentation of B3. This indicated that the fermentation process by Fungi A and Yeast B increased the total ash content of the food waste. The total ash content of commercial

chicken feed is approximately 8%. Standard deviation of total ash content after the fermentation among 4 batches was 0.18%. Fermentation could slightly increase the ash content which could be due to loss of dry matter caused by the activities of enzymes and microorganism during fermentation. It also could be due to reduction of certain chemical components such as carbohydrate, moisture and fat (Obadina et al. 2013).

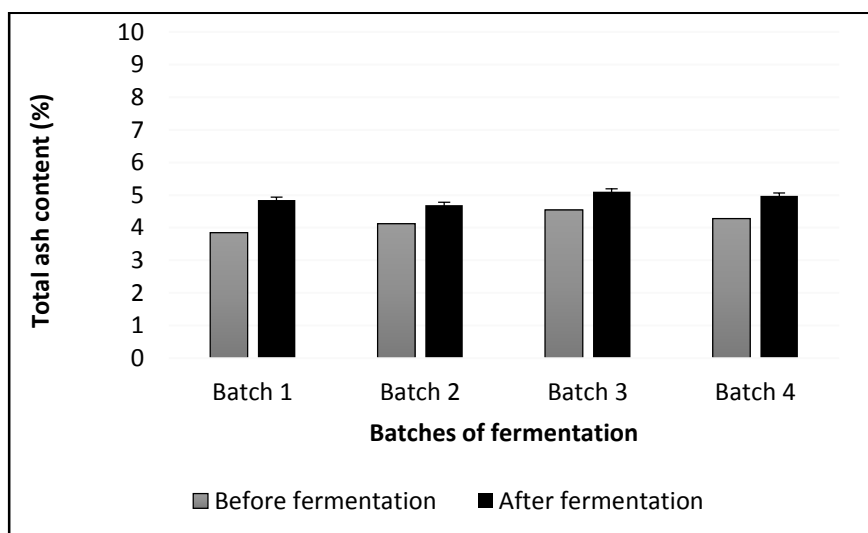


Fig. 7 Total ash content (%) of food waste fermentation batches. Std = 0.18 % (after fermentation)

Total Energy Content

Fig. 8 depicts that all 4 fermentation batches have higher total energy contents after fermentation compared to before fermentation. The fermentation process by Fungi A and Yeast B increased the total energy content of the food waste. The total energy content of commercial chicken feed is approximately

3 kcal/g. Standard deviation of total energy content after fermentation among 4 batches was 0.28%. Increase in total energy content was due to the increase in protein content and other sources of energy. High total energy content in chicken feed is good for the broilers as it could provide more energy for the metabolism and growth of the broiler.

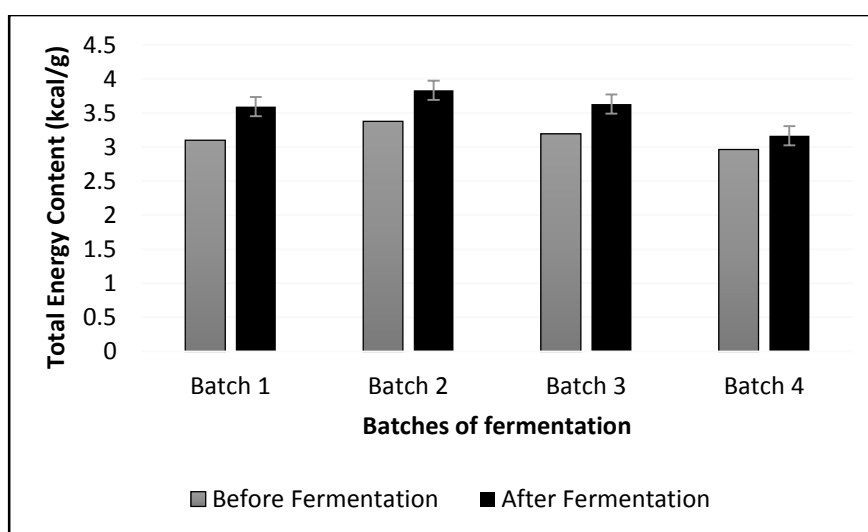


Fig. 8 Total energy content (kcal/g) of food waste fermentation batches. Std = 0.28 % (after fermentation)

Table 2 Characteristics of food waste before and after fermentation

Parameters	Food waste (Before fermentation)	Fermented and dried food waste (After fermentation)	Requirement range
pH	4.96 ± 1.40	4.14 ± 0.75	
Glucose	0.0790 g/g ± 0.0103	0.0500 g/g ± 0.2302	
Carbohydrate	0.1857 g/g ± 0.0040	0.1305 g/g ± 0.6235	
COD	0.1520 g/g ± 0.0055	0.1450 g/g ± 0.0045	
Total Kjeldahl Nitrogen (TKN)	2.31% ± 0.24	5.02% ± 0.45	
Crude Protein	14.07% ± 1.89	30.82% ± 1.21	18-23%
Crude Fat	20.59% ± 2.30	19.58% ± 1.30	> 5%
Crude Fiber	2.04% ± 0.45	0.98% ± 0.17	< 5.6%
Total Ash	4.20% ± 0.29	4.91% ± 0.18	< 10%
Total Energy	3.1587 kcal/g ± 0.1745	3.5555 kcal/g ± 0.2817	> 3.1 kcal/g
Moisture Content	81.32% ± 1.10	13.22% ± 0.40	< 13%
Phosphorus	-	0.1383% ± 0.0081	0.33-0.68%
Chloride	-	0.3369% ± 0.0381	0.12-0.20%

Notes: Data are means ± standard deviations (N=4). Requirement range refers to international standard of chicken feed

Table 2 shows the summary of characteristic of food waste before and after fermentation in terms of pH, COD, moisture content, phosphorus content and chloride content. Based on Table 1, the pH of food waste before fermentation was 4.96, while the pH of food waste after the fermentation was 4.14. Gupta et al. (2008) reported that the optimum pH for the production of amylase by Fungi A is pH 5.0, while the optimum range of pH for Yeast B is 4.0-6.0. Therefore, it was not necessary to adjust the pH value of the mixed food waste before the fermentation started as the pH value was in the optimum range for fermentation. In addition, Fungi A could stably grow at pH values ranging from below 2.0 to above 8.0 (Hesse et al. 2002). For Yeast B, it could grow in a pH range of 4.0 to 4.5 (Mountney and Gould 1988). This indicated that both Fungi A and Yeast B could grow well in the pH range of the mixed food waste. As shown in Table 1, the pH of food waste dropped from 4.96 (before fermentation) to 4.14 (after fermentation). The drop of pH after fermentation could be affected by the excretion of organic acids such as lactic acid by yeast cells during fermentation process (Coote and Kirsop 1976) and production of acid such as citric acid by Fungi A (Amal et al. 2018).

In Table 2, it can be seen that the chemical oxygen demand (COD) reduced after fermentation. COD measures the amount of oxygen needed to chemically oxidise a determined amount of organic matter and also the total organic material present in the substance (Tommaso 2011). Reduction in COD could be influenced by reduction of some of the organic materials present in the food waste throughout the fermentation process. The reduction of

COD in the food waste to an acceptable level will contribute the least side effect on the environment which is beneficial to our environment (Sheikh et al. 2012). Table 1 also shows the total Kjeldahl nitrogen content of food waste was 2.31% before fermentation, which is in agreement to that of reported by Kim et al. (2015) where the nitrogen content of their food waste was 2.50%.

The moisture content of food waste before fermentation was 81.32 %. Adjustment in moisture content of the mixed food waste for fermentation process was not carried out as the optimum moisture content required for fermentation in this study was 80% as reported by previous researcher. According to international standard (Table 1), the moisture content for chicken feed is not more than 13%. The moisture content for the dried fermented food waste was 13.22%. Despite the slight difference in moisture content between standard and this study, it could be improved by further drying of the fermented food waste in the oven. The dried chicken feed should also be kept and stored at cool and dry place with silica gels to keep the chicken feed dry. Low moisture content could prevent the growth of other microorganisms on the fermented food waste which would degrade the quality of the fermented food waste.

The phosphorus content of food waste after the fermentation was 0.14%. According to Applegate and Angel (2008), the requirement of phosphorus for broilers in the diet ranges from 0.33% to 0.68%. The phosphorus content in the chicken feed was not up to the requirement range, thus an appropriate amount of

supplement could be added into the fermented food waste to meet the standard.

The chloride content of fermented food waste was 0.34%. According to Chiba (2014), the requirement of chloride content in the diet of broilers ranges from 0.12% to 0.20%. The chloride content in the fermented food waste was higher than the requirement range. Themelis (2010) reported that high content of chloride comes from the salt and chloride contain in the mixed food waste, for example tomatoes, celery and lettuce. The electrolytes such as Cl⁻ are essential in osmotic pressure regulation and acid-based balance. Excess electrolyte could cause health and leg problems when the humidity of the environment is very high (Penz 1988). Cation and anion imbalance could also affect chicken growth and could influence the incidence of leg problems (Nesheim et al. 1964). Therefore, the chloride content

of the fermented food waste must be well controlled to meet the requirement range to avoid diseases which could be detrimental to the broilers growth and health.

Table 3 shows the result of Tukey test. The aim of this test was to determine significant difference between every single batch (B1 until B4). In terms of nutrients such as crude protein, fat and fibre, there is clearly not a significant difference (A>B) between every single batch. Meanwhile for total ash only B2-B3 is not significant (A<B) and others are significantly different. Meanwhile for energy, B1-B4, B2-B4 and B3-B4 are not significantly different, but for B1-B2, B1-B3, and B2-B3, they are significantly different. However, those nutrients are still in the accepted range of chicken feed which is highlighted in MS20:2008 (refer Table 1).

Table 3 Tukey Test analysis to determine which means amongst a batch of means differ from the other batches

Nutrient	Comparison of Every Batch	(A) Absolute difference	(B) Critical Range	Means different
Crude Protein	B1-B2	1.37	1.06	A>B :Not significant different
	B1-B3	4.00	1.06	A>B : Not significant different
	B1-B4	2.80	1.06	A>B : Not significant different
	B2-B3	2.62	1.06	A>B : Not significant different
	B2-B4	4.17	1.06	A>B : Not significant different
	B3-B4		1.06	A>B : Not significant different
Crude Fat	B1-B2	2.24	6.80	A>B : Not significant different
	B1-B3	0.55	0.47	A>B : Not significant different
	B1-B4	3.35	0.47	A>B : Not significant different
	B2-B3	2.79	0.47	A>B : Not significant different
	B2-B4	5.59	0.47	A>B : Not significant different
	B3-B4	2.81	0.47	A>B : Not significant different
Crude Fibre	B1-B2	0.11	0.08	A>B : Not significant different
	B1-B3	0.25	0.08	A>B : Not significant different
	B1-B4	0.39	0.08	A>B : Not significant different
	B2-B3	0.14	0.08	A>B : Not significant different
	B2-B4	0.28	0.08	A>B : Not significant different
	B3-B4	0.14	0.08	A>B : Not significant different
Total Ash	B1-B2	0.12	0.32	A<B : Significant different
	B1-B3	0.26	0.32	A<B : Significant different
	B1-B4	0.13	0.32	A<B : Significant different
	B2-B3	0.38	0.32	A>B : Not significant different
	B2-B4	0.25	0.32	A<B : Significant different
	B3-B4	0.13	0.32	A<B : Significant different
Total Energy	B1-B2	0.24	0.26	A<B : Significant different
	B1-B3	0.04	0.26	A<B : Significant different
	B1-B4	0.43	0.26	A>B : Not significant different
	B2-B3	0.20	0.26	A<B : Significant different
	B2-B4	0.66	0.26	A>B : Not significant different
	B3-B4	0.46	0.26	A>B : Not significant different

Viability of Fungi A and Yeast B in Chicken Feed

The viability of microorganism in chicken feed of Batch 1, Batch 2, Batch 3 and Batch 4 fermentation based on the presence of Fungi A and Yeast B at 3rd,

7th, 21st after fermentation of food waste is shown in Table 4. The viability of probiotics in chicken feed was tested to ensure that the probiotics will still present in the chicken feed even after it is stored for prolong time.

Table 4 Viability of Fungi A and Yeast B in chicken feed of Batch 1, Batch 2, Batch 3 and Batch 4 fermentation based on the presence of Fungi A and Yeast B at 3rd, 7th, 21st after the fermentation of food waste (✓ indicates the presence of probiotics in chicken feed)

Presence of probiotics in chicken feed								
Batch 1		Batch 2		Batch 3		Batch 4		
Day	Fungi A	Yeast B	Fungi A	Yeast B	Fungi A	Yeast B	Fungi A	Yeast B
3 rd	✓	✓	✓	✓	✓	✓	✓	✓
7 th	✓	✓	✓	✓	✓	✓	✓	✓
21 st	✓	✓	✓	✓	✓	✓	✓	✓

Based on Table 4, it could be observed that the probiotics were still present in the dried fermented food waste of all 4 fermentation batches up to 21 days. The viability test was carried out up to 21st day as the farmers at the chicken farm reported that normally the chicken feed will not be stored for more than 2 weeks (only 14 days). Therefore, viability test of 21 days or 3 weeks was enough to prove that the probiotics were still present in the chicken feed 14 days after fermentation. It is important to maintain the viability of probiotics in chicken feed as probiotics can improve the health of chicken and maintain the standard and quality of the chicken feed.

Fungi A has dark brown to black color, spore formation, spherical shape, and able to degrade starch, cellulose, xylose and a few complex carbohydrates. It only can survive in the aerobic condition.

Meanwhile, yeast B has a yellowish color to light brown and ellipsoid shape. Yeast B is also able to degrade glucose, fructose, lactose and growth. It can grow in the facultative condition.

Conclusion

In conclusion, the fermentation of mixed food waste from different sources by Fungi A and Yeast B was successfully carried out. The profiles of parameters such as carbohydrate and glucose contents during fermentation of food waste via two stage fermentation by Fungi A and Yeast B were successfully investigated and determined. The results showed that the carbohydrate content decreased from 0.1857 g/g (before fermentation) to 0.1305 g/g (after

fermentation) as Fungi A consumed carbohydrates and converted it to glucose. Glucose content was found to be increasing at the first 48 hours and then dropped until the fermentation completed. The highest glucose content recorded was 0.099 g/g for B2 at 48th hour. This was due to the consumption of glucose by Yeast B for its own biomass production from 48th hours onwards. The process control of elements of food waste and bioreactors to control the quality of end products was successfully validated. The C:N ratio, pH, aeration, agitation, temperature, antiseptic technique and sterilization were controlled accordingly throughout the fermentation process and the end products which was the chicken feed were found to have consistent quality and nutrients value. The standard of the fermented food waste or chicken feed was also compared with the standard of chicken feed used in Malaysia and internationally, especially for the crude protein content. The crude protein content, crude fat content, crude fiber content, total ash content and total energy content of chicken feed have met the requirement set by the Malaysian standard. Phosphorus content could be supplemented to meet the required range and moisture content could be lowered down by further drying. While for international standard, the chicken feed met the requirement of 18% crude protein content. This is very crucial because the chicken feed produced can be used both in Malaysia and internationally. The food waste has a massive potential in the production of chicken feed which in turn will reduce production cost and preserve the environment.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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APPENDIX 1

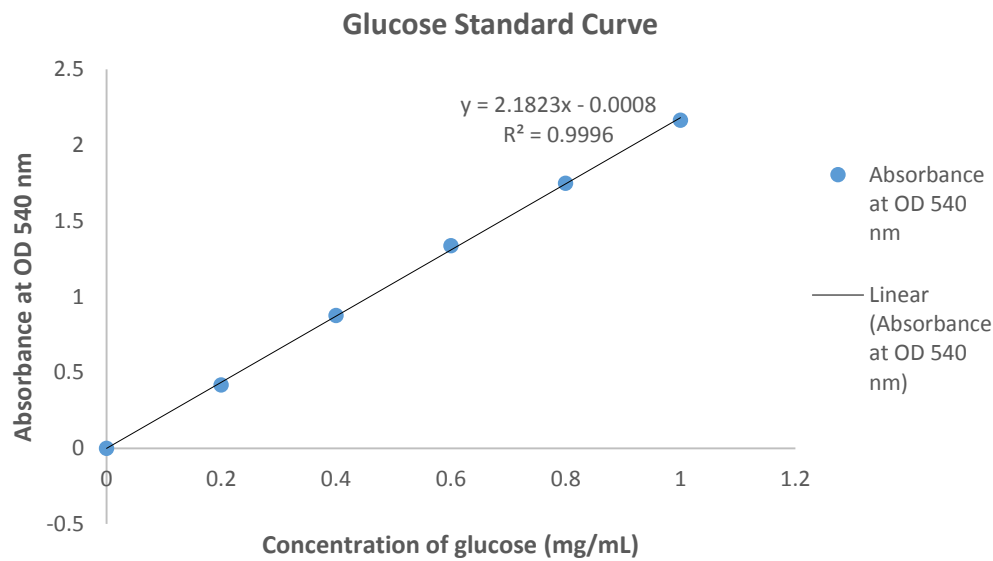


Fig A.1 Glucose standard curve

APPENDIX 2

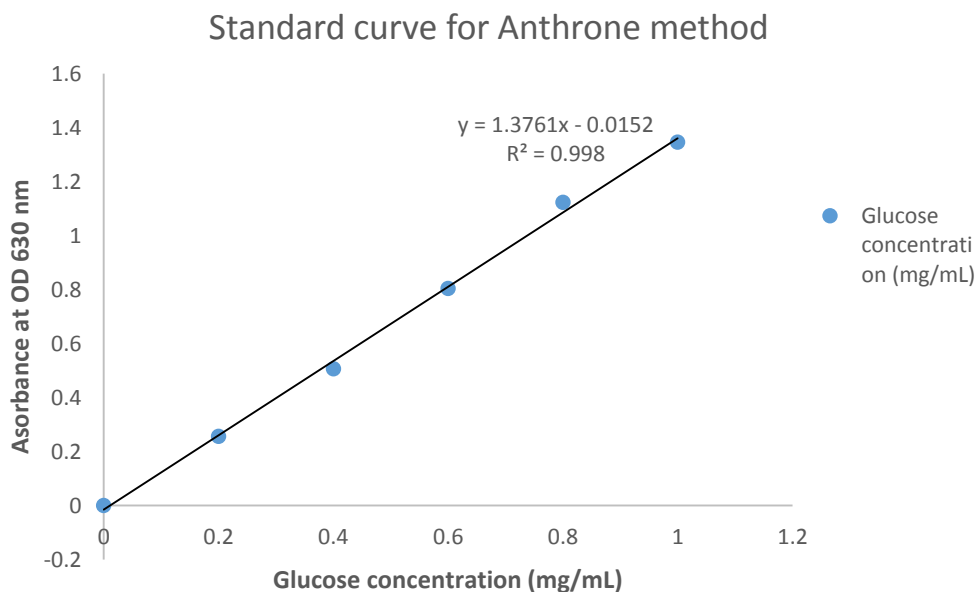


Fig. A.2 Standard curve for carbohydrate content determination by Anthrone method

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