

HYDROLYSIS OF CHICKEN INTESTINE FOR FLAVOR DEVELOPMENT IN
CAT FOOD

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DEDICATION

*Kusembahkan syukur padaMu Ya Allah,
Untuk nama, harta, dan keluarga yang mencinta,
Dan perjalanan yang sejauh ini tertempa,
Pilihan dan kesempatan,
Yang membuat hamba mengerti tentang makna diri,
Semua lebih berarti akan mudah dihayati...*

Alhamdulillah

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ABSTRACT

Increasing number of chicken slaughterhouse in Malaysia has produced a tremendous amount of waste that can be potentially utilized as a source for manufacturing a value added product. However, due to lack of scientific study, the waste is normally discarded which may give negative impacts to the environment. Thus, this study focuses on utilizing by-product from poultry industry such as chicken intestine for chicken flavour development, which cysteine and methionine are the precursor for the chicken flavour. In this study, chicken intestines were firstly characterized and their shelf life were studied before being hydrolysed using chemical and commercial enzyme protease to obtain chicken flavoured hydrolysate. Raw chicken viscera show significant amount of crude protein 9.1% w/w and crude fat 3.0% w/w. It was observed that fresh intestines have the highest protein content 8g/L while storage at -20°C sustained the protein concentration up to 88%. In contrast, storage at 0°C reduced the protein concentration for about 28% and 50% following one and two weeks of storage, respectively. Three different conditions of hydrolysis were studied; i) hydrolysis with indigenous protease without adjusting pH, ii) hydrolysis with indigenous protease with pH adjusted to 2.8 using 1N HCl and iii) hydrolysis with addition of 1% (v/v), 2% (v/v), and 3% (v/v) commercial protease. Results from this study showed that hydrolysis using 1N HCl at initial pH 2.8 produced protein content 19.12% higher than hydrolysis without pH adjusted where the initial pH recorded was 6.3. Meanwhile, hydrolysis using 1% (v/v), 2% (v/v) and 3% (v/v) commercial protease at buffer pH 6.4 produced protein content ranging from 3.85 g/L to 5.05 g/L, which the highest protein concentration was obtained from hydrolysis with addition of 2% (v/v) commercial protease. In addition, the detection of cysteine and methionine as precursor for chicken volatiles known as 2-methyl-3-furanthiol and 2-methyl-3-furanthiol using HPLC confirmed the production of the chicken flavour. From this study, it could be concluded that the highest amount of cysteine (0.44 mg/mL) and methionine (0.67 mg/mL) produced are from hydrolysis with indigenous protease with pH adjusted to 2.8 using 1N HCl and could be potentially used as key ingredients in developing chicken flavour for cat food industries. With thorough optimization, this research will benefit the rapidly-growing cat food industry in Malaysia.

ABSTRAK

Penambahan bilangan pusat penyembelihan ayam di Malaysia telah mengeluarkan banyak jumlah sisa buangan berpotensi untuk digunakan sebagai sumber bagi menghasilkan bahan nilai tambah. Walaubagaimanapun, disebabkan kekurangan kajian saintifik, sisa buangan tersebut biasanya dibuang sahaja dan memberikan impak negatif kepada alam sekitar. Oleh itu, kajian ini tertumpu kepada menggunakan sisa buangan seperti usus ayam untuk penghasilan perasa ayam dengan sisteina dan metionina sebagai petunjuk kepada perasa ayam. Perut ayam dicirikan dan jangka hayat perut ayam dikaji sebelum hidrolisis kimia dan hidrolisis menggunakan enzim protease komersial dilakukan. Usus ayam mentah menunjukkan jumlah protein kasar 9.1% (j/j), dan lemak kasar 3.0% (j/j). Kesan tempoh penyimpanan terhadap kandungan protein menunjukkan bahawa usus segar mempunyai kepekatan protein tertinggi kira-kira 8 g/L dan penyimpanan pada -20°C mengekalkan kepekatan protein hingga 88%. Sebaliknya, penyimpanan pada suhu 0°C mengurangkan kepekatan protein kira-kira 28% hingga 50% selepas satu dan dua minggu penyimpanan. Tiga jenis kaedah hidrolisis berbeza telah dikaji; i) hidrolisis dengan protease asli tanpa mengubah pH, ii) hidrolisis dengan protease asli dengan pH diselaraskan kepada 2.8 menggunakan 1N HCl dan iii) hidrolisis dengan penambahan 1% (v/v), 2% (v/v) dan 3% (v/v) protease. Hidrolisis menggunakan 1N HCl dengan pH awal 2.8 menghasilkan kandungan protein 19.12% lebih tinggi berbanding hidrolisis tanpa pelarasan pH di mana pH awal yang direkodkan ialah 6.3. Hidrolisis menggunakan protease komersial 1% (v/v), 2% (v/v) dan 3% (v/v) pada buffer pH 6.4 menghasilkan kandungan protein antara 3.85 g/L hingga 5.05 g/L. Hasil protein yang tertinggi diperolehi daripada hidrolisis dengan penambahan protease komersial 2% (v/v). Kehadiran sisteina dan metionina sebagai petunjuk kritikal bahan ruap rasa ayam yang dikenali sebagai 2-metil-3-furanthiol dan 2-metil-3-furanthiol menggunakan HPLC mengesahkan penghasilan rasa ayam. Kesimpulannya, jumlah sisteina yang paling tinggi (0.44 mg/mL) dan metionina (0.67 mg/mL) terhasil daripada hidrolisis dengan protease asli dengan pH diselaraskan kepada 2.8 menggunakan 1N HCl dan berpotensi untuk digunakan sebagai bahan dalam penghasilan perasa ayam untuk industri makanan kucing. Dengan pengoptimuman secara menyeluruh, kajian ini akan bermanfaat kepada industri makanan kucing yang berkembang pesat di Malaysia.

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LIST OF ABBREVIATIONS

°C	-	Degree Celsius
HCl	-	Hydrochloric acid
g/L	-	Gram per liter
mg	-	Milligram

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Protein are needed in a balance diet. It can be abundantly obtained from fish and meats. In modern era, fish is costlier compared to meat. The price different are due to the availability of the source. Compare to other ruminants, chicken can be grown fast and easy indoor. Fish are different. Although several types of fish can be grown in freshwater, it requires high maintainence to sustain the environment (Reid et al., 2013). Same situation applies to the salt water fish. They are threatened by overfishing, pollution, habitat loss and climate change (Reid et al., 2013; Allen & Steeby, 2011). Not to mention that before going out to the sea, fisherman needs to consider the weather first which affect the amount of the fish caught on the respective day. Consecutively, this scenario creates a massive demand on chicken meat as well create dependency of people towards poultry based meat.

With high supply and high demand for the chicken, chicken slaughterhouse become one of the largest contributors for high protein waste in Malaysia. It is reported by Sari et al. (2016) that 25 to 30 percent of the birds' weight are considered as inedible waste. As meat of the chicken are utilised to fulfil the human needs, the by-products are not fully utilised (Lasekan *et al.*, 2013). Examples of poultry-by products are viscera, bones, skins and head. Only several inner parts of the chicken such as heart and liver are recovered for chicken based product such as fish meal, live feed stock and baits for fishing. Often, these are considered as an absolute waste and were just disposed to waste disposal from the poultry farms, markets, food processing industries and homes although it contains high amount of proteins, which are potentially can be recovered and utilised for various purpose.

Due to underutilised of these waste in developing country, it always results on improper disposal. This will affect the environment as well the health of the communities living near to the poultry farm. The environmental problem arises include unpleasant smell on that area, and increase in number of pests such as flies and rats as reported by Anuar *et al*, 2017. The aftermath of the environmental problem is the health problem and the most recent event is the epidemic of Bovine Spongiform in the year of 1990. Since that, the cleanliness, standard operating procedure (SOP) of handling poultry including managing the waste are always in review by the authorities. In Malaysia, other than being buried in the waste disposal or landfill, by-product from the poultry farm are usually burnt to generate electricity or biogas as guided by European Union Legislation document (European Union, Regulation (EC) No. 1096/2009).

The idea to utilise chicken waste product derived from a report by Intelligence & Partner, 2015, stating that the demand to have a distinguished quality of pet food are raising correspondingly with the number of pet owner especially cat owner. Cats and dogs are the common companion animals in the world. Some of the pet owners already acknowledge their companion animals as their family members. For that reason, they are willing to spend thousands of their wealth to ensure their companion are consuming healthy food. Cats are strict carnivore. They require food with high content of protein to sustain their health. Using cheap source such as 4D (dead, dying, disable and diseased) animal and rendered animal waste as main ingredients are common practice of many pet food industries as reported by Donna Specter in an article titled Pet Food (What You Need to Know) for Your Pet's Sake. For this reason, The Association of American Feed Control (AAFCO) and The European Pet Food Industry Federation (FEDIAF) has put strict regulation for the criteria of the ingredients to be used in pet food production. The ingredients are mainly for human consumptions. In a long term, this might cause food security issue. Thus, this can be the main reason to utilize the poultry by product in a correct manner for pet food production to solve the problem that arise.

Connecting these situations, there are potential on conducting a research which can solve the problem and fulfil the demand that arise from these situations. Waste from the poultry farm, especially from chicken slaughterhouse can be reduced and managed, by utilizing its protein waste for the development of pet food. The environmental and health problems can be reduced significantly. The pet food produced will be based on the regulatory set by the regulators such as The Association of American Feed Control (AAFCO) and The European Pet Food Industry Federation (FEDIAF). It is important to ensure the safety and the healthiness of the food produced. Other issue that indirectly being solved is food security. Less competition of edible meat between human food industry and pet food industry.

Understanding this situation, the poultry by-products from chicken slaughterhouse have potential to be utilized as high protein product that can be used in pet food industries. The high protein product which the source and the method of production are according to the regulation set by the regulators will increase the confidence of the pet owner. To be added, this study has high potential to penetrate and create a new alternative of production in the pet food industries.

1.2 Problem Statement

Chicken slaughterhouse in Malaysia are producing numerous amount of protein waste which also known as poultry by-product. Disappointedly, most of the by-product were discharged into waste disposal or burnt instead of utilizing them for development of value added product. In the meantime, the number of pet owner especially cat owner in Malaysia increased. This lead to high demand of distinguished quality of pet food. In addition, the increased number of pet owner upsurges the concerns of the sources used for pet food production. Number of cases exposes that the sources of pet food production are from 4D (dead, dying, disable and diseased) animals thus forcing the industry to use sources similar to human food production. Eventually, food security issue will rise as human are competing the same sources of food with pets. Therefore, this study aims to utilize poultry by products specifically

chicken intestine as alternative for cat food production to fulfil the demand in the market.

1.3 Objectives of the Study

The objectives of this study are:

- (a) To characterize the composition of raw chicken intestine.
- (b) To study the effect of temperature on the shelf life and protein content of chicken intestine.
- (c) To study factors that influence cysteine and methionine production during enzymatic hydrolysis of chicken intestine using commercial protease.

1.4 Scope of the Study

In this study, raw chicken intestine is characterized using proximate analysis. The storage quality of the chicken intestine before being hydrolysed are studied. Different condition of intestine with different condition of hydrolysis are tested to select the best condition of achieving high protein content which contribute to the rise of chicken aroma and this can be detected via performic analysis of high performance liquid chromatography.

1.5 Significances and Original Contributions of This Study

This study will contribute in cat food industries as it will replace the current meat source for flavouring purpose to chicken intestine. Consecutively, the environmental problem will be reduced and competition on having meat between human and pets will be reduced. Thus, the target of having less waste and less production cost from industry can be achieve.

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