Amino acid composition analysis of beef, mutton, chevon, chicken and Pork by HPLC method

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Abstract— **Food plays important roles in social, cultural and religious life style of every community throughout the world. Due to increasing health concerns and sensitivity among the consumers over the food quality, there is currently a great need for food analysis and authentication. The detection of adulteration is a technical problem. Indeed, we must wonder about the following question: how can we detect an adulterant having approximately the same chemical composition of the food product in which it is included?**

A main approach to this problem could be to search for a specific marker in the product, which could be a chemical constituents (complexes, molecules, nucleic acids) or morphological components (plant cells), that proves either the adulteration or authenticity of the food. The rapid and reliable detection of pork in various food products has been an important subject of study in many countries, especially where religious laws prohibit the consumption of pork products. The amino acids were determined in pork, beef, chicken, chevon and mutton using HPLC with pre-column derivatization based on OPA methods with FLD detector. Amino acids were used as chemical descriptors to differentiate the meat varieties. Since neither region nor sex had any influence on the amino acid content of the meat, it can be concluded that the method of detection in this study could be used to determine the status of the meat. Therefore, it can be a reliable technique to investigate the presence of pork in different items such as processed meats and meat products.

Keywords— **HPLC, OPA, adulteration.**

I. INTRODUCTION

The adulteration and the authenticity of food and the methodology for testing are well known topics in food science since many years. It is a commonly encountered problem in food trade and industry. It is sometimes deliberate, sometimes accidental. Food adulteration for economic gain is a well-established malpractice. Authenticity covers many aspects, including adulteration, mislabeling, characterization and misleading origin [1]. Adulteration of food products is of primary importance for both consumers and industries, at all levels of the production process, from raw materials to finished products, Food products are generally of plant or animal origin, therefore the reliable identification of species is a key issue for food authenticity and should preferably be based on parameters which do not undergo too many adulterations during food processing [2; 3]. From the legislative point of view, quality standards have been established through the requirement of quality labels that specify the chemical composition of each product. From the economic point of view, product authentication is essential to avoid unfair competition that can create destabilized market and disrupt the regional economy and even the national economy [4]. The detection of pork and lard as adulterants has gained considerable importance and interest in many parts of the world. The Islamic, Judaism and Orthodox Jewish religion prohibit the consumption of both pork and lard derived from pigs in any products. In view of the biological complications and risk of diseases associated with pork and lard and the restriction on their consumption by some religions, a reliable method is required for the detection of pork and lard in their various forms to enforce restriction of such products. Interspecies meat adulteration or preparation of meat products by mixing meats and fats of different origin is a common procedure in most countries. These facts are of major concern for many consumers, particularly in relation with ambiguous or improper labeling, adulteration with cheaper meats, or religious specifications such as halal and kosher food for Muslim and Jews respectively. A significant problem on its own, too, is the use of pork in beef or fowl meat products. Therefore there is a need for rapid and reliable methods for species identification in such varieties of food commodities.

II. MATERIAL AND METHODOLOGY

Acetonitrile, hydrochloric acid and methanol (HPLC grade) were obtained from Merck KGaA (Darmstadt, Germany). *o*-phthaldialdehyde 3-mercaptopropionic acid (OPA/3-MPA) (Agilent PN 5061-3335), 100-µL of the 1-mL of OPA reagent was poured into conical insert, capped immediately and refrigerated (4°C). Borate buffer (Agilent PN 5061-3339) pH was adjusted by adding NaOH to make pH 10.2 from 0.4 N boric acid. Chromatographic grade water produced by purification system (Milli-Q system, Sartorius 611) was used throughout the study. Amber wide-opening vials, glass conical inserts with polymer feet and screw caps were purchased from Agilent. Solutions of 17 amino acids standard in five concentrations (10 pmol/µL, 25 pmol/µL, 100 pmol/µL, 250 pmol/µL and 1nmol/µL) were obtained from Agilent (Agilent PN 5061- 3330 through 5061-3334) for calibration curves. 100-µL of the 1-mL of each was poured into conical insert, capped immediately and refrigerated (4°C).

A. Meat Samples

Authentic samples of beef, mutton, chevon and chicken from animals, raised under check conditions, were obtained from the Dept of Animal Science, Faculty of Agriculture, University Putra Malaysia, Serdang, Malaysia. Samples of pork were purchased from the local wet market.

B. HPLC Analysis

Amino acids were determined using an Agilent 1100 HPLC system (Agilent Technologies), equipped with a quaternary pump delivery system, robotic autosampler , thermostatted column compartment and a Fluorescence detector (FLD). The samples were submitted to automatic pre-column derivatization with OPA-3MPA by programming the robotic autosampler. After derivatization, an amount equivalent to 3.5 µL of each sample was injected on a Zorbax Eclipse-AAA column, 4.6 × 150 mm, 3.5 µm. Mobile phase A was 40 mM NaH_2PO_4, adjusted to pH 7.8 with NaOH, while mobile phase B contained 45% acetonitrile, 45% methanol, and 10% deionized water. The chromatographic column temperature was set at 40 °C with a flow rate of 1.5 mL/min with a gradient program.

C. Statistical Analysis

The data taken were analyzed using One-way analysis of variance (ANOVA) to determine the significant differences of amino acids among the various meats studied. Subsequently, the Duncan's New Multiple Range Test (DNMRT) was applied for comparison of means of the amino acids measured in different meats. Principal Component Analysis (PCA) was performed to determine the main directions of variations among the meats. The sample meats were located in a graph based on the first three PCs. The analysis of variance was done using the General Linear Model (PROC GLM), while principle component analysis (PCA) was performed using PROC PRINCOMP of Statistical Analysis System (SAS) computer package.

III. RESULTS

Results of the analysis showed that the meats investigated were distinguishable from each other by measuring the percentage of their corresponding amino acids (Table 1). GLY was found to have the highest concentration compared to the other amino acids measured in all the meats sampled, while its concentration was not different between different meats. Similarly, Aristory and Toldra [5] reported that GLY had the highest value compared to the other amino acids studied in pork. In contrast, the lowest percentage of amino acid was obtained from MET in beef, mutton, chevon and chicken, except pork. Similar results were informed by many investigators indicating low percentage of MET in beef, mutton, chevon and chicken [6-11]. Schuster [12] reported that MET was not the lowest amino acid among the other amino acids studied in pork. The lowest quantity of the amino acids measured in pork was obtained from TYR. Flores et al. [13] reported the same result in which TYR was the lowest in pork.

The amino acid VAL was found to be a promising marker for differentiating pork from the other meats studied. The percentage of VAL was significantly lower

than those in beef, mutton, chevon and chicken. Aristoy and Toldr [14] reported that the quantity of VAL was found to be lower than the other amino acids measured in pork, while, Gilka et al. [8] and Webb et al. [15] showed high quantity of VAL in mutton and chevon. This indicates that the higher quantity of VAL could be found in mutton and chevon meats compared to that of pork. GLU was found to be significantly different between chevon and chicken, while HIS significantly differentiated chevon from pork. Webb et al [9] reported low value of HIS in chevon compared to that of pork investigated by Cornet and Bousset [16].

Table 1. Mean values for the amino acids measured from different raw meats

Treatment	Amino Acid Mean Values (%)														
	ASP	**GLU**	**SER**	**HIS**	**GLY**	**THR**	**ARG**	**ALA**	**TYR**	**VAL**	**MET**	**PHE**	**ILE**	**LEU**	**LYS**
Beef	2.82^{a}	7.81^{ab}	4.06^{ab}	5.89^{bc}	20.18^{a}	3.03^{a}	8.66^{ab}	6.93^{a}	2.13^{a}	4.33^{b}	2.03^{a}	3.05^{a}	3.47^{a}	7.08^{a}	8.05^{a}
mutton	2.92^{a}	7.65^{ab}	4.16^{a}	6.24^{abc}	18.26^{a}	2.88^{a}	9.95^{a}	6.50^{b}	2.36^{a}	4.63^{ab}	2.19^{a}	3.27^{a}	3.35^{a}	5.82^{a}	8.53^{a}
chevon	2.98^{a}	8.26^{a}	4.20^{a}	5.28^{c}	18.47^{a}	3.42^{a}	8.37^{b}	6.55^{ab}	2.45^{a}	4.50^{ab}	1.77^{a}	3.25^{a}	3.64^{a}	7.13^{a}	8.42^{a}
Chicken	2.70^{a}	7.11^{b}	3.81^{b}	7.29^{a}	19.10^{a}	3.82^{a}	9.27^{ab}	6.11^{c}	2.14^{a}	5.20^{a}	1.97^{a}	3.01^{a}	3.36^{a}	6.94^{a}	7.83^{a}
Pork	2.86^{a}	7.67^{ab}	4.15^{a}	6.74^{ab}	18.88^{a}	2.76^{a}	8.54^{b}	6.72^{ab}	2.27^{a}	4.05^{c}	2.29^{a}	3.14^{a}	3.35^{a}	7.26^{a}	8.23^{a}
Mean	2.86	7.70	4.08	6.29	18.98	3.18	8.96	6.56	2.27	4.54	2.05	3.14	3.43	6.85	8.21

Note: Means followed by the same letter in the same column are not significantly different at $p \leq 0.05$ based on DNMRT, ASP= Aspartic acid, GLU=Glutamic acid, SER= Serine, HIS= Histidine, GLY= Glycine, THR= Threonine, ARG= Arginine, ALA= Alanine, TYR= Tyrosine, VAL= Valine, MET= Methionine, PHE= Phenylalanine, ILE= Isoleucine, LEU= Leucine, and LYS= Lysine.

IV. DISCUSSION

Up to now, several methods have been described for the determination of amino acids. The classical approach to amino acid analysis is separation on a sulphonate cation-exchange resin, followed by derivatization with ninhydrin and spectrophotometric detection [17; 18]. These methods are adequate but generally time consuming and in addition, they require substantial amounts of sample. The use of RP-HPLC permits amino acid determinations in a relatively short time on small samples and with good sensitivity and specificity [19; 20]. In this study, we selected a method that employs pre-column derivatization with OPA/ 3-MPA. OPA have been used to react with primary amino acids [12; 21-23]. This chemical is more sensitive and easier to use than fluorescamine and 10 times more sensitive than ninhydrin [16; 24]. amino acids were identified with pre-column derivatization (OPA/ 3-MPA) by comparing retention times with those obtained from amino acid standard solutions in our study, with an injection time of 26 min preceded by a derivatization step of which 16 min were for analysis time. The sensitivity of the method was highly enough to estimate amino acid levels injected onto the column with a lower limit.

V.CONCLUSION

It can be concluded that a simple HPLC analysis by derivative formation with OPA and FLD detection was performed for more than a dozen amino acids with resolution and sensitivity high enough to measure the very low levels of the amino acids in the meats mentioned. OPA is a fluorophore and reacts with the amino acids to form an isoindole. The isoindole derivatives are very amenable to HPLC and sensitive to small changes in mobile phase conditions. it can be concluded that this method could be used for fast, simple and cost effective separation of pork as a non-halal meat from the other halal meats measured in this study.

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