Characterization of the ribose-induced maillard reaction in minced chicken and minced pork: a potential means of species differentiation

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Abstract: The addition of ribose to minced chicken or minced pork followed by heating at 95°C yielded minced meat with different pH, colour (CIE L^*, b^*) and absorbance values that can be used as indicators for species differentiation. The higher intensity of the Maillard reaction parameters in minced chicken was due to the higher protein and lysine contents, and the presence of more water-soluble proteins within the minced chicken during heating. Cluster analysis using Maillard reaction parameters showed that the two types of minced meat could be classified into two different groups. A confidence interval (95% confidence) analysis revealed that the absorbance, CIE L^* values, and CIE b^* values could be used as indicators for differentiation between the two types of minced meat, as the intervals between these Maillard reaction parameters for the two minced meats were far apart.

Introduction

Unlike in the past, when meats were mostly purchased from wet markets, there is an increased demand for convenient, ready-to-cook processed meat and meat products in both developing and developed countries. In the meat processing industry, minced meats are widely used in comminuted meat products, such as sausages, patties, meatballs, and surimi (Tornberg, 2005). Due to the unknown origin of minced meat, the public and authorities are concerned about the reliability of the labels on meat products. In most Islamic countries, pork is one of the main concerns in food, as it is considered "Haram" (forbidden) for Muslims. For example, surimi, which is typically prepared from fish, has been made with added chicken (Jin *et al*., 2009) or pork (Park *et al*., 1996).

Minced chicken and minced pork can be very similar in their appearance and physicochemical properties, which makes their differentiation very difficult. Even if it is sometimes possible to differentiate these meats visually, Muslim consumers need better assurance of species authenticity. Most studies of species differentiation were conducted based on electrophoretic techniques, and DNA techniques in order to search for viable tools for Halal verification (Aida *et al*., 2005; Che *et al*., 2007; Murugaiah *et al*., 2009). These identification methods require expensive equipment, well-trained operators, and a long analysis time.

The application of the Maillard reaction (non-enzymatic browning) to minced meat was shown by Meinert *et al*. (2009), with the aim of studying flavour formation. Because the Maillard reaction yields various physicochemical changes in meat, it could provide a novel method of species differentiation and identification. The Maillard reaction is the condensation reaction of a reducing sugar with amino acids or proteins. Upon heating with the reducing sugar, different Maillard reaction products are generated depending on the types of reactants and the heating conditions used (Ames, 1998). Ultimately, brown nitrogenous polymers and co-polymers, known as melanoidins, that can be measured at a wavelength of 420 nm are formed (Carabasa-Giribet and Ibarz-Ribas, 2000). The Maillard reaction parameters, such as browning, pH values, and colour values, have the potential to be used as an indicator for species differentiation.

To date, the use of the Maillard reaction as a means of species differentiation and identification has not been studied. Because pork and chicken have different chemical compositions, the extent of the Maillard reaction will be different when a reactive reducing sugar is heated with these meats. The aim of this paper is to assess the potential for using the Maillard reaction as an alternative method of differentiation and identification of minced chicken and minced pork.

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Materials and Methods

Materials

D-glucose, D-fructose, D-xylose, and D-ribose were purchased from Sigma-Aldrich Co., St. Louis, USA. Other chemicals (analytical grade) used were obtained from Sigma-Aldrich and Fluka Chemical Corp., Ronkonkoma, USA.

Sampling plan

Five fresh cuts of chicken breast (from Ross chickens) and five fresh blade shoulder cuts of pork (from Large White pigs) were purchased on 5 different days from a local wet market in Gelugor, Penang, Malaysia. The meat samples were cleaned, and visible fats were removed before cutting into cubes (2 cm³). The meat cubes were kept in a refrigerator (Toshiba Corp., GR-M48MP refrigerator, Minato-ku, Japan) at 4°C for 2 h before grinding in a meat mincer (Kenwood Limited, MG470 meat grinder, Hampshire, UK) using a 4.5 mm plate to produce minced chicken or minced pork.

Nutritional composition of raw materials

Proximate analysis

Proximate analysis (of moisture, crude protein, and crude fat) for both minced meats was done according to the standard AOAC method (AOAC, 1998). Mosture content was determined using Laboratory Dry Matter by Oven Drying for 2 h at 135°C (AOAC 930.15). Crude protein was determined using Nitrogen Determination by Kjeldahl (Block Disgestion) (AOAC 981.10). Conversion factor used in calculating the crude protein content was 6.25. Crude fat content was determined using Soxhlet extraction with diethyl ether (AOAC 920.39). The results were reported on a percentage-wet basis.

Amino acid analysis

Freeze-dried for both minced meats (0.1 g) were carefully weighed into tubes, and 5 mL of 6 N HCl was added. After heating at 110°C for 24 h, hydrolysates were added to 400 µL of 50 µmole/mL AABA (L-α-amino-n-butyric, as internal standard) and topped up to 100 mL using deionised water (ELGA LabWater, PURELAB Option-Q, High Wycombe, UK). The samples were then filtered through filter paper (Whatman plc, Whatman No. 1 filter paper, Maidstone, UK) and, subsequently, through 0.22 µm PTFE membrane filters (Millipore Corp., LCR Membrane Filters, Billerica, USA) prior to derivatisation. The filtrates (10 µL) were mixed with 70 µL borate buffer and allowed to derivatise with 20 µL AccQ.Fluor™ reagent (AQC: 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate) for 1 min at room temperature followed by 10 min at 55°C, prior to injection into a reverse phase HPLC (Waters Corp., Waters 1525 Binary HPLC Pump coupled with Waters 2475 Multi-Wavelength Fluorescence Detector and Waters 717 Plus Autosampler, Milford, USA) for amino acid analysis. Seperation was done using a reversed phase HPLC column (Waters Corp., Waters AccQ.Tag™ Amino Acid Analysis column, Milford, USA).

Slight modifications to the above methodology were made for cysteine and methionine determinations. The samples (0.1 g) were carefully weighed in tubes cooled with ice cubes prior to the addition of 2 mL of freshly prepared performic acid (formic acid mixed with 30% hydrogen peroxide at a ratio of 9:1). After the mixture had cooled in a refrigerator for 16 h, 0.4 mL of chilled HBr was added before cooling in the refrigerator for another 30 min. The samples were then dried using a rotary evaporator at 80°C prior to hydrolysis with 5 mL of 6 N HCl at 110°C for 24 h. The subsequent procedures were similar to those described above.

Maillard reaction

All minced meat samples purchased on a particular date were prepared in triplicate. Fructose, glucose, ribose and xylose were used to induce the Maillard reaction in the minced meat samples, while sucrose was used as a control. The sugars (10% w/w) and minced meat were manually mixed. Seven g of the sugar-minced meat mixtures were transferred into a 30-mL glass universal bottle and heated in a water bath (Memmert, WB22 water bath, Schwabach, Germany) at 95°C for 15, 30, 45, and 60 min. The heated samples were immediately cooled in ice water. The samples were left at room temperature for up to 1 h before analysis.

Measurement of pH

The pH of each sugar-induced Maillard reaction sample and control sample was determined with pH meter (Mettler-Toledo Inc., Delta 320 pH meter, Greifensee, Switzerland).

Extraction of water-soluble brown polymers (Maillard reaction products)

Heated samples from each universal bottle were removed individually and comminuted using a mortar and pestle to increase the surface area for better extraction. Approximately 1 g of the comminuted samples was transferred into 15 mL polypropylene test tubes, and 5 mL of distilled water was added.

The mixture was mixed by Vortexing (Gilson Inc., GVLab Vortex, Middleton, USA) for 15 s prior to mixing on a platform shaker (Janke and Kunkel, KS 501 D shaker, Staufen, Germany) at a speed of 300 x/min for 1 h. After mixing for 1 h, the mixture was centrifuged (Kubota Corp., 2100 centrifuge, Bunkyo-ku, Japan) at 3000 g for 15 min at room temperature. The supernatants were transferred into new 15-mL polypropylene test tubes, and 1 mL of n-hexane was added. The mixture was Vortexed for 15 s and mixed on a platform shaker at a speed of 300 x/min for 10 min, and, finally, centrifuged at 3000 g for 15 min. The supernatants were stored in new 15-mL polypropylene test tubes at 4°C prior to spectrophotometric analysis.

Measurement of Maillard extract absorbance

The absorption of the brown polymers formed in the samples was measured at 420 nm using spectrophotometer (Konica Minolta Holdings Inc., Spectrophotometer CM-3500d, Chiyoda, Japan). Water-soluble extracts were transferred into a quartz cell (Konica Minolta Holdings Inc., CM-A97 2 mm Cell, Chiyoda, Japan) to measure their absorbance at least twice, from both the front and back sides of the cuvette. The absorbance at 550 nm was also measured to correct for any turbidity in the extracts (Morales and van Boekel, 1998).

$$A_{420*} = A_{420} - A_{550} \qquad (1)$$

where 420^* is the corrected absorption at 420 nm.

Measurement of colour

The colours of the supernatants were recorded with a Konica Minolta CM-3500d spectrophotometer. The instrument was calibrated with zero and white calibration prior to use. The colour measurement were obtain in the CIE (Commission Internationale de l'Eclairage) system with $L^*a^*b^*$ values for lightness (L^*, black-white axis), redness (a^*, red-green spectrum), and yellowness (b^*, yellow-blue spectrum).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Maillard reaction

Electrophoretic ribose-minced meat samples were prepared in a similar way as described in Section 2.4. Pure minced meat samples were used as control.

Extraction of water-soluble proteins

Extraction of water-soluble proteins was based on a method as reported by Kim and Shelef (1986).

Heated samples from each universal bottle were removed individually and were blended (Panasonic Corp., MX-337 blender, Kadoma, Japan) with 2.1 mL of distilled water for 15 s. Blended samples were centrifuged at 1,500 g for 20 min. Supernatants were collected and stored at -20°C. The thawed samples were centrifuged again at 5,000 g for 10 min prior to testing.

Electrophoretic procedure

SDS-PAGE was done using a 4% stacking gel and an 8% separating gel according to the method of Laemmli (1970) with a vertical gel electrophoresis unit (Bio-Rad Laboratories Inc., Mini-Protean Tetra Cell, Hercules, USA). Gel samples were mixed with samples buffer containing 2% SDS and 5% β-mercaptoethanol (ratio 1:2). The mixtures were then heated at 95°C for 10 min before loading 10 μL of the mixtures to the gels. The samples were run at 200 V constant for approximately 35 min. Subsequently, the gels were soaked in fixative solution of 10% (v/v) acetic acid and 40% (v/v) methanol for 15 min. The gels were then rinsed with distilled water and stained with staining solution (Bio-Rad Laboratories Inc., Coomassie Brilliant Blue R-250 Staining Solution, Hercules, USA). After about 2 h of staining, the gels were rinsed with distilled water and destained with destaining solution of 10% acetic acid (v/v) for 24 h. The gels were photographed using an imaging system (Fujifilm Holdings Corp., Luminescent Image Analyzer-3000, Minato, Japan). The protein fractions were identified using Bio-Rad Prestained SDS-PAGE Standards, Broad Range (Bio-Rad Laboratories Inc., Hercules, USA).

Statistical analysis

Factorial experiment

A 2×4 design was used to study the effects of two factors, type of meat (X_1) and duration of heating (X_2), on five different outputs (pH, CIE L^*, CIE a^*, CIE b^* and A_{420*}). Two types of minced meats (chicken and pork) and four heating times (15, 30, 45 and 60 min) were tested. Five replicates were carried out for a total of 40 runs.

Hierarchical Cluster Analysis (CA)

Cluster analysis is a multivariate technique, the primary purpose of which is to classify the objects of a system into clusters based on their similarities. The objective is to find an optimal grouping in which the objects within each cluster are similar but the clusters are dissimilar. The most similar objects are first grouped, and these initial groups are merged according to their similarities. Eventually, as the

similarity decreases, all subgroups are fused into a single cluster. Cluster analysis was applied to the data for pH, CIE components, and A_{420*} using a single-linkage method (Richard and Dean, 2002; Alvin, 2002). In the single-linkage method, the similarity between two clusters, A and B, is defined as the minimum distance between a point in A and a point in B:

$$D(A, B) = \min\{d(y_i, y_j), \text{ for } y_i \text{ in A and } y_j \text{ in B}\} \quad (2)$$

where $d(y_i, y_j)$ is the Euclidean distance in Eq. 2.

At each step, the distance between every pair of clusters is found, and the two clusters with the smallest distance (largest similarity) are merged. After two clusters are merged, the procedure is repeated. The result of the hierarchical clustering procedure was displayed using a dendrogram (Richard and Dean, 2002; Alvin, 2002).

Confidence Intervals

Confidence intervals were built based on the pH, CIE components, and A_{420*} data. The main objective of calculating confidence intervals is to provide a reliable interval estimate (at a significance level of α=0.05) to differentiate between the two types of meats.

Analysis of variance

Analysis of variance (ANOVA) and Tukey's test for multiple comparisons were used for analysing the data. SPSS version 16 (SPSS Inc., Chicago, USA) was used for statistical analysis.

Results and Discussion

Preliminary study

In the preliminary phase of this study, four types of reducing sugars were used to react with the proteins in the minced meat: two hexoses (fructose and glucose) and two pentoses (ribose and xylose). A non-reducing sugar, sucrose, served as a control. Upon heating, the sugar-minced meat systems changed from disintegrated minced form into self-standing "gel-like" structures. The hexose-minced meat and sucrose-minced meat systems obtained after heating for 60 min maintained their original white-yellowish colour and mild "roasted" aroma. In contrast, the pentose-minced meat systems darkened in colour (from yellowish after 15 min of heating to brownish after 60 min of heating), and their aromas strengthened (from a mild "roasted" aroma at 15 min to a strong "roasted" aroma at 60 min).

Two distinct characteristics changed upon heating the different types of sugars with minced meat at 95°C: pH and colour values. In the presence of sucrose and hexoses, slight increases in pH values were observed after 15 min of heating, and the pH values remained almost unchanged at the remaining time points (Figure 1). There was no appreciable level of browning observed during heating, and this could be attributed to the low reactivity of these reducing sugars with meat proteins (Ashoor and Zent, 1984; Laroque *et al.*, 2008). This observation is similar to the results obtained upon heating 3% bovine serum albumin without reducing sugars or with sucrose (Easa *et al.*, 1996). In contrast, the pH values decreased throughout the 60 min heating time in minced meat heated with pentoses (Figure 1), and a noticeable level of Maillard browning was observed. The reduction in pH could be attributed to several factors: loss of basic amino groups, formation of less basic compounds by amines (Beck *et al.*, 1990), and condensation between the free amino and carbonyl groups of the substrates, yielding organic acids (Martins *et al.*, 2003). The pH and colour parameters have previously been used as indicators of the extent of the Maillard reaction (Koca *et al.*, 2003). Thus, these results confirm the occurrence of the Maillard reaction during heating of the pentose-minced meat systems. However, in all cases, the ribose-minced meat systems exhibited more browning and caused a lower decrease in pH values than the xylose-minced meat systems. This result is acceptable, because ribose is known to be more reactive than xylose in inducing the Maillard reaction (Laroque *et al.*, 2008).

(a) Minced chicken
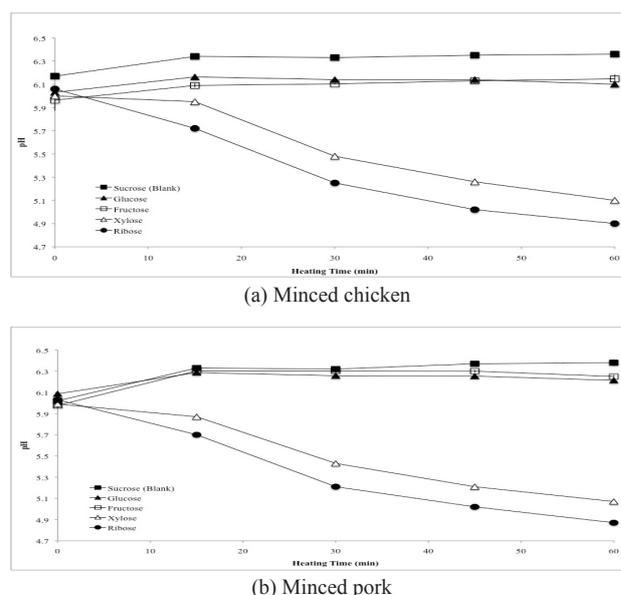
(b) Minced pork

Figure 1. pH as a function of heating time for (a) minced chicken and (b) minced pork in the presence of different types of sugars; ribose (●), xylose (Δ), glucose (▲), fructose (□), and sucrose (■). Bars indicate the standard deviation based on five replicates

Table 1. Maillard reaction parameters for ribose-minced chicken and ribose-minced pork systems as a function of heating time at 95°C

Responding Variables	Samples	Heating Time (min)			
		15	30	45	60
pH	Chicken	5.606 ± 0.030[aA]	5.160 ± 0.039[aB]	4.937 ± 0.020[aC]	4.813 ± 0.041[aD]
	Pork	5.700 ± 0.050[bA]	5.242 ± 0.004[bB]	5.022 ± 0.047[bC]	4.895 ± 0.038[bD]
L^*	Chicken	99.429 ± 0.110[aA]	98.261 ± 0.192[aB]	96.819 ± 0.303[aC]	96.234 ± 0.239[aC]
	Pork	99.808 ± 0.039[bA]	99.241 ± 0.055[bB]	98.804 ± 0.065[bC]	98.551 ± 0.078[bD]
a^*	Chicken	-0.351 ± 0.035[aA]	-1.087 ± 0.060[aB]	-1.430 ± 0.049[aC]	-1.834 ± 0.037[aD]
	Pork	-0.356 ± 0.027[aA]	-1.082 ± 0.059[aB]	-1.524 ± 0.065[bC]	-1.911 ± 0.067[bD]
b^*	Chicken	2.105 ± 0.192[aA]	7.504 ± 0.539[aB]	12.592 ± 0.652[aC]	16.032 ± 0.837[aD]
	Pork	1.311 ± 0.152[bA]	5.113 ± 0.396[bB]	8.722 ± 0.531[bC]	10.975 ± 0.671[bD]
A_{420*}	Chicken	0.025 ± 0.002[aA]	0.097 ± 0.006[aB]	0.165 ± 0.007[aC]	0.215 ± 0.012[aD]
	Pork	0.018 ± 0.002[aA]	0.062 ± 0.006[aB]	0.116 ± 0.006[bC]	0.143 ± 0.007[bD]

Different superscripts (a-b) for the same responding variables indicate significant differences between minced chicken and minced pork at the P<0.05 level.
Different superscripts (A-D) within the row indicate significant differences between the heating time at the P<0.05 level.

Characterisation of the ribose-induced Maillard reaction

Because a preliminary study indicated that the ribose-minced meat system exhibited the highest Maillard reaction yields, in terms of pH reduction and browning intensity, the ribose-induced Maillard reaction was considered for the differentiation of minced chicken and minced pork. The pH of both minced meats decreased throughout the 60 min of heating, and browning of both minced meat samples was indicated by the decrease in CIE L^* and increase in b^* values over the 60 min heating time (Table 1). With longer heating times, the Maillard extracts became darker and more yellowish in colour. As reported by Morales and van Boekel (1998), the formation of browning polymers (melanoidins) led to a decrease in the CIE L^* component and an increase in the CIE b^* component. Ribose-induced minced chicken showed significantly lower (P<0.05) pH and CIE L^* values but significantly higher (P<0.05) CIE b^* values than ribose-induced minced pork over the 60 min of heating. In addition to the direct colour analysis, the browning intensity was also assessed through the spectrophotometric measurement of extract absorbance. The A_{420*} has been widely used as an indicator for melanoidin formation in Maillard systems. The gradual increase in the A_{420*} for both minced meat samples (Table 1) indicates the formation of melanoidin or Maillard reaction products (Morales and van Boekel, 1998) during heating. The A_{420*} for the ribose-minced chicken system was significantly higher (P<0.05) than that of the ribose-minced pork system for 30-min or greater heating times. The low standard deviations obtained for all the readings (Table 1) indicate good reproducibility of the method, even though the minced meat samples were purchased on different days.

Various studies have shown that the Maillard reaction is influenced by the type of substrates and duration of heating (Ames, 1998). Therefore, a factorial experiment was carried out to study the effects of two factors, type of minced meat (X_1) and duration of heating (X_2), on ribose-induced Maillard reaction outputs (pH, CIE L^*, a^*, b^*, and A_{420*}). The results are given in Table 2. The effects of X_1 and X_2 were significant for all the variables. The interaction between the type of minced meat and duration of heating had a significant effect on the Maillard reaction parameters, except for pH. This significant interaction indicates that the factors (type of minced meat and heating time) do not work independently, and, thus, changes in the CIE components and A_{420*} were mainly due to interactive effects. Thus, at a given heating time, the differences in Maillard reaction parameters were attributed to the Maillard reaction substrates in the minced meat, which were mostly the added ribose as well as the meat proteins and amino acids. The coefficients of determination (R^2) for all variables were high (close to 1). This result indicates that the statistical model explained more than 98% of the total variation for all the dependent variables and that it would be possible to use the ribose-induced Maillard reaction to differentiate between the two minced meat types.

Table 2. The results of the factorial experiment for pH, CIE components, and A_{420*} of ribose-induced Maillard reactions in minced chicken and minced pork

Source[a]	Sum of Squares	df	Mean Square	F	P-value
pH					
X_1	0.076	1	0.076	51.710	<0.0001
X_2	3.763	3	1.254	857.036	<0.0001
$X_1.X_2$	0.0001	3	0.00003	0.102	0.958
Error	0.047	32	0.001		
Total	3.886	39			
CIE L^*					
X_1	20.035	1	20.035	1822.857	<0.0001
X_2	29.702	3	9.901	900.787	<0.0001
$X_1.X_2$	6.001	3	2.000	182.007	<0.0001
Error	0.352	32	0.011		
Total	56.090	39			
CIE a^*					
X_1	0.018	1	0.018	13.676	0.001
X_2	12.580	3	4.193	3112.337	<0.0001
$X_1.X_2$	0.019	3	0.006	4.695	0.008
Error	0.043	32	0.001		
Total	12.661	39			
CIE b^*					
X_1	91.684	1	91.684	545.854	<0.0001
X_2	797.917	3	265.972	1583.503	<0.0001
$X_1.X_2$	25.564	3	8.521	50.733	<0.0001
Error	5.375	32	0.168		
Total	920.540	39			
A_{420*}					
X_1	0.016	1	0.016	939.233	<0.0001
X_2	0.143	3	0.048	2740.121	<0.0001
$X_1.X_2$	0.006	3	0.002	108.136	<0.0001
Error	0.001	32	1.742E-5		
Total	0.607	40			

[a] X_1, type of meat; X_2, duration of heating

The chemical compositions of minced chicken and minced pork are shown in Table 3. The compositions

Table 3. Proximate (expressed as % wet basis) and amino acid composition (expressed as g/100 g freeze-dried minced meat) of minced chicken and minced pork

Proximate	Chicken	Pork
Moisture	75.924 ± 0.284[a]	70.503 ± 1.456[b]
Protein	18.587 ± 0.326[a]	16.267 ± 1.901[b]
Fat	5.202 ± 0.038[a]	13.781 ± 0.173[b]
Amino acid		
Aspartate	6.962 ± 0.240[a]	6.336 ± 0.410[b]
Serine	3.750 ± 0.173[a]	3.560 ± 0.132[a]
Glutamate	11.292 ± 0.856[a]	10.978 ± 0.495[a]
Glycine	3.754 ± 0.025[a]	4.254 ± 0.244[b]
Histidine	3.182 ± 0.297[a]	3.612 ± 0.143[b]
Arginine	6.228 ± 0.137[a]	5.854 ± 0.352[a]
Threonine	4.376 ± 0.441[a]	3.870 ± 0.223[a]
Alanine	4.700 ± 0.300[a]	4.344 ± 0.214[a]
Proline	3.266 ± 0.221[a]	3.598 ± 0.311[a]
Tyrosine	3.680 ± 0.285[a]	3.682 ± 0.232[a]
Valine	4.216 ± 0.080[a]	3.736 ± 0.223[b]
Methionine	3.036 ± 0.356[a]	2.774 ± 0.402[a]
Lysine	6.636 ± 0.303[a]	5.688 ± 0.425[b]
Isoleucine	4.512 ± 0.273[a]	3.746 ± 0.182[b]
Leucine	6.528 ± 0.503[a]	6.338 ± 0.155[a]
Phenylalanine	4.088 ± 0.558[a]	3.594 ± 0.272[a]
Cysteine	1.094 ± 0.162[a]	1.006 ± 0.184[a]
Total	81.300 ± 0.637[a]	76.970 ± 1.319[b]

Different superscripts (a-b) within the same row indicate significant differences at P<0.05 level.

of minced chicken and minced pork were similar to those determined by Elgasim and Alkanhal (1992) and Clausen and Ovesen (2001), respectively. Sales and Hayes (1996) indicated that the moisture content was inversely related to the fat content of minced meat. This conclusion is in agreement with our findings that minced chicken with a high moisture content had a low fat content, and the reverse is true for the moisture and fat contents of minced pork. The protein content of minced chicken was significantly higher (P<0.05) than that of minced pork. Higher protein contents contribute to higher browning intensities, because protein is the major substrate of the Maillard reaction (Ames., 1998). The higher protein content of minced chicken could explain the lower CIE L^* values and the higher CIE b^* and A_{420^*} values found in minced chicken samples compared to minced pork samples. In addition, the significantly (P<0.05) lower fat content of minced chicken compared to minced pork (Rhee *et al*., 1996) could also contribute to the different browning intensities of the minced meat samples. Fat content does not play a direct role in the Maillard reaction, but its presence could affect the browning intensity, as it could affect ribose solubility and availability for the Maillard reaction.

The second reason for the different browning intensities of chicken and pork samples is the composition and level of amino acids in the meat. The amounts of aspartate, valine, lysine, and isoleucine were significantly higher (P<0.05) in the minced chicken than in minced pork (Table 3). In contrast, the amounts of glycine and histidine were significantly lower (P<0.05) in minced chicken than in minced pork. Lysine is a basic amino acid that has been regarded as the most reactive amino acid involved in the Maillard reaction, due to the presence

of available amino groups on its side chains (Ashoor and Zent, 1984). Amino acid analysis indicated that the lysine content of minced chicken was significantly higher (P<0.05) than that of minced pork, yielding a higher browning intensity when ribose is heated with minced chicken compared to minced pork.

Another probable reason for the different browning intensities of minced chicken and minced pork is due to the content of water-soluble proteins in the minced meat's myowater. SDS-PAGE was used to examine the water-soluble proteins of control samples (minced meat heated without ribose) that illustrated the density and pattern variations in the protein bands (Figure 2). The variations clearly show the effect of heating at 95°C on the region around 29 kDa. The gradual increase of the band intensity with heating time indicates the progressive release of low molecular weight water-soluble proteins into the minced meat myowater during heating in minced chicken samples. Even though the protein bands were also present in minced pork's myowater, their quantity was less. When ribose was introduced, the low molecular weight protein bands abruptly faded upon heating (Figure 3) that are due primarily to the involvement of the proteins in Maillard reaction with ribose. The higher molecular weight protein bands, above 98k Da, started to fade at heating time above 45 min. Thus the decrease in the water-soluble proteins may have been due to the ribose-induced Maillard reaction and crosslinking (Gerrard *et al*., 2003). Disappearance of the protein regions also reveals that the effects of ribose-induced Maillard reaction were non-subjective to specific proteins.
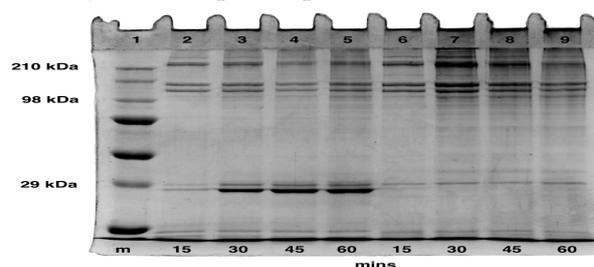
Figure 2. Typical SDS-PAGE results for minced meat samples heated without ribose; minced chicken (lane 2 – 5) and minced pork (lane 6 – 9), heated at 95 ºC for 15, 30, 45 and 60 min. Lane 1 (standard protein marker)
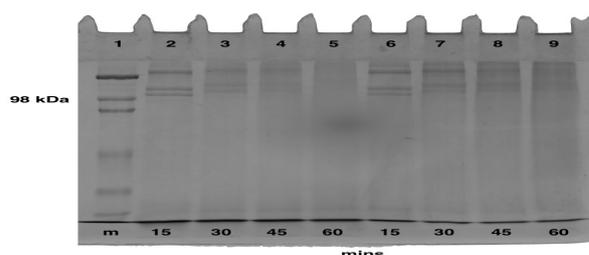
Figure 3. Typical SDS-PAGE results for ribose-induced minced meat samples; minced chicken (lane 2 – 5) and minced pork (lane 6 – 9), heated at 95ºC for 15, 30, 45 and 60 min. Lane 1 (standard protein marker)

Species differentiation and identification

Cluster analysis is a useful statistical tool for distinguishing between physiochemical properties and chemical compositions of foods. It has been used to distinguish the physical properties of Cavendish and Dream banana flours (Abbas *et al.*, 2009) and antioxidant compounds in Bam and Kharak dates (Biglari *et al.*, 2009). Cluster analysis was used to identify similarity groups from the ribose-induced Maillard reaction data of both meat systems. For each heating time, a dendrogram was rendered using the data obtained from the Maillard reactions (results not shown). All the dendrograms rendered showed similar patterns, grouping all ten samplings into two statistically significant clusters, but with different degrees of similarities. Similarities between the two clusters decreased with increased heating time. A dendrogram rendered based on a 60 min heating time that grouped the two types of minced meats into two statistically significant clusters, Cluster 1 (samples 1-5 of minced chicken) and Cluster 2 (samples 6-10 of minced pork), with the lowest similarity (slightly more than 23%) between these two clusters is shown in Figure 4. This analysis revealed that the two types of minced meats show different Maillard reaction parameters (pH, CIE components, and A_{420*}), thus suggesting the potential use of the Maillard reaction to differentiate between the two types of minced meat.
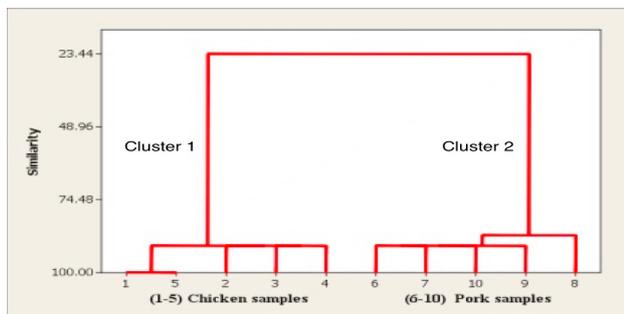
Figure 4. Dendrogram showing clustering of Maillard reaction parameters of ribose-minced chicken and ribose-minced pork systems obtained from a heating treatment of 60 min at 95ºC

Confidence interval analysis has been used for differentiation purposes by other researchers (Kumar *et al.*, 2008; Virkler and Lednev, 2009). Based on the significant difference (P<0.05) between the ribose-induced minced chicken and minced pork after 60 min of heating, as shown in Table 1 and Figure 4, potential differentiation between the two types of minced meats was further assessed by building confidence intervals for all the measured Maillard reaction parameters after 60 min of heating (Table 4). No overlap was observed for the confidence intervals of the two types of minced meat. Thus, there is strong potential to

Table 4. Summary of 95% confidence intervals analysis for Maillard reaction parameters in ribose-minced chicken and ribose-minced pork systems after heating for 60 min at 95ºC

Responding Variables	Chicken			Pork		
	Lower bound	Upper bound	Mean	Lower bound	Upper bound	Mean
pH	4.78	4.83	4.81	4.86	4.93	4.90
CIE L^*	96.16	96.31	96.23	98.50	98.60	98.55
CIE a^*	-1.85	-1.81	-1.83	-1.96	-1.87	-1.91
CIE b^*	15.53	16.54	16.03	10.48	11.47	10.97
A_{420*}	0.21	0.22	0.21	0.14	0.15	0.14

differentiate between the two types of minced meats using the confidence intervals built. The A_{420*}, CIE L^*, and CIE b^* confidence intervals were far apart and, therefore, more suitable for differentiating between the two types of minced meat than the pH and CIE a^* confidence intervals.

Colour spectrophotometry is sensitive, fast, and non-destructive (Norman *et al.*, 2004) and, thus, offers a viable alternative method for minced meat analysis. Because pork is avoided for religious reasons in Muslim countries, it would be beneficial to develop a simple and fast spectrophotometric analysis to determine the types or origins of minced meats. It is possible to use the A_{420*} confidence intervals after 60 min of heating for minced chicken and minced pork to differentiate between the two types of minced meats. The confidence interval analysis showed that the A_{420*} obtained from minced chicken was higher than that obtained from minced pork with 95% confidence. This result indicates that differentiation between minced chicken and minced pork can be done based on the A_{420*} for minced meat samples heated for 60 min with ribose. In this case, samples in which the A_{420*} ranges from 0.21 to 0.22 would be identified as minced chicken, while those with an A_{420*} ranging from 0.14 to 0.15 would be identified as minced pork. A similar strategy could be applied for the confidence intervals after 60 min of heating for CIE L^* and b^*. Further simplification of the analysis could lead to a reduction in cost, which would be valuable to minced meat-related industries and authorities.

Conclusion

Cluster analysis based on data obtained from the ribose-induced Maillard reaction parameters in minced chicken and minced pork revealed that the two types of minced meats exhibited different characteristics that could be classified into different groups. These groupings could be due to the significant differences (P<0.05) in the protein and lysine contents of the minced meats, and the presence of more water-soluble proteins within the minced chicken during heating. Confidence intervals analysis exhibited the potential for utilising Maillard reaction parameters, namely CIE L^*, CIE b^*, and

A_{420*} as indicators for differentiation between the two types of minced meats. Besides the composition of the minced meat, the physicochemical properties, structure, and myowater composition of the minced meat could also play some roles in Maillard reaction that require further investigation.

Acknowledgments

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