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Identification of Waste Chicken Feathers Degradation Products using Pyrolysis Gas Chromatography/Mass Spectrometry

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Abstract

Chicken feathers were separated into barb and rachis fractions and subject to analytical pyrolysis at 550°C in order to identify the protein degradation products and potential toxic compounds that could arise. Under these conditions, cyanide contain compounds, benzene and toluene were identified in each of the chicken feather fractions. The amino acids and the constitutive degradation products of the chicken feather protein were also identified, i.e., alanine, proline, valine, isoleucine and their respective degradation products piperidine, pyrroline, propanenitrile, butanenitrile. Rising concern for the environment and growing demand for safe and sustainable bio-based materials are prompting the search for environmentally friendly and "green" methods to exploit available natural by-products. The chemical, physical, morphological, thermal, electrical and mechanical properties of the chicken feathers and related potential valorization routes have been described previously by the authors. However, identification of their degradation products is necessary to complete the comprehensive description of chicken feather fractions. Hence, this research aims to evaluate the degradation properties of chicken feathers for the production of high value materials. The chicken feather fractions were thoroughly characterized by Py-GC/MS.

Keywords: Chicken feather fraction; Py-GC/MS; Amino acids; Toxic compounds

Introduction

Recycling of waste biomass is an important aspect to protect the human ecological environment and realize the full utilization of resources, especially the recycling and utilization of waste biological polymers [1,2]. Recycling of waste natural polymers can reduce the price of raw materials, but also alleviate the depletion of oil resources, and also ease the human exploitation of the environment. The most abundant natural polymers based on renewable, plant and microbial resources are polysaccharides (cellulose, starch, keratin and chitin) and protein resources [1]. These biological resources can be recycled, with the

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production of biodegradable materials, unlike synthetic polymer materials applied to many areas of human life. For example, keratin which widely exists in the skin and skin derivatives due to its special molecular structure can be used in cosmetics and pharmaceutical industries [1,3]. Feathers contain ~91% keratin protein [4,5] and thus, potentially, feathers can be beneficiated into high-value compounds or products comprised of keratin proteins or keratin fibres [6]. A report in 2014 by the South African Department of Agriculture, Forestry and Fisheries showed that chicken farming activities generated more than 258×10^6 kg of feathers with waste disposal being particularly problematic. Thus, valorization of feathers could be a viable option for sustainable beneficiation of the waste. Characterisation and analysis of the chicken feathers fractions, to assess their suitability as a keratin protein fibre source for high-value applications, are the first steps for valorisation. Thus, physical and morphological properties [7], chemical properties [8] and mechanical properties [6] of chicken feathers have been studied with the objective of ascertaining their valorisation based on the properties. The aim of this study is identification of degradation products of waste chicken feather fractions (barb and rachis) with the ultimate aim of developing valorisation routes for the waste feathers depending on their characteristics.

Materials and Methods

Materials

Sample collection: Chicken feathers were collected from 3-week old broiler/meat chickens at a slaughterhouse in Durban, South Africa.

Preparation of chicken feathers waste: On collection, the feathers were a wet mass of blood, faeces, skin, flesh and other slaughterhouse residues. They were washed with water at 50°C to remove easily removable matters and then dried at 105ºC for 24 hr and conditioned at a relative humidity of 65 ± 2% and a temperature of 20 ± 2°C. After drying, barbs were separated by manual stripping from the rachis. A portion of the chicken feather fractions (barb and rachis) as milled into powder, and the rest left intact. The chicken feather fractions powder as then packed and stored at normal room temperature (20-25°C) into three groups (whole feather, rachis and barb).

Methods

Detailed characterisation using Pyrolysis-Gas Chromatography/Mass Spectrometry: The samples were pyrolysed using a multi-shot pyrolyzer, EGA/PY-3030 D, (Frontier Lab, Japan) attached to a Shimadzu gas chromatograph/mass spectrometer (QP2010 SE). Approximately 100 to 150 µg of the sample were pyrolysed at 550°C for 20 seconds and the interface temperature to the analytical column was set at 350°C. The chromatographic separation of the pyrolysis products was performed using an ultra-alloy capillary column (Frontier Lab, Japan) (30 m × 0.25 mm, 0.25 µm). The injection temperature was set to 280°C and the column flow rate was set to 1.0 mL/min with helium used as a carrier gas. The GC temperature programme used was: (i) hold at 50°C for 2 min; (ii) ramp from 50°C to 200°C at a rate of 3°C/min; (iii) then hold for a further 4 min. The ion source and interface temperatures in the mass spectrometer were set to 200°C and 300°C, respectively. The scan range used for the mass selective detector was from m/z 40-650. The pyrolysis products were identified by comparing their mass spectra with the mass spectra in a NIST library.

Results and Discussion

Py-GC/MS analysis

Typical pyrograms of chicken feather fractions, displayed in FIG. 1 and 2, revealed that the degradation products from both chicken feather fractions are essentially identical with slight differences in the relative amounts of the degradation products.

The main degradation products, shown in TABLE 1, can be roughly classified into four groups, namely, those that contain cyanide groups, benzene groups, amino acid residuals, and amino acid degradation products as shown in TABLES 1 and 2). The mass spectra in FIG. 1 and 2 show that both chicken feather fractions have a sharp peak at around retention time 5 and 14 minutes due to toluene and phenol respectively. In addition, there is a sharp peak at around 4-minute retention time due to pyrolysis of amide groups and a peak at around retention time 3 minutes due to the imine and amine group. Pyrolysis of chicken feather fractions produced many Nitrogen containing (amines, amides, pyridines, nitriles, pyrazoles, pyrroles, pyrazines, indoles and imidazoles) and Sulphur containing (thiazoles) compounds.

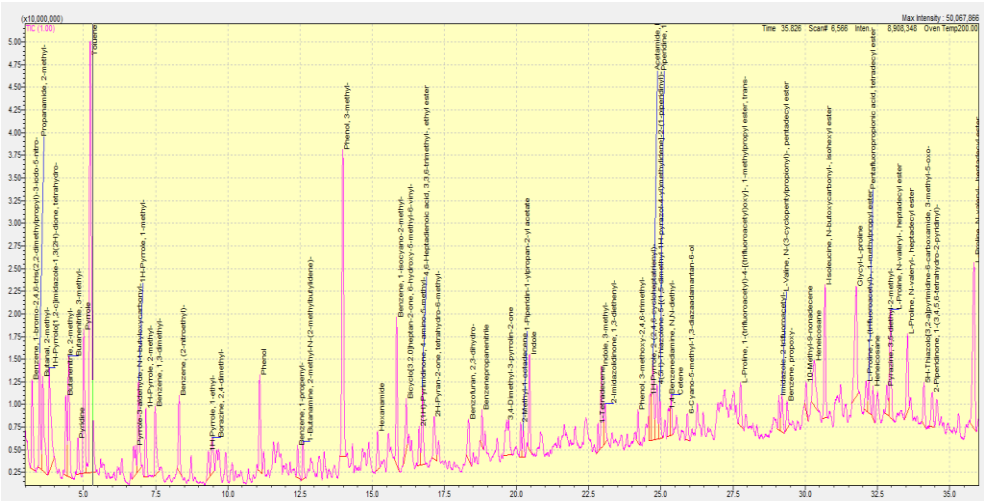

FIG. 1. **Py-GC/MS images of chicken feather barb.**

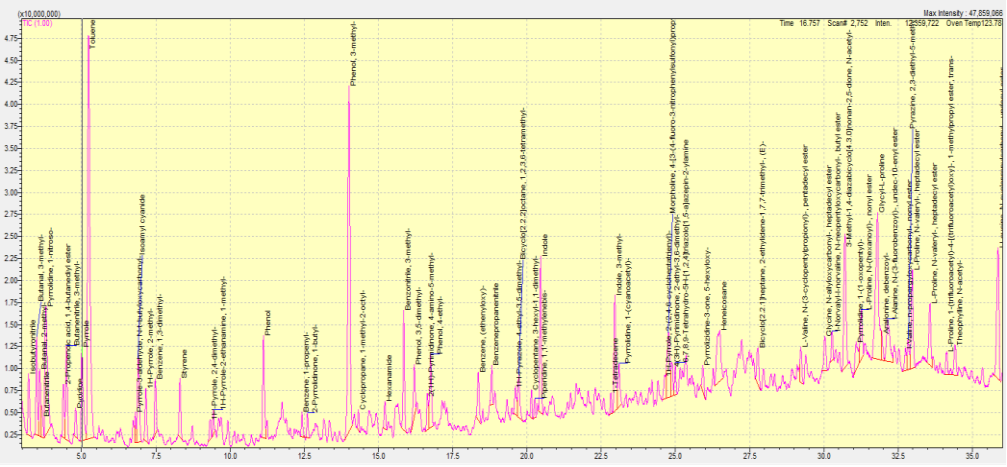

FIG. 2. **Py-GC/MS images of chicken feather rachis.**

TABLE 1. **Area percentage of different compounds found in chicken feather barbs.**

Compound	**Area%**
Cyanide containing compounds	
Benzene, 1-isocyano-2-methyl-	2.7
Cyanotoluene	1.72
Cyanoacetylpiperidine	0.78
Cyano	0.57
Benzene containing compounds	
Benzene, 1-bromo-2,4,6-tris(2,2-dimethylpropyl)-3-iodo-5-nitro-	1.79
Benzene, 1,3-dimethyl-	1.62

Benzene, (2-nitroethyl)-	1.42
Benzene, 1-propenyl-	0.6
Benzene, propoxy-	0.6
Toluene	12.52
Amino Acid Residues	
L-Proline, 1-(trifluoroacetyl)-4-[(trifluoroacetyl)oxy]-, 1-methylpropyl ester, trans-	1.01
L-Valine, N-(3-cyclopentylpropionyl)-, pentadecyl ester	0.84
l-Isoleucine, N-butoxycarbonyl-, isohexyl ester	2.73
Glycyl-L-proline	3.57
L-Proline, 1-(trifluoroacetyl)-, 1-methylpropyl ester	0.7
L-Proline, N-valeryl-, heptadecyl ester	2.47
L-Proline, N-valeryl-, heptadecyl ester	1.83
L-Proline, N-valeryl-, heptadecyl ester	4.71
Amino acid degradation products and other	
Propanamide, 2-methyl-	1.93
Butanal, 2-methyl-	2.42
1H-Pyrrolo[1,2-c] imidazole-1,3(2H)-dione, tetrahydro-	3.05
Butanenitrile, 2-methyl-	1.52
Butanenitrile, 3-methyl-	2.5
Pyridine	0.75
Pyrrole	3.04
Pyrrole-3-aldehyde, N-t-butyloxycarbonyl-	1.03
1H-Pyrrole, 1-methyl-	2.05
1H-Pyrrole, 2-methyl-	1.26
1H-Pyrrole, 1-ethyl-	0.59
Borazine, 2,4-dimethyl-	1.01
Phenol	2.05
1-Butanamine	0.66
Phenol, 3-methyl-	7.37
Hexanamide	0.83
Bicyclo [3.2.0] heptan-2-one, 6-hydroxy-5-methyl-6-vinyl-	1.17
2(1H)-Pyrimidinone, 4-amino-5-methyl-	0.75
4,6-Heptadienoic acid, 3,3,6-trimethyl-, ethyl ester	1.66
2H-Pyran-2-one, tetrahydro-6-methyl-	1.16
Benzofuran, 2,3-dihydro-	0.98
Benzenepropanenitrile	0.53
3,4-Dimethyl-3-pyrrolin-2-one	1.82
2-Methyl-1-octadecene	0.51
1-Piperidin-1-ylpropan-2-yl acetate	0.52
1-Tetradecene	0.39
Indole, 3-methyl-	1.82
2-Imidazolidinone, 1,3-diethenyl-	0.93
Phenol, 3-methoxy-2,4,6-trimethyl-	0.57
1H-Pyrrole, 2-(2,4,6-cycloheptatrienyl)-	1.21
Acetamide, N-(5-methylisoxazol-3-yl)-2-morpholin-4-yl-	2.07
4(5H)-Thiazolone, 5-[(1,5-dimethyl-1H-pyrazol-4-yl) methylidene]-2-(1-piperidinyl)-	1.46
1,4-Benzenediamine, N, N-diethyl-	0.84
Cetene	0.7
Imidazole, 2-trifluoroacetyl-	0.65
10-Methyl-9-nonadecene	0.46
Heneicosane	1.78
Pentafluoropropionic acid, tetradecyl ester	0.68
Heneicosane	0.51
Pyrazine, 3,5-diethyl-2-methyl-	0.72
5H-Thiazolo[3,2-a] pyrimidine-6-carboxamide, 3-methyl-5-oxo-	0.91
2-Piperidinone, 1-(3,4,5,6-tetrahydro-2-pyridinyl)-	0.96

TABLE 2. **Area percentage of different compounds found in chicken feather rachis.**

Compound	Area%
Cyanide containing compounds	
Isopropyl cyanide	1.31
Propyl cyanide	0.61
Isoamyl cyanide	2.12
Benzene containing compounds	
Benzene, 1,3-dimethyl-	1.11
Benzene, 1-propenyl-	0.43
Benzene, (ethenyloxy)-	1.09
Toluene	11.71
Amino acid residues	
L-Valine	0.78
l-Norvalyl-l-norvaline	0.58
L-Proline	1.28
Glycyl-L-proline	6.07
l-Alanine	1.8
l-Valine	0.57
L-Proline	2.42
L-Proline	1.64
L-Proline	0.5
l-Leucine, N-cyclopropylcarbonyl-, undecyl ester	3.68
Amino acid degradation products and other	
Indole	2.96
1-Tetradecene	0.31
Indole, 3-methyl-	2.66
Pyrrolidine, 1-(cyanoacetyl)-	1.09
1H-Pyrrole, 2-(2,4,6-cycloheptatrienyl)-	0.71
Morpholine, 4-[3-(4-fluoro-3-nitrophenylsulfonyl) propyl]-	2.2
4(3H)-Pyrimidinone, 2-ethyl-3,6-dimethyl-	0.81
6,7,8,9-Tetrahydro-5H-[1,2,4] triazolo [1,5-a]azepin-2-ylamine	0.58
Pyrrolizidine-3-one, 5-hexyloxy-	0.58
Heneicosane	2.68
Bicyclo[2.2.1]heptane, 2-ethylidene-1,7,7-trimethyl-, (E)-	0.98
Glycine, N-allyloxycarbonyl-, heptadecyl ester	0.91
3-Methyl-1,4-diazabicyclo [4.3.0] nonan-2,5-dione, N-acetyl-	3.73
Pyrrolidine, 1-(1-oxopentyl)-	1.08
Aralionine, debenzoyl-	0.47
Pyrazine, 2,3-diethyl-5-methyl-	0.7
Theophylline, N-acetyl-	0.92
Butanal, 3-methyl-	1.68
Butanal, 2-methyl-	1.24
Pyrrolidine, 1-nitroso-	4.28
2-Propenoic acid, 1,4-butanediyl ester	1.09
Butanenitrile, 3-methyl-	1.9
Pyridine	0.58
Pyrrole	2.44
Pyrrole-3-aldehyde, N-t-butyloxycarbonyl-	0.48
1H-Pyrrole, 2-methyl-	1.06
Styrene	1.14
1H-Pyrrole, 2,4-dimethyl-	0.41
1H-Pyrrole-2-ethanamine, 1-methyl-	0.4
Phenol	2.16
2-Pyrrolidinone, 1-butyl-	0.4
Phenol, 3-methyl-	9.62
Cyclopropane, 1-methyl-2-octyl-	0.29
Hexanamide	0.5
Benzonitrile, 3-methyl-	2.25

Phenol, 3,5-dimethyl-	1.2
2 (1H)-Pyrimidinone, 4-amino-5-methyl-	0.9
Phenol, 4-ethyl-	1.69
Benzenepropanenitrile	0.7
1H-Pyrazole, 4-ethyl-3,5-dimethyl-	0.7
Bicyclo [2.2.2] octane, 1,2,3,6-tetramethyl-	1.07
Cyclopentane, 3-hexyl-1,1-dimethyl-	0.43
Piperidine, 1,1'-methylenebis-	0.32

TABLE 3 shows a summary of the compounds present in the chicken feather barbs and chicken feather rachis. It is evident from TABLE 1 that the barb has a higher amount of cyanide containing compounds, benzene and toluene. It is also showing that the amino acids are higher in the rachis than the barb.

TABLE 3. **Summary of compounds present in chicken feather barbs and chicken feather rachis.**

Compounds	**Area %**	
	Barb	**Rachis**
Cyanide containing compounds	5.77	4.04
Benzene containing	6.03	2.63
Toluene	12.52	11.71
Amino acid residuals	17.86	19.32
Amino acid degradation products and others	57.82	62.30

Degradation mechanisms of chicken feather amino acids

Apart from the parent amino acids that were present in the pyrolysates, most of the frequently occurring pyrolysis products (hydrogen sulphide, aniline, carbon monoxide, carbon disulphide, carbonyl sulphide, benzonitrile, benzene, acetonitrile, and cyanide containing compounds) are easily explained based on cleavage reactions as illustrated in FIG. 3. Rearrangement reactions shown in FIG. 3a and 3b and bimolecular reactions depicted in FIG. 3c. The presence of sulphur dioxide, carbon disulphide, or carbonyl sulphide is indicative of the presence of sulphur-containing amino acids in protein and peptide materials [9-11].

The fact that derivatives of proline, glycine, alanine, and hydroxyproline are prominent in the pyrolysates confirms that these amino acids are major components in feather keratin. The presence of C16 and C18 fatty acids, n-alk-1-enes and n-alkanes, alkyl-nitriles, and amides is presumable due to degradation of lipid components in feathers since lipds are present in feathers.

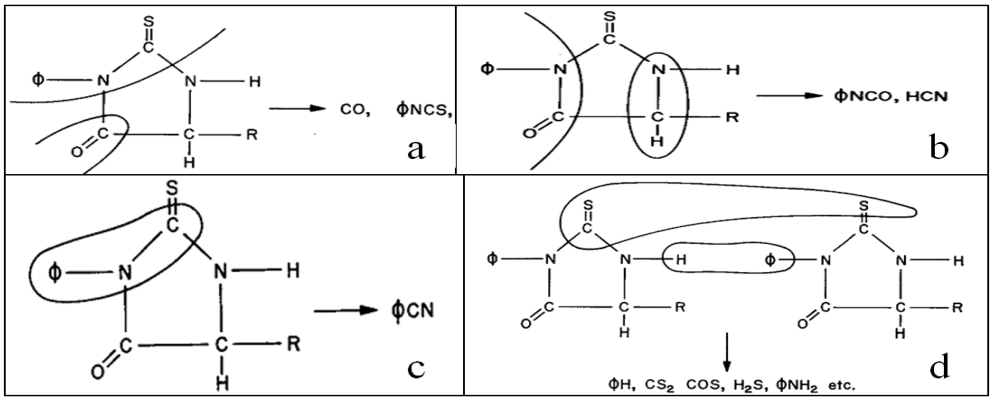

FIG. 3. **Formation mechanism of minor cyclic pyrolysis product.**

In addition to the most common pyrolysis components, there are numerous pyrolysate products derived from the chicken feather fractions that could be regarded as unique and specific for the parent amino acid. These are formed by cleavage of the R group of particular amino acids [10]. For example, as seen in FIG. 4, a cleavage reaction of alanine yields methane whereas ring closure following the loss of the thiocyanate moiety from alanine yields 2-methyl-6 propylamine, pyrimido-pyrimidine, pyrimidinedione, and piperidine derivatives [9].

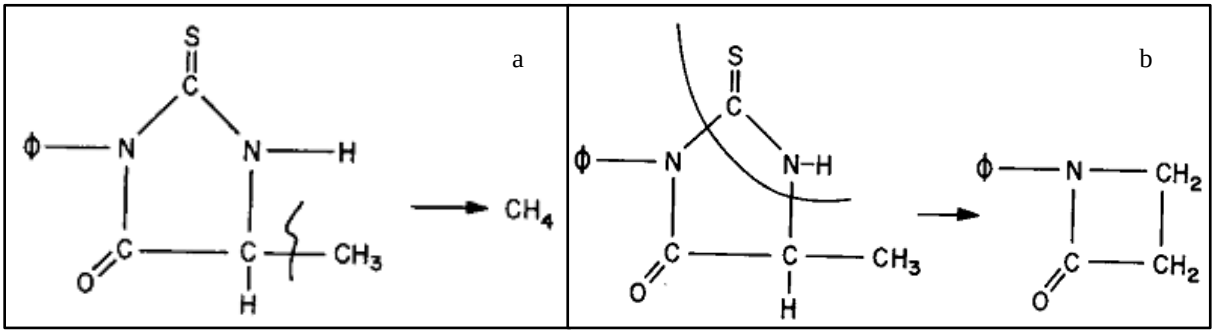

FIG. 4. **Formation mechanism of cyclic pyrolysis product of alanine.**

The presence of phenylisocyanate, benzonitrile, and phenylisothiocyanate derivatives can be used as an indicator of glycine in the pyrolysis of the chicken feather fraction protein (FIG. 5). The other peaks are due to mixture of unresolved components comprised of hydrogen cyanide, hydrogen sulphide and carbonyl sulphide and carbon monoxide [12,13].

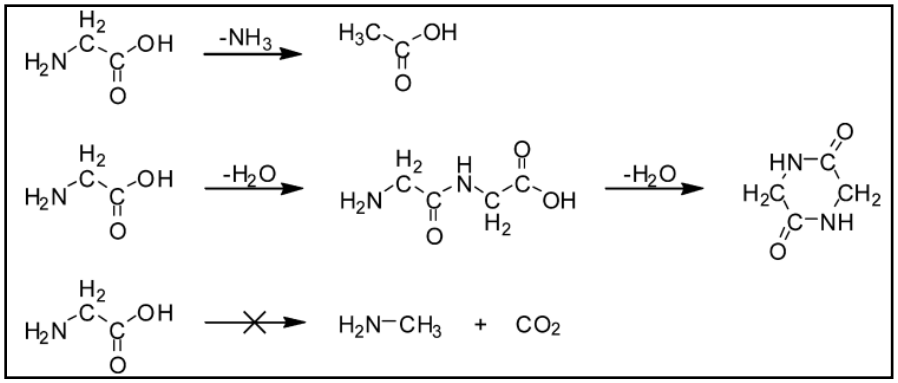

FIG. 5. **Formation mechanism of cyclic pyrolysis product of glycine.**

Phenol, p-cresol, 4-methylphenol, 4-ethylphenol, benzene-acetaldehyde, 4 hydroxyphenethylamine are indicative of tyrosine amino acid (FIG. 6a) [14,15] whereas the presence of toluene, ethylbenzene, ethenylbenzene, benzene-ethane-amine, diphenylethane in the pyrolysate product of chicken feather fractions is indicative of phenylalanine amino acid (FIG. 6b) [10,11,14,15].

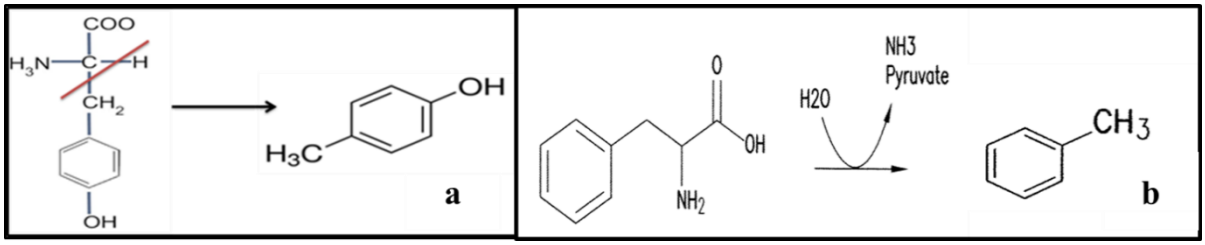

FIG. 6. **Formation mechanism of cyclic pyrolysis product of Tyrosine and Phenylalanine.**

The formation of indole, 3-ethylindole, 3-methylindole, 2,3-dimethylindole, and imidazole as pyrolysate compounds is indicative of the degradation of tryptophan amino acids (FIG. 7a) [16]. The serine protein yields acetonitrile as a pyrolysate compound, this suggests a mechanism that entails dehydration and ring cleavage as illustrated in FIG. 7b [13,16].

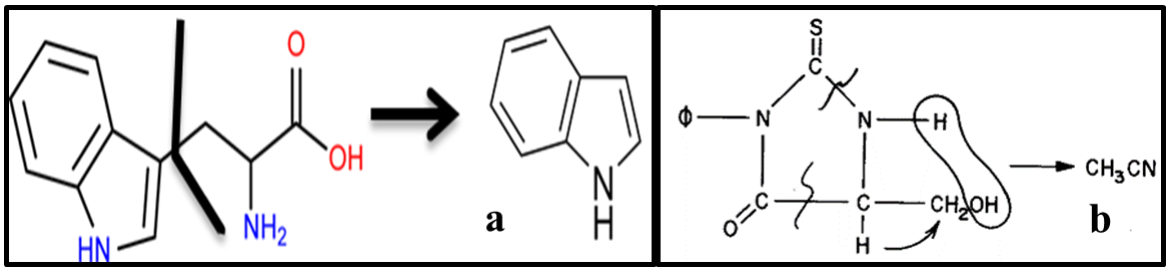

FIG. 7. **Formation mechanism of cyclic pyrolysis product of Tryptophan and Serine.**

Threonine protein yields acetaldehyde instead of ethanol, this could be ascribed to cleavage followed by dehydration. In addition to acetaldehyde, the pyrolysate of threonine amino acid yields acetonitrile and propionitrile in accordance to the mechanism shown in FIG. 8a [13,16]. The presence of pyrroline, pyrrolidine, 2,5 diketopiperazine as degradation products of pyrolysis can be attributed to the decomposition of proline amino acids as shown in FIG. 8b [13,15].

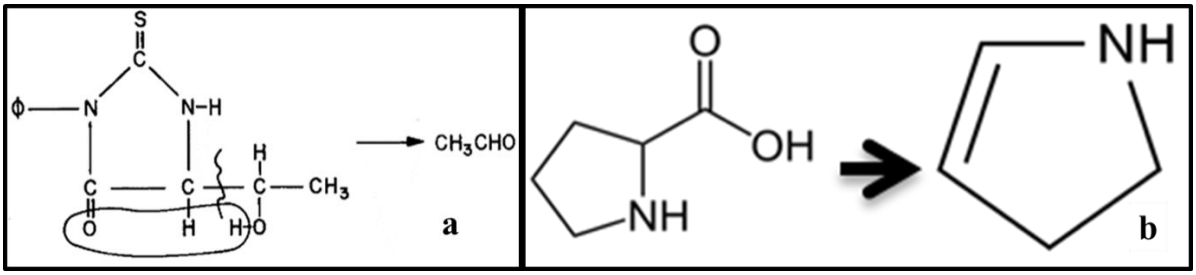

FIG. 8. **Formation mechanism of cyclic pyrolysis product of Threonine and Proline.**

The presence of 2-methyl-propanentrile as a pyrolysate product is an indication of the presence of valine amino acid (FIG. 9a). The presence of five-membered rings (Isobutyronitrile, 2-methyl-1-propanamide, methylamine-N-(2-methyl-propylidene), ethylamine-N-(2-methyl-propylidene), 2-methylpropylamine-N-(2-methyl-propylidene)) is also an indication of valine amino acid in the feathers (FIG. 9b and FIG. 9c) [13,15].

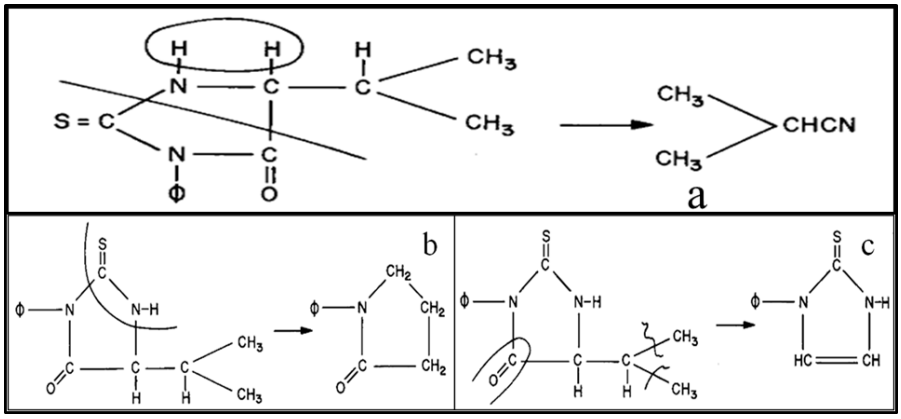

FIG. 9. **Formation mechanism of cyclic pyrolysis product of Valine.**

Methanethiol, vinyl-1-metylthioether, propane-1-methylthioether, 3-methylthio-1-propylamine, 1-propanamide-N-(3-methylthiopropylidene) were the pyrolysate/degradation compounds of methionine (FIG. 10) [15].

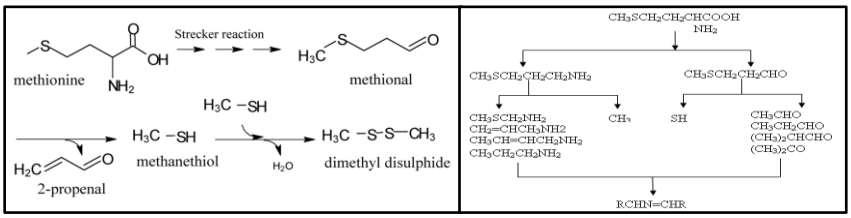

FIG. 10. **Mechanisms of formation of cyclic pyrolysis products of methionine [17].**

Pyrolysis of leucine amino acid in the chicken feathers yields isovaleronitrile, butanenitrile, 3-methylbutylamine,1-butanamide-3-methyl-N-(3-methyl-butylidene), 3,6-isobutyl-2,5-diketopiperazine as pyrolysate compounds (FIG. 11a) [9,18] Whereas that of isoleucine yields 2-methylbutyronitrile, butanenitrile, 2-methylbutylamine, 1-butanamine-2-methyl-N-(2-methyl-butylidene), 3,6-(2-methylpropyl)-2,5-diketopiperazine (FIG. 11b) [18].

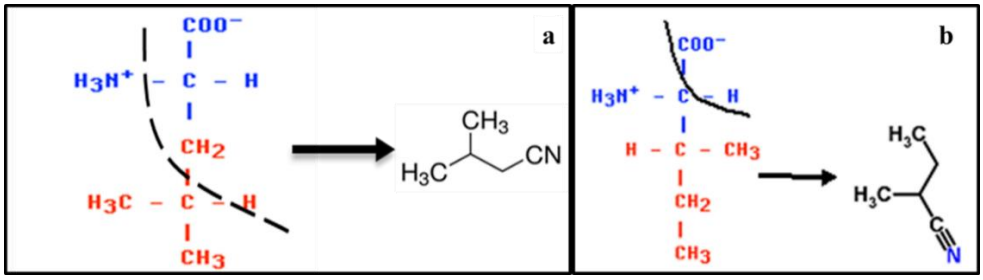

FIG. 11. **Formation mechanism of cyclic pyrolysis product of Leucine and Isoleucine.**

The presence of propane, propene, pyroglutamic acid, pyrroles, 2,3-dehydro-2-piperidone, 2,3-dehydromethyl-2-piperidone can be attributed to the decomposition of glutamic acid/glutamine amino acid present in the proteins of feather (FIG. 12) [18].

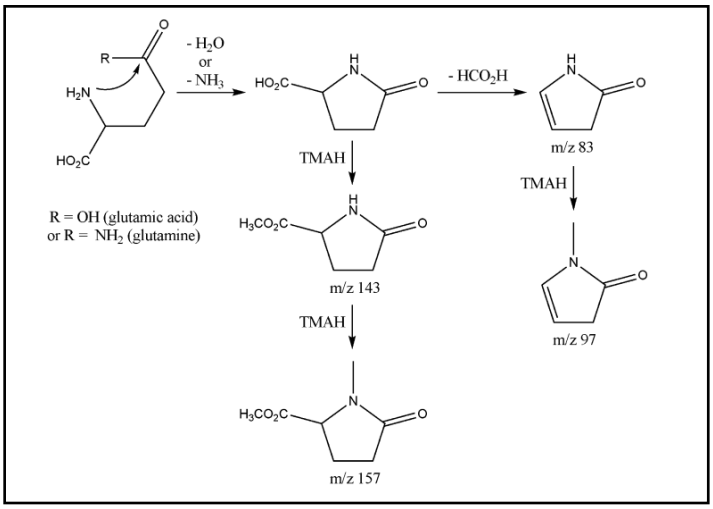

FIG. 12. **Formation mechanisms of cyclic pyrolysis products of glutamic acid and glutamine (Adapted from Gallois, 2007).**

From an extensive report [12] on Py-GC/MS analysis of 19 amino acids: tryptophan resulted in generation of a series of indoles; hydroxyproline gave a dehydration product similar to that of proline; glycine, alanine, histidine, and threonine yielded pyrograms of little diagnostic significance. Fifteen of the 19 amino acids analysed provided some very interesting spectra that perhaps could be used for diagnostic purposes.

The presence of cyanide and benzene containing degradation products implies that pyrolysis of feathers could be further proof of the hazards of burning as a means of disposal of waste chicken feathers. Also, use of feathers for insulation applications may be hazardous during disposal.

Conclusion

The Py-GC/MS pyrograms of the chicken feather fractions revealed the presence of a large variety of degradation products including hazardous/toxic containing cyanide, and benzene moieties. The confirmed the existence of amino acids in feathers that included valine, proline, cysteine, glutamic acid, threonine, leucine, aspartic acid and other amino acids. Future studies will entail ascertaining if Py-GC/MS can be used for diagnostic determination for the presence of feathers in composite materials made from beneficiation of waste chicken feathers.

Acknowledgments

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