A COMPREHENSIVE STUDY ON MICROBIAL ACTIVITY AND LIPIDS EXTRACTION FROM CHICKEN BY-PRODUCT WASTE VIA SUPERCRITICAL CARBON DIOXIDE FOR BIO-DIESEL PRODUCTION

MUHAMMAD KHALISH BIN MOHAMMAD ILIAS

UNIVERSITI SAINS MALAYSIA

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by

MUHAMMAD KHALISH BIN MOHAMMAD ILIAS

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TABLE OF CONTENTS

ACKNOWLEDGEMENT .... **ii**

TABLE OF CONTENTS .... **iii**

LIST OF TABLES .... **vii**

LIST OF FIGURES .... **ix**

LIST OF SYMBOLS .... **xi**

LIST OF ABBREVIATIONS .... **xii**

LIST OF APPENDICES .... **xiii**

ABSTRAK .... **xiv**

ABSTRACT .... **xvi**

CHAPTER 1 INTRODUCTION **1**

1.1 Background 1

1.2 Problem statement 4

1.3 Objectives of study 5

1.4 Scope of study 6

CHAPTER 2 LITERATURE REVIEW **7**

2.1 Chicken by-product waste generation 7

2.2 Existing disposal methods 11

2.2.1 Impact on existing disposal practices 12

2.2.1(a) Antibiotic 12

2.2.1(b) Antibiotic resistance bacteria 16

2.2.2 Environmental and human health concerns 20

2.3 Physicochemical compositions of chicken by-product waste 21

2.4 Biodiesel and biodiesel feedstock 22

2.4.1 Edible oil 23

2.4.2 Non-edible oil 24

2.4.3 Waste sources 25

2.4.4 Chicken by-product waste as a biodiesel feedstock 26

2.5 Lipids extraction technology 26

2.5.1 Mechanical extraction 27

2.5.2 Chemical extraction 29

2.5.3 Physical extraction 31

2.5.4 Advantages and limitations of existing lipids extraction 35

2.6 Supercritical CO_2 technology 37

2.6.1 Supercritical CO_2 as a sterilization technology 37

2.6.2 Supercritical CO_2 as lipids extraction technology 41

2.7 Potential of supercritical CO_2 technology in sustainable utilization of chicken by-product waste 45

CHAPTER 3 METHODOLOGY 47

3.1 Overview of methodology 47

3.2 Sample collection and preparation 47

3.3 Analyses of bacteria in chicken by-product waste 48

3.3.1 Identification of bacteria 48

3.3.2 Antibiotic susceptibility test on the identified bacteria 49

3.4 Sterilization of chicken by-product waste using $scCO_2$ technology 50

3.4.1 Enumeration of viable cells 51

3.4.2 Design of experiment 51

3.4.3 SEM analysis of bacteria 53

3.4.4 Cellular protein analysis of the bacteria 53

3.4.5 Evaluation of the $scCO_2$ sterilization of chicken by-product waste 54

3.4.5(a) Crude fat content 54

3.4.5(b) Protein content 55

3.4.5(c) Moisture content 55

3.4.5(d) Ash content 55

3.5 $scCO_2$ extraction in chicken by-product waste 56

3.5.1 Design of experiment 57

3.5.2 Kinetic and thermodynamic model 59

3.6 Biodiesel production from $scCO_2$ extracted lipids 61

3.6.1 Acid esterification pre-treatment 61

3.6.2 Transesterification 62

3.7 Analyses of lipids and biodiesel from chicken by-product waste 63

3.7.1 Physicochemical properties 63

3.7.1(a) Density 63

3.7.1(b) Acid value and FFA 64

3.7.1(c) Saponification value 64

3.7.1(d) Peroxide value 65

3.7.1(e) Iodine value 65

3.7.1(f) Cloud and pour point 66

3.7.1(g) Calorific value 67

3.7.2 Fatty acids composition 67

CHAPTER 4 RESULTS AND DISCUSSION 69

4.1 Analyses of bacteria in chicken by-product waste 69

4.1.1 Identification of bacteria 69

4.1.2 Antibiotic susceptibility on the identified bacteria 72

4.2 Sterilization of chicken by-product waste using $scCO_2$ technology 76

4.2.1 Factors affecting the $scCO_2$ sterilization 76

4.2.2 Response surface methodology (RSM) 80

4.2.2(a) Regression model of response $scCO_2$ sterilization 80

4.2.2(b) Response surface analyses of $scCO_2$ sterilization 84

4.2.3 Cellular bacteria analyses in sterilized chicken by-product waste 87

4.2.3(a) Morphological analysis ... 87

4.2.3(b) SDS-PAGE analysis ... 89

4.2.4 Evaluation of the $scCO_2$ sterilization of chicken by-product waste ... 90

4.3 Lipids extraction of chicken by-product waste using $scCO_2$ technology ... 91

4.3.1 Factors affecting the $scCO_2$ extraction lipids ... 91

4.3.2 Response surface methodology (RSM) ... 95

4.3.2(a) Regression model of response in $scCO_2$ extraction lipids ... 95

4.3.2(b) Response surface analyses of $scCO_2$ extraction lipids ... 99

4.3.3 Kinetic and thermodynamic modelling of $scCO_2$ extraction lipids ... 101

4.3.4 Analyses of lipids and biodiesel from chicken by-product waste ... 106

4.3.4(a) Physicochemical properties ... 106

4.3.4(b) Fatty acids composition ... 111

CHAPTER 5 CONCLUSION AND FUTURE RECOMMENDATIONS ... 114

5.1 Conclusion ... 114

5.2 Recommendations for future research ... 116

REFERENCES ... 117

APPENDICES

LIST OF TABLES

Page

Table 2.1 Maximum residue limits for each type of antibiotic used in chicken livestock in Malaysia (Hassali et al., 2018). 14

Table 2.2 Antibiotic residue found in various chicken sources worldwide. 15

Table 2.3 Antibiotic resistance bacteria found in various chicken sources worldwide. 18

Table 2.4 Physicochemical composition of chicken by-product waste 21

Table 2.5 Properties of ASTM, CEN, and Malaysia standards for biodiesel production (Chin, 2011). 23

Table 2.6 Advantages and limitations of existing lipids extraction methods..... 36

Table 2.7 $scCO_2$ sterilization technology on various types of materials for inactivation of microorganisms. 40

Table 2.8 Existing $scCO_2$ extraction process from various sources. 44

Table 3.1 Gravimetric composition of chicken by-product waste used in the present study. 48

Table 3.2 The coded and uncoded levels of the independent variables for $scCO_2$ sterilization. 52

Table 3.3 The coded and uncoded levels of the independent variables for $scCO_2$ extraction. 58

Table 4.1 Identification of bacteria in chicken by-product waste using selective and differential agar media. 71

Table 4.2 Antibiotic susceptibility test of the identified bacteria in chicken by-product waste. 74

Table 4.3 The central composite design of experiments for the inactivation of bacteria in $scCO_2$ sterilized chicken by-product waste. 82

Table 4.4 Regression coefficient and their significance of the quadratic model for the $scCO_2$ inactivation of bacteria in chicken by-product waste....83

Table 4.5 Analysis of the variance (ANOVA) of the response surface quadratic model for the inactivation of bacteria in $scCO_2$ sterilized chicken by-product waste....83

Table 4.6 Proximate analyses of untreated and treated chicken by-product waste....91

Table 4.7 Central composite design arrangement and responses....97

Table 4.8 Regression coefficient and their significance of the quadratic model for the $scCO_2$ extraction of lipids yield in chicken by-product waste....98

Table 4.9 Analysis of the variance (ANOVA) of the response surface quadratic model in $scCO_2$ extracted lipids yield in sterilized chicken by-product waste....98

Table 4.10 The second-order kinetic model and thermodynamic parameters for the separation of lipids from chicken by-product waste using $scCO_2$ extraction....105

Table 4.11 Properties of lipids and biodiesel from sterilized chicken by-product waste....110

Table 4.12 FAME composition of lipids and biodiesel from sterilized chicken by-product waste....113

LIST OF FIGURES

		Page
Figure 2.1	Chicken meat production and estimation of chicken by-product waste worldwide from 2018-2020 (OECD/FAO, 2019).	9
Figure 2.2	Chicken meat consumption and estimation of chicken by-product waste worldwide from 2016-2018 (OECD/FAO, 2019).	10
Figure 2.3	Potential of $scCO_2$ technology in sustainable utilization of chicken by-product waste.	46
Figure 3.1	Overview of methodology by objectives in the present study	47
Figure 3.2	Chicken by-product waste used in the present study.	48
Figure 3.3	Schematic diagram for the supercritical CO_2 sterilization unit	50
Figure 3.4	Schematic diagram of the supercritical carbon dioxide extraction system.	57
Figure 3.5	Chemical structure of transesterification reaction using NaOH as catalyst.	62
Figure 4.1	Effect of (a) pressure (temperature: 32 °C, time: 30 min), (b) temperature (pressure: 10 MPa, time: 30 min), and (c) treatment time (pressure: 10 MPa, temperature: 32 ℃) on the inactivation of bacteria in chicken by-product waste using $scCO_2$.	79
Figure 4.2	Response surface plots for the interaction effects of the variables (a) pressure and temperature, (b) pressure and time, and (c) temperature and time, on the inactivation of bacteria in chicken by-product waste using $scCO_2$.	87
Figure 4.3	SEM image of (a) untreated and (b) $scCO_2$ treated bacteria in sterilized chicken by-product waste.	88
Figure 4.4	SDS–PAGE untreated and $scCO_2$ treated bacteria in chicken by-product waste.	89

Figure 4.5 Single parameter studies on (a) effect of pressure, (b) effect of temperature, and (c) effect of treatment time in $scCO_2$ extraction. ...94

Figure 4.6 Interaction effect of (a) temperature and pressure, (b) time and pressure, and (c) time and temperature for lipids extraction............101

Figure 4.7 Lipids separation from chicken by-product waste using $scCO_2$ at pressure 20 MPa with varying temperature and separation time. 103

Figure 4.8 Second-order kinetic model for the separation of lipids from chicken by-product waste using $scCO_2$ with varying temperatures, ranging from 32 to 80 °C. 103

Figure 4.9 Relationships between the absolute temperature *(1/T)* and the second-order rate constant, (a) *ln(k)* and (b) *ln(k/T)* for the separation lipids from chicken by-product waste using $scCO_2$........104

LIST OF SYMBOLS

cm	Centimeter
°C	Degree Celsius
g	Grams
h	Hours
kg	Kilogram
L	Liter
mg	Milligram
mm	Millimeter
min	Minutes
nm	Nanometer
%	Percentage
P	Pressure
sec	Seconds
T	Temperature
t	Time
λ	Wavelength
MPa	Mega Pascal
W	Watt

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
EtOH	Ethanol
EU	European Union
FAME	Fatty Acid Methyl Ester
HCl	Hydrochloric acid
mag.	Magnification
CH_4	Methane gas
MUFA	Mono-unsaturated fatty acids
ppm	Part per million
PUFA	Polyunsaturated fatty acids
RSM	Response surface methodology
SFA	Saturated fatty acids
SEM	Scanning electron microscopy
NaOH	Sodium hydroxide
H_2SO_4	Sulfuric acid
wt.%	Weight percent

LIST OF APPENDICES

Appendix A	Identification of bacteria and antibiotic susceptibility
Appendix B	$scCO_2$ sterilization of chicken by-product waste
Appendix C	$scCO_2$ extraction of lipids from chicken by-product waste
Appendix D	Gas chromatography (GC-FID) of lipids and biodiesel

KAJIAN KOMPREHENSIF MENGENAI AKTIVITI MIKROB DAN PENGEKSTRAKAN LIPID DARIPADA SISA PRODUK SAMPINGAN AYAM MELALUI KARBON DIOKSIDA LAMPAU GENTING UNTUK PENGELUARAN BIO-DISEL

ABSTRAK

Terdapat kebimbangan yang semakin meningkat mengenai pelupusan yang selamat dan penggunaan mampan sisa produk sampingan ayam untuk meminimumkan kesan buruk alam sekitar. Pembuangan sisa produk sampingan ayam ke tapak pelupusan membawa kepada pembebasan bakteria tahan antibiotik ke dalam persekitaran. Walau bagaimanapun, sisa produk sampingan ayam mengandungi kira-kira 50% lemak. Kehadiran isipadu kandungan lemak yang besar (kira-kira 50 wt.%) dalam sisa produk sampingan ayam menunjukkan bahawa ia boleh digunakan sebagai bahan mentah kos rendah yang berpotensi untuk pengeluaran biodiesel. Dalam kajian ini, karbon dioksida lampau genting (KDLG) telah digunakan untuk mensterilkan sisa produk sampingan ayam bagi memastikan pengendalian yang selamat untuk pemprosesan seterusnya ke arah penggunaan mampan sisa produk sampingan. Kehadiran bakteria dalam sisa produk sampingan ayam dan kerentanan antibiotiknya telah dikenalpasti. Keadaan eksperimen pensterilan karbon dioksida lampau genting telah dioptimumkan berdasarkan ketidakaktifan bakteria rintangan antibiotik dalam sisa produk sampingan ayam menggunakan Kaedah Permukaan Tindak Balas (KPTB). Keadaan eksperimen optimum untuk penyahaktifan sepenuhnya bakteria rintangan antibiotik dalam sisa produk sampingan ayam yang disteril menggunakan karbon dioksida lampau genting ialah pada tekanan 18 MPa, suhu 60 ^{o}C, dan masa rawatan 45 min. Karbon dioksida lampau genting (KDLG) digunakan untuk mengasingkan lipid daripada sisa produk sampingan ayam yang disterilkan untuk pengeluaran biodiesel. Keadaan eksperimen pengasingan karbon dioksida lampau genting telah dioptimumkan berdasarkan pengasingan lipid yang maksimum daripada sisa produk sampingan ayam menggunakan Kaedah Permukaan Tindak Balas (KPTB). Pemisahan lipid maksimum yang diperoleh ialah 49.61% pada keadaan eksperimen optimum pemisahan karbon dioksida lampau genting iaitu tekanan 20 MPa, suhu 60 °C, dan masa pemisahan 60 min. Selain itu, model kinetik tertib kedua dan teori eyring telah digunakan untuk menentukan tingkah laku kinetik dan termodinamik dalam pemisahan lipid menggunakan karbon dioksida lampau genting daripada sisa produk sampingan ayam. Kira-kira 79% biodiesel telah disintesis daripada lipid yang diasingkan menggunakan karbon dioksida lampau genting daripada sisa produk sampingan ayam dengan proses transesterifikasi konvensional menggunakan sodium hidruksida sebagai pemangkin. Analisis komposisi sifat fizikokimia dan asid lemak bagi lipid dan biodiesel mendedahkan bahawa lipid sisa produk sampingan ayam boleh digunakan sebagai bahan mentah yang berpotensi untuk pengeluaran biodiesel.

A COMPREHENSIVE STUDY ON MICROBIAL ACTIVITY AND LIPIDS EXTRACTION FROM CHICKEN BY-PRODUCT WASTE VIA SUPERCRITICAL CARBON DIOXIDE FOR BIO-DIESEL PRODUCTION

ABSTRACT

There is an increasing concern on the safe disposal and sustainable utilization of chicken by-product waste to minimize adverse environmental impacts. The disposal of chicken by-product waste into the landfill leads to the release of antibiotics resistant bacteria into the environment. However, chicken by-product waste contains about 50% fat. The presence of the enormous volume of fat content (about 50 wt.%) in the chicken by-product waste indicates that it could be utilized as a potential low-cost feedstock for biodiesel production. In the present study, the supercritical CO_2 ($scCO_2$) technology was utilized to sterilize the chicken by-product waste to ensure safe handling for the subsequent processing towards sustainable utilization of the waste by-product. The presence of bacteria in the chicken by-product waste and their antibiotics susceptibility were identified. The experimental conditions of $scCO_2$ sterilization were optimized based on the inactivation of the antibiotics resistance bacteria in chicken by-product waste using Response Surface Methodology (RSM). The optimum experimental conditions for the complete inactivation of the antibiotics resistance bacteria in sterilized chicken by-product waste were $scCO_2$ pressure 18 MPa, temperature 60 ^oC, and treatment time 45 min. The supercritical CO_2 ($scCO_2$) extraction is employed to separate lipids from sterilized chicken by-product waste for biodiesel production. The experimental conditions of $scCO_2$ separation were optimized based on the maximum separation lipids from chicken by-product waste using Response Surface Methodology (RSM). The maximum lipids separation

obtained was 49.61% at the optimized experimental conditions of $scCO_2$ separation: pressure 20 MPa, temperature 60 °C, and separation time 60 min. Moreover, a second-order kinetics model and Eyring theory were utilized to determine the kinetics and thermodynamics behaviour of $scCO_2$ separation of lipids from chicken by-product waste. Approximately 79% of biodiesel was synthesized from the $scCO_2$ separated lipids from chicken by-product waste with a conventional catalytic transesterification process using sodium hydroxide as a catalyst. Physicochemical properties and fatty acids compositions analyses of lipids and biodiesel reveal that the chicken by-product waste lipids could be utilized as a potential feedstock for biodiesel production.

CHAPTER 1

INTRODUCTION

1.1 Background

There is an increasing interest in sustainable waste materials management to minimize adverse environmental impacts and produce value-added products. Chicken by-product waste is a part of the municipal solid waste, generating a massive amount every day in chicken slaughterhouses. Generally, chicken meat is one of the significant protein sources for human consumption (Górska-Warsewicz et al., 2018). Chicken farming is also increasing rapidly worldwide, with growing demand for animal-source protein for human consumption. Therefore, the generation of chicken by-product waste has also risen promptly. The compositions of the chicken by-product waste include chicken skin, feather, intestine, gizzard, head, blood, and kidney. The most common chicken by-product waste disposal practice is open dumping in a landfill (Shrestha et al., 2017).

The application of veterinary antibiotics in large-scale chicken farming has increased sharply to meet the growing demand for animal protein in human food consumption. Antibiotics as the expedient therapeutic agent are used in treating human infectious diseases. However, there are increasing concerns about human health risk assessment with the antibiotics resistance bacteria in the environment. The antibiotics resistance bacteria can enter the environment through various pathways, including antibiotics disposal, municipal sewage discharge, animal husbandry, and contaminate waste disposal (García et al., 2020; He et al., 2020; He et al., 2021). Since an excessive amount of antibiotics are used as a growth promotor and treating infectious diseases in chicken production, there is ample possibility of remaining unutilized antibiotics and antibiotics resistance bacteria in the chicken by-product waste (Shrestha et al., 2017; He et al., 2021). Therefore, the inappropriate disposal practices of chicken by-product waste lead to the release of antibiotics and antibiotic-resistant bacteria into the environment.

Studies have been conducted on the excretion of the antibiotics residue and antibiotics resistance bacteria in the environment, the potential impact on the environment and human health, and the possible way to minimize the impact (Azzam et al., 2017; García et al., 2020; Shrestha et al., 2017). However, no study has yet to be conducted to determine the potential threat of the existing disposal practice of chicken by-product waste. This is because it was considered safe due to the chicken from slaughterhouse has been undergone cleaning and disinfection protocols to avoid any contamination (Kim et al., 2017).Therefore, it urges to assess the presence of the bacteria and their antibiotics susceptibility in chicken by-product waste and determine an effective alternative disposal method for the safe disposal of the waste. Supercritical CO_2 ($scCO_2$) technology is an effective sterilization method for complete microorganisms inactivation without harming or lowering the product quality (Hossain et al., 2015; Mohd Omar et al., 2018; Soares et al., 2019). It physicochemical breaks down the bacteria's cell walls and extracts the cytoplasmic materials from the bacteria cell (Hossain et al., 2015; Norsalwani et al., 2020; Allafi et al., 2020). Mohd Omar et al. (2017) implemented the $scCO_2$ technology to sterilize oil palm fruits (OPFs). It was found that the $scCO_2$ completely inactivated the lipase-producing bacteria in OPFs at pressure 10 MPa, temperature 80 ^{o}C, and at sterilization time of 60 min.

Global concerns for energy security have increased immensely with the rapid depletion of fossil fuel-based energy (Syimir Fizal et al., 2021; Shaah et al., 2021). Moreover, the rapid development in urbanization, industrial activity, and rapid population growth significantly increases energy demand (Shaah et al., 2021). This consequence has led to moving towards renewable and eco-friendly fuels. Biodiesel is viewed as promising renewable energy, particularly an alternative to fossil fuel energy in the transportation sector. Accordingly, various feedstock such as edible oil, non-edible oil, and animal fats are utilized to produce biodiesel (Syimir Fizal et al., 2021; Shaah et al., 2021; Rajak and Verma, 2018). The utilization of edible feedstock for biodiesel production affects the supply chain of food, edible oil, and animal feed globally (Shrestha et al., 2017; Shaah et al., 2021; Kuleshov and Mahkamov, 2008). Therefore, it urges to search for vast availability and sustainability of adequate supplies and less expensive feedstock for competitive biodiesel production commercially (Shaah et al., 2021; Harish et al., 2021).

Studies reported that the utilization of environmental waste materials for biodiesel production offers various benefits, including cheap biodiesel feedstock, reducing the biodiesel production cost, and minimizing the environmental burden (Rajak and Verma, 2018; Liu et al., 2021). Besides, the advantage of using animal fats for biodiesel production is that the animal fats are highly saturated; therefore, biodiesel produced from animal fats has high cloud points (Liu et al., 2021; Toldrá-Reig et al., 2020). The chicken by-product waste contains over 50 wt.% of chicken fat. Thus, it bears considerable interest in utilizing chicken by-product waste as a feedstock for biodiesel because of its enormous generation and high-fat content. However, it urges the pre-treatment of extracted lipids from waste materials to reduce the free fatty acids content to enhance the produced biodiesel quality (Toldrá-Reig et al., 2020).

The existing methods for separating the lipids from animal fats or stabling the animal prior to biodiesel conversion are rendering process, pyrolysis, distillation, and microwave heating (Liu et al., 2021; Toldrá-Reig et al., 2020). There are several limitations of existing thermal-based animal fats separation processes for biodiesel production, high energy consumption, time-consuming, requires further purification process, high viscosity, and high FFAs content (Toldrá-Reig et al., 2020; Foroutan et al., 2021; Chowdhury et al., 2021). Supercritical carbon dioxide (scCO_2) extraction technology has been successfully used to extract and separate lipids from various matrices (Syimir Fizal et al., 2021; Hossain et al., 2016; Hogan et al., 2021). The fluid CO_2 in a supercritical state is viewed as an ideal solvent for the extraction and separation of lipids because of its low critical temperature (31.1 °C) and moderate critical pressure (7.4 MPa). Besides, the CO_2 is non-toxic, non-flammable, available in abundance, low cost, and environmentally friendly (Hossain et al., 2016). The scCO_2 is a waterless technology, and the extracted lipids do not require any separation process since the CO_2 remains a gas at ambient temperature (Shaah et al., 2021).

1.2 Problem statement

Chicken by-product waste may contain antibiotic and antibiotic resistance pathogenic bacteria. Studies reported that there are several types of antibiotics are mixed with chicken feed. Besides, chicken is a host for some pathogenic bacteria. Therefore, there is a possibility of remaining these pathogenic bacteria in the chicken by-product waste. Thus, it urges to identify the type of antibiotic-resistant bacteria present in chicken by-product waste.

If the chicken by-product waste is disposed of in landfill without treatment, it will pose a potential threat to human health and the environment. Thus, there is an urgency to sterilize chicken by-product waste prior to disposal in order to preserve human health and the environment. Existing sterilization technologies (steam autoclave, microwave, and ethylene oxide) are not effective in denaturing bacteria. Wherein, the $scCO_2$ is an effective method to sterilize heat-sensitive material without degrading the quality. This technology effectively kills bacteria by dissolving cytoplasmic substances in the fluids CO_2 at a supercritical state.

Chicken by-product waste contains fat, carbohydrate, and protein. The fat can be extracted to produce biodiesel, the residue could be utilized as fish feed. The existing solvent extraction method is not eco-friendly because of using toxic solvents. Besides, the extracted crude oil requires further purification and separation process to obtain the fresh oil. $scCO_2$ is an effective extraction method. The distinct advantage of this technology is that lipids extracted using $scCO_2$ do not require further purification and separation process, since CO_2 is gas in ambient temperature.

1.3 Objectives of study

In view of the existing literature review and the problem statements, some important areas for further research on sterilization and extraction of chicken by-product waste are identified. The present work, therefore, focused on the following objectives:

1. To identify the presence of bacteria chicken by-product waste and their susceptibility in various antibiotics.

2. To sterilize chicken by-product waste on the inactivation antimicrobial resistance bacteria using $scCO_2$ sterilization technology.

3. To extract lipids from chicken by-product waste using $scCO_2$ extraction technology.

4. To characterize $scCO_2$ extracted lipids towards the potential of biodiesel production.

1.4 Scope of study

In the present study, the influence of the $scCO_2$ pressure, temperature, and treatment time were determined on the inactivation of the bacteria in chicken by-product waste was evaluated. Subsequently, the experimental; conditions of the $scCO_2$ sterilization technology for the inactivation of the antibiotics resistance bacteria were optimized using response surface methodology. The $scCO_2$ inactivation mechanisms of the bacteria were assessed based on the morphological alteration of bacteria and deactivation of the cellular protein of the sterilized bacteria. Consequently, chicken by-product waste was sterilized at the optimum experimental condition and conducted to separate lipids from chicken by-product waste using $scCO_2$ with varying pressure, temperature, and treatment time. Kinetics and thermodynamics behaviour of the $scCO_2$ separation of lipids from chicken by-product waste was determined. The experimental condition of $scCO_2$ separation of lipids was optimized using response surface methodology. Moreover, the conversion of lipids for biodiesel production was conducted using the catalytic transesterification method. The physicochemical and fatty acids composition in lipids extracted from chicken by-product waste and biodiesel were assessed and compared with biodiesel standards specification. The present study's finding would be harmony to inactive the antibiotics resistance bacteria in chicken by-product waste to conduct safe handling and management of the waste and determine sustainable utilization of the chicken by-product waste towards low-cost biodiesel production.

CHAPTER 2

LITERATURE REVIEW

2.1 Chicken by-product waste generation

Chicken by-product waste (CBW) is considered municipal waste, and its generation depends on chicken consumption and production and is related to consumer demand. The poultry industry recently produced about 243.073 million tons of broiler meat products from 2018 to 2020 (OECD/FAO, 2019). The chicken industry has a massive level of total production that involves large volumes of by-products, including manure, mortalities, and processing wastes requiring regular and prompt disposal. Inedible parts account for approximately 28% of the live weight of a chicken (Sari et al., 2016). Figure 2.1 shows chicken meat production from 2018 to 2020 and its estimated chicken by-product waste. Based on that, the total availability of solid by-products worldwide generated from total production from 2018 to 2020 was estimated to be around 94.53 million tonnes. The USA has higher chicken production and consumption per capita rate worldwide, which is 59.565 million tonnes and 49.1 kg/cap, respectively.

Narrow it down, development countries, cultural, and economic aspects are influenced by chicken consumption and production. Most developed countries have higher production of chicken compared to less developed countries. Developed countries have mature and sophisticated economies, implying that a more significant percentage of the population can afford to consume and have easy access (Nigatu and Seeley, 2015). Thus, higher production significantly affects the waste generation rate in developed countries. When looking into the social aspect, chicken has been commercialized successfully and already became a staple diet worldwide. There were no boundaries to eat chicken among the religion and races compared to pork and beef for the culture. Jayaraman et al., (2012) has found that Malaysia, as a mixed-race country, possesses higher chicken meat consumption due to its religious acceptance of meat commodities. This also might be why Malaysia's chicken meat consumption is higher, which is 49.1 kg/cap, shows in Figure 2.2. The chicken was easy to farm, fast growth rate and optimum cost needed to be contributed to the high economic aspect compared to other animal farms. Consequently, these factors significantly impact the high consumption and the production of chicken meat, thus generating a higher waste from the chicken.

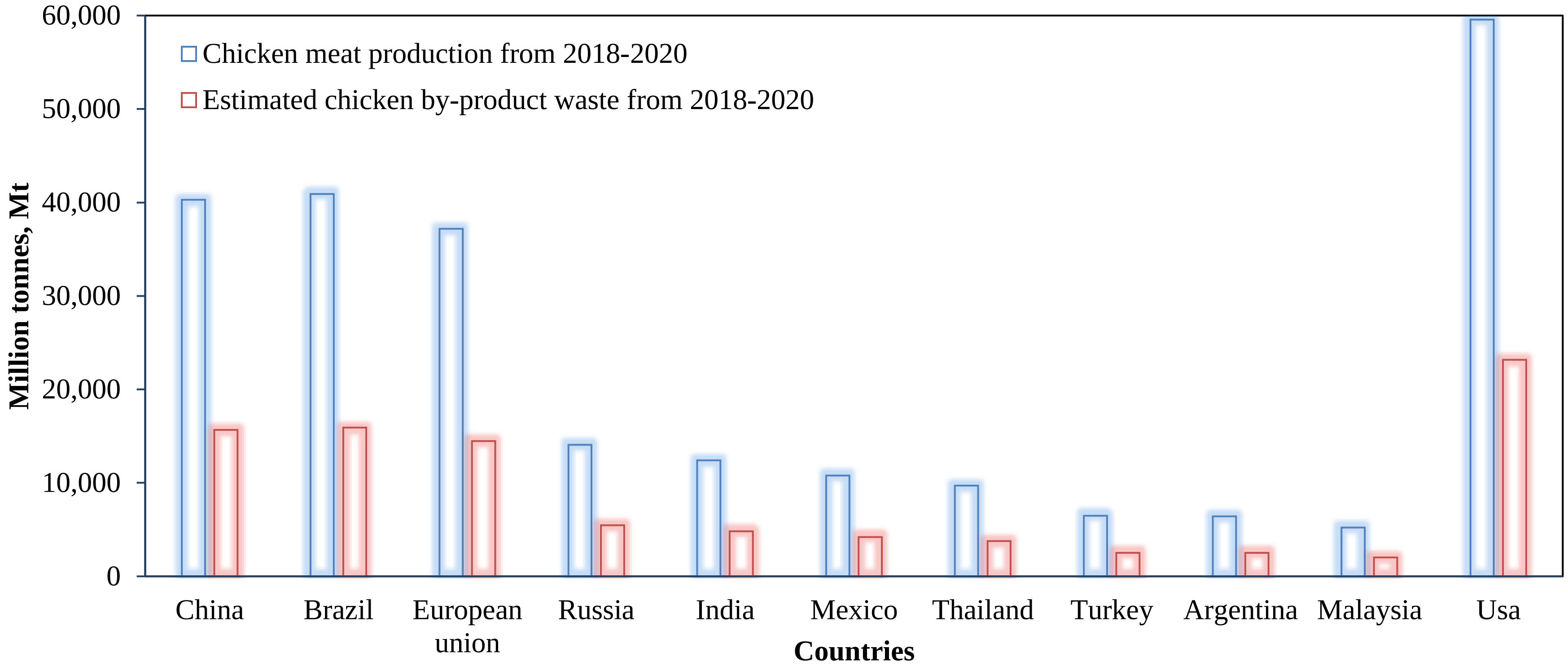

Figure 2.1 Chicken meat production and estimation of chicken by-product waste worldwide from 2018-2020 (OECD/FAO, 2019).

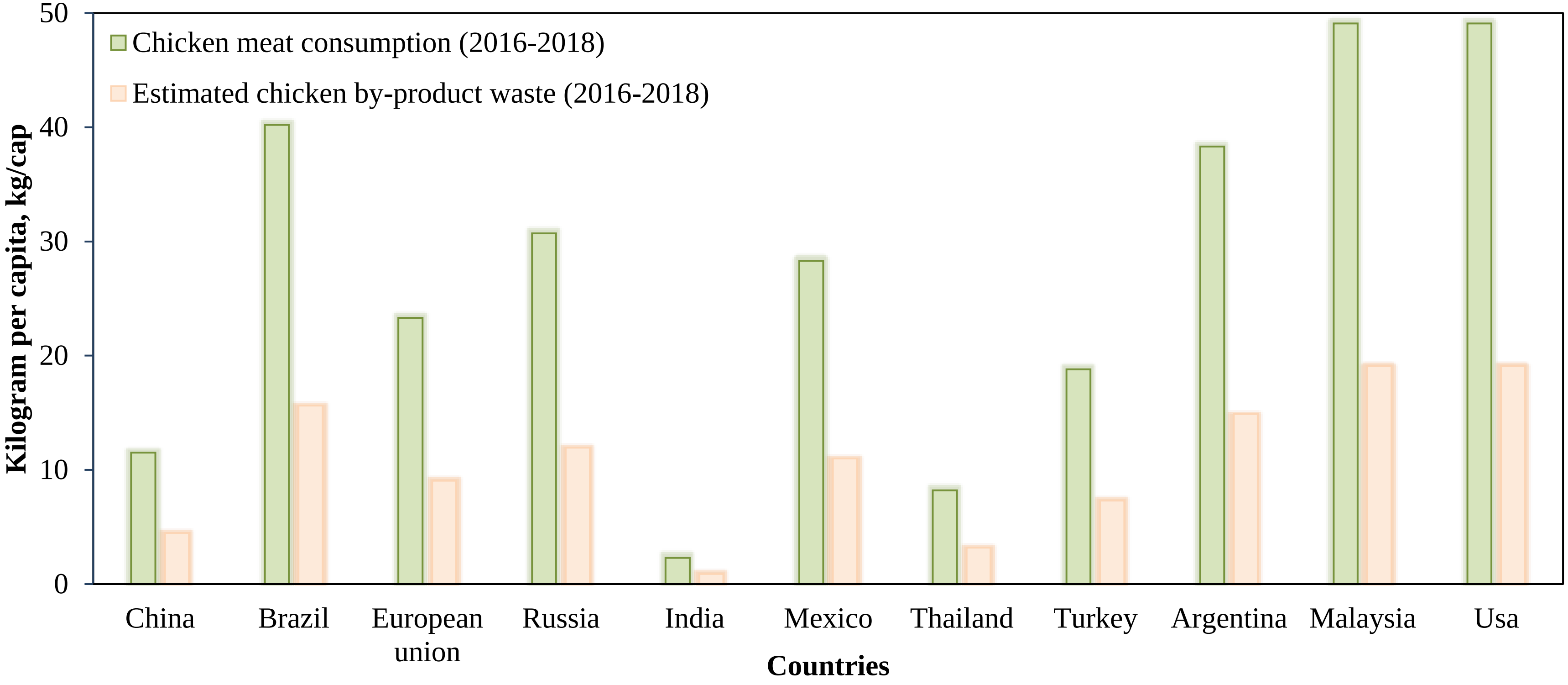

Figure 2.2 Chicken meat consumption and estimation of chicken by-product waste worldwide from 2016-2018 (OECD/FAO, 2019).

2.2 Existing disposal methods

Currently, chicken by-product waste is disposed of via landfill and rendering into fish meals. By-product waste disposal is a significant daily issue for chicken meat producers. Open dumping in landfill areas causes a negative impact on surface water and groundwater contamination, dangerous odour, releases of the greenhouse effect, acidity to soil, and epidemics through stray animals (Koka et al., 2020). In European countries, rendering for feed ingredients is prohibited due to disease transmission, meat hygiene, and ethical concerns about cannibalism (Ozdemir and Yetilmezsoy, 2020). Several existing disposal methods such as incineration and composting given disadvantages. Incineration is considered as a safe method of disposal for biological hazards waste, but it is relatively slow and costly, even when highly efficient incinerators are used (Tápparo et al., 2019). Additionally, it emits greenhouse gases such as CO_2 and CO, as well as particulate matter and soot (Shestakova et al., 2021). According to Prabakaran and Valavan (2021), Composting was introduced to waste management; because it is suitable for soil amendment. But it may lose beneficial sources such as protein and fats content in chicken by-product waste (Lasekan et al., 2013).

However, using protein-rich wastes as a feed ingredient following homogenization via rendering is the most profitable application. European legislation on animal feed establishes a framework for ensuring that feedstuffs do not endanger the health of humans, animals, or the environment (Campos et al., 2020). The legislation establishes rules governing the circulation and use of feed materials, feed hygiene requirements, prohibitions on undesirable substances in animal feed, and genetically modified foods and feed, and conditions for the beneficial use of additives in animal nutrition. The EU prohibits the use of meat and bone meals due to bovine spongiform encephalopathy (BSE) epidemic in 1986 (Woodsgate and Wilkinson, 2021). Subsequently, to reduce the risk of disease transmission by the feed and food chain, legislation has become more stringent regarding slaughter by-products for animal feed. In 2002, the European Union prohibited chicken by-product meals for livestock feed (Regulation (EC) No 1774/2002) (Ozdemir and Yetilmezsoy, 2020). Alternative methods to consider for wastes that are not suitable for processing into the food chain include burying, aerobic composting, or treating for energy production or processing for use as agricultural fertilizers. There is an urgent need to recover feed materials that affect the supply of feed ingredients and manage poultry slaughter waste sustainably and economically.

2.2.1 Impact on existing disposal practices

2.2.1(a) Antibiotic

Antibiotics were used in chickens for three purposes: as a treatment for chickens exhibiting symptoms of infectious disease, as metaphylaxis, which means treating a group of healthy chickens to minimize an expected outbreak of a disease, as prophylaxis to prevent those at risk from becoming infected, and as a growth promoter to increase the weight or size of the chicken (Hassali et al., 2018). According to the Organization for Economic Cooperation and Development (OECD), antibiotic use in food animals will grow globally from 63,151 tonnes in 2010 to 105,596 tonnes by 2030, a 67% increase (Hassali et al., 2018). Globally, the average annual antibiotic dose used to produce one kilogram of chicken meat is approximately 148 mg/kg. USA, China, Brazil, India, and Germany were the top five countries that used antibiotics in their food-animal production, at 23%, 13%, 9%, and 3%, respectively (Laxminarayan et al., 2015; Van Boeckel et al., 2015).

In 2016, agriculture contributed 8.1 percent (MYR89.5 billion) to Malaysia's Gross Domestic Product (GDP), while the livestock industry contributed 11.6 percent. Chicken is the most produced livestock at 305.06 million, a 6.4 percent increase over 2015 (Hassali et al., 2018). Thus, chicken was the most consumed food animal product in Malaysia compared to other food animal products. As a result, antibiotics were used to keep up with demand. The National Pharmaceutical Regulatory Agency (NPRA), Malaysia's drug control authority, was the primary organization responsible for establishing regulation for all antibiotics. Nonetheless, antibiotics used for disease prevention and growth promotion were placed under the control of the Ministry of Agriculture's Department of Veterinary Services (DVS) under the Feed Act 2009. According to the NPRA's 2017 list of registered veterinary products, 458 antibiotics (66.6 percent) were registered for use in livestock industries. Nonetheless, the World Health Organization (WHO) has designated a portion of the registered antibiotics as critical for human health, and their use in the veterinary sector should be restricted. Table 2.1 describes the type of antibiotic used in chicken livestock and its maximum residue limit (μg/kg).

According to EU regulations, a maximum residue level (μg/kg) for permitted antibiotics in animal products should not exceed. Antibiotic residue in chicken meat is a source of concern for human health because it has a detrimental effect on consumer health (Jammoul and El Darra, 2019). Previous studies have found antibiotic residue in various chicken samples worldwide, shows in Table 2.2. Despite this, it has been revealed that several antibiotics exceeded the allowed limit. Additionally, an illegal antibiotic, chloramphenicol, was discovered in the chicken product in Iran (Tajik et al., 2010). The use of chloramphenicol in chickens is restricted by EU regulation due to its adverse effect on the human. Thus, it suggested that antibiotics have been used abusively and in violation of existing legislation. These findings showed high possibilities that chicken by-product waste may also contain antibiotic residue. For this reason, when animals orally consume or are injected with antibiotics, antibiotic residues accumulate in muscle tissues, blood, internal organs, eggs, and even fluids such as milk.

Table 2.1 Maximum residue limits for each type of antibiotic used in chicken livestock in Malaysia (Hassali et al., 2018).

Type of antibiotic used in chicken livestock in Malaysia	Maximum residue limits (MRLs) (μg/kg)
Aminoglycoside	50
Amphenicol	100
β-lactam	10
Carboxylic	100
Cephalosporin	100
Fluoroquinolone	100
Lincosamide	150
Sulphonamide	100
Tetracycline	100

Table 2.2 Antibiotic residue found in various chicken sources worldwide.

Countries	Samples	Antibiotic	Level of residues (μg/kg)	References
Nigeria	Frozen chicken	Tetracycline	1,046.3–1,158.9	Olusola et al. (2012)
Malaysia	Breast	Sulfonamides	6–62	Cheong et al. (2010)
Egypt	Liver	Oxytetracycline	80–193	Hussien et al. (2013)
	Fresh and frozen broiler fillet		156–900	
	Breast	Tetracycline	124–5,812	Salama et al. (2011)
	Thigh		107–6,010	
	Liver		107–6,010	
Pakistan	Breast	Sulfonamides	20–800	Mehtabuddin et al. (2012)
	Kidney	Oxytetracycline	30–3,880	Shahid et al. (2007)
	Muscle	Sulfonamides	0.02–0.52	Mehtabuddin et al. (2012)
Iran	Kidney	Chloramphenicol	0.54	Tajik et al. (2010)
	Liver		155	
	Liver	Enrofloxacin	18.34	Salehzadeh et al. (2007)
	Kidney		26.06	
	Muscle		18.32	
Portugal	Muscle	Fluoroquinolones	37.6–164.7	Pena et al. (2010)
China	Muscle	Quinolones	0.7–43.6	Zhao et al. (2009)
Turkey	Muscle	Quinolones	31	Er et al. (2013)

2.2.1(b) Antibiotic resistance bacteria

In recent years, much evidence suggests the contribution of antibiotic use and antimicrobial resistance (AMR) from animals to the overall AMR burden has emerged (WHO, 2014; Marshal and Levy, 2011). Excessive use of antimicrobials in animal food production is a factor. The magnitude of demand is likely to grow significantly in the coming years, as agricultural techniques in most developing countries become more intensive (Van Boeckel et al., 2015). Generally, AMR is a term that refers to the ability of certain bacteria to thrive and protect themselves despite being subjected to the antibiotic. When an antibiotic is used, it kills only susceptible bacteria; other bacteria can survive and grow into resistant bacteria because of genetic mutation. Then, resistant bacteria multiplied, and some bacteria can transmit their drug-resistance DNA to other bacteria (Subedi et al., 2018; Pang et al., 2019). When these antibiotic-resistant bacteria were treated again, only a tiny number of bacteria were killed, while others developed resistance. As a result, several antibiotics were rendered ineffective in treating diseases caused by these bacteria. Thus, the chicken can be found as a host to the multidrug resistance bacteria (García et al., 2020; Hasan et al., 2011; Xiong et al., 2018).

Poultry is one of the most widely farmed food sectors worldwide, with over 90 billion tonnes of chicken meat produced yearly (FAOSTAT, 2017). The primary reasons for this are the low manufacturing costs and the lack of cultural or religious constraints on its consumption. In most countries, a wide variety of antimicrobials are used to produce poultry (Agunos and Carson, 2012; Landoni and Albarellos, 2015), primarily oral, to prevent and treat disease and enhance growth and productivity (Page and Gautier, 2012). Antimicrobial indiscriminate usage in animal agriculture is anticipated to promote AMR development in pathogens and commensal organisms.

Apart from the worries concerning the rise of AMR in microorganisms associated with chicken farming, there are also issues about antibiotic residues in manure (Ngogang et al., 2021) and eggs (Agunos et al., 2021). Additionally, AMR in chicken pathogens is anticipated to result in economic losses due to antimicrobial resistance and the burden of untreated chicken disease.

Undoubtedly, the presence of antibiotic residue in the chicken sample leads to the revolution of antibiotic resistance bacteria. Previous studies have found *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella spp.*, *Campylobacter spp.*, *Yersinia enterocolitica*, *Enterococcus spp.*, *Proteus spp.*, *Staphylococcus aureus*, and *Vibrio cholarae* are resistant to multi-drug, is a host in the various sample of chicken, shown in Table 2.3. Due to the diversity and dispersion of cross-contamination in slaughterhouses, preventing or eliminating it is difficult and requires detailed information about the slaughterhouse and contamination routes at various points along the production chain (Shang et al., 2019). Thus, chicken by-product waste may be contaminated with these antibiotic-resistant bacteria.

Table 2.3 Antibiotic resistance bacteria found in various chicken sources worldwide.

Sources	Bacteria	Type of drug resistance	References
Diseased and freshly dead chicken	Pseudomonas aeruginosa	Ampicillin, Lincomycin, Nalidixic acid	Farghaly et al. (2017)
Diseased chicken		Ampicillin, Cefoxitin, Lincomycin, florofenicol, Tetracycline, Sulfamethoxazole-trimethoprim	Hassan et al. (2020)
Environmental and chicken from the farm	Escherichia coli	Ampicillin, Erythromycin, Tetracycline	Enany et al. (2019)
visceral organs of sick chickens		Amoxicillin, Doxycycline, Sulphamethoxazole-trimethophorim, Spectinomycin, Florfenicol	Ibrahim et al. (2019)
Chicken cloacal, litter, and feed	Salmonella spp.	Tetracycline, Chloramphenicol, Ampicillin	Alam et al. (2020)
Chicken meat from retail		Sulfisoxazole, Ofloxacin, Chloramphenicol, Tetracycline	Zhang et al. (2018)
Cloacal, water supply, and meat	Campylobacter spp.	Amoxicillin, erythromycin, tetracycline, ciprofloxacin, norfloxacin, azithromycin	Neogi et al. (2020)
Chicken cloacal		Erythromycin, azithromycin, Nalidixic acid, ciprofloxacin, Tetracycline, clindamycin	Tang et al. (2020)

Table 2.3 (Continued)

Sources	Bacteria	Type of drug resistance	References
Retail poultry meat	*Yersinia enterocolitica*	Ampicillin, Amoxicillin/Clavulanic acid, Cefazolin	Peng et al. (2018)
Retail chicken and processed meat		Ciprofloxacin, Gentamicin, Cefotaxime, Streptomycin, Cephalothin, Ampicillin	Younis et al. (2019)
Conventional and organic chicken carcasses	*Enterococcus spp*.	Tetracycline, Erythromycin, Rifampicin	Kin et al. (2018)
Chicken farm	*Proteus spp.*	Ampicillin, Gentamicin, Nalidixic acid, Tetracycline, Trimthophorim	Nahar et al. (2014)
Breeding, Laying hen and broilers	*Staphyloccocus aureus*	Penicillin, Amoxicillin/Clavulinic acids, Oxacillin, Cefoxitin, Tetracycline, Ciprofloxacin, Erythromycin, Clindamycin	Benrabia et al. (2020)
Chicken carcasses		Penicillin, Ciprofloxacin, Gentamicin, Levofloxacin, Erythromycin, Clindamycin, Tetracycline	Okorie-Kanu et al. (2020)
Poultry sources	*Vibrio cholerae*	Erythromycin, Ampicillin, Rimphamicin, Kanamycin, Penicillin, Tetracycline	Akond et al. (2008)

2.2.2 Environmental and human health concerns

Antimicrobial resistance (AMR) is becoming an increasingly severe public health problem. Recent research indicates that interactions between pathogens and antibiotic residues in various environmental matrices contribute to the development and spread of AMR. Antibiotic residue levels in chicken products have been analyzed globally. Once in the environment, antibiotic residues can have a deleterious impact on biota at various trophic levels and on human health through the consumption of contaminated food and water, by contributing to the growth of the resistant bacterial population, and by maintaining the selective pressure that results in the development and/or spread of resistance in various compartments of the environment (Jechalke et al., 2014; Yannarell and Mackie, 2012).

Antibiotic residues can be absorbed by plants, interfering with physiological processes and causing potential ecotoxicological effects. As a result of this, numerous chronic and acute toxicity tests revealed the impact of antibiotics on photosynthesis (chloroplast gene expression and cell proliferation) and mitochondria (oxidative stress response in plants) (Wang et al., 2015). Antibiotics in agricultural soils may also delay germination or reduce biomass, reducing yield in farmland fertilized with contaminated manure (Minden et al., 2017). Additionally, antibiotic residues can disrupt the human microbiome and result in health problems such as allergic reactions, chronic toxic effects from prolonged exposure, and disruption of digestive system functions (Ben et al., 2019; Van Boeckel et al., 2015; Larramendy and Soloneski, 2015). This highlighted existing disposal practices that have contributed to the transmission of antibiotic-resistant bacteria from chickens to humans (Achi et al., 2021; Alam et al., 2019). Ergo, a proper technique to overcome these problems should be implied for disposal and utilization of chicken by-product waste.

2.3 Physicochemical compositions of chicken by-product waste

Chicken by-product waste (CBW) is inedible or unwanted parts, processed from slaughterhouses to produce ready meat chicken. In general, there were a few crucial steps to process live chicken which were receiving and slaughter; cleaning and evisceration processing and preparation, and packaging and shipping (Mahler et al., 2015). Through out of these processes, a different by-product produced and the composition of chicken by-product waste from average l.9 kg live weight of chicken is showed in Table 2.4 (Sari et al., 2016). Based on the overall composition of chicken by-product waste, it was found that chicken by-product waste roughly consisted of 34.2% dry matter, including 51.88% crude protein, 41.08% fat, and 6.32% ash (El Boushy et al., 2000).

Table 2.4 Physicochemical composition of chicken by-product waste (Sari et al., 2016; El Boushy et al., 2000).

Physicochemical composition	Percentage, %
Physical	
Feather and blood	7.38
Head	2.55
Feet	4.23
Internal package	6.15
Heart, liver, gizzard	4.36
Abdominal fat	1.59
Miscellaneous	1.64
Chemical	
Protein	51.88
Fat	41.08
Ash	6.32

2.4 Biodiesel and biodiesel feedstock

Biodiesel is an alternative to diesel fuel that has been produced from edible oil, non-edible oil, animal fat, and sources from wastes and is viewed as non-toxic and biodegradable (Kiran and Hebbar, 2021). Furthermore, biodiesel burns cleaner than traditional petrol diesel, means it appreciably produced less harmful exhaust emission and increase the energy security (Yaqoob et al., 2021). Thus, it may improve air and lower the greenhouse effect. The existing diesel engine is compatible with biodiesel for the engine prospect, where no modification is needed (Uludamar, 2018). Biodiesel contains a high cetane number that gives better engine combustion and is less explosive than diesel (Bemani et al., 2020). Utilizing biodiesel can broaden the economic opportunity because it can be produced in various types of sources. Therefore, it gives potential markets for rural communities, farmers, and industries.

To produce quality biodiesel, standards have been established to qualify the specification of biodiesel produced. The standards for biodiesel are critical for its commercialization and market entry. As a result, it is critical to have a strong standard in place to regulate quality and provide assurance to engine manufacturers and end-users (Yusoff et al., 2021). ASTM D6751-America Standard Testing Materials (ASTM), and EN 14214-European committee for standardization (CEN) is an international standard that has been extensively referred to in biodiesel production research in the various source. The Standard and Industrial Research Institute of Malaysia (SIRIM Berhad) established the quality standards for palm biodiesel in Malaysia, where the MS 2008:2008 was published in 2008. The MS 2008:2008 is largely based on European Standards (EN 14214), with some minor modifications suggested by the Technical Committee on Petroleum Fuels (Chin, 2011). Table 2.5 shows the comparison between ASTM D6751, EN 14214, and MS 2008:2008.

Table 2.5 Properties of ASTM, CEN, and Malaysia standards for biodiesel production (Chin, 2011).

Properties	Unit	ASTMD 6751	EN 14214	MS 2008: 2008
Viscosity, 40 °C	mm^2/sec	1.9-6.0	3.5-5.0	3.5-5.0
Density	kg/m^3	-	860-900	860-900
Cetane number	-	47 min.	51 min.	51 min.
Flash point	°C	130 min.	120 min.	120 min.
Acid number	mg KOH/g	0.80 max.	0.5 max.	0.5 max
Free glycerine Total glycerine	wt.% wt.%	0.02 max. 0.24 max.	0.02 max. 0.25 max.	0.02 max. 0.25 max.
Iodine number	-	-	120 max.	110 max.
Cold filter plugging point (CFPP)	°C	-	Location and season dependent	-
Methanol content	% mass	0.2 max.	0.2 max.	0.2 max.
Phosphorus content	% mass	0.001 max.	0.001 max.	0.001 max.
Ester content	% mass	-	96.5 min.	96.5 min.
Water content	mg/kg	-	500 max.	500 max.

2.4.1 Edible oil

Edible oil is referred to any fat from sources that are suitable for food use. Mainly, sources from vegetable oil are used as biodiesel feedstock. Edible oil is also known as first-generation biodiesel. The use of edible oil as a biodiesel feedstock was the most favored at the beginning of the biodiesel production era due to the accessibility of the feedstock sources and the relatively simple conversion required and suitable substitute for diesel fuel (Lukić et al., 2016). Currently, over 90% of total biodiesel is derived from edible biomass (Oh et al., 2018). But, the risk arises because edible oil was the primary source in the food supply, and it became a competition (Keneni and Marchetti, 2017). High usage of edible oil as a biodiesel feedstock causes an increase in the cost of food products. Furthermore, appropriate environmental conditions and limited area to grow the crops are also contribute to complications for the production of biodiesel from edible oil (Singh et al., 2020). Thus, these downsides became an inconvenience and shifted to further substitution of new sources for biodiesel production.

2.4.2 Non-edible oil

Non-edible oil is the second generation of biodiesel, and castor oil, neem oil, Karanja Oil, rubber seed oil, and jatropha oil were categorized as non-edible oil. The presence of toxic components in the oil has made it unsuitable for human consumption (Demirbas et al., 2016). Non-edible oil as biodiesel feedstock seems very promising in overcoming the obstacles from first-generation feedstock, such as competition between food and fuel and environmental and economic issues related to edible oil sources (Mahdavi et al., 2015; Aransiola et al., 2014; Tariq et al., 2012). Furthermore, the main advantage of using second-generation feedstock for biodiesel production is that the crop itself did not rely on food supply and is not specific to agricultural land required (Singh et al., 2020). However, it still needs area to grow the non-edible crop and compete with food crops for land. Therefore, a new source to overcome this problem has been looking for economically feasible and simply accessible to a greater extent.

2.4.3 Waste sources

The increase in the global population increases the volume of food industry wastes and by-products (Pikula et al., 2020). Biodiesel production from waste sources is labeled as third-generation feedstock. The foremost advantage of utilizing third-generation feedstock for biodiesel production is the lesser impact on farming land and barely affecting the food supply. Biodiesel production from waste sources enables their effective utilization while also providing additional environmental, economic, and food security benefits (Foteinis et al., 2020). Waste sources also contain a high amount of oil compared to other generation feedstock (Fasanya et al., 2021). The use of waste sources as biodiesel feedstock positively impacts the environment by decreasing the amount of waste being disposed to landfills and reducing the greenhouse effect. Plus, increasing animal consumption and production of meat shows as a possible and dependable waste source for producing low-cost biodiesel (Barua et al., 2020).

Even though oil recovered from waste sources usually contains high free fatty acid (FFA) that might affect biodiesel production, it can be solved by using the pre-treatment process (Idowu et al., 2019). Overall, these possible sources seem to beat all the issues that occurred on previous generation feedstock related to the food chain, economic feasibility, availability, and flexibility with environmental parameters. However, the lack of a centralized system is the main impediment to the industrial application of fat and oil wastes, and biosafety concerns arise from using animal fats as a biodiesel feedstock since they may come from infected animals (Matthew et al., 2021).