

**JOURNAL  
OF HALAL QUALITY AND CERTIFICATION**

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**ČASOPIS  
ZA HALAL KVALITET I CERTIFICIRANJE**

## JOURNAL OF HALAL QUALITY AND CERTIFICATION

Printed edition | Štampano izdanje ISSN 2831-1469 (Print)

Online edition | Online izdanje: <https://congress.halal.ba/#journal> ISSN 2831-1477 (Online)

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| Technical preparation and design  <br>Tehnička priprema i dizajn | Nermin Hadžić, Repro Karić d.o.o   |
| Publisher B&H   Izdavač u BiH                                    | Islamic Community in Bosnia and Herzegovina, Agency for halal quality certification  |
| Co-Publisher B&H   Suizdavač u BiH                               | University of Sarajevo - Faculty of Islamic Studies<br>University of Tuzla – Faculty of Technology   |
| Co-Publisher Croatia   Suizdavač u<br>Hrvatskoj                  | Josip Juraj Strossmayer University in Osijek Faculty of Food Technology  |
| Print   Štampa   | ELAK d.o.o. Gradačac   |
| Frequency of Publication   | Two issues per year  |

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## Foreword

Dear Readers,

We are pleased to present the latest issue of the *Journal of Halal Quality and Certification*, Volume 3, Issue 1, which once again features a diverse range of current scientific contributions in the fields of halal standardization, food safety, cosmetics, pharmaceuticals, nutritional habits, and sustainable technologies within the halal framework.

Today, the halal concept is increasingly recognized not only as a religious norm but also as a comprehensive model that integrates health, ethical, technological, and social values. Accordingly, the papers published in this issue explore halal from a multidisciplinary perspective—covering topics such as microbiological safety of traditional products, metabolomics-based methods for gelatin authentication, development of halal cosmetics and personalized medicine, as well as research on consumer habits and sustainable approaches in the food and pharmaceutical industries.

We are confident that this issue will serve as a valuable resource for researchers, professionals, regulators, and all stakeholders interested in the modern development of the halal sector, both locally and globally.

We sincerely thank all authors and reviewers for their valuable contributions to the quality of this edition and warmly invite researchers to continue enriching the scientific foundation and practical application of halal standards through their future work.

Prof. Dr. Midhat Jašić  
Editor-in-Chief

Dr. sci. Damir Alihodžić  
Editor

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**The influence of *Lactobacillus* spp. on the hygienic safety of dry cheese - a traditional halal product of Bosnia and Herzegovina**

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Original scientific paper



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**ABSTRACT**

The research focuses on dry cheese, an important part of the cultural and gastronomic heritage of Bosnia and Herzegovina, especially in the northeastern part of the country. Produced according to traditional methods that are passed down from generation to generation, dry cheese not only reflects a rich culinary tradition, but also provides potential health benefits due to the presence of probiotic bacteria. The aim of this research is to examine the antimicrobial activity of *Lactobacillus* spp. isolated from dry cheese against pathogenic microorganisms: *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Aerobic mesophilic bacteria*. For the purposes of the research, were analyzed 33 samples of dry cheese. Microbiological analyses were performed according to ISO accredited methods. Samples were analyzed at different stages of production: after smoking, after 7 days of ripening at a temperature of 15 °C +/- 1 °C and after 7 days of storage at a temperature of +4 to 8°C. The results showed that dry cheese remains microbiologically intact and safe for consumption after ripening and storage. The presence of *Lactobacillus* spp. confirms the probiotic properties of cheese, which can positively affect digestive health. Additionally, the production of dry cheese in accordance with Halal standards ensures its safety and quality, providing added value to Muslim consumers. This research contributes to the understanding of the microbiological safety of dry cheese and its health benefits, and highlights the importance of preserving traditional production methods in the modern context of food safety.

**Keywords:** *Lactobacillus* spp., dry cheese, microbiological safety, halal product

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## **Introduction**

In the insurance of quality, health and hygienic correctness of food, a number of standards that can be implemented in production plants are used. One of the insurance systems and management of health and hygiene of food is the Halal Standard bass 1049: 2023 - halal food, demands and measures.

Halal generally means permitted, authorized, approved, sanctioned, lawful, legal, legitimate or permitted. Consumption of halal food and goods is obligatory for all Muslims. In fact, the Holy Quran has addressed all human beings, not just Muslims, to seek halal and this is for their own benefit. Halal food requires preparation under hygienic conditions that meet international food safety standards and should not be considered offensive to any religious belief. The fundamental issue in the production of halal food is cleanliness, free from 'contamination' and wholesome food as defined in the Quran (AmalMerge (M) Sdn. Bhd., 2004). Halal is not only considered to be part of the adherence of faith, is considered to be a standard of choice of life -style for Muslims (Golniz, Zainalabidin, Mad Nasir, & Chiew, 2010), whether in the kingdom of business, trade or in other areas (Omar, Nik Mat, Imhemed and Ali, 2012). Halal as a symbol becomes a barometer to determine the quality of goods or services, ranging from safety and hygiene to well -being or benefits (Golnaz et al., 2010).

The report on the state of the global Islamic economy (SGIE) regularly monitors the trends and development of the Islamic market globally. In ten years since the first edition of the 2013 report, the Islamic consumption market increased with \$ 1,62 trillion, according to estimates from 2012, to \$ 2,29 in 2022 trillion.

According to the definition of Cac/GL 24-1997 Halal food is food allowed under Islamic law and must meet the following conditions: that it does not contain anything that is against Islamic law, not prepared, processed, transported or stored using any devices or objects that are against Islamic laws, and that during preparation, processing, transportation and storage it was not

in direct contact with non-halal food. Halal milk and dairy products must be manufactured in accordance with the regulations and rules that ensure that all procedures and ingredients are production in accordance with halal requirements. Halal additives in milk processing and dairy production exclude the use of any haram raw materials (pork, alcohol, carrion and blood, and intoxicating and toxic plants) in the complete food chain. It is difficult to classify additives in advance as halal, haram or mesbuh, so in most cases their halal status needs to be proven. Proofing the presence of haram additives in concrete production processes is carried out by proactive analysis of the processes that prevent their use. Complex analytical methods (PCR, ELISA, HPLC, electrophoresis) are used to validate the status of additives on the identification of the origin of the origin (Midhat Jašić et al., 2007).

The microbial quality of raw milk is key to the production of quality dairy products. Trashing is a term used to describe the exacerbation of the texture, color, aroma or taste of food to a point in which it becomes tasteless or inappropriate for human nutrition. Microbial shattering of food often involves decomposing protein, carbohydrates and fat with microorganisms or their enzymes. Milk is sterile when secreting in udder, but bacteria contaminate it even before leaving the udder. Further infection of milk with microorganisms can occur during masculine, handling, storage and other activities before processing. The dominant human bacterial pathogens that can be potentially transferred to milk include mostly listeria monocytogenes, Salmonella spp., Staphylococcus aureus and pathogen Escherichia coli. Raw milk provides potential medium for growth of these bacteria (Farrokh C., et al., 2013). Hygiene practices of milk production, proper handling and storage of milk, and mandatory pasteurization have reduced the risk of milk that is transmitted by milk, such as tuberculosis, brucellosis and typhoid. There are a number of foods that are transmitted by food, which are due to the consumption of raw milk or dairy products made of milk that was not properly pasteurized or was poorly done with it, which caused contamination after processing.

The following bacterial pathogens are still worrying today in raw milk and other dairy products: *Bacillus cereus*, *Listeria monocytogenes*, *Yersinia Enterocolitica*, *Salmonella* spp., *Escherichia coli* O157: H7, *Campillobakter Jejuni* (Dairy Science and Technology).

In today's society, an increasingly expressive awareness of the importance of healthy and proper diet, which encourages consumers to seek to reconcile their eating habits to achieve optimal health and reduce the risk of illness. This trend results in increased consumption of products that positively affect human health. Given changes in consumer habits, the food industry adapts its processing and production processes. In response to these changes, the development of the modern food industry is globally directed to the production of functional food - food that not only meets the basic nutritional needs but also contributes to the health of humans. The term functional food was first introduced in Japan in the mid-1980s, and since then the functional food market has been constantly developing (Kobayashi, et al., 2017). Some functional food has been known since ancient times and is traditionally consumed because of its health benefits. Many of them, due to the biologically active substances they contain, have been identified and characterized only in recent years. The category of functional foods are probiotics, which are used thousands of years, and whose health benefits have been proven over time.

Given that consumers today are increasingly aware of the importance of diet and its effect on health and quality of life, additional requirements for a wide range of quality food products are created, among which cheese is in an ideal position to play an important role. Compared to milk, it contains a higher amount of protein and fat, and is especially significant by high calcium content. In addition to quantitative changes in milk components, in cheese, during the ripening, there are also complex biochemical processes that change some ingredients significantly to the substitutes of the components, which allows for easier digestion and better resorption in the human body. Therefore, today the cheese

represents an important food in the diet of people, but it is increasingly being wider significance by presenting the cultural and traditional mirror of a country (Bijeljic and Sarić 2005). Homemade cheeses are often produced from raw milk on farms or in small dairies, following special protocols in accordance with traditional heritage. They differ in taste characteristics and are generally associated with a particular region or earth (Montel M.-C., et al., 2014). The production of traditional dry cheese begins with male milk. The milk is muttered by hand, with prior to wash the udder with water and wiping a cloth. Milk is cut into a clean enamel bin. Today, there are increasingly used music for masculine. The grated milk is strained and poured into the sherry and allowed to stand at room temperature for two to three days. At the top of the sherpa appears the kajmak that must be picked up before the cheese starts. Put the sauce on the furnace, so that the peel is sowed over low heat. The cheese is then picked up and drained well. The well -drained cheese is salted and hand over, then placed in a gauze, for better squeezing, and manually forms. The prepared and shaped cheese thus smoked and dried in simple dried, while earlier drying was done above the stove and Sulnar in the attic of old houses (Jasic et al., 2012).

Homemade cheeses have a complex microflora characterized by succession of various microorganisms during cheese production (Irlinger F., Mounier J., 2009). These microorganisms are an essential component of all mature cheeses, playing an important role in cheese matting by influencing the sensory and physicochemical properties of the final product (Yeluri Jonnala B., et al., 2018). The presence of various nonstarter lactic acid bacteria was observed in the production of traditional cheeses. It is important to isolate them and identify them as well as to study their different characteristics (dancesas et al., 2017).

Lactic acid bacteria are found in local cheeses and play an important role in ripening through biochemical reactions. Since auxiliary cultures play an important role in the creation of flavor in cheese, the identification of this group of bacteria for industrial application is very important

(Guarrasi et al., 2017). Lactic acid bacteria comprise a wide range of genera including a significant number of species. The most important genera of these bacteria are: *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus* and others. The strains used as probiotics usually belong to species of the genera *Lactobacillus*, *Enterococcus* and *Bifidobacterium*. *Lactobacillus* species are gram-positive, non-spore-forming rod-shaped bacteria that ferment carbohydrates into lactic acid as a major end point (Goldstein et al., 2015). *Lactobacillus* are crucial in various industries, especially in food production and healthcare applications. They play an important role in the fermentation of dairy products such as yogurt and cheese. Their ability to produce lactic acid during fermentation not only preserves food, but also improves flavor and texture. Application of lactic acid bacteria that produce antimicrobial agents or dietary ferments in the production of dairy products, which can be installed into fermented or unfermented dairy products, implies an additional advantage in processing to improve safety and increase the quality of dairy products, which is an additional obstacle to reducing the likelihood of foods transmitted by food. Bacteriocins are ribosomally synthesized bioactive peptides that produce bacteria that show antimicrobial action against related (narrow spectrum) or unstable (wide spectrum) bacteria. These peptides are considered to be natural bioconzers, and their potential use in the food industry has attracted great interest (Cotter PD et al., 2005).

*Lactobacillus* are key in different industries, especially in food production and using health care. They play an important role in fermentation of dairy products such as yogurt and cheese. Their ability to produce lactic acids with tokom fermentation not only preserves food but also improves the taste and texture. As probiotics, lactobacils contribute to the health of the intestines by balance of microflora, supporting digestion and even strengthening immunity. Their recognition by the US FDA (Food and Medicines Administration) and the European Food Safety Agency (EFSA) testifies to their safety and well-established use in food and

health products. The fact that lactobacils are well studied in terms of genomics and interaction with the human body makes them an ideal candidate for probiotic administration, where they are used in dietary supplements, functional foods and even in clinical therapies for different conditions such as irritable bowel syndrome (IBS) or infection. Their ability to survive in different conditions, such as acidic conditions in the stomach, further contributes to their versatility as probiotics. (Elaine Dempsey and Sine' ad C. Corr, 2022).

### Material and methods

Before to collecting dry cheese samples, an analysis of the harmony of this product was carried out with the Halal standard, which included an overview of the raw material, additives and the overall technological process in order to determine whether the final product meets the requirements of the Halal Norm BAS 1049: 2010. The first step in the verification process is the inspection of all ingredients and raw materials. It is necessary to find that raw milk for the production of dry cheese is halal and does not contain forbidden elements such as alcohol, medicines and other forbidden substances. The equipment used to produce dry cheese should be clean and should not be used to produce haram products and must be thoroughly cleaned. Production must be in line with halal mtodama, which means that there is no contamination of haram materials during the production process. After finding the manufacturer of the craft type that fulfills these conditions, for the needs of microbiological analysis, we took 33 samples of dry cheese. The analyzes were made in three periods of time: after completing the production process (after smoking), after 7 days of ripening at 15 °C +/- 1 °C, a relative humidity of 85° C to 95° C and after 7 days of storage at temperatures + 4° C to + 8° C. At each phase, microbiological analysis is performed to follow the changes in microbiology. Dry cheese during these stages. The samples were kept in the above controlled conditions until the analysis of the analysis. Microbiological analysis was carried out in the Laboratory for Microbiology of Food and Animal Feed, Faculty of Veterinary Medicine, University of Sarajevo, according to accredited methods.

## Experimental part

The preparation of samples for microbiological analysis was conducted in accordance with the standard of BAS EN ISO 6887-1: 2018 (ISO 6877-1: 2018), microbiology of food chain-preparing samples for testing, initial suspension and decimal dilution for microbiological trials - DIO 1: General rules for the preparation of initial suspension and decimal dilution and part of the milk and part of the milk and part of the milk and part of the milk and part. BAS EN ISO 6887-5: 2022 (ISO 6887-5: 2020), microbiology of food chain-preparation of test samples, initial suspensions and decimal dilution for microbiological tests-part 5: specific rules for milk and dairy preparation. The samples were prepared in aseptic conditions. The samples were prepared in aseptic conditions. First, the samples were weighed and an initial dilution of 1:10 was made. After the preparation of the decimal dilution, seeding was carried out on appropriate selective media to determine the following microbiological parameters: *Lactobacillus* spp., *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Aerobic mesophilic bacteria*.

For each batch of dry cheese samples (after smoking, the seventh day of ripening and the seventh day of storage), microbiological analyzes were performed according to the methods of the BAS EN ISO standard.

### Methods for determining the number of bacteria

Horizontal method for counting mesophilic lactic acid bacteria - *Lactobacillus* spp. Technique of counting colonies at 30 degrees ° C - BAS ISO 15214: 2008 (ISO 15214: 1998)

Horizontal method for detection and counting *Listeria monocytogenes* and *Listeria* spp.

Horizontal method for counting *Escherichia coli*-Part 2: The technique of counting colonies at 44 ° C using 5-bromo-4-chlorine-3-indolile- $\beta$ -D-Gluconide BAS EN ISO 16649-2: 2008 (ISO 16649-2: 2001).

Horizontal method for counting coagulase-positive *staphylococcus* (*Staphylococcus aureus*

and other species)-Part 1: The use of Baird-parker agar Media bas iso 6888-1: 2022 (ISO 6888-1: 2021).

Horizontal method for determining the number of microorganisms -part 1: counting colonies at 30 ° C of the substrate spilling technique -with amendment bass en ISO 4833 -1/A1: 2023 (ISO 4833-1: 2013/AMD 1: 2022).

## Results

The results of microbiological analyses of dry cheese for the presence of *Lactobacillus* spp. and pathogenic bacteria such as: *aerobic mesophilic bacteria*, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*, conducted in three time periods (after smoking, after 7 days of ripening and after 7 days of storage) are presented in Tables 1, 2, and 3.

The following microbiological parameters are recorded in Table 1:

*Lactobacillus* spp: Values range from  $1.2 \times 10^3$  to  $5.3 \times 10^3$  CFU(g). The lowest value was recorded in sample 11, the highest value was recorded in sample 6. These results indicate the presence of lactic acid bacteria that are crucial for the preservation and maturation of cheese. *Aerobic mesophilic bacteria*: Values range from  $3.3 \times 10^2$  to  $6.2 \times 10^2$  CFU(g). The highest value was recorded in sample 11, and the lowest value was recorded in sample 6. *Escherichia coli*: Values range from  $9.1 \times 10$  to  $2.1 \times 10^2$  CFU/g. The highest value was recorded in sample 7, the lowest in samples 5 and 6. *Staphylococcus aureus*: Values range from  $5.5 \times 10$  to  $1.4 \times 10^2$  CFU/g. The highest value was recorded in sample 3, and the lowest in samples 1 and 5. The values are below the critical limit of  $10^3$  CFU/g often used for pathogenic strains, indicating a satisfactory hygienic status of dry cheese. *Listeria monocytogenes*: The samples were negative for the presence of this pathogenic bacterium. This is of utmost importance from a food safety perspective because *Listeria* poses a serious risk to human health in unpasteurized milk products, such as dry cheese.

Table 1. Results of microbiological analysis of dry cheese after smoking

| Microorganisms CFU/g |                           |                                    |                         |                              |                               |
|----------------------|---------------------------|------------------------------------|-------------------------|------------------------------|-------------------------------|
| Number of samples    | <i>Lactobacillus</i> spp. | <i>Aerobne mezofilne bakterije</i> | <i>Escherichia coli</i> | <i>Staphylococcus aureus</i> | <i>Listeria monocytogenes</i> |
| 1.                   | 4,4x10 <sup>3</sup>       | 3,6x10 <sup>2</sup>                | 1,1x10 <sup>2</sup>     | 5,5x10                       | 0                             |
| 2.                   | 4,2x10 <sup>3</sup>       | 5,0x10 <sup>2</sup>                | 1,4x10 <sup>2</sup>     | 9,1x10                       | 0                             |
| 3.                   | 2,6x10 <sup>3</sup>       | 5,4x10 <sup>2</sup>                | 1,0x10 <sup>2</sup>     | 1,4x10 <sup>2</sup>          | 0                             |
| 4.                   | 2,7x10 <sup>3</sup>       | 3,5x10 <sup>2</sup>                | 1,4x10 <sup>2</sup>     | 7,3x10                       | 0                             |
| 5.                   | 4,3x10 <sup>3</sup>       | 4,8x10 <sup>2</sup>                | 9,1x10                  | 5,5x10                       | 0                             |
| 6.                   | 5,3x10 <sup>3</sup>       | 3,3x10 <sup>2</sup>                | 9,1x10                  | 8,2x10                       | 0                             |
| 7.                   | 2,7x10 <sup>3</sup>       | 5,2x10 <sup>2</sup>                | 2,1x10 <sup>2</sup>     | 1,0x10 <sup>2</sup>          | 0                             |
| 8.                   | 2,5x10 <sup>3</sup>       | 4,4x10 <sup>2</sup>                | 1,4x10 <sup>2</sup>     | 8,2x10                       | 0                             |
| 9.                   | 2,0x10 <sup>3</sup>       | 5,5x10 <sup>2</sup>                | 2,0x10 <sup>2</sup>     | 1,1x10 <sup>2</sup>          | 0                             |
| 10.                  | 1,9x10 <sup>3</sup>       | 5,8x10 <sup>2</sup>                | 1,0x10 <sup>2</sup>     | 1,1x10 <sup>2</sup>          | 0                             |
| 11.                  | 1,2x10 <sup>3</sup>       | 6,2x10 <sup>2</sup>                | 1,1x10 <sup>2</sup>     | 1,3x10 <sup>2</sup>          | 0                             |

Table 2. Results of microbiological analysis of dry cheese after 7 days of ripening

| Microorganisms CFU/g |                           |                                    |                         |                              |                               |
|----------------------|---------------------------|------------------------------------|-------------------------|------------------------------|-------------------------------|
| Number of samples    | <i>Lactobacillus</i> spp. | <i>Aerobne mezofilne bakterije</i> | <i>Escherichia coli</i> | <i>Staphylococcus aureus</i> | <i>Listeria monocytogenes</i> |
| 1.                   | 7,2x10 <sup>3</sup>       | 1,8x10 <sup>2</sup>                | 1,8x10                  | 0                            | 0                             |
| 2.                   | 4,9x10 <sup>3</sup>       | 3,0x10 <sup>2</sup>                | 7,3x10                  | 0                            | 0                             |
| 3.                   | 5,0x10 <sup>3</sup>       | 3,2x10 <sup>2</sup>                | 2,7x10                  | 1,8x10                       | 0                             |
| 4.                   | 6,7x10 <sup>3</sup>       | 2,5x10 <sup>2</sup>                | 6,4x10                  | 0                            | 0                             |
| 5.                   | 8,5x10 <sup>3</sup>       | 1,0x10 <sup>2</sup>                | 0                       | 0                            | 0                             |
| 6.                   | 9,1x10 <sup>3</sup>       | 1,2x10 <sup>2</sup>                | 0                       | 0                            | 0                             |
| 7.                   | 5,2x10 <sup>3</sup>       | 2,9x10 <sup>2</sup>                | 8,2x10                  | 0                            | 0                             |
| 8.                   | 6,8x10 <sup>3</sup>       | 2,3x10 <sup>2</sup>                | 3,6x10                  | 0                            | 0                             |
| 9.                   | 5,0x10 <sup>3</sup>       | 2,7x10 <sup>2</sup>                | 8,2x10                  | 0                            | 0                             |
| 10.                  | 6,5x10 <sup>3</sup>       | 1,5x10 <sup>2</sup>                | 0                       | 1,8x10                       | 0                             |
| 11.                  | 5,7x10 <sup>3</sup>       | 2,0x10 <sup>2</sup>                | 1,8x10                  | 1,8x10                       | 0                             |

The following microbiological parameters are recorded in Table 2:

*Lactobacillus* spp: After ripening of dry cheese, a significant increase in *Lactobacillus* spp was recorded. This indicates active reproduction of beneficial bacteria during ripening, which contributes to further fermentation, aroma development and increased microbiological stability. *Aerobic mesophilic bacteria*: A

The following microbiological parameters are recorded in Table 3:

decrease of more than 50% was recorded, which can be attributed to competition from lactic acid bacteria. *Escherichia coli*: A significant decrease was recorded, in some samples a complete absence, which indicates inhibition of the growth of this bacteria during ripening. *Staphylococcus aureus*: Most samples were negative after ripening, except for 3 (very low values). *Listeria monocytogenes*: The presence of this pathogenic bacterium was not recorded in any sample.

*Lactobacillus* spp.: A continuous increase was recorded throughout all phases, compared to the

initial one. This indicates that fermentation activity continues during storage, which is beneficial for ripening, aroma and stability of the product. *Aerobic mesophilic bacteria*: Their abundance decreases significantly throughout all phases. This is a sign that lactic acid bacteria dominate the microflora, preventing the growth of these spoilage bacteria. *Escherichia coli*: After storage, this bacterium is completely eliminated.

Which confirms good microbiological safety and efficiency of fermentation, smoking and ripening. *Staphylococcus aureus*: Completely absent after storage, which is an indicator of the hygienic safety and sustainability of dry cheese in storage conditions. *Listeria monocytogenes*: Not detected in any sample. A key indicator of safety and hygienic safety for raw milk products, such as dry cheese.

Table 3. Results of microbiological analysis of dry cheese after 7 days of storage

| Number of samples | Microorganisms CFU/g      |                                    |                         |                              |                               |
|-------------------|---------------------------|------------------------------------|-------------------------|------------------------------|-------------------------------|
|                   | <i>Lactobacillus</i> spp. | <i>Aerobne mezofilne bakterije</i> | <i>Escherichia coli</i> | <i>Staphylococcus aureus</i> | <i>Listeria monocytogenes</i> |
| 1.                | 1,5x10 <sup>4</sup>       | 1,0x10 <sup>2</sup>                | 0                       | 0                            | 0                             |
| 2.                | 8,9x10 <sup>3</sup>       | 1,2x10 <sup>2</sup>                | 0                       | 0                            | 0                             |
| 3.                | 1,0x10 <sup>4</sup>       | 8,2x10                             | 0                       | 0                            | 0                             |
| 4.                | 1,9x10 <sup>4</sup>       | 5,5x10                             | 0                       | 0                            | 0                             |
| 5.                | 2,0x10 <sup>4</sup>       | 4,5x10                             | 0                       | 0                            | 0                             |
| 6.                | 1,5x10 <sup>4</sup>       | 7,3x10                             | 0                       | 0                            | 0                             |
| 7.                | 8,5x10 <sup>3</sup>       | 1,2x10 <sup>2</sup>                | 0                       | 0                            | 0                             |
| 8.                | 9,2x10 <sup>3</sup>       | 1,0x10 <sup>2</sup>                | 0                       | 0                            | 0                             |
| 9.                | 8,7x10 <sup>3</sup>       | 1,5x10 <sup>2</sup>                | 0                       | 0                            | 0                             |
| 10.               | 9,8x10 <sup>3</sup>       | 7,3x10                             | 0                       | 0                            | 0                             |
| 11.               | 1,4x10 <sup>4</sup>       | 8,2x10                             | 0                       | 0                            | 0                             |

## Discussion

Dairy products are an excellent medium for the growth of a wide range of microorganisms and therefore have a reduced shelf life (Ruegg, 2003). The microbiological quality of dairy products is influenced by the initial flora of raw milk, processing conditions and subsequent heat treatment. Spoilage bacteria and various bacteria that are dangerous for public health can be found in these products, and their concentrations should be kept as low as possible (Varga, 2007). In contrast, *lactic acid bacteria*, which are found in the indigenous microflora of raw milk and are the main components of starter cultures used in fermentation, contribute to the quality of fermented cheese-type products by improving taste and texture and inhibiting spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid (Jana and Mandal, 2011).

The study of the influence of *Lactobacillus* spp. on the hygienic safety of dry cheese - a traditional halal product of Bosnia and Herzegovina was conducted in three time periods: after smoking, after 7 days of ripening at a temperature of 15 °C +/- 1 °C with a relative humidity of 85° C to 95° C and after 7 days of storage at temperatures of + 4° C to + 8° C. The values of *Lactobacillus* spp. in dry cheese after smoking range from 1.2 x 10<sup>3</sup> to 5.3 x 10<sup>3</sup> CFU(g). After 7 days of ripening, a significant increase in *Lactobacillus* spp. was recorded, indicating the active reproduction of beneficial bacteria during this process, which contributes to further fermentation, aroma development and increased microbiological stability. Fermentation activity continues during product storage. The range of values from 3.3 x 10<sup>2</sup> to 6.2 x 10<sup>2</sup> CFU(g) for aerobic mesophilic bacteria indicates a good microbiological status and effective smoking that limited the

development of these bacteria in dry cheese. During ripening, a decrease of more than 50% in aerobic mesophilic bacteria was recorded, which can be attributed to competition from lactic acid bacteria and a decrease in available nutrients. The number of *aerobic mesophilic bacteria* also decreases significantly during the storage phase. This is a sign that lactic acid bacteria dominate the microflora, preventing the growth of these spoilage bacteria. *Escherichia coli* values range from  $9.1 \times 10$  to  $2.1 \times 10^2$  CFU/g. The presence of *E. coli* in dry cheese indicates possible contact with fecal contamination during production, but the values are in a decreasing range and do not exceed the limits of acceptability for most fermented products, assuming that they belong to non-pathogenic strains. A significant decrease was recorded during ripening, and in some samples the complete absence of *E. coli* indicates inhibition of the growth of this bacterium during ripening, probably due to the antimicrobial activity of lactic acid bacteria and low pH values. After storage, this bacterium was completely eliminated. This confirms good microbiological safety and efficiency of fermentation, smoking and ripening. Values for *Staphylococcus aureus* range from  $5.5 \times 10$  to  $1.4 \times 10^2$  CFU/g, which is in accordance with the values prescribed by the current Regulation. Most samples were negative after ripening, except for 3 (very low values). This further confirms the safety of the product after seven days of ripening. Coagulase-positive staphylococci are completely absent after storage, which is an indicator of hygienic correctness and sustainability of dry cheese in storage conditions. *Listeria monocytogenes* was not found in any sample of dry cheese, which shows the hygienic safety and security of this product.

Cheese production using natural microflora of lactic acid bacteria is more acceptable compared to cheese production using commercial dairy cultures, as is evident from the results of research conducted by Renata Pyz-Lukasik et al., (2018) in the production of traditional short- and long-maturing cheeses from Poland. Commercial dairy cultures were used in the production of this cheese, and the temperature in the production and maturing room was 18-20°C and 14-20°C,

respectively. The tested cheeses did not meet the microbial criteria for food safety (presence of *L. monocytogenes*) and process hygiene (exceeded permissible levels of *E. coli* and coagulase-positive *Staphylococcus*).

*Lactobacillus* spp. isolated from Iranian local raw milk cheese from Ahvaz province, have in vitro studies that make them potential candidates for probiotic and technological applications. The results showed that these strains have good probiotic and technological potential. The results of safety aspects also showed that these strains can be used for human nutrition. Therefore, in vivo studies are needed to investigate their effectiveness in real-life situations (Hasan B., et al., 2021).

Monitoring the concentration of lactic acid bacteria in Mozzarella cheese shows that the results confirmed the correlation between the concentration of lactic acid bacteria and the quality of the process: a high concentration of lactic acid bacteria in the raw materials and whey starter ensured safe and good final products (Francesca L., et al., 2014).

According to Cretenet et al., (2011) adequate growth of milk bacteria during milk farming contributes to the control of the reproduction of potentially pathogenic bacteria, with special reference to staphylococci that produce enterotoxins, and can help in the production of safer cheese.

According to the research of Angeliki D. et al., (2024) the application of bioprotective strains of lactic acid can result in an extension of the shelf life of feta and provide a mild antimicrobial effect against *L. monocytogenes*.

The results of a study conducted on the microbiological diversity and physicochemical properties of Kurdish cheese from Iran during ripening (1, 20, 40 and 60 days) showed that the type and number of microorganisms were most affected by the ripening time. Lactic acid bacteria and *Enterobacteriaceae* dominated during the first 20 days of ripening, and *lactobacillus* were the most common microorganisms found during ripening. The initial number of coliforms and *E.*

coli decreased rapidly, while a gradual increase in the number of molds and yeasts was observed in the early days of ripening. *Coliform bacteria*, *Salmonella* and *coagulase-positive Staphylococcus* spp. could not be detected in the ripened cheese (Elnaz Milani et al., 2014).

Brooks JC., et al., (2012) analyzed the presence of pathogenic bacteria in raw milk cheese produced in the USA. They analyzed them for the presence of *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Campylobacter*. Five samples contained coliforms; two of them contained *E. coli* less than 10(2) cfu/g. Three other cheese samples contained *S. aureus*. Research by Brooks JC., et al., (2012) supports the theory that with adequate control of microbiological parameters and proper aging, cheeses produced from unpasteurized milk can be safe for consumption, although there is a risk of the presence of pathogens such as *S. aureus* and *E. Coli*.

By evaluating the interaction (growth behavior and survival) of *Listeria monocytogenes* and *Lactobacillus acidophilus* in different stages of production, ripening and storage of Iranian white cheese, changes in pH values were recorded at different stages of cheese ripening, as well as a positive effect on the sensory characteristics of the cheese. The decrease in the number of *L. monocytogenes* during the ripening and storage period of probiotic Iranian white cheese may be due to the combined effect of the lowered pH and the antimicrobial activity of the starter and probiotic bacteria used in this study (R. Mahmoudi et al., 2012).

In Minas cheeses (type Canastra) made from raw or pasteurized milk, selected strains of lactic acid bacteria showed bacteriostatic effects and inactivation of *L. monocytogenes* in soft cheese, and in semi-hard cheese, respectively. Inactivation of *L. monocytogenes* was significantly higher in semi-hard cheeses made with raw milk and the addition of selected indigenous lactic acid bacteria. Strains isolated from artisanal Minas cheeses may provide an additional barrier to the growth of *L. monocytogenes* during refrigerated storage of soft

cheese and help shorten the ripening period of semi-hard cheeses aged at room temperature (Fernanda B. Campagnollo et al., 2018).

## Conclusion

Based on the results of the research on the microbiological safety of dry cheese through three phases, it was concluded that the safety of dry cheese after smoking improves through the ripening and storage process. Potentially dangerous microorganisms are eliminated. Fermentation continues, which is visible through the growth of *Lactobacillus* spp., which also contributes to the development of the texture and taste of dry cheese. The technological process (smoking, ripening and storage) has proven to be effective for the microbiological safety and safety of dry cheese. These results confirm that dry cheese can be a safe, healthy and halal product, which not only meets religious requirements, but also meets high food safety standards through the presence of probiotic bacteria, such as *Lactobacillus* spp. that act as a natural protection against pathogens. These properties make traditional dry cheese from Bosnia and Herzegovina an even more valuable product on the market.

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## Uticaj *Lactobacillus* spp. na higijensku ispravnost suhog sira - tradicionalnog halal proizvoda Bosne i Hercegovine

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Originalni naučni rad

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### Sažetak

Istraživanje je usmjereno na suhi sir, važan dio kulturnog i gastronomskog naslijeđa Bosne i Hercegovine, posebno u sjevernoistočnom dijelu zemlje. Proizveden po tradicionalnim metodama koje se prenose na generacije na generaciju, suhi sir ne samo da odražava bogatu kulinarsku tradiciju, već i pruža potencijalne zdravstvene koristi zahvaljujući prisutnosti probiotskih bakterija. Cilj ovog istraživanja je ispitati antimikrobnu aktivnost *Lactobacillus* spp. izoliranih iz suhog sira protiv patogenih mikroorganizama: *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* i aerobnih mezofilnih bakterija. Za potrebe istraživanja analizirano je 33 uzorka suhog sira. Mikrobiološke nalize su rađene prema ISO akreditovanim metodama. Uzorci su analizirani u različitim fazama proizvodnje: nakon dimljenja, nakon 7 dana zrenja na temperaturi 15 °C +/- 1 °C i nakon 7 dana skladištenja na temperaturi +4 do 8°C. Rezultati su pokazali da suhi sir ostaje mikrobiološki isparavan i siguran za konzumaciju nakon zrenja i skladištenja. Prisustvo *Lactobacillus* spp. potvrđuje probiotska svojstva sira, koja mogu pozitivno uticati na zdravlje probave. Dodatno, proizvodnja suhog sira u skladu sa Halal standardima osigurava njegovu sigurnost i kvalitetu, pružajući dodatnu vrijednost potrošačima islamske vjeroispovijesti. Ovo istraživanje doprinosi razumijevanju mikrobiološke ispravnosti suhog sira i njegovih zdravstvenih koristi, te ističe važnost očuvanja tradicionalnih metoda proizvodnje u modernom kontekstu sigurnosti hrane.

**Ključne riječi:** *Lactobacillus* spp., suhi sir, mikrobiološka ispravnost, halal proizvod

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## JOURNAL OF HALAL QUALITY AND CERTIFICATION

### Metabolomics-Based Profiling of Porcine and Bovine Gelatin for Halal Authentication

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#### ABSTRACT

Gelatin is widely used in the food, pharmaceutical, and cosmetic industries, where its source is critical for halal authentication and adulteration detection. This study employs a metabolomics-based approach using liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (LC-QTOF-MS/MS) to comprehensively profile the metabolic differences between porcine and bovine gelatin. Untargeted metabolomics was conducted to identify species-specific metabolic signatures. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were applied to detect metabolic variations and classify gelatin sources with high accuracy. The results showed that PCA clearly distinguished between bovine and porcine gelatin. Several metabolites with high variable importance in projection (VIP) scores, including sphingomyelin SM(d18:0/18:1(9Z)), cytidine 5'-diphosphocholine, 3-methylcytidine, NAD<sup>+</sup>, ATP, and Tyr-Tyr, were found in higher abundance in porcine gelatin, whereas cytidine-5'-triphosphate, tryptophan, and phenylalanine were more prevalent in bovine gelatin. These metabolic differences arise due to species-specific variations in collagen breakdown and nucleotide turnover pathways. The LC-QTOF-MS/MS-based metabolomics workflow provides high sensitivity and a non-targeted analytical platform for gelatin authentication, preventing fraudulent substitutions in the food supply chain. This method offers a robust, data-driven solution for distinguishing gelatin sources and could also be used in halal applications.

**Keywords:** *Metabolomics, Bovine Gelatin, Porcine Gelatin, Halal, LC-QTOF-MS/MS*

#### Introduction

Gelatin is a biopolymer obtained through the partial hydrolysis of collagen, a structural protein found in animal tissues such as skin, bones, and connective tissue (Yörük et al., 2024). It is widely used in the food, pharmaceutical, and cosmetic industries due to its excellent gelling, stabilizing, emulsifying, and texturizing properties (Rohman

et al., 2020). However, the animal origin of gelatin is a major concern for specific consumer groups, particularly those adhering to halal, kosher, or vegetarian/vegan dietary restrictions (Mahamud et al., 2023).

The global gelatin market is projected to reach USD 5.0 billion by 2025, driven by increasing demand across various industries. The majority of

commercially available gelatin is derived from porcine skin (46%), bovine hides (29.4%), porcine and bovine bones (23.1%), and to a lesser extent, fish skin (1.5%) (Rather et al., 2022). Among these, porcine and bovine sources represent the dominant share of the market (Adel & Sheet, 2025). Given their widespread use, accurately identifying the animal origin of gelatin is crucial for ensuring proper labeling, maintaining consumer confidence, and supporting regulatory and religious compliance.

As a result, the authentication of gelatin sources is essential to ensure compliance with religious and ethical dietary laws and to prevent fraudulent substitution or mislabeling in commercial products. The most analytical methods for gelatin source determination include electrophoresis (Abdullah Amqizal et al., 2017), enzyme-linked immunosorbent assays (ELISA) (Tukiran et al., 2015), Fourier-transform infrared spectroscopy (FTIR) (Cebi et al., 2019), DNA-based techniques (Sultana et al., 2018), LC-MS/MS (Kwon et al., 2025). While these approaches have been widely applied, many suffer from limitations such as low sensitivity, insufficient specificity, and lacking comprehensiveness.

In recent years, comprehensive metabolomics analysis of small-molecule metabolites within biological systems has emerged as a promising strategy for food authentication (Selamat et al., 2021). Among the analytical platforms available, LC-QTOF-MS/MS enables untargeted metabolomic profiling with high sensitivity, resolution, and reproducibility (Zhong et al., 2022). This technique allows for the detection of

a broad range of metabolites, making it particularly suitable for differentiating gelatin sources based on their distinct biochemical signatures.

This study aims to develop a metabolomics-based approach using LC-QTOF-MS/MS to discriminate between porcine and bovine gelatin sources. The application of PCA and PLS-DA enables the identification of metabolic markers, offering a robust solution for halal authentication.

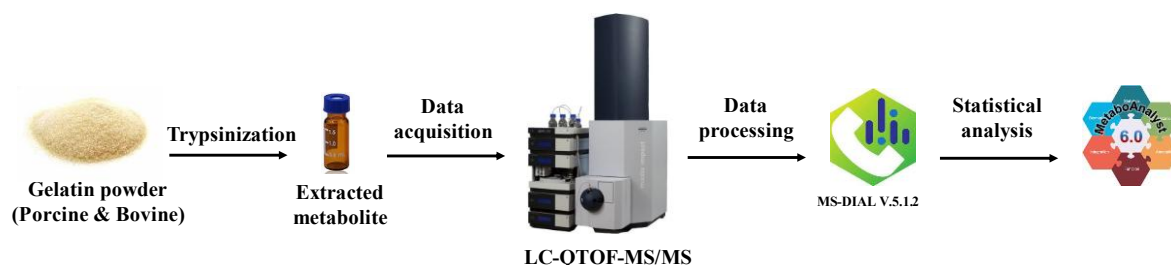
## Materials and Methods

### Materials

Porcine gelatin standard was obtained from Sigma-Aldrich (St. Louis, MO, USA), and bovine gelatin was sourced from Cartino Gelatin Co., Ltd. (Thailand). Trypsin from bovine pancreas and ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) was obtained from Merck (Darmstadt, Germany), and formic acid was sourced from Fisher Scientific (Buchs, Switzerland).

### Sample Preparation

10 mg of bovine gelatin and porcine gelatin standard were each weighed into separate 2 mL microcentrifuge tubes. Next, 500  $\mu\text{L}$  of a working solution (pH 8.0) was added to each sample. The mixtures were incubated in a shaking incubator at 600 rpm and 37 °C for 15 minutes. Subsequently, 10  $\mu\text{L}$  of trypsin enzyme solution (1 mg/mL) was added to each tube, followed by incubation in the shaking incubator at 600 rpm and 37 °C for 16 hours.



**Figure 1.** Schematic overview of the metabolomics analysis process for gelatin.

After enzymatic digestion, the samples were centrifuged at 13,000 rpm for 5 minutes. A volume of 400  $\mu\text{L}$  of the supernatant was

discarded, and 400  $\mu\text{L}$  of the working solution was added to each tube. Finally, the resulting solutions were filtered through a 0.45  $\mu\text{m}$  syringe

filter and transferred into 1.5 mL LC vials. The samples were stored at  $-80\text{ }^{\circ}\text{C}$  until LC-MS/MS analysis. The workflow of metabolomics analysis in gelatin is shown in Figure 1.

### LC-QTOF-MS/MS Analysis

Metabolomic profiling was performed using a Dionex Ultimate 300 UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) connected to a QTOF impact II mass spectrometer (Bruker Daltonics, Bremen, Germany). Metabolite separation was carried out on a C18 reversed-phase column ( $2.1 \times 100\text{ mm}$ ,  $1.9\text{ }\mu\text{m}$  particle size; Thermo Scientific, Sunnyvale, CA, USA). The column oven was maintained at  $40\text{ }^{\circ}\text{C}$ , while the autosampler was set to  $7\text{ }^{\circ}\text{C}$  to preserve sample integrity. The gradient elution method was employed using mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile, v/v). The gradient program was as follows: The mobile phase B gradually increased from 0% to 10% over the first 10 minutes, then to 20% by 30 minutes, followed by 35% at 40 minutes and 45% at 45 minutes. It was then rapidly decreased to 1% at 50 minutes, with a total run time of 50 minutes. The flow rate was maintained at 0.3 mL/min throughout the run.

Mass spectrometric data were acquired using electrospray ionization (ESI) in positive ionization modes, with a mass scan range of  $m/z$  50–1000. Nitrogen served as both the nebulizing and collision gas. Collision energy was set at 20.0 eV and the dry gas temperature was maintained at  $250\text{ }^{\circ}\text{C}$  with a flow rate of 8.0 L/min. Capillary voltages were adjusted to 3800 V for positive mode.

### Data Processing and Metabolite Identification

Raw LC-QTOF-MS/MS data were converted into ABF (Analysis Base File) format and imported into MS-DIAL version 5.1.2 for data processing, including deconvolution, peak detection, alignment, and compound identification. Compound annotation was performed using an authentic standards library in MSP format (last updated in August 2024) for positive ionization modes. The resulting peak area data were then

uploaded to MetaboAnalyst 6.0 for statistical analysis. Prior to analysis, the data were log-transformed and Pareto-scaled. Multivariate analysis was conducted using PLS-DA, and potential biomarkers were identified based on VIP scores and statistically significant differences in metabolite levels between groups ( $p < 0.05$ ).

## Results and Discussion

### Metabolic Profiling and Sample Discrimination

A total of 49 metabolites were detected through untargeted metabolomics analysis using LC-QTOF-MS/MS in both porcine and bovine gelatin samples. Subsequent multivariate analysis using PCA revealed a clear and distinct separation between the porcine and bovine gelatin groups, as shown in Figure 2. This distinct clustering indicates significant differences in the metabolic profiles of the two gelatin sources. The separation observed in the PCA two-dimensional (2D) score plot suggests that the detected metabolites can effectively discriminate between the origins of the gelatin samples.

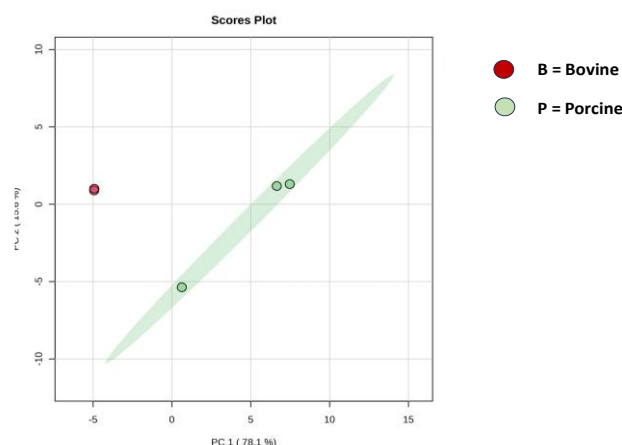


Figure 2. PCA 2D score plot of metabolites in porcine and bovine gelatin.

PCA facilitates the visualization and interpretation of underlying patterns, such as sample groupings or outliers, by transforming the original, potentially correlated variables (metabolites) into a smaller set of uncorrelated variables known as principal components (PCs). In this study, the group separation along the first two principal components PC1 and PC2

highlights the substantial variation in metabolite composition between the two species. PC1 and PC2 accounted for 78.1% and 15.6% of the total variance, respectively, further confirming the discriminatory power of the metabolite profiles.

To identify the most discriminative metabolites contributing to the differentiation between

porcine and bovine gelatin, VIP scores were calculated using PLS-DA. Among the identified metabolites, 25 exhibited VIP scores greater than 1.0, indicating their significant contribution to group separation. These metabolites are summarized in Table 1.

Table 1. Metabolites with VIP scores > 1.0 identified in porcine and bovine gelatin.

| No. | VIP Scores | Metabolite name                              | Formula   | RT(min) | Detected m/z |
|-----|------------|--|---|---------|--------------|
| 1   | 1.5880     | Cytidine-5'-triphosphate                     | C <sub>9</sub> H <sub>16</sub> N <sub>3</sub> O <sub>14</sub> P <sub>3</sub>    | 9.270   | 484.2588     |
| 2   | 1.4870     | Tryptophan                                   | C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>                   | 11.686  | 205.0927     |
| 3   | 1.3654     | Sphingomyelin SM(d18:0/18:1(9Z))             | C <sub>41</sub> H <sub>83</sub> N <sub>2</sub> O <sub>6</sub> P                 | 14.219  | 731.5930     |
| 4   | 1.3393     | Cytidine 5'-diphosphocholine                 | C <sub>14</sub> H <sub>26</sub> N <sub>4</sub> O <sub>11</sub> P <sub>2</sub>   | 1.088   | 489.4421     |
| 5   | 1.3168     | Phenylalanine                                | C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>                                  | 1.005   | 166.1049     |
| 6   | 1.2970     | 3-Methylcytidine                             | C <sub>10</sub> H <sub>15</sub> N <sub>3</sub> O <sub>5</sub>                   | 0.988   | 258.1050     |
| 7   | 1.2890     | NAD <sup>+</sup>                             | C <sub>21</sub> H <sub>28</sub> N <sub>7</sub> O <sub>14</sub> P <sub>2</sub>   | 15.613  | 664.1122     |
| 8   | 1.2468     | Adenosine triphosphate                       | C <sub>10</sub> H <sub>16</sub> N <sub>5</sub> O <sub>13</sub> P <sub>3</sub>   | 11.634  | 505.9886     |
| 9   | 1.2432     | Tyr-Tyr                                      | C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub>                   | 1.325   | 345.1384     |
| 10  | 1.2332     | Coenzyme Q10                                 | C <sub>59</sub> H <sub>90</sub> O <sub>4</sub>                                  | 21.447  | 869.6860     |
| 11  | 1.2310     | 3-Methyl-N-phenylaniline                     | C <sub>13</sub> H <sub>13</sub> N   | 19.623  | 184.1109     |
| 12  | 1.2299     | FAD  | C <sub>27</sub> H <sub>33</sub> N <sub>9</sub> O <sub>15</sub> P <sub>2</sub>   | 15.709  | 786.1673     |
| 13  | 1.2242     | Coenzyme A                                   | C <sub>21</sub> H <sub>36</sub> N <sub>7</sub> O <sub>16</sub> P <sub>3</sub> S | 18.861  | 768.5505     |
| 14  | 1.1842     | Vitamin B12                                  | C <sub>63</sub> H <sub>88</sub> N <sub>14</sub> O <sub>14</sub> P               | 1.111   | 678.3049     |
| 15  | 1.1614     | 5-Hydroxylysine                              | C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub>                    | 1.055   | 163.1048     |
| 16  | 1.1331     | Nicotinamide<br>hypoxanthine<br>dinucleotide | C <sub>21</sub> H <sub>27</sub> N <sub>6</sub> O <sub>15</sub> P <sub>2</sub>   | 16.221  | 665.5268     |
| 17  | 1.1243     | N-2-Hydroxycyclopentyladenosine              | C <sub>15</sub> H <sub>21</sub> N <sub>5</sub> O <sub>5</sub>                   | 0.915   | 352.1648     |
| 18  | 1.0990     | Acetyl-CoA                                   | C <sub>23</sub> H <sub>38</sub> N <sub>7</sub> O <sub>17</sub> P <sub>3</sub> S | 19.563  | 810.1216     |
| 19  | 1.0961     | PC (16:0/18:1(9Z))                           | C <sub>42</sub> H <sub>82</sub> NO <sub>8</sub> P                               | 21.447  | 760.5999     |
| 20  | 1.0760     | D-Ala-D-ala                                  | C <sub>6</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub>                    | 19.612  | 161.0959     |
| 21  | 1.0733     | Leucine_Enkephalin                           | C <sub>28</sub> H <sub>37</sub> N <sub>5</sub> O <sub>7</sub>                   | 1.056   | 556.2841     |
| 22  | 1.0493     | putative heterocyst glycolipid               | C <sub>32</sub> H <sub>64</sub> O <sub>8</sub>                                  | 24.234  | 576.4599     |
| 23  | 1.0470     | N-L-Leucyl-L-leucine                         | C <sub>12</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>                   | 7.973   | 245.1809     |
| 24  | 1.0449     | Betaine                                      | C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>                                  | 0.946   | 257.1433     |
| 25  | 1.0311     | S-Adenosyl-L-homocysteine                    | C <sub>14</sub> H <sub>20</sub> N <sub>6</sub> O <sub>5</sub> S                 | 1.056   | 385.3889     |

The PLS-DA model showed excellent performance, with a goodness of fit ( $R^2$ ) of 0.99 and a predictive ability ( $Q^2$ ) of 0.79. These values demonstrate that the model provides a highly accurate representation of the data, explaining a large proportion of variance ( $R^2 = 0.99$ ) and exhibiting strong predictive power ( $Q^2 = 0.79$ ). Such metrics indicate that the model is both robust and reliable in distinguishing the metabolic profiles of the sample groups. The model's ability to accurately classify samples underscores its suitability for metabolomic discrimination and biomarker identification in this study.

### Identification of Metabolites

The identified metabolites highlight underlying biochemical differences in the source origins used for gelatin production. These variations may arise from species-specific metabolic pathway and differences in protein composition during the gelatin extraction process. Several metabolites with high VIP scores including sphingomyelin SM(d18:0/18:1(9Z)), cytidine 5'-diphosphocholine, 3-methylcytidine, NAD<sup>+</sup>, ATP, and Tyr-Tyr were found in greater abundance in porcine gelatin. In contrast, cytidine 5'-triphosphate, tryptophan, and phenylalanine

were more prevalent in bovine gelatin, as shown in Figure 3.

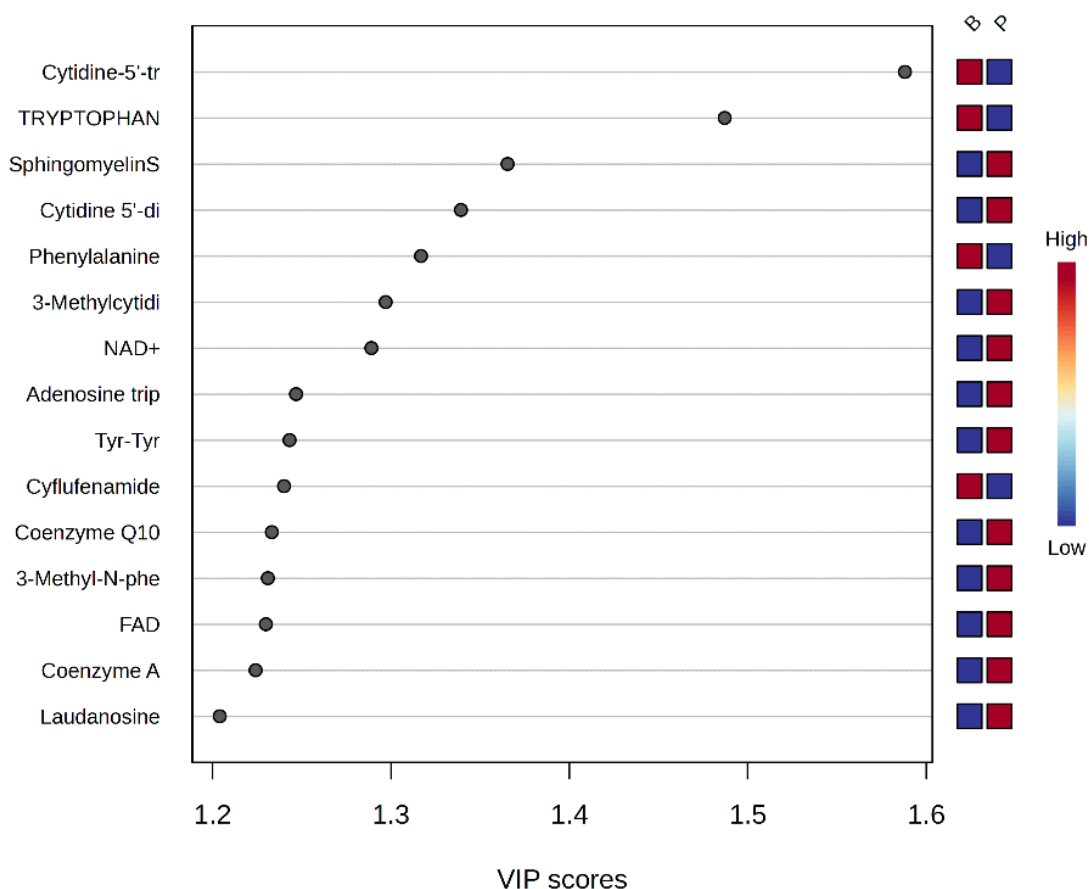


Figure 3. VIP scores plot of metabolites in porcine and bovine gelatin.

Porcine gelatin was characterized by elevated levels of sphingomyelin SM(d18:0/18:1(9Z)), a structural lipid commonly found in animal cell membranes. The presence of this lipid in porcine gelatin may reflect the origin of the raw materials that are rich in sphingolipids. According to a study by (Enomoto et al., 2019), various sphingomyelin species, including SM(d18:0/18:1), were identified in pork meat using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS), supporting the finding of SM enrichment in porcine-derived products. In Addition, several studies have reported that tyrosine is found in higher abundance in porcine tissues compared to bovine sources (Hassan et al., 2025; Kumar et al., 2015). This difference may be attributed to variations in the protein composition and amino acid profiles of the two species,

particularly in collagen-rich tissues commonly used for gelatin production.

Higher levels of cytidine-5'-triphosphate were observed in bovine gelatin samples. The presence of tryptophan is consistent with the study conducted by (Jamaluddin et al., 2024), as indicated by peaks at 1335.45 and 1336.42  $\text{cm}^{-1}$  in bovine gelatin, attributed to the C–N vibrations of tryptophan. In addition, the elevated level of phenylalanine in bovine gelatin aligns with findings from studies by (Harlina et al., 2024; Hassan et al., 2025; Maritha et al., 2023), which reported that bovine gelatin contains a higher concentration of phenylalanine compared to porcine gelatin.

The hierarchical clustering heatmap generated using MetaboAnalyst 6.0 provided a visual overview of the metabolic differences between porcine and bovine gelatin samples, is shown in

figure 4. This analysis simultaneously clustered both samples and metabolites based on similarities in metabolite abundance patterns, allowing for intuitive interpretation of species-specific metabolic profiles.

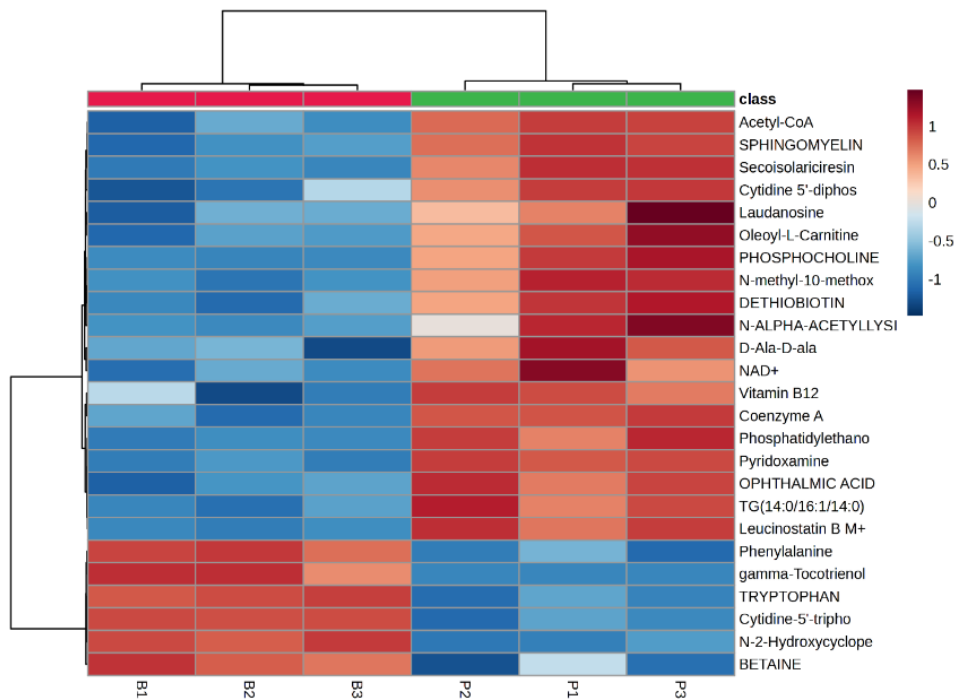


Figure 4. Heatmap analysis in porcine and bovine gelatin.

In the heatmap, color intensity represents the relative abundance of each metabolite across the samples: red indicates higher relative abundance, blue indicates lower relative abundance, and intermediate shades (white or light red/blue) represent moderate metabolite levels. This color gradient facilitates the rapid identification of biochemical patterns. For example, a cluster of red-colored cells corresponding to porcine samples suggests that certain metabolites are consistently more abundant in porcine gelatin, while a blue pattern for the same metabolites in bovine samples indicates lower abundance.

The results revealed a clear separation between porcine and bovine gelatin samples, as evidenced by the distinct clustering observed in the sample dendrogram. Samples derived from the same species clustered closely together, indicating consistent and species-specific metabolic signatures. These findings align with the PCA and PLS-DA results, further confirming that the metabolomic profiles of bovine and porcine gelatin are significantly different.

## Conclusion

This study presents a comprehensive metabolomics-based approach for gelatin authentication using LC-QTOF-MS/MS. The results demonstrate that clear metabolic signatures can effectively differentiate between porcine and bovine gelatin, offering a powerful tool for halal adulteration prevention. The identification of species-specific metabolites, particularly those with high VIP scores, provides a robust biochemical foundation for gelatin source differentiation. This is especially significant for halal authentication, as the consumption of porcine-derived products is prohibited in Islam. By enabling accurate discrimination between gelatin sources, this metabolomics approach offers a valuable analytical tool for regulatory agencies, food manufacturers, and certification bodies to ensure compliance with halal standards.

In a broader context, the application of metabolomics contributes to enhanced

transparency, traceability, and consumer confidence in the global food supply chain. This is particularly relevant in multicultural markets where religious or ethical dietary restrictions such as halal, kosher, or vegetarian play a critical role in consumer decision-making. As such, metabolomics-based techniques have strong potential to become standard practice for the authentication of animal-derived ingredients in processed foods and nutraceuticals. Future research may extend this method to complex food matrices containing gelatin and further validate the identified metabolic markers across a wider range of samples and processing conditions.

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## Profiliranje svinjskog i govedeg želatina zasnovano na metabolomici za halal autentifikaciju

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Originalni naučni rad

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### Sažetak

Želatin se široko koristi u prehrambenoj, farmaceutskoj i kozmetičkoj industriji, gdje je njegovo porijeklo ključno za halal autentifikaciju i otkrivanje falsifikata. Ova studija koristi pristup zasnovan na metabolomici, koristeći tečnu hromatografiju u kombinaciji s kvadrupolnim vremenski-of-flajt tandem masenim spektrometrom (LC-QTOF-MS/MS), kako bi se detaljno profilisale metaboličke razlike između svinjskog i govedeg želatina. Neselektivna (untargeted) metabolomika je provedena s ciljem identifikacije metaboličkih potpisa specifičnih za vrstu. Primijenjene su analiza glavnih komponenti (PCA) i parcijalna najmanje kvadrata diskriminantna analiza (PLS-DA) za otkrivanje metaboličkih varijacija i klasifikaciju izvora želatina s visokom tačnošću. Rezultati su pokazali da PCA jasno razlikuje goveđi od svinjskog želatina. Nekoliko metabolita s visokim VIP vrijednostima, uključujući sfingomijelin SM(d18:0/18:1(9Z)), citidin 5'-difoskokolin, 3-metilcitidin, NAD<sup>+</sup>, ATP i Tyr-Tyr, pronađeni su u većoj količini u svinjskom želatinu, dok su citidin-5'-trifosfat, triptofan i fenilalanin bili prisutniji u goveđem želatinu. Ove metaboličke razlike proizlaze iz specifičnih varijacija po vrstama u razgradnji kolagena i putevima razmjene nukleotida. Metabolomički pristup zasnovan na LC-QTOF-MS/MS nudi visoku osjetljivost i neselektivnu analitičku platformu za autentifikaciju želatina, sprječavajući prevarne zamjene u lancu prehrambene opskrbe. Ova metoda predstavlja robusno, na podacima zasnovano rješenje za razlikovanje izvora želatina i može se koristiti i u halal kontekstu.

**Ključne riječi:** Metabolomika, goveđi želatin, svinjski želatin, halal, LC-QTOF-MS/MS

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## JOURNAL OF HALAL QUALITY AND CERTIFICATION

### Exploring Halal Cosmetics: *Morus Nigra* As An Alternative To Halal Coloring In Lipstick And Antimicrobial Cream Formulations

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Original scientific paper



#### ABSTRACT

The demand for halal-certified cosmetics has significantly increased due to consumer awareness of ethical, safe, and Shariah-compliant ingredients. Conventional cosmetic colorants often raise concerns regarding their synthetic origin, potential toxicity, and non-halal sources. *Morus nigra* (*M. nigra*), or black mulberry, is a flowering plant native to southwestern Asia, valued for its nutritious fruits with antioxidant, anti-inflammatory, and antibacterial properties. The high anthocyanin content in *M. nigra* provides an intense natural pigment of suitable for cosmetic applications while offering antioxidant and antimicrobial properties. The aim of this study is to explore *M. nigra* extract as a natural halal alternative for lipstick coloring and antimicrobial cream formulation. *M. nigra* was extracted using a maceration method. Antimicrobial cream and lipstick containing *M. nigra* extract were formulated using halal-certified ingredients, supported by relevant documentation, including the Halal Certificate, Certificate of Analysis (CoA), International Nomenclature of Cosmetic Ingredients (INCI), and Material Safety Data Sheet (MSDS). Formulated lipstick and antimicrobial cream were tested using disk diffusion test. The antioxidant properties of *M. nigra* cream and lipstick (5, 10, 15 % W/W) were tested by using DPPH and FRAP method and UV protection properties were tested using SPF test. Furthermore, physicochemical properties of lipstick and antimicrobial cream were also evaluated. The lipstick formulation was assessed for color intensity, pH stability, and texture, while the antimicrobial cream was tested for its antimicrobial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results showed that *M. nigra* lipsticks exhibit a purplish red color with a fruity odour. Lipstick has uniform aspect, good spreadability and stable at pH 5.72 to 6.02 making it suitable to be applied onto the lip. *M. nigra* extract (100µg/ml) have great antioxidant activity of 85.86% in DPPH and 2.409 µmol Fe (II)/g in FRAP assay and have UV protection percentage of 37.23% on SPF test. The antimicrobial testing showed that *M. nigra* cream was able to inhibit the growth of *S. aureus* and *P. aeruginosa*. This study highlights *M. nigra* as a sustainable, halal-friendly, and bioactive alternative to synthetic dyes and antimicrobial agents in the cosmetic industry. The findings contribute to the development of natural and halal-certified beauty products, catering to the growing market demand.

**Keywords:** Halal cosmetics, *Morus nigra*, natural colorant, antimicrobial cream

## Introduction

The global demand for halal cosmetics has significantly increased, driven by the rising awareness among Muslim consumers regarding the permissibility and ethical sourcing of cosmetic ingredients. Halal cosmetics must comply with Islamic principles, ensuring that products are free from prohibited (haram) substances such as porcine-derived ingredients, alcohol, and animal-derived colorants that are not slaughtered according to halal guidelines (Ali et al., 2016). As a result, the cosmetic industry is increasingly exploring natural, plant-based alternatives that meet both regulatory and religious standards. Among these, *Morus nigra* (*M nigra*) (black mulberry), a fruit known for its rich anthocyanin content and deep pigmentation, has emerged as a promising candidate for use in natural colorant applications (Khoo et al., 2017). Its vibrant hue and halal-friendly botanical origin make it a potential substitute for synthetic dyes and animal-derived pigments commonly used in lipstick and topical formulations.

In addition to its coloring properties, *M nigra* has also attracted attention for its antimicrobial and antioxidant activities, which are beneficial for skin care and therapeutic cosmetic formulations. Studies have shown that extracts of *M nigra* possess broad-spectrum antimicrobial properties, which can be harnessed in the development of antimicrobial creams aimed at preventing or managing skin infections (Hemavathi et al., 2024). This dual functionality—providing both pigmentation and bioactivity—aligns with the growing consumer preference for multifunctional, clean-label, and ethically produced cosmetics. Therefore, investigating the potential of *M nigra* in halal-certified lipstick and antimicrobial cream formulations not only addresses religious and ethical concerns but also supports the development of sustainable and health-promoting cosmetic products.

## Methodology

### *Identification of Halal Ingredients*

A halal certificate was provided by the supplier during the purchase of raw material. The raw material without halal certificate was checked using Certificate of Analysis (CoA), Material Safety Data Sheet (MSDS) and International Nomenclature Cosmetic Ingredient (INCI) to ensure the safeness and the sources of the material purchase are from plant based and safe to be used.

### *Morus Nigra Extraction*

2 kg of *M nigra* fruits was dried using an oven at 50°C for 3 days until completely dry. After drying, the fruits were blended and sieved into fine powder. The dried fruit powder was extracted using the maceration method. The crude methanolic extract was obtained, weighed, and stored in refrigerator for lipstick and cream preparation (Arif et al., 2019).

### *Formulation of M nigra Lipstick Formulation*

White beeswax and white soft paraffin were categorized in phase A, olive oil and glycerin were categorized in phase B, *Morus Nigra* extract, methylparaben and propylparaben were categorized in phase C. Then, melted phase A ingredients by heating up, then added in phase B ingredients. Phase C was added to phase A and B mixture after and then poured in the lipstick molds. It was kept in a laboratory refrigerator for 15 minutes for solidification and then the lipstick was removed from the mold (Saied et al., 2022).

### *Formulation of M nigra Cream Formulation*

40 g cream containing different weight of *M. nigra* extract (0 g, 2 g, 4 g, and 6 g) were incorporated into the cream. The formulation process started with the emulsifier (stearic acid) and other oil soluble components were dissolved in the oily phase and heated to 75 °C using water bath. Then, the preservatives and other water-soluble components were dissolved in the aqueous phase in a separate beaker followed by heating at 75°C using water bath. After the heating process was completed, the aqueous

phase was mixed with the oily phase with continuous stirring until the mixture cooled. Lastly, the formulated cream was transferred into a plastic container and labeled for further analysis.

#### *Physical evaluation of M nigra Lipstick and Cream*

The physical evaluation of the lipstick and cream formulations was conducted by recording their organoleptic after one month of storage. The cream properties such as appearance, colour, odour, texture, phase separation, pH and homogeneity were observed following the method by Viswanad et al. (2012), meanwhile stability, spreadability and breaking point was measured in lipstick.

#### *Lipstick Sun Protection Factor (SPF) Test*

SPF value in lipstick was measured with a UV-vis spectrophotometer to analyze UV absorbance of samples. 1 mg of lipstick sample was prepared by dissolving in hexane and washed with distilled water. The absorbances of the lipstick samples (0.1 mg/mL) were measured in the range of 290–320 nm, with 5 nm increments, and three determinations were made at each point (Sadeghifar & Ragauskas, 2020).

#### *Antioxidant Activity of M nigra Lipstick*

##### *DPPH (1, 1-diphenyl-2-picryl hydroxyl) Radical Scavenging Assay*

0.1 mM DPPH solution was prepared. 1 mL of lipstick samples with different concentrations added to different test tubes. Each test tube contains different concentrations of sample added with 3 mL of DPPH solution. A test tube containing 3 mL of solution containing DPPH in 1mL of distilled water without sample was used as the negative control while 3mL of DPPH solution and 1mL of ascorbic acid was used as positive control. The tubes were then kept in the darkroom for 30 min. The absorbance was determined at 517 nm using UV-vis spectrophotometer. Lipstick samples were prepared and measured in triplicates (Baliyan et al., 2022).

##### *Ferric Reducing Antioxidant Power (FRAP) Assay*

1 mL of lipstick samples with different concentrations added to different test tubes. Each test tube contains different concentrations of lipstick samples added with 2.5 mL of the FRAP reagent. The prepared lipstick samples were incubated at 37 °C 30 minutes in complete darkness. A test tube containing 2.5 mL of solution containing FRAP in 3mL of distilled water without lipstick sample was used as the negative control while 2.5 mL of FRAP solution and 1 mL of ascorbic acid was used as positive control. Absorbance was then measured at 593 nm using a UV-vis spectrophotometer. Lipstick samples were prepared and measured in triplicates (Wojtunik-Kulesza, 2020).

#### *Antimicrobial Screening of M nigra Cream*

The antimicrobial activity of *M nigra* cream against *S aureus* and *P aeruginosa* was evaluated using the disk diffusion method. Agar plates were first inoculated by evenly spreading the test microorganisms across the surface. Filter paper discs (6 mm) soaked with the extract were then placed on the agar, and the plates were incubated at 37°C for 24 hours to facilitate bacterial growth. Following incubation, clear areas surrounding the discs indicating suppression or absence of bacterial growth were measured in millimeters. Each extract was tested in four replicates against each microorganism, and the results were reported as mean ± standard deviation (Pawar et al., 2019).

## **Results**

#### *Physical evaluation of M nigra Lipstick and Cream*

Figure 1 shows the *M nigra* cream in different concentration. The colour increased in intensity in the order of 2 g < 4 g < 6 g of *M. nigra* extracts cream formulations. All the cream formulations with *M. nigra* extract had a berry like smell. The odour of the cream became stronger as the weight of the active ingredient increased. Based on the results, the four creams showed smooth texture and homogenous with no sign of phase

separation. They are also moisturizing, non-greasy, light and washable upon application which made it suitable to be applied onto the skin. A study conducted by Aswal et al. (2013) on polyherbal cosmetic cream indicated that a stable formulation has almost constant pH, homogenous, emollient, non-greasy and easily removed after application.



Figure 1. The *M nigra* cream in different concentration

Figure 2 shows the *M nigra* lipstick in different concentration. The colour increased in intensity in the order of 2 g < 4 g < 6 g of *M. nigra* extracts lipstick formulations. Over a 30-day stability test, no physical changes at room temperature ( $24.0 \pm 3.0$  °C) was observed in all formulation. The colour and odour of all formulations remained purplish red and emitted a fruity smell, consistent with Susmiatun et al. (2018), indicating good product durability. Spreadability remained good at room temperature but was impaired under high temperatures, melting points for all formulations ranged from 52.3°C to 60.2°C, within the acceptable range for hot climates (50–75°C) as supported by Rigano (2021), indicating satisfactory thermal stability.



Figure 2. The *M nigra* lipstick in different concentration

The pH values of the lipstick formulations ranged from 5.72 to 6.02, which is within the safe range for lip application (pH 4–7) (Saeid et al., 2022). The pH decreased as more *M nigra* extract was added, due to its acidic nature, as supported by Okatan et al. (2016), who reported an average pH of 3.85 for the fruit.

For the breaking point test, Formulation 1 (2g *M nigra* extract) had the highest breaking point at 133.33 g, while Formulation 2 (4g extract) was slightly lower, and Formulation 3 (6g extract) had the lowest breaking point at 70 g. This indicates that increasing the amount of extract reduced the hardness of the lipstick. According to Chee et al., (2018), a higher percentage of base content results in a harder lipstick with a greater breaking point.

#### Lipstick SPF Test

The highest percentage of UV protection was F3 as shown in Table 1. This was in line with study by Nareswari et. al. (2019), which uses crude palm oil as an active ingredient to produce natural lip balm with 5 formulations (15, 12.5, 10, 7.5, 5 % b/b). The study found that the higher active ingredients are used, the higher the percentage of UV protection produced.

**Table 1.** UV Protection Percentage of Lipstick Formulations

| Formulation | UV Protection (%) |
|-------------|-------------------|
| F1 (2g)     | 32.22             |
| F2 (4g)     | 35.38             |
| F3 (6g)     | 37.07             |

#### Antioxidant Activity of *M nigra* Lipstick

The antioxidant activity of the lipstick formulations was confirmed through both DPPH and FRAP assays. In DPPH, all formulations showed radical scavenging ability with Formulation 3 (F3), exhibiting the greatest antioxidant activity. In the FRAP assay, F3 showed the highest ferric reducing power (0.379  $\mu\text{mol Fe(II)/g}$ ), followed by F2 and F1. The results indicated a dose-dependent relationship, where increasing extract concentration led to higher antioxidant effects. These findings align

with research conducted by Pamungkas et al., (2013), which also demonstrated a similar pattern in lipstick formulated with 8g of mangosteen rind extract had the highest antioxidant activity compared to lower concentration of extract.

**Table 2:** FRAP and DPPH of *M nigra* Lipstick

| Lipstick Formulation | FRAP Value (Mmol Fe(ii)/G) | DPPH (%) |
|----------------------|----------------------------|----------|
| Formula 1 (2g)       | 0.282                      | 10.56    |
| Formula 2 (4g)       | 0.322                      | 11.40    |
| Formula 3 (6g)       | 0.379                      | 12.38    |

#### Antimicrobial Activity of *M nigra* Cream

From the study, only the cream formulation with 6 g of *M nigra* extract showed zone of inhibition against *S. aureus*. However, there is no zone of inhibition recorded against *P. aeruginosa* after the *M nigra* extract had been formulated into cream as dosage form. The results were in-line to the study conducted by Melo et al. (2022), in which the emulsion with *M nigra* extract possessed antimicrobial activity against *S. aureus*, but there was no activity recorded against *P. aeruginosa*. This may due to different variations in the performance of the tests, such as different sizes of bacterial inoculum, volume, and type of agar, well size, disc size, incubation period and type of extraction. Thus, standardization through guidelines is the way to reduce the number of conflicts. The findings of this study showed that the *M nigra* extract lost its antimicrobial properties against *P. aeruginosa* after it was incorporated into a cream base. The loss of antimicrobial property in this study might be due to the interaction between the preservatives and active ingredient used in the cream formulation or due to the low concentration of *M nigra* extract used in cream formulation.

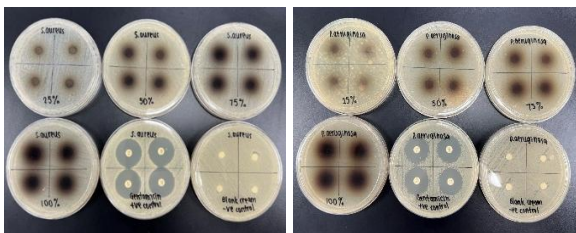


Figure 3. Disk diffusion test *S. aureus* and *P. aeruginosa*

## Conclusion

The use of *M nigra* addresses the need for halal-certified natural colorants in cosmetics. Its incorporation into lipstick not only meets aesthetic needs but also fulfills halal requirements for safe, clean, and ethical product development. This study successfully formulated two halal-compliant cosmetic products, lipstick and cream using *M nigra* fruit extract that demonstrated desirable physical and functional properties. Thus, *M nigra* presents a promising multifunctional ingredient offering natural colour, antioxidant and antimicrobial benefits, and compliance with halal cosmetic standards aligning with growing consumer demand for Shariah-compliant, plant-derived cosmetic ingredients.

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## Istraživanje halal kozmetike: *Morus nigra* kao alternativa halal bojilima u formulacijama ruža za usne i antibakterijske kreme

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Originalni naučni rad

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### Sažetak

Potražnja za kozmetikom s halal certifikatom značajno je porasla zbog sve veće svijesti potrošača o etičkim, sigurnim i šerijatski prihvatljivim sastojcima. Konvencionalna kozmetička bojila često izazivaju zabrinutost zbog svog sintetičkog porijekla, potencijalne toksičnosti i izvora koji nisu halal. *Morus nigra* (*M. nigra*), odnosno crni dud, je cvjetnica porijeklom iz jugozapadne Azije, cijenjen zbog svojih hranjivih plodova s antioksidativnim, protuupalnim i antibakterijskim svojstvima. Visok sadržaj antocijanina u *M. nigra* pruža intenzivnu prirodnu boju pogodnu za kozmetičke primjene, uz dodatne antioksidativne i antimikrobne efekte. Cilj ove studije je istražiti ekstrakt *M. nigra* kao prirodnu halal alternativu za bojenje ruža za usne i formulaciju antimikrobne kreme. *M. nigra* je ekstrahirana metodom maceracije. Antimikrobna krema i ruž za usne s ekstraktom *M. nigra* formulirani su koristeći sastojke s halal certifikatom, uz prateću dokumentaciju uključujući Halal certifikat, Certifikat o analizi (CoA), Međunarodnu nomenklaturu kozmetičkih sastojaka (INCI) i Sigurnosno-tehnički list (MSDS). Formulirani ruž za usne i antimikrobna krema testirani su metodom difuzije na disku. Antioksidativna svojstva *M. nigra* kreme i ruža (5, 10, 15 % W/W) testirana su pomoću DPPH i FRAP metode, a UV zaštitna svojstva su ispitana putem SPF testa. Također su ispitana i fizičko-hemijska svojstva ruža i kreme. Formulacija ruža ocijenjena je po intenzitetu boje, stabilnosti pH i teksturi, dok je antimikrobna krema testirana na djelovanje protiv *Staphylococcus aureus* i *Pseudomonas aeruginosa*. Rezultati su pokazali da ruž s *M. nigra* ima purpurno-crvenu boju s voćnim mirisom. Ruž ima ujednačen izgled, dobro se razmazuje i stabilan je pri pH vrijednostima od 5,72 do 6,02, što ga čini pogodnim za nanošenje na usne. Ekstrakt *M. nigra* (100 µg/ml) pokazao je visoku antioksidativnu aktivnost od 85,86% u DPPH testu i 2.409 µmol Fe(II)/g u FRAP analizi, te UV zaštitu od 37,23% prema SPF testu. Antimikrobno testiranje pokazalo je da krema s *M. nigra* može inhibirati rast *S. aureus* i *P. aeruginosa*. Ova studija ističe *M. nigra* kao održivu, halal-prihvatljivu i bioaktivnu alternativu sintetičkim bojilima i antimikrobnim agensima u kozmetičkoj industriji. Nalazi doprinose razvoju prirodnih i halal-certificiranih kozmetičkih proizvoda, odgovarajući na rastuću potražnju tržišta.

**Ključne riječi:** halal kozmetika, *Morus nigra*, prirodno bojilo, antimikrobna krema

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## JOURNAL OF HALAL QUALITY AND CERTIFICATION

### Nutritional habits of respondents who consume only halal food aged 30–60 in the Tuzla Canton

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Original scientific paper



#### ABSTRACT

A proper and healthy diet should be a priority for individuals who consume only halal food to reduce the risk of diseases linked to poor dietary habits. However, a fast-paced lifestyle has led to the neglect of nutrition, causing deviations from proper halal nutrition dietary principles. Halal nutrition is not just about choosing permissible foods but also about their proper preparation and consumption in line with health and well-being principles. The aim of this study was to determine the dietary habits of consumers who exclusively consume halal food and to analyze the extent to which these habits align with the principles and recommendations of halal nutrition. The research methodology involved the collection and analysis of data through an online questionnaire distributed among respondents aged 30 to 60 from the Tuzla Canton. The study results revealed significant deviations in the application of proper halal nutrition among a large number of respondents. The analysis of responses showed that a considerable number of respondents do not pay sufficient attention to nutritional balance and dietary diversity, while many reported only occasionally considering the nutritional aspect of their diet. This indicates that halal nutrition is often perceived solely through the lens of food permissibility. Additionally, the results demonstrated that most respondents only occasionally pay attention to moderation in food consumption. These findings suggest a lack of awareness regarding the importance of moderation as a fundamental principle of halal nutrition. The findings highlight the need for further education on the principles of proper halal nutrition to improve understanding and application. The gap between theoretical knowledge and practical implementation suggests opportunities for further research and educational initiatives. This study can serve as a foundation for broader research on the dietary habits of the halal population and the factors influencing their food choices.

**Keywords:** proper halal nutrition, nutritional intake, dietary habits

## Introduction

In contemporary society, dietary habits play a key role in maintaining health and preventing numerous chronic diseases. The fast-paced lifestyle increasingly leads to the neglect of dietary quality, characterized by the predominance of high-energy food intake, saturated and trans fats, sugars and salt. This dietary pattern contributes to the development of obesity, metabolic syndrome, type 2 diabetes, cardiovascular diseases and even certain types of cancer (WHO, 2021). The World Health Organization (WHO) has long warned of these risks, emphasizing the direct connection between diet and an individual's quality of life. On the other hand, a growing body of research highlights the positive impact of religious principles on health behaviors and psychophysical well-being. In this context, halal nutrition, based on Islamic regulations, does not solely imply the consumption of permissible food in the religious sense, but also a broader dietary concept that includes cleanliness, moderation, balanced nutrient intake and awareness of the harmful effects of excess (Tan et al., 2013; Pranić et al., 2023). Halal nutrition promotes a responsible attitude towards the body and health, whereby religious regulations complement modern nutritional recommendations (Čanković et al., 2011). However, in contemporary settings, there is a growing imbalance between the declarative commitment to halal food and the actual application of halal dietary principles. The consumption of certified halal products, especially industrially processed fast food, sweets and snacks, often does not align with the broader concept implied by halal nutrition, which includes moderation, nutritional value and awareness of food's impact on health (Pranić et al., 2023). This highlights the need for a deeper understanding of actual dietary patterns and the motivations of consumers who declare themselves as exclusive halal food consumers. Based on available literature, no systematic research has been conducted in the Tuzla Canton context to examine consumers' attitudes and behaviors towards halal nutrition in its broader

sense. The aim of this paper is to analyze the attitudes of consumers who consume exclusively halal food and to determine the extent to which they adhere to other principles of halal nutrition, including awareness of moderation, food nutritional quality and the connection between dietary choices and health.

## The Relationship between Health and Nutrition

Throughout history, dietary recommendations have been passed down through generations, often mixing scientifically based advice with potentially harmful practices. According to the World Health Organization, health is not merely the absence of disease but complete physical, mental and social well-being (WHO, 2020). In professional circles, this concept is often referred to as "*wellness*" or "*complete well-being*." The individual's goal should be to achieve balance in bodily, mental and spiritual health, which can be attained through proper, varied nutrition, avoiding harmful habits such as smoking and alcohol consumption, regular physical activity and maintaining a healthy body weight. Nutrition primarily refers to food intake aimed at meeting the body's needs for energy and nutrients. However, the balance in the quality and quantity of nutrients consumed is crucial. Inadequate nutrition can seriously impair immune function, increase susceptibility to infections and lead to various forms of malnutrition and undernourishment (Thompson & Manore, 2018). Nutrition's role in disease development is multi-layered, it can be a direct cause or an indirect risk factor. For instance, deficiencies in certain nutrients can cause diseases such as scurvy and pellagra, while general nutritional imbalance weakens the body and increases vulnerability to infections. Thanks to nutritional initiatives, food fortification with essential micronutrients has significantly reduced the incidence of these conditions in developed countries, although significant challenges remain in developing nations. An unbalanced diet can also be a factor in the development of osteoporosis, certain cancers and chronic non-communicable diseases

such as heart disease, stroke and diabetes. Obesity, most often resulting from excessive caloric intake relative to expenditure, is a dominant risk factor for these diseases and its prevalence has been increasing globally for decades (Thompson & Manore, 2018).

It is estimated that the average person, if they reach the age of 65, will consume more than 70,000 meals or approximately 50 tons of food throughout life. Each of these meals leaves a mark on the body, with dietary effects becoming more evident with age. Our bodies are constantly regenerating: new muscles, bones, blood and skin form, while old tissues break down and are replaced. The body also stores or uses fat according to intake and energy needs. The food consumed today directly becomes part of the body tomorrow. Optimal nutrition enables proper growth and function of the muscular and skeletal systems and contributes to healthy skin and blood. Therefore, it is important that the diet provides all essential nutrients: water, carbohydrates, fats, proteins, vitamins and minerals (Sizer & Whitney, 2021). A deficiency or excess of certain nutrients can quickly lead to mild health disturbances, while long-term imbalances may cause serious illnesses. Proper nutrition that ensures an appropriate intake of energy and nutrients is essential for preventing various forms of malnutrition, from nutrient deficiencies to imbalances and excesses. Poor dietary habits, along with smoking and alcohol consumption, further increase health risks. Four of the leading causes of death, cardiovascular diseases, cancers, stroke and diabetes, are directly linked to dietary patterns. In addition, alcohol-related accident mortality accounts for a significant proportion. Although genetics, physical activity and lifestyle play roles, it is everyday dietary and behavioral choices that fundamentally shape the risks of these diseases. Besides nutrition, other factors also significantly influence health: avoidance of tobacco and alcohol, sleep quality, stress levels and environmental conditions can either mitigate or

exacerbate health outcomes (Sizer & Whitney, 2021).

### **Obesity and Obesity-Related Diseases**

In contemporary society, obesity is one of the most widespread chronic diseases. The World Health Organization (WHO) defines it as abnormal or excessive fat accumulation that poses a risk to health. Four of the top ten causes of mortality, heart disease, stroke, cancer and diabetes, are directly linked to diet (Brown, 2017). Obesity is recognized as a key risk factor for the development of these conditions, alongside insulin resistance and hypertension. Body mass is assessed using the Body Mass Index (BMI), which allows for classification into categories: underweight, normal weight, overweight and obese. Its advantage lies in its universal applicability across both genders (Brown, 2017). Statistics show that overweight individuals have an increased risk of mortality, second only to underweight individuals. Excess body weight negatively affects health by causing metabolic disorders such as hypertension, elevated triglycerides, glucose and insulin levels, fatty liver and increased C-reactive protein, a marker of inflammation. These changes develop gradually but can be prevented with timely preventive measures. Obesity results from the combined effects of multiple factors, including genetic predisposition, high sugar intake, low socioeconomic status, sleep deficiency, exposure to polychlorinated biphenyls (PCBs), smoking cessation and the use of certain medications (Brown, 2017).

The most common risk factors for chronic diseases such as hypertension, stroke, heart disease, type 2 diabetes, gallbladder disease, osteoarthritis and certain cancers include excessive body weight, unbalanced diet, physical inactivity, smoking, genetic predisposition, stress and hormonal changes. Obese individuals often face discrimination, which negatively affects their mental health, increasing the risk of anxiety, depression and reduced life satisfaction (Brown,

2017). Along with obesity, cigarette and alcohol consumption seriously endanger health. Although their harmful effects are well known, their usage trends do not decline. Smoking harms nearly every organ and passive smoking especially endangers children, increasing the risk of respiratory diseases such as asthma, bronchitis and pneumonia (Ayad, 2012). Alcohol has severe consequences, particularly for the liver, but also for other organ systems. Nutritional guidelines increasingly recommend its complete exclusion. Alcoholism harms the individual's physical and mental health and disrupts family and social relationships (Ayad, 2012; Hammaker, 2023).

### **Key Nutrients in Halal Nutrition and Their Role**

Halal nutrition provides clear guidelines for healthy and balanced food intake. It emphasizes the consumption of pure, high-quality foods that do not harm the body and actively contribute to its health. Furthermore, it underscores the importance of moderation; portions should be small and balanced, yet nutritionally rich and beneficial for the body (Saeed, 2022). Understanding the basic nutrients essential for normal bodily function is of fundamental importance. There are six basic categories of nutrients consumed through food. Of these, four are organic and contain carbon (Sizer & Whitney, 2021). Three organic nutrients have energy potential: carbohydrates, as the primary energy source; fats, as a significant energy source and carrier of fat-soluble vitamins; and proteins, which, in addition to providing energy, play a key role in building and repairing body tissues. The remaining two nutrients: vitamins and minerals, do not provide energy but are crucial in regulating numerous biological processes in the body. Minerals such as calcium and phosphorus, for example, contribute to bone formation, while vitamins act as coenzymes in various metabolic reactions (Sizer & Whitney, 2021). Finally and by no means less important, is water—the most abundant nutrient in the human body by quantity. The body constantly loses water through sweating, breathing and excretion,

making regular hydration essential for the function of every cell. Essential nutrients are those that the body cannot produce on its own and must be obtained through food. Such nutrients exist across all six basic groups: water, certain carbohydrates and fats, essential amino acids from proteins, all vitamins and numerous minerals (Sizer & Whitney, 2021). Many foods consumed by Prophet Muhammad (peace be upon him), when analyzed through the lens of modern nutritional science, exhibit anti-inflammatory properties. Since inflammation is a common cause of various chronic diseases, a diet rich in anti-inflammatory foods may play a significant role in preventing and treating such conditions. Adherence to dietary and lifestyle guidelines from the Qur'an and Sunnah can help the body restore its balance. Such a diet may reduce inflammatory processes, improve hormonal balance, optimize brain function, support a healthier gut microbiome and positively influence gene expression (Saeed, 2022; Ali et al., 2018).

### **Halal Diet as a Model for Healthy Living**

Islam is not only a religion of peace but also a complete way of life. Allah has provided clear instructions on how to eat, drink, sleep, pray, behave toward others and even how to use the restroom. These guidelines are not tied to transient social norms but are universal and enduring, forming a foundational guide to success in both worlds. As with other aspects of life, when it comes to food, a Muslim should strive for what benefits the body and avoid what harms the body entrusted to them by Allah. The focus should be on food that provides energy, improves posture, supports weight maintenance or loss, aids in treating chronic diseases and generally leads to a healthier and more successful life. When Allah, exalted is He and His Messenger (peace be upon him) speak about food, the emphasis is always on three principles: quality (ṭayyib), moderation and nutritional density (Saeed, 2022). Such food directly supports gut health, glucose regulation, reduction of insulin resistance and hormonal balance. Nutrient-dense foods assist the body in

regenerating internally and externally, building resistance to disease. The Prophet Muhammad (peace be upon him) set a clear example in his dietary practices and when his practices are compared to modern science, it becomes evident that his nutritional recommendations were based on anti-inflammatory principles. Contemporary research confirms that inflammation is the root cause of most chronic diseases, thus logically supporting the recommendation to reduce or eliminate foods that promote inflammation, not only in those diagnosed with illness but also as a preventive measure. Living in accordance with the Qur'an and Sunnah restores the body's natural balance. Quality, pure food carries biological signals that support health, reduce inflammation, stabilize hormones, optimize brain function, improve the gut microbiome and influence gene expression in a positive way.

High-quality food works from the inside out, but it also has broader ecological implications: it can contribute to soil preservation, improve the nutrient content of plants, restore ecosystems, protect water resources and reduce negative impacts on the climate. These foods contain over 25,000 known plant-based phytochemicals essential for health, which directly affect all biochemical processes in the body. A deficiency of these substances in the diet is associated with the development of various chronic diseases (Saeed, 2022). The benefits of these foods can only be realized when applied according to the principles of the Qur'an and Sunnah. One should not overlook the important hadith which states: "The worst vessel the son of Adam can fill is his stomach..." (Ibn Majah). This hadith reminds us of the dangers of overeating, something modern science now confirms. Scientific research has clearly shown that the digestive system is key to health, as the intestines serve as the body's first line of defense against the external environment. Approximately 70–80% of the immune system resides in the gut, which also houses billions of microorganisms actively involved in regulating health and behavior. For the gut to function efficiently, it requires a diverse and balanced

microbiota. These microorganisms even influence gene expression and every meal, environmental interaction and lifestyle choice impacts the gut microbiome (Saeed, 2022). Our health is no coincidence, it is built with every decision we make, starting with what we put in our mouths.

## **Methods and Materials**

### ***Methods***

The study was conducted as quantitative research with a descriptive-analytical approach. The primary method of data collection was an online survey. The research aim was to examine attitudes, dietary habits and the level of awareness regarding proper and halal nutrition among the adult population.

The obtained data were processed using descriptive statistics, including frequency distributions, percentages and mean values. The analysis was carried out in the Microsoft Excel software environment. The results are presented in tabular and graphical formats and the interpretation of findings was aligned with the defined research objectives and relevant theoretical framework.

### ***Materials***

For the purpose of this study, a survey questionnaire was created using the online platform Google Forms. The questionnaire contained a total of 51 questions, structured as a combination of closed and open-ended questions and divided into six thematic sections. It was distributed electronically via social media to reach the adult population of the Tuzla Canton, aged between 30 and 60 years. The research is part of a broader study involving a total of 206 respondents, of whom 157 were women and 49 men. For the purposes of this paper, a subsample of 176 respondents who stated that they consume exclusively halal food was analyzed. Participation was voluntary and all respondents were previously informed about the purpose of the research. Anonymity and confidentiality of

the collected data were ensured in accordance with ethical research principles. Additionally, a review of relevant available literature was

conducted to establish a scientifically grounded framework based on which the dietary habits of respondents were assessed.

**Table 1.** Respondents' Attitudes toward Halal Nutrition

| Question   | Response Option           | % of Responses |
|--|---------------------------|----------------|
| Is it sufficient for halal nutrition that only the food being prepared is halal?   | 1) Yes                    | 66.5 %         |
|  | 2) Not sure               | 30.1 %         |
|  | 3) No                     | 3.4 %          |
| How often do you check nutritional information on halal products?                  | 1. Never                  | 14.2 %         |
|  | 2. Occasionally           | 59.1 %         |
|  | 3. Regularly              | 26.7 %         |
| I consume various types of halal food while ensuring nutritional balance.          | 1) I do not pay attention | 9.1 %          |
|  | 2) Sometimes              | 63.1 %         |
|  | 3) Always                 | 13.1 %         |
| When consuming halal food, I make sure not to overeat.                             | 1) I do not pay attention | 23.9 %         |
|  | 2) Sometimes              | 59.7 %         |
|  | 3) Always                 | 31.3 %         |
| Practicing halal nutrition can improve health related to diet-associated diseases. | 1) Not sure               | 14.2 %         |
|  | 2) Strongly agree         | 79.5 %         |
|  | 3) Strongly disagree      | 6.3 %          |
| Is health the most common reason for choosing halal food?                          | 1) Not sure               | 22.7 %         |
|  | 2) Strongly agree         | 18.8 %         |
|  | 3) Strongly disagree      | 58.5 %         |
| Is religion the most common reason for choosing halal food?                        | 1) Not sure               | 4.5 %          |
|  | 2) Strongly agree         | 83.5 %         |
|  | 3) Strongly disagree      | 11.9 %         |

**Results and Discussion**

Respondents' attitudes toward halal nutrition are presented in Table 1. The analysis of collected data clearly indicates a strong connection to halal principles, with religious motivation prevailing as the primary reason for choosing this dietary approach. Specifically, 83.5% of respondents strongly agree that religion is the most common motive for consuming halal food, whereas only 18.8% cite health as the main reason, which is significantly lower. These findings confirm that most respondents perceive halal nutrition primarily through a spiritual and religious lens. It is noteworthy that, although health is not the leading motivation, there is a strong belief in the positive health impact of halal nutrition. As many

as 79.5% of respondents believe that practicing halal nutrition can improve health status, particularly in the context of preventing diet-related diseases. This finding opens space for greater educational engagement, as awareness of health relevance exists even if it is not the dominant initial motive. This represents a valuable entry point for nutritionists and health educators aiming to strengthen the connection between religious principles and modern nutritional science. In this sense, halal nutrition can serve as a strong platform for preserving identity and promoting health and chronic disease prevention, provided it is practiced in accordance with the principles of moderation, balance and variety.

**Table 2.** Nutritional Habits of Respondents

| <b>Nutritional Habits</b>  | <b>Occasionally</b> | <b>Daily / More than 3 times per week</b> |
|--|---------------------|---|
| Consumption of white flour products  | 61.90%              | 13.10%                                    |
| Frequency of consuming sweets, cakes, pastries, chocolate, candy, puff pastries      | 53.41%              | 39%                                       |
| Frequency of consuming fried food  | 64.77%              | 9.10%                                     |
| Consumption of diet beverages, carbonated and non-carbonated                         | 46.02%              | 5.50%                                     |
| Frequency of consuming food containing margarine, vegetable fat, beef tallow         | 32.95%              | 3.97%                                     |
| Consumption of processed meats and salty snacks                                      | 81.81%              | 7.95%                                     |
| Frequency of consuming whole milk and full-fat dairy products (butter, fatty cheese) | 63.10%              | 27.80%                                    |
| Frequency of consuming sunflower, sesame, pumpkin seeds, soy, nuts                   | 68.75%              | 23.90%                                    |
| Consumption of fruits and vegetables   | 37.50%              | 61.40%                                    |
| Frequency of consuming healthy dairy products such as whey, kefir, fresh cheese      | 61.40%              | 22.30%                                    |
| Frequency of drinking 100% natural juices  | 79%                 | 21%                                       |
| Consumption of whole grain products  | 49.40%              | 39.70%                                    |
| Consumption of berries   | 75%                 | 13.10%                                    |
| Consumption of leafy and cruciferous vegetables                                      | 76.70%              | 14.20%                                    |
| Consumption of legumes   | 86.93%              | 6.25%                                     |
| Frequency of fish consumption (not fried)  | 51.70%              | 0   |
| Frequency of drinking unsweetened green tea  | 55.26%              | 6.25%                                     |

Although awareness of the importance of halal nutrition is high, 66.5% of respondents believe that it is not sufficient for food to be formally halal, but that it should encompass broader principles. At the same time, a concerning low level of knowledge and practice regarding the nutritional aspects of the diet is observed. Only 26.7% of respondents regularly check nutritional information on halal products, while 59.1% do so occasionally and 14.2% never. These figures clearly show that, despite a general commitment to the halal way of life, there is a significant gap in understanding proper and balanced nutrition within this concept. This gap is further supported by data related to dietary balance and portion control. Only 13.1% of respondents consistently ensure nutritional balance in their food, while 63.1% do so occasionally. Regarding moderation in food intake, a core principle of both Islamic and modern nutrition, only 31.3% report it as a regular practice. More than half (59.7%) pay attention to it only occasionally and 23.9% not at

all. These results suggest that the majority fall into a so-called "comfort zone" of superficial knowledge and practice, a common occurrence in nutritional studies when respondents feel uncertain about their knowledge but wish to demonstrate some level of engagement.

These data can also be viewed in the context of global and local studies showing low frequency of reading nutritional labels. For example, in the Una-Sana Canton, only 2.7% of respondents reported always reading labels, while over 49% do so rarely or never (Muhamedbegović et al., 2018). In France, in 2004, only 22% of consumers actively monitored nutritional information (Temple et al., 2014), while in Croatia, only 39% read labels regularly and as many as 60% never or rarely pay attention to them. This clearly indicates that the issue is not a lack of willingness, but rather a complex combination of insufficient knowledge, time constraints, and lack of interest, factors also identified by other authors

as contributing to 'information overload' and 'time pressure' during decision-making in stores. Therefore, although halal nutrition is deeply rooted as part of identity and religious practice, it is evident that there is room for further development of awareness regarding its health and nutritional aspects. These findings point to an urgent need for additional education and more accessible information about what halal nutrition truly entails, not only that food is technically permissible, but also that it aligns with modern principles of healthy eating: diverse, moderate and nutritionally valuable.

### **Nutritional Habits of Respondents Who Consume Only Halal Food**

The analysis of the data on the respondents' nutritional habits indicates the presence of significant challenges when it comes to the daily consumption of foods that contribute to health, while simultaneously highlighting the excessive use of food products that may potentially jeopardize health. According to the collected data, 61.4% of respondents consume fruits and vegetables daily or more than three times a week, which represents a slightly more favorable indicator compared to the previous sample. However, 37.5% of respondents still consume fruits and vegetables only one to three times a week, a significant percentage considering the importance of these foods for the prevention of chronic diseases and strengthening the immune system. In contrast, concerning data shows that 81.81% of respondents consume processed meats and salty snacks one to three times a week, while 7.95% consume them daily. These foods, rich in saturated fats, salt and additives, negatively impact health. Excessive consumption of processed meat is classified as carcinogenic, while red meat is considered probably carcinogenic, especially in the context of colorectal cancer (IARC, 2015). Additionally, the reduced consumption of berries (only 13.1% daily) and leafy and cruciferous vegetables (14.2%) further deteriorates the nutritional balance of the diet. Phytochemicals from these foods have been recognized as agents against

obesity, as they reduce oxidative stress and inflammation (Pem and Jeewon, 2015).

Another notably problematic data point concerns the high intake of refined products, with 61.9% of respondents consuming white flour products daily, while only 39.7% consume whole grain products daily or more than three times a week. Refined grains have a high glycemic index, causing disruptions in glucose and insulin levels, thereby increasing the risk of metabolic diseases, including type 2 diabetes and cardiovascular diseases (Suárez et al., 2022). In contrast, whole grains are rich in fiber and indigestible carbohydrates, which contribute to gut health and the reduction of inflammatory processes. Excessive sugar intake is clearly expressed, with 39% of respondents consuming sweets, cakes, chocolate, candies and similar products daily. Excessive consumption of simple sugars is directly associated with increased body weight, the development of insulin resistance and type 2 diabetes and an elevated risk of cardiovascular diseases. Halal nutrition can play an important role in changing habits, as it promotes the consumption of natural and whole foods. The Quran emphasizes the importance of consuming various herbs, fruits and natural resources: "He sends down water from the sky and with it We make grow all kinds of plants... and vineyards planted with vines, especially olives and pomegranates..." (Al-An'am, 99).

It is also concerning that only 21% of respondents consume 100% natural juices daily and no respondents consume fish daily, while 51.7% consume fish 1 to 3 times a week. Daily consumption of legumes is present in only 6.25% of respondents. These foods are essential for a balanced diet, as they are rich in proteins, healthy fats, fiber, phytonutrients and micronutrients. Their absence can lead to long-term nutritional deficiencies and the development of chronic diseases. A very small number of respondents (6.25%) regularly drink unsweetened green tea, which is known for its antioxidant and anti-inflammatory properties. All of this further confirms that there is significant room for

nutritional interventions and education that would enable the formation of healthier dietary patterns. Healthy dietary patterns include high consumption of fruits, vegetables and whole grains; moderate intake of dairy products, fish and poultry; and low consumption of sugar, saturated fats and processed foods (Yeung et al., 2021). Cultural factors, ethnicity and geographic context significantly influence dietary habits, so recommendations must be adapted accordingly to be effective.

Ultimately, these data indicate that although there is awareness of the importance of certain healthy habits, there is simultaneously a strong pattern of dietary behavior characterized by high intake of processed, fatty, sugary and nutritionally poor food. For this reason, education and the application of halal nutrition principles can be key tools in shaping a healthier society.

## Conclusion

The research results on the nutritional habits of individuals who consume exclusively halal food aged 30 to 60 in the Tuzla Canton area revealed a significant gap between declarative religious practice and actual dietary behavior. Although the respondents consistently consume halal-certified products, the nutritional analysis reveals a low intake of key food groups and excessive consumption of highly processed foods, which is not in line with the core principles of halal nutrition. These findings highlight the need for education and the introduction of a holistic approach to halal nutrition, based on scientific and religious foundations. The research provides a valuable contribution to understanding the dietary practices of this population and points to directions for future multidisciplinary interventions aimed at improving public health.

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## Prehrambene navike ispitanika koji konzumiraju isključivo halal hranu u dobi od 30 do 60 godina na području Tuzlanskog kantona

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Originalni naučni rad

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### Sažetak

Pravilna i zdrava ishrana trebala bi biti prioritet za osobe koje konzumiraju isključivo halal hranu kako bi se smanjio rizik od bolesti povezanih s lošim prehrambenim navikama. Međutim, užurban način života doveo je do zanemarivanja ishrane, uzrokujući odstupanja od principa pravilne halal ishrane. Halal ishrana ne podrazumijeva samo odabir dozvoljenih namirnica, već i njihovu pravilnu pripremu i konzumaciju u skladu s principima zdravlja i blagostanja. Cilj ove studije bio je utvrditi prehrambene navike potrošača koji isključivo konzumiraju halal hranu te analizirati u kojoj mjeri su te navike u skladu s principima i preporukama halal ishrane. Metodologija istraživanja uključivala je prikupljanje i analizu podataka putem online upitnika distribuiranog među ispitanicima uzrasta od 30 do 60 godina s područja Tuzlanskog kantona. Rezultati studije pokazali su značajna odstupanja u primjeni principa pravilne halal ishrane kod velikog broja ispitanika. Analiza odgovora pokazala je da značajan broj ispitanika ne pridaje dovoljno pažnje nutritivnoj ravnoteži i raznolikosti ishrane, dok su mnogi naveli da samo povremeno razmatraju nutritivne aspekte svoje prehrane. Ovo ukazuje na to da se halal ishrana često posmatra isključivo kroz prizmu dozvoljenosti hrane. Također, rezultati su pokazali da većina ispitanika samo povremeno obraća pažnju na umjerenost u konzumaciji hrane. Ovi nalazi ukazuju na nedostatak svijesti o važnosti umjerenosti kao osnovnog principa halal ishrane. Nalazi studije naglašavaju potrebu za dodatnom edukacijom o principima pravilne halal ishrane s ciljem poboljšanja razumijevanja i primjene. Jaz između teorijskog znanja i praktične primjene sugerše mogućnosti za dalja istraživanja i obrazovne inicijative. Ova studija može poslužiti kao temelj za šira istraživanja prehrambenih navika halal populacije i faktora koji utiču na njihov izbor hrane.

**Ključne riječi:** pravilna halal ishrana, nutritivni unos, prehrambene navike

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## JOURNAL OF HALAL QUALITY AND CERTIFICATION

### Developing A Halal Built-In Work System For Pharmaceutical Manufacturing

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Original scientific paper



#### ABSTRACT

Halal built-in is a systematic approach which integrates the requirement of halal in all aspect of manufacturing from research and development to raw material sourcing until delivery of finished products. It is a concept that is still under-developed and not a well-established in the pharmaceutical sciences industry despite the establishment of the MS 2424:2012, Malaysian Standard in Halal Pharmaceuticals-General Guidelines in 2012. Despite the introduction of the MS 2424:2012 Malaysian Standard on Halal Pharmaceuticals, its implementation remains inconsistent due to fragmented guidelines, a lack of integration between regulatory and Shariah principles, and an absence of a comprehensive work system framework. This qualitative case study investigates current Halal Built-In practices in Malaysia's pharmaceutical industry, identifies key Shariah-compliant references, explores challenges in its adoption, and proposes an integrated work system framework. Data collection involved semi-structured interviews with four key stakeholder groups: fiqh scholars, JAKIM certification officers, Ministry of Health (MoH) regulators, and pharmaceutical industry representatives. Using Steven Alter's Work System Method, nine critical elements—process, information, participants, technologies, products, customers, environment, infrastructure, and strategies—were analyzed through thematic analysis. Findings reveal varying levels of understanding and differing priorities among stakeholders. Fiqh scholars emphasize Shariah compliance and raw material purity, while MoH regulators focus on labeling policies and consumer preferences. Meanwhile, JAKIM officers and industry players demonstrate a more comprehensive grasp of halal certification requirements but highlight gaps in technical knowledge among manufacturers. Key challenges include the absence of a dedicated Halal Act, misalignment between regulatory policies and halal requirements, and limited cross-disciplinary collaboration. To address these challenges, the study proposes a Halal Built-In Work System Framework, integrating Islamic principles with operational efficiency, ethical values, and regulatory compliance. This framework offers a structured approach for pharmaceutical companies to seamlessly implement halal requirements, ensuring both compliance and market

competitiveness. Ultimately, the study underscores the growing importance of a holistic Halal Built-In system in strengthening Malaysia's position as a global leader in halal pharmaceuticals

**Keywords:** *Halal Built-In, Halal Pharmaceuticals, Work System Framework, Halal Certification, Shariah Compliance*

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## Introduction

The concept of halal has long extended beyond food to encompass pharmaceuticals, cosmetics, logistics, and services. The global halal pharmaceutical market continues to grow, driven by increasing awareness and demand for Shariah-compliant products. In the pharmaceutical industry, halal compliance requires stringent control over sourcing, processing, and certification to ensure that the final product meets both Shariah and regulatory standards. Despite Malaysia's proactive stance in introducing the MS2424:2012 Halal Pharmaceuticals - General Guidelines, (2012), the industry still faces challenges in its consistent implementation.

A systematic "Halal Built-In" approach that fully integrates halal requirements into every aspect of pharmaceutical production is essential to fill this gap. However, the concept of Halal Built-In—a systematic approach to integrating halal requirements throughout the manufacturing process—is still underdeveloped within the pharmaceutical sector.

### *Regulatory and Shariah Frameworks*

The whole teaching of Islam consisting of laws and way of life prescribe by Allah including the laws on halal and haram. The law of Allah and its inner meaning is not easy to grasp. Therefore, *Sharī'ah* is needed to regulate all human actions (Laldin, 2011). The main goal of the *Sharī'ah* is to bring about benefits for the people in regard to their issues in this life and the next. It is commonly accepted that the *Sharī'ah* works to secure advantages for the people or to keep them safe from evil and corruption in all its components (Afridi, 2016).

### *Concept of Halal Built-In Work System*

Halal Built-In emphasizes embedding halal principles into every phase of pharmaceutical manufacturing, from research and development to product delivery. Unlike traditional post-production certification, this approach ensures that halal considerations are intrinsic to the entire work process. The Work System Method by Steven Alter serves as a foundation for analyzing and designing this integrated system, focusing on nine critical elements: processes, information, participants, technologies, products, customers, environment, infrastructure, and strategies (Alter, 2013).

This article explores the development of a Halal Built-In Work System Framework, addressing current gaps, stakeholder perspectives, and proposing a structured solution embedding the essence of shariah to enhance compliance and spiritual elements to the proposed framework.

## Methodology

This study employed a qualitative case study design to explore the implementation of the Halal Built-In system in Malaysia's pharmaceutical industry. The case study approach was selected to gain in-depth insights from multiple stakeholders and to capture the complexity of integrating Shariah principles with technical manufacturing processes and regulatory requirements. The qualitative case study design was guided by Steven Alter's Work System Method (WSM). A total of 12 semi-structured interviews were conducted with four stakeholder groups.

### *Participant Selection and Sampling*

A purposive sampling strategy was used to select participants with specific expertise and roles in the halal pharmaceutical ecosystem. A total of 12 participants were selected and grouped into four categories:

- Fiqh Scholars (n = 3): Experts in Islamic jurisprudence and Shariah law.
- JAKIM Certification Officers (n = 3): Authorities responsible for halal certification processes.
- Ministry of Health (MoH) Regulators (n = 3): Policy makers and compliance officers overseeing drug safety and labeling.
- Pharmaceutical Industry Representatives (n = 3): Individuals from manufacturing firms with halal certification or actively pursuing it.

Participants were selected based on their experience, involvement in halal policy development, and engagement in pharmaceutical operations or certification.

### *Data Collection Procedures*

Data were collected through semi-structured, face-to-face interviews, guided by a protocol developed using the nine WSM elements: processes, participants, information, technologies, products/services, customers, environment, infrastructure, and strategies.

Each interview lasted between 60–90 minutes and was conducted in either English or Malay, depending on the participant's preference. Interviews were audio-recorded with informed consent, transcribed verbatim, and anonymized for confidentiality.

Supplementary data were collected from relevant documents and policy manuals, including:

- Malaysian Halal Pharmaceutical Standard (MS 2424:2012 and 2019)
- Halal Certification Procedures Manual (MPPHM)
- Drug Registration Guidance Document (DRGD)

- Fatwas and circulars relevant to pharmaceutical ingredients and processes

### *Data Analysis*

Data were analyzed using thematic analysis, with a deductive-inductive coding approach. Initial codes were developed based on the WSM framework, and new codes were added inductively as themes emerged from the data. The initial theme used the nine WSM elements: processes, participants, information, technologies, products, customers, environment, infrastructure, and strategies (Suraiya Abdul Rahman, 2023).

### *Data Triangulation and Validation*

Triangulation was employed to enhance the trustworthiness and validity of the study's findings. The following types of triangulations were applied:

**Data Source Triangulation:** Data were gathered from four distinct stakeholder groups to capture varied perspectives across religious, regulatory, and industrial domains.

**Methodological Triangulation:** Multiple data sources—interviews, document reviews, and policy analysis—were used to validate emerging themes.

**Analyst Triangulation:** Coding and interpretation were peer-reviewed by two qualitative researchers with expertise in Islamic jurisprudence and pharmaceutical policy, respectively.

**Theory Triangulation:** Findings were interpreted using both local guidelines, fiqh references (e.g., MHMS and shariah publications) and systems thinking (Alter's, 2002), allowing for a multi-dimensional understanding of the halal built-in system.

These triangulation strategies ensured rigor, credibility, and dependability, and helped mitigate potential researcher bias.

## Results

The qualitative case study revealed rich insights into the current state of Halal Built-In practices within Malaysia's pharmaceutical industry. Analysis of data highlighted variations in stakeholder understanding, critical challenges, and the necessity for an integrated framework to streamline halal compliance in pharmaceutical manufacturing.

- Fiqh Experts focused on upstream Sharī'ah issues such as fatwa, najis avoidance, and ingredient permissibility.
- MoH Regulators prioritized consumer protection, labeling constraints, and medication compliance challenges.
- JAKIM Officers demonstrated cross-disciplinary understanding of halal certification, Shariah rulings, and quality systems.
- Industry Players showed practical knowledge of supply chains and GMP but lacked depth in *Sharī'ah* knowledge.

### *Challenges in Implementing Halal Built-In Work System*

Across all stakeholder groups, several key challenges emerged:

1. Absence of a Dedicated Halal Pharmaceuticals Act: The lack of a specific legislative framework for halal pharmaceuticals limits enforcement and standardization.
2. Misalignment of Regulatory and Shariah Requirements: Differences between NPRA's focus on product safety and JAKIM's halal compliance create conflicting priorities.
3. Fragmented Guidelines and Multiple Standards: Diverse and sometimes contradictory halal guidelines complicate implementation efforts.
4. Technical Knowledge Gaps: Limited understanding of pharmaceutical processes among Shariah scholars, and insufficient fiqh

knowledge among industry players, hampers holistic compliance.

5. Resource Constraints: SMEs struggle with the financial and human resources needed to adopt comprehensive halal built-in systems

The outcome from the study also shows that the current practice of halal Built-in and halal certification requirements focuses more on the physical and mechanistic paradigm and lacks spiritual and ethical values. Halal is a faith-based practice as a sign of obedience to Allah, is not only about meeting material and documents requirements related to the demands of producing halal products but also needs to showcase comprehensive Islamic values. It should be understood that 'halal' does not just cover things related to the physical substance of the product but also includes a comprehensive and unique network and a mutually integrated cycle.

### *Proposed Halal Built-In Work System Framework*

To bridge the gap, a Halal Built-In Work System Framework is proposed, incorporating:

1. Islamic legal foundations (Sharī'ah and Usul al-Fiqh) as the core reference
2. The nine elements of the Work System Method (WSM) for operational structuring
3. Integration with Good Manufacturing Practice (GMP) and Halal Assurance System (HAS)
4. Emphasis on conscience-based values and ethical decision-making.

The halal built-in work system integrates the following WSM as per Alter's (2013) elements with Shariah-based values:

Table 1. Halal Built-In Work System Elements

| WSM Element    | Halal Built-In Integration Focus  |
|----------------|---|
| Processes      | Embed halal checkpoints at each manufacturing stage—R&D, sourcing, production, packaging, and distribution.   |
| Participants   | Multi-disciplinary teams including Shariah advisors, QA/QC staff, R&D chemists, procurement officers, and regulators.                                 |
| Information    | Establish centralized halal knowledge management (including fiqh references, fatwas, ingredient databases, and SOPs).                                 |
| Technologies   | Use halal-compliant materials, validated systems for traceability, and software to support decision-making and audits.                                |
| Products       | Ensure every output (active ingredients, intermediates, and finished products) adheres to halal standards and maqasid.                                |
| Customers      | Design patient-centered strategies to meet religious sensitivities and improve trust, especially for chronic medications.                             |
| Environment    | Align company policy with both national halal strategy and global pharmaceutical regulations, as well as cultivate spiritual and value-based culture. |
| Infrastructure | Institutionalize Halal Internal Committee (IHC) and audit mechanisms aligned with MS2424 and HAS.   |
| Strategies     | Integrate halal policy in business models; pursue R&D for halal alternatives; advocate for Halal Act enactment.                                       |

This above holistic elements can be integrated into a work system snapshot (Alter, 2013) for easy reference and ensures continuity of compliance across the value chain, replacing the ad hoc and reactive approach currently practiced in many firms.

The snapshot can be seen in table 2. This framework guides organizations to internalize

halal as a management philosophy, rather than a compliance burden. It promotes traceability, accountability, and continuous improvement through training, cross-functional alignment, and spiritual motivation. The framework is intended to serve as both a guiding blueprint and a compliance tool, enabling pharmaceutical companies to internalize halal requirements throughout their supply and production chain, not merely at the certification endpoint.

Table 2: Proposed Halal built-in Work System Snapshot

| Proposed Halal built-in Work System Snapshot   |  |   |  |  |
|--|--|---|--|--|
| Adapted Work System Snapshot Template  |  |   |  |  |
| Customers  |  | Products  |  |  |
| <ul style="list-style-type: none"> <li>Government Hospitals</li> <li>MINDEF/Armed forces Tengkuz Mizan hospital</li> <li>Patients/end user.</li> <li>Mass market</li> <li>Export to Middle East and OIC countries</li> <li>Independent pharmacy</li> <li>High traffic outlet like 7Eleven.</li> </ul>  |  | <ul style="list-style-type: none"> <li>Pharmaceuticals products such as over the counter products, health supplements, traditional products and ethical products. Eg: Metformin, the diabetes drug, which is already halal, the doctor will prefer to take Halal</li> </ul> |  |  |
| Major Activities and Processes   |  |   |  |  |
| In-bound phase   | Production phase   | Out-bound Phase   |  |  |
| <ul style="list-style-type: none"> <li>Raw Material Selection</li> <li>Packaging Materials</li> <li>Materials/ Raw Material Control/Istihalah</li> </ul>   | <ul style="list-style-type: none"> <li>Sertu (Islamic Cleansing)</li> <li>Processing/production</li> <li>Halal Internal Audit/self-inspection</li> <li>Quality Control</li> <li>Halal Certification</li> </ul>   | <ul style="list-style-type: none"> <li>Complaints and Product Recall</li> <li>Transportation &amp; Distribution</li> </ul>  |  |  |
| Spiritual And Values Building Campaign and Activities<br>MHMS 2020/ Halal Built-in HAS<br>Traceability<br>Halal Risk Control & HCP Identification  |  |   |  |  |
| Participants   | Information  |   | Technologies   |  |
| <ul style="list-style-type: none"> <li>JAKIM</li> <li>JAIN</li> <li>Mufti</li> <li>Workers</li> <li>Dedicated Halal Executive</li> <li>Head of the site.</li> <li>Internal Halal committee</li> <li>The Halal representative from every department.</li> <li>Vendors, suppliers, logistics provider.</li> <li>Health care provider and private health care providers.</li> <li>Relevant Stakeholders, academicians, government.</li> <li>Leaders &amp; Top Management team</li> <li>Shari'ah advisors</li> </ul> | <b>Document for HAS Manual</b><br>HAS Manual<br>Halal Policy<br>IHC organization Chart<br>SOPs to explain implementation of the activity for every process in detail.<br>Halal Critical Control Point (HCP)<br>Training SOPs/Plan<br>Raw Material<br>Halal certificate,<br>Material specification,<br>production process flow chart,<br>Certificate of Origin/declaration of the ingredient source (free from animal and alcohol from khamar).<br>MSDS<br>COA<br>GMP statement<br>TSE/BSE statement.<br>Drug Master File (DMF)<br>Product Technical Information<br>Manufacturing Formula<br>CEP if any<br>Critical Ingredients<br>Suppliers contact number<br>supplier audit report<br>Supplier Approved List<br>SOPS<br>Integrated with GMP<br>material purchasing,<br>new material purchasing,<br>production,<br>co-production &<br>non-conformance.<br>Dedicated Halal SOP<br>SOP for halal material selection,<br>Sertu, halal certification application,<br>product recall & internal halal audit (IHA) |   | <b>Document for Halal File</b><br>Halal File<br>application form<br>address and contact<br>Product List<br>Labelling information<br>Licences<br>SSM,<br>PBT<br>Manufacturing licence from NPRA<br>employee training and check-up<br>Certificates<br>GMP<br>Register with NPRA (MAL number)<br>Records<br>Batch Manufacturing Record<br>Training records<br>Pest Control<br>Cleaning, record<br>Others<br>Internal Halal Committee meeting report & minutes<br>Audit Report<br>Lab test result for porcine if necessary.<br>Information on Muslim worker<br>Financial reports,<br>Sertu history | <ul style="list-style-type: none"> <li>Use existing GMP certified production tools and technology.</li> <li>Internet connectivity</li> <li>Social media platform</li> <li>Telephones</li> <li>Email</li> <li>Database of halal raw materials from JAKIM</li> </ul> |

From the snapshots, a summary of the recommended Halal Built-in Work System, can be easily referred as a quick guidance for a new beginner to get an overview of the elements that they need to put in place in order to produce a halal pharmaceutical that meet the Shari'ah requirement and qualify for halal certification. This finding firstly reveals that, to establish a Halal built-in Work system that comply to

JAKIM halal certification requirement is not very complicated and can be viewed with a one-page summary snapshot, and secondly there are room for improvement in the current halal pharmaceutical industry practice to make the halal built-in work system more seamless and encourage more pharmaceutical companies to go for halal.

## **Conclusion**

This study proposes an integrated Halal Built-In Work System Framework tailored for pharmaceutical manufacturing, designed to operationalize Shariah principles while fulfilling regulatory and quality requirements. The proposed framework draws from the nine core elements of the Work System Method (WSM), combining them with Islamic jurisprudence and industrial best practices. With enhanced collaboration, targeted training, and policy alignment, Malaysia can reinforce its leadership in the global halal pharmaceutical sector. Future research should focus on piloting this framework in pharmaceutical manufacturing environments and assessing its impact on compliance and market competitiveness.

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## Izrada halal integrisanog sistema rada za farmaceutsku proizvodnju

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### Sažetak

Halal integrisani sistem rada predstavlja sistematski pristup koji integriše zahtjeve halala u sve aspekte proizvodnje – od istraživanja i razvoja, preko nabavke sirovina, pa sve do isporuke gotovih proizvoda. Riječ je o konceptu koji je još uvijek nedovoljno razvijen i nije čvrsto uspostavljen u industriji farmaceutskih nauka, uprkos uvođenju malezijskog standarda MS 2424:2012 Halal Pharmaceuticals – Opće smjernice još 2012. godine.

Iako je standard MS 2424:2012 uspostavljen, njegova implementacija ostaje nedosljedna zbog fragmentiranih smjernica, nedostatka integracije između regulatornih i šerijatskih principa, kao i odsustva sveobuhvatnog okvira radnog sistema.

Ova kvalitativna studija slučaja istražuje postojeće prakse halal integrisanog pristupa u farmaceutskoj industriji Malezije, identifikuje ključne šerijatski usklađene izvore, analizira izazove u njegovoj primjeni i predlaže integrisani okvir radnog sistema. Prikupljanje podataka obuhvatilo je polustrukturirane intervjue s četiri ključne grupe učesnika: učenjaci fikha, službenici za halal certifikaciju iz JAKIM-a, regulatori iz Ministarstva zdravstva (MoH) i predstavnici farmaceutske industrije.

Koristeći Steven Alter-ovu metodu radnog sistema (Work System Method), analizirano je devet ključnih elemenata: proces, informacije, učesnici, tehnologije, proizvodi, korisnici, okruženje, infrastruktura i strategije, putem tematske analize.

Rezultati ukazuju na različite nivoe razumijevanja i različite prioritete među akterima. Učenjaci fikha naglašavaju šerijatsku usklađenost i čistoću sirovina, dok regulatori iz MoH-a fokus stavljaju na politike označavanja i preferencije potrošača. U međuvremenu, službenici JAKIM-a i predstavnici industrije pokazuju šire razumijevanje zahtjeva za halal certifikaciju, ali ističu nedostatke u tehničkom znanju među proizvođačima.

Ključni izazovi uključuju nedostatak posebnog zakona o halalu, neusaglašenost između regulatornih politika i halal zahtjeva, te ograničenu interdisciplinarnu saradnju.

Kao odgovor na ove izazove, studija predlaže halal integrisani sistem u okviru radnog sistema koji integriše islamske principe s operativnom efikasnošću, etičkim vrijednostima i regulatornom usklađenošću. Ovaj okvir nudi strukturiran pristup farmaceutskim kompanijama za dosljednu implementaciju halal zahtjeva, osiguravajući pritom i usklađenost i tržišnu konkurentnost.

Zaključno, studija naglašava rastuću važnost holističkog Halal integrisanog sistema u jačanju pozicije Malezije kao globalnog lidera u halal farmaceutici.

**Ključne riječi:** Halal Built-In (halal integrisani sistem), halal farmaceutski proizvodi, okvir radnog sistema, halal certifikacija, šerijatska usklađenost.

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## JOURNAL OF HALAL QUALITY AND CERTIFICATION

### Future Foresight of Halal in the Context of Sustainable Technologies with Focus to Animal Waste Index

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Original scientific paper



#### ABSTRACT

The future of Halal sustainable technologies presents significant potential in addressing the growing demand for ethical, environmentally friendly solutions, particularly within Muslim communities and the global market. As the world becomes more aware of sustainability challenges, there is an opportunity to integrate Islamic principles of halal (permissible) and tayyib (pure, wholesome) into emerging technologies, especially in sectors like agriculture, pharmaceuticals, cosmetics, and finance. These sectors can benefit from innovations that adhere to sustainability standards while aligning with Halal practices. This paper focuses on evaluating the Animal Waste Index (AWI) as a tool to measure the management and impact of animal waste in agricultural settings. AWI aims to improve sustainability and waste management by tracking how animal waste is converted into compost, using efficient methods that align with Halal principles. The potential for real-time data collection and monitoring technologies would further enhance this process, leading to more sustainable and Halal-compliant agricultural practices. One solution in food waste management involves using bioreactors, which break down organic waste through controlled biological processes. These devices, which function both aerobically (with oxygen) and anaerobically (without oxygen), can convert food waste into compost, enriching soil and supporting agricultural production. The integration of bioreactors offers an efficient, scalable solution to waste management, reducing environmental impact and enhancing sustainability while meeting Halal standards. As Halal-certified products are increasingly in demand across various industries, the alignment of Halal practices with environmentally responsible solutions creates an opportunity to expand Halal standards into sustainable technologies. The growing application of Halal principles in new sectors such as animal welfare, cosmetics, and bio-based products suggests a broader potential for Halal sustainability efforts. The development of AWI as a tool for tracking animal waste conversion is vital in guiding agricultural practices toward more sustainable, ethical, and Halal-compliant methods. Composting food waste not only reduces

environmental footprints but also turns waste into valuable resources, enriching soil and supporting food security. Looking ahead, technologies like bioreactors will play a crucial role in addressing global waste management challenges, sustainability goals, and the increasing demand for bio-based products. By fostering innovations that meet both ethical and environmental standards, the future of Halal sustainable technologies promises a more responsible and sustainable global market.

**Keywords:** *Halal technologies, sustainability, waste management, bioreactors, Animal Waste Index (AWI)*

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## **Introduction**

As the global population grows and environmental challenges intensify, the demand for ethical, sustainable, and health-conscious solutions continues to rise. (1) Within this evolving landscape, the integration of Islamic principles; particularly those related to Halal (permissible) and Tayyib (pure and wholesome), into technological and environmental frameworks offers an innovative and culturally rooted approach to sustainability. The Halal industry, traditionally associated with food, is now expanding its influence into diverse sectors such as agriculture, pharmaceuticals, cosmetics, and biotechnology, calling for robust systems that align with both religious compliance and environmental stewardship. (2)

One pressing issue in the sustainability discourse is the management of animal waste, especially in agricultural systems. Poor waste management contributes significantly to greenhouse gas emissions, water pollution, and soil degradation. To address these challenges, there is a growing need for tools that can effectively monitor and enhance the use of animal by-products in a way that supports environmental goals while adhering to Halal standards. (3)

This paper introduces the concept of the Animal Waste Index (AWI): a proposed metric for evaluating the sustainability and compliance of animal waste management systems within the Halal framework. AWI is typically refers to a measure or metric used to assess the amount and environmental impact of waste produced by livestock and other animals, particularly in

agricultural settings. It's not a universally standardized term, so its meaning can vary depending on the context (e.g., academic research, environmental policy, or local regulations). It might include; waste management practices (e.g., composting, lagoon storage, runoff control), and it is more matters in regulatory compliance: used by environmental agencies to ensure farms meet waste disposal standards. (4)

By incorporating technologies such as bioreactors, precision agriculture, and real-time monitoring, AWI aims to serve as both an evaluative and operational tool to guide more responsible agricultural practices. (5) The use of bioreactors, in particular, offers a scalable and efficient solution for converting animal waste into high-quality compost, supporting circular economy principles and reducing environmental impact.

This study explores how the convergence of Halal ethics and sustainable technology can shape future waste management strategies. (6) It emphasizes the potential of AWI to become a standard benchmark for Halal-compliant, eco-friendly agricultural operations, ultimately contributing to broader goals such as food security, soil health, and ethical environmental practices.

## **Materials and Methods**

This study proposes the development and application of the Animal Waste Index (AWI) as a framework for evaluating and managing animal waste in alignment with Halal and sustainability principles. (12) The methodology combines

technological, environmental, and religious dimensions to establish a practical and ethical waste management approach.

The primary material used in this study was raw animal waste, including manure and organic by-products collected from livestock farms. The waste was free from non-organic contaminants and stored in sealed containers before processing. The treatment system used was a closed-loop **bioreactor unit**, equipped with temperature regulation, aeration controls, and compost collection chambers.

#### **Additional materials included:**

- Water is needed in old fashion windrows as well as for other technologies, for moisture control. With Bioreactors this is absolutely avoided. A very big advantage of the Bioreactor is that there is no need for water at all, saving lot of water and costs. In addition to this, the Bioreactor generates water vapor that can be captured and re-used for irrigation of greenhouses or for any other water needs (for example, the mod size Bioreactor generates about 100L of H<sub>2</sub>O a day that can be reused for many different needs).
- Temperature sensors (to monitor internal conditions).
- Aeration system (to maintain aerobic microbial activity); as well as to control temperature levels and appropriate internal moisture levels generated as heat and water vapor during the composting process.
- pH meter (to monitor acidity during composting): pH is measured for the organic waste to be composted as well as from the produced compost. The Bioreactor does not measure the pH during the composting process unless set-up for a specific and customized study for specific testing and analysis.

#### **Conceptual Framework of the Animal Waste Index (AWI)**

The AWI is designed as a composite index that measures the efficiency, compliance, and

environmental impact of animal waste management systems. It integrates indicators from three main dimensions:

**Halal Compliance:** Assesses whether waste handling, transformation, and byproduct usage adhere to Islamic principles (e.g., cleanliness, animal welfare, and the avoidance of najis materials in contamination).

**Environmental Sustainability:** Evaluates reduction of greenhouse gas emissions, soil and water conservation, and minimization of harmful runoff.

**Technological Efficiency:** Measures the operational effectiveness of applied technologies such as bioreactors, including waste conversion rates, compost quality, and system scalability.

#### **UN Data on Animal Waste Consumption**

The United Nations provides data on animal waste consumption by households and other sectors across different countries. (17)

- Bangladesh: Household consumption of animal waste has been consistently high, with figures around 99,863 terajoules in 2021.
- Armenia: Household consumption has fluctuated, reaching 2,863 terajoules in 2020. (17)

#### **Global Residue and Manure Data**

Research consolidating global residue data from agriculture, fisheries, and forestry provides insights into livestock manure: (15 and 16)

- Slaughterhouse By-products: The inedible parts of slaughtered animals vary by species, e.g., 22% for turkeys, 37% for broilers, 38–40% for pigs, 47% for sheep and goats, and 49% for cattle.
- Fish Processing By-products: These include trimmings like heads, frames, skin, and tails, constituting up to 70% of fish and shellfish after processing. (15 and 16)

This data aids in understanding the volume and potential utilization of animal waste.

### **UAE Animal Waste Statistics**

In the United Arab Emirates, specifically in Abu Dhabi Emirate, the volume of non-hazardous solid animal waste generated was approximately 386,440 tons in 2018. (10) This statistic highlights the scale of animal waste management challenges in urban settings. (11)

### **Bioreactor Integration**

The bioreactor is a controlled, thermophilic composting unit designed to accelerate the decomposition of organic animal waste. (Figure

1) The reactor is made of corrosion-resistant material; it is made from pipes used for drinking water and coated for anti-corrosion and anti-rust, following corresponding regulations, insulated to retain heat, and sealed to prevent odor emissions. The internal mixing and uniformity of the organic matrix being composted is ensured thanks to the continuous, non-stop gentle low pace rotation of 10 rotations per hour, which is a real competitive advantage of this technology. This approach avoids the constant and repeated intermittent mechanical disruption of the natural and biological occurring composting process and all the negative impact that this type of interruptions generates; to ensure even distribution of heat and microbial activity. (6) Halal process compliance (as reviewed by Sharia auditors).



Figure 1: Bioreactor Device

### **Discussion**

The integration of Halal principles into modern sustainability technologies presents a promising avenue for addressing global environmental challenges while respecting cultural and religious values. The findings and observations from applying the Animal Waste Index (AWI) framework highlight several key insights into how Islamic ethics and technological innovation can jointly shape the future of sustainable agriculture. (12)

### **Alignment of Halal and Sustainability Objectives**

One of the most compelling outcomes of this study is the clear compatibility between Halal requirements and sustainable practices. Islamic teachings emphasize cleanliness, resource conservation, and humane treatment of animals all of which align with global sustainability goals. By embedding Halal compliance into waste management systems, the AWI helps ensure that both spiritual and ecological integrity are maintained. (13)

The bioreactor system, for instance, fulfils multiple objectives: it minimizes environmental impact, transforms waste into beneficial compost, and operates under conditions that are transparent and controllable important aspects for Halal certification. The monitoring of inputs and processes reduces the risk of contamination and supports traceability, which are critical in Halal assurance systems. (14)

### **Efficiency and Impact of Bioreactor Technology**

Bioreactors have proven to be effective in managing animal waste with minimal environmental harm. The transformation of raw

manure into nutrient-rich compost within a relatively short time frame (less than 20 days) supports their suitability for both small-scale and industrial farming operations. (Figure 2) In the pilot case, the compost output showed significant improvement in soil nutrient levels and reduced odor and pathogenic risks key concerns in both sustainable agriculture and Halal practices. (18)

Furthermore, the ability to control oxygen levels, temperature, and microbial activity allows for greater standardization and reproducibility, which is critical for scaling the technology across regions with varying climates and agricultural needs. (19 and 20)



Figure 2: The input to the Bioreactor device (animal manure, waste)

### **Challenges and Limitations**

Despite its promise, the implementation of AWI and bioreactor technology faces several challenges:

**Regulatory and Certification Gaps:** Current Halal certification systems are not universally equipped to evaluate sustainability-oriented technologies, particularly in waste processing. There is a need

for updated guidelines that integrate environmental metrics with Halal verification.

**Cost and Accessibility:** Initial investment in bioreactor systems and real-time monitoring tools may be prohibitive for smallholder farmers, particularly in developing countries.

**Knowledge and Training:** Adoption of the AWI framework requires training for farmers,

certifiers, and policymakers to understand its components and implications.

**Broader Implications for the Halal Industry**

As Halal-certified products expand into sectors such as bio-based materials, pharmaceuticals, and cosmetics, the demand for ethically sourced and environmentally friendly inputs will continue to grow. Tools like AWI can serve as a model for other indices that evaluate sustainability and Halal compliance in different contexts, including water use, packaging, and energy consumption.

Moreover, incorporating digital technologies such as blockchain for traceability and sensors for monitoring can further enhance the transparency and trustworthiness of Halal su Results. (21)

The implementation of the Animal Waste Index (AWI) in the pilot study produced meaningful insights into the feasibility and effectiveness of integrating Halal compliance with sustainable waste management technologies, particularly the use of bioreactors. (6 and 22)

**Table1:** Compost Testing Parameters:

| Category                        | Parameter   | Purpose / Significance                           | Acceptable / Target Range   |
|---------------------------------|---|--|---|
| <b>Maturity &amp; Stability</b> | Temperature Profile                                 | Confirm completion of thermophilic phase         | ≥ 55°C for ≥ 3 days (EPA 503 standard)                              |
|                                 | Respiration Rate (CO <sub>2</sub> /O <sub>2</sub> ) | Measure microbial activity; low = stable compost | < 4 mg CO <sub>2</sub> -C/g OM/day (or similar O <sub>2</sub> rate) |
|                                 | Ammonia Levels                                      | High NH <sub>3</sub> = immature compost          | Low or non-detectable   |
|                                 | Germination Index (Phytotoxicity)                   | Assess seed germination and root growth          | ≥ 80% (ideal ≥ 90%)   |
| <b>Hygiene</b>                  | Fecal Coliforms                                     | Public health safety                             | < 1000 MPN/g (per EPA 503)  |
|                                 | Salmonella spp.                                     | Indicator of pathogen risk                       | Absent in 50g sample  |
|                                 | Helminth Ova (if required)                          | Particularly for biosolid compost                | Country-specific (usually < 1–3 ova/g)                              |
| <b>Nutrients</b>                | Total Nitrogen (N)                                  | Plant nutrition                                  | Typically 1–3% dry weight   |
|                                 | Total Phosphorus (P)                                | Plant nutrition                                  | Typically 0.3–1.5%  |
|                                 | Potassium (K)                                       | Plant nutrition                                  | Typically 0.5–2%  |
|                                 | Secondary & Micronutrients                          | Support plant health                             | Trace levels; avoid excess  |
| <b>Heavy Metals</b>             | Arsenic (As), Cadmium (Cd), etc.                    | Environmental and crop safety                    | Comply with local/EPA/EU/GCC limits                                 |
| <b>Physical Properties</b>      | Moisture Content                                    | Ideal composting & usability                     | 40–60%  |
|                                 | Bulk Density  | Affects transport & application                  | 400–700 kg/m <sup>3</sup> (typical range)                           |
|                                 | Particle Size                                       | Consistency & application method                 | < 25 mm (customizable)  |
|                                 | Odor  | Indicator of aerobic/anaerobic status            | Earthy (no sour or rotten smell)                                    |
| <b>Chemical Properties</b>      | pH  | Affects nutrient availability                    | 6.0–8.0   |
|                                 | Electrical Conductivity (EC)                        | Indicates salt levels                            | < 4 dS/m (preferably < 2 dS/m for sensitive crops)                  |

**Compost Output and Quality:**

The bioreactor successfully converted over 80% of raw animal waste into usable compost within 15-20 days. (Table 2 and 3)

The final compost met safety standards for use in organic farming and was deemed suitable as animal bedding, showing no harmful pathogens or chemical residues.

**Table 2:** Laboratory analysis of the compost revealed favorable nutrient content:

| Unit        |                               | %   | —     | dS/m   | %         | %     | %     | —         | %     |
|-------------|-------------------------------|---|-------|--------|-----------|-------|-------|-----------|-------|
| No.         | Standard S.Name               | < 25.0  | < 7.5 | < 10.0 | > 50 - 60 | < 0.8 | < 1.2 | < 25 : 1  | > 2.0 |
| 2           | compost (animal waste origin) | 23.50   | 6.20  | 17.25  | 57.00     | 0.53  | 0.80  | 15.38 : 1 | 2.15  |
| Lab Remarks |                               | (pH & EC & CL & Na) is measured on 1:5 (W/V) Extract. |       |        |           |       |       |           |       |

**Table 3:** Laboratory analysis of the compost revealed favorable nutrient content:

|             |                |                                     | Organic Fertilizer Analysis                           |       |        |       |       |       |           |       |
|-------------|----------------|-------------------------------------|---|-------|--------|-------|-------|-------|-----------|-------|
| 2023        | Parameter      |                                     | Moisture  | PH    | Ec     | O.M   | Na    | Cl    | C:N ratio | N     |
|             | Units          |                                     | %   | —     | dS/m   | %     | %     | %     | —         | %     |
| Sr.No.      | No. of Samples | Standard Sample Name                | < 25.0  | < 7.5 | < 10.0 | > 40  | < 0.8 | < 1.2 | < 25 : 1  | > 1.0 |
| 13          | 1              | Animal and plant fertilizer         | 21.92   | 6.50  | 11.88  | 41.20 | 0.48  | 0.72  | 14.06 : 1 | 1.70  |
| 14          | 2              | Planet fertilizer (under treatment) | 25.10   | 6.80  | 8.71   | 42.00 | 0.48  | 0.70  | 22.15 : 1 | 1.10  |
| Lab Remarks |                |                                     | (pH & EC & CL & Na) is measured on 1:5 (W/V) Extract. |       |        |       |       |       |           |       |

**Environmental Impact:**

A 35–40% reduction in greenhouse gas emissions (methane and ammonia) was recorded compared to conventional waste storage methods. (17)

Water runoff from the composting process contained lower levels of contaminants, aligning with environmental protection guidelines and Islamic values of resource preservation. (2 and 23)

**Halal Compliance Evaluation:**

- Process audits conducted in coordination with Shariah advisors confirmed:
- No cross-contamination with non-Halal or najis materials.

- Ethical treatment of animals and sanitary handling of waste.
- Use of pure, non-toxic materials and no harmful additives.

The overall process scored above 90% on a compliance checklist developed in line with Halal assurance criteria. (6 and 24)

**Stakeholder Feedback:**

Interviews with farm operators and Halal certifiers highlighted strong support for integrating sustainability measures into Halal processes.

Farmers noted increased soil fertility and improved public perception of eco-conscious practices.

Certifiers expressed interest in adapting the AWI model for broader application in the Halal industry.

### **Conclusion**

The integration of Halal principles with sustainable technologies presents a transformative opportunity to address environmental challenges while adhering to ethical and religious values. This study introduced the Animal Waste Index (AWI) as a novel tool for evaluating the sustainability, efficiency, and Halal compliance of animal waste management systems, particularly through the use of bioreactor technology.

The quality of the compost produced was notably high. Laboratory analysis confirmed the absence of harmful contaminants and a nutrient-rich composition, making it a safe and beneficial input

for agricultural applications. This supports the growing demand for organic fertilizers that enhance soil health without introducing chemical residues into the environment. The compost was suitable for use in both conventional and organic agriculture, providing a sustainable alternative to chemical fertilizers

Moreover, the system adhered strictly to halal and Sharia-compliant practices. No impure (najis) or prohibited (haram) substances were introduced during the process, and animals were not subjected to harm or distress at any stage. This ensures that the final compost product aligns with Islamic ethical standards, making it suitable for use in halal-certified agricultural systems.

The AWI framework proved effective in offering a multidimensional assessment, combining religious, environmental, and technological criteria into a single evaluative tool. Its application supports a more structured approach to sustainable agricultural practices in Muslim-majority and Halal-conscious communities, offering both practical and spiritual benefits.



**Figure 14:** Plant growing from the compost in Turkey

### **Recommendations**

Based on the findings and insights of this study, the following recommendations are proposed to support the wider adoption and development of Halal-aligned sustainable technologies,

particularly in the area of animal waste management:

Looking ahead, scaling up the AWI framework and integrating it with digital monitoring tools can further enhance its applicability across

diverse sectors. Establishing formal guidelines and engaging Halal certification bodies will be critical to legitimizing and expanding the use of such models. By continuing to align religious values with environmental innovation, Halal sustainable technologies have the potential to shape a more ethical, resource-conscious global market.

Provide specialized training for farmers, waste management personnel, and Halal certifiers to understand the technical and ethical dimensions of AWI-based systems and bioreactor technologies.

Policy Support and Incentives; Encourage governments and Halal regulatory bodies to support sustainable Halal technologies through policy frameworks, financial incentives, and research funding, particularly in rural and developing regions.

Public Awareness and Market Development; Launch awareness campaigns to educate consumers and producers about the value of Halal-compliant sustainable practices. Promote products made using AWI-compliant compost to enhance consumer trust and demand.

Further Research and Piloting; Conduct additional studies in different environmental and agricultural contexts to refine the AWI model, evaluate long-term impacts, and explore its adaptability across sectors such as aquaculture, bioenergy, and Halal pharmaceuticals.

Collaboration with Certification Bodies; Partner with recognized Halal certification authorities to validate and adopt AWI into broader Halal standards, helping bridge the gap between traditional certification processes and emerging environmental technologies.

s good microbiological safety and efficiency of fermentation, smoking and ripening. Values for *Staphylococcus aureus* range from  $5.5 \times 10$  to  $1.4 \times 10^2$  CFU/g, which is in accordance with the values prescribed by the current Regulation. Most

samples were negative after ripening, except for 3 (very low values). This further confirms the safety of the product after seven days of ripening. Coagulase-positive staphylococci are completely absent after storage, which is an indicator of hygienic correctness and sustainability of dry cheese in storage conditions. *Listeria monocytogenes* was not found in any sample of dry cheese, which shows the hygienic safety and security of this product.

Cheese production using natural microflora of lactic acid bacteria is more acceptable compared to cheese production using commercial dairy cultures, as is evident from the results of research conducted by Renata Pysz-Lukasik et al., (2018) in the production of traditional short- and long-maturing cheeses from Poland. Commercial dairy cultures were used in the production of this cheese, and the temperature in the production and maturing room was 18-20°C and 14-20°C, respectively. The tested cheeses did not meet the microbial criteria for food safety (presence of *L. monocytogenes*) and process hygiene (exceeded permissible levels of *E. coli* and coagulase-positive *Staphylococcus*).

*Lactobacillus* spp. isolated from Iranian local raw milk cheese from Ahvaz province, have in vitro studies that make them potential candidates for probiotic and technological applications. The results showed that these strains have good probiotic and technological potential. The results of safety aspects also showed that these strains can be used for human nutrition. Therefore, in vivo studies are needed to investigate their effectiveness in real-life situations (Hasan B., et al.,2021).

Monitoring the concentration of lactic acid bacteria in Mozzarella cheese shows that the results confirmed the correlation between the concentration of lactic acid bacteria and the quality of the process: a high concentration of lactic acid bacteria in the raw materials and whey starter ensured safe and good final products (Francesca L., et al., 2014).

According to Cretenet et al., (2011) adequate growth of milk bacteria during milk farming

contributes to the control of the reproduction of potentially pathogenic bacteria, with special reference to staphylococci that produce enterotoxins, and can help in the production of safer cheese.

According to the research of Angeliki D. et al., (2024) the application of bioprotective strains of lactic acid can result in an extension of the shelf life of feta and provide a mild antimicrobial effect against *L. monocytogenes*.

The results of a study conducted on the microbiological diversity and physicochemical properties of Kurdish cheese from Iran during ripening (1, 20, 40 and 60 days) showed that the type and number of microorganisms were most affected by the ripening time. Lactic acid bacteria and *Enterobacteriaceae* dominated during the first 20 days of ripening, and *Lactobacillus* were the most common microorganisms found during ripening. The initial number of coliforms and *E. coli* decreased rapidly, while a gradual increase in the number of molds and yeasts was observed in the early days of ripening. *Coliform bacteria*, *Salmonella* and *coagulase-positive Staphylococcus* spp. could not be detected in the ripened cheese (Elnaz Milani et al., 2014).

Brooks JC., et al., (2012) analyzed the presence of pathogenic bacteria in raw milk cheese produced in the USA. They analyzed them for the presence of *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Campylobacter*. Five samples contained coliforms; two of them contained *E. coli* less than 10(2) cfu/g. Three other cheese samples contained *S. aureus*. Research by Brooks JC., et al., (2012) supports the theory that with adequate control of microbiological parameters and proper aging, cheeses produced from unpasteurized milk can be safe for consumption, although there is a risk of the presence of pathogens such as *S. aureus* and *E. Coli*.

By evaluating the interaction (growth behavior and survival) of *Listeria monocytogenes* and *Lactobacillus acidophilus* in different stages of production, ripening and storage of Iranian white cheese, changes in pH values were recorded at different stages of cheese ripening, as well as a

positive effect on the sensory characteristics of the cheese. The decrease in the number of *L. monocytogenes* during the ripening and storage period of probiotic Iranian white cheese may be due to the combined effect of the lowered pH and the antimicrobial activity of the starter and probiotic bacteria used in this study (R. Mahmoudi et al., 2012).

In Minas cheeses (type Canastra) made from raw or pasteurized milk, selected strains of lactic acid bacteria showed bacteriostatic effects and inactivation of *L. monocytogenes* in soft cheese, and in semi-hard cheese, respectively. Inactivation of *L. monocytogenes* was significantly higher in semi-hard cheeses made with raw milk and the addition of selected indigenous lactic acid bacteria. Strains isolated from artisanal Minas cheeses may provide an additional barrier to the growth of *L. monocytogenes* during refrigerated storage of soft cheese and help shorten the ripening period of semi-hard cheeses aged at room temperature (Fernanda B. Campagnollo et al., 2018).

## Conclusion

Based on the results of the research on the microbiological safety of dry cheese through three phases, it was concluded that the safety of dry cheese after smoking improves through the ripening and storage process. Potentially dangerous microorganisms are eliminated. Fermentation continues, which is visible through the growth of *Lactobacillus* spp., which also contributes to the development of the texture and taste of dry cheese. The technological process (smoking, ripening and storage) has proven to be effective for the microbiological safety and safety of dry cheese. These results confirm that dry cheese can be a safe, healthy and halal product, which not only meets religious requirements, but also meets high food safety standards through the presence of probiotic bacteria, such as *Lactobacillus* spp. that act as a natural protection against pathogens. These properties make traditional dry cheese from Bosnia and Herzegovina an even more valuable product on the market.

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## Predviđanje budućnosti halala u kontekstu održivih tehnologija s fokusom na Indeks životinjskog otpada

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Originalni naučni rad

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### Sažetak

Budućnost halal održivih tehnologija pokazuje značajan potencijal u odgovoru na rastuću potražnju za etičkim i ekološki prihvatljivim rješenjima, posebno unutar muslimanskih zajednica i globalnog tržišta. Kako svijet postaje sve svjesniji izazova održivosti, otvara se prilika za integraciju islamskih principa halala (dozvoljeno) i tayyiba (čisto, zdravo, korisno) u nove tehnologije, posebno u sektorima kao što su poljoprivreda, farmacija, kozmetika i finansije. Ove oblasti mogu imati koristi od inovacija koje poštuju standarde održivosti, a istovremeno su usklađene s halal praksama. Ovaj rad fokusira se na evaluaciju Indeksa životinjskog otpada (AWI) kao alata za mjerenje upravljanja i uticaja životinjskog otpada u poljoprivrednim uslovima. Cilj AWI indeksa je poboljšanje održivosti i upravljanja otpadom kroz praćenje procesa pretvaranja životinjskog otpada u kompost, koristeći efikasne metode koje su u skladu s halal principima. Potencijal za korištenje tehnologija za prikupljanje i nadzor podataka u realnom vremenu dodatno bi unaprijedio ovaj proces, vodeći ka održivijim i halal-kompatibilnim praksama u poljoprivredi. Jedno od rješenja u upravljanju prehrambenim otpadom uključuje korištenje bioreaktora, koji razgrađuju organski otpad putem kontrolisanih bioloških procesa. Ovi uređaji, koji mogu raditi i aerobno (uz prisustvo kisika) i anaerobno (bez kisika), pretvaraju prehrambeni otpad u kompost, obogaćujući zemljište i podržavajući poljoprivrednu proizvodnju. Integracija bioreaktora nudi efikasno i skalabilno rješenje za upravljanje otpadom, smanjujući negativan uticaj na okoliš i povećavajući održivost, uz ispunjenje halal standarda. Kako potražnja za halal-certificiranim proizvodima raste u različitim industrijama, usklađivanje halal praksi s ekološki odgovornim rješenjima stvara priliku za širenje halal standarda u domenu održivih tehnologija. Sve šira primjena halal principa u novim sektorima kao što su dobrobit životinja, kozmetika i bio-bazirani proizvodi ukazuje na veći potencijal za razvoj halal održivosti. Razvoj AWI indeksa kao alata za praćenje pretvaranja životinjskog otpada od suštinske je važnosti za usmjeravanje poljoprivrednih praksi ka održivijim, etičnijim i halal-kompatibilnim metodama. Kompostiranje prehrambenog otpada ne samo da smanjuje ekološki otisak, već otpad pretvara u vrijedne resurse, obogaćuje zemljište i podržava sigurnost hrane. Gledajući unaprijed, tehnologije poput bioreaktora igraće ključnu ulogu u rješavanju globalnih izazova upravljanja otpadom, ispunjavanju ciljeva održivosti i rastuće potražnje za bio-baziranim proizvodima. Podsticanjem inovacija koje zadovoljavaju i etičke i ekološke standarde, budućnost halal održivih tehnologija obećava odgovornije i održivije globalno tržište.

**Ključne riječi:** Halal tehnologije, održivost, upravljanje otpadom, bioreaktori, Indeks životinjskog otpada (AWI)

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## JOURNAL OF HALAL QUALITY AND CERTIFICATION

### The Impact of Ethical and Health Guidelines of Halal Standards on Lifestyle and the Global Growth of the Halal Market

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#### ABSTRACT

The Halal standard constitutes a comprehensive framework of ethical, religious, health, and safety guidelines that delineate what is permissible (halal) and prohibited (haram) under Islamic law. While its primary function is to ensure that products and services adhere to Islamic principles and fulfill defined quality attributes, the Halal concept has increasingly acquired universal significance, particularly in the sectors of food, cosmetics, pharmaceuticals, hospitality, and tourism. The global Halal market sector is witnessing robust growth, driven not only by the rising Muslim population but also by heightened consumer awareness regarding quality, hygiene, and ethical considerations. This study examines the influence of the ethical and health guidelines embedded within the Halal standard on consumer lifestyles worldwide, and their pivotal role in the exponential expansion of the global Halal market. A survey was conducted on a sample of 101 participants, addressing key dimensions of Halal as a lifestyle choice. By analyzing the ways in which these principles inform consumer behavior, dietary practices, and perceptions of well-being, the research aims to offer a comprehensive insight into the intersection of religious values, ethical commitments, and economic opportunities within the framework of Halal standards.

**Keywords:** food safety, halal, haram, tourism, hospitality, lifestyle, ethical guidelines, BAS 1049:2023

#### Introduction

In the contemporary global landscape, characterized by increasingly pronounced ethical, cultural, and religious consumer preferences, the halal industry has recorded substantial growth, extending beyond the food sector into the broader domains of tourism and hospitality services. Within this framework, halal food assumes a central role, representing not merely a matter of dietary compliance but embodying the profound religious, cultural, and health values of the Islamic community. Halal dietary practices, which require that all ingredients and production

processes align with Shariah principles, constitute a fundamental component of any halal tourism offering and serve as a critical indicator of the authenticity and credibility of the services provided.

Standards related to halal food, such as OIC/SMIIC 1 and BAS 1049:2023, therefore play a crucial role in shaping tourism offerings targeted at Muslim consumers. Accommodation facilities seeking to carry the "halal-friendly" designation must ensure not only physical and spiritual comfort but also complete safety and full compliance of food products with halal

requirements. This includes supply chain control, specific conditions for food storage and preparation, and the strict prohibition of the introduction and use of haram substances, such as alcohol and pork. According to the OIC/SMIIC 1 standard and the national BAS 1049:2023 standard, a halal restaurant must not serve alcoholic beverages, must clearly label halal products, and the staff must be familiar with basic halal principles. Staff training is also recommended to enable them to answer guests' questions and prevent unintentional non-compliance. In the contemporary context, a halal restaurant does not necessarily have to be exclusively religious in nature. An increasing number of non-Muslim consumers are choosing halal restaurants due to:

- the guarantee of food quality and safety (particularly regarding meat, additives, and hygiene),
- the absence of alcohol and additives associated with health concerns,
- the ethical and environmental aspects of production.

Restaurants that pursue halal certification can significantly expand their target audience, particularly in urban areas and tourist destinations, where the number of Muslim travelers actively seeking halal options is steadily growing.

Furthermore, the availability of halal-certified restaurants is considered a key element of halal tourism, as it provides a sense of security and trust for consumers who adhere to a halal lifestyle.

The growing halal tourism market, coupled with the rising awareness of ethical consumption and the health benefits of halal food, makes this topic particularly relevant for research. The aim of this paper is to analyze the interdependence between the concept of halal nutrition and halal tourism, through the lens of Shariah, technological, and regulatory requirements, and to highlight the challenges and opportunities that arise in the process of

developing and standardizing services in accordance with halal principles.

### **Materials and methods**

In order to assess the role of halal nutrition in the daily lives of Muslim consumers, as well as perceptions of halal tourism as a growing sector within the broader halal industry, an empirical study was conducted using a structured survey method. The focus of the research was on examining the habits, attitudes, and knowledge of end users regarding halal products and their availability, as well as their interest in halal tourism services. A quantitative approach was applied, utilizing a structured questionnaire with predefined answer options. This approach enabled the standardization of data and its statistical processing, ensuring objectivity and comparability of responses among participants.

The survey was completed by 101 respondents, mostly from Bosnia and Herzegovina. The targeted sample included individuals of various age groups, educational backgrounds, and levels of religious practice, ensuring diversity in the perception of and attitudes toward halal products.

The questionnaire was distributed through:

- social media platforms,
- university mailing lists and organizations associated with halal-related topics.

The questionnaire consisted of four thematic sections:

- Demographic profile (gender, age)
- Halal dietary habits (place of purchase, frequency, brand awareness)
- Perception and knowledge of halal certification and companies
- Attitudes toward halal tourism and the halal industry as a whole (key services, preferences, economic potential)

The survey included 24 questions, predominantly of a closed-ended type (one or more offered answers), and to a lesser extent, semi-open-ended (allowing additional comments or the naming of specific companies/brands). Participants were informed about the purpose of the study and voluntarily agreed to participate. Data collection was conducted anonymously, without recording any personal information.

## Results and discussion

A total of 101 respondents participated in the study, with women being significantly more represented than men. This finding may indicate a stronger role of women in decisions related to nutrition and consumer habits within the household, which is consistent with the results of similar studies in the fields of food safety and family consumption.

The most represented age group was 31–45 years (48%), which corresponds to the so-called active, working-age, and family-oriented population. This group is also the one most often responsible for purchasing, nutrition, and travel planning, thus having the most direct impact on the market demand for halal products and services. Other age groups were more evenly represented: 18–30 years (13%) – often considered the “emerging segment” with a strong interest in sustainable, ethical, and healthy lifestyles; 46–60 years (35%) – a generation that typically has stable income and well-established consumer habits; and 61+ years (5%) – showing a somewhat lower level of engagement, likely due to lower digital literacy or limited access to online surveys. This demographic profile confirms that interest in the halal lifestyle is strongest among individuals in their life and professional maturity, further supporting the argument for a more institutionalized and standardized approach to the halal market. These consumers, in particular, demand transparency, safety, and consistency in the quality of the services and products they consume.

The majority of respondents (59.4%) indicated that they partially practice a halal lifestyle, while 35.6% stated that they fully adhere to it. This highlights a high level of awareness but also points to the need for greater availability of halal products to enable more consistent practice.

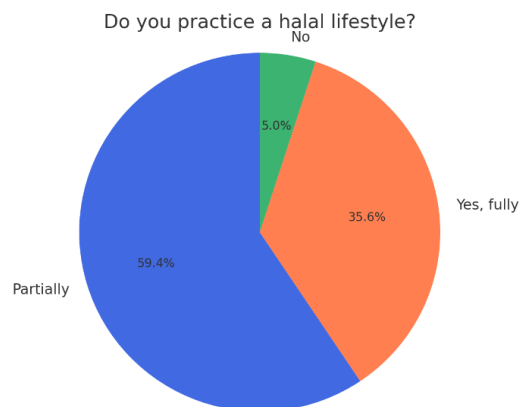


Diagram 1. Respondents' Answers to the Question: Do You Practice a Halal Lifestyle?

This distribution, as shown in Diagram 1, indicates a high level of awareness and knowledge regarding Islamic regulations related to nutrition and daily habits. However, the fact that the majority falls into the "partial practice" category also points to certain barriers in the consistent application of a halal lifestyle – which may include the insufficient availability of halal-certified products, limited offerings in local stores, or the absence of clearly labeled services in the hospitality and tourism sectors. Considering the different age groups, when compared to the practice of a halal lifestyle, the data presented in Diagram 2 emerge.

Based on Diagram 2, it is evident that the largest group of respondents who fully adhere to a halal lifestyle falls within the 31–45 age range.

This result confirms expectations, as it encompasses a population with greater independence in decision-making. Among younger respondents aged 18 to 30, a higher proportion chose "partial practice," which can be interpreted as a generation in transition, balancing between traditional values and modern lifestyles.

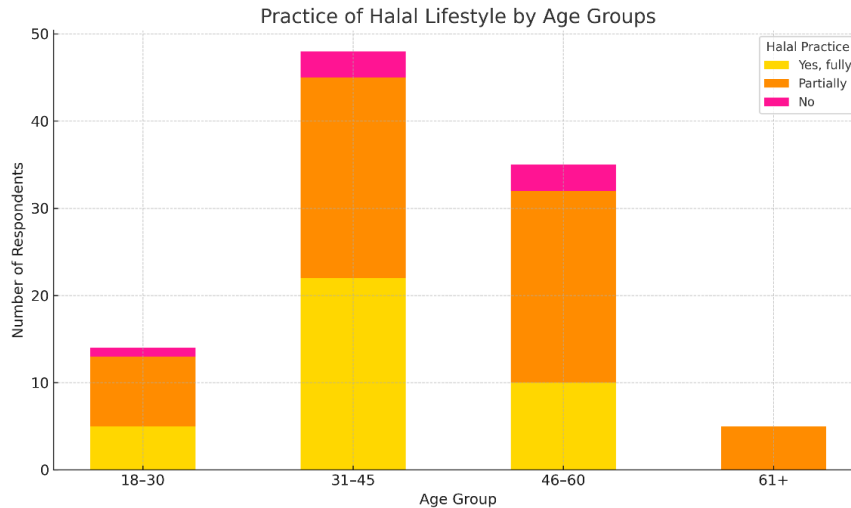


Diagram 2. Practice of a Halal Lifestyle by Age Groups

The analysis of responses to the question "Which brands of halal products do you purchase?" (Diagram 3) indicates a relatively high level of respondent awareness regarding the halal food market. The majority of survey participants cited specific and recognizable companies such as

Akova Impex d.o.o., Solana d.d. Tuzla, MADI d.o.o., Klas d.d., and Vispak d.d., confirming that halal consumers do not purchase blindly – they know what they are looking for and whom they trust.

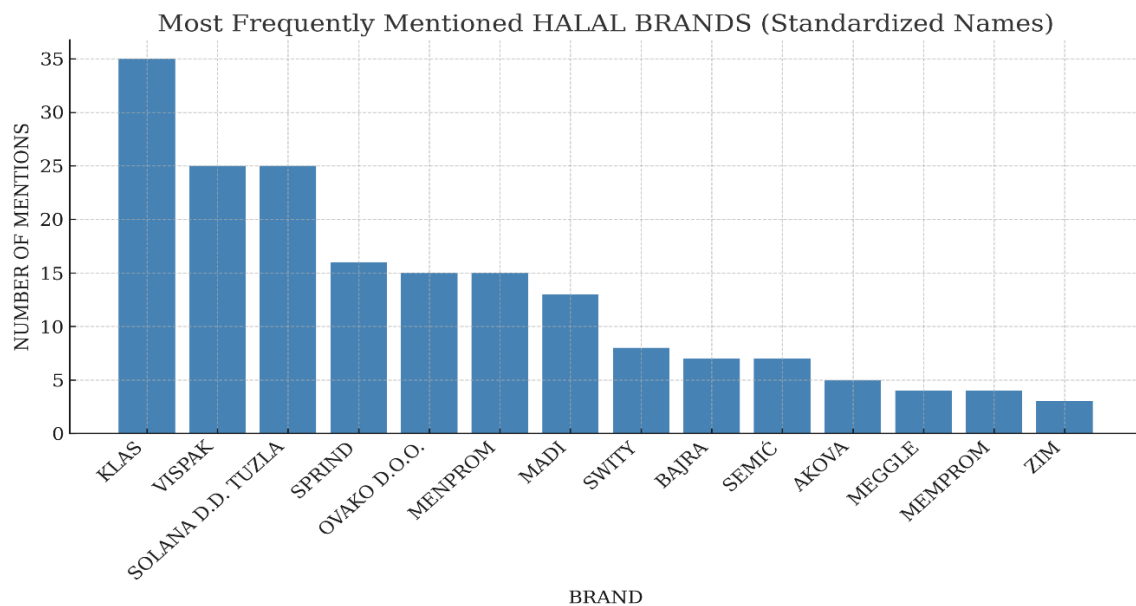


Diagram 3. Results of Responses to the Question: Can You Name Some Brands or Companies Whose Halal Products You Purchase?

This demonstrates that:

- The halal certificate holds value as an indicator of trust and quality;
- Respondents do not choose products solely based on religious affiliation, but

often also due to safety, verified origin, and an ethical approach to food production.

Surveyed consumers indicated that supermarkets with halal sections are the primary source of halal

products, cited by 71.3% of respondents, highlighting the importance of availability and clear labeling of halal products within existing retail chains.

More than 90% of respondents expressed support for the expansion of halal offerings in their local areas, stating that greater availability of halal-certified products would significantly contribute to their daily choices and lifestyle. This result confirms the market potential for the development of additional halal product lines in both the food and tourism sectors.

AS Holding, as a group, has a remarkably strong presence in the minds of halal product consumers, primarily through the tradition of its well-known brands.

An analysis of the conducted survey shows that 31.4% of the mentioned brands belong to the AS Holding group. This indicates a high potential for unified branding and for strengthening the message regarding the application of halal standards.

The growing demand for halal tourism is directly linked to the demographic and economically stronger Muslim population, as well as to the increasing awareness of the values promoted by halal tourism—such as hygiene, ethics, family orientation, and social responsibility. According to reports from international tourism agencies and forums, the halal tourism market is experiencing an annual growth rate of approximately 5%, with Malaysia, Turkey, Indonesia, and the United Arab Emirates emerging as the most promising destinations, along with some EU countries introducing halal-friendly offerings. In response to the question about interest in traveling to countries known for their developed halal tourism sectors (e.g., Malaysia, UAE, Turkey), 49% of respondents answered "Yes, definitely," while the remainder expressed moderate interest. This indicates strong potential for the development of specialized travel packages that include halal dining, religious facilities, and privacy features.

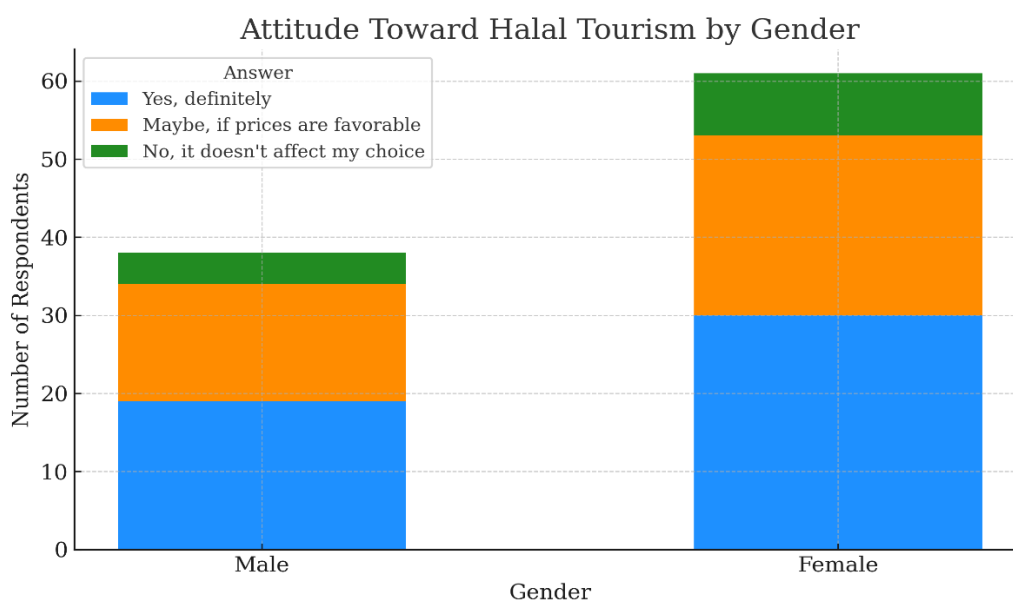


Diagram 4. Results of the Conducted Research on Halal Tourism

Based on the data from Diagram 4, it can be concluded that women show a stronger inclination toward halal tourism, with a higher number clearly choosing the option "Yes, definitely."

Price plays an important role in the decision-

making process, indicating the need for promotional or budget-friendly packages; however, it is evident that overall interest in the selected destinations remains high.

Halal restaurants represent a crucial link in the halal value chain—they are the point where

religious principles, safety standards, and market needs merge into a coherent and sustainable offering. In addressing the needs of the hospitality sector, restaurant owners face significant challenges, ranging from securing raw materials from certified suppliers, managing the costs and administrative procedures associated with halal certification, to addressing the lack of awareness and knowledge about halal requirements among hospitality workers. According to the conducted survey (Diagram 5), the services that respondents would most like to see in halal restaurants, listed in descending order, are:

- Better labeling of halal products – indicating the need for transparency and clearer communication with guests.
- A wider variety of dishes – reflecting the desire for a more diverse and creative menu offering.
- A greater number of halal restaurants, designated prayer spaces, and affordable prices are additional aspects that shape respondents' expectations.



Diagram 5. Services Respondents Would Like to See in Restaurants

The vast majority of respondents support the expansion of halal requirements to cosmetics, pharmaceuticals, and fashion, indicating a growing awareness that halal is no longer limited solely to the food sector.

This reflects a global trend toward a holistic approach to the halal lifestyle, associated with ethics, health, and sustainability.

The results confirm that the halal lifestyle transcends purely religious boundaries and is becoming integrated into broader consumer behavior patterns. The strong support for expanding halal offerings and the high interest in halal tourism highlight the need for halal standards to be applied not merely declaratively,

but as a systematically integrated approach to food production and service delivery. Halal certification of restaurants and hotels, along with better consumer awareness of existing brands and certifications, represent key points for the development of the halal sector in Bosnia and Herzegovina and the region.

**Conclusion**

The results of this study clearly confirm that the halal lifestyle in the contemporary context transcends the boundaries of religious practice and is evolving into a comprehensive value system—rooted in trust, ethics, quality, and safety. The majority of respondents practice halal either partially or fully, with the highest level of

adherence observed among those aged 31 to 45. This indicates a high level of awareness and readiness to implement halal principles in daily life, but also highlights the need for additional education among both younger and older generations. Respondents predominantly recognized and cited well-established brands such as Akova Impex d.o.o., Solana d.d. Tuzla, MADI d.o.o., Klas d.d., and Vispak d.d., including members of AS Holding as well as other companies, collectively accounting for over 30% of all mentioned brands. This reflects both consumer trust and loyalty, as well as the importance of clear and visible halal certification.

Supermarkets with halal sections dominate as the most frequent place of purchase (71.3%), while in the hospitality sector, the most desired improvements are better product labeling, a wider selection of dishes, and more affordable prices. It is evident that consumers expect transparency, diversity, and accessibility—both in retail and dining sectors.

Halal tourism enjoys strong support, particularly among women and middle-aged individuals. Almost all respondents support the expansion of the halal economy into sectors such as cosmetics, pharmaceuticals, and fashion. The economic potential of the halal market for Bosnia and Herzegovina is assessed as moderate to very high, clearly indicating that halal represents not only a spiritual but also a significant economic value.

Key factors influencing the decision to purchase halal products include: trust in the brand, visibility of halal labeling, previous positive experiences, and availability in supermarkets.

This research confirms the existence of a stable consumer base that approaches halal thoughtfully, modernly, and strategically. For companies, producers, and policymakers, this represents a call to view investment in halal certification, availability, and promotion not as an option, but as a development priority. A halal product is not merely a matter of religion—it is a culture of quality, safety, and identity.

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## Uticaj etičkih i zdravstvenih smjernica halal standarda na stil života i globalni rast halal tržišta

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Originalni naučni rad

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### Sažetak

Halal standard predstavlja sveobuhvatan okvir etičkih, vjerskih, zdravstvenih i sigurnosnih smjernica koje određuju šta je dozvoljeno (halal), a šta zabranjeno (haram) prema islamskom pravu. Iako mu je primarna funkcija osigurati da proizvodi i usluge budu u skladu s islamskim principima i zadovolje definisane attribute kvaliteta, koncept halala sve više dobija univerzalno značenje, posebno u sektorima hrane, kozmetike, farmacije, ugostiteljstva i turizma. Globalno halal tržište bilježi snažan rast, potaknut ne samo rastućom populacijom muslimana, već i povećanom svijješću potrošača o kvaliteti, higijeni i etičkim aspektima. Ova studija ispituje uticaj etičkih i zdravstvenih smjernica sadržanih u halal standardu na stil života potrošača širom svijeta i njihovu ključnu ulogu u eksponencijalnom širenju globalnog halal tržišta. Anketa je sprovedena na uzorku od 101 učesnika i obuhvatila je ključne dimenzije halala kao životnog stila. Analizom načina na koje ovi principi utiču na ponašanje potrošača, prehrambene navike i percepciju dobrobiti, istraživanje ima za cilj da pruži sveobuhvatan uvid u spoj vjerskih vrijednosti, etičkih opredjeljenja i ekonomskih prilika unutar okvira halal standarda.

**Ključne riječi:** sigurnost hrane, halal, haram, turizam, ugostiteljstvo, stil života, etičke smjernice, BAS 1049:2023

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## JOURNAL OF HALAL QUALITY AND CERTIFICATION

### Halalopathy and Lifestyleopathy: Integrating Ethical and Scientific Principles in Personalized Medicine

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Preview paper



#### ABSTRACT

Personalized medicine has significantly advanced through genetic and molecular precision but often overlooks the ethical, cultural, and spiritual dimensions that shape patient engagement and therapeutic success. This manuscript introduces two integrative frameworks—Lifestyleopathy, which views health as the dynamic regulation of potential energy and entropy through lifestyle choices, and Halalopathy, which extends this model within an Islamic ethical paradigm by aligning medical interventions with the principles of Halal (permissible) and Tayyib (pure and clean). To operationalize these concepts, we propose the Epigenetic Faith-Lifestyle Resonance (EFLR) to describe the dynamic synergy fostering therapeutic coherence. Halalopathy emphasizes faith-compatible pharmacotherapy, integrating therapeutic efficacy, ethical compatibility, and placebo-trust effects to enhance immune function and support complete recovery. Furthermore, institutional strategies for global Halal pharmaceutical certification, ethical pharmaceutical innovation, and professional education are outlined to mainstream Halalopathy as a scalable model of ethical pluralism in healthcare. By harmonizing scientific rigor with spiritual integrity, this approach redefines healing as a collaborative process of biological, ethical, and cultural coherence, fostering trust, adherence, and improved health outcomes.

**Keywords:** Lifestyleopathy, Halalopathy, Personalized Medicine, Entropy regulation, Halal pharmaceutical certification

#### Introduction

Personalized medicine has transformed healthcare by enabling treatments to be tailored to each patient's unique genetic profile, molecular markers, and risk factors. This has led to more precise diagnoses and improved treatment outcomes. Yet, an important dimension is often overlooked: the ethical, cultural, and spiritual values that deeply influence how patients

experience illness and recovery (Chadwick, 2017). For many people, healing goes beyond simply addressing biological problems. Effective care often requires a holistic approach that respects personal beliefs and lifestyle choices. When healthcare professionals overlook these aspects, it can erode trust, reduce adherence to treatment, and ultimately impact health outcomes.

In many communities, religious and ethical values are central to daily life. Ignoring these factors can make even the most advanced medical care feel incomplete or disconnected from patients' real needs. Despite significant scientific progress, the current biomedical model sometimes fails to consider the moral and cultural frameworks that shape health behaviors (Caplan, 2012). Recognizing this gap, researchers have called for a more inclusive approach that integrates scientific knowledge with ethical and cultural sensitivity. In response, two innovative frameworks have emerged: Lifestyleopathy and Halalopathy (Alzeer, 2025a; Alzeer, 2024a).

Lifestyleopathy focuses on the balance between order and disorder in the body, emphasizing how lifestyle choices, environmental factors, and personal habits affect health. It encourages individuals to take an active role in managing their health by making informed decisions about diet, stress, and other daily behaviors (Alzeer, 2023b).

Halalopathy builds on this concept within the context of Islamic healthcare. It stresses the importance of treatments that are both Halal (permissible) and Tayyib (pure and clean), addressing concerns about religious and culturally sensitive practices. For many Muslims, medical care is most effective when it aligns with their moral values as well as scientific evidence.

Both frameworks highlight the need for a more holistic, patient-centered approach to healthcare—one that respects the diverse ethical, cultural, and spiritual dimensions that matter to individuals. By bridging the gap between advanced science and personal values, we can foster greater trust, improve adherence, and ultimately achieve better health outcomes for all.

### **Conceptual foundations**

#### *Lifestyleopathy: Health as entropy regulation*

Lifestyleopathy is based on the idea that health is fundamentally governed by the second law of thermodynamics. In this view, living organisms are open systems that constantly exchange energy and matter with their environment. Health is not a fixed state, but rather a dynamic balance between high potential energy and low entropy,

reflecting the ongoing effort to maintain internal order against the natural tendency toward disorder (Alzeer, 2024b). Every metabolic process—such as breathing, digestion, nutrient absorption, and movement—involves converting potential energy into work, which inevitably produces entropy as a byproduct (Alzeer, 2022a). The body's ability to capture and use energy efficiently while minimizing entropy is central to maintaining order and resilience.

From this thermodynamic perspective, Lifestyleopathy serves as a framework for managing energy inputs and outputs to support optimal biological function. Lifestyle factors such as nutrition, physical activity, rest, stress management, and environmental exposures are not just healthy habits—they are essential regulators of the body's energy reserves and entropy levels (Alzeer, 2022b).

Making positive lifestyle choices helps maximize the intake of high-quality energy sources and supports efficient energy use for growth, repair, and maintenance. This leads to a state of lower entropy and greater order. On the other hand, unhealthy lifestyle patterns can result in poor energy utilization, increased entropy, and greater risk of inflammation, metabolic dysfunction, and overall decline in health (Mak et al., 2018). By addressing the root causes that affect energy balance and entropy, Lifestyleopathy offers a proactive approach to maintaining health and preventing disease, rather than simply reacting to illness after it occurs (Natalucci et al., 2023).

The strength of Lifestyleopathy lies in its grounding in basic thermodynamic principles, which apply to all living systems regardless of culture, geography, or socioeconomic status. The goal is to maintain a balance between energy input and entropy production—a universal challenge for all organisms. This framework is adaptable, drawing from concepts like core bioenergetic balance, energy efficiency, and waste removal, and can be tailored to individual and community needs.

Scientific evidence increasingly supports the importance of lifestyle in regulating energy and entropy. For example, regular physical activity

boosts energy production and reduces entropy, while nutrient-rich diets support efficient metabolism and cellular repair. By contrast, sedentary behavior and poor nutrition increase entropy and contribute to disease (Alzeer, 2024c).

In summary, Lifestyleopathy provides a scientific, actionable approach to health that empowers individuals and communities to make informed choices. By focusing on the regulation of energy and entropy through lifestyle, it offers a holistic strategy for promoting resilience, preventing disease, and supporting overall well-being.

### *Halalopathy: Faith-compatible healing in islamic healthcare*

Halalopathy is grounded in the same fundamental scientific principles as Lifestyleopathy, emphasizing the importance of entropy regulation and the impact of lifestyle choices on health outcomes. However, Halalopathy adapts these principles to address the unique ethical, spiritual, and practical framework of Islam, aiming to support disease prevention and promote a more holistic recovery process for Muslim individuals. Recognizing that health-related decisions are often closely linked to religious beliefs, Halalopathy posits that aligning healthcare practices with these values can significantly enhance both physical and spiritual well-being (Alzeer, 2024a).

Similar to Lifestyleopathy, Halalopathy emphasizes optimizing nutrition, physical activity, rest, stress management, and environmental factors. However, it ensures that these domains are approached in accordance with Halal and Tayyib standards (Alzeer et al., 2018). This involves not only choosing permissible foods, but also engaging in physical and spiritual activities that are consistent with Islamic teachings. For example, Halalopathy encourages practices such as prayer, mindfulness, and seeking environments that support spiritual reflection. It also emphasizes managing stress in ways that are compatible with Islamic values, thereby supporting resilience and well-being. By integrating these faith-specific considerations, Halalopathy aims to create a lifestyle that is

harmoniously aligned with Islamic teachings, thus strengthening the body's natural defenses and promoting a more comprehensive healing process when illness occurs (Alzeer, 2022c).

The ultimate goal of Halalopathy, through its targeted application of Lifestyleopathy principles, is twofold: first, to empower Muslims to adopt preventive lifestyles by adhering to Islamic guidance on purity, moderation, and well-being; and second, to ensure that when medical treatment is required, it is delivered in a manner that respects religious beliefs and fosters trust and adherence. This approach contributes to a more holistic and spiritually integrated healthcare experience. By addressing both physical and spiritual dimensions, Halalopathy seeks to optimize overall health outcomes and support greater well-being in accordance with Islamic values (Miller and Thoresen, 2003).

### **Clinical implications & application**

#### *Faith-compatible pharmacotherapy in practice*

Halalopathy extends beyond simply prescribing permissible substances; it seeks to build a strong, collaborative relationship between patients and their medical treatment, promoting active patient engagement in the recovery process. This approach recognizes that healing involves more than just addressing biological factors-it also requires alignment with the patient's deeply held values and lived experiences (Alzeer, 2021).

A key aspect of Halalopathy is ensuring that medical treatments are compatible with Halal and Tayyib principles. When therapies are consistent with daily routines, ethical beliefs, and spiritual practices, they foster internal harmony and create a supportive environment that enhances the effectiveness of medical interventions. This alignment can improve patient trust and satisfaction, which are crucial for optimal therapeutic outcomes. Halalopathy also emphasizes that achieving complete recovery involves the patient's conscious and active participation in their own healing. By empowering patients to take an active role, the approach incorporates additional therapeutic dimensions that go beyond the direct biochemical effects of medicine.

Active patient involvement can facilitate beneficial interactions among various healing mechanisms. Integrating medical treatment with ethical and lifestyle values produces a “compatibility effect,” which supports psychological coherence and emotional engagement. At the same time, the trust developed through respectful and transparent communication can strengthen the “placebo effect”—a well-documented phenomenon known to positively influence immune function and neurobiological pathways (Finniss et al., 2010; Colloca and Miller, 2011).

Halalopathy conceptualizes these effects not as isolated events, but as interconnected processes, where:

- The therapeutic effect can directly address biological dysfunction at the molecular level.
- The compatibility effect also integrates treatment with the patient’s personal and spiritual identity.
- The placebo effect, supported by patient trust and belief, enhances the body’s overall readiness to heal.

While pharmacotherapy is essential for treating specific medical conditions, Halalopathy recognizes that the primary driver of recovery is the body’s innate capacity for self-healing, particularly through the immune system. This coordinated synergy between medicine and faith-based practices supports and boosts immune efficiency (Figure 1).

Furthermore, Halalopathy considers the influence of psychosocial and spiritual factors on healing. These factors can modulate immune function, promote biological order, and accelerate the recovery process.

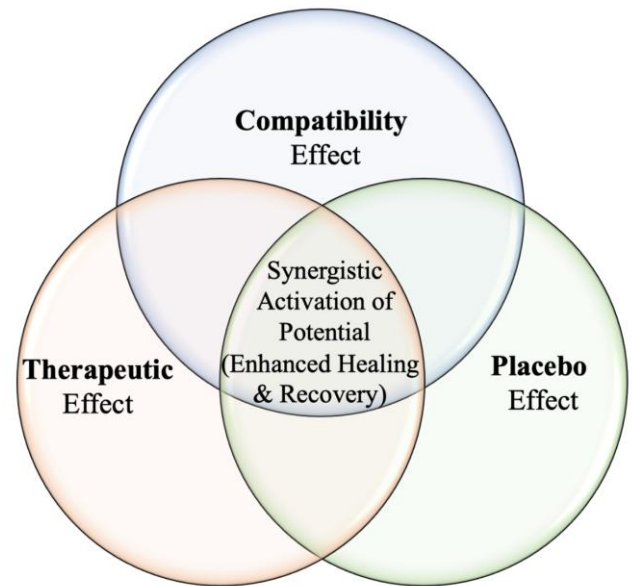


Figure 1: Synergy Model of Halalopathy: Therapeutic, Compatibility, and Placebo-Trust Effects

In summary, Halalopathy promotes a holistic, cooperative approach to healing—one that integrates medical interventions with patients’ values and beliefs, aiming for comprehensive recovery rather than just symptom management. This model highlights the importance of addressing biological, psychological, and spiritual factors together. By supporting both the physical and non-physical aspects of health, Halalopathy contributes to improved well-being and long-term recovery (Zhang et al., 2020).

Ultimately, Halalopathy underscores that true healing is not limited to treating disease, but involves fostering a therapeutic alliance that respects and incorporates the patient’s ethical and spiritual framework, leading to more meaningful and sustained health outcomes.

*The Bio-ethical alignment of healing: Introducing the Epigenetic Faith-Lifestyle Resonance (EFLR)*

Personalized medicine has made significant advances by tailoring treatments to individual genetic profiles. However, it often overlooks the profound impact of lifestyle choices, personal values, and beliefs on health outcomes. The Epigenetic Faith-Lifestyle Resonance (EFLR) framework addresses this gap by integrating genetic insights with lived experiences, aiming to

ensure that therapies are aligned not only with biological needs but also with psychological, social, and spiritual dimensions.

Recent research highlights that aligning genetic information with individual experiences can enhance both the efficacy and adherence of therapeutic interventions (Alzeer, 2025b). EFLR specifically maps how gene expression is influenced by lifestyle and faith-based behaviors. Factors such as nutrition, physical activity, sleep, and stress management have demonstrated direct effects on the epigenome, revealing limitations in conventional personalized medicine approaches that do not sufficiently account for these variables (Alzeer and Benmerbat, 2023).

Faith-based practices can also modulate stress and promote resilience, potentially influencing biological processes through mechanisms such as hormonal regulation and neuroendocrine stability. For example, religious rituals like fasting can enhance metabolic efficiency and support cellular repair mechanisms, while structured prayer may help regulate circadian rhythms and hormonal balance (Jaber et al., 2024). These observations suggest that belief-driven behaviors act as biological modulators, underscoring the need for healthcare frameworks—such as Halalopathy—that integrate religious and spiritual dimensions into patient care (Figure 2) (Alzeer, 2023b).

The EFLR framework offers a model for biologically effective, lifestyle-compatible, and ethically sensitive care (Alzeer, 2025c). By recognizing the interplay between physiological processes and emotional, spiritual, and cognitive factors, EFLR aims to optimize healing and improve patient adherence. Treatments designed with EFLR principles may yield more meaningful and sustainable outcomes by honoring individual belief systems and supporting holistic well-being (Sloan et al., 2022). When healthcare providers respect the values and faith of their patients, they foster a therapeutic alliance that supports not only biological healing but also emotional and spiritual resilience (Seetharaman et al., 2021). This multifaceted approach may reduce stress, enhance immune function, and accelerate

recovery, ultimately resulting in improved patient outcomes.

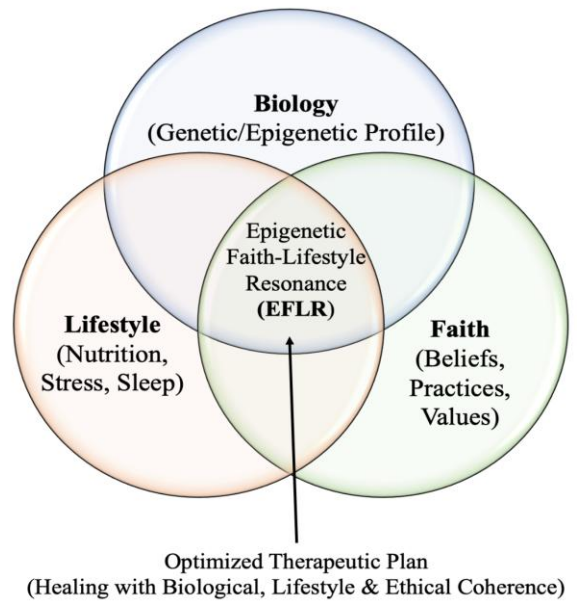


Figure 2: Epigenetic Faith-Lifestyle Resonance (EFLR): Integrating Biology, Lifestyle, and Faith for Value-Aligned Therapeutic Decisions

By integrating genetic, lifestyle, and faith-based factors, the EFLR framework provides a comprehensive, patient-centered approach to healing. This alignment with patients’ ethical and spiritual convictions can profoundly enhance the effectiveness and personal relevance of medical care (McSwan et al., 2021).

### Institutional Strategies for Halalopathy Integration

Building on the foundational concepts of Lifestyleopathy and the faith-based framework of Halalopathy, fully realizing the potential of these approaches requires their systematic integration into the broader healthcare landscape. For lifestyle-compatible, ethically grounded medicine to move beyond theory, it must become an integral part of research protocols, regulatory standards, educational programs, and patient engagement strategies. This section identifies and discusses the key strategic pillars necessary for positioning Halalopathy as a central model of ethical and personalized healthcare.

### *Global Halal pharmaceutical certification*

Building on the principles of Lifestyleopathy and Halalopathy, fully realizing their potential requires addressing the current fragmentation in the Halal pharmaceutical sector. This can be achieved by developing a globally harmonized certification system that integrates recent research on comprehensive Halal-Tayyib positive list for both ingredients and manufacturing processes. Such a framework should go beyond basic ingredient verification to include rigorous standards for manufacturing integrity, supply chain traceability, and ethical sourcing, thereby enhancing transparency and trust throughout the pharmaceutical supply chain (Alzeer et al., 2025).

Implementing this vision will require close collaboration among Islamic scholars, pharmaceutical scientists, bioethicists, and regulatory authorities. These stakeholders must work together to establish clear guidelines and secure regulatory approval, paving the way for the widespread clinical adoption of this ethical and personalized healthcare model. Ultimately, the goal is to improve access to medications that are consistent with Islamic values, thereby supporting the health and well-being of Muslim patients worldwide.

### *R&D Priorities: Ethical pharmaceutical innovation*

Building on the principles of ethical and personalized healthcare, the future of faith-aligned pharmaceuticals requires a proactive and comprehensive approach to innovation—one that goes beyond simply identifying permissible substitutes. Research and development should prioritize the design of pharmaceuticals that comply with Halal-Tayyib standards from the outset, integrating ethical considerations early in the drug development process. This approach is particularly relevant in the application of synthetic biology, which offers innovative methods to create medications that meet dietary laws while also addressing evolving consumer needs (Turpal, 2023).

A key aspect of advancing ethical pharmaceutical innovation is the development of synthetic and plant-based excipients to replace animal-derived

components—a topic that has generated significant interest in recent scientific literature. The move toward natural polymers as pharmaceutical excipients is especially promising, as these materials offer sustainable alternatives to conventional excipients often sourced from animals. Natural polymers are valued for their abundance, affordability, and biodegradability, and their use represents an ethical advancement in pharmaceutical formulation (Saha et al., 2018). This shift toward plant-based materials also aligns with increasing consumer demand for cruelty-free and Halal products (Constantinides et al., 2016).

Recent progress in biological therapies, such as nanomedicine for targeted drug delivery using biocompatible and ethically sourced materials, further highlights the potential for innovation in this field. Advances in synthetic biology have enabled the engineering of genetic circuits and biotherapeutics that provide targeted treatments, reduce side effects, and enhance therapeutic efficacy (Jain et al., 2012). Additionally, green chemistry principles for sustainable pharmaceutical development complement Halal standards by ensuring that production processes are environmentally friendly and uphold ethical values.

The development of biologically based Halal therapeutics, including advanced therapies such as biologics and nanomedicines, represents a significant advancement by enabling tailored treatments that address disease mechanisms without ethical dilemmas. Current frameworks are already demonstrating success in developing emerging therapeutics that adhere to Halal standards while improving bioavailability and minimizing adverse reactions.

To fully realize the potential of Halalopathy in this innovative landscape, it is imperative to establish a supportive research infrastructure guided by Halal-Tayyib principles. This will enable the development of ethically sound and scientifically validated medicines, ultimately facilitating the availability of faith-compatible pharmaceutical solutions for a wide range of patient populations (Charbonneau et al., 2020).

### *Academic & professional education*

Building on the ethical and personalized healthcare frameworks established by Lifestyleopathy and Halalopathy, advancing clinical implementation requires a fundamental shift in academic and professional education. To ensure healthcare professionals possess the necessary expertise—an area currently underserved in formal training on Halal medical ethics—it is essential to integrate Halalopathy into medical and pharmacy curricula.

This progressive educational approach should encompass dedicated courses on Islamic bioethics and Halal-compliant therapeutics, as well as mandatory continuing education programs for practicing clinicians. Additionally, the development of advanced clinical decision support tools for prescribing faith-compatible medications will further enhance clinical practice. By elevating provider knowledge in this domain, Halalopathy can move beyond being a mere accommodation, becoming an intrinsic component of truly patient-centered care.

### *Patient empowerment & public health engagement*

To optimize patient engagement in their healing process within the Halalopathy framework, it is essential to prioritize active patient participation in health management. The most effective initial digital health tools to support this goal include AI-powered medication checkers that provide immediate, faith-based verification of pharmaceutical products; secure tele-consultation platforms that connect patients with culturally competent healthcare practitioners; and interactive symptom trackers that incorporate faith-based reminders into daily health routines. These tools collectively ensure that patients have access to clear, reliable information regarding faith-compatible pharmaceutical options, thereby empowering them to make informed and autonomous health decisions.

### **Global implications & ethical pluralism in healthcare**

The frameworks of Lifestyleopathy and Halalopathy exemplify the movement toward

ethical and personalized healthcare. Halalopathy, in particular, offers a scalable approach for a globally connected world characterized by diverse ethical, cultural, and spiritual identities. This approach aligns with the concept of **medical pluralism**, which recognizes the coexistence of multiple medical systems—such as conventional biomedicine, traditional medicine, and complementary and alternative therapies—within a single society. Medical pluralism emphasizes the importance of patient autonomy, advocating for individuals to make informed choices based on their own ethical and cultural values (Lahey and Nelson, 2019).

Integrating various healthcare paradigms is essential for addressing the complex and multifaceted needs of patients. This integration enhances patient-centered care by respecting individual beliefs and preferences, ultimately leading to improved health outcomes (Wedgeworth and Cody, 2023).

Halalopathy, grounded in Islamic ethical principles, serves as a concrete example of how belief-based medical systems can be incorporated within a pluralistic healthcare framework. By aligning healthcare delivery with patients' spiritual and moral values, Halalopathy fosters therapeutic relationships that support physiological, psychological, and spiritual well-being. Recognizing the influence of cultural context, this approach demonstrates how values-driven care can address the holistic needs of patients. Evidence suggests that attending to patients' spiritual and emotional dimensions can significantly enhance satisfaction and health outcomes (Morland et al., 2022).

Importantly, the principle of aligning healthcare with patient values is not exclusive to Islam. It sets a precedent for developing similar belief-based models across different traditions, enabling diverse populations to maintain their health without compromising their conscience in a pluralistic society (Surbakti et al., 2024).

Halalopathy also contributes to the evolution of global bioethics by challenging the dominance of secular biomedical models and advocating for ethical pluralism. This approach stresses the

importance of moral coherence-integrating ethical considerations into pharmaceutical development and redefining quality in healthcare to include alignment with human values. By engaging underserved communities and respecting their cultural and spiritual needs, Halalopathy helps to reduce global health disparities and build trust in healthcare systems.

For academia and research, models like Halalopathy encourage critical exploration at the intersection of belief, biology, and clinical outcomes. They demonstrate that scientific rigor and spiritual integrity can coexist, fostering more inclusive, effective, and humane healthcare solutions. As global medicine increasingly prioritizes personalized and patient-centered care, operationalizing ethical pluralism is becoming essential for establishing best practices in healthcare delivery (Rykkje et al., 2021).

### Conclusion

The integration of ethical, cultural, and spiritual dimensions into personalized medicine is no longer optional but essential for delivering truly patient-centered care. This manuscript has introduced Lifestyleopathy as a universal framework for health optimization through the regulation of potential energy and entropy via lifestyle choices, and Halalopathy as its faith-centered extension, aligning medical interventions with the principles of Halal and Tayyib. By proposing the Epigenetic Faith-Lifestyle Resonance (EFLR) to capture the dynamic synergy between belief systems, lifestyle behaviors, and biological processes, this approach redefines healing as a collaborative process of biological, ethical, and cultural coherence. Beyond the Muslim context, Halalopathy offers a scalable model for ethical pluralism in healthcare, fostering trust, enhancing treatment adherence, and improving health outcomes across diverse populations. Institutional strategies such as global Halal pharmaceutical certification, ethical pharmaceutical innovation, and faith-aligned education are crucial for mainstream adoption. Ultimately, by harmonizing scientific precision with spiritual integrity, Halalopathy and Lifestyleopathy bridge the gap between modern

medicine and human values, advancing a holistic, inclusive vision of health and healing in the 21st century.

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## Halalopatija i Lifestyleopatija: Integracija etičkih i naučnih principa u personaliziranoj medicini

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Pregledni rad

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### Sažetak

Personalizirana medicina značajno je napredovala kroz genetsku i molekularnu preciznost, ali često zanemaruje etičke, kulturne i duhovne dimenzije koje oblikuju angažman pacijenata i uspješnost terapije. Ovaj rukopis predstavlja dva integrativna okvira—Lifestyleopathy (bolesti stila života), koji zdravlje posmatra kao dinamičku regulaciju potencijalne energije i entropije putem životnih navika, i Halalopathy, koji proširuje ovaj model unutar islamskog etičkog okvira usklađujući medicinske intervencije s principima Halal (dozvoljeno) i Tayyib (čisto i dobro). Kako bismo operacionalizirali ove koncepte, predlažemo model Epigenetske Rezonance Vjere i Životnog Stila (EFLR), koji opisuje dinamičnu sinergiju koja podstiče terapijsku usklađenost. Halalopatija naglašava farmakoterapiju usklađenu s vjerom, integrirajući terapijsku efikasnost, etičku kompatibilnost i placebo-efekte povjerenja u cilju jačanja imunološke funkcije i potpune oporavka. Nadalje, izložene su institucionalne strategije za globalnu certifikaciju halal farmaceutskih proizvoda, etičke farmaceutske inovacije i profesionalno obrazovanje, s ciljem da se Halalopatija uvede kao skalabilni model etičkog pluralizma u zdravstvenoj zaštiti. Usklađujući naučnu preciznost s duhovnim integritetom, ovaj pristup redefiniše iscjeljenje kao zajednički proces biološke, etičke i kulturne usklađenosti, podstičući povjerenje, pridržavanje terapije i bolje zdravstvene ishode.

**Ključne riječi:** *Lifestyleopathy, Halalopatija, personalizirana medicina, regulacija entropije, halal certifikacija lijekova.*

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## JOURNAL OF HALAL QUALITY AND CERTIFICATION

### Rigorous Sanitary Measures to Reduce *Campylobacter* in Chicken Production as an Alternative to the Prophylaxis Use of Antibiotics

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Review paper 

#### ABSTRACT

Campylobacteriosis is a leading cause of food poisoning in Europe, the USA, Australia and New Zealand, and identifying reservoirs of infection is important in disease prevention. Although campylobacteriosis is considered rare in Africa, research indicates that chicken meat is contaminated in African countries as well. Monitoring the prevalence of *Campylobacter jejuni* and *Campylobacter coli* in broiler meat production indicates variability depending on the country, season, source of the sample and hygiene in primary production (farms) and broiler meat processing (slaughterhouses/production plants). Therefore, the purpose of this review was to improve the understanding of the impact of implementing appropriate sanitation measures on reducing contamination with *Campylobacter* species in primary production and processing of chicken meat, in facilities with implemented HACCP and Halal control systems. The alignment with EU legislation regulates hygiene criteria in primary production and processing of chicken meat. The application of rigorous hygiene measures at all stages of chicken meat production can reduce the number of thermophilic *Campylobacter* species on broiler chicken carcasses. By setting the recommended EU critical limit of 1000 CFU/g for contamination of broiler neck skin in slaughterhouses, the incidence of campylobacteriosis can be reduced by 40% to 90%.

**Keywords:** Antibiotics, *Campylobacter*, primary production and processing of chicken meat, sanitary measures

## Introduction

The cessation of the use of antibiotics in broiler farming would contribute to reducing the spread of antibiotic resistance, which together with campylobacteriosis represents a serious threat to public health. The application of rigorous sanitary measures, although a long-known procedure, can now be said to be a novelty in the production process because it contributes to reducing the spread of campylobacteriosis and antibiotic resistance. Today, rigorous hygiene measures in broiler farming and slaughterhouses are used as an alternative to the prophylactic use of antibiotics in broiler farming, but also as a means of biostimulation of chickens and preventing the spread of campylobacteriosis and antibiotic resistance (WHO 2023).

The Nordic countries have perfected a control system that includes prevention at the level of primary production (farm), which is in line with the European Food Criteria (EU) 2017/1495. Preventive biosecurity measures (vaccination, carvacrol in feed and drinking water, water disinfection) and hygiene can be implemented on broiler farms. Broilers are tested for the presence of *Campylobacter* near slaughter age in order to further implement preventive measures depending on the status of the flock. In the case of a positive flock, it is recommended that the meat of the positive flock be frozen (Olsen et al. 2024).

Meunier et al. (2016) state in their study that primary broiler production is crucial in ensuring the sanitary quality of meat and that it is necessary to ensure good sanitation of the facilities where chickens are kept. Chicken farms are a major source of *Campylobacter* spp. because they colonize the cecum of chickens. Control at the farm level is very important, but also very difficult to implement (Baali et al. 2020). On the other hand, negative broiler flocks, which do not have *Campylobacter* species in the intestines, after slaughtering without strict hygiene at different stages of primary processing, can produce carcasses contaminated with *Campylobacter* species above the limit values,

indicating subsequent contamination via workers' hands or from surfaces in slaughterhouses (Wieczorek & Osek 2015).

In slaughterhouses and production plants, the most important measure for controlling campylobacteriosis remains the application of rigorous sanitary measures, which some authors' state can produce broiler carcasses free of *Campylobacter* spp. even in positive flocks (Szott et al. 2020). Hue et al. (2010) investigated the risk factors that lead to increased contamination of broiler carcasses with *Campylobacter* species during slaughter and found that the most critical stage in the slaughterhouse is evisceration. As a solution to the problem, it is proposed to rinse broiler carcasses after evisceration and perform slaughter according to the contamination status of the broiler flock with *Campylobacter* spp.

Positive flocks result in carcass contamination. It is important that farms and slaughterhouses implement strict biosecurity and hygiene measures in accordance with European food criteria. The limit value for contamination of the neck skin of broiler chickens with *Campylobacter* spp. in slaughterhouses should not exceed 1000 CFU/g (EU 2017/1495 & EFSA 2011). Therefore, the purpose of this review was to improve the understanding of the impact of implementing appropriate sanitation measures on reducing contamination of *Campylobacter* species in primary production and processing of chicken meat, in facilities with implemented HACCP and Halal control systems.

## Epidemiology

Campylobacteriosis, epidemiologically speaking, occurs during periods of high air temperatures and high humidity. It can be said that it is a seasonal disease (Cortés et al. 2022). The reproduction of *Campylobacter* spp. (*Campylobacter jejuni*, *Campylobacter coli*) in the intestines of chickens is most intense in late summer and early autumn in temperate climates. While in subtropical and tropical areas, high temperatures and high humidity are almost always present, so campylobacteriosis occurs in

these areas throughout the year (Urdaneta et al. 2023).

Chicken meat is one of the important sources of protein for people around the world, therefore the health safety of chicken meat is of great importance. Biosecurity and hygiene measures in the production and processing of chicken meat must be at an enviable level in accordance with the recommended hygiene criteria for the chicken meat production process in accordance with European regulations, especially during the campylobacteriosis epidemic season (Olsen et al. 2024).

The Nordic and Baltic countries have developed the best control system for *Campylobacter* spp. in the chicken meat production chain, where in Norway, but also in Iceland, microbiological control of meat is carried out weekly during the campylobacteriosis season. In Iceland, the limit for *Campylobacter* spp. in neck skin is set at 500 CFU/g instead of 1000 CFU/g (EU) 1495/2017, and for this purpose, rigorous hygiene measures are implemented in slaughterhouses and production plants (Olsen et al. 2024). Campylobacteriosis outbreaks have long been associated with developed European countries,

Sweden, Denmark, then the United States and Australia (Hanafy et al. 2022), and outbreaks have been rarely reported in Africa, Asia and the Middle East, with the exception of Thailand (Mason et al. 2017). In the region, in Croatia, an increase in campylobacteriosis epidemics has been recorded since 2007, when the reporting of campylobacteriosis became a legal obligation (Furmeg et al. 2021).

According to reports from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), the number of cases of campylobacteriosis in Europe has been increasing annually since 2005 (Table 1) (EFSA & ECDC 2009–2021). Today, the Nordic countries have developed the best control system for *Campylobacter* spp. at the farm, slaughterhouse and production plant levels, where rigorous sanitary measures are applied in the chicken meat production process, and without the use of antibiotics in chickens in breeding (Lopes et al. 2018; Olsen et al. 2024). The limit for contamination of chicken neck skin for *Campylobacter* spp. in slaughterhouses is set at 1000 CFU/g in accordance with EU 2017/1495.

Table 1. Number of reported cases of campylobacteriosis in Europe per year

| Annual reports         | Growth in campylobacteriosis cases from 2005 to 2021 |                 |                    |
|------------------------|--|-----------------|--------------------|
|                        | Annual growth (%)                                    | Number of cases | Sources            |
| <b>2005</b>            | 6-7  | 195 426         | EFSA & ECDC (2009) |
| <b>2007</b>            | 2-3  | 200 507         | EFSA & ECDC (2009) |
| <b>2010</b>            | 5-6  | 212 064         | EFSA & ECDC (2012) |
| <b>2011</b>            | 3-4  | 220 209         | EFSA & ECDC (2013) |
| <b>2014</b>            | 7-8  | 236 851         | EFSA & ECDC (2015) |
| <b>2016</b>            | 3-4  | 246 307         | EFSA & ECDC (2017) |
| <b>2018</b>            | 0-1  | 246 571         | EFSA & ECDC (2019) |
| <b>2019 (COVID-19)</b> | Recorded decline since 2019                          | 120 946         | EFSA & ECDC (2021) |
| <b>2021</b>            | /  | /               | /                  |

Rigorous sanitary procedures applied in primary production and processing of chicken meat, based on control systems best developed in Scandinavian countries, and based on compliance

with EU legislation, have been proven to contribute to protection against campylobacteriosis and prevent the spread of antibiotic resistance. Based on research to date,

these methods have proven effective in most EU countries. By setting the recommended EU critical limit of 1000 CFU/g for contamination of the neck skin of broilers in slaughterhouses, the incidence of campylobacteriosis can be reduced by 40% to 90%.

### **Biological characteristics**

#### ***Campylobacter***

The pathogenicity of *Campylobacter* species depends on the degree of virulence of the bacterial species/strain. Thermophilic *Campylobacter* spp. (mainly *C. jejuni* and *C. coli*) are extremely virulent and only a small number of bacteria are required to cause campylobacteriosis in consumers of chicken meat (Finsterer 2022). These bacterial species possess virulence factors, i.e. a capsule, polysaccharide and oligosaccharide membranes, toxin production, various biochemical properties, protease enzymes, etc. All of the above virulence factors can encode genes for toxins, as well as genes for acquiring antibiotic resistance. On the other hand, there are mechanisms that pathogenic *Campylobacter* species use to exert their pathogenicity. *Campylobacter* species have the ability to invade, produce toxins, adhere to the intestinal mucosa and initial reproduction (the mechanism by which they colonize the intestines of chickens) (Habib et al. 2023).

In order for bacteria to possess these pathogenic mechanisms at all, they must possess genes: genes for invasion and colonization such as *cadF*, *ciaB*, *pldA* (Buiatte et al. 2023). The presence of flagella is also one of the mechanisms of bacterial pathogenicity by which bacteria move to their final destination, in this case the intestinal epithelium. Chemotaxis represents directed movement, translocation is movement towards organs, and invasion is passage through the epithelium (Habib et al. 2023). Pathogenesis is the way in which infection occurs and depends on virulence factors, the infectious dose of bacteria and the immunity of the attacked macroorganism (Jama & Ketley 2023).

### **Prophylaxis use of antibiotics in broiler farming**

The use of antibiotics (AB) in primary broiler production is present worldwide. Antibiotics are used as a supplement to the diet of chickens in order to biostimulate, increase growth and shorten fattening time (Santos-Ferreira et al. 2022). The use of non-resorbable antibiotics bacitracin and spiramycin in broiler farms reduced the risk of infection and cross-contamination within the flock (Milanov et al. 2016). By slaughtering a healthy flock of broilers in slaughterhouses, the risk of cross-contamination of chicken meat is reduced (Wieczorek & Osek 2015). Non-absorbable antibiotics perform enterodisinfection in broilers, improve their performance, their health, the microbiological correctness of chicken meat and protect the health of consumers. Many authors warn that the benefits of using antibiotics in broiler prophylaxis are much smaller than the negative effects (Santos-Ferreira et al. 2022). Denmark and Sweden, countries where campylobacteriosis was a pressing public health problem, saw the disadvantages of this practice and were the first to ban the use of AB in broilers for prophylactic purposes (Olsen et al. 2024).

Antibiotic residues in chicken meat can lead to the spread of antibiotic resistance (ABR). Cooking chicken meat containing antibiotic residues in the meat can create more dangerous chemical structures, and the consumption of such meat can lead to autoimmune disorders in consumers (Senčić et al. 2021). Antibiotic residues in chicken meat are not the only danger for consumers. Antibiotic resistance genes can spread through microbial contaminants of chicken meat to bacteria of human origin. Also, intestinal bacteria can develop various mechanisms of antibiotic resistance. The use of antibiotics can cause mutations. Antibiotic resistance genes can be transmitted to offspring vertically and to almost all bacteria by horizontal transfer (Gharbi et al. 2021). The European Union has banned the use of antibiotics in broiler prophylaxis since 2006 (FDA 2017), so finding an alternative to the prophylactic use of

antibiotics is of crucial importance for broiler chicken producers. A natural agent that should have a biostimulating effect should not be harmful to the health of chickens, consumers, and should also not pollute the environment (Santos-Ferreira et al. 2022).

### Antibiotic resistance

Thermophilic *Campylobacter* spp. can acquire antibiotic resistance genes from other bacteria in the immediate vicinity. They have the ability to accumulate genes in the genome, but they are also prone to mutations caused by the use of antibiotics because they do not possess genes that protect bacterial DNA (*umuCD*, *vsr*) or genes that serve for DNA repair (*mutH*, *mutL*) (Al-Khresieh et al. 2022). Vertical gene transfer from parent to offspring and horizontal gene transfer to all bacteria in the immediate vicinity are known. Horizontal gene transfer is enabled by conjugation, transformation and transduction

(Habib et al. 2023). Bacterial conjugation occurs with the help of sexual cells, and is enabled by plasmids, transposons and integrons. For horizontal gene transfer to occur at all, bacteria must possess conjugated *tet(O)* genes within the *pTet* plasmid. Transformation is possible after the death of the bacterial cell, where the bacterial DNA is released through the lysis process and gene transfer to other competent bacteria can occur, only 1% of bacteria have this ability. Transduction is the transfer of bacterial DNA with the help of phage bacterial viruses to other competent bacteria (Habib et al. 2023).

In *Campylobacter* species, DNA mutations are not uncommon and can be caused by various mutagens, most often antibiotics. Therefore, uncontrolled use of various types of antibiotics, e.g. from the fluoroquinolone class, can cause mutations of local *gyrA* genes associated with fluoroquinolone resistance.

Table 2. Mechanisms of acquisition of antibiotic resistance in *Campylobacter* species (Markey et al. 2013)

| Antibiotics:<br>class/type   | Antibiotic resistance of <i>Campylobacter</i> species  |
|--|--|
|  | Biochemical mechanisms of antibiotic resistance  |
| <b>I <math>\beta</math> lactams: penicillins, amoxicillin, amoxicillin with clavulanic acid, ampicillin, ampicillin sulbactam, cephalosporins I, II, III and IV generation, reserve carbapenems: carbapenem, imipenem, meropenem, ertapenem.</b><br><b>Glycopeptides: vancomycin, teicoplanin, telavancin, antitumor antibiotic belomycin.</b><br><b>Polypeptides: polymyxin B, polymyxin E or colistin, bacitracin.</b> | I Enzyme alteration and modification   |
| <b>II Quinolones and fluoroquinolones: ciprofloxacin, norfloxacin, marbofloxacin.</b><br><b>Aminocoumarins: novobiocin.</b><br><b>Rifamycins: rifampicin or rifampin.</b>  | II Change in DNA gyrase and topoisomerase IV   |
| <b>III and IV Nitrofurans: nitrofurantoin.</b><br><b>Tetracyclines: tetracycline, doxycycline.</b><br><b>Aminoglycosides: gentamicin, reserve amikacin, kanamycin, neomycin, etc.</b><br><b>Lincosamides: clindamycin, lincomycin.</b><br><b>Macrolides: azithromycin, erythromycin, clarithromycin.</b>   | III Target modification or change of the target enzyme and IV Modification of ribosome structure |
| <b>V To all antibiotic classes and disinfectants</b>   | V Active efflux (pump or carrier protein)  |
| <b>VI Sulfonamides and trimethoprim</b>  | VI Alteration of metabolic pathway   |
| <b>VII To all antibiotic classes</b>   | VII Alteration of cell membrane permeability   |

On the other hand, uncontrolled use of macrolide antibiotics can cause mutations in 23S rRNA, which is associated with macrolide resistance.

Since *Campylobacter* spp. can accumulate genes in the genome, there is a possibility that they can acquire antibiotic resistance genes from other

competent bacteria, mainly from gram-positive bacteria, by horizontal transfer. They can possess *ermB*, *optrA*, *fexA* and *cfrC* genes in their genome. The *erm(B)* genes are associated with acquired resistance to erythromycin (macrolides), *tet(O)* to tetracycline (tetracyclines) (Qin et al. 2023). Sometimes it is only the presence of the gene that is at issue, which is why it is important to examine the presence of phenotypic, not just genotypic, resistance. The presence of the gene indicates the possibility of *Campylobacter* spp. resistance to antibiotics, and indicates the possibility of preventing antibiotic resistance, which is achieved by reducing or completely stopping the use of a particular antibiotic in chickens, even for therapeutic purposes. It is important to use antibiotics responsibly and rationally in agriculture, veterinary medicine and medicine. To date, *Campylobacter jejuni* has ~1650 conjugated genes in its genome, and this number is increasing (Hitchcock et al. 2022). Bacteria can also possess antibiotic resistance mechanisms without the presence of resistance genes in the genome of the bacterial cell. Resistance mechanisms are mainly related to the mechanism of action of the antibiotic whose use has caused resistance (Table 2) (Markey et al. 2013).

### **Control of Campylobacteriosis along the Food Chain**

#### ***Biological contaminants and preventive measures***

The improved European system for the control of *Campylobacter* spp. in raw chicken meat sets a limit for contamination of chicken neck skin with *Campylobacter* spp. in slaughterhouses of 1000 CFU/g based on Regulation (EU) 2017/1495. Preventive measures to prevent contamination of

chicken meat with *Campylobacter* spp. can be divided into three levels of protection. The first and most important level of safety that ensures the health safety of chicken meat is control at the farm level. Sanitary control in chicken farming is the most important preventive measure with a strong effect, because flocks positive for *Campylobacter* spp. produce positive broiler carcasses in slaughterhouses and production plants (Sibanda et al. 2018).

The second measure is the serological categorization of *Campylobacter* species from chicken meat, since campylobacteriosis is mainly caused by the consumption of infected chicken meat (Taha-Abdelaziz et al. 2023). The third measure is the implementation of hygiene and sanitation measures in slaughterhouses, but with a somewhat smaller impact on the safety of chicken meat if it is a positive flock slaughtered. It includes the application of hygiene and sanitation standards during slaughter, carcass processing and packaging (Sibanda et al. 2018).

The fourth measure is of medium effect, which includes washing broiler carcasses under a strong stream of drinking water, which is important during evisceration because it leads to decontamination of the carcass. The fifth preventive measure is cooling the meat to +4°C of medium effect, because *Campylobacter* spp. are mesophilic bacteria (Olsen et al. 2024). On farms, aldehydes and cresols are most commonly used in the sanitation process (Table 3) (Butucel et al. 2022). The second line of defense in the fight against human campylobacteriosis is represented by hygienic and sanitary measures applied during transport, processing of broiler chicken carcasses and during packaging (Table 4) (Perez-Arnedo & Gonzalez-Fandos 2019; Olsen et al. 2024).

Table 3. Disinfectants used on chicken farms (Butucel et al. 2022)

| Disinfectant  | Characteristics of disinfectants   |
|---|--|
| Aldehydes, a well-known representative of formaldehyde (HCHO)                         | Positive and negative properties<br>Bactericide, fungicide, virucidal. Disinfection of floors, walls and equipment is achieved at a concentration of 1-2%. It leaves no residue, but is toxic, corrosive, has a pungent odor and is inhibited by organic detritus. Neutralization of too pungent odor is carried out with 25% ammonia.<br>Formaldehyde is the only one in a gaseous state, and in combination with potassium permanganate it is used for fumigation of eggs. |
| Monohydroxyl phenols – Cresols, representative of carboric acid (C6H5OH)              | Bactericides, fungicides, virucides and sporocides. Used for disinfection of floors, walls and equipment. Leaves a residue. Mildly toxic and inexpensive, but corrosive and moderately inactivated by organic detritus. A solution of cresol in potassium soap is often used for disinfection.   |
| Carboxylic acids, the main representative is acetic acid (CH3COOH)                    | Bactericides, fungicides, virucides, but not sporicides. Insignificant residual activities. They are not inhibited by organic detritus. They have a good synergism of action in combination with detergents.<br>Disadvantages: toxicity, corrosion, high cost and strong smell.  |
| Halogen elements, the main representative is chlorine (Cl), chlorine lime (CaCl(OCl)) | It has an antimicrobial effect on almost all microorganisms, but it is the best bactericide. Chlorine is a cheap disinfectant, leaves no residue, but is toxic, corrosive and inhibited by organic detritus. In its gaseous state, it is used for water disinfection, and in the form of chlorine lime/lime, calcium hypochlorite is used for sanitation.  |
| Halogen elements, represented by iodine (I)   | Bactericide, fungicide and virucide. It leaves no residue. Disadvantages are toxicity, medium corrosivity and inhibition in organic detritus.  |

HCHO = molecular formula for formaldehyde

C6H5OH = molecular formula for carboric acid

CH3COOH = structural formula for acetic acid

Cl = symbol for the chemical element chlorine

CaCl(OCl) = structural formula for chlorine lime, calcium chloride hypochlorite/caporite

I = symbol for the chemical element iodine

Table 4. Hygienic and sanitary measures applied during slaughter and carcass processing (Perez-Arnedo & Gonzalez-Fandos 2019; Olsen et al. 2024)

| Slaughter and primary processing stages | Hygienic and sanitary measures during slaughter and carcass processing   |  |
|---|--|--|
|   | Dirty phases   | Pure phases  |
| <b>I phase</b>                          | The slaughter<br>"Eat the meat of animals whose blood has been shed and over which the name of Allah has been mentioned" (Bukhari and Muslim) (Halal slaughter). | Sanitary processing of the cages, healthy flocks are slaughtered, the reception/slaughter house is separated, the slaughtering is done immediately. Bleeding 1-3 min without treatment.  |
| <b>I phase</b>                          | Skinning<br>Plucking<br>Hygiene washing of carcasses with removal of head and legs   | The pools must be hygienically clean, dried and the water used must be potable. The pools for skinning and plucking represent an important risk factor for cross-contamination of carcass skin with <i>Campylobacter</i> spp. and it is very important that the pools and the water are prepared with strict biosecurity measures recommended by the veterinary service. Before processing the carcasses, they are washed under a stream of clean potable water. |
| <b>II phase</b>                         | Evisceration   | Evisceration is a dirty stage with a high risk of cross-contamination. It is important to prevent gastric and intestinal effusion. When removing organs, sterile knives are used, and all working surfaces must be treated sanitarly, as well as the workers' hands.   |
| <b>III phase</b>                        | Draining<br>Cooling<br>Confectionery   | Correct carcasses go for cooling, and defective ones are discarded. Minor damage can be cut/trimmed. Final wash. Strain and cool for 1 hour at 4°C, without tying. Packaging (name, address of the manufacturer and veterinary control number).  |

## Sanitation and Risk Analysis

Sanitation in broiler chicken production should be performed only by trained workers. Sanitation, which is commonly used in European countries, reduces the bacterial load by 95% and involves mechanical washing with detergent, followed by the use of disinfectants (Table 5) (Quinn et al. 2011). Hot air drying reduces the number of bacteria (Butucel et al. 2022). Another method of sanitation involves mechanical cleaning, without the use of disinfectants (Burbarelli et al. 2015). Sanitation removes microorganisms, which contributes to the control of the environment in which broiler chicken meat is produced. Sanitation is a legally prescribed hygiene and health measure in chicken meat production that improves the safety of the final product (Olsen et al. 2024).

Mechanical cleaning is an important phase of sanitation, especially in broiler farming (farms). The purpose of mechanical cleaning is to remove organic matter that inactivates disinfectants. In the dry phase, the equipment is first dismantled and moved. Then, the equipment and parts of the facility are blown out with air using a compressor. Coarse, polypropylene brushes and brooms are

used for mechanical brushing of floors, walls and equipment (Luyckx et al. 2015). In the wet phase, detergents that break down organic and mineral deposits are used. Basic and acidic detergents are used. By breaking down organic matter, the full antimicrobial potential of the disinfectant is used. After washing and drying, disinfectants are used. An atomizer (sprayer) is used to disinfect the facility. In this way, the facility is prepared for the reception of one-day-old chicks (Gichure et al. 2022).

The implementation of hygiene and sanitation measures must be constantly monitored and controlled (Olsen et al. 2024). After proper sanitation, swabs are taken to show the effectiveness. In case of positive swabs, disinfection should be repeated until the standard is achieved (Luyckx et al. 2015). In EU countries, producers are required to submit results on the number of *Campylobacter* spp. in chicken meat to the government, which improves meat safety control. Chicken meat contaminated with *Campylobacter* spp. above the limit value is more often subject to controls on the implementation of the HACCP system, until the analysis results are satisfactory (Olsen et al. 2024).

Table 5. Disinfectants and mechanisms of action (Quinn et al. 2011)

| Disinfectant   | Different mechanisms of action of disinfectants  |
|--|--|
| <b>Alcohols and aldehydes</b>  | Mechanisms of action<br>They coagulate bacterial proteins                                    |
| <b>Monohydroxyl phenols – Cresols, carbolic acid (C<sub>6</sub>H<sub>5</sub>OH), Halogen elements: fluorine (F), chlorine (Cl), bromine (Br), iodine (I), astat (At) Peroxide halogen elements: hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and potassium permanganate (KMnO<sub>4</sub>), Carboxylic acids: acetic (CH<sub>3</sub>COOH), lactic (CH<sub>3</sub>CH<sub>2</sub>COOH), etc.</b> | They damage the bacterial cell through the oxidation process                                 |
| <b>Cationic (quaternary ammonium compounds), anionic, nonionic and amphoteric surfactants Detergents (acids and bases)</b>   | They damage the bacterial cell by surface activity. They hydrolyze internal cell structures. |

## Monitoring Genotypic Antibiotic Resistance

The presence of antibiotic resistance determinants in *Campylobacter* spp. isolates from chicken meat, such as antibiotic resistance genes, various mutations, pose a challenge for food safety. The importance of monitoring *Campylobacter* spp. isolates from chicken meat, changes within the genome, the presence of new antibiotic resistance genes is important to prevent the spread of antibiotic resistance. The presence of antibiotic resistance genes in *Campylobacter* spp. indicates uncontrolled use of antibiotics, which, in the case of genotypic resistance, should be withdrawn from use (Habib et al. 2023).

In a study on the association between genotypic and phenotypic antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolates from meat and cecum of various animals in the United States, Zhao et al. (2015) reported whole genome sequencing data from 2000 to 2013, where 114 isolates (82 *C. coli* and 32 *C. jejuni*) were characterized for the detection of antibiotic resistance genes and mutations. In resistant isolates of *C. jejuni* and *C. coli*, 18 acquired antibiotic resistance genes were detected, *tet*(O), *bla*OXA-61, *cat*A, *lnu*(C), *aph*(2'')-Ib, *aph*(2'')-Ic, *aph*(2'')-If, *aph*(2'')-Ig, *aph*(2'')-Ih, *aac*(6')-Ie-*aph*(2'')-Ia, *aac*(6')-Ie-*aph*(2'')-If, *aac*(6')-Im, *aad*E, *sat*4, *ant*(6'), *aad*9, *aph*(3')-Ic i *aph*(3')-IIIa. Mutations in the regions of the native *gyrA* gene and in the 23S rRNA were recorded. Genotypes were tested by whole genome sequencing, and phenotypes were determined by microdilution. A high degree of agreement was found between the results of genotypic and phenotypic methods used to determine antibiotic resistance (99.2%), for fluoroquinolones and tetracyclines (100%), macrolides: erythromycin (95.4%), azithromycin (98.7%), aminoglycosides: aminoglycosides: amicosides.78%. clindamycin: (98.7%), ketolides: telithromycin (98.7%). The results of the study suggest that whole genome sequencing is a reliable method for monitoring antibiotic resistance.

In a 16-year retrospective study examining the genotypes and phenotypes of antibiotic resistance

in *Campylobacter coli* isolates from different sources in Brazil, Gomes et al. (2023) reported that the antibiotic resistance genes *bla*OXA-605 / *bla*OXA-61/ (54%), *tet*(O) (22.2%), *cme*B (9.5%), *aad*E-Cc (6.3%), *aph* (3') – IIIa (1.6%), *sat*4 (1.6%) i *aad*9 (1.6%) were found to be co-resistant. Mutations in the *gyrA* gene regions (T86I in QRDR) were detected in 8 (12.7%) *C. coli* strains. Genotypes were tested using whole genome sequencing (WGS), and phenotypes were examined using disk diffusion. Comparison of genotypic and phenotypic methods revealed concordance for fluoroquinolones (100%), tetracyclines (92.9%),  $\beta$ -lactams (82.4%), and aminoglycosides (80%).

## Monitoring the Impact of Sanitation on the Reduction of *Campylobacter*

The purpose of this review is to improve the understanding of the impact of implementing appropriate sanitation measures on reducing *Campylobacter* species contamination in primary chicken production and processing, in facilities with implemented HACCP and Halal control systems. Also, this research can improve the understanding of the global public health problem, which is antibiotic resistance of *Campylobacter* spp. isolates from chicken meat. By implementing rigorous sanitation in primary chicken production of broilers, it is possible to stop the use of antibiotics in primary chicken production (farms) in countries where this is still the practice.

In accordance with the EU Regulation, which prescribes hygiene criteria in the primary production and processing of chicken meat, antibiotics are not used on chicken farms, but other biosecurity measures (vaccines, carvacrol in chicken feed, monoclonal antibodies) and rigorous hygiene and sanitary measures. In order to check the hygiene of chicken meat production and processing, a critical limit of 1000 CFU/g (EU 2017/1495) has been set for contamination of chicken neck skin in slaughterhouses. Laboratory tests for the presence of *Campylobacter* spp. in chicken meat, in slaughterhouses with HACCP and Halal standards must be performed once a week. In case

the 52 analyses are satisfactory, laboratory tests are performed every other week. Only drinking water may be used for the purpose of decontamination of chicken carcasses (EU 2017/1495). Based on a report by the European Food Safety Authority, by setting a critical limit of 1000 CFU/g for contamination of broiler neck skin in slaughterhouses, the incidence of campylobacteriosis can be reduced by 40% to 90% (EFSA 2011).

In order to produce healthy chicken meat in slaughterhouses, it is very important that strict hygiene and sanitation measures are implemented on chicken farms. These are prophylactic measures to prevent the spread of diseases in chickens, while at the same time omitting the prophylactic use of antibiotics in chickens, which contributes to the spread of antibiotic resistance of bacteria (Umaraw et al. 2017). Appropriate sanitary conditions on chicken farms improve the intestinal microbiota of chickens. When a large number of pathogens are present in the intestines of chickens, 20% of energy is consumed. If there is a healthy microbiota of chickens, feed conversion is improved and chickens gain weight (biostimulation) even when antibiotics are not used (de Castro Burbarelli et al. 2017). Cessation of the use of antibiotics in chickens for the purpose of protection against campylobacteriosis, other bacterial diseases, and for the purpose of biostimulation (antibiotics as growth promoters) prevents the spread of antibiotic resistance, a global public health problem (Nastasijević et al. 2020). Based on monitoring the spread of antimicrobial resistance in *Campylobacter* spp., there are still no such reports of antimicrobial resistance to currently available disinfectants, but the possibility of the emergence of disinfectant resistance can never be ruled out. For this purpose, it is recommended not to use the same disinfectant for a long period of time (Davies & Wales 2019).

In Bosnia and Herzegovina and the region, such research has not been conducted, and we do not have data on the impact of sanitary measures on chicken growth, health and ensuring the health safety of chicken meat in slaughterhouses. It has

not yet been investigated whether chicken producers have stopped using antibiotics in primary production (farms) in Bosnia and Herzegovina and turned to other alternative solutions to antibiotics in order to ensure the biostimulation of chickens and the health safety of meat. Although it seems that sanitation is something that is already known and has been used for a long time in the chicken meat production process, reporting on the prevalence of *Campylobacter* spp. in chicken meat tells us that some European countries have seriously approached solving this public health problem, while some have not yet achieved satisfactory results. Therefore, it is important to apply rigorous hygiene and sanitary measures in the process of primary production and processing of chicken meat, in order to achieve satisfactory results.

The best system for controlling *Campylobacter* spp. in chicken meat, according to EU criteria, developed by the Nordic countries, where campylobacteriosis has been one of the biggest public health problems for many years. Today, the Nordic and Baltic countries can boast a significantly lower prevalence of *Campylobacter* spp. in chicken meat, because they have developed the best control system (EU 2017/1495). According to the latest reports, the prevalence of *Campylobacter* spp. in chicken meat in Estonia were very low, the results are excellent (1.8%), followed by Iceland, which also recorded a low prevalence (2.1%) (Lopes et al. 2018; Mäesaar et al. 2014). On the other hand, some European countries that also act according to the latest Regulation (EU) 2017/1495, do not have satisfactory results in the prevalence of *Campylobacter* spp. in chicken meat. High prevalences were recorded in France (76%), Spain (70%), Turkey (56.1%), Poland (50%), Italy (34.1%) and Belgium (29%) (Ozbey & Tasdemi 2014; Tedersoo et al. 2022; Wiczorek et al. 2012). Such results indicate inadequate biosecurity measures, poor hygiene in the chicken meat production process, poor control of insects, rodents that serve as vectors on farms and

slaughterhouses (Horvat et al. 2022; Olsen et al. 2024).

It is most important that good hygiene and sanitation standards are applied on farms, but that these measures continue to be applied during transport, slaughter, primary processing of broiler carcasses and packaging. Proper sanitation of broiler chicken farms will ensure a healthy microbiota of chickens, prevent colonization of *Campylobacter* spp. 42 days of fattening. If a healthy chicken flock is produced, healthy chicken meat will also be ensured, if hygiene and sanitation measures are continued along the entire chicken meat production chain. However, if a sick chicken flock is produced, highly contaminated with *Campylobacter* spp. and the most rigorous chicken meat control measures in slaughterhouses will rarely have satisfactory results in terms of the prevalence of *Campylobacter* spp. in chicken meat (Olsen et al. 2024).

If proper sanitation is not provided in the facilities where chickens are kept (sanitation of floors, walls, equipment, drinkers and feeders), the proper application of Good Manufacturing Practices and Good Hygiene Practices, the HACCP concept in slaughterhouses, will not have much significance in ensuring the sanitary quality of chicken meat. The application of the HACCP concept in slaughterhouses prevents cross-contamination and is very important for ensuring the microbiological safety of chicken meat if healthy flocks are slaughtered (Castañeda-Gulla et al. 2020). Protection against cross-contamination of chicken meat is an important segment in ensuring the sanitary quality of meat (Borges et al. 2020).

Meunier et al. (2016) state in their research that primary broiler production is key to ensuring the sanitary quality of meat and that it is necessary to ensure good sanitation of facilities where chickens are kept, because positive flocks affect increased contamination during slaughter and carcass processing. They also state that the application of nutrients, organic, inorganic acids, probiotics, bacteriocins, bacteriophages, essential

oils and immunization of chickens contributes to the biosecurity of meat.

Burbarelli et al. (2015) analyzed two models of farm cleaning that would affect the microbiological status of the environment and the increase in growth in broilers. In the first model, they used mechanical cleaning, dry and wet, and in the second model, they used high-pressure water washing with detergents and, after rinsing, they used two disinfectants from the aldehyde and cresol group: glutaraldehyde 250g/L, formaldehyde 185g/L and p-chloro-m-cresol 210 g/L, which represents the European cleaning model that has proven to be more effective in reducing pathogens.

Luyckx et al. (2015) in their study of the effectiveness of four cleaning models implemented in broiler houses with the aim of preventing zoonoses, discovered critical points of disinfection: drinkers, cracks in the floors and holes for water drainage. Such studies indicate that before sanitation, all holes in chicken houses that are difficult to access for sanitation and allow the development of microorganisms should be sanitized. Drinkers, due to the constant presence of water, often due to the presence of biofilm, can be a source of pathogenic microorganisms and it is important to remove organic matter, biofilms, by pressure washing, which will increase the effectiveness of disinfectants.

Hue et al. (2010) investigated the risk factors that lead to increased contamination of broiler carcasses with *Campylobacter* species during slaughter and found that the most critical stage in the slaughterhouse is evisceration. As a solution to the problem, it is proposed to rinse broiler carcasses after evisceration and perform slaughter according to the *Campylobacter* contamination status of the broiler chicken flock. Positive flocks determine carcass contamination. Cross-contamination occurs during transport, slaughter and primary processing of broiler carcasses (Perez-Arnedo & Gonzalez-Fandos 2019).

## Conclusions

Rigorous sanitary procedures applied in primary production and processing of chicken meat, based on control systems best developed in the Nordic and Baltic countries, and based on compliance with EU legislation, have been proven to contribute to protection against campylobacteriosis and prevent the spread of antibiotic resistance. Testing the correlation of genotypic and phenotypic acquired antibiotic resistance in *Campylobacter* spp. isolates from raw chicken meat may be an appropriate control measure, in case of suspicion of misuse of antibiotics in chicken farming (farms) as growth promoters. Based on previous research, these methods have proven effective in most EU countries. By setting the recommended EU critical limit of 1000 CFU/g for contamination of the neck skin of broilers in slaughterhouses, the incidence of campylobacteriosis can be reduced by 40% to 90%.

## Acknowledgment

This study was made possible thanks to a grant from the Ministry of Science, Higher Education and Youth of the Sarajevo Canton in 2022 and a grant for doctoral students from the Federal Ministry of Education and Science in 2021.

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## Stroge sanitarne mjere za smanjenje kampilobaktera u proizvodnji pilića kao alternativa profilaktičkoj upotrebi antibiotika

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Pregledni rad

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### Sažetak

Kampilobakterioza je vodeći uzrok trovanja hranom u Europi, SAD-u, Australiji i Novom Zelandu, a otkrivanje rezervoara infekcije važno je u prevenciji bolesti. Iako se kampilobakterioza smatra rijetkom u Africi, istraživanja ukazuju na kontaminaciju pilećeg mesa i u afričkim zemljama. Praćenje prevalencije *Campylobacter jejuni* i *Campylobacter coli* u proizvodnji mesa brojlera ukazuje na varijabilnost u zavisnosti od zemlje, sezone, porijekla uzorka i higijene u primarnoj proizvodnji (farme) i preradi mesa brojlera (klaonice/proizvodni pogoni). Stoga je svrha ovog preglednog rada bila poboljšati razumijevanje utjecaja koji primjena odgovarajućih sanitarnih mjera ima na smanjenje kontaminacije *Campylobacter* vrsta u primarnoj proizvodnji i preradi pilećeg mesa, u objektima sa implementiranim HACCP i Halal kontrolnim sistemima. Usklađivanjem sa zakonodavstvom EU uređuju se higijenski kriterijumi u primarnoj proizvodnji i preradi pilećeg mesa. Primjenom rigoroznih higijenskih mjera u svim fazama proizvodnje pilećeg mesa može se smanjiti broj termofilnih vrsta *Campylobacter* na trupovima brojlerskih pilića. Postavljanjem preporučene kritične granice EU od 1000 CFU/g za kontaminaciju kože vrata brojlera u klaonicama, incidencija kampilobakterioze može se smanjiti za 40% do 90%.

**Ključne riječi:** Antibiotici, *Campylobacter*, primarna proizvodnja i prerada pilećeg mesa, sanitarne mjere

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## JOURNAL OF HALAL QUALITY AND CERTIFICATION

### Halal In The Context Of Sustainability

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#### ABSTRACT

In the context of sustainable technologies and lifestyle, “halal” refers to practices, products and technologies that are consistent with Islamic principles and ensure that they are not only ethical but also environmentally and socially responsible. Given that the concept of halal encompasses more than food and drink, it expands to several aspects of life, including finance, clothing and indeed technologies. However the traditional halal practices do not directly address the environmental issues such as, greenhouse gas emissions, water adequate risks and natural impacts there is a growing movement to incorporate sustainable practices into halal food production. The halal approach which contains sustainable technologies that prioritize reducing environmental damage, with renewable energy and environmentally-friendly materials, sustainable farming conserving water and organic farming, protecting cultural heritage, animal welfare and social justice covering fair labor practices welfare workers, equal opportunity, fair wages, health and safety of the employees, ethical communication, are highly compatible with Islamic values. Considering the significant growth of the halal industry, this review aims to provide an overview the long-term impacts of sustainability on society, ecosystem, cultural heritage and the sustainable halal food industry.

**Keywords:** *halal, sustainability, environment, social*

#### Introduction

In an increasingly interconnected world, the industry faces numerous challenges, including the need for sustainable practices and ethical standards. As consumers become more conscious of the environmental and social implications of their choices, the demand for products that adhere to both Halal standards and sustainability efforts have grown (İdris et al. 2025).

On the other hand, sustainability in the food industry is concerned with practices that ensure food production meets present needs without

compromising the ability of future generations to meet their own needs.

#### Understanding Sustainability

The most influential definition of sustainability is that of the World Commission on Environment and Development (WCED 1987). According to WCED, " Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs". Although this definition is simple in construction, it is multifaceted in scope.

A crucial Aspect of sustainability is recognizing that there is a limit to the natural resources of the Earth. It has been a matter of some concern that certain quality variables like atmospheric concentration of CO<sub>2</sub>, GHG emissions, temperature changes, biodiversity loss, human exploitation of flora, fauna, per capital energy use, water scarcity and deforestation rates were more deteriorated than in before.

Since the Rio Summit in 1992, governments, multinational companies, and consumers have been interested in sustainability. As of 2015, world leaders adopted the 2030 Agenda for Sustainable Development and the 17 Sustainable Development Goals to reduce poverty and inequality within and among countries, and to tackle climate change and environmental degradation (UN) (2015).

### **The Intersection of Halal and Sustainability**

Sustainability is a multidimensional attribute where economic, environmental, and social components cannot be separated but work together to ensure long-term growth and survival. Sustainability can be also defined as halal food, places, or actions that are kept on the fundamental aspects required by halal principles and the supporting systems or mechanisms that guarantee the preservation of the halal authenticity (Nizam et al., 2019) . In short, sustainability in halal indicates that the halal must be preserved and passed on from generation to generation in line with progress in technology and society.

The intersection of Halal standards and sustainability presents a unique opportunity as food as nonfood producers to meet the expectations of consumers while also addressing global challenges such as climate change and resource protection.

### **Animal Welfare in Halal Practices**

Halal food systems have the potential to contribute meaningfully to a more sustainable global paradigm. The most commonly understood connection between the halal concept and sustainable practices is welfare through avoiding cruelty, especially in the case of animals. Such consideration can extend well beyond animal welfare.

Halal certification lays down a strict set of requirements for any farm seeking halal certification. As with all certifications, this acts as a simple checklist for guidelines that enhance sustainability in these hydrocarbon-heating, fertilizer-dependent, pesticide-dependent, monocropping, soil-depleting, biodiversity-irrigation land practices. For example, a halal-certified farm is not allowed to inject fetuses of cows or sheep with hormones to fatten their bodies up. The stress of cramped quarters in feeding lots is prohibited. Halal farms are also encouraged to follow the spirit of organic agriculture and are discouraged from using chemicals, altering of genes or developing synthetic substitutes for products that are otherwise provided by ecosystems.

Halal slaughter principle is not causing suffering to animals is based on ethical considerations, in addition to the legal requirement. The authorities and organizations on animal welfare recognize halal practitioners' goals of reducing the suffering of animals in general and suggest that the controversies on halal slaughter practices be resolved through evidence-based science (Farouk et al, 2016)

### **Environmental Impact of Halal Practices**

Halal practices has significant positive environmental impacts. Halal products are often produced more environmentally friendly than conventional methods since the processes often focus on optimizing resource use, which reduces overall carbon footprints. Halal-certified products are less likely to contain synthetic chemicals, including pesticides, preservatives, and artificial colors, making them a more sustainable choice for consumers. Eco-friendly packaging materials that are used by halal producers, such as biodegradable or recyclable packaging also reduce the environmental impact.

### **Sustainable Agriculture Practices**

The characteristics of halal agri-food practices associated with sustainability considerations have more or less similar conclusions. Halal agriculture can have long-term benefits, for example, eliminating the often dangerous, complete erasing of nutrient content caused by synthetic fertilizers, or organic alternatives for weed and pest control. However, considerations

such as have little meaning in a world of rising food production requirements. It is time increasing number of halal-certified producers explore emerging techniques to reduce their carbon footprint or for their consumption, product origin transparency, maybe even alternative protein production pathways to claim their unique product characteristics should they wish to enter local organic markets.

Sustainable halal agriculture has the following characteristics: conservation of natural resources, conservation of energy, minimal use of chemical inputs, care of animal welfare, food diversity, economic feasibility, and support for rural development. Moreover, sustainable agriculture follows the principle of fair, which refers to prohibition on depriving people of others' rights. While halal certification covers requirements such as Islamic slaughter and animal feeding, supporting other characteristics of sustainable agriculture is voluntary in halal standards.

### **Waste Reduction Strategies**

Halal food practices pay considerable attention to waste management and disposal, including the correct methods for animal slaughter and consumption, along with by-product utilization for food and feed. Halal slaughter rituals call for specific waste treatment methods, and their implementation is believed to ensure food safety and prevent negative external influences to guarantee the physical, mental, and spiritual soundness of consumers, thereby rendering the entire food system sanitary and halal. "Waste Management" is also a problem in the production stage which consists not only of the product of the industry, but also of the raw materials, and in the use stage which results in spent/used products

Food waste in halal slaughterhouses comprise valuable by-products for the production of feed supplements and high-nutrient and organic fertilizer for plant growth. Halal slaughtering items such as the skin, feet, hairs, blood, lymphatic fluids, horns, claws, eyeballs, internal organs, and bones can be utilized to manufacture leather, beauty products, nutritious fertilizers, vitamin feed additives.

### **Food Waste**

Food waste constitutes an important issue of public concern. Wasted food is either discarded or inedible, and food that is unfit for human consumption still often has an environmental footprint ( Usubiaga et al., 2017) . Reducing the waste of resources linked to food should be one priority in the context of sustainability.

Focusing on consumer food waste, four environmental categories in EU28: greenhouse gas emissions, land use, blue water consumption, and material use. These environmental categories link food waste reductions to the scarce natural resources with high social costs, since at the input stage of the food chain.

### **Social Dimensions of Halal Sustainability**

Halal food production and preparation has the potential to become a global market that goes well beyond fulfilling Muslim Dietary requirements (Adams, 2011). " Halal " can also be made into a customizable niche through analysis of consumer demand and knowledge of the global supply chain.

As Muslim consumers become more experienced, both their requirements and those of the producers increase. This is perhaps most clearly demonstrated with the efforts of halal companies producing not just "good" halal meat, but "tayyib" halal food that promotes the buying of meats from inspected companies, feeding animals a wholesome diet, and humane killing, and transporting the animals long enough distances to lessen their cruelty (Claire Hawthorne , 2014 ) . As these positive and ethical disambiguations become more prevalent, Halal companies must increasingly become cognizant of them to retain and grow their consumer base (Ummesalma Husein et al., 2017)

### **Community Engagement**

Sustainable development recognizes the need to engage multiple stakeholders to better understand expectations. In addition to regulators and companies, communities are increasingly regarded as important stakeholders in the discourse on sustainable development. Community engagement refers to a process of interaction and collaboration between organizations and a community. Community engagement is important because communities are actively involved in defining their own

development priorities and monitoring the commitment and impact of development priorities.

If well-planned, community engagement can be an effective means of learning about a community's history, culture, issues, interests, priorities, and perspectives, as well as of building trust and rapport, thereby increasing ownership and accountability for issues of concern.

Community engagement can also provide valuable knowledge to organizations, serving to better inform decision-making and programming. In addition, the achievement of development goals, however defined, largely depends on the participation and support of stakeholders (Ummesalma Husein et al., 2017). In particular, social engagement is increasingly viewed as an important means of responding to sustainability challenges.

### **Ethical Labor Practices**

Halal is based on principles of justice and balance. These principles direct actors within the halal business model towards an attitude in which no party may benefit from or at the expense of another party. Social legitimacy is the basis for all activities in halal, protecting the actor against harm and ensuring equity throughout all halal business operations which has both business opportunities and sustainability built into its core.

Halal in the context of sustainability, the literature nevertheless highlights the realistic issues of ethical labor practices relevant to halal food production, processing and selling. As with many industrial food systems, the halal food supply chain is steeped in human rights violations outlined by the United Nations Human Rights Council's Guiding Principles on Business and Human Rights (Claire Hawthorne, 2014) .

Moreover, many of the literature's overall reports of Muslim consumer humanity around the world, particularly the Middle East and Southeast Asia, suggest that better ethical labor standards in halal food production and processing (preferably independent of certification) are a rapidly growing demand and market opportunity (Ummesalma Husein et al., 2017) . Halal standards provide a holistic view of the ethical dimensions of sustainable consumption. This

approach demonstrates how Halal practices can promote not only religious compliance but also broader social and economic justice.

Decent work is defined by the International Labor Organization (ILO) as productive work that delivers a fair income, security in the workplace, and social protection for families (ILO, 2012)

Halal standards emphasize ethical practices that extend beyond food production to include labor practices. Companies that adhere to Halal regulations are often bound to uphold labor rights, ensuring that workers are treated fairly and provided with safe working conditions. The integration of decent work principles into Halal practices can lead to improved labor conditions in the food industry, supporting the overall goals of sustainable development.

Equal opportunity refers to the fair treatment of all individuals, irrespective of their background, gender, or ethnicity, particularly in employment and access to services. Halal standards can promote equal opportunity in the workforce by encouraging diverse hiring practices and ensuring that all workers receive the same rights and benefits.

Fair trade is a social movement aimed at ensuring that producers in developing countries receive fair prices for their goods, promoting sustainable farming practices, and improving social and environmental standards. As consumers become more aware of ethical consumption, there is a growing demand for Halal products that also adhere to Fair trade standards (White et al., 2016). There is a natural synergy between Halal standards and fairtrade principles. Fair Trade attempts to achieve several goals; the primary and best-known is to provide prices that deliver a basic livelihood for producers. Both frameworks prioritize ethical sourcing and seek to improve the livelihoods of producers. Halal-certified products can incorporate fair trade principles by ensuring that farmers and producers are compensated fairly, thus contributing to sustainable consumption. (Dragusanu et al., 2014).

### **Economic Aspects of Sustainable Halal**

The halal economy is a burgeoning and multifaceted global economic sector. Halal food forms only a part of the halal economy, as this

sector is anticipated to expand rapidly to embrace halal cosmetics and pharmaceuticals, hygiene items, clothing, and financial products and services that conform to Islamic law (Zubaidi Baharumshah, 2014). Consistent with this trend, the halal market is not confined to Muslim societies, as both non-Muslims and Muslims have an interest in it, and many recognize the advantages of consuming halal products and services (Salman et al, 2019)

### **Market Demand for Halal Products**

Many companies worldwide are seeking halal certification to gain access to this market, as many believe millions will join the ranks of formal halal issuers by 2030.

First, the observance of halal laws positively affects halal product consumption. This is because the observance of halal laws determines whether a product is classified as halal or haram (unlawful). Ingredients must combine to form food meat products that are lawful and pure. Ethical and religious obligations compel halal observance among ultra-observant Muslims and concern for the welfare of all conscious and sensitive humans (Nizam et al., 2019). Second, factors associated with halal observance, such as peer group pressure, availability, observational learning, and the pressure to belong to in-groups (not social networks), create obligations. Third, distrust among consumers regarding certification laws affects halal observance and halal consumptions independently. Low consumer trust in farmers and suppliers entails low consumption norms. A consumer who believes halal certification trusts the government and authorities will consume halal products because the law imposes consequences on falsifiers (Yusuf et al., 2016).

### **Resource Management**

The Implications of sustainability and Halal are fairly consistent. The principles of sustainability are also consonant with the morals and ethics/accompanying principles of Sharia compliant practices which provide guidance for the management and investments industries, reporting & publishing practices in the construction industry. It is noted that in addition to preventing the company from investing in “haram” revenue, a positive point of Halal

consultations is that the screening often results in identifying industry sectors with positive ethical muster such as the environmental, 'green' sector. It is therefore concluded that it is necessary and prudent for organizations to embrace and invest in sustainability as part of their corporate social responsibilities, and report on the positive values, impacts, and acts of sustainability (Ummesalma Husein et al., 2017).

### **Cultural Preservation, Sustainable and Halal Tourism**

Sustainable tourism is a great alternative, which aims to minimize the negative impact and maximize the positive, thus ensuring the sustainability of the tourism industry (GSTC,2016). In addition, sustainable tourism also sets a real example of sustainable development that benefits the local community. Halal tourism is a sub-type of sustainable tourism which provides a model for practicing sustainable principles within the region (Sye et al, 2024). Halal tourism activities can enhance the quality of preservation of the local culture of the local community.

Battour et al. (2022), highlight how the United Nations and media have shed light on the negative environmental impacts of tourism, particularly in relation to cultural preservation.

### **Challenges in Implementing Halal Sustainability**

Despite the growing awareness of sustainability issues, halal industry stakeholders still face challenges transitioning to sustainable halal practices. Halal and Islamic organizations must focus on building awareness and incorporating concerns into halal assurance systems and sustainability education. (Nizam et al., 2019).

Sustainable halal implementation challenges are exacerbated by the lack of involvement of the halal ecosystem. To effectively leverage sustainability efforts, halal food needs to be a collective effort of regulators, organizations, producers, and buyers. Halal standards need to be upgraded quickly to provide guidance for sustainability. The limited presence of natural sustainable halal practices in the food chain hinders the transition to sustainable halal.

## **International Standards for Halal Sustainability**

The establishment of international standards related to Halal and Sustainability are still limited in number. This standard integrates National Halal Standards and other halal industry standards. It consists of food, personal care, cosmetics, bio-pharmaceutical, agriculture, tourism, logistics, supply chain, and maritime sections (Abas Siti et.al., 2021).

## **Sustainable Supply Chain Management**

Since Halal encompasses all aspects of a person's physical, mental, and spiritual well-being, it is crucial that sufficient confidence exists in both the integrity and impeccability of the method for handling the Halal status of goods offered for trade. Tampering with a Halal product would fatally breach both contract and Islamic Law. Nevertheless, at times "obscure" or "unforeseen" circumstances and logistic disruptions could leave the security of products and cargo vulnerable to "empire of trade". In addition to sabotage, a host of sanitizing delays, exposure to questionable parties, and adjunction to non-savory goods shed doubt on the current halal industry's exposure or capability to preserve the halal integrity and / or awareness ( Zailani et al., 2017). Therefore , halal integrity means to uphold the halal status for foods and cargo; and thoyyib for the manner the foods and cargo being handled along the supply networks by service providers in the halal industry ( Zulfakar et al., 2012)

The demand for Halal products extends beyond food and healthcare to also include cosmetics, personal care, and pharmaceuticals, among others. Countries that have embraced globalization must ensure their products are Halal-compliant in order to remain competitive in international markets. Thus, the emergence of a new Halal transport system for the international trade of Halal products is urgent. (Mohd Saifudin et al., 2018) .

As Halal food is rapidly growing and has become a prominent industry over the last few years, challenges and opportunities related to the Halal food supply chain are also mushrooming. A common Halal certification standard and regulation for logistics and transportation will greatly improve the effectiveness of Halal food

supply chain and logistics operations by eliminating Halal food handling liabilities at all prospects from the origin to the delivery point. The government may also play a more active role in providing Halal niche market incentive programs for both consumers and suppliers and increasing public awareness of Halal food industry through effective information dissemination campaigns. The logistic service providers may also invest more on technology and infrastructure developments to enhance their capabilities of providing Halal food supply chain and logistics services (Mohd Saifudin et al., 2018) .

## **Consumer Perspectives**

Halal market is not just limited to Muslims, but also caters to non-Muslim consumers, who form a vital market niche, looking for healthy and safe food products, produced with ethical and environmentally-friendly considerations. Countries with Halal certification systems have promoted acceptance not just among domestic Muslim consumers, but also by Non-Muslim consumers, who feel that Halal offers them meat and meat products that are healthy and free of any prohibited contaminants, such as drugs in the form of chemical additives, hormones or toxins (Fakir et al, 2023). The growth of the meat industry towards consolidation has led to rising concerns among consumers over cruelties and the sickness of animals housed at industrial farms. Consumers today are increasingly suspicious of the food they consume, questioning how farm animals are raised and processed, how food production and distribution consequences for our health, environment and society. There is also growing awareness among consumers regarding the ecological consequences of locally sourced products, of minimizing miles of purchasing. This consumer behavior is part of the "Marketplace Transformation" phenomenon. Consumers demand ethical, sustainable, and innovative brands, products, and services more than ever. The halal market can be viewed as an illustration of how consumers actively create social worlds based on their values and the information they carry. (Claire Hawthorne, 2014).

The emerging halal food market offers a good example of how marketers are seeking to

understand consumer behavior within a new contextual discussion. As the halal market expands, it is crucial to understand how Muslim consumers behave toward food marketed as halal. Specifically, marketing questions can be asked: What do consumers prefer? What do they value about halal? Consumer studies are open for discussion of the halal market and Muslim consumers' behavior regarding this product category (Rezai et al., 2010).

### Future Trends in Halal and Sustainability

The combination of Halal and sustainability is an essential research field. Future trends and challenges can be identified as literature under the Halal market trend, role of media, and future challenges with stakeholders.

Food is one of the fundamental needs of humans. It is paramount for the sustainability of humanity. The food production methods are no longer sustainable to the earth. Naturally, compliance with the concept of sustainability is now the prime concern of food producers and marketers (Muhammad et al., 2013)

The integration of information technologies may enhance Halal practices to minimize bribery and corruption, voluntary tax collection and tax reporting, reduction of excess consumerism, and rampant illicit activities. Digitalization and the circular economy (CE) are two emerging technological and organizational trends with the potential to improve productivity and sustainability performance (Khan et al., 2021).

### Conclusion

In conclusion this paper sheds light on the role of Islam and the halal ecosystem in leading the sustainability movement towards realizing a sustainable future. Drawing from the findings of this study, halal has been identified as an avenue for sustainable development practices for socio-economic and environmental issues facing the Islamic world. The global halal food is anticipated to expand into a multi trillion-dollar market by 2030. Global businesses and stakeholders in food production, financial services, logistics, travel, hospitality, and events should be innovating their services and products with halal values. Solutions could include business and financial digitalization, industrialization, and financing that are halal

compliant. Halal has the support of more than two billion people which is a seventh of the global population, establishing an ecosystem of trusted partners. In the face of global challenges, collaborative actions are essential to provide the sustainable future.

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## Halal u kontekstu održivosti

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### Sažetak

U kontekstu održivih tehnologija i načina života, "halal" se odnosi na prakse, proizvode i tehnologije koje su u skladu s islamskim principima i koje osiguravaju ne samo etičnost, već i ekološku i društvenu odgovornost. S obzirom na to da koncept halala obuhvata više od hrane i pića, on se širi na različite aspekte života, uključujući finansije, odjeću, pa čak i tehnologije. Međutim, tradicionalne halal prakse ne bave se direktno ekološkim pitanjima kao što su emisije stakleničkih plinova, rizici vezani za vodne resurse i prirodni uticaji, pa sve više raste pokret za uključivanje održivih praksi u halal proizvodnju hrane. Halal pristup, koji uključuje održive tehnologije usmjerene na smanjenje štete po okoliš, obnovljive izvore energije i ekološki prihvatljive materijale, održivu poljoprivredu sa očuvanjem vode i organskom proizvodnjom, zaštitu kulturnog naslijeđa, dobrobit životinja i društvenu pravdu — uključujući poštene radne prakse, dobrobit radnika, jednake mogućnosti, poštene plate, zdravlje i sigurnost zaposlenih te etičku komunikaciju — visoko je kompatibilan s islamskim vrijednostima. Uzimajući u obzir značajan rast halal industrije, ovaj pregled ima za cilj pružiti uvid u dugoročne uticaje održivosti na društvo, ekosistem, kulturno naslijeđe i održivu halal industriju hrane.

***Ključne riječi:*** *halal, održivost, okoliš, društvo*

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