**Journal of Food Science and Technology (Iran)**

**Homepage:**www.fsct.modares.ir

**Scientific Research**

# Evaluation of image analysis software competency in skeletal muscle percentage assessment in meat products

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**ARTICLE INFO**

**Article History:**

Received:2024/4/17

Accepted:2024/7/7

**Keywords:**

Meat Products,

Histology,

Image Analysis,

Graphic Software

DOI: **10.22034/FSCT.21.157.123.**

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**ABSTRACT**

In recent years, there has been a growing interest in methods for assessing the percentage of meat (skeletal muscle) in meat products. Given the high margin of error in methods such as chemical analysis, the most reliable and accurate approach for assessing the percentage of skeletal muscle in meat products is histology and subsequent use of image analysis. Due to limited research in this field and the not-so-easy access to some image analysis software, the present study, for the first time, examines the percentage of skeletal muscle in meat products and the time spent on analyzing each sample using two freely accessible graphic software programs (Adobe Photoshop and ImageJ) and two non-free graphic software programs (Clemex and Image Pro-Plus). For this purpose, 100 samples of meat products (30 Kielbasa, 30 sausages, 20 hamburgers, 10 kebab bite, and 10 chicken nuggets) with a known skeletal muscle content were used. After transferring the samples to the laboratory and preparing tissue sections using the Hematoxylin-Eosin staining method, the images of tissue sections were analyzed using the mentioned software programs. The results showed almost equal accuracy of all four software programs assessing skeletal muscles. However, the time required to analyze each ImageJ sample was significantly lower than the other software programs ($p < 0.05$). Based on the results of this study, it appears that ImageJ software offers greater competence for image analysis of tissue sections and determining the percentage of skeletal muscle in meat products.

## 1- Introduction

The consumption of meat products has significantly increased in recent years. Therefore, the quality and safety of these food products are of utmost importance to consumers. Meat products are defined as products in which at least 30% of their composition is made up of meat. In addition to meat, other ingredients are used in the production of these products. These products are prepared and manufactured according to national regulations and standards in each country [1].

According to Iranian national standard No. 2303, sausages and cold cuts consist of a stable mixture of halal-slaughtered meat, fat, water, and other ingredients such as caseinates, casein, gluten, dried cheese, egg yolks, oils, spices, etc. These products are categorized into seven groups based on their meat content, ranging from 40% meat to 90% meat, as well as cold cuts with added chicken paste [2].

According to Iranian national standard No. 2304, hamburgers are made from ground red meat from halal-slaughtered animals and contain other ingredients such as oil, spices, fillers (e.g., egg yolks, dried milk, wheat flour, breadcrumbs, gluten, salt, and vegetables). They are classified into various categories based on the type and percentage of red meat, ranging from 30% to 95% red meat [3].

Based on standard No. 6938, kebab meat consists of a minimum of 70% ground red meat from halal-slaughtered animals or poultry, along with other ingredients such as egg yolks, dried milk, wheat flour, breadcrumbs, gluten, salt, spices, edible vegetables, and oils. It is categorized as kebab meat with red meat and kebab meat with poultry meat [4].

As per the Iranian national standard No. 9868, chicken nuggets are composed of a minimum of 70% chicken meat, which may contain tenderizing agents such as phosphates, wheat flour, egg powder, isolated soy concentrates, and flavor enhancers. Chicken nuggets are further divided into chicken meat nuggets and mixed meat nuggets based on their composition [5].

Histology, as a diagnostic and segregating tool, has been proven to be an efficient method for detecting and separating various components in meat products. Over the past decade, the incorporation of this discipline into food quality control has significantly reduced the use of unauthorized tissues in some meat product manufacturing companies [1]. However, the absence of an efficient and cost-effective method for determining the composition of ingredients in meat products has created a significant gap in the quality control process [6].

In recent years, there has been significant attention given to methods aimed at assessing the percentage of meat (skeletal muscle) in processed meat products. Due to the high margin of error associated with techniques such as chemical analysis, histology combined with image analysis has emerged as the most reliable and accurate approach for determining the skeletal muscle content in these products.

It should be noted that image analysis is a component of the broader field called image processing, where the main goal is to improve the visual quality of an image or extract useful information and features.

In a study conducted by Francisco and colleagues in 2004 used version 5.4 of the Image-Pro Plus software for the automatic counting of PCNA-stained nuclei in immunohistochemistry [7]. In another report by Fernandes-Santos and colleagues in 2013, factors influencing quantitative histological studies and modern methods used in this approach were discussed. This study highlighted factors such as the thickness of prepared sections, tissue staining capacity, and the type of method used as influential factors in the results [8].

In a study, histological sections of Langerhans islands and adipose tissue stained with Hematoxylin-Eosin were examined. The diameter of Syrian mouse fat tissue cells was measured using Image Pro-Plus software [9]. In other study, the thickness of the middle layer (tunica media) and the external elastic layer of the aorta in transverse sections of Syrian mice was measured [10]. In another research study, images of aortic cross-sections in Sprague-Dawley rats and the percentage of elastic fibers using Image Pro-Plus software were reported [7]. Additionally, the study investigated the level of hepatic fat changes in cross-sections





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stained with Oil-Red O. The accumulation of fat tissue, indicated in the black and white images by appearing white, was expressed as a percentage in each microscopic field [7].

In another study, histology and image analysis were used for the detecting and grading of cancer, alongside other non-immunohistochemical and radiological methods. The results of this research were very promising in providing more modern, faster, and more reliable methods [11]. However, there are very few studies available on the use of image analysis for detecting the percentage of skeletal muscle tissue in meat products. The study reported by Ghisleni and colleagues in 2010 may be the sole research conducted in this particular area. In this report, the quality of a canned food product called tortellini was examined using histology and image analysis. The study focused on identifying the presence of various animal tissues in the prepared food and measuring the percentage of skeletal muscle tissue in it. The study's results, obtained from analyzing four brands of tortellini production units, clearly indicate that histology and image analysis are effective methods for determining the percentage of skeletal muscle tissue and assessing meat product quality [12].

The use of ImageJ software for histological image analysis is quite common. In a study, the automated classification and analysis of retinal microglia cells were examined using ImageJ software [13]. In another study, quantitative analysis of histological tissue staining and fluorescence was investigated using this software [14]. Using this software, changes in spleen tissue resulting from a high-fat and high-sugar diet and its treatment with exercise were studied [15]. In another study, image analysis was used for detecting diabetes by examining tomographic images of light-emitting bodies [16].

Regarding the Clemex software, there have been relatively few studies on using it to analyze structural tissue. While this software is mostly used in materials science and metallurgy, it can be a powerful tool for image analysis, especially for tissue sections. In a study, the effects of the antimicrobial rifamycin on the bone structure of mice were evaluated using Clemex software [17]. Additionally, the effects of PRP on wound size in bone lesions were also examined using this software [18].

Saad and colleagues introduced the use of Adobe Photoshop software in 2008 as an easy and cost-effective method for quantitative analysis of damaged endothelial tissue sections [19]. Furthermore, in a report, the use of Photoshop for quantitative analysis of tissue-stained sections in histochemical, immunocytochemical, and hybridocytochemical staining was highlighted [20]. In another study, this software and its image analysis were used for the quantitative analysis of muscle cell components in a fluorescent-stained culture medium [21].

## 2- Materials and Methods

For the current research, 100 samples of meat products with a specified percentage composition of their constituents were needed. To achieve this, in collaboration with the production unit of Sina Protein Gooshtiran, a total of 30 samples of Kielbasa, 30 samples of sausages, 20 samples of hamburgers, 10 samples of kebabs bite, and 10 samples of chicken nuggets were produced. The exact percentages of components in these products were provided by the manufacturer, with precision to the mg/kg. After production, the samples were transferred to the Faculty of Veterinary Medicine's histology laboratory at Bu-Ali Sina University. The samples were coded upon arrival, and a double-blind study method was applied. According to the national standard method with the code 6103, sample collection was carried out.

For samples such as Kielbasa and sausages, three sections were taken from each roll, and four sections were taken from each cut, irrespective of whether the sample was from the background material or display meat. The selection of sections was done entirely randomly. In cases of samples like hamburgers and kebabs bite, 12 sections were taken from 12 pieces, and in cases of samples like chicken nuggets, 12 sections were randomly selected from 12 pieces for the tissue fixation stage.

A buffered formalin solution of 10% was used for sample fixation. Adequate time for tissue fixation was set at a minimum of 24 to 48 hours





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for products such as Kielbasa and sausages, and a minimum of 4-5 days for products like hamburgers, kebab bits, and chicken nuggets. The fixative solution was changed once every 24 hours if its color changed.

After fixation, the samples entered the tissue processing stage, which included steps of dehydration, clearing with xylene, and infiltration with paraffin. Then, 12 paraffin blocks were prepared from each sample, followed by the tissue sectioning stage. Three 5-7 µm thick tissue sections were obtained from each block [22].

In total, 36 tissue sections were prepared from each sample, resulting in a total of 3600 tissue sections. For this research, it was necessary to view the tissue sections on a display. Therefore, after drying, the sections were scanned using a slide scanner, and images were obtained. These images were analyzed using four graphic software programs, including Image Pro Plus (V.6), ImageJ/FIJI (V. 1.53 c), Clemex (V.8), and Adobe Photoshop (2020).

### 2-1- The procedure with Image Pro Plus (V.6.0)

To analyze images in this software, after opening the image in the software environment, the image contrast was adjusted to "Best fit" mode using the Contrast settings in the menu. After drawing a histogram chart and creating appropriate color contrasts to aid in differentiating various animal and plant components present in each microscopic field, the different tissues were marked using multiple colors, and the changes were confirmed using the annotation tools in the "Examine & Count" toolbar.

In the Hematoxylin and Eosin (H&E) staining method, skeletal muscle structures typically appear as strong red color, plant structures as violet to blue, and the background material as white or pale pink. In the final stage, with the presence of food materials pathologists, a table related to the percentage of different components present in the microscopic field based on the type and degree of staining was drawn.

The results obtained from this analysis were compared with the reports provided by the production unit, and the margin of error in the test was determined.

### 2-2- The procedure with ImageJ/FIJI (V. 1.53c)

Start by loading the image into the ImageJ software. Go to the "Image" menu and under "Type," change the image type to 8-bit. Calibrate the image as needed. To enhance the image quality, navigate to the "Image" menu and select "Adjust," then choose "Brightness and Contrast" to adjust the image clarity. To remove image noise, go to the "Process" menu and use the "Smooth" and "Sharpen" options to further refine image clarity. For thresholding, select "Image" from the menu, then choose "Adjust," and select "Threshold." In the thresholding window, mark the area of the image that you want to measure using a red overlay. Finally, measure the area of the selected region as a percentage of the total image size.

### 2-3- Procedure with Clemex (V.4)

Begin by loading the image into the Clemex software. Adjust the contrast of the image using the "toolbox" and the available options in the contrast window. Navigate to the "Gray/binary transformations" section and use the "gray threshold" option to threshold the regions of interest. Mark the areas of interest with different colors and labels. Finally, measure the area of the selected regions in micrometers or square millimeters using the "Area" option in the "Field measure" section.

### 2-4- Procedure with Adobe Photoshop (2020)

Start by determining the image dimensions recorded with the microscope camera. In Photoshop, you can access the image length and width in pixels by going to the "Window" menu and selecting "Properties". Calculate the image area in pixels by multiplying the length and width. You can also view the dimensions of the entire image under "Window" by selecting "Histogram". To select the desired cross-section, use the "Magic Wand Tool" with the shift key to carefully select the areas of interest. Once you have selected all the areas of interest, go to the "Window" menu and choose "Histogram." In the opened window, you can see the dimensions of the selected areas in pixels. Calculate the cross-sectional area as a percentage of the total image area by dividing





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the dimensions of the selected regions by the dimensions of the entire image. In the end, the results obtained from this analysis were compared with reports from the production unit, and the error coefficient was determined. The results were then statistically analyzed using statistical software for each food item. The main variables among the food items were compared through statistical analysis. The time required for the analysis of each sample using different software was noted, and the average time for sample analysis by different software was compared. The study employed SPSS version 21 and the T-Test statistical method.

## 3- Results and Discussion

### 3-1- Examination of Kielbasa samples using four software applications: Adobe Photoshop, Clemex, ImageJ, and Image-Pro Plus:

The results of examining the Kielbasa samples with all four of the mentioned software applications indicate no statistically significant difference between the factory-declared percentage of meat and the results obtained from image analysis of tissue cross-sections ($p > 0.05$). This is while for plant-based additives, a statistically significant difference was observed between the factory-declared percentage and the results obtained from image analysis ($p < 0.05$). Details of the declared percentages by the factory and the results obtained from image analysis are presented in Tables 1 and 2 (Figure 1).

Numerous reports exist on the use of histological methods for analyzing the contents of meat products, especially in Iran, exist. All these studies mention histology as a powerful and efficient method for identifying and differentiating tissue structures used in the production of meat products [1, 6 and 23].

In a study conducted in 2004, Francisco et al. utilized Image-Pro Plus software (V. 4.5) to count labeled nuclei using the immunohistochemistry method (PCNA) [7]. In another separate study in 2013, Fernandes-Santos et al. investigated factors affecting histomorphometry and image analysis, identifying variables such section thickness, the tissue coloration, and methodology as factors affecting the results [8]. Another investigation involved the analysis of tissue sections from Langhans islands and stained fat tissue, where the diameter of fat tissue cells in Syrian mice was measured using Image-Pro Plus software. Additionally, the thickness of the middle layer (tunica media) and the outer elastic layer in the aortic tissue sections of Syrian mice were assessed in a different report [9]. In another investigation, involved the analysis of tissue sections from Langhans islands and stained fat tissue, where the diameter of fat tissue cells in Syrian mice was measured using Image-Pro Plus software. The amount of hepatic fat change in Oil-Red O stained sections was examined, and the accumulation of fat tissue was expressed as a percentage in each microscopic field [7].

Furthermore, histological and image analysis methods were used for cancer detection and grading alongside biochemical methods and radiological images. The results obtained from this research were very promising in providing more modern, faster, and more reliable methods [11].

All these studies confirm the accuracy and efficiency of Image-Pro Plus software for analyzing tissue sections. Just as in the present study, the accuracy of this software in assessing the percentage of skeletal muscles in meat products was evident, aligning with previous reports.

However, there are very few studies available on using image analysis to determine the percentage of skeletal muscle tissue in meat products. Perhaps the only global study in this regard is the one reported by Ghisleni and colleagues in 2010 [12]. This report examines the quality of a certain canned food product called tortellini use histological examination and image analysis. The study involved investigating and identifying various animal tissues in ready-made food products with minced meat and assessing the percentage of skeletal muscle tissue in these products. According to the results of this report, which was conducted on four brands of tortellini production units and the criteria for investigation included the percentage of skeletal muscle and their quality, the study demonstrated that the use of histology and image analysis could be a suitable solution for determining the percentage of skeletal muscle tissue and subsequently determining the quality of meat products, making it highly practical and beneficial.

**3-2- Examination of sausage samples using four software applications: Adobe Photoshop, Clemex, ImageJ, and Image-Pro Plus:**

The results of examining the frankfurter samples with all four of the mentioned software applications indicate no statistically significant difference between the factory-declared percentage of meat and the results obtained from image analysis of tissue cross-sections ($p > 0.05$). However, for plant-based additives, a statistically significant difference was observed between the factory-declared percentage and the results obtained from image analysis ($p < 0.05$). Details of the declared percentages by the factory and the results obtained from image analysis are presented in Tables 3 and 4 (Figure 1).

In Iran, a single study conducted by Asadi and colleagues in 2023 employed Image-Pro Plus software for the analyzing of tissue sections from meat products. The results of this study demonstrated the high accuracy and capability of this software in image analysis of tissue sections from meat products, which aligns with the findings of the current study. However, there was a significant difference observed between the percentage of plant-based additives measured using this software in the current study and the percentage declared by the factory, which was not consistent with previous studies. This discrepancy might be attributed to the diversity of plant-based additives and their varied and distinct structures [23].

**3-3- Examination of hamburger samples using four software applications: Adobe Photoshop, Clemex, ImageJ, and Image-Pro Plus:**

The results of examining hamburger samples using Adobe Photoshop indicate no statistically significant difference between the factory-declared percentage of meat and the results obtained from image analysis of tissue cross-sections ($p > 0.05$). However, a statistically significant difference was observed between the factory-declared percentage and the results obtained from image analysis for plant-based additives ($p < 0.05$). Details of the declared percentages by the factory and the results obtained from image analysis are presented in Tables 5 and 6 (Figure 1).

The use of ImageJ software in histological image analysis is quite common. In one study, automated classification and analysis of retinal microglia cells were carried out using ImageJ software [13]. Another study focused on quantitative analysis of histological and fluorescence staining using this software [14]. Additionally, changes in the tissue structure of the spleen due to a high-fat and high-carbohydrate diet and its treatment with exercise were examined with ImageJ software [15]. The results of the current study confirmed the high accuracy and capabilities of this software in image analysis of tissue sections from meat products, which aligns with previous studies. However, the accuracy in measuring the percentage of plant-based additives showed a significant difference between the results of the current study and the percentage declared by the factory, which was inconsistent with previous studies. This discrepancy is likely due to the diversity of plant-based additives and their varying structures.

**3-4- Examining kebab bite samples using four Adobe Photoshop, Clemex, Image J, and Image-Pro Plus software:**

The results of the examination of kebab samples using Adobe Photoshop software showed no significant difference between the percentage of meat declared by the factory and the result of the image analysis of tissue sections ($p > 0.05$). Meanwhile, in the case of plant additives, a significant difference was observed between the percentage declared by the factory and the result of image analysis ($p < 0.05$). The details of the percentages announced by the factory and the result of the image analysis are presented in Tables 7 and 8 (Figure 1).

Regarding the use of Clemex software in the analysis of tissue structures, there are relatively few studies available. This software is mainly employed in materials science and metallurgy but is undoubtedly a powerful tool for image





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analysis, particularly in histological analysis. A study evaluated the effects of the disinfectant rifamycin on bone tissue structure in mice using Clemex software [17]. Furthermore, the influence of PRP on the volume of bone lesions was also examined using this software [18]. The accuracy in assessing the percentage of skeletal muscle in meat products confirmed in the current study was in line with previous research. However, this was not the case concerning plant-based additives.

**3-5- Examination of chicken nugget samples using four software applications: Adobe Photoshop, Clemex, ImageJ, and Image-Pro Plus:**

The results of examining chicken nugget samples using Adobe Photoshop indicate no statistically significant difference between the factory-declared percentage of meat and the results obtained from image analysis of tissue cross-sections ($p > 0.05$). However, a statistically significant difference was observed between the factory-declared percentage and the results obtained from image analysis for plant-based additives ($p < 0.05$). Details of the declared percentages by the factory and the results obtained from image analysis are presented in Tables 9 and 10 (Figure 1).

In 2008, Saad and colleagues introduced the use of Adobe Photoshop as a simple and cost-effective method for quantitative analysis of damaged endothelial tissue sections [19]. Another report mentioned the application of Photoshop for histological tissue section analysis to determine the colorimetry of histostaining, immunocytochemistry, and hybridocytochemistry [20]. In another study, Photoshop was used to quantitatively analyze muscle cell components in fluorescent-stained cell culture media [21]. The high accuracy and capabilities of this software for assessing the percentage of skeletal muscles observed in the current study were in agreement with previous research. However, this did not hold for plant-based additives. In conclusion, this study underscores the utility of Image-Pro Plus, Adobe Photoshop, Clemex, and Image J software for accurately analyzing the percentage of skeletal muscle in meat products, without significant differences in accuracy between the programs. However, Image J stood out as the software requiring less time for image analysis compared to others, which had statistically significant differences in this aspect. The study emphasizes that image analysis and histological methods can be effective tools for assessing the quality and composition of meat products, and the use of these methods can be valuable in the food industry.



[ DOI: 10.22034/FSCT.21.157.123 ]

**Table 1:** Percentage of skeletal muscle in Kielbasa samples calculated with Image-Pro Plus, Image J, Clemex and Adobe Photoshop software.

| Product Type | Product code | Factory-declared skeletal muscle (%) | Image-Pro Plus (%) | Clemex (%) | Image J (%) | Adobe Photoshop (%) |
|---|---|---|---|---|---|---|
| Kielbasa | 1 | 41.32 | 41.66 | 40.35 | 42.97 | 40.21 |
| | 2 | 27.91 | 26.95 | 26.55 | 26.07 | 26.54 |
| | 3 | 81.62 | 82.26 | 81.12 | 80.17 | 80.63 |
| | 4 | 54.01 | 55.98 | 55.60 | 55.46 | 55.06 |
| | 5 | 58.49 | 58.78 | 56.27 | 57.89 | 58.24 |
| | 6 | 59.66 | 58.07 | 58.41 | 60.01 | 59.88 |
| | 7 | 34.67 | 34.68 | 35.98 | 35.55 | 33.46 |
| | 8 | 68.61 | 69.61 | 38.92 | 37.43 | 69.28 |
| | 9 | 77.52 | 77.84 | 76.14 | 75.08 | 78.18 |
| | 10 | 46.05 | 47.17 | 47.01 | 47.44 | 45.55 |
| | 11 | 42.36 | 41.03 | 41.36 | 45.35 | 41.34 |
| | 12 | 82.84 | 80.60 | 81.12 | 81.02 | 83.17 |
| | 13 | 79.71 | 79.16 | 77.29 | 80.05 | 78.50 |
| | 14 | 52.19 | 51.38 | 53.32 | 51.49 | 51.03 |
| | 15 | 52.21 | 51.84 | 53.12 | 53.10 | 53.36 |
| | 16 | 27.12 | 25.46 | 29.06 | 26.77 | 26.41 |
| | 17 | 33.15 | 32.56 | 32.09 | 32.08 | 32.46 |
| | 18 | 68.78 | 68.29 | 67.83 | 67.28 | 69.69 |
| | 19 | 54.80 | 55.52 | 53.16 | 53.91 | 54.00 |
| | 20 | 47.98 | 49.73 | 47.11 | 46.74 | 48.15 |
| | 21 | 63.53 | 62.88 | 64.70 | 62.82 | 62.78 |
| | 22 | 53.77 | 52.42 | 52.64 | 52.46 | 54.50 |
| | 23 | 71.21 | 70.66 | 70.19 | 69.76 | 71.52 |
| | 24 | 39.37 | 40.90 | 38.43 | 42.68 | 38.08 |
| | 25 | 82.56 | 81.09 | 81.09 | 80.26 | 81.32 |
| | 26 | 27.48 | 26.77 | 26.31 | 29.56 | 26.42 |
| | 27 | 30.22 | 29.69 | 31.50 | 31.03 | 31.36 |
| | 28 | 58.00 | 57.36 | 58.52 | 57.16 | 59.63 |
| | 29 | 45.16 | 45.97 | 46.28 | 44.67 | 44.06 |
| | 30 | 81.65 | 81.19 | 81.02 | 80.12 | 80.47 |
| | ***p*-value** | | **0.963** | **0.765** | **0.784** | **0.951** |

- $p < 0.05$ indicate a significant difference



[ DOI: 10.22034/FSCT.21.157.123 ]

**Table 2:** Percentage of plant-based additives in kielbasa samples calculated with Image-Pro Plus, Image J, Clemex and Adobe Photoshop software.

| Product Type | Product code | Factory-declared Plants (%) | Image-Pro Plus (%) | Clemex (%) | Image J (%) | Adobe Photoshop (%) |
|---|---|---|---|---|---|---|
| Kielbasa | 1 | 30.42 | 48.63 | 42.71 | 35.91 | 38.98 |
| | 2 | 35.04 | 48.30 | 40.94 | 39.03 | 41.11 |
| | 3 | 4.42 | 17.39 | 8.66 | 9.54 | 12.17 |
| | 4 | 29.41 | 41.20 | 33.17 | 36.95 | 39.76 |
| | 5 | 25.07 | 21.74 | 36.08 | 30.36 | 36.59 |
| | 6 | 23.35 | 22.88 | 35.76 | 31.96 | 35.30 |
| | 7 | 37.89 | 55.22 | 47.21 | 45.87 | 49.09 |
| | 8 | 16.64 | 17.44 | 29.39 | 22.42 | 29.56 |
| | 9 | 5.40 | 17.54 | 9.64 | 10.71 | 18.68 |
| | 10 | 38.83 | 50.15 | 45.51 | 43.06 | 48.79 |
| | 11 | 33.50 | 50.09 | 44.48 | 44.97 | 47.66 |
| | 12 | 17.61 | 17.46 | 19.74 | 14.11 | 29.50 |
| | 13 | 3.28 | 15.78 | 7.57 | 5.37 | 16.23 |
| | 14 | 30.59 | 50.94 | 43.89 | 37.35 | 43.93 |
| | 15 | 22.43 | 21.70 | 28.70 | 31.19 | 35.92 |
| | 16 | 37.63 | 52.86 | 46.21 | 45.05 | 47.74 |
| | 17 | 40.93 | 54.13 | 45.24 | 48.18 | 51.14 |
| | 18 | 16.49 | 29.15 | 23.12 | 55.41 | 28.01 |
| | 19 | 23.94 | 23.62 | 35.65 | 28.09 | 39.61 |
| | 20 | 34.13 | 23.90 | 40.95 | 42.14 | 48.33 |
| | 21 | 26.27 | 21.54 | 32.96 | 31.01 | 37.44 |
| | 22 | 23.17 | 27.11 | 28.99 | 16.23 | 31.95 |
| | 23 | 12.39 | 23.49 | 18.73 | 21.32 | 27.58 |
| | 24 | 30.67 | 43.38 | 40.82 | 37.52 | 46.45 |
| | 25 | 3.74 | 14.68 | 8.58 | 11.70 | 16.50 |
| | 26 | 45.55 | 60.20 | 45.99 | 57.06 | 55.16 |
| | 27 | 41.62 | 38.35 | 49.27 | 45.88 | 52.71 |
| | 28 | 22.28 | 26.42 | 30.17 | 32.00 | 38.75 |
| | 29 | 38.68 | 39.12 | 50.63 | 41.93 | 48.37 |
| | 30 | 19.02 | 33.78 | 24.52 | 25.64 | 29.82 |
| | ***p*-value** | | **0.028** | **0.023** | **0.041** | **< 0.001** |

- $p < 0.05$ indicate a significant difference





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**able 3:** Percentage of skeletal muscle in sausage samples calculated with Image-Pro Plus, Image J, Clemex and Adobe Photoshop software.

| Product Type | Product code | Factory-declared skeletal muscle (%) | Image-Pro Plus (%) | Clemex (%) | Image J (%) | Adobe Photoshop (%) |
|---|---|---|---|---|---|---|
| Sausage | 1 | 78.48 | 78.60 | 77.25 | 78.96 | 79.52 |
| | 2 | 50.48 | 51.17 | 49.33 | 51.11 | 49.25 |
| | 3 | 45.30 | 44.76 | 46.21 | 44.60 | 44.79 |
| | 4 | 77.50 | 76.66 | 77.28 | 76.56 | 78.53 |
| | 5 | 28.16 | 30.26 | 29.24 | 30.25 | 27.62 |
| | 6 | 35.61 | 34.52 | 34.84 | 36.05 | 35.73 |
| | 7 | 50.52 | 48.34 | 51.58 | 50.22 | 49.49 |
| | 8 | 75.20 | 74.11 | 74.14 | 74.57 | 76.21 |
| | 9 | 43.35 | 44.28 | 42.11 | 45.83 | 42.13 |
| | 10 | 40.55 | 42.85 | 41.68 | 39.69 | 39.45 |
| | 11 | 39.50 | 39.36 | 38.01 | 40.53 | 40.94 |
| | 12 | 43.69 | 41.16 | 42.79 | 41.84 | 42.43 |
| | 13 | 30.99 | 31.55 | 32.17 | 31.12 | 31.22 |
| | 14 | 68.97 | 69.26 | 68.13 | 67.34 | 67.34 |
| | 15 | 49.53 | 50.39 | 48.63 | 49.04 | 49.16 |
| | 16 | 45.55 | 46.72 | 44.40 | 44.11 | 47.60 |
| | 17 | 70.28 | 70.01 | 71.28 | 69.34 | 69.56 |
| | 18 | 79.02 | 78.44 | 78.08 | 77.23 | 80.44 |
| | 19 | 45.90 | 46.19 | 46.16 | 47.88 | 46.31 |
| | 20 | 30.62 | 31.61 | 31.35 | 31.05 | 29.43 |
| | 21 | 40.44 | 39.38 | 41.14 | 41.39 | 41.29 |
| | 22 | 28.53 | 29.16 | 27.23 | 30.71 | 29.64 |
| | 23 | 65.86 | 64.30 | 64.01 | 63.11 | 46.04 |
| | 24 | 45.98 | 44.20 | 44.67 | 45.40 | 46.40 |
| | 25 | 52.52 | 51.54 | 52.02 | 51.11 | 51.67 |
| | 26 | 33.12 | 34.59 | 32.84 | 35.84 | 32.74 |
| | 27 | 32.66 | 32.63 | 33.42 | 33.07 | 30.60 |
| | 28 | 35.44 | 35.73 | 36.00 | 34.84 | 36.95 |
| | 29 | 78.65 | 77.39 | 77.60 | 77.36 | 77.34 |
| | 30 | 29.73 | 30.09 | 30.55 | 31.18 | 29.18 |
| | ***p*-value** | **0.982** | **0.951** | **0.995** | **0.969** | |

- $p < 0.05$ indicate a significant difference



[ DOI: 10.22034/FSCT.21.157.123 ]

**Table 4:** Percentage of plant-based additives in sausage samples calculated with Image-Pro Plus, Image J, Clemex and Adobe Photoshop software.

| Product Type | Product code | Factory-declared Plants (%) | Image-Pro Plus (%) | Clemex (%) | Image J (%) | Adobe Photoshop (%) |
|---|---|---|---|---|---|---|
| Sausage | 1 | 8.59 | 4.31 | 12.62 | 11.09 | 13.67 |
| | 2 | 10.57 | 19.62 | 16.74 | 9.85 | 12.70 |
| | 3 | 6.02 | 18.36 | 14.09 | 12.46 | 17.41 |
| | 4 | 7.38 | 12.26 | 13.77 | 12.92 | 14.34 |
| | 5 | 23.63 | 37.29 | 32.61 | 30.14 | 26.34 |
| | 6 | 20.79 | 32.11 | 29.01 | 27.17 | 37.88 |
| | 7 | 15.13 | 24.86 | 22.29 | 24.13 | 28.40 |
| | 8 | 6.04 | 14.39 | 13.12 | 12.90 | 9.76 |
| | 9 | 14.84 | 24.62 | 20.38 | 19.61 | 15.77 |
| | 10 | 16.36 | 27.43 | 20.88 | 19.45 | 19.82 |
| | 11 | 25.63 | 34.21 | 32.25 | 33.07 | 28.34 |
| | 12 | 25.21 | 12.05 | 30.87 | 31.64 | 38.82 |
| | 13 | 19.00 | 28.24 | 24.04 | 22.97 | 20.74 |
| | 14 | 7.42 | 4.43 | 12.98 | 11.00 | 12.78 |
| | 15 | 15.12 | 22.17 | 27.54 | 22.35 | 21.33 |
| | 16 | 11.73 | 20.15 | 19.55 | 18.42 | 17.76 |
| | 17 | 4.93 | 9.38 | 10.94 | 7.64 | 8.09 |
| | 18 | 5.41 | 2.13 | 10.67 | 8.09 | 12.30 |
| | 19 | 15.45 | 25.24 | 20.91 | 25.73 | 30.24 |
| | 20 | 26.56 | 32.51 | 31.31 | 36.14 | 39.50 |
| | 21 | 18.50 | 26.06 | 33.94 | 27.40 | 21.22 |
| | 22 | 20.65 | 34.33 | 36.90 | 29.97 | 26.76 |
| | 23 | 6.69 | 12.29 | 14.85 | 12.15 | 14.53 |
| | 24 | 16.26 | 9.16 | 31.27 | 22.51 | 19.51 |
| | 25 | 23.11 | 31.06 | 29.19 | 30.58 | 30.66 |
| | 26 | 18.20 | 34.88 | 22.64 | 22.64 | 24.68 |
| | 27 | 19.28 | 37.09 | 29.71 | 32.51 | 27.46 |
| | 28 | 22.62 | 15.92 | 35.84 | 38.69 | 27.83 |
| | 29 | 5.04 | 8.93 | 16.19 | 15.73 | 13.12 |
| | 30 | 23.60 | 39.81 | 32.79 | 30.31 | 29.49 |



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| | | | | | | |
|---|---|---|---|---|---|---|
| | ***p*-value** | | **0.009** | **<0.001** | **0.002** | **0.002** |

- $p< 0.05$ indicate a significant difference

**Table 5:** Percentage of skeletal muscle in hamburger samples calculated with Image-Pro Plus, Image J, Clemex and Adobe Photoshop software.

| **Product Type** | **Product code** | **Factory-declared skeletal muscle (%)** | **Image-Pro Plus (%)** | **Clemex (%)** | **Image J (%)** | **Adobe Photoshop (%)** |
|---|---|---|---|---|---|---|
| **Hamburger** | 1 | 30.52 | 31.94 | 29.75 | 31.00 | 25.54 |
| | 2 | 26.16 | 25.39 | 27.64 | 25.94 | 25.66 |
| | 3 | 63.66 | 54.47 | 54.28 | 54.46 | 53.12 |
| | 4 | 49.77 | 50.83 | 49.52 | 50.63 | 49.51 |
| | 5 | 70.69 | 69.62 | 69.39 | 69.11 | 71.95 |
| | 6 | 75.10 | 74.68 | 74.18 | 74.56 | 75.33 |
| | 7 | 75.88 | 75.47 | 73.12 | 73.19 | 72.61 |
| | 8 | 60.79 | 59.96 | 61.41 | 60.09 | 57.73 |
| | 9 | 50.18 | 50.74 | 51.63 | 49.31 | 49.68 |
| | 10 | 75.94 | 74.13 | 74.05 | 75.00 | 70.32 |
| | 11 | 33.59 | 34.63 | 35.44 | 35.86 | 31.24 |
| | 12 | 30.50 | 31.91 | 30.52 | 29.07 | 29.48 |
| | 13 | 80.62 | 79.94 | 80.24 | 79.74 | 81.52 |
| | 14 | 66.22 | 66.82 | 65.46 | 65.19 | 61.07 |
| | 15 | 75.89 | 74.72 | 76.44 | 75.06 | 71.42 |
| | 16 | 78.09 | 85.41 | 87.26 | 86.08 | 80.43 |
| | 17 | 78.90 | 87.50 | 86.16 | 87.64 | 88.64 |
| | 18 | 52.11 | 54.70 | 53.75 | 53.76 | 51.45 |
| | 19 | 71.60 | 70.16 | 70.70 | 73.97 | 66.43 |
| | 20 | 80.33 | 79.69 | 78.63 | 80.41 | 80.93 |
| | ***p*-value** | | **0.988** | **0.968** | **0.971** | **0.791** |

- $p< 0.05$ indicate a significant difference



[ DOI: 10.22034/FSCT.21.157.123 ]

**Table 6:** Results of investigating the percentage of plant-based additives in hamburgers samples using Adobe Photoshop, Clemex, Image J, and Image-Pro Plus software.

| Product Type | Product code | Factory-declared Plants (%) | Image-Pro Plus (%) | Clemex (%) | Image J (%) | Adobe Photoshop (%) |
|---|---|---|---|---|---|---|
| Hamburger | 1 | 48.01 | 56.64 | 54.86 | 43.58 | 62.34 |
| | 2 | 45.51 | 54.21 | 61.21 | 53.03 | 62.02 |
| | 3 | 37.39 | 47.24 | 54.30 | 47.21 | 57.56 |
| | 4 | 32.04 | 55.84 | 47.41 | 48.44 | 48.04 |
| | 5 | 25.54 | 29.67 | 32.08 | 36.08 | 39.73 |
| | 6 | 15.18 | 25.90 | 28.12 | 22.36 | 29.41 |
| | 7 | 5.14 | 25.87 | 17.64 | 14.80 | 17.53 |
| | 8 | 37.00 | 45.44 | 42.69 | 51.32 | 48.08 |
| | 9 | 40.44 | 47.85 | 23.40 | 48.30 | 59.33 |
| | 10 | 10.99 | 21.01 | 21.16 | 22.03 | 23.60 |
| | 11 | 40.56 | 62.63 | 55.27 | 55.77 | 54.48 |
| | 12 | 44.20 | 53.70 | 55.12 | 59.53 | 58.77 |
| | 13 | 1.55 | 18.19 | 16.30 | 7.69 | 5.36 |
| | 14 | 15.35 | 23.04 | 23.02 | 34.12 | 25.11 |
| | 15 | 21.15 | 31.00 | 35.88 | 33.37 | 36.15 |
| | 16 | 3.64 | 17.81 | 11.28 | 15.42 | 14.76 |
| | 17 | 2.24 | 15.70 | 14.50 | 7.43 | 4.99 |
| | 18 | 35.59 | 29.18 | 44.11 | 57.32 | 53.42 |
| | 19 | 20.28 | 26.31 | 38.71 | 33.28 | 37.34 |
| | 20 | 4.29 | 17.67 | 13.87 | 13.33 | 7.21 |
| | ***p*-value** | | **0.037** | **0.049** | **0.048** | **0.031** |

- $p < 0.05$ indicate a significant difference



[ DOI: 10.22034/FSCT.21.157.123 ]

**Table 7:** Results of investigating the percentage of skeletal muscle in kebab bite samples using Adobe Photoshop, Clemex, Image J, and Image-Pro Plus software.

| Product Type | Product code | Factory-declared skeletal muscle (%) | Image-Pro Plus (%) | Clemex (%) | Image J (%) | Adobe Photoshop (%) |
|---|---|---|---|---|---|---|
| **Kebab bite** | 1 | 55.56 | 56.48 | 56.60 | 57.12 | 55.03 |
| | 2 | 59.37 | 59.03 | 60.34 | 58.20 | 58.25 |
| | 3 | 54.18 | 53.82 | 54.72 | 54.69 | 54.54 |
| | 4 | 50.73 | 51.76 | 49.09 | 52.10 | 49.82 |
| | 5 | 50.11 | 49.90 | 49.55 | 48.62 | 51.54 |
| | 6 | 53.33 | 53.89 | 52.42 | 52.70 | 53.48 |
| | 7 | 55.47 | 45.52 | 53.28 | 55.09 | 45.16 |
| | 8 | 60.40 | 61.39 | 61.17 | 59.34 | 59.77 |
| | 9 | 53.54 | 53.52 | 52.08 | 52.01 | 54.05 |
| | 10 | 52.13 | 51.47 | 52.76 | 52.35 | 53.71 |
| | ***p*-value** | | **0.658** | **0.869** | **0.864** | **0.058** |

- $p < 0.05$ indicate a significant difference

**Table 8:** Results of investigating the percentage of plant-based additives in Kebab bite samples using Adobe Photoshop, Clemex, Image J, and Image-Pro plus software.

| Product Type | Product code | Factory-declared Plants (%) | Image-Pro Plus (%) | Clemex (%) | Image J (%) | Adobe Photoshop (%) |
|---|---|---|---|---|---|---|
| **Kebab bite** | 1 | 27.80 | 22.00 | 21.19 | 34.64 | 33.94 |
| | 2 | 29.41 | 30.37 | 34.81 | 35.74 | 34.17 |
| | 3 | 19.08 | 32.88 | 26.97 | 35.06 | 23.73 |
| | 4 | 22.19 | 24.60 | 31.21 | 36.75 | 28.13 |
| | 5 | 20.56 | 40.22 | 27.86 | 31.24 | 27.86 |
| | 6 | 20.50 | 35.28 | 28.20 | 32.14 | 27.94 |
| | 7 | 19.46 | 35.28 | 25.81 | 29.79 | 24.41 |
| | 8 | 30.16 | 36.36 | 36.29 | 24.90 | 37.59 |
| | 9 | 21.44 | 22.13 | 31.44 | 30.84 | 33.30 |
| | 10 | 23.08 | 40.39 | 33.66 | 33.47 | 30.67 |
| | ***p*-value** | | **0.012** | **0.005** | **<0.001** | **0.003** |

- $p < 0.05$ indicate a significant difference



[ DOI: 10.22034/FSCT.21.157.123 ]

**Table 9:** Results of investigating the percentage of skeletal muscle in Chicken Nugget samples using Adobe Photoshop, Clemex, Image J, and Image-Pro Plus software.

| Product Type | Product code | Factory-declared skeletal muscle (%) | Image-Pro Plus (%) | Clemex (%) | Image J (%) | Adobe Photoshop (%) |
|---|---|---|---|---|---|---|
| Chicken Nugget | 1 | 69.14 | 70.02 | 68.13 | 67.55 | 69.24 |
| | 2 | 64.13 | 65.42 | 64.87 | 65.98 | 63.56 |
| | 3 | 60.55 | 60.14 | 59.24 | 59.58 | 59.50 |
| | 4 | 66.19 | 65.71 | 65.07 | 65.18 | 65.42 |
| | 5 | 67.23 | 66.89 | 67.77 | 67.10 | 48.37 |
| | 6 | 74.22 | 75.15 | 75.54 | 73.34 | 74.75 |
| | 7 | 70.19 | 71.41 | 69.43 | 69.92 | 69.69 |
| | 8 | 66.52 | 67.23 | 67.60 | 65.14 | 65.70 |
| | 9 | 65.63 | 65.40 | 66.79 | 65.33 | 66.41 |
| | 10 | 65.07 | 64.32 | 64.02 | 66.89 | 64.23 |
| | ***p*-value** | | **0.874** | **0.892** | **0.862** | **0.910** |

- $p < 0.05$ indicate a significant difference

**Table 10:** Results of investigating the percentage of plant-based additives in chicken nugget samples using Adobe Photoshop, Clemex, Image J, and Image-Pro Plus software.

| Product Type | Product code | Factory-declared Plants (%) | Image-Pro Plus (%) | Clemex (%) | Image J (%) | Adobe Photoshop (%) |
|---|---|---|---|---|---|---|
| Chicken Nugget | 1 | 25.78 | 22.35 | 32.28 | 30.74 | 29.79 |
| | 2 | 19.05 | 19.67 | 25.09 | 23.17 | 23.33 |
| | 3 | 19.46 | 27.42 | 26.11 | 24.06 | 21.71 |
| | 4 | 19.15 | 14.83 | 25.29 | 27.05 | 23.14 |
| | 5 | 18.55 | 27.97 | 29.48 | 28.20 | 26.47 |
| | 6 | 19.63 | 20.36 | 23.86 | 22.07 | 19.04 |
| | 7 | 21.37 | 22.52 | 24.01 | 25.16 | 27.98 |
| | 8 | 18.70 | 29.64 | 22.74 | 24.15 | 26.43 |
| | 9 | 24.99 | 27.70 | 29.34 | 30.11 | 25.58 |
| | 10 | 19.55 | 29.66 | 27.97 | 26.82 | 23.27 |
| | ***p*-value** | | **0.046** | **<0.001** | **<0.001** | **0.006** |

- $p < 0.05$ indicate a significant difference



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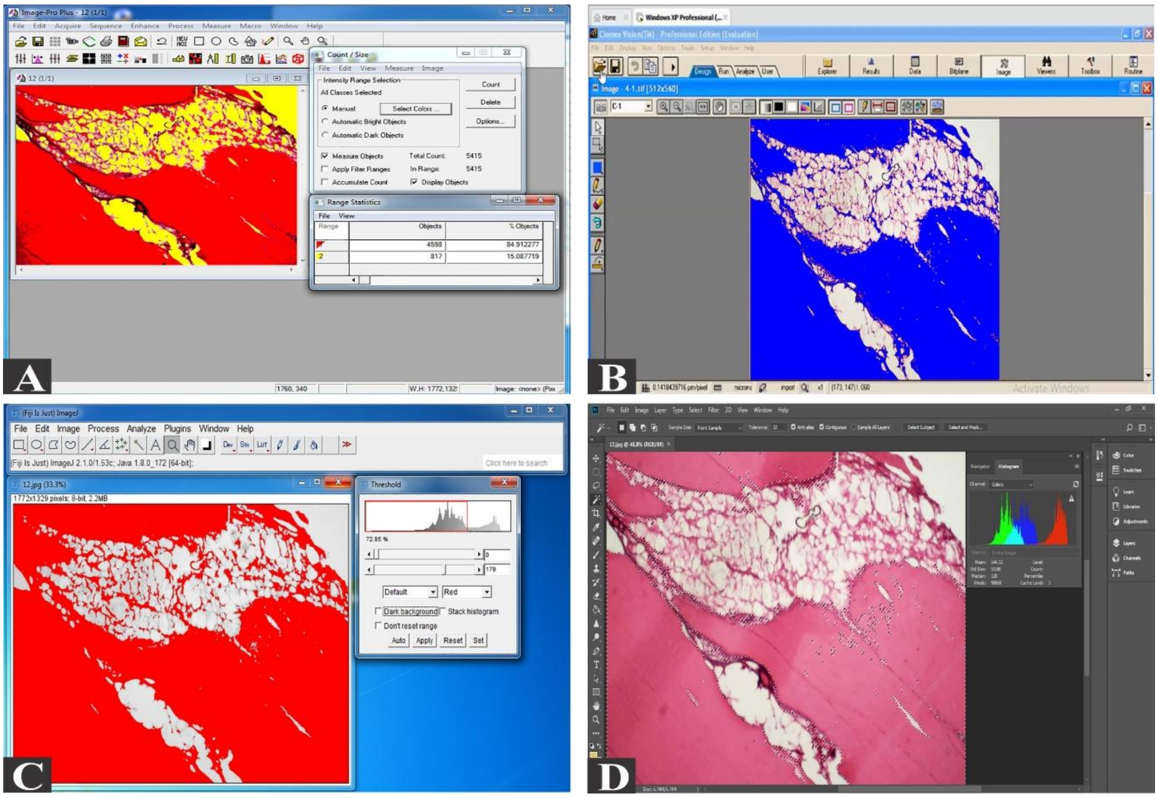


**Figure 1**: Images from Image-Pro Plus (A), Adobe Photoshop (B), Image J (C) and Clemex (D) that used in this research.

**3-6- The amount of time spent on each sample**:

The study revealed the time spent on image analysis of meat products using various software for the samples of Kielbasa, sausage, kebab lumps, and chicken nuggets. In all four mentioned samples, the duration of analysis in Image J software was statistically less than in other software ($p<0.05$). However, in the case of burger samples, the shortest time was related to Adobe Photoshop software, which had a significant difference compared to other software in this regard ($p<0.05$). The details of the results are visible in Table 11.

In the context of the average time spent for image analysis of tissue sections from meat products, the results showed that only Image J software took significantly less time compared to other software programs, and this difference was statistically significant. This might be due to the simpler interface of Image J for such tasks.

**Table 11:** Table of the time spent on image analysis of meat products using different software.

| Software Name | Kielbasa Samples (seconds | Sausage Samples (seconds) | Hamburger Samples (seconds) | Kebab bite Samples (seconds) | Chicken Nugget Samples (seconds) |
|---|---|---|---|---|---|
| **Image-Pro Plus** | 2723± 112$^{a}$ | 2687± 118$^{a}$ | 2592± 113$^{a}$ | 2633± 68$^{a}$ | 2446± 45$^{a}$ |
| **Adobe Photoshop** | 2699± 48$^{a}$ | 2635± 63$^{a}$ | 2289± 53$^{b}$ | 2521± 61$^{a}$ | 2393± 102$^{a}$ |
| **Image J** | 2346± 30$^{b}$ | 2308± 75$^{b}$ | 2324± 44$^{a}$ | 2212± 49$^{b}$ | 2199± 53$^{b}$ |
| **Clemex** | 2649± 82$^{a}$ | 2503± 113$^{a}$ | 2446± 58$^{a}$ | 2431± 76$^{a}$ | 2424± 55$^{a}$ |

Dissimilar letters indicate a significant difference ($p< 0.05$).

**3-7- Comparison of Software Sufficiency**

In the comparison of accuracy and overall time spent on image analysis of tissue sections obtained from meat products, no significant difference in accuracy was observed between different software. However, in general, the





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time spent on image analysis of meat products in the Image J software was statistically less compared to other software examined in this study ($p$<0.05) (Figure 2).

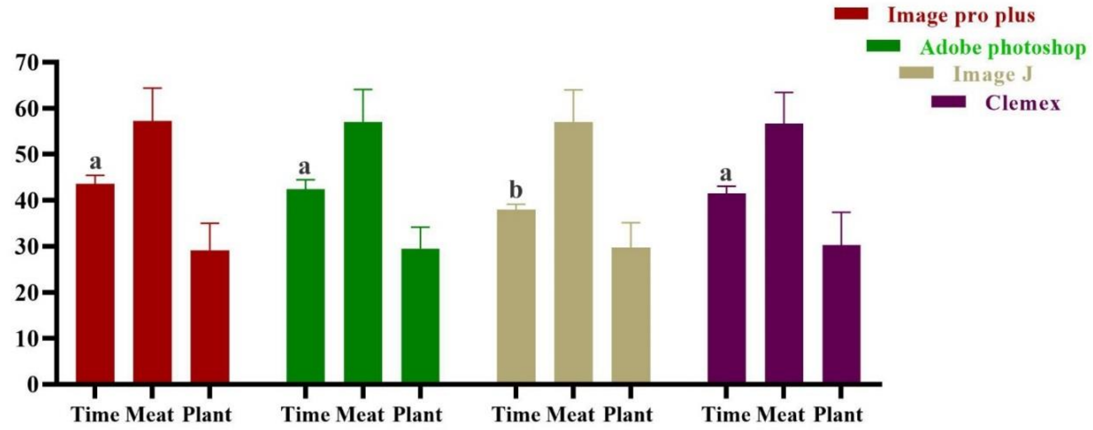


**Figure 2:** Comparison of accuracy and total time spent for image analysis of tissue sections obtained from meat products. Dissimilar letters indicate a significant difference ($p$< 0.05).

## 4- Conclusion

In the analysis of the results from all four software programs, no statistically significant differences were observed between the obtained percentage and the declared meat content by the factory. Moreover, the study revealed a statistically significant difference only in the average time spent on image analysis of tissue sections from meat products when comparing Image J software to the other software programs. The other remaining software programs exhibit significant differences in terms of time spent. In conclusion, all four software programs, Image-Pro Plus, Adobe Photoshop, Clemex, and Image J, used in the current study are capable of accurately assessing the percentage of skeletal muscles used in the production of meat products. In the estimate of skeletal muscle percentage in Kielbasa and Hamburger samples the Image-pro plus software, in Sausage samples the Image J software, in Kebab bite samples the Clemex software and in Chicken Nugget samples the Adobe Photoshop software showed higher accuracy. While, the studied software could not estimate the amount of plant-based additives in different meat products. This seems to be due to the variety of herbal additives tisuue. However, the comparison in general showed that Image J stands out as the preferred option due to its open accessibility and shorter time required for image analysis. The comparison of Image J with other related software programs that have not been studied to date can be valuable in furthering the current study.

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[ DOI: 10.22034/FSCT.21.157.123 ]

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مقاله علمی_پژوهشی

# بررسی کفایت نرم‌افزارهای آنالیز تصویری در سنجش درصد عضلات اسکلتی فرآورده‌های گوشتی

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**اطلاعات مقاله**

**تاریخ های مقاله :**

**تاریخ دریافت:** ۱٤۰۳/۱/۲۹

**تاریخ پذیرش:** ۱٤۰۳/٤/۱۷

**کلمات کلیدی:**

فرآورده‌های گوشتی،

بافتشناسی،

آنالیز تصویری،

نرم افزار گرافیکی

**DOI:10.22034/FSCT.21.157.123.**

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**چکیده**

در سال‌های أخیر توجه ویژه‌ای به روش‌های سنجش درصد گوشت (عضلات اسکلتی) در فرآورده‌های گوشتی شده است. با توجه به ضریب خطای بالای روش‌هایی مانند روش شیمیایی، به نظر می‌رسد مطمئن‌ترین و دقیق‌ترین روش برای سنجش درصد عضلات اسکلتی در فرآورده‌های گوشتی روش بافت‌شناسی و متعاقب آن استفاده از آنالیز تصویری برای تفکیک اجزای مختلف این فرآورده‌ها باشد. از این رو با توجه به مطالعات اندک انجام گرفته در این زمینه و دسترسی نه چندان آسان به برخی از نرم‌افزارهای آنالیز تصویری، در مطالعه حاضر برای اولین بار درصد عضلات اسکلتی فرآورده‌های گوشتی و مدت زمان صرف شده برای بررسی هر نمونه، با استفاده از دو نرم افزار گرافیکی با دسترسی رایگان (Adobe Photoshop و ImageJ) و دو نرم افزار گرافیکی با دسترسی غیررایگان (Clemex و Image pro-plus) مورد بررسی قرار گرفته است. برای این کار از تعداد ۱۰۰ نمونه فرآورده گوشتی (۳۰ عدد کالباس، ۳۰ عدد سوسیس، ۲۰ عدد همبرگر، ۱۰ عدد کباب لقمه و ۱۰ عدد ناگت مرغ) با میزان مشخص عضله اسکلتی استفاده شده بهره برده شد. نمونه‌ها پس از انتقال به آزمایشگاه و تهیه مقاطع بافتی توسط روش هماتوکسیلین–ائوزین رنگ آمیزی شدند. تصاویر حاصل از مقاطع بافتی با استفاده از نرم‌افزارهای مذکور آنالیز شد. نتایج نشان‌دهنده دقت تقریباً یکسان هر چهار نرم‌افزار در سنجش عضلات اسکلتی بود. در حالی که مدت زمان مورد نیاز برای بررسی هر نمونه در نرم‌افزار ImageJ نسبت به سایر نرم‌افزارها بطور معنی‌داری کمتر بود ($p<$ ۰/۰۵). با توجه به نتایج مطالعه حاضر به نظر می‌رسد نرم‌افزار ImageJ کفایت بیشتری برای آنالیز تصویری مقاطع بافتی و تعیین درصد عضلات اسکلتی فرآورده‌های گوشتی دارد.