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EVALUATION OF THE DEGREE OF SNAIL MEAT FRESHNESS BY THE PHOTOMETRIC METHOD

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Abstract. This work focuses on developing a method of determining the degree of snail meat freshness by means of a photoelectric photometer. For this purpose, measurements of the optical density of the filtered meat-water extract with the addition of Nessler's reagent was measured. The method can be used in laboratories to determine the quality of meat of snails during their processing and sale. The results of using this method allow obtaining quantitative values when assessing the quality of snails of different degrees of freshness. A selection of 180 *Helix pomatias* snails was studied. They were divided into three groups, 60 snails in each, in accordance with the meat freshness degree. Three degrees of meat freshness were distinguished: fresh, questionably fresh, and stale. Meat samples were taken from all the snails of each group. Then the meat was ground, and 2.00 g portions were taken from each sample. 20.00, 30.00, 40.00, and 50.00 cm³ of distilled water were added successively to the weighed portions. They were infused for 15 minutes and then filtered. 0.50 cm³ of Nessler's reagent was added to 3.00 cm³ of the filtrate. The absorbance of the colour intensity of the meat-water extracts was measured with a concentration photocolourimeter extended in the range of light waves from 400 nm to 490 nm. It has been established that the highest absorbance indicates stale meat, the lowest is characteristic of fresh meat, and the value of absorbance for questionably fresh meat lies in between. Thus, when the concentration of snail meat extract was 1:10, at the wavelength 440 nm, the absorbance values were 1.243±0.031 for stale meat, 0.262±0.034 for fresh meat, and 0.804±0.054 for questionably fresh meat. This may be due to an increase in the ammonia concentration during the meat spoilage process. The three degrees of meat freshness were best determined at the wavelengths 440 nm and 490 nm. The results obtained are qualitatively and quantitatively in line with those of photometric studies of the freshness degree in beef, poultry, and fish.

Key words: snail, meat, freshness degree, photometric method, research.

Introduction. Formulation of the problem

Today, snail meat is considered an expensive gourmet product, but the methods of controlling its quality and safety have not yet been developed. Ukraine is trying to become one of the leading exporters of Roman snails (*Helix pomatia*) in Europe [1]. There are large farms specialising in heliciculture, in processing, refrigeration, and packing of this species of edible snails [2]. However, in fact, methods of quality and safety control of this delicacy product have not been elaborated yet. At the same time, veterinary and sanitary expert examination of snail meat quality at different stages of processing and storage is a crucial issue [3]. Correct evaluation of

meat purity is essential to assess the condition of meat, its shelf life, and methods of processing. Numerous studies are dedicated to elaboration techniques of quick and affordable objective evaluation of the primary stages of meat and meat products spoilage [4].

Organoleptic methods allow distinguishing between the three degrees of snail meat freshness: fresh, questionably fresh, and stale [5]. Fresh snail meat has a light grey colour with a dense consistency. Depending on the type of snail, it has a tone from light to dark. It is very slippery [6]. The smell is specific, with the scent of the herb that the snail has been eating for the last 5–7 days. Questionably fresh meat has the signs of the initial stage of spoilage. The surface of the meat becomes greyer, there is less slime in it, and it

gradually turns into a clear liquid [7]. Herbal smell is not so pronounced. The texture is slightly doughy [8]. It is not permitted to sell questionably fresh meat, and it is sanitary inspection authorities who decide about its further use for processing [9]. Stale meat is recognised by distinctive offensive, sharp odour and dark grey colour. There is no slime in it at all, and it turns into turbid liquid. The consistency of meat is doughy.

Analysis of recent research and publications

By the colour of solutions of coloured substances, it is possible to determine the concentration of a particular component either visually or with the help of photocells, devices that convert light energy into electrical energy. In accordance with this, a distinction is made between the photometric visual method of analysis, often called colourimetric, and the method of analysis using photocells – the photometric method of analysis as such. The photometric method is an objective method, since its results do not depend on the abilities of the observer, unlike the results of the subjective colourimetric method. However, there is an urgent need to apply modern objective quantitative methods of scientific research to evaluate snail meat freshness. One of these methods is the photometric method [10]. It is widely used to determine reliably the degree of freshness of beef [11], poultry meat [12,13], and fish [14].

V. Kasyanchuk and N. Bogatko developed a method to determine the degree of freshness of beef and pork, which involved the use of Nessler's reagent and extracts from meat. According to their method, to the meat-water extract prepared in the ratio 1:4, Nessler's reagent is added, and by the changes in the colour intensity and consistency, the freshness of meat is rated [11].

The authors N. Bogatko, N. Bukalova, T. Prilipko, T. Gruba, and D. Bogatko developed a method to determine the degree of freshness of poultry meat by photometry. It involves using meat-water extract (in the ratio 1:10) prepared as follows. 20.0–20.2 cm³ of distilled water is added to 2.0–2.2 g of crushed poultry meat, the mixture is infused for 10–12 minutes, and 1.0–1.1 cm³ of Nessler's reagent is added to 3.0–3.5 cm³ of filtered meat-water extract, which is then rested in a tripod for 2–3 minutes. This is followed by centrifugation for 1–2 minutes at 1000 rpm. Further, the absorbance of the colour intensity of the supernatant is measured with a photoelectric photometer in a cuvette with the absorption layer thickness 1.0 cm at the wavelength 450–455 nm [12].

The freshness of fish, too, can be determined by the photometric method. It involves using 2.0–2.2 g of chopped fish meat and infusing the meat-water extract for 12–15 minutes. To 4.0–4.2 cm³ of the filtered meat-water extract, 1.0–1.2 cm³ of Nessler's reagent is added. This is followed by resting in a tripod for 4–5 minutes and centrifugation for 1–2 minutes at 2000 rpm. Then the absorbance of the colour intensity of the

supernatants measured with a photoelectric photometer in a cuvette with the absorption layer thickness 1.0 cm at the wavelength 455–460 nm [14].

Therefore, the first attempt to apply photometry to determine snail meat freshness was made in the patent [15].

Meat of edible snails is considered a delicacy and is part of Ukrainian food exports. After snails are raised to the required state, they are frozen and in this form transported to their destinations. Sometimes fresh snails are immediately cooked and then consumed in catering outlets. Before preparing and eating dishes from snails, it is quite important to determine how fresh their meat is. But in the current circumstances, these data are not available.

So, **the purpose** of this work is a detailed study of the possibilities of using the photometric method for reliable quantitative evaluation of freshness of edible snail meat.

The objectives:

- to prepare extracts from snail meat in different ratios: 1:10, 1:15, 1:20, 1:25, to add 0.5 cm³ of Nessler's reagent to filtered meat-water extract.
- to measure the absorbance of the colour intensity of the filtered meat-water extract at different wavelengths: 400, 440, 490, 540 nm.
- to compare the data obtained by us with the data on other types of meat.

Research materials and methods

180 Roman snails (*Helix pomatia*) collected on a summer morning in a forest near the Kharkiv State Zooveterinary Academy campus (Kharkiv Region, Ukraine) were used as research material. Then the snails were divided into three groups with 60 snails in each according to the degree of meat freshness. The first group consisted of freshly collected live snails, the second group of snails after storage in the refrigerator at +4°C for 5 days, the third of snails after storage in the refrigerator at +4°C for 7 days.

After refrigeration, the snails were slaughtered by breaking the shell mechanically and separating the pedal mass and visceral mass. Meat samples were taken from all the snails of each group. Then the meat was ground, and 2.00 g portions (in total 60 weighed portions for each group) were taken from each sample. All weighing was done on electronic scales ScoutProSPS202 F(OHAUS, US) accurate within 0.01 g. Distilled water was successively added, in volumes of 20.00, 30.00, 40.00, and 50.00 cm³, to the weighed portions taken (in total, 12 subgroups with 4 samples in each of them). They were infused for 15 minutes, then filtered (like it was done in the studies [5-7]), and 0.50 cm³ of Nessler's reagent (alkaline aqueous solutions of potassium tetraiodomercurate dehydrate) was added to 3.00 cm³ of the filtrate. All aliquots were taken with Eppendorf Research plus pipettes (Eppendorf, Germany) with an accuracy of at least ±0.01 cm³. The absorbance of the colour intensity of the meat-water

extract was measured on a concentration photocolourimeter Apel AP-120 (Japan) in a wide range of light waves (400 nm to 980 nm). In our previous work [8], we measured the absorption spectrum of solutions and selected the optimal wavelength range 400–540 nm. Quartz cuvettes with the optical path length 10.075 mm and external dimensions 24.00×16.00×40.00 mm with the thickness 3.00 mm were used.

The results were processed by the methods of variation statistics and biometrics using the application software package MS Excel2012 [16].

Results of the research and their discussion

The APEL AP-120 Photocolourimeter is a microprocessor-controlled general-purpose photoelectric colourimeter that is easy to operate for reliable and accurate results in a variety of research applications.

It is used to determine the concentration of various substances in solutions. The photocolourimeter is used in medical laboratories to determine biochemical parameters in biological fluids; in production laboratories for quality control of drinking water, waste water, industrial waste; in the food industry (sugar factories, enterprises manufacturing juices, alcohol, confectionery shops, etc.) to control the quality of purchased raw materials and that of finished products.

The advantages of the AP-120 photoelectric colourimeter: ergonomics, simplicity and ease of use, cost-effectiveness of reagent consumption, open system for any methods and reagents, ability to use round and square cuvettes, no need to calculate using graphs. Advantages: Compact device; open system for any methods and reagents; small sample volume (minimum sample volume 0.5–1 cm³); direct determination of concentration; use of square cuvettes; automatic zeroing; a new highly stable type of silicon photodiode, providing high measurement accuracy; after setting the standard value, the concentration of each sample can be directly changed; long life of the lamp.

The photoelectric concentration colourimeter is designed to measure, in certain parts of the wavelength range 315–980 nm emitted by light filters, the transmittance and absorbance of liquid solutions and solids, and to determine the concentration of substances in solutions by constructing calibration graphs. The colourimeter also makes it possible to measure the transmission coefficients of scattering suspensions, emulsions, and colloidal solutions in transmitted light.

The devices for photocolourimetry are photoelectric colourimeters. They are characterised by the simplicity of optical and electrical circuits. Most photometers have a set of 10–15 light filters and are double-beam devices where the light beam from the radiation source (incandescent lamp) passes through a light filter and a light beam splitter, usually a

prism (which divides the beam into two), and is directed through the cuvettes with the test solution and with the reference solution. After the cuvettes, the parallel light beams pass through calibrated attenuators (diaphragms) designed to equalise the intensities of light fluxes and fall on two radiation receivers (photocells) connected in a differential circuit to a null indicator (galvanometer, indicator lamp). The advantages of the device are the simplicity of design and high sensitivity due to the large luminosity. The measured range of absorbance is approximately 0.05–3.0, which makes it possible to determine many elements and their compounds in a wide range of contents: from $\sim 10^{-6}$ to 50% by weight. For better sensitivity and selectivity of determinations, essential factors are the appropriate selection of reagents forming intensely coloured complex compounds with analytes, the choice of the composition of solutions, and the conditions of measurement.

Comparing the results obtained with those of applying the photometric method to determine the degree of freshness of beef [11], it can be noted that we used meat-water extracts of a lower concentration (1:10, 1:15, 1:20 and less) than 1:4 used for beef in our studies. This is results from the presence of a large amount of mucus in snail meat. This fact creates certain difficulties in filtering high concentration solutions. The two working wavelengths 440 nm and 490 nm are somewhat larger, as compared with 420 nm for beef. But the fact that there are two of them allows making more reliable expert conclusions about the freshness of snail meat, possibly using the discriminant and cluster analysis methods [16]. Higher concentrations of the extracts result in higher average values of the absorbance of the beef extract (0.990±0.084 for fresh meat, 2.413±0.277 for stale meat, and 1.537±0.140 for meat of questionable freshness). Still the results are qualitatively consistent with the results of determining the degree of freshness of snail meat. An increase in the absorbance of the extract with Nessler's reagent is due to an increase in the concentration of ammonia in the course of deterioration of meat quality.

Studies conducted by domestic and foreign scientists have revealed that snails contain much larger quantities of complete proteins, vitamins, minerals, and other bioactive substances necessary for the human body than many livestock products do. Snail meat does not contain cholesterol and harmful fats, which distinguishes it from the meat of farm mammals and poultry [9,17]. Currently, the range of applications is expanding, as well as the commercial value of this mollusc in Europe, Central Asia, North Africa, North and South America, the Baltics, and in other regions. Snail meat is most intensively consumed in France, Italy, Spain, Belgium, Switzerland, Germany, USA, Austria, Hungary [2].

In Ukraine, snail farms are becoming more active every year. They actively sell snails to domestic

markets and export to other countries. However, despite this, Ukraine currently lacks regulations on the system of controlling the safety and quality of snail meat, and has no developed rules for procurement, acceptance, transportation, storage and veterinary examination of this delicacy at all stages of its cultivation and processing [8]. In connection with the above, determination of the safety of snail meat, in particular, the degree of its freshness, has both theoretical and applied significance [3].

The results of studies of snail meat on a photoelectric concentration colourimeter are summarised in Table 1 as $M \pm m$, where M is the average value of the absorbance of the colour intensity of meat-water extract for the group, and m is the average error.

The data in Table 1 indicate that the maximum absorbance is characteristic of stale meat in a wide range of meat-water extract concentrations. Fresh meat has the lowest absorbance. And meat of questionable freshness takes an intermediate position in the values of absorbance.

The greatest reliability of the differences between fresh meat, stale meat, and meat of questionable freshness for Roman snails is achieved by performing photometry at waves with the lengths 440 nm and 490 nm using the meat-water extract at the concentrations 1:10, 1:15, and 1:20 with the addition of 0.50 cm³ of Nessler's reagent ($p < 0.001$). Low concentrations of the extract (1:25) are non-informative for determining the degree of meat freshness by photometry.

It should be noted that the values of M for all subgroups at different wavelengths are significantly different according to Student's test [9] ($p < 0.05$). The exception is the 4th subgroup (1:25 + 0.50 cm³ of Nessler's reagent), the concentration of which is considered non-informative, for fresh and stale meat at the wavelength 540 nm, which may be due to statistical fluctuations.

Table 2 shows the ranges of changes in the absorbance of the colour of the meat-water extract at the concentrations 1:10, 1:15, and 1:20 with the addition of 0.50 cm³ of Nessler's reagent at the two most informative wavelengths 440 nm and 490 nm.

As seen in Table 2, the variation ranges of absorbance for snail meat of different freshness degrees do not overlap ($p < 0.001$ according to Student [16]). Therefore, Table 2 can be directly applied for rapid veterinary examination of snail meat by photometry.

The comparative data on meat from different animal species are shown in Fig. 1.

Fig. 1 shows that in all animal species, the indicator of meat freshness is directly proportional. That is, the fresher the meat, the lower the indicator is, and vice versa [11,12,14].

Comparing our findings with the results of determining the degree of freshness of poultry [12], let us focus on the concentration of meat-water extracts 1:10, which was used in the studies of both poultry and snails.

Table 1 – Average values and average errors of the absorbance of the colour intensity of the extract in the groups

Groups	Wavelength, nm			
	400	440	490	540
Fresh meat				
1. (1:10 + 0.50 cm ³ of Nessler's reagent)	1.283±0.112	0.262±0.034	0.304±0.033	0.424±0.052
2. (1:15 + 0.50 cm ³ of Nessler's reagent)	0.654±0.064	0.463±0.035	0.421±0.041	0.123±0.023
3. (1:20 + 0.50 cm ³ of Nessler's reagent)	0.521±0.073	0.532±0.061	0.543±0.082	0.103±0.022
4. (1:25 + 0.50 cm ³ of Nessler's reagent)	0.522±0.061	0.273±0.033	0.155±0.023	0.064±0.014
Stale meat				
1. (1:10 + 0.50 cm ³ of Nessler's reagent)	1.381±0.061	1.243±0.031	1.803±0.053	1.461±0.074
2. (1:15 + 0.50 cm ³ of Nessler's reagent)	1.802±0.072	1.374±0.062	1.912±0.055	1.404±0.063
3. (1:20 + 0.50 cm ³ of Nessler's reagent)	1.103±0.033	1.461±0.023	2.104±0.061	0.953±0.051
4. (1:25 + 0.50 cm ³ of Nessler's reagent)	0.684±0.073	0.364±0.032	0.295±0.031	0.103±0.021
Questionable freshness				
1. (1:10 + 0.50 cm ³ of Nessler's reagent)	0.693±0.081	0.804±0.054	0.851±0.048	0.843±0.049
2. (1:15 + 0.50 cm ³ of Nessler's reagent)	0.932±0.072	0.833±0.041	1.584±0.072	0.971±0.075
3. (1:20 + 0.50 cm ³ of Nessler's reagent)	1.181±0.083	1.172±0.033	1.633±0.061	1.352±0.093
4. (1:25 + 0.50 cm ³ of Nessler's reagent)	1.002±0.074	0.993±0.072	0.864±0.049	0.695±0.061

Table 2 – Ranges of change in the absorbance values of the colour intensity of the extracts in accordance with the degrees of snail meat freshness

Wavelength, nm	Degrees of snail meat freshness		
	Fresh meat	Questionable freshness	Stale meat
The meat-water extracts at the concentration 1:10			
440	0.103–0.427	0.544–1.063	1.082–1.405
490	0.125–0.481	0.536–1.074	1.444–1.983
The meat-water extracts at the concentration 1:15			
440	0.333–0.593	0.615–1.052	1.074–1.672
490	0.217–0.634	1.374–1.791	1.843–2.365
The meat-water extracts at the concentration 1:20			
440	0.241–0.824	0.954–1.313	1.355–1.572
490	0.157–0.933	1.412–1.853	1.915–2.291

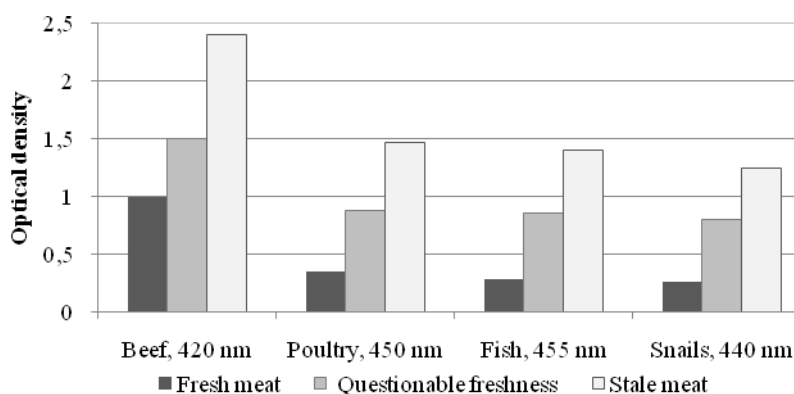


Fig. 1. Data on different types of meat by the photometric method [11,12,14]

The study [12] determined a narrow range of working wavelengths 450÷455 nm, which is between the values of our working wavelengths 440 nm and 490 nm. We compare the average absorbance for poultry meat and snail meat at the same concentration of the extract 1:10. Fresh meat: poultry 0.350 ± 0.102 (450÷455 nm); snails 0.262 ± 0.034 (440 nm) and 0.304 ± 0.033 (490 nm). Stale meat: poultry 1.456 ± 0.087 (450÷455 nm); snails 1.243 ± 0.031 (440 nm) and 1.803 ± 0.053 (490 nm). Meat of questionable freshness: poultry 0.865 ± 0.075 (450÷455 nm); snails 0.804 ± 0.054 (440 nm) and 0.851 ± 0.048 (490 nm). The results are not only qualitatively, but also quantitatively consistent with each other. Apparently, an increase in the absorbance of the extract with Nessler's reagent also results from an increase in the concentration of ammonia in the course of deterioration of the meat quality (meat spoilage).

Let us compare our findings with the results of measurements of the freshness degree of fish flesh [14] at the concentration of meat and water extracts 1:10, which was present in studies both for fish and for snails. The paper [14] determined the narrow operating wavelength range 455÷460 nm, which is between the values of our working wavelengths 440 nm and 490 nm. We compare the average absorbance for fish flesh and snail meat at the same concentration of the extract 1:10. Fresh meat: fish 0.279 ± 0.104 (455÷460 nm); snails 0.262 ± 0.034 (440 nm) and 0.304 ± 0.033 (490 nm). Stale meat: fish 1.403 ± 0.185 (455÷460 nm); snails 1.243 ± 0.031 (440 nm) and 1.803 ± 0.053 (490 nm). Meat of questionable freshness: fish 0.848 ± 0.169 (455÷460 nm); snails 0.804 ± 0.054 (440 nm)

and 0.851 ± 0.048 (490 nm). The results are qualitatively and quantitatively well consistent with each other, which is apparently due to the closeness of the ammonia composition of fish meat and snail meat.

Conclusion

Summing up the results of the research conducted, we can draw the following conclusions:

1. The authors are the first who proposed using the photometric method to evaluate the freshness degree of Roman snail (*Helix pomatia*) meat.

2. The photometric method of determining the freshness of snail meat is advantageous, as it allows clearly determining the degree of freshness of meat in numerical terms.

3. The photometric method of determining the freshness of snail meat is proposed by us as a qualitative method along with other methods of determining its safety.

4. This method allows reliably distinguishing three degrees of snail meat freshness (fresh, stale, and questionably fresh) according to the results of measuring the optical absorption density (absorbance) of light with the wavelengths 440 nm and 490 nm at the concentrations of meat-water extract 1:10, 1:15, and 1:20, with the addition of 0.50 cm^3 of Nessler's reagent ($p < 0.001$).

5. The presence of two working waves allows drawing more reliable expert conclusions about the degree of snail meat freshness with the possibility of using discriminant and cluster analysis.

6. When the freshness of snail meat is determined photometrically during storage, the indicator increases.
7. The results obtained are quantitatively and qualitatively quite in line with other authors' results on the degree of freshness determined photometrically for beef, poultry and fish.
8. The method is simple to implement and rapid, since the analysis takes less than an hour.

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ОЦІНКА СТУПЕНЯ СВІЖОСТІ М'ЯСА РАВЛИКІВ ФОТОМЕТРИЧНИМ МЕТОДОМ

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Анотація. В основу даної роботи поставлено задачу розробити спосіб визначення ступеня свіжості м'яса равликів на фотометрі фотоелектричному. Для цього проводили вимірювання оптичної густини профільрованої м'ясо-водної витяжки з додаванням реактиву Несслера. Даний спосіб може бути використаний при визначенні якості м'яса равликів у лабораторіях при їх переробці, реалізації. За результатами цього методу можна отримати кількісні значення при оцінці якості равликів різного ступеня свіжості. Досліджено 180 равликів *Helix pomatia*. Їх розділили на три групи по 60 равликів у кожній за ступенем свіжості м'яса. Розрізняли три ступені свіжості м'яса: свіже, сумнівно свіже і несвіже. Від всіх равликів кожної групи відбирали проби м'яса. Потім м'ясо подрібнювали і з кожної проби відбирали по 2,00 г. До обраних проб послідовно додавали 20,00, 30,00, 40,00 і 50,00 см³ дистильованої води. Настоювали протягом 15 хвилин, потім фільтрували. До 3,00 см³ фільтрату додавали 0,50 см³ реактиву Несслера. Вимірювання оптичної густини інтенсивності забарвлення м'ясних та водних витяжок проводили на концентраційному фотоколориметрі, розширеному в діапазоні світлових хвиль від 400 нм до 490 нм. Встановлено, що найбільша оптична густина є показником для несвіжого м'яса, найменша – для свіжого м'яса, а значення оптичної густини для сумнівно свіжого м'яса займає проміжне положення. Так, при концентрації витяжки м'яса равликів 1:10 в діапазоні світлових хвиль 440 нм показник оптичної густини несвіжого м'яса дорівнює 1,243±0,031, свіжого м'яса – 0,262±0,034, а сумнівної свіжості – 0,804±0,054. Очевидно, це пов'язано з підвищенням концентрації аміаку в процесі псування м'яса. При цьому три ступеня свіжості м'яса визначати найкраще при довжині хвиль 440 нм і 490 нм. Отримані результати якісно та кількісно співпадають з результатами фотометричних досліджень ступеня свіжості яловичини, птиці та риби.

Ключові слова: равлик, м'ясо, ступінь свіжості, фотометричний метод, дослідження.