MICELLAR EXTRACTION OF ACTIVE INGREDIENTS OF PLANT RAW MATERIALS AS A TOOL FOR IMPROVING THE QUALITY OF DIET SUPPLEMENTS AND ADDITIONAL SUBSTANCES

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ABSTRACT:

Micellar extraction method (MME - Micellar Mediated Extraction) is an alternative method of classical extraction, which takes place with the participation of surfactants. In this method, instead of an organic solvent, a highly efficient solution of surfactants is used, which dissolves the desired component in hydrophobic micelles. Moreover Micellar extraction is often used for separating analytes from complex matrices, enriching analytes in environmental research, as well as for determining trace amounts of heavy metals or toxins in biological samples.

Main goal of presented study was to compare conventional extraction (using ethanol solutions) with micellar extraction (carried out with Whey Protein Isolated - WPI solutions and Whey Protein Concentrated - WPC). The test material consisted of dried elderberry (*Sambucus nigra*) fruits. The comparison of the obtained extracts was performed by analyzing the content of reducing compounds, the flavonoid content and the ability to reduce iron (III) ions in a system of three variables: temperature, concentration and time.

The obtained results clearly shows that, the factor influencing the efficiency of micellar extraction using WPC and WPI was time. The catechin content in the studied WPC extracts from WPI. The higher ability to reduce iron (III) ions was characterized by WPC protein extract as opposed to WPI extracts. The content of flavonoids in micellar extraction using protein extracts from WPC was higher than with the use of protein extracts from WPI.

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Keywords: micellar mediated extraction, elderberry, micelle, polyphenols

Introduction

Micellar extraction method (MME) is an alternative method of classical extraction, which takes place with the participation of surfactants. In this method, instead of an organic solvent, a highly efficient solution of surface-active substances is used in hydrophobic micelles and it dissolves the desired component [Paul and Moulik 2001]. Moreover, micellar extraction is often used for separating analytes from complex matrices, enriching analytes in environmental research, as well as for determining trace amounts of heavy metals or toxins in biological samples [Madej 2009]. In comparison with liquid-liquid or solid-liquid extraction methods, it does not require the use of toxic organic solvents, and the parameters that distinguish it from others are the simplicity of its implementation and the speed of the process [Szymanowski 2000]. In addition, it can be used for the extraction of biological active substances, such as: vitamins A, E, K, B_1, salicylic acid from plant material. The efficiency of this method means that the extracted substances can be safely used in food, pharmaceuticals or cosmetics [Madej 2009]. Due to low values of critical micellar concentration (CMC), neutral surfactants (nonionic and anionic/cationic systems) used in very small quantities, is an additional advantage of this method. Nonionic surfactants have the best solubilizing properties. Factors that influence the dissolution capacity of a solution of surfactants include: structure, type of surfactant, pH of the test sample, presence of electrolytes, presence of other organic materials (polymers and monomers) and temperature and time of process [Quina and Hinze 1999]. The use of an appropriate surfactant for micellar extraction depends on the type of substance being tested. The structure of the surfactant used should enable its connection with the analyte to be determined. For this purpose, anionic, cationic, nonionic and amphoteric surfactants are used. The concentration of a given surfactant is equally important, because in the case of exceeding the critical concentration of micellisation (CMC) and reaching the cloud point micelles are formed, which combine with the analyte, as it is with nonionic compounds.

The formation of micellar systems is typical for surface-active substances (surfactants), whose molecules are composed of parts that differ in polarity. In diluted solutions, surfactants exist in the form of monomers, i.e. single molecules. As the colloidal solution develops, the concentration increases above a certain threshold, called critical micellar concentration. Surfactant monomers accumulate spontaneously and form colloidal sizes, called micelles. The mechanism of micelle formation is associated with hydrophobic interactions [Lindman 2001]. The micelle structure consists of three surfaces: the surface area, the micelle core and the volume phase area. The micelle core consists of nonpolar hydrocarbon chains, of which the first four methyl groups form the outer core and the remaining inner core. The surface area is built from polar groups of nonionic surfactants. Micelles are usually composed of 50 - 200 monomeric molecules, and their size and shape is energy dependent and geometrically [Eastone 2005]. The size characteristic of a particular surfactant is the average number of aggregations corresponding to the average number of micelle surfactant molecules. Depending on the particular surfactant and the solution conditions, micelles can take various

shapes, from spherical to ellipsoidal beads [Evangelos et al. 2005]. Such a structure allows micellar aggregates to improve the solubility of hydrophobic materials and modify environmental features such as viscosity and polarity [Myers 1992].

Therefore, in order to give the highest process efficiency and carry out the extraction separation as easily as possible, a concentration of surfactants not lower than its CMC values is required. Another important factor is the pH of the test sample, which regulates the transfer of analytes to the micellar phase, when the appropriate concentration of hydrogen ions is applied. The highest extraction efficiency is achieved at the pH value at which the uncharged form of the analyte is predominant [Carabias - Martinez et al. 2000]. Only pH does not affect the efficiency of the process, as the extracted substance does not occur as jn, which strongly interacts with micellar aggregates [Ferrera et al. 2004]. A particularly important factor affecting the critical micellar concentration (CMC) in the micelle extraction is the proportion of neutral salt (e.g. $CaCl_2$, Na_2SO_4, NaCl). During the addition of salt, the sizes of the micelles formed increases as well as the number of aggregates. It affects the better extraction efficiency of polar substances as opposed to hydrophobic compounds. This factor is applicable in the extraction of analytes from complex matrices (environmental samples) [Kiszkiel and Hryniewicka 2011]. An important role during the extraction process is heating the sample at a given temperature, during which separation of the analytes under study occurs. Micelles are dehydrated by breaking the hydrogen bonds and thus the number of water molecules in the micellar phase is reduced. At the end of the process, the sample is still spinning, which speeds up the separation of both phases, affecting the percentage of extraction efficiency [Evangelos et al. 2005].

The basic concept describing the process of micelle is critical micellar concentration (CMC). In colloidal and surface chemistry, CMC is defined as the concentration above which micelles are formed. At low concentration of surfactant, surfactant molecules are deposited on the surface. When more surfactant is added, the surface tension of the solution starts to drop sharply because more and more surfactant molecules are on the surface. As the surface becomes saturated, the addition of surfactant molecules leads to the formation of micelles. This concentration point is called the critical concentration of micelles. Three different phases can be identified: at a very low concentration of surfactant, only a small change in surface tension is detected, the addition of surfactant drastically reduces surface tension and at the CMC point the surface becomes saturated and surfactant molecules are added do not affect the surface tension. [Mukherjee and Mysels 1971]. The lower critical micelle concentration is generally preferred because it ensures that the micelles will not destabilize during dilution. There are different methods for measuring CMC, these methods take advantage of the fact that the macroscopic properties of the aqueous dispersion of micellar aggregates change deeply into critical micellar concentrations. For example, the surface tension gradually decreases when molecules bind to the aqueous air interface, this continues until the surface tension reaches the limit value and the point at which the addition of further molecules to the aqueous dispersion will not result in a combination of molecules on the surface but will aggregate the molecules in the micelles. Measurements of

surface tension as a function of concentration thus provide for the determination of CMC [Nagadome et al. 1992].

The whole process resembles traditional liquid-liquid extraction, the only difference being that the "organic" phase is produced in the aqueous phase, transforming the previously homogeneous solution into a heterogeneous one, simply by collecting previously dispersed hydrophobic suspensions. When the solution conditions, such as temperature and pressure, are changed accordingly, phase separation occurs for the aqueous micellar solution. In other words, surfactant monomers aggregate and separate from water during the scattering of visible light. This turbid, surfactant rich phase is loaded with the hydrophobic load of the initial solution, while the aqueous supernatant inhibits the concentration of the surfactant near CMC (*Critical Micellar Concentration*) [Evangelos et al. 2005].

Main goal of presented study was to compare conventional extraction (using ethanol solutions) with micellar extraction (carried out with Whey Protein Isolated - WPI solutions and Whey Protein Concentrated - WPC). The test material consisted of dried elderberry (*Sambucus nigra*) fruits..

Experimental

Materials and methods

The research material was extracts made of elderberry. Commercial, dried fruits of elderberry were used for the preparation of extracts

For the preparation of extracts, commercial WPI and commercial WPC were used at different concentrations levels (0,1%, 0,15%, 0.2% (m/v).

Methods

Content of reducing compounds

Extracts were transferred quantitatively from 0,2 cm^3 of the solution and diluted with 9,8 cm^3 of distilled water. The solutions thus obtained were taken 5 cm^3 and mixed with 0,25 cm^3 of Folin-Ciocalteau reagent and 0.5 cm^3 of a 7% Na_2CO_3 solution was added and the mixture was stirred. Samples were incubated for 30 minutes in a dark place and after this time the absorbance of samples was tested. Measurement of absorbance was done with Spectro UV - VIS Dual Beam UVS – 2800spectrophotometer, (Labomed inc. USA), at wavelength λ = 760 nm. The calibration curve was made from a solution of gallic acid in the concentration range of 0,01-0,11 mg/ml and the equation describing it was:

$y = 0{,}078x + 0{,}0552$ with $R^2 = 0{,}9994$. The study was performed in duplicate [Duda-Chodak et al. 2011].

Content of flavonoids compounds

The extract was diluted with 1 cm^3 and stirred with 1 cm^3 of distilled water. The solution thus obtained was taken 1 cm^3 into a 10 cm^3 volumetric flask, 5 cm^3 of redistilled water and 0,3 cm^3 of 5% (w/w) aqueous sodium nitrate solution was added. The resulting solution was stirred and allowed to stand for 5 minutes, 0,6 cm^3 10% (w/w) aqueous hexahydrated aluminum chloride solution and remixed. After 5 minutes 2 cm^3 of a 1M aqueous NaOH solution was added and the water was redistilled to the mark. The absorbance of the samples so prepared was measured at wavelength λ = 510 nm against the zero test. Calibration curve was made from quercetin solution in the concentration range 0,07-0,36 mg / ml and the equation describing it was: $y = 0.0021x + 0.1345$ at $R^2 = 0,9959$. The study was performed in duplicate [Cieszynska et al. 2011].

The ability to reduce iron (III) ions

The study of the iron ions reduction capability was determined using the spectrophotometric method using the FRAP reagent [Benzie et al. 1996].

Preparation of the FRAP reagent:

The acetate buffer: TPTZ: $FeCl_3 \times 6H_2O$ was mixed in a ratio of 10: 1: 1 + 10% distilled water. The whole was incubated for 10 min in a shaking water bath at 37 ° C.

Performing the analysis:

To 6 cm^3 of the sample, 6 cm^3 of the mixture was added, and a blank sample was prepared (instead of 0,2 cm^3 of the sample, distilled water was added) to which the test solutions were measured. Subsequently, they were incubated 10 minutes in a water bath with a shaker at 37 ˚C. Absorbance was then measured at 595 nm.

Results and discussion

Conventional extraction

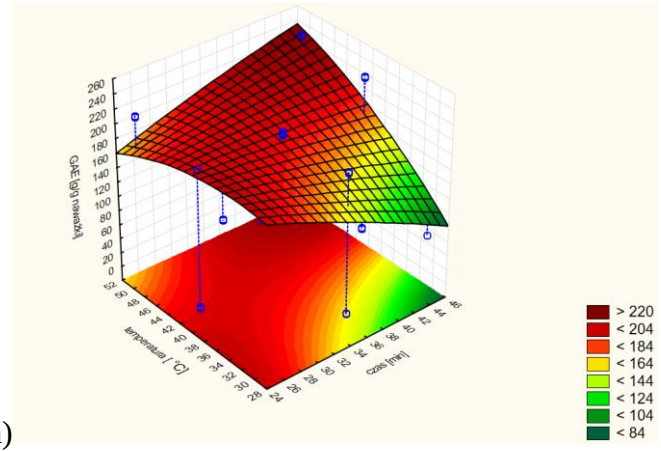
The aim of the study was to compare conventional extraction with micellar extraction (using WPI and WPC) by analysis of the content of reducing compounds, flavonoids content and ability to reduce iron (III) ions on the basis of three factors: temperature, concentration and time. The following graphs show the effectiveness of conventional extraction.

Content of reducing compounds

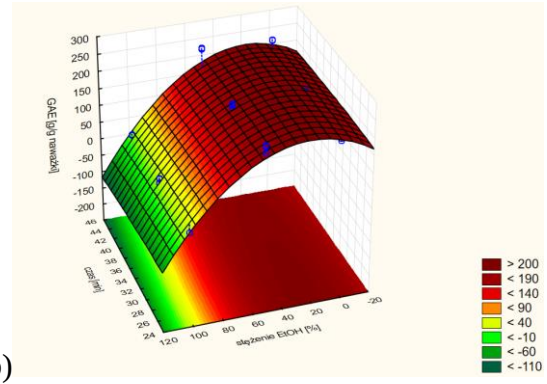
Comparing the results of the content of reducing compounds, it can be observed that for the lowest

temperature (30 ° C), the highest value was obtained in 45 min and the eluent at 50% while the lowest was in 25 min and the eluent equal to 50%. However, at the middle temperature (40 ° C) it can be seen that the highest value was obtained at the longest time and the lowest concentration of the eluent. At the highest temperature, the highest value was determined at 45 min and the eluent at 50%.

a)



b)



c)

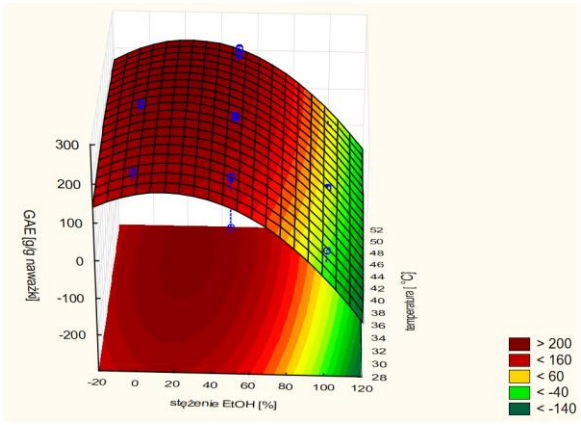


Fig. 1 a) A surface graph of the content of reducing compounds (GAE) depending on temperature and extraction time at an average concentration of 50% EtOH.

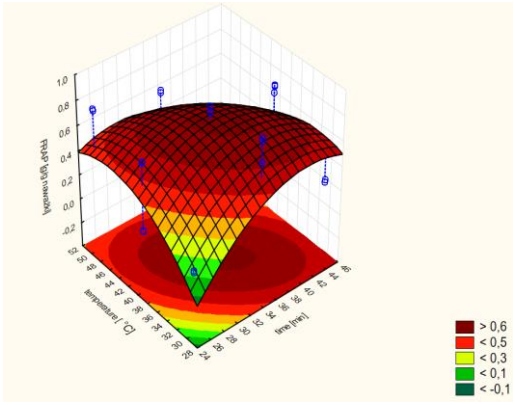
b) A surface graph of reducing contents (GAE) depending on the time of extraction and the concentration of EtOH at an average temperature of 40 ° C.

c) A surface graph of the content of reducing compounds (GAE) depending on the temperature and the concentration of EtOH with an average extraction time of 35 min.

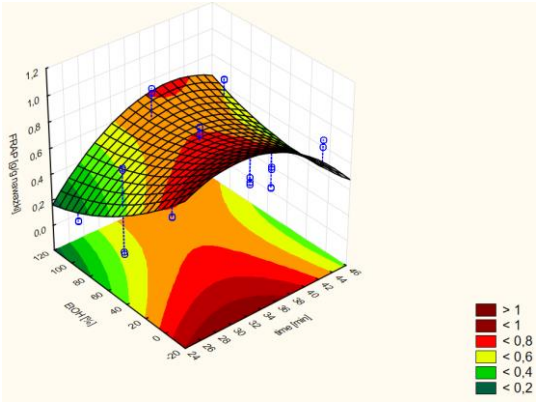
The ability to reduce iron (III) ions

The situation is different when determining the ability to reduce iron ions, because at the lowest temperature, the lowest result was obtained for 25 min and the eluent 50% and the highest for 35 min and 100% concentration. At the highest temperature, the lowest value was tested at 45 min and 50% solvent and the highest at 25 min and 50% solvent.

a)



b)



c)

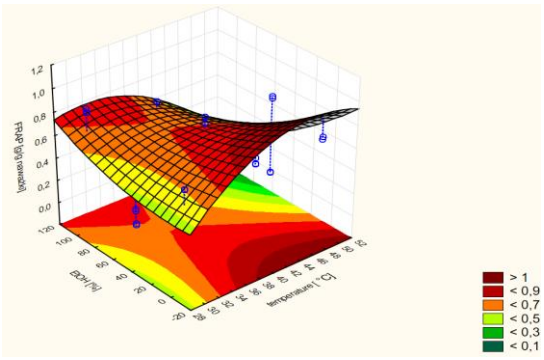


Fig. 2 a) Surface diagram of the ability to reduce iron ions depending on temperature and extraction time at an average concentration of 50% EtOH.

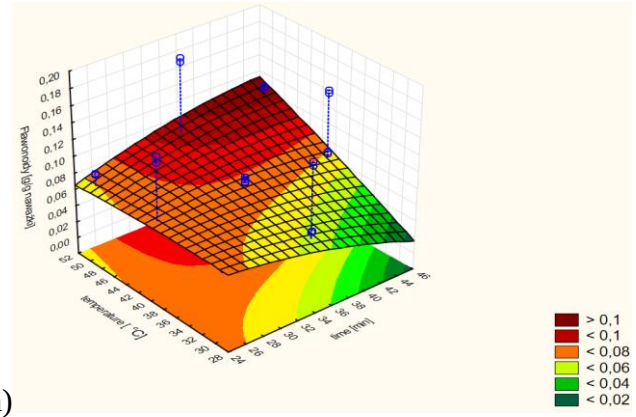
b) Surface diagram of the ability to reduce iron ions depending on the time of extraction and the concentration of EtOH at an average temperature of 40 ° C.

c) Surface diagram of the ability to reduce iron ions depending on the temperature of extraction and the concentration of EtOH at an average time of 35min.

Content of flavonoids compounds

The lowest content of flavonoids, at the lowest temperature, was characterized by a sample with an extraction time of 45 min and an eluent of 50%, and the highest determined flavonoid content with a extraction time of 35 min and 100% concentration. At the middle temperature, the lowest value was for the time of 25 min and a concentration of 0% and the highest value for 35 min and 50% of the eluent. During the extraction at the highest temperature, the lowest value was obtained during 35 min and 0% concentration and the highest at 35 min and 100% concentration.

a)



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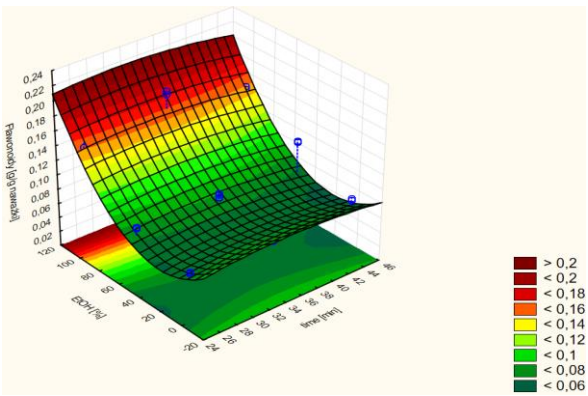


c))

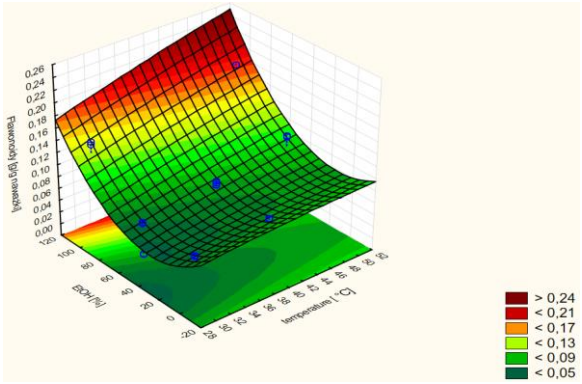


Fig. 3 a) Surface diagram of the content of flavonoids depending on the temperature and extraction time at an average concentration of 50% EtOH.

b)Surface diagram of the content of flavonoids compounds depending on the time of extraction and the concentration of EtOH at an average temperature of 40 °C.

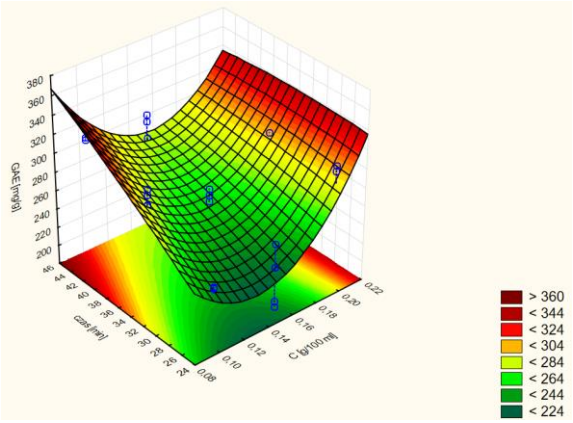
c) Surface diagram of the content of flavonoids compounds depending on the temperature of extraction and the concentration of EtOH at an average time of 35min.

Micellar Mediated Extraction

Content of reducing compounds

When testing the content of reducing compounds with the extraction with WPC at the lowest temperature at which the extract was prepared, the highest result was demonstrated by a solution whose extraction lasted 35 min and 0.1 g / 100 ml and the lowest 45 min and 0 , 2 g / 100 ml. At the middle temperature, the highest value was obtained at 35 min and 0.15 g / 100 ml and the lowest at 25 min and 0.2 g / 100 ml (in the micellar extraction with WPI also the lowest value was obtained at 25 min and 0.1 g / 100 ml). At the highest temperature, the highest result was obtained at 45 min and 0.15 g / 100 ml and the lowest at 35 min and 0.1 g / 100 ml.

a)



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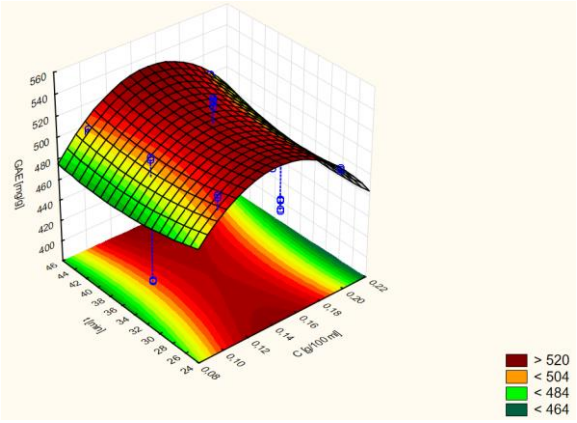
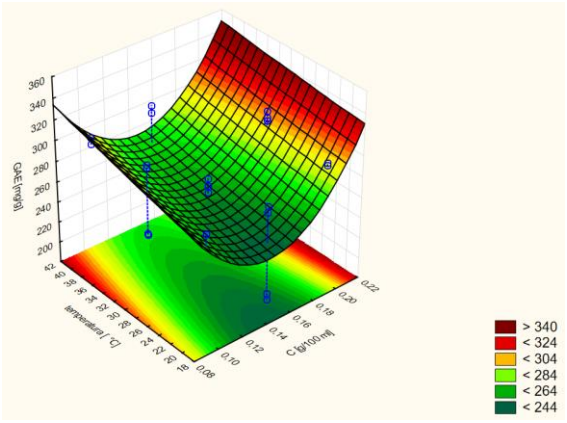


Fig. 10 Surface diagram of the content of reducing compounds (GAE) depending on time and concentration of WPI(a) and WPC (b) at an average temperature 30 °C.

a) 

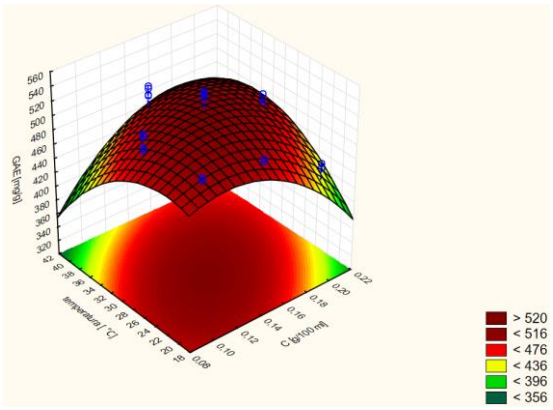
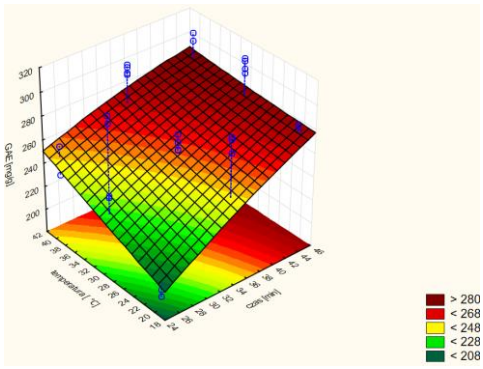
b) 

Fig. 11 Surface diagram of the content of reducing compounds (GAE) depending on temperature and concentration of WPI(a) and WPC(b) at an average time 35 min.

a) 

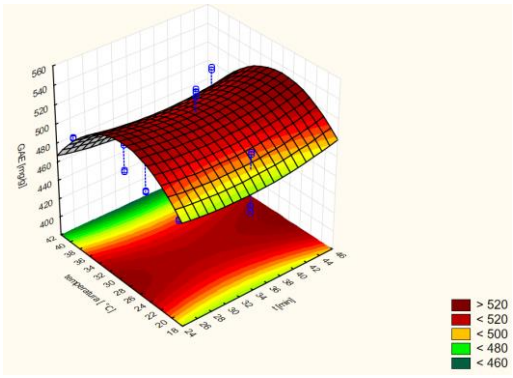
b) 

Fig. 12 Surface diagram of the content of reducing compounds (GAE) depending on time and temperature of extraction at an average concertation of WPI(a) and WPC(b) 0,15 g/100 ml.

The ability to reduce iron (III) ions

In the analysis of the ability to reduce iron ions on extracts obtained by micellar extraction at the lowest temperature, the highest yield was obtained after 45 min and 0.2 g / 100 ml, the lowest obtained after 25 min and 0.15 g / 100 ml (in micellar extraction from WPI at the same temperature the values obtained were the same). However, at the middle temperature, the highest result was obtained at 45 min and 0.1 g / 100 ml, and the lowest at 25 min and 0.1 g / 100 ml (in the micellar extraction also the highest value was obtained at 45 min and 0.1 g / 100 ml). When testing the highest temperature, it turned out that the highest value was obtained with a process lasting 25 min and 0.15 g / 100 ml, and the lowest lasting 35 min and 0.1 g / 100 ml (in the micellar extraction using WPI the highest value had an extract lasting 35 min and 0.1 g / 100 ml and the lowest 25 min and 0.15 g / 100 ml).

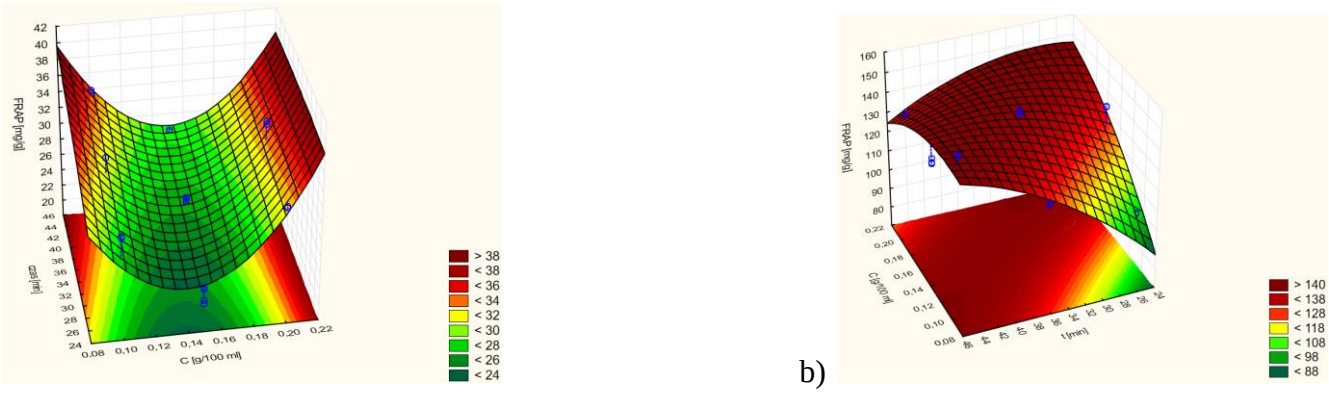


Fig. 13 Surface diagram of the ability to reduce iron (III) ions depending on the concentration of WPI(a) and WPC(b) and time at an average temperature 30 ºC.

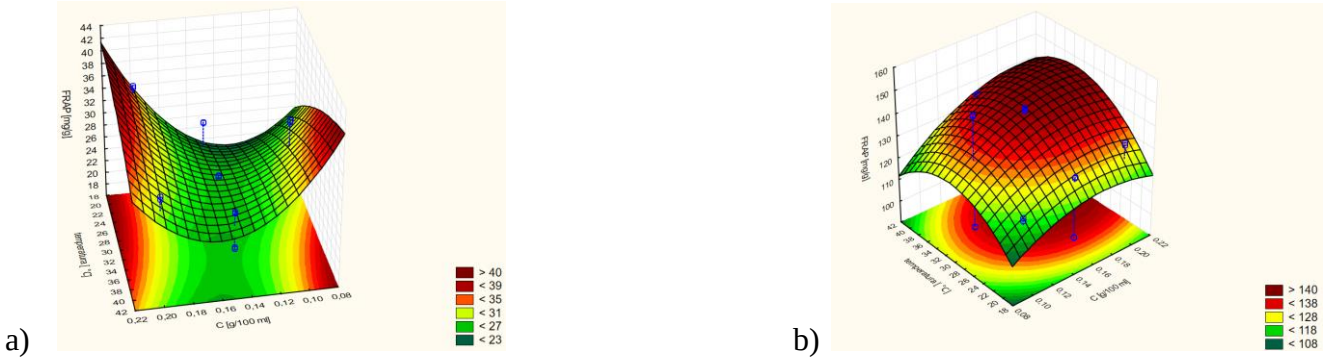


Fig. 14 Surface diagram of the ability to reduce iron (III) ions depending on the concentration of WPI(a) and WPC(b) and temperature at an average time 35 min.

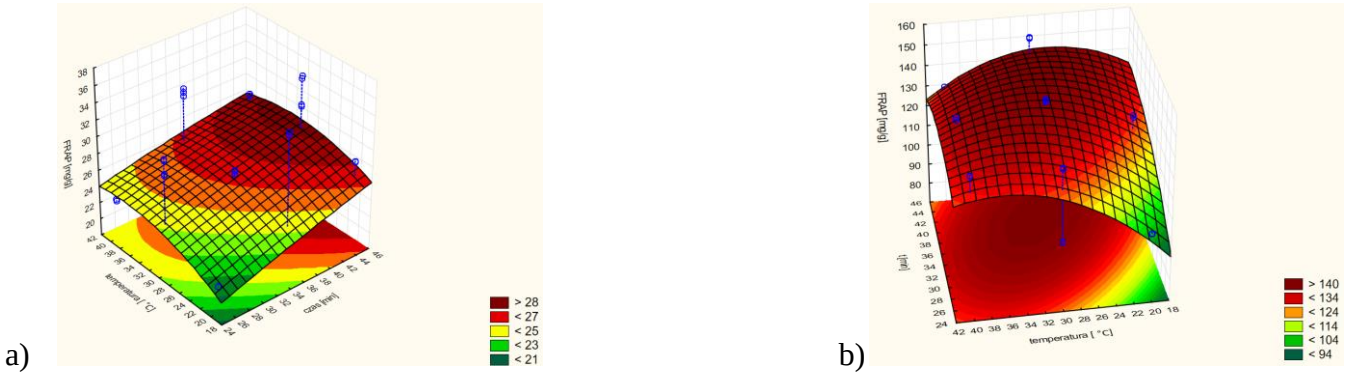


Fig. **15** Surface diagram of the ability to reduce iron (III) ions depending on the time and temperature of extraction at an average concentration of WPI 0,15g/100ml.

Content of flavonoids compounds

In the analysis of the content of flavonoids at the lowest temperature, the highest result had an extract of concentration 0.2 g / 100 ml and after 45 min and the lowest result was 0.1 g / 100 ml and time 35 min. At the middle temperature, the highest result was obtained at 45 min and 0.2 g / 100 ml and the lowest at 35 min and 0.15 g / 100 ml (in the micellar extraction from WPI the highest result was obtained for the 0.2 g / 100 ml extract and time: 25 and 45 min). At the highest temperature, the highest value obtained was obtained at 45 min and 0.15 g / 100 ml and the lowest at 25 min and 0.15 g / 100 ml.

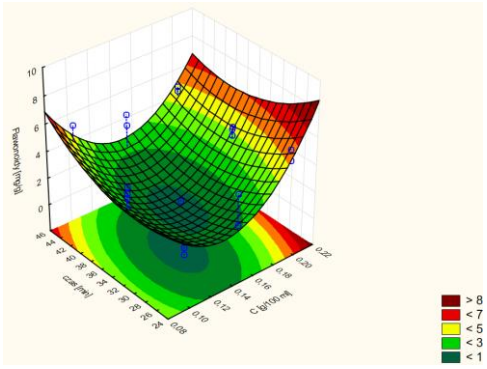
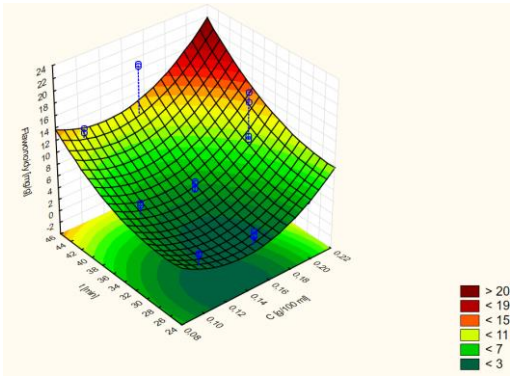
a)  b) 

Fig. 16 Surface diagram of the content of flavonoids compounds depending of the time and concentration of WPI(a) and WPC(b) at an average temperature 30 °C.

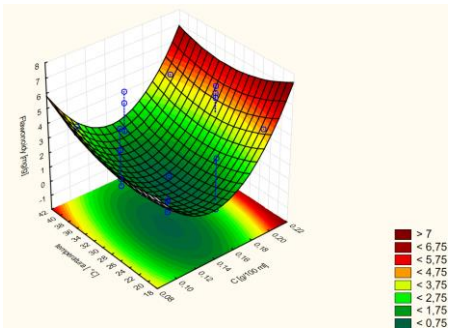
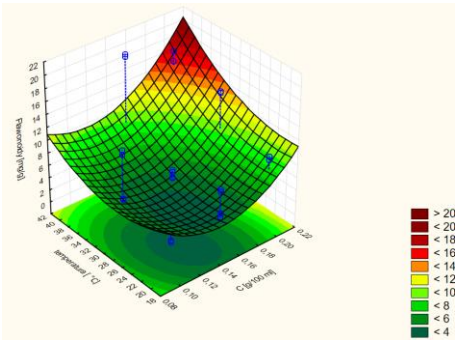
a)  b) 

Fig. 17 Surface diagram of the content of flavonoids compounds depending of the temperature and concentration of WPI(a) and WPC (b) at average time 35 min.

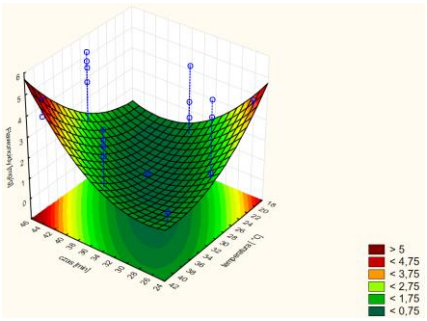
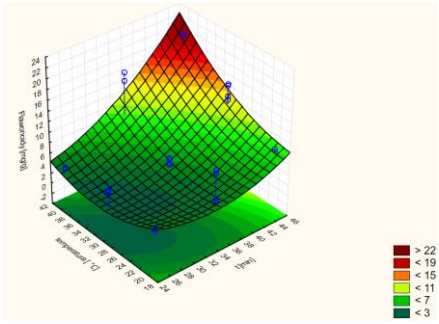
a)  b) 

Fig. 18 Surface diagram of the content of flavonoids compounds depending on the time and temperature of extraction at an average concentration of WPI(a) and WPC(b) 0,15 g/100 ml.

Conclusions

1. The total catechin content in the analyzed protein extracts (WPC and WPI) was higher than in the case of ethanol extracts. In comparison to micellar extraction, more flavonoid compounds have been extracted in conventional extraction. Higher reduction capacity of iron (III) ions was characterized by protein extract from WPC in contrast to extracts from WPI.

2. The concentration of the solution was of significant importance for the conventional extraction efficiency.

3. Time was the factor influencing the efficiency of micellar extraction using WPC and WPI.

4. During the micellar extraction using WPC and WPI proteins, the content of flavonoid compounds increases with increasing time.

Literature

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