Micellar-mediated extraction of green tea containing *Chrysanthemum morifolium* flowers

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Article history:
Received 1 December 2017
Received in revised form
22 December 2017
Accepted 23 December 2017
Available online 27 December 2017

Abstract
Micellar mediated extraction (MME) is a new and alternative method of obtaining biologically active substances, such as flavones, flavanones, anthocyanins, triterpene from plant material. In this study a series of polyethoxylated sorbitan esters of fatty acids (Tween 20, 40, 60 and 80) have been applied for the extraction of green tea containing *Chrysanthemum morifolium* flowers. Results showed that all tested surfactants were effective in the solubilisation of flavonoids and phenolic compounds. Probably, because of too high hydrophilicity, Tween 20 was not sufficient to obtain antioxidants, in contrast to another Tween. It has been shown that method is economical, attractive and ecological.

Key words: Micellar-mediated extraction, *Chrysanthemum morifolium*, non-ionic surfactants

Introduction

Micellar mediated extraction (MME) is an alternative method to the classical extractions using organic solvents. In this method, an aqueous solution of surfactant is used and the desirable substances are solubilized/adsorbed into the micelles [1]. The non-ionic or anionic/cationic systems of surfactants are used in low concentrations, which is an additional advantage of this method. The non-ionic surfactants exhibit the best solubilizing properties. The dissolving capacity of the surfactant solution depends on following factors: the structure and type of surfactant, the presence of electrolytes, presence of other organic materials (e.g. polymers or monomers) and the temperature [2].

The MME was used for the enrichment of analytes in environmental studies, for the determination of trace amounts of heavy metals or toxins in biological samples. Moreover, MME have been successfully applied to obtain biologically active substances, such as flavones, flavanones [5–7], anthocyanins [5], triterpene saponins [8], vitamins A, E, K, B1 [3, 9], paraffin [10], dyes [11–15], coumarin [16], anthraquinones [4] and salicylic acid [3, 9] from the plant material So far, a number of non-ionic surfactants have been used, such as very popular Triton X-100 [4, 11, 17], Genapol X-080 [4], mixture of SFAE surfactants (sucrose esters of fatty acids) with sodium lauryl sulphate [12–14] and the commercial surfactants such as Steareth-2 with Steareth-21 (stearyl alcohol ethoxylates), PEG-5 Glyceryl Stearate (polyethylene glycol stearate, glycerol-5), POE-5 Stearyl Stearate (polyoxyethylene stearyl-5), Glycerin Sorbitan Fatty Acid Ester (a mixture of fatty acid esters of glycerol and sorbitol), Triceteareth-4 Phosphate (tri-ethoxylated cetyl alcohol), the mixture Glyceryl Stearate and PEG-100 Stearate (glyceryl stearate and polyethylene glycol stearate), and the mixture of Ceteareth-6 , Stearyl Alcohol and Ceteareth-25 (ethoxylated cetyl alcohol, stearyl alcohol) [18] and finally silicone surfactants such as DC-190 and DC-193 [15]. However, the general mechanism of the MME is known, the detailed analysis of the relationship between the concentration of the acquired active substance and the structure of the surfactant has not been yet explained.

This study is a continuation of studies related to the use of MME for the extraction of plant material [6, 7]. Non-ionic ethoxylated fatty alcohols with different hydrophilic and hydrophobic moieties (series of Rokanol) have been used so far. Therefore, in this work, a series of Tween, polyethoxylated sorbitan esters of various fatty acids have been used. They contain the same hydrophilic fragment consisting of a sorbitan substituted with 20 oxyethylene units. As a plant material, a tea blend consisting of green tea and flowers of *Chrysanthemum morifolium* was used.

Chrysanthemum morifolium is a species of perennial plant from Asteraceae family and is a very popular plant used in Chinese medicine for many diseases. It was used to treat headaches, allergies and eye disease [19–21]. Chrysanthemum flower is rich in many chemical compounds such as flavonoids, sesquiterpenes, triterpenes and unsaturated fatty acids [22].

Materials and Methods

Chemicals

Non-ionic surfactants Tween 20, 40, 60 and 80 were purchased from Croda Poland. These surfactants are hydrophilic with HLB value: 16.7, 15.6, 14.9 and 15.0 respectively. The plant material

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for the extraction was the mixture of green tea and *Chrysanthemum morifolium* flower (1:5 w/w) purchased from local ecostore. The citric acid and sodium benzoate used for extraction were from POCH S.A. The aluminium chloride, sodium nitrite, sodium hydroxide, Folin–Ciocalteu reagent and sodium carbonate used to analysis were also from POCH S.A. The quercetin and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) were from Sigma-Aldrich.

Micelle-mediated extraction

The 1% w/w solutions of Tween 20, 40, 60 and 80 were used for extraction. The right samples were prepared in triplicate by mixing respectively 0.05 g sodium benzoate, 0.25 g citric acid, 7.5 g of herb and 100 ml of appropriate surfactant solution. The mixture was then sonicated at 300 W for 30 minutes using Ultrasonic Bath Inter Sonic. A blank sample was also prepared with demineralized water instead of surfactant solution. The samples were stored in dark glass bottles in temperature 4–8 °C. Analysis was performed after 24 hours.

Determination of flavonoid content

The quantitative determination of flavonoid compounds in extracts was carried according to Christ-Müller's method [23], by formation of a complex with aluminum chloride. The sample containing 1 ml of extract, 5 ml of demineralized water and 0.3 ml of 5% $NaNO_2$ was incubated for 5 minutes in room temperature. After that time 0.6 ml of 10% $AlCl_3$ was added and left again for 6 minutes. Next, 2 ml of 1 M NaOH was added, filled with demineralized water to 10 ml and immediately measured absorbance at λ = 510 nm (Spectrophotometer UV/VIS Macherey-Nagel). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. It means the average of nine measurements for each surfactant. The contents of flavonoids were expressed as quercetin equivalents (mgQ/ml).

Determination of polyphenol content

The content of polyphenol was determined using a color reaction with Folin-Ciocalteau reagent [24, 25]. The sample containing 1 ml of extract and 5 ml of 10% Folin-Ciocalteau reagent was incubated for 4 minutes in room temperature. After that time 4 ml of 7.5% Na_2CO_3 was added and was left again for 2 hours in dark. Then the absorbance was measured at λ = 750 nm. The samples were prepared in triplicate and the final results was the mean value of nine absorbance. The contents of polyphenols were expressed as quercetin equivalents (mgQ/ml).

Evaluation of antioxidant activity

The DPPH free radical scavenging potential was determined using methodology describe by Shabir et al. [26] The 2 ml of 0.01% DPPH solution was added to sample with 4 ml of extract and 4 ml of demineralized water. The sample was then incubated in the dark at room temperature for 30 minutes and after that time absorbance was measured at λ = 517 nm. The blank sample containing all the reagents except the extract was also prepared. The samples were prepared in triplicate and the percentage of inhibition was calculated using equation 1:

$$\%inhibition = \frac{A_0 - A_i}{A_0} \times 100\% \quad (1)$$

where: A_0 – the absorbance of blank, A_i – absorbance of sample

Determination of critical micellar concentration (CMC)

For every surfactant, the critical micellar concentration (CMC) was determined using the surface tension method (tensiometer STA1 with thermostat Huber-Ministat 125).

Results and Discussion

Micellar and aqueous extracts of *Chrysanthemum* flower were prepared to examine the total phenolic content, flavonoid concentration and antioxidant activity. The results of experiments are shown in Table 1. Generally, all studied extracts contained flavonoids and phenolic compounds. Except the most hydrophilic Tween 20 and water, obtained extracts were characterized by very good antioxidant properties. The concentration of surfactants used in micellar extraction was much above CMC, that was certainly aggregated (Table 2).

Table 1. The flavonoid content (C_f, mgQ/ml), the total polyphenol content (C_p, mgQ/ml) and the percentage of DPPH free radical inhibition (%inhibition) determined for studied extracts prepared with different surfactant solutions or demineralized water. The results are shown with standard deviation

surfactant	C_f [mgQ/ml]	C_p [mgQ/ml]	%inhibition
Tween 20	1.365±0.046	0.342±0.018	16.7
Tween 40	1.266±0.042	0.320±0.011	83.2
Tween 60	1.254±0.039	0.330±0.011	83.6
Tween 80	1.257±0.049	0.335±0.012	80.6
Water	1.980±0.126	0.352±0.011	29.1

The flavonoid content in examined plant ranged from 1.25 to 1.98 mgQ/ml. The highest amount of flavonoid was measured in aqueous extract. The extract with Tween 20 contained the highest content of flavonoids among the studied surfactants. For others, the concentration of flavonoids was comparable. These

results have correlated with the HLB of surfactants and with increased hydrophilicity the content of flavonoids also increased. The studied surfactant group was definitely small and represented only an o/w emulsifiers, so it is not possible to conclude on the global dependency of flavonoid content from hydrophobicity of surfactants. However, this is consistent with previous work for non-ionic surfactants where this tendency was maintained [6, 7].

The concentration of polyphenols in studied extracts was almost the same for every extraction medium. The total phenolic contents in plant extracts of the species *C. morifolium* did not depend on the type of extract.

The DPPH free radical scavenging potential measurements showed that extracts with Tween 40, 60 and 80 have significant (great than 80%) and comparable antioxidant properties. The other authors used Tween 20 and 80 directly as potential antioxidant and for both achieved only 3% of DPPH inhibition and noticed any effects associated with used surfactant [27]. However, another work form the same year [28] showed that value of HLB influenced on efficiency of extraction and it was the highest for surfactants with HLB between 13 and 15. The results of this work indicated that surfactants with HLB higher than 16 are ineffective in the solubilisation of compounds with antioxidant properties (other than flavonoids or phenols, e.g. terpenes).

Table 2. Determined values of critical micellar concentration (CMC) of Tweens

surfactant	CMC [%]	CMC [mM]
Tween 20	0.024	0.21
Tween 40	0.075	0.68
Tween 60	0.068	0.64
Tween 80	0.054	0.47

Conclusions

In this work, four non-ionic surfactants belonging to the group of ethoxylated sorbitan esters (Tween 20, 40, 60 and 80) were applied for MME extraction of tea mixture (green tea and *Chrysanthemum* flowers). Results showed that all tested surfactants were effective in the solubilisation of flavonoids and phenolic compounds. Probably, because of too high hydrophilicity Tween 20 was found to be not sufficient to obtain antioxidants, in contrast to another Tween. The relation between the length of hydrophobic chain of surfactants and concentration of bioactive compounds was insignificant. It seems that in the case of MME, the structure and size of the hydrophilic part, which for the surfactants tested was the same, may be more important, but further studies are needed. Despite this, it has been proved that a diluted water solutions of non-ionic surfactants were efficient for performing MME. It has been shown that method is economical, attractive and ecological. The active substances extracted by this methodology could be added directly to product, e.g. cosmetics, without purification from surfactants.

Acknowledgments

The study was financed from statutory funds of Faculty of Chemical Engineering and Technology, Cracow University of Technology.

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