Zinc-quercetin complex – from determination to bioactivity

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A B S T R A C T

Flavonoids are a group of polyphenolic compounds widely present in the herbal world and playing an important role in the human diet. The flavanol quercetin makes up 70% of the total daily intake of flavonoids. Quercetin is able to complex with many metal ions, and it exhibits potent antioxidative ability. Using the equimolar solution variation method, it was confirmed that quercetin makes a complex with the zinc(II) ion at pH 5.25, in a stoichiometric ratio quercetin:zinc(II) ion = 2:1, with the absorption maximum λmax=363 nm. This fact was used to develop a simple, precise and accurate assay to determine the content of quercetin in various samples of heterogeneous composition. The proposed indirect spectrophotometric method can selectively determine quercetin in concentrations ranging from 0.1 to 6.0 mgL−1, with LOD and LOQ estimated as 0.03 mgL−1 and 0.1 mgL−1, respectively. The reliability of the proposed method was confirmed by a previously developed RP-HPLC/UV method. The proposed method was successfully used to determine the quercetin content in dietary supplement tablets, capsules and two onion extracts, with high reproducibility. The antioxidative ability of quercetin and the zinc(II)-quercetin complex was determined using DPPH and FRAP methods. The same samples were tested for antimicrobial activity against seven laboratory control strains of bacteria and one strain of yeast. As a result of those tests, there are no obstacles to combine quercetin and zinc in the same supplement formulation.

Keywords: quercetin, zinc(II)-ion complex, antioxidative activity, antimicrobial activity, spectrophotometry; onion extracts.

1. Introduction

In the present development stage of the world population, it is necessary to recognize the health benefits and potential of agricultural products regularly present in the human diet. Quercetin (Fig. 1) is a flavonoid (plant pigment) commonly found in fruits and vegetables, especially onions, citrus and apples. Other sources include dark berries, grapes and olive oil. Green tea and red wine have also been pointed out as having notable amounts of quercetin.

![Figure 1. The chemical structure of quercetin](image-url)
The primary benefit of quercetin is that it possesses potent antioxidant properties. Antioxidants fight against free radicals - chemically reactive compounds that damage cell membranes and DNA and may cause cell death. Actually, many of the benefits that are often attributed to antioxidants refer to the effects related to quercetin. Quercetin possesses anti-inflammatory and anti-allergic abilities, realized through the inhibition of lipooxygenase and cyclooxygenase effects (Li et al., 2016). Quercetin has been reported to have an antihistamine effect, i.e. to alleviate the effects of histamine, such as the irritation of the respiratory system, redness and swelling. This is because quercetin influences numerous intracellular enzymes and may even help inhibit the release of histamine (Mcek et al., 2016). Quercetin can also be successfully applied in the treatment of late-phase and late-late-phase bronchial asthma.

Studies have shown that the consumption of flavonoids, specifically quercetin, offers at least a twofold benefit in promoting overall cardiovascular health, such as encouraging blood flow (Serban et al., 2016). Further, due to its antioxidant action, quercetin prevents LDL cholesterol oxidation, which is a very important effect because oxidation causes the sticking of LDL cholesterol to artery walls (Egert et al., 2009). In addition, a reported study confirmed that quercetin exhibits a significant reduction in triglycerides at doses above 50 mg/day (Sahebkar, 2017). Furthermore, the study performed by Milenkovic et al. (2010) reported that quercetin ameliorates experimental autoimmune myocarditis in rats, giving its role as an interfering agent in the production of proinflammatory (TNF-a and IL-17) and/or anti-inflammatory (IL-10) cytokines. In addition to supporting cardiovascular health, quercetin naturally promotes balanced blood pressure. A randomized, double-blind, placebo controlled, crossover study evaluated the effect of quercetin supplementation and reported that the participants experienced a stabilization in systolic, diastolic and average arterial pressure (Anand David et al., 2016).

If the stress levels are high and ongoing, overproduced cortisol can damage muscle tissue, leading to protein breakdown in the body. Quercetin can fight these effects during times of extended stress as it suppresses the enzyme necessary for cortisol release (Cheng and Li, 2012). Further, quercetin (a major bioflavonoid in the human diet) is a polyphenolic flavonoid with potential chemopreventive activity.

Besides the potent antioxidative ability, which is responsible for its wide spectrum of bioactivities, quercetin, like other flavonoids, has the ability to complex with many metal ions. Those complexes may be the basis for many methods developed for the determination of both flavonoids and ions. In our previous research, we have developed spectrofluorimetric and spectrophotometric methods based on the complexes formed with metal ions, and successfully applied them for the determinations of several flavonoids in fruit juices and/or pharmaceutical dosage forms (Pavun et al., 2018, Pavun et al., 2016, Pavun et al., 2014).

Having in mind the constantly present necessity of finding improved procedures applicable in routine quality control laboratory work, the aim of this work was to develop an appropriate simple, accurate and low-cost spectrophotometric method for the determination of quercetin in various samples, such as onion extracts and dietary supplements. To obtain a better look into the potential of the formatted complex, we tested antimicrobial and antioxidative activities of quercetin and its complex with the zinc(II) ion.

2. Experimental

2.1. Materials and Methods

Reagents and Materials
Quercetin (Fluka AG), methanol, NaOH, CH₃COOH, CH₃COONa, zinc-chloride, (Merck), all p.a. grade, were used. The stock solution of zinc-chloride (1.0 × 10⁻⁴ mol L⁻¹) was prepared by dissolving ZnCl₂ in double distilled water. The stock solution of quercetin (1.0 × 10⁻⁴ mol L⁻¹) was prepared by dissolving quercetin in methanol (70% v/v) and was stored in a refrigerator.

Working solutions of the zinc (II)-quercetin complex were prepared by diluting the stock solutions of zinc (II) (2.5 × 10⁻⁵ mol L⁻¹ ZnCl₂) and quercetin (5.0 × 10⁻⁷ to 1.0 × 10⁻⁵ mol L⁻¹). Acetate buffers (in 70 wt% methanol and pH 5.18), previously prepared according to the literature (Perrin and Dempsey 1974), were used for all spectrophotometric measurements.

Instruments
Spectrophotometric measurements were performed on a Beckman DU-650 spectrophotometer, using 1 cm of quartz cells. Measurements of pH were carried out using a Mettler Toledo mp 120 pH meter, equipped with a combination electrode.

2.2. Determination procedures

Spectrophotometric determination of flavonoids
The calibration curve method was used, and it required the preparation of solutions containing a constant concentration of ZnCl₂ and different concentrations of quercetin in acetate buffer (in 70 wt% methanol) pH 5.18; the blank was acetate buffer in 70% methanol pH 5.18.

The obtained data were used to calculate analytical validation parameters for the spectrophotometric method according to the literature (Miller and Miller, 2010, ICH Guideline Q2B, 1997). The limit of detection (LOD) was calculated by establishing the minimum level at which quercetin can be detected, according to the following formula:

\[ \text{LOD} = 3.3 \frac{S_b}{a} \]

where: \( S_b \) – standard deviation of the intercept; \( a \) – slope of the calibration line

The limit of quantification (LOQ) was determined using the following formula:

\[ \text{LOQ} = 10 \frac{S_b}{a} \]

Procedure for the analysis of quercetin in capsules
The chosen dosage forms (Quercetin + C capsules Twinlab, USA; Quercetin Plus, tablets, Nature Plus, New York, USA; Quercetin Nettle Complex, capsules, Magnifood) were prepared for analysis according to the following procedure. Ten tablets or capsules were weighed and powdered using a pestle and mortar. A portion of the powder, equivalent to the weight of one tablet, was dissolved in 100 mL 70 v/v% of methanol and kept in an ultrasonic bath at 25 °C for 30 min, after which the solution was filtered through a Millipore membrane filter with a 0.45 μm pore size.
Appropriate volumes of each filtrate and a 3.0 mL portion of $1.0 \times 10^{-5} \text{ mol L}^{-1} \text{ ZnCl}_2$ were mixed in a 10 mL volumetric flask and diluted to the mark with 70 v/v% methanol. The expected concentration of quercetin in prepared solutions was approximately $5.0 \times 10^{-5} \text{ mol L}^{-1}$. The pH values of these solutions were adjusted to 5.18. The absorbances of prepared solutions were measured at $A_{\text{abs}}=363 \text{ nm}$ against acetate buffer in 70 v/v% methanol at pH 5.18 as blank.

All measurements were performed in triplicate. Origin 8 Pro software was used for all necessary calculations and to obtain appropriate analytical parameters.

**Procedure for quercetin determination in onion extracts**

Onion extracts were obtained by a modified procedure (Lu et al. 2011) as follows: Onions (red and yellow varieties) were crushed in a blender, soaked in 70% methanol (1 g onions treated with 10 mL 70% methanol) and left in the dark for 12-24 hours. The mixture was stirred with a magnetic stirrer for 15 minutes at 900 rpm. Then, the extract was filtered using filter paper (Millipore Membrane Filter with a 0.45 μm pore size), and the filtrate was centrifuged for 15 minutes at 3000 rpm to precipitate any impurities present at the bottom. After completion of the centrifugation step, the supernatant was filtered using filter paper. The extract was stored at 4 °C in a dark place.

An aliquot of 0.25 mL of the tested onion extract was mixed with 2.5 mL ZnCl$_2$ of a concentration of $2.5 \times 10^{-5} \text{ mol L}^{-1}$ and 7.25 mL of acetate buffer pH 5.18 in methanol, and the mixture was centrifuged at 900 rpm for 5 min. The absorbance of the transparent supernatant measured at $A_{\text{abs}}=363 \text{ nm}$ with 70 v/v% methanol at pH 5.18 as blank was used with the previously obtained calibration curve to calculate the quercetin content in tested samples.

**DPPH photometric assay**

The free radical-scavenging activity of the quercetin and zinc(II)-quercetin complex solutions, as well as of onion extracts, was evaluated using the stable DPPH radical (Gardner et al., 2000). The hydrogen atom or electron donation abilities of the tested juices and pure quercetin were measured from the bleaching of the purple-colored methanol solution of DPPH.

To perform the DPPH test, it was necessary to construct the calibration curve, starting by preparing the stock solution of Trolox, c = 100 mg L$^{-1}$, and the stock solution of DPPH, c = 40 mg mL$^{-1}$. Six volumetric flasks (V=25 mL) were used to mix 12.5 mL of the stock solution of DPPH and appropriate volumes of Trolox solution to reach the trolox concentration of 0.5; 1.0; 1.5; 2.0; 2.5 and 3.0 mg L$^{-1}$, and the flasks were filled to the mark with methanol. The control solution was prepared by dissolving 12.5 mL of DPPH solution in methanol. The flasks were filled to the 25 mL mark with methanol. The absorbances of the prepared working solutions were read after 60 min at $\lambda=517 \text{ nm}$, against methanol. The inhibition of the DPPH free radical was calculated from Equation 3:

$$I (\%) = \frac{A_C - A_t}{A_C} \times 100 \quad (3)$$

where: $A_C$ – absorbance of the control mixture (containing all reagents except the test compound); $A_t$ – absorbance of the prepared sample or standard.

One milliliter from each tested sample was added to 12.5 mL of c = 40 mg L$^{-1}$ methanol solution of DPPH, and the volume was made up to the 25 mL mark with methanol. After 60 min, the absorbance was recorded at 517 nm, and the inhibition of the DPPH free radical was calculated from Equation 3.

**FRAP photometric assay**

Another test applied for the determination of the antioxidative ability was a slightly modified FRAP test (Benzie and Strain 1996). Although the FRAP test was firstly used for the determination of reducing substances in blood plasma, after the modification it can be successfully applied to test the antioxidative ability of plant extracts. The antioxidative entity can donate electrons to complex iron(III)-2,4,6-Tris(2-pyridyl)-s-triazine [Fe$^{3+}$-TPTZ] and reduce it to intensively blue colored iron(II)-2,4,6-Tris(2-pyridyl)-s-triazine [Fe$^{2+}$-TPTZ], in an acidic medium. The reaction can be spectrophotometrically followed at $\lambda=593 \text{ nm}$ (that is the absorption maximum of the reduced product).

To construct the calibration curve, five standard solutions containing increasing concentrations of Fe$^{2+}$ in the range 0.2 – 1 mmol L$^{-1}$ and a constant concentration of the FRAP reagent were prepared by mixing acetate buffer (300 mmol L$^{-1}$, pH 3.6), TPTZ reagent (10 mmol L$^{-1}$ in 40 mmol L$^{-1}$ HCl) and FeCl$_3$·6 H$_2$O (20 mmol L$^{-1}$) in a volume ratio of 10:1:1. The absorbance was measured at $\lambda=593 \text{ nm}$ against blank prepared by mixing 3 mL FRAP reagent and 0.1 mL water.

**Antimicrobial activity – determination of MIC**

The antimicrobial activity of quercetin and the zinc(II)-quercetin complex were investigated against eight different laboratory control strains of microorganisms (KWIKSTiK™, Microbiologics, USA). We tested seven bacterial strains, including the Gram-positive: *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* (ATCC6633), and the Gram-negative: *Escherichia coli* (ATCC 10536), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 9027), and *Salmonella abony* (ATCC 6017), and one strain of yeast *Candida albicans* (ATCC 10251).

The tests for the determination of the minimum inhibitory concentration were performed in Mueller Hinton broth (Torlak, Serbia).

The minimum inhibitory concentration (MIC) was determined by the broth microdilution method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2018). The tested compounds were suspended in distilled water, homogenized and then diluted to the highest concentration. Twofold serial concentrations of the compounds were prepared in a 96-well microtiter plate (ranging from 31.2–1000 μg mL$^{-1}$). The MIC determinations were performed in duplicate, and two positive growth controls were included. Each test was repeated three times. Tests were performed in the presence of triphenyltetrazolium chloride (TTC, Sigma-Aldrich, USA) as a microbial cell growth indicator.
3. Results and discussion

3.1. Complex formation between quercetin and the zinc(II) ion

The study of the complexation between quercetin and the zinc(II) ion started by testing the dependence of absorbance intensity on pH in the acetate buffers (in 70 v/v % methanol) of different pH values, prepared according to the literature (Perrin and Dempsey 1974). Fig. 2 presents the absorbance as a function of solution pH, showing a strong pH dependence.

Figure 2. pH dependence of absorbance

To select the optimum wavelength for the proposed method, it was necessary to prepare the solution of quercetin (c = 5 × 10⁻⁵ mol L⁻¹), and the solution obtained by mixing quercetin solution (c = 5 × 10⁻⁵ mol L⁻¹) and ZnCl₂ solution (c = 2.5 × 10⁻⁶ mol L⁻¹); the excess of quercetin made the Zn²⁺-quercetin complex formation quantitative. The recorded absorption spectra of two prepared solutions in the region 300–500 nm, against 70% V/V methanol as blank, showed that the absorbance maximum of the zinc(II)-quercetin complex was λmax = 363 nm, at pH 5.25, Figure 3. The solution of ZnCl₂ did not exhibit significant absorbance in the range 250–400 nm.

Figure 3. Absorption spectra of the Zn–quercetin complex, pH = 5.25

The equimolar solution variation method (Irving H., Pierce 1959) was used to determine the ratio of the components in the Zn²⁺-quercetin complex. The absorbances of a series of solutions formed by mixing equimolar solutions of Zn²⁺ and quercetin (5.0 × 10⁻⁵ mol L⁻¹), at pH= 5.25, were recorded at 363 nm. The dependence of the absorbance of tested solutions on molar fractions of the Zn²⁺ ion gave a curve with the maximum at X₉₀₅ = 0.67, Fig. 4, which showed that the complex composition was Zn²⁺: quercetin = 1:2.

Figure 4. Determination of the Zn²⁺–quercetin complex composition by the eqimolar solutions method

The composition of the complex was also checked by the mole ratio method (Yoe and Jones 1944). The absorbances of solutions containing a constant concentration of Zn²⁺ (5.0 × 10⁻⁵ mol L⁻¹) and different concentrations of quercetin (2.5–40 × 10⁻⁵ mol L⁻¹) were measured at a constant pH (5.25) at 363.0 nm. A straight line, A = f (c₉₀₅ / c₉₀₅Zn²⁺), with the intercept at c₀₉₀₅ / c₀₉₀₅Zn²⁺ = 2, was obtained, Figure 5, and also proved that the stoichiometric ratio of Zn²⁺: quercetin in the complex was 1:2.

Figure 5. The mole ratio method applied for the Zn²⁺–quercetin complex at pH 5.25

The conditional stability constant of the Zn(quercetin)²⁺ complex at pH = 5.25, calculated according to the literature (Inczédy, 1976), provided a value logβ² = 10.24 ± 0.02. Such a high stability constant proves the possibility of using this complex as
the basis for developing a spectrophotometric determination of quercetin.

As natural sources of flavonoids, agricultural products often contain a number of these compounds at the same time. In supplementary products, quercetin is often combined with other flavonoids. Therefore, it is important to be sure that the proposed method for the determination of quercetin in natural sources as well as in supplements is selective towards the most common and most abundant flavonoids, such as hesperidin and rutin. Rutin builds complexes that are stable in a basic environment and which exhibit maximum absorbance in the visible part of the spectrum. In contrast, quercetin (as well as hesperidin) builds complexes with zinc that are more stable in acidic solutions and that exhibit maximum absorbance in the UV part of the spectrum. This method allows the two bioflavonoids (quercetin and rutin) to be determined in parallel using buffers of different pH values. Further, our previous work (Pavun and Uskoković-Marković, 2019) determined the maximum absorbance of the zinc-hesperidin complex at λmax = 283 nm, at pH 3.12, thus confirming that hesperidin will not interfere with the zinc(II) complex based spectrophotometric determination of quercetin at λmax = 363 nm, and pH 5.25.

3.2. Spectrophotometric determination of quercetin - method development

Linearity, LOD (Limit of Detection) and LOQ (Limit of Quantification), Precision

The proposed indirect spectrophotometric method can selectively determine quercetin in concentrations ranging from 0.1 to 6.0 mg L\(^{-1}\) (Eq. 4). LOD and LOQ were derived from the calibration curve, Eqs. 1 and 2, and estimated as 0.03 mg L\(^{-1}\) and 0.1 mg L\(^{-1}\) respectively. The good linearity of the calibration curve and the small scatter of experimental points brought about a high coefficient of determination, \(r^2=0.99992\).

\[
A = (19.92 \pm 0.07) \times 10^3 \cdot c - (6.0 \pm 0.6) \times 10^{-4}, \quad (c \text{ is expressed as mol L}^{-1}) \quad (4)
\]

The satisfactory accuracy and repeatability of the method were confirmed according to the obtained results for five different concentrations of quercetin in aqueous-methanolic solutions. The recovery values were in the range 96.8–99.7%, while the coefficient of variation was within the range 0.4–1.6%.

3.3. The spectrophotometric determination of quercetin in samples

Quercetin content in tablets and capsules

The ability of quercetin to make a stable complex with the zinc(II) ion was used to develop a simple, precise and accurate assay to determine the content of quercetin in various samples of heterogeneous composition.

The linearity in such a low concentration range provides the use of highly diluted samples, which leads to the avoidance of matrix effects, which is confirmed by the standard addition method in our preliminary experiments to verify the method applied to chosen dietary samples (tablets and capsules) and onion extracts. The reliability of the method was also checked by the previously developed RP-HPLC/UV method for methanol solutions of quercetin and capsules with direct determination of quercetin after separation from other compounds. The results of the spectrophotometric determination of quercetin in dietary supplements are presented in Table 1.

| Sample                                      | Measured content of quercetin dihydrate (mg) | Recovery (%) | SD (%) |
|---------------------------------------------|---------------------------------------------|--------------|--------|
| Quercetin + C capsules Twinlab, USA         | 242.8                                       | 97.12        | 0.33   |
| Quercetin Plus, tablets, Nature Plus, New York, USA | 124.8                                       | 99.04        | 0.18   |
| Quercetin Nettle Complex, capsules, Magnifood | 197.2                                       | 98.60        | 0.16   |

Dietary supplements, prescribed for therapeutic and/or prophylactic purposes, usually contain quercetin combined with other flavonoids and ascorbic acid. After checking the selectivity of the proposed method towards other components, the method was successfully used to determine the quercetin content in dietary supplement dosage forms.

Quercetin content in onion extracts

The concentrations of quercetin in onion extracts were calculated, and they were as follows: for yellow onions \(c = 2.33 \times 10^{-4}\) mol L\(^{-1}\) and for red onions \(c = 1.23 \times 10^{-4}\) mol L\(^{-1}\).

Using these results, the content of quercetin per 1 kg of sample was calculated, namely 704 mg kg\(^{-1}\) in yellow onions and 372 mg kg\(^{-1}\) in red onions. The average amount of quercetin in onions was 284–486 mg kg\(^{-1}\) with the total content of polyphenols in natural sources varying depending on variety, geographic area, number of sunny days, soil properties, climate and other conditions (Ou et al. 2002).
3.4. Antioxidative ability tests

In addition, the antioxidative ability of quercetin, zinc (II)-quercetin complex and tested onion extracts was determined using the oxidation-reduction standardized methods DPPH and FRAP. According to the results of the applied methods, both quercetin and its complex with the zinc(ii) ion possess similar antioxidative properties in tested concentrations. The calibration curves for both DPPH and FRAP tests were obtained by the procedures explained in detail in the Experimental section 2.2.

The antioxidative ability of the samples was estimated by the DPPH test according to the equation obtained by the procedure explained in detail in the Experimental section 2.2: \( RSC = 13.65 \cdot c - 1.13 \), where RSC is the neutralization rate of DPPH, while c is the trolox concentration expressed as mg L\(^{-1}\), and \( r^2 = 0.9864 \). For the evaluation of the antioxidative effect of the samples by the FRAP test, the following equation was applied: \( A = 0.8268 \cdot c + 0.0096 \), where \( A \) is absorbance and \( c \) is the concentration of Fe\(^{2+}\), expressed as mmol L\(^{-1}\), with \( r^2 = 0.9992 \).

Antioxidative ability of quercetin and zinc(II)-quercetin complex

A solution of quercetin at a concentration of \( c_{\text{querc}} = 5 \cdot 10^{-4} \) mol L\(^{-1}\) was used for DPPH assays. After 60 min, the absorbance value was measured. The calculated neutralization rate of DPPH reagents was RCS = 29.09%, which was equivalent to the concentration of trolox \( c = 2.21 \) mg L\(^{-1}\).

A quercetin solution of a concentration \( c_{\text{querc}} = 7.5 \times 10^{-5} \) mol L\(^{-1}\) was used to test the antioxidative capacity of the FRAP test. The measured absorbance value corresponds to the antioxidative capacity of the Fe\(^{2+}\) ion with a concentration \( c = 0.267 \) mmol L\(^{-1}\).

The analysis of the antioxidant activity of the zinc-quercetin complex revealed that zinc did not significantly affect the degree of neutralization of DPPH and the antioxidative capacity of quercetin in the FRAP test. Therefore, it was found possible to use quercetin and the zinc ion in the same oral formulations.

Antioxidative ability of onion extracts

Extracts of yellow onions \( c_{\text{querc}} = 2.33 \times 10^{-4} \) mol L\(^{-1}\) were made to determine the antioxidative capacity via DPPH and FRAP assays. A solution of \( 10^{-5} \) mol L\(^{-1}\) quercetin was prepared for the DPPH test. The absorbance of the solution was measured after an hour, corresponding to a neutralization rate of RCS = 17.86% and a trolox concentration of 1.39 mg L\(^{-1}\). A solution of \( 7.5 \times 10^{-5} \) mol L\(^{-1}\) quercetin was prepared for the FRAP test. The absorbance of this solution corresponds to the antioxidative capacity of the Fe\(^{2+}\) ion solution of \( c = 0.277 \) mmol L\(^{-1}\).

Extracts of red onions \( c_{\text{querc}} = 1.23 \times 10^{-4} \) mol L\(^{-1}\) were used to make solutions of \( 10^{-5} \) mol L\(^{-1}\) for the DPPH test and \( 7.5 \times 10^{-5} \) mol L\(^{-1}\) for the FRAP test. The absorbance of the solution measured after an hour in the DPPH test corresponded to a neutralization rate of RCS = 37.70% and a trolox concentration of 2.84 mg L\(^{-1}\). The absorbance of this solution in the FRAP test corresponded to the antioxidative capacity of the Fe\(^{2+}\) ion solution of \( c = 0.338 \) mmol L\(^{-1}\).

The DPPH test showed that 1 g of onion sample had an antioxidative activity corresponding to a concentration of 3.08 mg L\(^{-1}\) trolox for yellow onions and 3.43 mg L\(^{-1}\) for red onions.

3.5. Antimicrobial activity of quercetin and zinc(II)-quercetin complex

Natural products, widely available and affordable to people all around the world, attract the attention of experts, who bring them into the focus of studies with the aim of investigating numerous potential bioactivities such as antimicrobial activity (Adamczak et al. 2020). Supplementary products often contain oligo elements, such as zinc, and flavonoids in the same dosage forms, and therefore it was interesting to test the antimicrobial potential of the zinc(II)-quercetin complex. The antimicrobial effects of quercetin and its zinc complex against tested yeast and bacteria, expressed as Minimum Inhibitory Concentration (MIC), are presented in Table 2.

| Microorganisms | MIC [µg/mL] | Quercetin | Zn(II)-quercetin |
|----------------|------------|-----------|-----------------|
| Staphylococcus aureus ATCC 6538 | 500 | 500 |
| Enterococcus faecalis ATCC 29212 | 250 | 500 |
| Bacillus subtilis ATCC 6633 | 125 | 125 |
| Escherichia coli ATCC 10536 | 500 | 250 |
| Klebsiella pneumoniae ATCC 13883 | 500 | 500 |
| Pseudomonas aeruginosa ATCC 9027 | 500 | 500 |
| Salmonella aborty NTCT6017 | 500 | 500 |
| Candida albicans ATCC 10251 | 500 | 125 |

All seven laboratory control strains of bacteria and one yeast were sensitive to quercetin and the zinc-quercetin complex, with MIC values ≤ 500 µg mL\(^{-1}\). For most of the tested bacteria, both quercetin and its zinc complex exhibited the same effects. Bacillus subtilis was the most sensitive bacteria, with the MIC of 125 µg mL\(^{-1}\) for both compounds. The same MIC value was found for the zinc-quercetin complex against the yeast Candida albicans.

The slightly better activity of the zinc complex compared with quercetin itself was expected, considering the previous report indicating a better anticancer and anti-metastasis effects of this complex on human bladder cancer cells (Lee and Tuyet 2019).
4. Conclusion

The ability of quercetin to make complex compounds with the zinc(II) ion was used to develop a simple, precise, accurate, and low-cost assay to determine the content of quercetin in various samples of heterogeneous composition. The proposed spectrophotometric method for the determination of quercetin based on the formation of the Zn\(^{2+}\)-ion-quercetin complex proved to be reproducible and accurate, with a high value of the coefficient of determination \(r^2 = 0.99992\). The method was successfully applied to determine the content of quercetin in dietary supplement tablets.

The method was also applied to determine the content of quercetin in two varieties of onions, using the adapted sample preparation method. A significantly higher content of quercetin in yellow onions compared with red ones was obtained. Using DPPH and FRAP assays, the antioxidant capacity of the red onion extract was found to be higher than that of the yellow onion extract, which could be attributed to the antioxidant capacity of other compounds in onion.

Based on the obtained MIC values, the strongest antimicrobial activity for both quercetin and the zinc(II)-quercetin complex was exhibited against Bacillus subtilis (MIC=125 \(\mu\)g mL\(^{-1}\)), while the complex had the same activity against the yeast Candida albicans. These findings and the results of the antioxidative tests indicate that, because zinc does not decrease the antimicrobial and/or antioxidative potentials of quercetin, it is possible to have both quercetin and zinc in the same dosage form of dietary supplements.

Having in mind the importance of natural products in the human diet nowadays, a particular focus should be placed on finding an appropriate method for the determination of nutriceuticals, such as flavonoids. The spectrophotometric method reported herein should be promising for this purpose, bearing in mind that selectivity to other flavonoids can be achieved by choosing an appropriate transition metal able to form complexes with flavonoids, a pH range of complex stability, different wavelengths for spectra recording, etc., with the aim of developing simple methods for the determination of numerous flavonoids.

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References

Adamczak, A., Ozarowski, M., Karpinski, T.M. (2020). Antibacterial activity of some flavonoids and organic acids widely distributed in plants. Journal of Clinical Medicine, 9, 109, 1‒17.

Anand, David, A.V., Arulmoli, R., Parasaruman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacognosy Reviews, 10(20), 84–89.

Benzie, I.F.F., Strain, J.J. (1996). The ferric reducing ability of plasma (Frap) as a measure of “Antioxidant power”: The Frap Assay. Analytical Biochemistry, 239, 70–76.

Cheng, L.C., Li, L.A. (2012). Flavonoids exhibit diverse effects on CYP11B1 expression and cortisol synthesis. Toxicology and Applied Pharmacology, 258(3), 349–50.

CLSI M07, Clinical Laboratory Standards Institute (2018). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-11th edition; January 2018, Wayne, PA, USA.

Egert, S., Bosy-Westphal, A., Seibelfl, J., Kürbitz, C., Settler, U., Plachta-Danièlek, S., Wagner, A.E., Frank, J., Schrezenmeir, J., Rabenach, G., Wolfgram, S., Müller, M.J. (2009). Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. British Journal of Nutrition, 102, 1065–74.

Gardner, P., White, T., McPhail, D., Duthe, G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chemistry, 68, 471–476.

ICH Guideline Q2B (1997). “Validation of analytical procedures: Methodology”. Federal Register, 62, 96, 27463–27467.

Inczydy, J. (1976). Analytical Applications of Complex Equilibria; Horword and Willy, New York, 137.

Irving, H., Pierce, T. (1959). Observations on Job’s method of continuous variations and its extension to r tp phase systems. Journal of Chemical Society, 2565–2574.

Lee, Y., Tuyet, P. (2019). Synthesis and biological evaluation of quercetin-zinc (II) complex for anti-cancer and anti-metastasis of human bladder cancer cells. In Vitro Cellular and Development Biology - Animal, 55, 395–404.

Li, Y., You, J., Han, C., Yang, J., Chaudhry, M.T., Wang, S., Liu, H., Yin, Y. (2016). Quercetin, inflammation and immunity. Nutrients, 8, 167–180.

Lu, X., Wangb, J., Al-Qadiri, H.M., Ross, C.F., Powers, J.R., Tang, J., Rasco, B.A. (2011). Determination of total phenolic content and antioxidant capacity of onion (Allium cepa) and shaBot (Allium oschaninii) using infrared spectroscopy. Food Chemistry, 129, 637–644.

Milenković, M., Arsenović-Ranin, N., Stojč-Vukanči, Z., Bufan, B., Vučević, D., Jančić, I. (2010). Quercetin ameliorates experimental autoimmune myocarditis in rats. Journal of Pharmaceutical Science, 13(3), 311–319.

Miller, J.N., Miller, J.C. (2010). Statistics and Chemometrics for Analytical Chemistry. 6th ed., Harlow, England, Prentice Hall/Pearson.

Micek, J., Juríkova, T., Skrovankova, S., Sochor, J. (2016). Quercetin and its anti-allergic immune response. Molecules, 21, 623–637.

Pavun, L., Uskoković-Marković, S. (2019). Spectrophotometric determination of hesperidin in supplements and orange juices. Hrana i Ishrana, 60(1), 18–23.

Pavun, L., Uskoković-Marković, S., Jelikić-Stankov, M., Dijkanović, D., Durdjjević, P. (2018). Determination of flavonoids and total polyphenol contents in commercial apple juices. Czech Journal of Food Science, 36(3), 233–238.

Pavun, L., Jelikić-Stankov, M., Durdjjević, P., Ćirić, A., Uskoković-Marković, S. (2016). Zinc complex-based determination of rutin in dietary supplements. Macedonian Journal of Chemistry and Chemistry Engineering, 35(1), 13–18.

Pavun, L., Durdjjević, P., Jelikić-Stankov, M., Dijkanović, D., Ćirić, A., Uskoković-Marković, S. (2014). Spectrofluorimetric determination of quercetin in pharmaceutical dosage forms. Macedonian Journal of Chemistry and Chemistry Engineering, 33(2): 209-215.

Perrin D.D., Dempsey, B. (1974): Buffers for pH and metal ion control. 77–94, London: Chapman and Hall.

Ou, B.X., Huang, D.J., Hampsch-Woodill, M., Flanagan, J.A., Deemer, E.K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comprehensive study. Journal of Agricultural and Food Chemistry, 50(11), 3122–3128.
Sahebkar, A. (2017). Effects of quercetin supplementation on lipid profile: A systematic review and meta-analysis of randomized controlled trials. *Critical Revision in Food Science and Nutrition*, 57, 666-676.

Serban, M.C., Sahebkar, A., Zanchetti, A., et al. (2016). Effects of quercetin on blood pressure: A systematic review and meta-analysis of randomized controlled trials. *Journal of American Heart Association*, 5, e002713.

Yoe, J., Jones A. (1944). Colorimetric determination of iron with disodium-1,2-dihydroxybenzene-3,5-disulfonate. *Industrial Engineering Chemistry and Analytical Edition*, 16, 111-115.