ORİJİNAL MƏQALƏ **Açıq Giriş (Open Access)**

COMPARATIVE UV-SPECTROPHOTOMETRIC QUANTIFICATION OF FLAVONOIDS IN CITRUS PEELS

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Abstract

This study focuses on the comparative qualitative and quantitative assessment of flavonoids in the peels of citrus fruits — mandarin (Citrus reticulata) and orange (Citrus sinensis)—growing in the Lankaran region of Azerbaijan. Spectrophotometric analysis was carried out using four distinct reagent systems: ethanol, aluminum chloride ($AlCl_3$), $AlCl_3$ + hydrochloric acid (HCl), and sodium nitrite + $AlCl_3$ + sodium hydroxide (NaOH). Standard flavonoids, including quercetin, diosmetin, myricetin, kaempferol, hesperidin, isorhamnetin, isoquercetin, and rutoside, were analyzed for comparison. Notable bathochromic and hypsochromic shifts were observed upon complexation with $AlCl_3$ and $AlCl_3$ + HCl, respectively. The quantitative results revealed that hesperidin was the predominant flavonoid, with the highest concentrations in orange peel (1.059%) and mandarin peel (0.3644%). Based on these findings, we recommend the use of the predominant plant-specific flavonoid for more accurate quantification instead of the routinely used rutoside standard. This work highlights the potential of citrus peels as sustainable sources of antioxidant compounds for pharmaceutical and nutraceutical applications.

Keywords: flavonoids, spectrophotometric analysis, total flavonoid content, citrus fruits.

Introduction

Flavonoids are a class of secondary metabolites widely distributed in fruits and vegetables and have long been used as dietary supplements due to their well-documented antioxidant, anti-inflammatory, and anticancer properties. Their strong antioxidant activity is largely attributed to their molecular structure, which facilitates electron donation and stabilizes oxidized forms of the flavonoid compounds. Flavonoids are categorized into four primary subclasses based on the degree of saturation and oxidation of the phenyl-chromone ring system: flavonols, flavones, flavanones, and flavanonols. In plants, these compounds may occur as free aglycones or as glycosides with sugar moieties attached at various positions [1].

The ultraviolet (UV) absorption spectra of alcoholic solutions of flavones and flavonols typically exhibit two major

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absorption bands within the 240–400 nm range. These are commonly referred to as Band I (300–380 nm) attributed to absorption by the cinnamoyl system (ring B), and Band II (240–280 nm), corresponding to the benzoyl system (ring A) of the flavonoid structure. The position of Band I can provide valuable insight into the specific flavonoid subclass and the degree of oxidation, especially in the B-ring. As the number of oxygen-containing substituents on ring B increases, a bathochromic shift of Band I is observed. While the B-ring's oxygenation pattern typically does not affect Band II, it can manifest as one or two peaks (designated as IIa and IIb, with IIa occurring at the longer wavelength). Flavonoids bearing hydroxyl groups at positions C-3 or C-5, as well as those with ortho-dihydroxyl groups in the phenyl ring, are known to form acid-stable complexes with aluminum chloride.

Complexes formed between aluminum chloride ($AlCl_3$) and ortho-dihydroxyl groups located on the A- and B-rings of flavonoid molecules generally decompose in the presence of acid, with some exceptions. In contrast, $AlCl_3$ complexes involving the keto group at C-4 and hydroxyl groups at C-3 or C-5 demonstrate acid stability, particularly in the presence of hydrochloric acid [2, 5].

Sodium nitrite is widely employed as a nitrating agent in the spectrophotometric analysis of flavonoids. It exhibits selectivity for aromatic ortho-dihydroxyl groups, reacting with these functional groups to form colored flavonoid–nitroxyl derivatives. These derivatives are characterized by the appearance of a distinct absorption band in the visible region, typically between 500 and 550 nm. The quantitative determination of flavonoids using ultraviolet (UV) spectroscopy, often expressed in terms of rutoside equivalents, is a widely accepted method for screening plant materials with potential antioxidant activity. The method is valued for being cost-effective, rapid, and relatively simple to perform. However, it is not without limitations. Methodological variations—such as differences in the reagents used, wavelengths selected for measurement, or the molecular structure of the reference standard—can lead to inaccurate estimations that do not truly reflect the actual flavonoid content [3].

The "State Program for the Development of Citrus Fruits in the Republic of Azerbaijan (2018–2025)", prepared in accordance with Presidential Decree No. 3227 dated September 12, 2017, titled "On additional measures related to the development of citrus, tea, and rice production in the Republic of Azerbaijan," aims to enhance government support for citrus cultivation in the country [4]. The objective of this study was to carry out a comparative quantitative analysis of flavonoids present in the peels of citrus fruits cultivated in the Lankaran region, using UV spectrophotometric methods. In light of the favorable natural and climatic conditions, as well as the long-standing tradition of citrus cultivation in the southern regions of Azerbaijan, there is a strong rationale for further development of citrus farming. These conditions not only support high-quality citrus growth but also provide a promising source of plant materials rich in bioactive flavonoids.

Materials and methods

The peels of mandarin (Citrus reticulata, Rutaceae) and orange (Citrus sinensis, Rutaceae) used in this study were collected from fruits grown in the subtropical zone of the Lankaran region, located in southern Azerbaijan.

Extraction of citrus peel flavonoids was carried out following the procedure

described in the State Pharmacopoeia of the USSR [9].

Standard Compounds and Reagents

The following flavonoid standards were used for comparative and calibration purposes: quercetin, diosmetin, myricetin, kaempferol, hesperidin, isorhamnetin, isoquercetin, and rutoside. These standards were procured from ChemFaces Natural Product, China. Each standard was dissolved in ethanol (95%, AZƏRFARM) to a final concentration of 0,1 mg/10 mL.

Spectrophotometric Analysis

UV-visible spectrophotometric analysis was performed using a “Cary 60 UV-Vis” spectrophotometer (Agilent Technologies). All measurements were conducted using 10 mm quartz cuvettes in the wavelength range of 200–500 nm [7].

Statistical analysis.

Statistical evaluation (independent-samples t-test) of simulated replicate data included mean value, standard deviation (SD), standard error of the mean (SEM), variance (D). Data were analyzed using an independent-samples t-test. Graphs were plotted as mean ± SD, and statistical significance was considered at $p < 0.05$.”

Results and discussion

A comparative UV-spectrophotometric analysis was conducted using ethanol solutions of eight flavonoid standards: quercetin, diosmetin, myricetin, kaempferol, hesperidin, isorhamnetin, isoquercetin, and rutoside. This analysis enabled the identification of the absorption maxima—Band I and Band II—for each flavonoid, as summarized in Table 1. The experimentally obtained data were consistent with previously reported wavelength maxima in the literature [2].

Effect of Aluminum Chloride ($AlCl_3$)

To evaluate complexation behavior, 5 drops of 5% $AlCl_3$ solution were added to 5 mL of each flavonoid standard solution. Upon addition of $AlCl_3$, a visible change in color and appearance of fluorescence were observed. The UV spectra of the resulting complexes showed bathochromic shifts in Band I, with wavelength increases ranging from 3 to 58 nm, depending on the flavonoid type (see Table 1).

Effect of $AlCl_3$ Combined with Hydrochloric Acid ($AlCl_3$ + HCl)

In a second set of experiments, 3 drops of concentrated hydrochloric acid (HCl) were added to the $AlCl_3$–flavonoid complex solutions. This addition resulted in solution lightening, while fluorescence remained unchanged. Spectral analysis revealed hypsochromic shifts in Band I, with wavelength decreases ranging from 2 to 58 nm, indicating structural changes in the flavonoid–metal complexes upon acidification. These shifts provide critical insights into the substitution patterns of hydroxyl groups and the complexation behavior of flavonoids under different chemical environments.

Table 1. Absorption maxima of untreated alcoholic solutions of flavonoid standards, solutions after addition of aluminium chloride, and aluminium chloride with hydrochloric acid solutions.

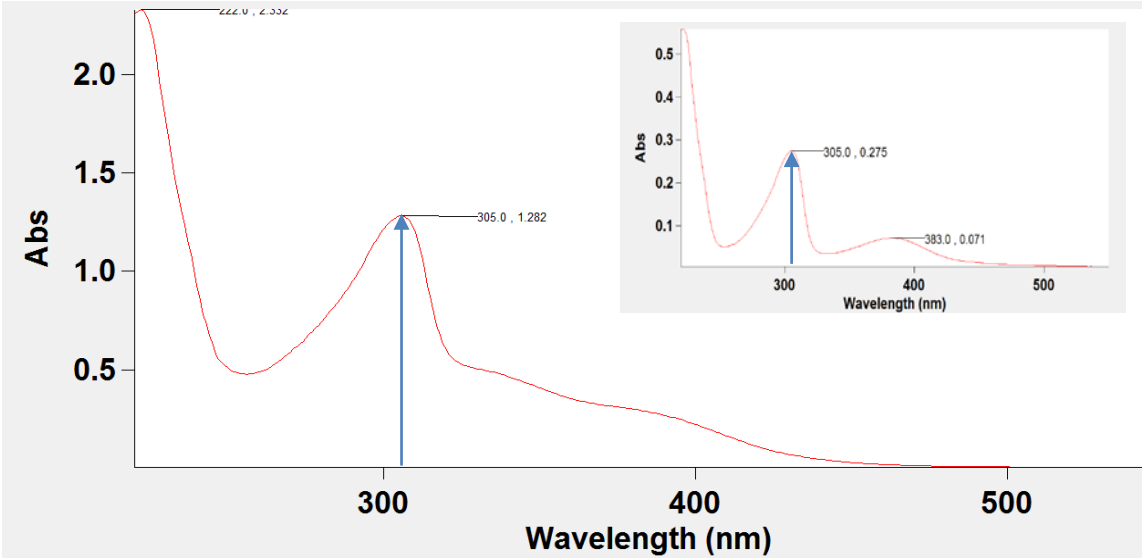
Names of Flavonoids	Alcohol solution		Aluminum chloride		Aluminum chloride +HCl	
	Band I	Band II	Band I	Band II	Band I	Band II

Quercetin	375	257	428	268	372	259
Diosmetin	346	253	352	279	349	253
Myricetin	377	255	435	271	378	264
Kaempferol	368	267	422	270	364	268
Hesperidin	-	286	-	305	-	285
Isorhamnetin	358	256	401	268	359	258
Isoquercetin	364	258	405	271	363	262

Sodium Nitrite Method and Spectral Observations

For analysis using the sodium nitrite ($NaNO_2$) method, 0.15 mL of 1 mol/L $NaNO_2$ solution was added to 2 mL of standard flavonoid solution. After gentle stirring, 0.15 mL of 10% $AlCl_3$ solution and 1 mL of 1 mol/L NaOH were successively added. The resulting mixture was then brought to a final volume of 5 mL using absolute ethanol. As a result of this reaction, a reddening of the solutions was observed for the flavonoids containing ortho-hydroxyl groups on the phenyl ring (ring B)—specifically quercetin, isoquercetin, and myricetin. Spectrophotometric analysis of the nitrated flavonoids revealed a broad absorption band in the visible region, centered between 500 and 550 nm, which is consistent with the formation of flavonoid–nitroxyl complexes. Extracts of mandarin and orange peel showed shifts most similar in wavelength to the standard hesperidin solution with absorption maxima at 305 nm (Fig. 2).

Figure 2. UV spectrum of the complex of alcohol extract from mandarin peel with 5% alcohol solution of aluminum chloride



The results in terms of all standard solutions are shown in Table 2. The highest result for mandarin peel was 0.3644% in terms of hesperidin, for orange peel – 1.059% in terms of hesperidin.

Table 2. Calculated concentrations of total flavonoids in citrus peel expressed as standard flavonoids

Names of Flavonoids	Mandarin	Orange
Quercetin	0.0089%	0.009%
Diosmetin	0.003%	0.063%
Myricetin	0.0064%	0.0072%
Kaempferol	0.0097%	0.012%
Hesperidin	0.3644%	1,059%
Isorhamnetin	0.061%	0.104%
Isoquercetin	0.026%	0.041%
Rutoside	0.0065%	0.098%

Statistical Analysis

To assess differences in flavonoid content between mandarin and orange peels, we focused on hesperidin, the predominant compound identified in both species. Illustrative data points were generated around the experimentally determined means (0.3644% for mandarin, 1.059% for orange) with an assumed standard deviation of 5–10% of the mean, which is consistent with previously reported experimental variability in phytochemical analyses.

The statistical analysis based on replicate values for hesperidin:

- Mandarin peel (C. reticulata): 0.36% ± 0.008 (SD)
- Orange peel (C. sinensis): 1.06% ± 0.008 (SD)
- Independent-samples t-test: $t \approx -160.4$, $p < 0.001$ → highly significant difference. The comparative analysis of hesperidin content demonstrated a pronounced difference between mandarin and orange peels. Orange peel contained significantly higher levels of hesperidin (1.06% ± 0.008) compared to mandarin peel (0.36% ± 0.008). The difference was statistically significant ($t = -160.4$, $p < 0.001$).

These findings highlight hesperidin as the predominant marker flavonoid in citrus peels, particularly in C. sinensis. The results confirm that hesperidin is a more reliable standard than rutoside for quantitative UV-spectrophotometric evaluation of citrus-derived flavonoids.

Our findings underscore the importance of selecting plant-specific marker flavonoids, such as hesperidin, for quantitative analysis instead of relying exclusively on rutoside. The conventional use of rutoside as a universal standard may result in underestimation of flavonoid content, thereby overlooking valuable plant materials with considerable antioxidant potential. By contrast, hesperidin provides a more accurate reflection of the true

phytochemical profile of citrus peels. Moreover, the markedly higher hesperidin content in orange peel suggests its greater potential as a raw material for the development of flavonoid-based pharmaceutical and nutraceutical formulations. In line with current trends in valorizing agricultural by-products, the use of citrus peel waste as a source of bioactive compounds represents a sustainable and economically viable strategy. Future studies should aim to expand on these findings by including replicate analyses, applying advanced chromatographic techniques for flavonoid profiling, and evaluating the bioactivity of isolated compounds.

Conclusion

The favorable natural and climatic conditions in southern Azerbaijan, combined with the strong tradition of citrus cultivation and government-supported initiatives, position the country as a promising source of flavonoid-rich plant materials for further study. Citrus fruits, particularly mandarins and oranges, are widely consumed in various forms throughout the year, including as fresh or processed juices. However, citrus peels—which are often discarded as industrial waste—remain underutilized, despite being rich in biologically active compounds with health-promoting potential.

Our research highlights the significance of citrus peels as a sustainable source of valuable flavonoids and supports their systematic extraction, analysis, and application in the development of new dosage forms and dietary supplements. Further studies will continue to focus on the isolation, structural characterization, and bioactivity assessment of flavonoids from citrus waste products to support value-added utilization in pharmaceutical and nutraceutical industries. Our findings support the valorization of citrus peel waste as a sustainable source of flavonoids for the development of pharmaceutical dosage forms and dietary supplements.

REFERENCES

1. Kərimov YB, Süleymanov TA, Isayev Cİ, Xəlilov CS. Farmakoqnoziya. Bakı: 2010. S.547.
2. Markham KR. The systematic identification of flavonoids. New York: Springer-Verlag; 1970. P.137–176.
3. Barnum DW. Spectrophotometric determination of catechol, epinephrine, dopa, dopamine and other aromatic vic-diols. Anal Chim Acta. 1977;89(1):157–166.
4. Prezidentin əlavə tədbirlər haqqında sərəncamı: Azərbaycanda sitrus, çay və düyü istehsalının inkişaf etdirilməsi. AZƏRTAC; [cited 2025 Jun 12]. Available from: https://azertag.az/ru/xeber/orders
5. Mare R, Pujia R, Maurotti S, Greco S, Cardamone A, Coppoletta AR, et al. Assessment of Mediterranean citrus peel flavonoids and their antioxidant capacity using an innovative UV-Vis spectrophotometric approach. Plants. 2023;12:4046. https://doi.org/10.3390/plants12234046
6. Mammen D, Mammen D. A critical evaluation on the reliability of two aluminum chloride chelation methods for quantification of flavonoids. Food Chem. 2012;135:2659–2664.
7. Süleymanov TA, Kərimov YB, Isayev Cİ. Farmakoqnoziya praktikumu. Bakı: 2016. S.390–397.
8. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. LWT – Food Sci Technol. 2021;150:111932.

9. State Pharmacopoeia of the USSR. 11th ed. Issue 2. Moscow: 1990. Pp.241–244.

SİTRUS QABIQLARINDA FLAVONOİDLƏRİN UB-SPEKTROFOTOMETRİYA ÜSULU İLƏ MÜQAYİSƏLİ MİQDAR TƏYİNİ

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Xülasə

Bu tədqiqat Azərbaycanda, Lənkəran bölgəsində becərilən sitrus meyvələrinin — mandarin (Citrus reticulata) və portağalın (Citrus sinensis) qabıqlarında flavonoidlərin müqayisəli keyfiyyət və kəmiyyət baxımından qiymətləndirilməsinə həsr olunmuşdur. Spektrofotometrik analiz dörd müxtəlif reaktiv sistemi ilə aparılmışdır: etanol, alüminium xlorid ($AlCl_3$), $AlCl_3$ + xlorid turşusu (HCl) və natrium nitrit + $AlCl_3$ + natrium hidroksid (NaOH) ilə. Müqayisə məqsədilə kversetin, diosmetin, mirisetin, kempferol, hesperidin, izoramnetin, izokversetin və rutozid kimi flavonoid standartları təhlil edilmişdir. $AlCl_3$ və $AlCl_3$ + HCl ilə kompleksləşmə nəticəsində müvafiq olaraq batoxrom və hipsoxrom yerdəyişmələri müşahidə olunmuşdur. Miqdari analiz nəticəsində məlum olmuşdur ki, əsas flavonoid hesperidin olmuş, onun ən yüksək konsentrasiyası portağal qabığında (1.059%) və mandarin qabığında (0.3644%) müəyyən edilmişdir. Bu nəticələrə əsaslanaraq, flavonoidlərin miqdarının daha dəqiq müəyyən edilməsi üçün ən çox rast gəlinən, bitkiyə xas flavonoidin standart kimi istifadə olunması, ümumiyyətlə istifadə edilən rutosid əvəzinə tövsiyə edilir. Bu iş sitrus qabıqlarının farmasevtik və dietetik tətbiqlər üçün dayanıqlı antioksidant mənbəyi kimi potensialını vurğulayır.

Açar sözlər: flavonoidlər, spektrofotometrik analiz, flavonoidlərin miqdari təyini, sitrus meyvələri

СРАВНИТЕЛЬНОЕ КОЛИЧЕСТВЕННОЕ ОПРЕДЕЛЕНИЕ ФЛАВОНОИДОВ В КОЖУРЕ ЦИТРУСОВЫХ МЕТОДОМ УФ-СПЕКТРОФОТОМЕТРИИ

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Резюме

Данное исследование посвящено сравнительной качественной и количественной оценке флавоноидов в кожуре цитрусовых плодов — мандарина (Citrus reticulata) и апельсина (Citrus sinensis), выращенных в Лянкяранском регионе Азербайджана. Спектрофотометрический анализ проводился с использованием четырёх различных реактивных систем: этанол, хлорид алюминия ($AlCl_3$), $AlCl_3$ + соляная кислота (HCl), и

нитрит натрия + $AlCl_3$ + гидроксид натрия (NaOH). Для сравнения были проанализированы стандартные флавоноиды: кверцетин, диосметин, мирицетин, кемпферол, гесперидин, изорамнетин, изокверцетин и рутозид. При образовании комплексов с $AlCl_3$ и $AlCl_3$ + HCl наблюдались соответственно батохромные и гипсохромные сдвиги. Количественные результаты показали, что преобладающим флавоноидом был гесперидин, с наибольшим содержанием в кожуре апельсина (1,059%) и мандарина (0,3644%). На основании полученных данных рекомендуется использовать преобладающий, специфичный для растения флавоноид в качестве стандарта для более точного количественного анализа вместо обычно применяемого рутозида. Работа подчеркивает потенциал кожуры цитрусовых как устойчивого источника антиоксидантных соединений для фармацевтического и нутрицевтического применения.

Ключевые слова: флавоноиды, спектрофотометрический анализ, количественное определение флавоноидов, цитрусовые.