

COMPARATIVE ASSESSMENT OF SOLUBLE PROTEIN CONTENT IN *PRUNUS SPINOSA* L. PLANT SAMPLES USING THE BRADFORD METHOD

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Abstract. The article presents results of determining soluble protein content in plant samples of *Prunus spinosa* L. using the Bradford method and assessing their comparative characteristics. The study used samples obtained from the leaves of *P. spinosa*. Protein extraction was performed using a 50 mM phosphate buffer solution at pH 7.0. To reduce the potential influence of phenolic compounds in plant tissues, polyvinylpyrrolidone (PVPP) was used during extraction. The obtained extracts were centrifuged, and the soluble protein content in the clear supernatant was determined using the Bradford colourimetric method. A series of bovine serum albumin (BSA) standard solutions was used to calculate protein concentration and construct a calibration curve.

The study showed that soluble protein content in *P. spinosa* samples varies by plant organ and by individual sample characteristics. Optical density values of leaf and fruit extracts were compared, and protein concentrations were calculated from these measurements. The Bradford method enabled a comparative assessment of soluble protein content in plant extracts. The calibration curve constructed using BSA standards served as the basis for quantitatively determining protein concentrations in the studied samples.

The results provide an opportunity to assess the distribution patterns of soluble proteins in *P. spinosa* tissues and to compare leaf samples. The findings may serve as baseline data for characterising the biochemical properties of *P. spinosa* and for planning further biochemical studies of the protein composition of plant materials.

Keywords: *Prunus spinosa* L., soluble protein, Bradford method, BSA, phosphate buffer, PVPP, protein extraction, spectrophotometry.

PRUNUS SPINOSA L. ӨСІМДІК ҮЛГІЛЕРІНДЕГІ ЕРИТІН АҚУЫЗ МӨЛШЕРІН БРЭДФОРД ӘДІСІМЕН САЛЫСТЫРМАЛЫ БАҒАЛАУ

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Аңдатпа. Мақалада *Prunus spinosa* L. өсімдік үлгілеріндегі еритін ақуыздардың мөлшерін Брэдфорд әдісімен анықтау және олардың салыстырмалы ерекшеліктерін бағалау нәтижелері қарастырылды. Зерттеу нысаны ретінде *P. spinosa* өсімдігінің жапырақтарынан алынған үлгілер пайдаланылды. Ақуыздарды экстракциялау рН 7,0 деңгейіндегі 50 мМ фосфатты-буферлік ерітінді көмегімен жүргізілді. Өсімдік ұлпаларындағы фенолдық қосылыстардың ықтимал әсерін төмендету мақсатында экстракция барысында поливинилполипирролидон (PVPP) қолданылды. Алынған экстракттар центрифугаланып, мөлдір супернатанттағы еритін ақуыз мөлшері Брэдфордтың колориметриялық әдісі арқылы анықталды. Ақуыз концентрациясын есептеу үшін сиыр сарысуы альбумині (BSA) негізіндегі стандартты ерітінділер қатары пайдаланылып, калибрлеу қисығы құрылды.

Зерттеу барысында *P. spinosa* өсімдік үлгілеріндегі еритін ақуыз мөлшерінің зерттелген өсімдік мүшесіне және үлгі ерекшеліктеріне байланысты өзгеретіні анықталды. Жапырақ және жеміс үлгілерінен алынған экстракттардың оптикалық тығыздық көрсеткіштері салыстырылып, олардың негізінде ақуыз концентрациялары есептелді. Брэдфорд әдісі өсімдік экстракттарындағы еритін ақуыздарды салыстырмалы түрде бағалауға мүмкіндік берді. BSA стандарттары бойынша алынған калибрлеу қисығы зерттелген үлгілердегі ақуыз концентрациясын сандық анықтаудың негізі ретінде қолданылды.

Алынған нәтижелер *P. spinosa* өсімдік ұлпаларындағы еритін ақуыздардың таралу ерекшеліктерін бағалауға және жапырақ үлгілерін салыстыруға мүмкіндік береді. Зерттеу нәтижелері *P. spinosa* түрінің биохимиялық ерекшеліктерін сипаттауда, сондай-ақ өсімдік материалдарының ақуыздық құрамын әрі қарай зерттеуге арналған биохимиялық талдауларды жоспарлауда бастапқы дерек ретінде негіз бола алады.

Кілт сөздер: *Prunus spinosa* L., еритін ақуыз, Брэдфорд әдісі, BSA, фосфатты буфер, PVPP, ақуыз экстракциясы, спектрофотометрия.

СРАВНИТЕЛЬНАЯ ОЦЕНКА СОДЕРЖАНИЯ РАСТВОРИМОГО БЕЛКА В ОБРАЗЦАХ РАСТЕНИЙ *PRUNUS SPINOSA* L. МЕТОДОМ БРЭДФОРДА

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Аннотация. В статье представлены результаты определения содержания растворимых белков в образцах растения *Prunus spinosa* L. с использованием метода Брэдфорда и оценки их сравнительных особенностей. В качестве объекта исследования использовали образцы, полученные из листьев растения *P. spinosa*. Экстракцию белков проводили с использованием 50 мМ фосфатного буферного раствора с pH 7,0. С целью снижения возможного влияния фенольных соединений, содержащихся в растительных тканях, в процессе экстракции использовали поливинилполипирролидон (PVPP). Полученные экстракты центрифугировали, а содержание растворимых белков в прозрачном супернатанте определяли колориметрическим методом Брэдфорда. Для расчета концентрации белка использовали ряд стандартных растворов на основе бычьего сывороточного альбумина (BSA), по которым была построена калибровочная кривая.

В ходе исследования установлено, что содержание растворимых белков в образцах *P. spinosa* изменяется в зависимости от исследуемого органа растения и особенностей образцов. Были сопоставлены показатели оптической плотности экстрактов, полученных из образцов листьев и плодов, на основании которых рассчитаны концентрации белка. Метод Брэдфорда позволил провести сравнительную оценку содержания растворимых белков в растительных экстрактах. Калибровочная кривая, построенная по стандартам BSA, использовалась в качестве основы для количественного определения концентрации белка в исследуемых образцах.

Полученные результаты позволяют оценить особенности распределения растворимых белков в тканях *P. spinosa* и провести сравнительный анализ образцов листьев. Результаты исследования могут служить исходными данными при характеристике биохимических особенностей *P. spinosa*, а также при планировании дальнейших биохимических исследований белкового состава растительного материала.

Ключевые слова: *Prunus spinosa* L., растворимый белок, метод Брэдфорда, BSA, фосфатный буфер, PVPP, экстракция белка, спектрофотометрия.

Introduction. A significant portion of leaf proteins are enzymes of the photosynthetic apparatus, especially ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which binds carbon dioxide. In addition, the leaf contains antioxidant enzymes, membrane proteins, transport proteins, and special proteins that protect the cell from stress. Therefore, the amount of soluble protein in the leaf is considered one of the most important biochemical indicators of the plant's metabolic activity and physiological state.

Methods for determining protein in plant material were developed gradually in the late 19th and early 20th centuries. One of the first methods was J. Kjeldahl's method for determining total nitrogen content. Later, the Biuret and Lowry methods enabled direct colourimetric assessment of proteins. In 1976, M. Bradford proposed a rapid and sensitive method based on the binding of the Coomassie Brilliant Blue G-250 dye to protein molecules. When the dye binds to protein, its spectral properties change, and the optical density of the formed complex is measured at 595 nm. The method's simplicity, relatively high sensitivity, and suitability for analysing small sample volumes have made it widely used in plant biochemistry (Bradford et al., 1976). However, phenolic compounds, tannins, pigments, and polysaccharides in plant leaves can co-extract with proteins and interfere with colourimetric measurements. Therefore, using polyvinylpyrrolidone (PVPP), which binds phenolic compounds, increases the accuracy and reproducibility of the results.

Drought is a major abiotic factor limiting plant growth and biochemical processes. Decreased soil moisture reduces leaf water potential, leading to stomatal closure, limiting carbon dioxide absorption, and reducing photosynthetic intensity. Under prolonged or severe water deficit, protein synthesis slows, and the degradation of some structural and photosynthetic proteins may increase. As a result, the total amount of soluble protein in the leaf decreases. However, the drought response is not unidirectional: the plant activates the synthesis of protective proteins, such as dehydrins, late embryogenesis proteins (LEA), heat shock proteins, and antioxidant enzymes. These proteins help maintain enzyme and membrane stability, reduce the harmful effects of reactive oxygen species, and restore metabolism after water deficit. Therefore, soluble protein levels under drought can vary with plant genotype, stress intensity and duration, leaf age, phenological stage, and habitat characteristics. (Bannerjee et al., 2016)

Prunus spinosa L. is a thorny shrub belonging to the Rosaceae family. It contributes to shrub community formation in natural populations, protects soil surfaces, and provides food and habitat for various animals. Because the fruits, leaves, and flowers are rich in phenolic compounds, flavonoids, anthocyanins, and other biologically active substances, most previous studies have focused on determining its phytochemical composition and antioxidant properties. The soluble protein content in the leaves of *P. spinosa*, especially in populations growing in natural and arid environments, has been relatively poorly studied. Therefore, determining leaf protein provides additional data needed to assess the physiological state and interpopulation biochemical variability of this species (Buchanan et al., 2015)

Studies on the response of *O* to drought have shown that plant populations respond to water deficit in a heterogeneous manner. Wanjiku and Bohne compared three ecotypes and found that their growth, morphological, physiological and biochemical parameters changed depending on the irrigation regime (Wanjiku et al., 2017). In subsequent experiments, K. Vander Mijnsbrugge and colleagues showed that summer drought affects leaf senescence, post-stress regrowth and bud break in the following season in *P. spinosa*. These works prove that the drought tolerance of the species is not limited only to responses to water deficit, but also to the ability to recover from stress (Vander Mijnsbrugge et al., 2025). However, these studies did not fully characterise total soluble protein content in the leaf as a key indicator. In this regard, determining the protein parameters of natural populations is relevant for explaining the ecological and biochemical features of the species.

The Mangystau region is arid, with low rainfall, high summer temperatures, intense evaporation, and unstable soil moisture. Under these conditions, plants face the combined effects of long-term water deficit, high temperatures, and soil characteristics. The microclimate, soil properties, moisture supply, and anthropogenic load differ between the Kogez and Samal-sai sites on the Karatau ridge. These differences may affect metabolic intensity and the accumulation level of soluble proteins

in the leaves of *P. spinosa* L. Therefore, comparison of samples from these sites allows us to assess the physiological state and interpopulation biochemical variability of the species in the arid conditions of Mangystau. (Imanbayeva et al., 2018)

This study aimed to determine the amount of soluble protein in *P. spinosa* leaf samples collected from the Kogez and Samal-sai sites in the Karatau Range of the Mangystau region using the Bradford method and to compare indicators between the two populations. During the study, we extracted leaf proteins with phosphate buffer, reduced the influence of phenolic compounds using PVPP, created a calibration curve using BSA standards, and conducted descriptive statistical analysis of the results. The data can supplement the initial biochemical characterisation of *P. spinosa* under arid conditions and serve as a basis for subsequent ecological, physiological, and molecular studies.

Materials and methods. We collected wild leaf samples of *P. spinosa* from the Kogez and Samal-sai sites of the Karatau Range in the Mangystau region of Kazakhstan. We divided the samples into two populations and analysed three replicates from each population.



Figure 1. *P. spinosa* on the Karatau ridge

Table 1. Characteristics of the studied *P. spinosa* specimens

No.	Plant type	Member studied	Mountain range	Sampled area	Sample mass, g
1	<i>P. spinosa</i>	Leaf	Karatau	Kogez, Mangystau region	0.100
2	<i>P. spinosa</i>	Leaf	Karatau	Samal-sai, Mangystau region	0.100

Ecological characteristics of the study areas. The studied Kogez and Samal gorges are located in the Western Karatau system and are characterised by an arid, semi-desert climate. The region's lack of moisture, high temperatures, and uneven rainfall mainly determine the spatial structure of plant communities. Although drought-adapted xerophytic species prevail in these conditions, mesophytic and ruderal plants are also widespread, depending on local water sources and terrain. Although the two sites share a similar climatic background, their soil composition, moisture regime, and grazing load differ.

The length of the Koghez Gorge is about 2000 m, and the width is 50-100 m. The gorge has moderately steep slopes and gravelly brown soils. The soil is stony, well-drained and relatively poor in nutrients. The humus content is 0.47%, carbonates 7.9%, and water-soluble salts 0.18%. The low humus content indicates low organic matter accumulation, and the relatively high carbonate content indicates that soil formation occurs in arid climate conditions. The low level of water-soluble salts indicates that the soil is not significantly salinised.

In the Kogez Gorge, a mixed hawthorn-wormwood community - *Caragana grandiflora* – *Artemisia* spp. - *Crataegus ambigua* has formed. The community includes 56 plant species, with a total projective cover of 55%. The main species include *Acanthophyllum pungens* (Bunge) Boiss, *Achnatherum splendens* (Trin.) Nevski, *Agropyron fragile* (Roth) P. Candargy, *Artemisia austriaca* Jacq., *Artemisia gurganica* (Krasch.), *Atraphaxis replicata* Lam., *Caragana grandiflora* (M. Bieb.) DC, *Crataegus ambigua* C.A. Mey, *Ephedra distachya* L., *Rosa iliensis* Chrshan, *Rosa laxa* Retz, *Salix alba* L, *Stipa caucasica* Schmalh, *Ulmus pumila* L.

The absence of livestock grazing has created favourable conditions for preserving the natural structure of the vegetation cover and restoring shrub and herbaceous species. In this regard, the Kogez community can be described as a relatively stable ecosystem with low anthropogenic disturbance. (A.A Imanbayeva et al., 2025) The Samal Gorge is about 2500 m long and 100 m wide. Its slopes range from moderately steep to steep. The fresh water flow along the gorge bottom is an important source of moisture in the arid region, creating spatial differences in soil moisture. The soil of the Samal Gorge is brown, loamy and stony. Although clay particles help retain moisture, the stony layers accelerate water movement to the lower horizons. The soil has 0.39% humus, 7.3% carbonates, and 0.12% water-soluble salts. These indicators suggest the soil is poor in organic matter and carbonates, has low salinity, and is generally well-drained.

The *Crataegus ambigua*–*Herba xerophytica* hawthorn–xerophytic herbaceous community is widespread in the Samal Gorge. We recorded 55 plant species, with a total projective cover of 50%. The community includes xerophytic, mesophytic, and ruderal species, such as *Acanthophyllum pungens*, *Agropyron fragile*, *Artemisia austriaca*, *Artemisia gurganica*, *Caragana grandiflora*, *Crataegus ambigua*, *Ephedra distachya*, *Malus sieversii*, *M. longifolia*, *Pyrus communis*, *Salix alba*, *Stipa caspia*, *S. caucasica*, and *Ziziphora tenuior*. Although the freshwater flow supports hygrophilous plant species, grazing may influence vegetation cover density, species regeneration, and overall community structure. Comparing the two sites, Kogez had 0.08 percentage points higher humus content than Samal, 0.6 percentage points higher carbonate content, and 0.06 percentage points higher water-soluble salts content. The projected vegetation cover in Kogez was also 5 percentage points higher than in Samal. These differences likely reflect the absence of grazing pressure in Kogez and the preservation of the plant community in a relatively natural state. Although the water source in Samal provides additional moisture, grazing may limit natural regeneration. These environmental and soil features may affect the physiological state of the studied plants and the amount of soluble protein in leaf tissues. In this regard, we used the same standardised extraction method for all samples to comparatively assess the biochemical characteristics of leaf samples from Kogez and Samal populations. This approach allowed us to distinguish changes arising from ecological differences between the study sites from those due to laboratory processing conditions.

For protein extraction, we weighed 0.100 g of each leaf sample and added 2.0 mL of 50 mM phosphate buffer (pH 7.0). To reduce interference from phenolic compounds, we added 40 mg of polyvinylpolypyrrolidone (PVPP) to each sample. The samples were homogenised three times for 1 min, with a 15-s break between each stage. Cool the homogenates for 10–15 min, then centrifuge at 10,000–12,000 rpm for 10 min. Remove the resulting clear supernatant without disturbing the pellet.

For the Bradford assay, add 2.0 mL of Bradford reagent to 40 µL of supernatant. Mix the mixture thoroughly, allow the colour reaction to proceed at room temperature, then measure the optical density at 595 nm. Prepare a control solution with 40 µL of distilled water and 2.0 mL of Bradford reagent, and use it to zero the spectrophotometer.

BSA standards at concentrations of 0.125, 0.250, 0.500, 0.750, and 1.000 mg/ml were analysed in the same volume and under the same conditions as the test samples. The calibration equation was

$A = aC + b$, where A is the optical density at 595 nm; C is the protein concentration in the extract (mg/ml); a is the slope; and b is the intercept. From this, $C = (A - b)/a$ was calculated.

We calculated the protein content of the starting plant material using the formula $P = (C \times V \times DF)/m$, where P is the protein content (mg/g), V is the extraction volume (2.0 mL), DF is the additional dilution factor, and m is the sample mass (0.100 g). Because we performed no additional dilution, we calculated P as $(C \times 2.0)/0.100 = C \times 20$ mg/g.

For each population, we calculated the arithmetic mean, standard deviation (SD), standard error (SE), median, minimum and maximum values, and coefficient of variation (CV). Because of the small number of replicates ($n = 3$ per population), no statistical significance analysis was performed (Haslam et al., 1989).

Results and discussion. The mean soluble protein content in the Kogez population was 0.7877 mg/g, with individual values ranging from 0.6697 to 0.9266 mg/g. The standard deviation was 0.1297 mg/g, and the coefficient of variation was 16.47%. This indicates moderate variability within the population.

The average soluble protein content in the Samal-sai population was 0.2425 mg/g, with individual values ranging from 0.1794 to 0.3054 mg/g. The coefficient of variation was 25.98%, and the relative variability was higher than in the Kogez population. The average protein content in the Kogez samples was 3.25 times higher than the Samal-sai value, with an absolute difference of 0.5452 mg/g.

Table 2. Descriptive statistics of soluble protein content in *P. spinosa* leaf samples

Population	n	Average, mg/g	SD, mg/g	SE, mg/g	Median, mg/g	Min, mg/g	Max, mg/g	CV, %	Extract
Blue	3	0.7877	0.1297	0.0749	0.7668	0.6697	0.9266	16.47	
Windy	3	0.2425	0.0630	0.0364	0.2428	0.1794	0.3054	25.98	

The differences between the two populations may be due to the physiological state of the plants, microclimate, soil moisture and nutrient content, sampling period, and other environmental conditions. Soluble protein levels may vary with developmental processes and environmental stress. However, this study design does not allow for a clear determination of the influence of individual factors.

The differences in soluble protein content in the studied populations are consistent with their environmental conditions. The higher average protein content in the Kögöz samples was observed alongside relatively high levels of humus and carbonates in the soil, good preservation of vegetation cover, and the absence of grazing pressure. Low disturbance of the natural community may provide favourable conditions for normal plant growth, preservation of the leaf apparatus, and metabolic stability (Kumar et al., 2013).

Although the permanent water source in the Samal Gorge provided moisture, the soil humus content was lower than in the Kogez, and grazing effects were observed. Grazing can mechanically damage plant tissues, reduce leaf biomass, and increase regrowth. These effects may indirectly

affect soluble protein accumulation by changing the plant's physiological state. In addition, the high coefficient of variation in the Samal samples may reflect heterogeneity in microtopography, distance to the watercourse, and grazing load at this site.

However, soil or grazing conditions alone cannot explain the observed biochemical differences. Plant age, phenological stage, leaf position, harvesting time, temperature, and sample storage conditions also affect protein content. Therefore, this work treats the relationship between ecological parameters and protein content not as a cause-and-effect conclusion, but as a scientific hypothesis to be tested in further studies (Orazov A., et al., 2022). In the future, it is recommended to measure soil moisture, total nitrogen, available phosphorus, temperature, and grazing intensity simultaneously, and to increase the number of biological replicates.

PVPP extraction yielded supernatants suitable for analysis by the Bradford method, and it reduced the influence of phenolic compounds in the leaf composition. However, the influence of pigments and residual phenolic compounds cannot be ruled out completely. Future studies should include extraction controls, run technical replicates, and verify that sample optical density values fall within the linear range of the BSA calibration curve.

The main limitation of the study is the small sample size. Therefore, treat the identified differences as preliminary descriptive results. Future studies should include more populations and independent plants, standardise the phenological period, and simultaneously measure environmental indicators.

Conclusion. Phosphate buffer extraction, PVPP, and the Bradford method allowed us to quantitatively compare the soluble protein content of *P. spinosa* leaf samples. The protein content in the Kogez population was 0.7877 ± 0.0749 mg/g, and in the Samal-sai population it was 0.2425 ± 0.0364 mg/g. Although the mean value in Kogez samples was 3.25 times higher, relative variability was higher in Samal-sai. These results provide an initial biochemical characterisation of the two populations and should be confirmed with studies using more biological replicates. Ecological comparisons showed that the Kogez site had better-preserved vegetation, relatively high soil humus content, and no grazing pressure. Although a freshwater stream runs through the Samal gorge, humus content is low, and grazing effects are noticeable. These factors may contribute to the physiological state of both populations; however, extensive ecological and biochemical studies are needed to prove their effect on soluble protein content.

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