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Biochemical analyses in the ICARDA collection of unique dried materials of lentils

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ABSTRACT

Background: Among conventional grains, lentils stand out as a rich source of nutrients, boasting high levels of protein and low levels of carbohydrates. They serve as an excellent meat alternative and are abundant in macronutrients, micronutrients, and bioactive phytochemicals. The red lentil seed coat surpasses other legumes in phenol content, showcasing remarkable antioxidant activity. Recognized as a high-level functional crop, lentils demonstrate efficacy in mitigating various health issues, including hypertension, cardiovascular disease, diabetes, and cancer.

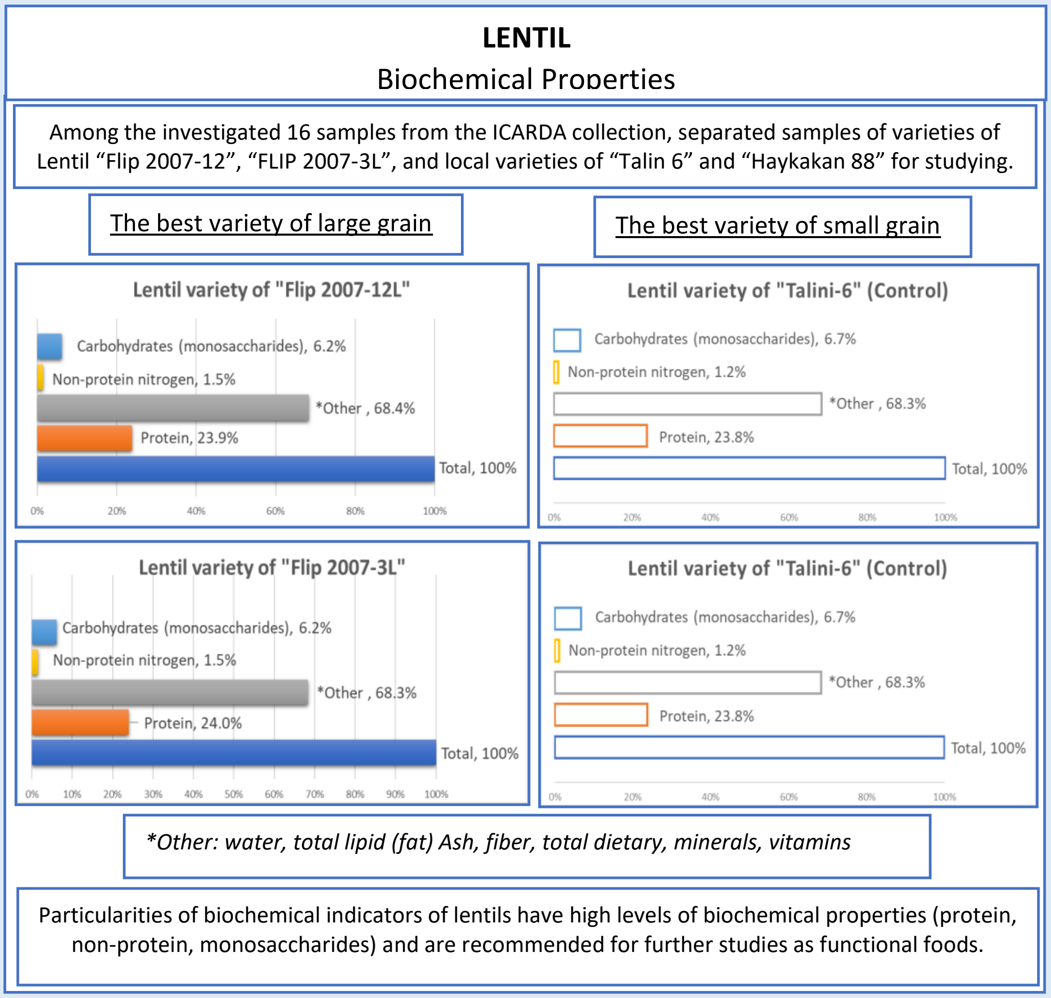
Objective: This study aims to analyze the biochemical and bioactive components, such as proteins and monosaccharides, in selected lentil varieties that thrive in the conditions of the Ararat Valley and hold economic value. The objective is to evaluate their potential as functional foods.

Methods: This study was conducted at the Scientific Centre of Vegetables and Industrial Crops in Darakert, Armenia, from 2021 to 2022, and used a block-randomized design with 4 replications. Lentil seeds were planted in mid-May, with proper care and optimal spacing. Harvested in mid-July, lentil fruits underwent biochemical analysis in the laboratory. Investigated lentil varieties included those from ICARDA collection and locally bred ones. Total protein, protein nitrogen, and carbohydrate content were determined using Kjeldahl's, Bernstein's, and Ofner's iodometric methods, respectively.

Results: The investigation into the biochemical properties of the selected lentil varieties revealed protein levels ranging from 20.5% to 23.9%, non-protein content between 1.2% and 1.6%, and monosaccharides ranging from 6.2% to 6.7%.

Conclusions: Economically valuable lentil varieties, both sourced from the ICARDA collection (Flip 2007-12L, Flip 2007-3L, Flip 2007-30L, EP-54, Sell from1767L) and locally bred varieties that are registered in the State Register of Breeding achievements of Armenia (Talini-6, Haykakan88), exhibit high concentrations of key biochemical properties such as proteins, non-proteins, and monosaccharides. When considering their potential as functional foods with various health benefits, these varieties align well with contemporary nutritional trends emphasizing the inclusion of health-promoting ingredients in diets. Further research and development in this area hold the promise of not only expanding the agricultural and culinary landscape in Armenia but also contributing to the broader conversation around nutrition and functional foods around the world.

Keywords: lentils, biochemical properties, protein, non-protein, monosaccharides



INTRODUCTION

Lentil (*Lens culinaris* Medik) is a very important and indispensable legume crop, providing nutritious, rich food for people in low- to middle-income countries worldwide. Last year's legumes have been increasingly used in various food products [1]. Creating a healthy and balanced diet includes a reduction in meat products and an increase in the use of plant-based foods. Functional foods can reduce the risk of disease [2-3]. Global production of lentils since 2000 has increased from 3.39 to 6.54 million metric tons [4]. The composition and nutritional value of lentils differ depending on genotypes [5-6]. One of the important features is that lentils can symbiotically fix atmospheric nitrogen, which provides a high amount of nitrogen to soil [7]. Esteemed lentil varieties from the ICARDA seed collections are rich in landraces and wild relative species that are vital for developing new crops by breeding in traits that can cope with the evermore harsh conditions driven by climate change. More than 85 percent of the accessions are studied for their different traits and advantages, and 75 percent are georeferenced which is a process important to developing new crops for use in similar settings. These techniques have been used to cultivate many varieties that are rich in biochemical compounds such as proteins and monosaccharides including Flip 2007-12L, Flip 2007-3L, Flip 2007-30L, EP-54, Sell From l1767L, Talini-6 and Haykakan 88. Notably, the Talini-6 variety boasts the highest monosaccharide level at 6.7%, markedly outperforming its counterparts. In terms of protein, the Flip 2007-3L variety leads with a 24% content, narrowly eclipsing the Flip 2007-12L and Talini-6 varieties. This groundbreaking study in Armenia offers the first glimpse into the biochemical profiles of these lentil varieties, setting the stage for the development of nutrient-packed functional foods. Studies show that a diet rich in legumes are directly related to health benefits in humans. [8-9] and that lentil is a crop that is rich in proteins, iron, fiber, and vitamin B. [10]. Plant proteins serve as the basis for sustainable food production and thus, secure our future [11]. When combined with rice, wheat, or other cereal grains high in sulfur-containing amino acids, lentil proteins can complete the daily essential amino acid requirements of people in developing countries and solve their problem of the need to consume animal proteins [12-13]. According to Tziouvalekas et.al, Dhull protein content in lentil seeds varies between 15.9% to 31.4%, carbohydrates between 43.4% to 74.9%, fat between 0.3% to 3.5%, total fiber between 5.1% to 26.6%, and ash between 2.2–6.4%. These wide ranges are because of the plant genetics, soil type, environmental temperature, rainfall, and use of fertilizers, herbicides, and pesticides [14-15].

Lentil polyphenols aren't just multitaskers with antidiabetic, cardioprotective, and anticancer effects; saponins are also beneficial might be able to reduce cholesterol in some patients. In order to increase bioactivity, there is a plethora of innovative techniques such as soaking, dehulling, pulsing them with electric fields, heating them up, putting them under high pressure, and using isoelectric precipitation [16,19]. Additionally, plant extracts loaded with polyphenols have sometimes been said to be a natural treatment for many chronic illnesses including cancer [20-21]. People with periodontitis might also benefit from these plants as well as people who are not consuming enough fiber in their diet. Finally, patients with heart problems might find that lentils help them immensely [22-23].

Lentils are self-pollinated crops and can be very sensitive to climatic conditions [24]. They are often adapted to cooler season conditions [24]. Often, they can be grown in soil with poor irrigation systems and their growth depends on the water that has been conserved in the soil after fall and winter rains. Modern genetic engineering and breeding approaches have been used to create stress-resistant (abiotic/biotic), climate-optimized, and high-yielding varieties of lentils with the best nutritional and functional characteristics [24-26].

The work aimed to study the samples obtained from ICARDA in the climatic conditions of Ararat Valley, Armenia to select from them high-yielding varieties with a high content of proteins and carbohydrates.

MATERIALS AND METHODS

The research was conducted at the experimental plot of the Scientific Centre of Vegetables and Industrial Crops in Darakert in the Ararat Marz of Armenia from 2021 to 2022. The experiment was set up using a block-randomized method with 4 replications. Lentil seeds

Image 1. lentil varieties both from ICARDA collection (Flip 2007-12L, Flip 2007-3L, Flip 2007-30L, EP-54, Sell From1767L) and of locally bred and registered in State Register of Breeding Achievements of Armenia (Talini-6, Haykakan88)

were planted in mid-May, with a spacing of 70 cm between rows and 20 cm between plants. Proper care, including irrigation, nutrition, fertilization, and pesticide use, when necessary, was provided to ensure optimal plant growth. The lentil fruits reached maturity in mid-July, and we collected them for biochemical analysis in the laboratory. The economically valuable lentil varieties both from ICARDA collection (Flip 2007-12L, Flip 2007-3L, Flip 2007-30L, EP-54, Sell From1767L) and of locally bred and registered in State Register of Breeding Achievements of Armenia (Talini-6, Haykakan88) were investigated under local agro-climatic conditions of Armenia Total protein determined using Kjeldahl's method, protein nitrogen using 'Bernstein's method, and carbohydrate content was measured using Ofner's iodometric method.

Determination of total protein: Lentil's seeds were crushed to produce flour. From the total mass of received flour, 1-1.5 g was transferred into the dry flask. 20 ml of concentrated sulfuric acid (relative density 1.84) was added into the same flask with further heating for moisture evaporation. 1 ml of ethanol was added to the flask. After cooling 8 g of potassium sulfate and 2-3 g of copper sulfate were added into the flask. The flask was closed, and the content was burned until it became completely white. The received white content was transferred into a 500-700 ml distillation apparatus, after distilled water was added, filling 1/3rd of the flask volume. 20-50 ml solution of sulfuric acid (0.1 N) was added to the distillation apparatus, then several pieces of pumice stone and 100 ml of 33% sodium hydroxide solution were added. The alkaline pH with litmus paper was checked. The content of the distillation apparatus was heated for 3-5 minutes. After sulfuric acid and sodium hydroxide (0.1 N) were added, a color change was recorded. For calculating the amount of nitrogen from the received results, the following formula:

$$x = \frac{(a-b)\cdot 1.4\cdot 100}{p}\,\%$$

a - quantity of 0.1 N sodium hydroxide solution used for titration of the control variant, in ml.

b - quantity of 0.1 N sodium hydroxide solution used for titration of the tested variant, in ml.

1.4 - quantity of nitrogen in milligrams, corresponding to 1 ml of 0.1 N sulfuric acid solution used to bind ammonia in the receiver.

p - weight of the test substance in grams.

Determination of protein nitrogen: 0.2 g of finely ground plant sample was added to a chemical glass with a capacity of 50-100 ml. Then, 10 ml of distilled water was carefully added along the walls of the glass, and the content was brought to a boil. The solution was mixed with a glass rod, followed by the addition of 10 ml of a 6% solution of sulfuric acid and 10 ml of a 2.5% solution of sodium hydroxide. This resulted in the formation of the alkaline salt of copper sulfate. The content in the flask was then filtered. The filter, which contained the protein precipitate, was transferred to a Keldahl flask, and washed with a mixture of sulfuric and perchloric acids. After determining the nitrogen through distillation, the protein content was calculated by multiplying the resulting number by a coefficient of 6.25.

Determination of carbohydrates: 5 ml of the studied solution, 45 ml of distilled water, and 50 ml of Ofner's reagent were added into a 300 ml flask and brought to a boil on a grid for 4-5 minutes. After that, the solution was moderately boiled for 5 minutes and cooled in cold water. 15 ml of hydrochloric acid solution was poured into the flask. Immediately after that, an iodine solution was added. The flask was capped, and the content was stirred occasionally by rotating the flask, left for 2 minutes, and titrated with hyposulfite. Each milliliter of hyposulfite corresponded to 1 mg of glucose. The amount of reducing sugar is calculated as a percentage using the following formula:

$$x = \frac{(a-b)K\cdot 100\cdot 1000V_1}{nV_2}$$

a - the amount of hyposulfite used for titration of the control variant, ml.

b - the amount of hyposulfite used for titration of the tested variant, ml.

K - coefficient of conversion of hyposulfite to the reducing sugar in mg.

n - measured quantity of sample

V_1 - volume of sample dissolution, ml.

V_2 - is the volume of the sample taken for titration, ml.

Statistical analysis: The experimental data was subjected to statistical processing using the ANOVA method.

RESULTS

From Fig. 1, it is clear the highest content of monosaccharides was in the Talini-6 variety (6.7%), which significantly exceeded the other varieties in monosaccharides content. Most of the other varieties had the same number of monosaccharides (6.2%). The lowest content was observed in the variety Sell from 1767 L (6%) (Fig. 1).

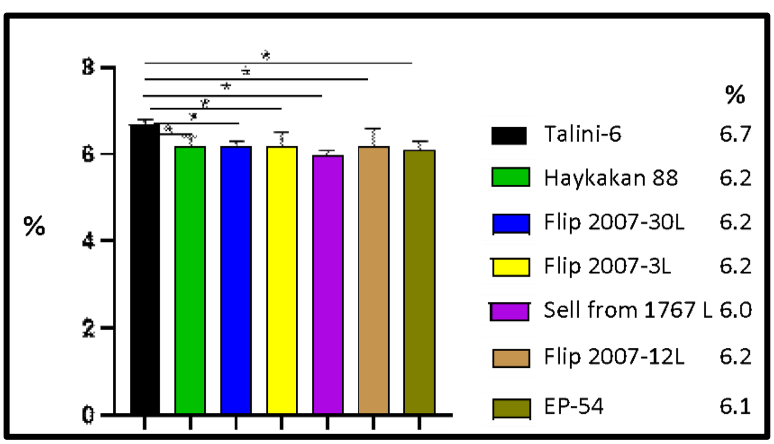


Figure 1. Content of monosaccharides in studied varieties of lentils. LSD_{05}=0.39%, S_x%=0.15

The highest content of non-protein nitrogen was observed in the variety Flip 2007-3L (1.6%), which is followed by the variety Flip 2007-12L (1.5%). The lowest values were detected for the variants Talini-6, Sell from 1767 L, and EP-54 (1.2%). The differences between the highest and lowest results were significant (Fig. 2).

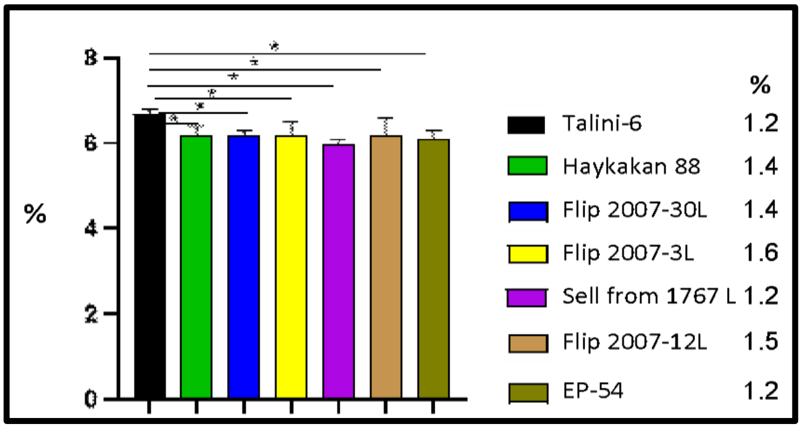


Figure 2. Content of non-protein nitrogen in studied varieties of lentils. LSD_{05}=0.19%

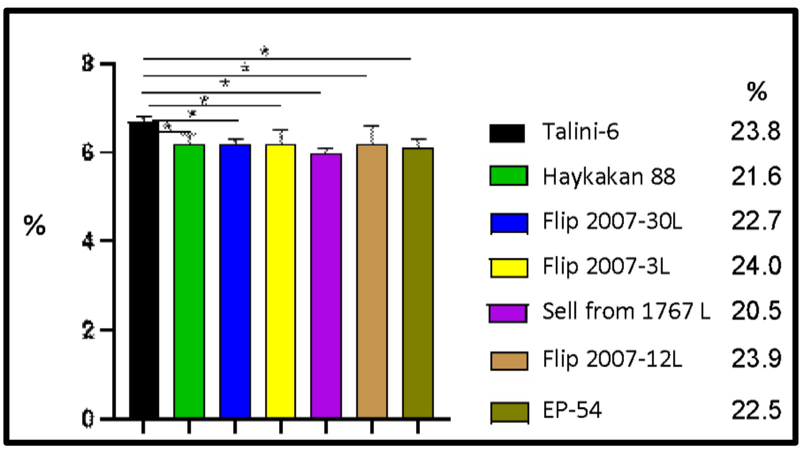


Figure 3. Content of crude protein in studied varieties of lentils. LSD_{05}=1.15%, S_X%=0.02

According to the results of biochemical analyses of the bioactive components, lentils contain 20.5-23.9% crude protein, 1.2-1.6% non-protein nitrogen, and 6.0-6.7% monosaccharides.

DISCUSSION

There is a wealth of information in the literature [16-18] about lentil composition, but usually, the results are very variable due to the genotype differences, environmental conditions, and analytical methods used. Protein content is sensitive to precipitation, light intensity, length of the growing season, day length, and environmental temperature, as well as agronomic factors such as planting density, weeds, or soil type. [23-27]. Several researchers [28-30] in their research came to the realization that the conditions of plant cultivation, ecology, and the use of different amounts of fertilizers contribute to the increase of bioactive properties in fruits, and the improvement of antioxidant properties. All this contributes to growing healthier food and making it available to the masses [31-36].

In a study by Wang and Daun, the crude protein content in four different varieties of lentils ranged from 24.3% to 30.2%. Our studied varieties showed a lower content of proteins compared with their results. According to Wang and Daun, the variational difference and environmental conditions may significantly influence the starch content, and there is an inverse correlation between the protein and starch content [37]. The studies done in western Canada between 1998 and 2003 showed that the annual mean crude protein content of lentils ranged from 25.8% to 27.1%. It was also shown that each year the range of crude protein content in the individual samples ranged from 21.4% to 30.0% [38-39]. Although the overall average crude protein content did not vary greatly during the years, factors such as environmental conditions, agronomic practices, and genetic factors impacted these results. In our study, protein content difference is in the range of 3.5%, depending on the genetic differences of the studied varieties. Flip 2007-3L and Flip 2007-12L varieties showed the highest content. The Talini-6 variety followed them with a slight difference. At the same time, the Flip 2007-3L and Flip 2007-12L varieties also showed the highest levels of non-protein nitrogen, which is not absorbed by human organisms. In contrast to them, Talini-6 showed the lowest level of non-protein nitrogen. In our study, monosaccharide content difference is in the range of 0.7% depending on the genetic differences of the studied varieties.

CONCLUSION

The economically valuable lentil varieties both from the ICARDA collection (Flip 2007-12L, Flip 2007-3L, Flip 2007-30L, EP-54, Sell from1767L) and of locally bred and registered in State Register of Breeding Achievements of Armenia (Talini-6, Haykakan88) investigated under local agro-climatic conditions of Armenia boast high levels of key biochemical components such as non-protein nitrogen, crude protein, and monosaccharides. Notably, the Talini-6 variety stands out with the highest monosaccharide level at 6.7%, surpassing its counterparts. Protein content peaks in the Flip 2007-3L variety at 24%, closely followed by Flip 2007-12L at 23.9% and Talini-6 at 23.8%. This pioneering research marks the first time in Armenia that this type a biochemical analysis of lentil genetic resources has been conducted, laying the groundwork for the development of functional food. The varieties Flip 2007-12L, and Flip 2007-3L will be submitted to the State Register of Breeding Achievements of Armenia for inclusion in the State variety testing.

Agro-biodiversity supports the livelihoods of millions of people living within rural communities around the globe. In the specific context of Armenia, it is imperative to not only protect genetic resources for global food security but also to strategically add varieties tailored to the unique climatic conditions. By prioritizing crops with high potentials for food functionality, we can enhance resilience in the face of climate change, ensure sustainable agricultural practices, and address the nutritional needs of our growing population.

List of Abbreviations: Glycemic index, GI; Nitrogen, N; International Center for Agricultural Research in the Dry Areas, ICARDA

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Competing Interests: There are no conflicts of interest to declare.

Authors' Contributions: G. Sh., K. S., designed the research. G. Sh., provided a variety Flip2007-12L, Flip2007-3L, Flip 2007-30L, Ep-54, Sell from1767L, and local sports Talini-6, and Haykakan 88 for research. L. M., G. Sh., performed biochemical analysis. G. M., performed statistical analyses. G.Sh., and G.M., wrote the article. All authors read and approved the final version of the manuscript.

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