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QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF SELECTED LETTUCE HYBRIDS (*LACTUCA SATIVA* L.) IN A PROTECTED AGROSPACE

SUMMARY

In order to solve the mentioned problem, two-factor experimental research was conducted with eight lettuce hybrids: Tonale, Donertie, Hetti, Limeria, Nolanie, Australiae, Cencibel and Saturdai. Lettuce was grown in the winter period in a protected agricultural area without a heating system, and the quantitative and qualitative component of the yield was monitored with an emphasis on the vitamin C content, and the amount of accumulated nitrates and nitrites depending on the hybrid, the technological maturity of the plant and the position of the leaf in the head. Based on the results of the analysis (ANOVA), a significant influence of the tested parameters (hybrid) on the quantitative characteristics of the plant was determined, the Hettie hybrid with a head weight of 633 g was rated as the best hybrid. Different time periods of plant development, as well as the type of hybrid, showed a statistically significant influence on the average nitrate content in the fresh mass of the plant, as well as the amount of nitrate depending on the part of the plant (outer rosette leaves, middle leaves and inner leaves), and the highest amount of nitrate recorded in all hybrids in the outer leaves of the leaf rosette and increased in relation to the extension of the plant's development time, ranging from 524,212 mg/kg hybrid Tonale to 2562,323 hybrid Hetti. In all tested hybrids, a drop in vitamin C concentration was recorded after 60 days of development.

Key words: nitrates, nitrites, *Lactuca sativa*, quality, vitamin C.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an annual herbaceous plant, a widespread type of leafy vegetable from the *Asteraceae* family. The leaf is consumed fresh, low in calories, rich in minerals and vitamins: C, B1, B2, carotene, etc., and mineral

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substances such as potassium, iron and phosphorus salts (Parađiković, 2009). Since lettuce is characterized by a high production of leaves in a short period of time, it is one of the leading vegetable species worldwide, and the growing demand for it on the market requires more intensive agricultural practices and abundant application of fertilizers. However, lettuce has the ability to accumulate and accumulate nitrates, which can potentially become harmful to human health. Nitrites oxidize the iron in hemoglobin, thus preventing the normal supply of blood with oxygen. As a consequence, poisoning occurs, which is especially dangerous for cardiovascular patients (Stephan, 2017). Numerous studies have confirmed that nitrates are the cause of methemoglobinemia in children (Addiscott & Benjamin, 2004). Methemoglobinemia is a disease in which hemoglobin is in an oxidized form and cannot bind oxygen. The amount of nitrate in vegetables is greatly influenced by environmental factors (atmospheric humidity, water content in the substrate, temperature, radiation and photoperiod), as well as agricultural factors (nitrogen doses and chemical forms, availability of other nutrients), (Santamaria, 2006). Light intensity is a key factor in the amount of nitrate in leafy vegetables as a result of nitrogen assimilation and electron transport during the process of photosynthesis in the leaves. Winter crops generally contain a higher amount of nitrates than summer crops grown in the same environment, and plants grown in the area of northern Europe contain higher levels of nitrates compared to areas of southern Europe (Weightman et al., 2006). The mentioned differences can be explained by the fact that greater lighting in summer reduces the nitrate content in favor of increasing plant growth (Kanaan & Economakis, 1992). According to the World Health Organization (WHO) and numerous epidemiological studies, a healthy diet with a daily intake of 400 to 500 g of fruit is recommended and vegetables. This can promote good health, reduce the risk of various diseases and strengthen immunity (FAO 2020; Leenders et al., 2013; Boffeta et al., 2010). The legislation of the European Union in Regulation 1258/2011 prescribes maximum permissible concentrations only for nitrates, while the legal regulation is limited only for three types of vegetables (spinach, lettuce and rocket), (European Commission 2011).

On the basis of Art. 16 and 72 of the Law on Food ("Official Gazette of BiH", No. 50/04) and Article 17 of the Law on the Council of Ministers of Bosnia and Herzegovina ("Official Gazette of BiH", No. 30/03, 42/03, 81/ 06, 76/07, 81/07, 94/07 and 24/08), the Council of Ministers of Bosnia and Herzegovina, on the proposal of the Agency for Food Safety of Bosnia and Herzegovina, in cooperation with the competent authorities of the entity and Brcko District of Bosnia and Herzegovina, passed regulation according to which the maximum allowed amount of nitrates for fresh lettuce (*Lactuca sativa* L.), harvested from October 1 to March 31, from greenhouses is 5000 mg or grown outdoors 4000 mg NO_3/kg, for lettuce harvested from April 1 to On September 30, from the greenhouse 4000 mg or grown outdoors 3000 mg NO_3/kg.

The purpose of the conducted research is to determine the differences in the quantitative and qualitative characteristics of the selected hybrids grown in a

protected agricultural area according to the principles of conventional agricultural production, and after the research to provide information about the most favorable harvest time, parts of consumption and the most adequate lettuce hybrid with regard to the investigated parameters.

MATERIAL AND METHODS

A two-factorial one-year study was carried out according to a random block arrangement in four repetitions, where factor A = hybrid, factor B = technological maturity. The experiment was done in a greenhouse in the northwestern part of Bosnia and Herzegovina, location Bihac (figure 1).

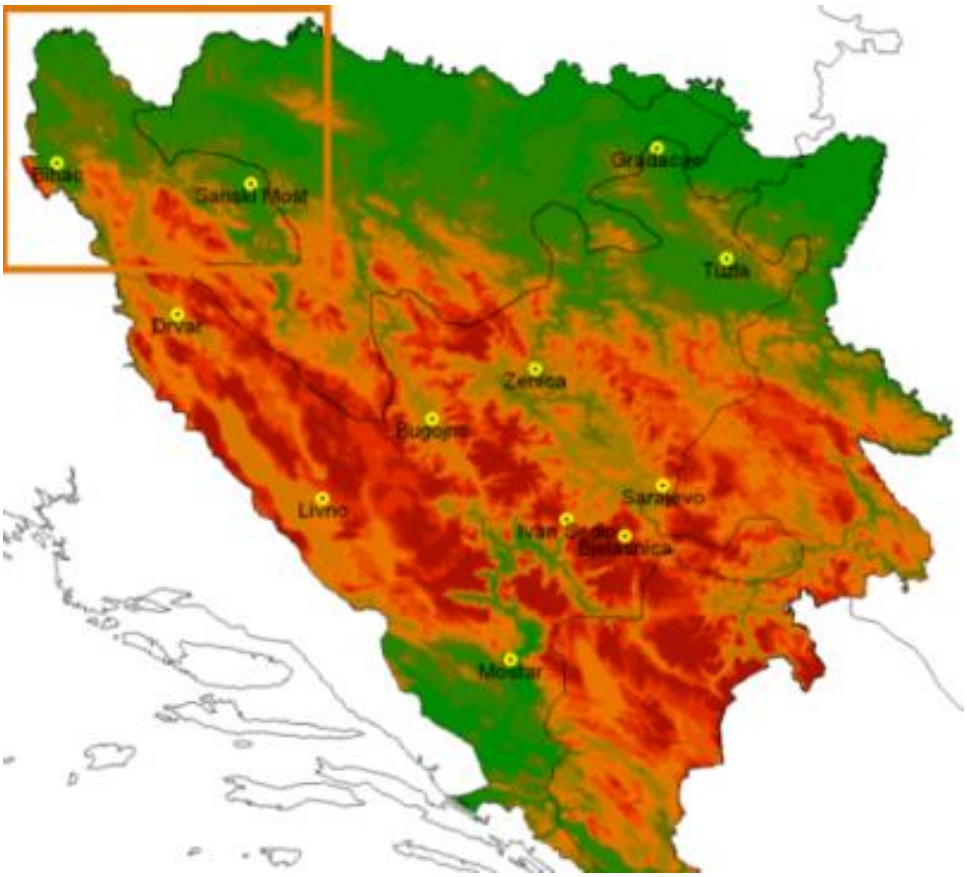


Figure 1. Map of Bosnia and Herzegovina, source: maps.google.com

Lettuce is grown according to the principles of conventional agriculture The total yield (biological and marketable mass for each tested hybrid) was determined, followed by the determination of mineral substances in the salad (Fe, P, K, Ca, Mg, content of dry matter, fiber, fat, protein, ash and UH, as well as water content, sugar and vitamin C), as well as nitrate and nitrite values, sampling was carried out over a period of 3 months, depending on the technological development of the

plant. Special emphasis is placed on determining the amount of nitrates in the plant material depending on the technological development of the plant (baby lettuce, medium-developed lettuce and lettuce at full technological maturity), as well as the hybrid used. The analysis were carried out in the laboratories of the Agricultural Institute of the Una Sana Canton, as well as the laboratory of the Faculty of Biotechnology of the University of Bihac (BiH). In all samples, dry matter was determined by drying at 105°C until constant weighing, amount of water, protein concentration according to Kjeldahl's principle, determination of vitamin C using the iodometric titration method, P concentration (by photometry with ammonium molybdate) and reading on a spectrophotometer by the International standard - Water quality - Spectrometric determination of phosphorus using ammonium molybdate -ISO 6878 (1998) method, and K, Ca and Mg by the flame photometry method according to International standard determination of sodium and potassium by flame emission - ISO 9964-3 (1996), total sugars by the Luff-Schoorl method, and the application of ion chromatography (eng. Ion chromatography, IC) in the analysis of nitrates and nitrites in leafy vegetables. All results were statistically processed (PAST 4.0) and SAS 9.4 (SAS, 2012).

RESULTS WITH DISCUSSION

Given that a greenhouse without an additional heating system and additional lighting was used for the experimental research, two parameters (temperature and light) that directly affect the quantitative and qualitative characteristics of the investigated species were processed in the further part of the paper for the time period October. 2022 until March. in 2023. Average monthly temperatures for the vegetation period for the Bihac locality were: 13,9 °C, 10,7 °C, 6,2 °C, 1,3 °C, 5,4 °C and 10,0 °C (min. -1,8° C, max. 20,9° C), while the temperature in the greenhouse is 7 °C higher on average. The determined air temperature is significantly above the standard normal value, and according to the percentile distribution of temperature conditions in the year of conducting the experiment, we classify autumn and the month of March in the extremely warm category, with only 4 cold days and no extremely cold days compared to an average of 22 cold days and 8 extremely cold days cold ones. The most pronounced monthly negative deviation of sunshine was recorded in February and March at the meteorological station Bihac thirty eight (38) hours of sunshine were recorded in Bihać, which is about 51% lower than the average. Comparing the research results with Cometti et al., (2013); Anusiya & Sivachandiran (2019), who investigated the influence of the amount, spectrum of light and the influence of shade on the quality of lettuce, proved that light plays a very important role in the life of the plant, and that light directly affects the amount of vitamin C in lettuce leaves.

Examining the total weight of the head per plant, and the yield per unit area, significant statistical differences were determined depending on the lettuce hybrid grown, but also the sampling time shown in table 1.

Statistically significant differences ($P<0,01$) were found in the average total mass of plants between lettuce hybrids, as well as the time of harvest. Namely, the

highest yield was achieved by Hetti with an average weight of piece of 633 g (90 days), 381 g (60 days) and 190 g (30 days) from sowing, while the lowest yield was recorded with the hybrid Saturdai 299 g (90 days), 163 g (60 days) and only 98 g (30 days) from sowing. Alisson Franco et al., (2017) studied the reaction of the plant and the influence of the organic fertilizer and variety on plant growth and development and yield. The experiment was conducted in an open field using the split plot method, with a combination of organic fertilizer of 0, 20, 40, 60 and 80 t/ha and three lettuce varieties Delícia, Babá de Verão and Itapuã 401. The influence of fertilization on yield was determined, but continuously good yields were achieved by the Delícia and Babá de Verãovarieties in all treatments and in 50% shade, which proves that the yield components are largely varietal characteristics. Therefore, the stated results are in accordance with our research.

Table 1. Yield per plant based on hybrids and technological maturity

tonale	donertie	hettie	limeria	nolanie	Ostralie	concibel	saturdai
173±1,26^{a}	131±1,86^{b}	190±1,17^{a}	132±1,86^{b}	113±2,11^{c}	100±2,43^{b}	125±1,93^{b}	98±2,96^{d}
245±4,98^{c}	361±5,81^{a}	381±5,71^{a}	248±4,96^{c}	380±5,71^{a}	357±5,81^{a}	252±4,91^{c}	163±3,96^{d}
460±7,89^{b}	496±7,12^{b}	633±6,49^{a}	396±8,67^{bc}	612±6,43^{a}	468±7,24^{b}	358±8,19^{bc}	299±12,02^{c}
p≤ 0,01	p≤ 0,01	p≤ 0,01	p≤ 0,01	p≤ 0,01	p≤ 0,01	p≤ 0,01	p≤ 0,01

ANOVA	Suma	df	Average value	F	p≤0,01
Hybrid	442227	2	221114	31,1	0,0338*
Tec. maturity	148937	21	7092,23	8	0,02961**

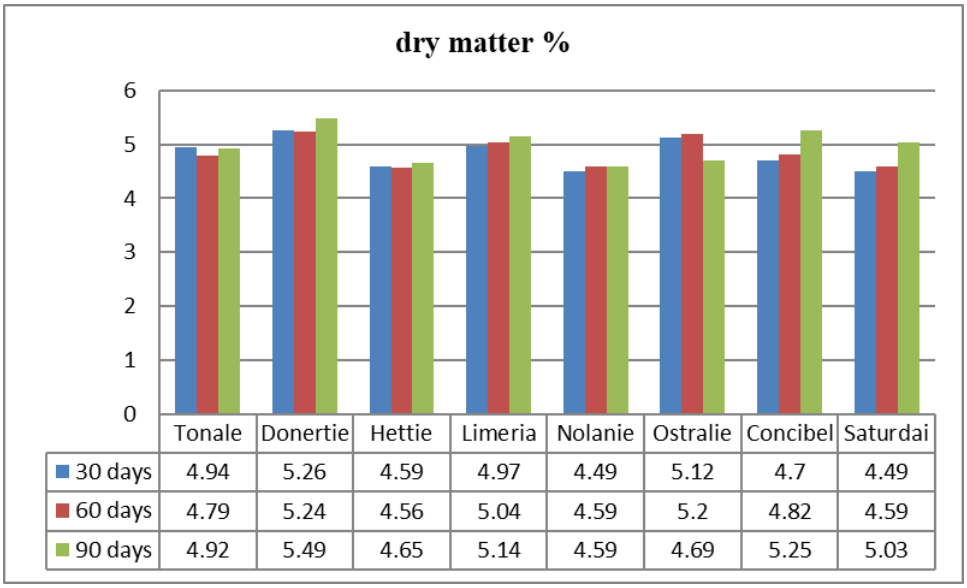
Kruskal - Walis test: H(ch^2):17,8 H_c (tie corrected):17,8 p (≤0,01):0,0001367
There is a statistically significant difference between the samples

Drăghici et al. (2016) investigating the effect of fertilizers on the yield components of lettuce, conducted experimental research with the use of four types of fertilizers: three organic fertilizers and a chemical one. The experimental fertilization variants were: V1 - organic Grow; V2 - Bio Leafez; V3 - Formulex; V4 - chemical fertilizer. The total cultivated area was 160 m^2 with 17,5 plants per m^2. Three varieties of lettuce were used: Markies, Lollo Bionda and Lollo Rosa.

The analysis of the results and their statistical processing showed that, regardless of the type of applied fertilizer, the cultivated varieties of lettuce and the genetic specificity were superior when all the investigated parameters, including the plant weight, were in question, hich is also in line with our research.

In addition to the examined yield parameters, analyzes of qualitative characteristics were also carried out on salad samples, and the content was determined: % protein, % fat, % water, % vitamin C mg/100, % ash, % UH, % fiber % and % sugar. Chemical analyzes were carried out in three repetitions (30, 60 and 90 days of development from the day of sowing) for all eight tested lettuce

hybrids. The absolute highest percentage of dry matter (graph 1) was measured in the Donertie hybrid: 5,26% (30 days), 5,25% (60 days) and 5,49% (90 days) from the day of sowing, while the lowest content recorded in the hybrid Hetti of only 4,59% (30 days), 4,56% (60 days) and 4,65 (90 days). Statistical processing of the data revealed a small statistical significance in the values of dry matter between different hybrids, but not in dependence on the technological age of the plant.



	Tonale	Donertie	Hettie	Limeria	Nolanie	Ostralie	Concibel	Saturdai
30 days	4.94	5.26	4.59	4.97	4.49	5.12	4.7	4.49
60 days	4.79	5.24	4.56	5.04	4.59	5.2	4.82	4.59
90 days	4.92	5.49	4.65	5.14	4.59	4.69	5.25	5.03

Graph 1. Dry matter content depending on the hybrid and harvest time

Slamet et al., (2017) determined the content of dry matter in the range of 5,56 to 6,98% when applying different organic fertilizers in the production of lettuce, which is a significantly higher content compared to our research. Similar research was carried out by Alaeddin et al., (2010) who, investigating the application of five types of organic fertilizer and the cultivation of lettuce according to the principles of conventional production, came to the conclusion that the content of dry matter in conventional production was around 5,6%, which is in accordance with our research, while when growing with compost, the content of dry matter increased up to 8,02%

Few researchers have analyzed the properties of raw proteins in lettuce, but Sularz et al., (2020) investigating the impact of iodine biofortification (potassium iodate/KIO_3/, 5-iodosalicylic acid /5-ISA/ and 3,5-diiodosalicylic acid /3, 5-diISA/) in the chemical composition of lettuce (*Lactuca sativa* L. *capitata*) state that the application of iodine compounds had an effect on the yield and content of dry matter, and thus the chemical composition of lettuce leaves. The recorded values of protein in our research, depending on the hybrid and the time of harvest, are from 1,48% to 2,10%, they are significantly lower than the values achieved in the research of Sularza et al., (2020) which ranged from 1,1 % control up to 5,1%.

Lettuce (*Lactuca sativa* L.) is a highly valued vegetable in the human diet not only because of its wealth of minerals and vitamins, but also because of the fact that today it is produced throughout the year and consumed fresh, so that all ingredients remain intact. Even if lettuce contains nutrients that promote health, according to research by Kim et al., (2016) the biosynthesis of such phytochemicals varies depending on the variety, leaf color and growing conditions. The average values of the concentration of vitamin C and the statistical processing of these values are shown in table 2. The highest concentration of vitamin C was recorded in the hybrid Nolania of 30,24% (30 days), 35,88% (60 days) and 34,33% (90 days), in terms of values, the Tonale hybrid is in second place at 32,75%, while the lowest amount of vitamin C was recorded in the Hetti hybrid at 22,1%. The specifics of the vitamin C content are reflected in the fact that in all hybrids the time period of plant development had an effect on the vitamin C content, whereby a statistically significant difference in values was determined at a time period of 60 days of development, after which a decrease in the amount of vitamin C was recorded.

Table 2. Vitamin C content % based on hybrids and technological maturity

ANOVA	Sum	df	Average value	F	p≤0,01
Hybrid	54,449	2	27,2245	0,758	0,7545
Tehn. maturity	2036,54	21	96,9569		0,02874*

Kruskal - Walis test: H(ch^2): 2,555 H_c(tie corrected):2,555 p(≤0,01): 0,2787
There is a statistically significant difference between the samples

Similar experimental research carried out by Aćamović-Djoković et al., (2011) investigating the content of vitamin C concluded changes in the value of vitamin C depending on the variety of cultivation, which is in accordance with our research and the research of other researchers. The research of Lozano et al., (2021) makes interesting conclusions, stating that until now lettuce breeding was mainly aimed at obtaining more fertile crops and more resistant to biotic stresses, but little attention was paid to its nutritional quality. By comparing the vitamin C content between wild and commercial lettuce varieties, they came to the conclusion that the same up to eight times higher vitamin C content is found in wild lettuce varieties compared to commercial varieties.

Koudela & Petříková (2008), studying the content and yield of nutrients in selected varieties of lettuce leaves (*Lactuca sativa* L. var. *crispa*), conducted experimental research with five varieties of lettuce, and after two years of experimental research determined a significant influence of the variety in the case of K content, Na and Ca, as well as the amount of dry matter and the weight of the leaf rosette, fiber is an exception.

Research by Bajwa & Kwatra (2013) states that dietary potassium is usually limited to 2000 to 3000 mg per day-1 for patients suffering from chronic kidney disease. In lettuce, the potassium content can be partially reduced by boiling or soaking in water. Unfortunately, other nutrients, such as ascorbic acids and

minerals, also lose their nutritional value during these processes. Thanks to the rapid increase in kidney disease patients Zhang et al., (2017); Zhang & Rothenbacher (2008) increased the demand for fresh lettuce with a low potassium content. In Japan, some companies are dedicated to the production of low-potassium salad. The accumulation of potassium in our research did not significantly depend on the time of harvest, and no statistically significant differences were found even in dependence on the hybrid, so the value of K ranged from 2,517 mg/100 g to 4,165 mg/kg.

The closing of the leaves and the formation of the head leads to a reduced concentration of Ca, which is often manifested by visually visible injuries of the tips of the leaves that look like a burn injury. Barta et al., (2000) studied the concentration of Ca in leaves depending on different nutrition systems, cultivars, as well as the rate of plant development, that is, the formation of the head. The first injuries on the tips of the leaves were noticed on the 22nd day of plant development from the time of emergence, when the leaves were about 3 cm long, however, in the phase of head formation, 83% of the leaves had visible injuries due to Ca deficiency. With the measured amounts of Ca from 261 mg/100 g to 449 mg/100 g, no differences were observed between the hybrids, but a decrease in Ca concentration depending on the time of harvest. Plants with deficiency of P form shorter leaves and deficiencies are visible after 2 weeks, and after four weeks, necrotic spots and the presence of anthocyanins are observed on the edges of the leaves. Since the land on which the experiment was built was rated as extremely well supplied with phosphorus in the amount of 72,43 mg/100 g of land, no deficiencies of P were observed in the plants. The recorded values were uniform based on both tested treatments and ranged from 31 mg/100 g to 48 mg/100 g.

Matson et al., (2015) conducted experimental research with one variety of Fladnria lettuce using the system of hydroponics and growing on rock wool, where they monitored the plant's development with different concentrations of nutrients. According to their research, approximately 10 days after Mg deficiency, the leaves show a slight chlorosis, and not long after, marginal necrosis becomes visible. Recorded Mg values ranged from 122 mg/100 g to 146 mg/100 g, and no significant statistical differences were found based on the tested treatments, as well as visible symptoms on the plant. Based on the recommended daily intake of lettuce, the most important bioactive components are: iron, folates, vitamin C, β-carotene, lutein, total phenol content, which play a prominent role in the prevention of many chronic diseases (Kim et al., 2016).

There were no statistically significant differences in Fe content depending on the tested treatment, and the recorded values ranged from Fe 1,3 mg/100 g to 3,2 mg/100 g.

Lettuce (*Lactuca sativa* L.) is one of the most popular vegetables in the world, but it is often considered to have a low nutritional value. However, lettuce contains health-promoting nutrients, and the biosynthesis of such phytochemicals varies depending on variety, leaf color, and growing conditions. Earlier research by numerous scientists showed that the content of nutritional components can

change depending on growing conditions, but the fiber content in lettuce is mainly a varietal characteristic, which is confirmed by these studies (tab. 4).

Table 3. Nutrient content mg/100 g based on hybrids and technological maturity

Hybrid	**K**	**Ca**			**P**	**Mg**	**Fe**
		I	II	III			
TONALE	**2,600**	**483**	469	**443**	40	142	2,9
DONERTIE	2,971	423	420	415	39	122	2,7
NOLANIE	2,770	442	439	432	41	139	3,2
CENCIBEL	3,062	312	309	289	**36**	146	2,5
OSTRALIE	**4,165**	475	467	412	**36**	141	2,4
LIMERIA	3,727	390	389	367	38	137	1,9
HETTIE	3,771	440	435	410	46	130	1,3
SATURDAI	3,888	**298**	287	**261**	**48**	133	1,5

Table 4. Fiber content % based on hybrids and technological maturity

ANOVA	Sum	df	Average value	F	p≤0,01
Hybrid	0,0384111	2	0,0192056	0,1607	0,0545*
Tehn. maturity	1,69463	15	0,112976		0,9537

Kruskal - Walis test: H(ch^2): 2,775 H_c (tie corrected): 2,783 p (≤0,01):0,2485
There is a statistically significant difference between the samples.

The results of the research showed that there are significant differences in the fat content depending on the hybrid and statistical differences of less significance when the question is about the fat content depending on the harvest time (tab. 5).

Table 5. Fat content % based on hybrids and technological maturity.

ANOVA	Sum	df	Average value	F	p≤0,01
Hybrid	0,0056333	2	0,000281667	8,349	0,003352**
Tehn. maturity	0,00501667	15	0,00033444		0,077658*

Kruskal - Walis test: H(ch2):9,026 Hc(tie corrected):9,207 p(≤0,01) 0,01002
There is a statistically significant difference between the samples.

The highest fat overestimation was recorded in the hybrid Hettie after 90 days of development (0,18 %) compared to the hybrid Limeria with only 0.09 % fat (30 days) and 0,13 % (90 days) of development. Most of the research is related to the research of the fat content in lettuce seeds, so Afsharypuor et al., (2018) in research on the analysis of the composition of fatty acids in the crude oil of *Lactuca sativa* L. GC-MS and GC methods identified ingredients that represented 98,20% of of total eluates were methyl esters of linoleic (52,38%), oleic (34,42%), palmitic

(7,25%), stearic (2,66%), arachidic (1,32%) and myristic (0, 17%) acid. The total percentage of saturated methyl esters and unsaturated fatty acids identified in the examined oil was 11,4 and 86,80%, respectively. At the end of the research, they conclude that the seeds and oil from the seeds of *Lactuca sativa* L., as well as many other vegetable fats, are rich in unsaturated fatty acids and as such should be included in the diet of the population of our regions, which was not the case until now.

Among the primary metabolites found in vegetables, soluble sugars and organic acids are important components and both contribute to flavor and nutritional value. Soluble sugars found in lettuce are glucose, fructose and sucrose in different concentrations. Research by Lopez (2014) was conducted in order to assess the difference in the chemical composition and sugar concentration of three varieties of lettuce (Romaine, Little Gem and Mini Romaine). All tested varieties were grown in a greenhouse according to conventional principles, but significant differences in sugar concentration were found depending on the variety, which is also confirmed by our research.

Similar experimental research was conducted by Shwerif et al., (2018) where they monitored the influence of different temperature regimes on the sugar content of two types of lettuce grown in a controlled environment. The results of the research proved that the temperature regimes had a very significant influence on the sugar content of both tested varieties of lettuce (Dixter and Exbury). The highest sugar content was recorded at low temperature (12/8 °C), and it decreased with increasing temperature, while Dixter lettuce had a higher concentration of sugar than Exbury lettuce.

We obtained similar results in our research, where it was found that the amount of sugar was statistically significant depending on the variety, and the interaction between temperature and amount of sugar was lower, because the lettuce was grown in the winter period, when the optimal temperatures for the development of lettuce were determined. The results of the research are shown in table 6.

Table 6. Sugar content mg/100 g based on hybrid and technological maturity

ANOVA	Sum	df	Average value	F	p≤0,01
Hybrid	12,261	2	6,13052	0,002401	0,09954*
Tehn. maturity	53627,8	15	2553,71		0,9963

Kruskal - Walis test: H(ch^2): 0,285 H_c (tie corrected): 0,285 p (≤0,01) 0,0867
There is a statistically significant difference between the samples.

No statistically significant difference was found in the concentration of sugar in the tested lettuce hybrids depending on the technological maturity at the level of significance (p≤0.01), while a smaller statistical difference was found depending on the cultivated hybrid, the highest sugar content was recorded in the

hybrid Cencibel 5,26 mg/100 g, and the lowest in Limeria at a value of 4,14 mg/100 g.

Leafy vegetables occupy a very important place in the human diet, but unfortunately, they form a group of foods that contribute to the intake of nitrates through food in the human body.

Under the excessive application of nitrogen fertilizers or in the case of a lack of light, these vegetables can accumulate a high level of nitrates and, when used in the diet, represent a serious health hazard. Shaid & Umar (2007) in their research on the impact of leafy vegetables on human health state that vegetables are the main source of nitrate intake in the human diet and nitrates and that people unknowingly consume more harmful and less useful substances for human health. Burns et al., (2004) investigating the accumulation of nitrates in lettuce under the influence of various factors, they state that nitrates tend to accumulate in lettuce shoots when the supply of nitrates exceeds the required amount of N crops, especially during the summer. It also accumulates when there is a reduction in solar radiation or when parts of the head of lettuce become susceptible to shading due to increased density and reduced vegetation space of the plant.

In winter, more nitrate accumulates because: the growth rate tends to decrease faster than the nitrate uptake rate; there is less light energy for the reduction of nitrates into organic forms of N; winter crops seem to use N more efficiently for dry matter production; and yields are often lower, so relatively more nitrates are available in the crops. Research also confirms that nitrate concentrations in heads of lettuce are more variable, depending on the variety and stage of development, which is in line with our research. As we assumed, we found a statistically significant difference, depending on the hybrid ($P<0,01$) and the time of harvest ($P<0,01$), in the average nitrate concentration (tab. 7).

Table 7. Nitrate content % based on hybrids and technological maturity

ANOVA	Sum	df	Average value	F	p≤0,01
Hybrid	3,08355	2	1,547706	5,22	0,0001093**
Tehn. maturity	6,20015	15	295245		0,0001482**

Kruskal - Walis test: H(ch^2): 7,505 H_c (tie corrected):7,505 p (≤0,01): 0,02346
There is a statistically significant difference between the samples.

The number of hours of sunshine in the months of research(October, November and December, as well as January) was higher in all analyzed stations compared to the thirty-year average (1961-1990), however, the most pronounced monthly negative deviation of sunshine was recorded at the end of February and March at the weather station Bihac 38 hours of sunshine were recorded in Bihac, which is about lower than 51% of the average value. As expected, the highest nitrate values (NO_2 mg/kg) were recorded in the month of March, ranging from 883,483 mg/kg in the Tonale hybrid to an enormously high 2540,138 mg/kg in the Hettie hybrid. Koudela & Petříková (2008) investigated 5 hybrids of lettuce: Lollo

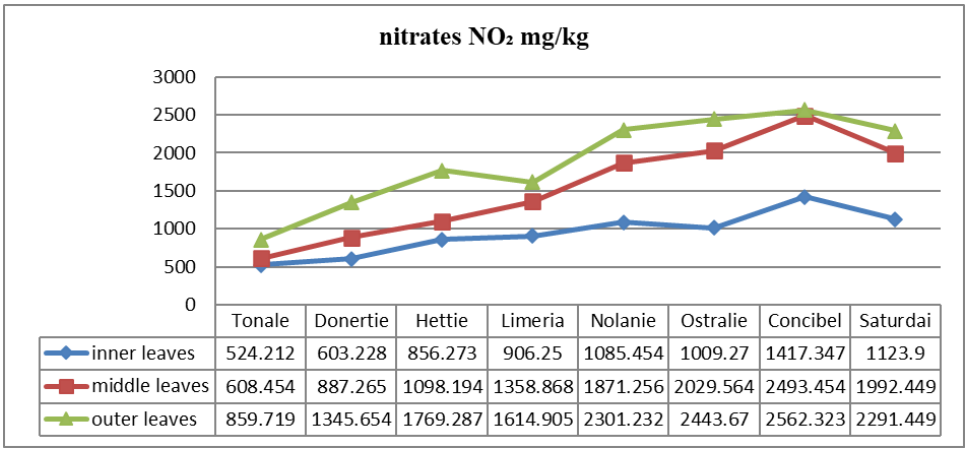
Rossa, Redin, Bergamo, Dubacek and Frisby, through two years of research and growing lettuce in three seasons, as expected, they recorded the highest nitrate content in autumn/winter cultivation in the hybrid Lollo Rossa with a value of 3817 mg/kg in autumn, 1993 mg/kg in spring/summer cultivation and 1193 mg/kg in summer/autumn cultivation, thus confirming the ditrect amount of nitrates in lettuce depending on the amount of light, but also on the variety. Tosun & Utson (2004) studied the nitrate content of lettuce grown in a greenhouse, they examined 20 varieties and concluded that in addition to the nitrate content depending on the lettuce variety, the amount of available light also plays a major role, so the nitrate content ranged from 425 mg/kg to 4040 mg/kg. Jernej & Osvald (2004). They conducted research with four varieties of lettuce, radish and spinach in greenhouse conditions and fertilized with different concentrations of N fertilizer. The experiment was carried out in the winter cultivation of lettuce, lower temperatures and less light. The highest concentration of nitrates was recorded in radish roots, then in lettuce leaves and finally in spinach (higher amount in the stem, lower in the stem).

As expected, the amount of nitrates in lettuce differed significantly from the variety itself, so the highest amount of nitrates was found in the Grand Rapids variety, and significantly lower in the Domineer and Kolrekt varieties.

Azmi Abu-Rayyan et al., (2004) conducted field research in two locations (Jordan Valley and Al-Jubeiha), which differ based on altitude, rainfall, and temperature range. The experiment was set up to evaluate the optimal planting density, form of nitrogen and level of irrigation in order to achieve the best quality of the lettuce crop in terms of minimum nitrate content and minimize the impact on the environment. Three forms of N fertilizer; (Ca $(NO_3)_2$, $(NH_4)_2SO_4$ and CO $(NH_2)_2$) were applied three times in the amount of 100 kg N ha/ha. Three row spacings (15, 20 and 25 cm) and two levels of irrigation were applied. Level one the usual amount of water m^2 and level two twice the amount of water than usual. The results showed that there was an increase in the amount of nitrate depending on the applied fertilization, but also that the outer lettuce leaves had a 5 times higher concentration of nitrate than the inner leaves, which proves to us that the plant loses the amount of vitamin C as it ages, and the nitrate content increases. which is consistent with our research.

The obtained results of this research proved that all varieties after 90 days of development from the time of sprouting have up to three times the amount of nitrates in the leaves, as well as the fact that the outer leaves have a higher content of nitrates compared to the inner younger ones, (graph 2).

The concentration of nitrates in lettuce leaves was statistically significantly affected by the hybrid, as well as the time of harvest, so statistically significant differences were found, as the average lowest amount of nitrates recorded in the Tonale hybrid was 613,954 mg/kg (30 days), 680,005 mg/kg (60 days) and 883,468 mg/kg (90 days), while the highest amount of nitrates was recorded in hybrid Saturdai 1834, 389 mg/kg (30 days), 2014, 389 mg/kg (60 days) and 2285, 562 mg/kg (90 days).



	Tonale	Donertie	Hettie	Limeria	Nolanie	Ostralie	Concibel	Saturdai
inner leaves	524.212	603.228	856.273	906.25	1085.454	1009.27	1417.347	1123.9
middle leaves	608.454	887.265	1098.194	1358.868	1871.256	2029.564	2493.454	1992.449
outer leaves	859.719	1345.654	1769.287	1614.905	2301.232	2443.67	2562.323	2291.449

Graph 2. Nitrate content mg/kg depending on the position of the leaf in the head

At the time of conducting the research, the highest NDK of nitrates were prescribed by the Ordinance on the maximum allowed amounts of contaminants in food (OG 154/2008. OG 78/2019), and for lettuce (*Lactuca sativa* L.) (grown in a greenhouse) and harvested up to 1. March, the permitted amount of nitrates was up to 4000 mg/kg, while in the open field that amount was 2500 mg/kg.
Nitrates and nitrites are on the list of human carcinogens. In 2012, the Scientific Committee on Food (SCF) determined the acceptable daily intake for nitrates (ADI) which is from 0 to 3,7 mg/kg of body weight/day, which is equivalent to the intake of 222 mg of nitrate for an adult a 60 kg person. The ADI for nitrites is 0,07 mg/kg/day. If an adult consumes only 400 g of different vegetables per day, the average nitrate intake is 157 mg/kg.

If we add fruits that contain significantly less, the intake of nitrates ranges from 81 to 106 mg/day for the majority of Europeans, which is within the acceptable daily intake. Exceptions are vegetarians and people who eat significantly more green leafy vegetables and may exceed these values. Recorded nitrite values are presented in table 9 and graph 3.

Table 9. Nitrite content % based on hybrids and technological maturity

Hybrid	0,823812	2	0,411906	5,837	0,000946**
Tehn. maturity	1,48198	21	0,0705707		0,00231**

Kruskal - Walis test: H(ch^2): 8,725 H_c (tie corrected): 8,728 p (same):0,01273
There is a statistically significant difference between the samples.

Based on the data from table 15, a statistically significant difference was found in the nitrite content in lettuce, depending on the hybrid, but also the time of harvest. Namely, it turned out that the hybrid factor had a greater influence on the nitrite content, so the highest average amount measured in the Limeria hybrid was 1,105 mg/kg, and the lowest recorded value in the Donertie hybrid was 0,657 mg/kg.

The amount of nitrites in vegetables is also affected by the storage method and the time that passes from the moment of harvesting to consumption, so research by Silalahi et al., (2016) on the topic of the influence of temperature and storage time on the amount of nitrates and nitrites in lettuce leaves proves the stated claim. Namely, the experimental research was conducted on freshly picked lettuce that was stored at two temperature values of 1 °C and 22 °C for 48 hours, and the nitrate and nitrite content was analyzed every 4 hours. The results of this research show that during storage for 48 hours at room temperature, the nitrate level increased from the initial value of 6,07 mg/kg to 70,83 mg/kg, and the nitrite level increased from 22,63 mg/kg to 48,14 mg/kg. The nitrate and nitrite levels in salads stored in the refrigerator increased from the initial level of 3,06 mg/kg to 64,42 mg/kg, and the nitrite level increased from 21,89 mg/kg to 40,08 mg/kg. Research recommends storing salad for no longer than 3 days in the refrigerator, because any longer storage leads to a deterioration in the quality of the food and a negative effect on human health when consuming it.

CONCLUSIONS

In the research, the most favorable harvest date was determined, as well as the type of hybrid for growing larger and better quality plants with the ultimate goal of a positive impact on human health. On the basis of the conducted study, it is evident that the greater amount of natural light resulted in positive effects on the growth and development of the plant in all measured parameters, but also the lack thereof in the increased amount of harmful substances in the leaves. The positive and negative influence of the harvest time was observed for most of the tested parameters when the question is the quantity and quality of the product. So we conclude that the hybrids Nolanie and Hetti are the hybrids with the most favorable characteristics for growing in the winter period, because the ratio of quantitative and qualitative characteristics is approximately the same. But it is recommended harvest after 60 days, because with a later harvest there is a sudden increase in nitrates and nitrites.

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