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In Loving Memory of Professor d-r Dana Prelik

With heavy hearts, we bid farewell to a remarkable educator, professor d-r Dana Prelik, whose indelible impact on Macedonian academic community will forever be cherished. A dedicated scholar, mentor, and advocate for knowledge, she illuminated the path of learning for countless students.

Her passion for her field and commitment to excellence inspired all who had the privilege of sitting in her classroom. Beyond the lecture halls, she fostered an environment of intellectual curiosity, encouraging her students to think critically and dream boldly.

As we reflect on her legacy, let us remember the profound influence she had on shaping minds and nurturing futures. Professor d-r Dana Prelik will be dearly missed, but her spirit lives on in the countless lives she touched.

May her memory continue to inspire us to pursue knowledge with the same vigor and dedication that she exemplified throughout her esteemed career.

Rest in peace, dear professor d-r Dana Prelik.

INTRODUCTION

It is true that uncertainty and instability are expected to mark the year 2024, taking into account a series of events with an unpredictable outcome – elections for the new European Parliament, presidential elections in the USA, the outcome of the current wars, in Ukraine and the Middle East, and the tensions between the main blocs in global geopolitics.

At the time, two relevant events had not yet occurred, with an impact on world markets: the end of the Black Sea grain agreement, relatively expected, considering Russia's demands, and the Israeli conflict in Palestine, with the horrors to come, that we have seen in Gaza, which took the focus and all mediatic attention away from the Russian invasion of Ukraine. Apparently, in recent days, the situation seems to have reversed, with Israel having mortgaged much of the sympathy in the West, and Ukraine once again gaining the interest of public and published opinion, whether due to the ongoing debate in the USA and the divisions between Democrats and Republicans – which will tend to be another theme for a strongly polarized campaign.

Recently, delegates from nearly 200 countries gathered in Dubai for the COP28 climate conference which resulted in nearly every country in the world agreeing to transition away from fossil fuels. It appears that this agreement represents a milestone for global climate progress. The main aim of this agreement is to decarbonize the real world, and this will require the support of all industries, including finance and investment. Better understanding climate-related risks and opportunities can drive the actions needed to achieve net-zero emissions.

These challenges will have an effect in the agriculture production that has to make enormous efforts and adaptations to the new uncertainties that we will face in 2024. Firstly, increasing agricultural production and food quality with the aim of "feeding the world" and then trying to respond to the challenges of environmental decarbonization on which modern agriculture depends. We hope that the next publications of JAPS Vol. 21, No. 2 will be the best contribution to a new agriculture full of challenges.

December, 2023

On behalf of JAPS Editorial Board,

**Prof. d-r Kiril Bahcevandziev
Coimbra Agricultural School, Portugal
Member of JAPS Editorial Board**



IMPACT OF INSECTICIDES ON BENEFICIAL ORGANISMS

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Abstract

In the process of increasing crop production, the use of insecticides is much greater than in the past. These chemicals have largely emerged since the introduction of synthetic insecticides in 1940, when organochlorine insecticides were the first used to control pests. Insecticides are now an integral part of our modern life and are used to protect agricultural production, storage products, as well as to destroy pests that transmit dangerous infectious diseases. It would be ideal if the applied insecticides are toxic only to the target organisms, biodegradable and environmentally friendly to some extent. But, the most of them are non-specific and, in addition to pests, destroy organisms that are harmless or beneficial to the ecosystem (predators, pollinators) or affect their proper growth and development.

Key words: *pests, insecticides, predators, pollinators, earthworms, biological control*

INTRODUCTION

Knowing the fact that the world's population is expected to grow to nearly 10 billion by 2050 (Gill & Garg, 2014), increasing food production is a major goal for all countries. According to the Food and Agriculture Organization (FAO) of the United Nations the world food production needs to increase by 70% to keep up with the demand of a growing population (Gill & Garg, 2014). In the process of increasing crop production, pesticides and fertilizers are now used in greater quantities than in the past. These chemicals have largely emerged since the introduction of synthetic insecticides in the 1940s, when organochlorine insecticides were first used to control pests (Gill & Garg, 2014).

Pesticides are an integral part of our modern life and are used to protect agricultural production, stored products, flowers, as well as to destroy pests that transmit infectious diseases. Approximately, 2 million tons of pesticides are utilized annually worldwide, where China is the

major contributing country, followed by the USA and Argentina, which is increasing rapidly. However, by the year 2020, the global pesticide usage has been estimated to increase up to 3.5 million tons (Sharma et al., 2019). Nowadays manufacturers and researchers are creating new pesticide formulations that are specific only to the target organisms, biodegradable and environmentally friendly to some extent. Although pesticides are beneficial for crop production of view, extensive use of pesticides can possess serious consequences because of their persistence. The continuous use of persistent and non-degradable pesticides pollutes various components of water, air and soil and indirectly affects the living organisms.

In this review paper, the impact of insecticides on beneficial organisms and possible ways to reduce the use of insecticides in the plant protection from pests will be presented.

EFFECT OF PESTICIDES ON BENEFICIAL ORGANISMS

The effect of pesticides on non-target organisms has been a source of worldwide attention and concern for decades. Adverse effects of applied pesticides on non-target arthropods have been widely reported (Ware, 1980). Unfortunately, the natural enemies of insects, for example, predators and parasitoids, are the most susceptible to insecticides and are severely affected (Gill & Garg, 2014).

Usually, if natural enemies are absent, additional insecticide sprays are required to control the target pest. Along with natural enemies, the population of soil organisms is also drastically disturbed due to indiscriminate application of pesticides in agricultural systems.

Effects of pesticides on earthworms

Earthworms represent the largest proportion of terrestrial invertebrates (>80%) (Yasmin and D'Souza, 2010) and play a significant role in improving soil fertility by decomposing organic matter into humus. They also play a major role in improving and maintaining soil structure by making channels in the soil that improve its water-air regime. They are also considered an important indicator of soil quality in agricultural ecosystems (Paoletti, 1999). However, their diversity, density and biomass are strongly influenced by the use of pesticides. Various studies confirm the harmful impact of carbamate and organophosphate insecticides on earthworms by reducing their population (Edwards, 1987). For example, carbamate insecticides are highly toxic to earthworms, and some organophosphates (chlorpyrifos and azinphos methyl) have been shown to reduce earthworm populations (Reinecke & Reinecke, 2007), and increased exposure periods and higher doses of insecticides can cause physiological damage to earthworms (Yasmin and D'Souza, 2010). Various scientific studies have reported that pesticides affect the growth and reproduction of earthworms, and a study conducted in France showed that a combination of insecticides and fungicides at different concentrations caused neurotoxic effects in earthworms (Schreck et al., 2008).

Effects of pesticides on predators

Predators are organisms that feed on other organisms and they play a very crucial

role in keeping pest populations under control. Predators (beneficial organisms) are also an important part of the "biological control" approach, which is one component of an integrated pest management strategy. Various comparative studies have been conducted, showing the negative impact of synthetic pyrethroids and neonicotinoids in relation to biopesticides. Namely, treatment with cypermethrin and imidacloprid caused higher mortality of ladybugs, parasitic wasps and predatory spiders compared to treatment with bio-pesticides and insecticides based on Neem (*Azadirachta indica*) (Ghananand et al., 2011). In foliar application, all systemic neonicotinoids such as acetamiprid, imidacloprid, thiamethoxam and thiacloprid were found to be highly toxic to natural enemies compared to buprofezin (IGR insecticides) and fipronil (phenylpyrazoles) (Kumar et al., 2012).

In addition, pesticides can affect both predator behavior and parameters that determine their lifespan, including growth rate, development time, and other reproductive functions. In Calabria, Italy, dimethoate has been shown to significantly reduce the body size and hemocyte count of the carabid beetle *Pterostichus melas italicus* (Giglio et al., 2011).

Effects of pesticides on pollinators

Pollinators are biotic agents that play a very important role in the pollination process. Some of the more important pollinators are various species of bees, bumblebees (*Bombus* spp.), honey bees (*Apis* spp.), fruit flies, some beetles, and birds. The use of pesticides causes direct loss of pollinating insects and indirect loss of crops due to lack of adequate pollinator population (Fishel, 2011). During our field research, we noticed that fertilization in pepper is reduced due to the absence of pollinators, which is the result of the use of insecticides.

Pesticide application also affects a variety of pollinator activities, including foraging, colony mortality, and pollen collection efficiency. Bees make up 80% of the insect pollinator population and the most of the current knowledge about the effects of pesticides on changing pollinator behaviour comes from various studies on bees. For example, many laboratory studies have demonstrated the

lethal effects of neonicotinoid insecticides (imidacloprid, acetamiprid, thiamethoxam, and thiacloprid), as well as their effects on bee behaviour, learning, and memory (Blacquière et al., 2012). Of the listed neonicotinoids, only acetaprimide is still used in plant protection, due to the minimal doses with which it is used and the immediate lethal effect it has on pests. Due to the application of neonicotinoids and pyrethroids mortality of worker bees, reduced pollen collection efficiency and eventual colony collapse occur. Lethal doses of imidacloprid

(the most commonly used pesticide in the world) affected longevity and foraging in bees (*A. mellifera*). Microsporidial infections in the gut of bees were significantly increased after treating plants with imidacloprid. It is expected that interactions between pathogens (bacteria, viruses...) and imidacloprid could be the main reason for the mortality of honey bee colonies worldwide (Gill & Garg, 2014). There are also reports that imidacloprid reduces the fecundity of bumblebees by decreasing fertility (Laycock et al., 2012; Whitehorn et al., 2012).

HOW TO PROTECT BENEFICIAL ORGANISMS FROM PESTICIDES

Today, it is almost impossible to imagine modern agricultural production without the use of chemicals to destroy harmful insects. The chemical method gives quick and visible results, but in addition to the positive effects of their application, negative consequences were also quickly determined: resistance in insects, disturbance of the balance in agro-ecosystems, toxic effects on humans and domestic animals, environmental pollution.

Contemporary directions in plant protection go in two ways, namely: the development of new methods for controlling harmful insects and the development of means of protection with favourable toxicological properties and a selective character against the natural enemies of insects.

New methods for controlling the population of harmful insects are based primarily on the cultivation of resistant crop varieties, natural bioregulatory systems in agrobiocenoses, new plant protection products, with active substances based on microorganisms or their metabolites, and on synthesized protection products with new mechanisms of action and with favourable toxicological properties. The installation of new systems in plant protection is the result of realizing the negative side of the one-sided application of chemical agents. Current concepts for combating harmful insects aim to integrate all existing possibilities for pest control and reduce negative consequences for the environment.

The fight against insects in agricultural production must be seen from an ecological point of view, because regardless of which method is applied, it means the application of

a certain environmental factor within the limits of the ecological valence for the population of the certain insect. In addition, the fight against insects automatically means a disturbance of the dynamic balance in agroecosystems. Therefore, the fight against harmful insects in agricultural production has an ecological content and must be based on solid knowledge of the biology and ecology of the individual harmful species and the knowledge of the individual agrobiocenoses.

Concern for the environment and the production of safe food drives academic, government and industry research towards the development and promotion of a new and safe strategy for the control of harmful organisms in agricultural production, known as Integral pest management.

Integral pest management is a plant protection system, which implies the use of all available methods of combating pests, pathogens and weeds (growing resistant varieties, agrotechnical, mechanical, biological, chemical and others) in order to prevent the increase in their number across the border above which significant economic damages occur.

By implementing integral protection measures, the number of chemical treatments is reduced, thus protecting and enabling greater activity of natural enemies. Their role is so important that many authors, under the term integral protection, mean only the application of natural enemies and chemical protection. When choosing pesticides for chemical control, one should take into account their selectivity in relation to the most important natural enemies of pests in a given crop. Also, unnecessary and

excessive use of chemicals should be avoided. By treating the entire surface to suppress soil pests, almost all natural enemies are destroyed, and the same effect is achieved by introducing chemical agents only in the rows of plants,

which preserves many beneficial organisms.

The application of the system of integral protection leads to less use of pesticides, and therefore to cheaper protection. In addition, environmental pollution is also reduced.

CONCLUDING REMARKS

Synthetic insecticides are used to control the weeds and insect pests, affecting the agricultural systems. Water, soil and air serve as an important medium for transportation of insecticides from one site to another. Insecticide contamination is a serious problem for each ecosystem and is harmful for all associated organisms. So, in order to control insecticide

usage, new methodologies and techniques are needed in assessing the effect of widespread use of insecticides on ecosystem and efforts should be made to provide awareness among public to minimize the application of harmful insecticides. Use of bioinsecticides should be encouraged over chemical pesticides.

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ВЛИЈАНИЕ НА ИНСЕКТИЦИДИТЕ ВРЗ КОРИСНИТЕ ОРГАНИЗМИ**Билјана Атанасова^{1*}, Душан Спасов¹, Драгица Спасова¹, Мите Илиевски¹**¹*Земјоделски факултет, Универзитет „Гоце Делчев“, ул. „Крсте Мисирков“ 10-А, 2000 Штип, Република Северна Македонија***Контакт автор: biljana.atanasova@ugd.edu.mk***Резиме**

Во процесот на зголемување на растителното производство, употребата на инсектицидите е многу поголема отколку во минатото. Овие хемикалии во голема мера се појавија по воведувањето на синтетичките инсектициди во 1940 година, кога органохлорните инсектициди беа првите користени за контрола на штетниците. Инсектицидите сега се составен дел од нашиот современ живот и се користат за заштита на земјоделското производство, складираните производи, како и за уништување на штетници кои пренесуваат опасни заразни болести. Би било идеално доколку применетите инсектициди се токсични само за целните организми, биоразградливи и до одреден степен еколошки. Но, повеќето од нив се неспецифични и, покрај штетниците, уништуваат и организми кои се безопасни или корисни за екосистемот (предатори, опрашувачи) или влијаат на нивниот правилен раст и развој.

Клучни зборови: *штетници, инсектициди, предатори, опрашувачи, дождевни црви, биолошка контрола.*



INTRODUCTION OF *ULOMOIDES DERMESTOIDES* (CHEVROLAT, 1878) (COLEOPTERA) TO NORTH MACEDONIA: UNVEILING THE FIRST HUMAN-INDUCED RECORD OF A NOVEL SPECIES – SHORT COMMUNICATION

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Abstract

This short communication presents first record of *Ulomoides dermestoides* (Coleoptera, Tenebrionidae, Diaperinae) in North Macedonia. The problem of the introduction of this non-native darkling beetle as alternative medicine is briefly discussed. Prior to this study, no data exist about the presence of *U. dermestoides* on the territory of the former Balkan countries as well.

Key words: *Tenebrionidae*, *Diaperinae*, new data, Balkan

INTRODUCTION

Brief taxonomic history: *Ulomoides dermestoides* is a cosmopolitan species, non-native for Europe, known under a variety of different names, according Flores et al. (2002), *Histeropsis dermestoides* by Chevrolat (1878). Later on, Doyen et al. (1989) named the species *U. dermestoides* (Chevrolat, 1878).

Brief history of species distribution: It is considered native for Asia, with prevalence in China, but meets other oriental countries: Japan, Taiwan and Australia as well (Löbl et al., 2008). Until 1988, it was only known for the Oriental region, while later it was introduced in the United States and Latin America, as well as in Europe (Lourenço et al., 2022), with first records in Sweden (Ferrer, 1988).

U. dermestoides is widely used as a traditional medicine in the countries of South-East Asia, Africa, Australia, and particularly in Latin America. There are many scientific papers related to their breeding behavior, biology and life cycle (Garcés Molina et al., 2009; Marinoni & Ribeiro-Costa 2001; Morillo-Garcia et al. 2016), as well as the effects caused by the beetle diet and their usage in traditional medicine (Aguilar-Toalá et al., 2022; Deyrup et al., 2021; Jasso-Villagomez et al., 2018). That is the main reason for its introduction in other countries of the world, including our country.

This paper presents the first record about the presence of the species *U. dermestoides* and its use as an alternative medicine in the Republic of North Macedonia.

[†] Deceased 11.12.2023

MATERIAL AND METHODS

During February 2023, specimens of *U. dermestoides* were brought at the Department of Animal Ecology (Institute of Biology, Faculty of Natural Sciences and Mathematics in Skopje, Ss. Cyril and Methodius University) for identification. The identification was made according to Gorham (1987). Studied specimens

are deposited in the Macedonian National Collection of Invertebrates. A stable colony of this species is still maintained under laboratory conditions at the same department (Fig.1).

Photographs of *U. dermestoides* (Fig. 1, 2, 3, 4) were taken under the WILD M3Z stereo microscope.

RESULTS AND DISCUSSION

The specimen was identified as *U. dermestoides* based on the head, which was widest at eyes; large eyes, separated by distance subequal to width of eye; asymmetrical and broad antennal segments; and the pronotum which was without anteromedial bead (Fig. 1, 2, 3, 4).

The colony of *U. dermestoides* was introduced in North Macedonia through illegal transport from Sweden and was deliberately grown and reared in captivity by the local people in Negotino city (41.48419, 22.09108) for alternative medical treatment of cancer and diabetes. Later on, locals were breeding and growing this beetle at home for personal use or commercial purpose.

Local people believe that consumption of *U. dermestoides* may treat some heavy diseases. According to the breeder, there is a protocol in the therapy of using beetles, in which the first day only one live beetle with a glass of water should be drunk, the second day two, the third day three, and then every day the number increases up to 40 beetles, then from 40 backwards. Thus, exactly 1,640 beetles should be consumed in 80 days. Although this practice is already well known among the local population, this paper reports the practice of using *U. dermestoides* as a medicine in North Macedonia for the first time.

The abdominal glands of the species *U. dermestoides* release secrete with quinones, which has some anti-inflammatory, cytotoxic,

genotoxic, antiproliferative, antidiabetic, antioxidant and antimicrobial effects causing reduced cell viability and DNA damage (Cázares-Samaniego et al. 2021). Therefore, these beetles are used by locals for medical treatments. But the problem is that the influence of quinones is not selective, i.e. they act both on damaged and healthy tissues, and long-term consumption of these beetles in larger amounts can lead to serious health problems. However, there are no data related to the negative effects of this species on human health in North Macedonia.

The presence of a new allochthonous species in the country is a matter of concern among the scientific community, especially regarding the ability of the species to live outside the controlled conditions created by humans. Due to people's inadequate knowledge of species ecology and biology and their strong beliefs that these beetles have healing power, they are mass selling and breeding the beetles in domestic conditions, thereby increasing the likelihood of species dispersal in the environment.

Unfortunately, there is currently no documentation on the species' occurrence outside of captivity or in any of the nearby Balkan countries. Therefore, in this paper we base our observations on the potential risk of adaptation of the species in its natural habitats in the country solely on the literature data that refer to its biology within its range of distribution.



Figure 1. Colony of *U. dermestoides*.

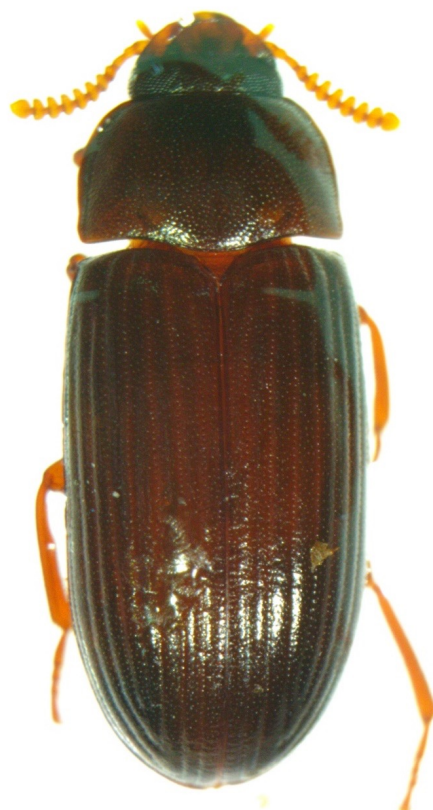


Figure 2. Dorsal view of *U. dermestoides*.



Figure 3. Dorsal part of the head of *U. dermestoides*.



Figure 4. Antennae of *U. dermestoides*.

Biology of the species: This species has complete metamorphosis with four life stages presented with egg, seven larval instars, pupa and adult stage (Morillo-Garcia et al., 2016). The average life cycle (from egg to adult stage) has duration of 67.63 days. The semi-mobile pupae stage lasts 3-5 days after the larval stage, which lasts about 60 days (Morillo-Garcia et al., 2016).

In its native range this species is typically found in warm and humid environments, such as tropical and subtropical forests, but it is able to thrive in areas with temperatures ranging from 20 to 30 °C and relative humidity above 50% (Marinoni & Ribeiro-Costa, 2001; Garcés Molina et al., 2009). Given that the climate of North Macedonia, with annual average temperature 15°C and humidity around 70% (Lazarevski, 1993), partially matches the climatic preferences of this species, climatic conditions are not anticipated to restrict the distribution of *U. dermestoides*. In addition to this statement is the presence of the species in Sweden (Ferrer, 1988), as an indication of its wide tolerance to different climatic conditions.

In general, the adult and larval stages of *U. dermestoides* are highly adaptable. They can persist high temperatures and low precipitations through morphological, physiological and behavioral adaptations. During the unfavorable winter conditions this beetle hibernates by burrowing in the soil (Garcés Molina et al., 2009).

Probably, fast growth and high reproductive potential contribute in overcoming physiological or other barriers in order to reproduce successfully in a wide range of climates and habitats, including stored products as secondary habitats. This behavior enhances potential persistence of the species and its invasiveness. However, little is known about species invasive potential (Lourenço et al., 2022). Therefore, the possibility of its survival in natural conditions is not excluded, especially in southern and eastern parts of North

Macedonia where the climate is continental-submediterranean. So, it is quite obvious that this species can maintain self-sustaining populations in the country, although there are no published data.

The dispersion of this species is mainly the result of human activity i.e. worldwide commerce. Potential pathways for dispersion are trade, transportation, use as pets' food, medical treatments, and climate change as well. The transport of the species is mainly as an adult, but the possibility of the larval presence in the containers in which the adults are kept is not excluded. Since North Macedonia is a small country, high rate of distribution and adaptability makes the presence of this species concerning by itself, because there is high probability that the species can very easily expand its distribution to other parts and habitats of the country. So far, this situation can only be assumed because no additional modelling of the possible spread (habitats, climate, altitude, etc.) was done.

It is also well known that the species that act as a strong allergen, can host endoparasites and infests stored products such as peanuts, oat, soybeans, corn, rice and bread (Vergara et al., 1997). Worldwide, this pest is treated with insecticides which can cause series of health problems in humans, biodiversity loss and reduced habitat quality (Plata-Rueda et al., 2020; Plata-Rueda et al., 2022).

So far, there is no literature data that *U. dermestoides* does not suppress the growth of native taxa, nor has large negative impact on flora and fauna, i.e. on the environment in general. But it possesses a strong biotic potential and great adaptability, and if it is introduced can very easily displace the native species from their ecological niches and cause disturbance in food chains and ecosystem functioning which will cause ecological as well as series of social and economic problems.

CONCLUDING REMARKS

This short communication presents first confirmed record of the presence and use of the species *U. dermestoides* as an alternative medicine in the Republic of North Macedonia. We truly hope that it will raise public awareness

and provoke a reaction among the competent institutions to solve the problem of species introduction in the country and its illegal commerce.

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**ИНТРОДУКЦИЈА НА *ULOMOIDES DERMESTOIDES* (CHEVROLAT, 1878) (COLEOPTERA)
ВО СЕВЕРНА МАКЕДОНИЈА: ПРВ ПОДАТОК ЗА НОВ ВИД ИНТРОДУЦИРАН
ОД СТРАНА НА ЧОВЕКОТ - КРАТКА КОМУНИКАЦИЈА**

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Резиме

Овој труд претставува прв запис за присуството на видот *Ulomoides dermestoides* (Coleoptera, Tenebrionidae, Diaperinae) во Северна Македонија. Даден е краток приказ на интродукцијата на видот во Република Северна Македонија и неговата примена како алтернативен лек. Вреди да се истакне дека досега не постојат податоци за присуство на *U. dermestoides* на територијата на останатите балкански земји.

Клучни зборови: *Tenebrionidae*, *Diaperinae*, нов податок, Балкан.

[†] Почината на 11.12.2023



CONSTRUCTIVE ALIGNMENT BETWEEN OBJECTIVES, TEACHING AND LEARNING ACTIVITIES, STUDENT COMPETENCIES AND ASSESSMENT METHODS IN HIGHER EDUCATION

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Abstract

A high-quality learning process in higher education properly and constructively aligns essential elements: study programs and course objectives, learning outcomes, content, student workload, teaching and learning activities, assessment methods, and acquisition of student competencies, which is known in the literature as constructive alignment (CA). In essence, CA is bringing into alignment the predetermined competencies, the learning and teaching activities, and the assessment types. Furthermore, CA is an outcomes-based approach to teaching in which the learning outcomes that students are intended to achieve are defined before teaching takes place.

Learning success at all levels of study primarily depends on constructive alignment as one of the most significant and influential principles in higher education. Detailed knowledge of this principle and consistent application is the basic obligation of teaching staff in higher education. To develop a “constructively aligned” course unit, a teacher should start from the intended course-specific competencies, after which they should choose the most appropriate learning, instructive, and teaching activities, and assessment methods for these specific competencies.

The paper explains in more detail the essence of CA between student workload expressed through ECTS, study programs, course and unit objectives, learning outcomes, theoretical and practical contents, teaching and learning methodology, formative and summative assessment methods, and effective acquisition of student generic and course-specific competencies.

Keywords: *constructive alignment, student workload, objectives, learning outcomes, learning and teaching activities, assessment, competencies*

INTRODUCTION

The roots of the most important elements of constructive alignment (CA) in education were laid by Ralph Tyler (1949) in his best-selling publication *Basic Principles of Curriculum and Instruction*. In the series of publications by Biggs from 1993 to 2013, cognitive systems approach (Biggs, 1993), enhancing teaching through constructive alignment (Biggs, 1996), teaching for quality learning at higher education institutions (Biggs, 1999, 2001), assuring and enhancing the quality of teaching and learning (Biggs, 2001) and many other aspects of changing higher education institutions (Biggs, 2013) were considered.

In addition, Biggs & Tang (2007; 2011; 2011a; 2011b) considered teaching for quality learning at Higher Education Institutions (HEIs) and CA to represent an outcomes-based approach to teaching in which the learning outcomes (LOs) that students are intended to achieve are defined before teaching takes place. The design of curriculum, assessment, and evaluation in Higher Education (HE) with CA were discussed in the papers by Ali (2018), Fotoh & Lorentzon (2021), and Maffei et al. (2022). Display of the sequence of constructive alignments that connects the explanations in the text of the paper is given in Figure 1.

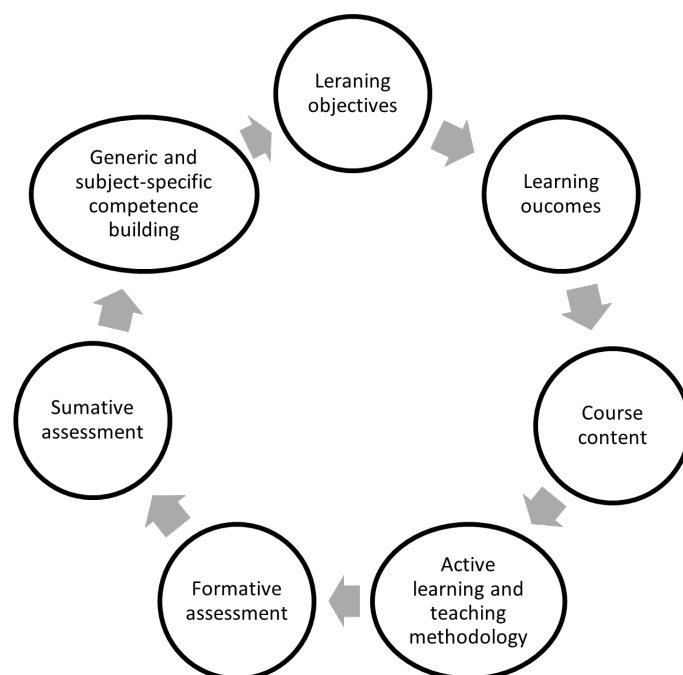


Figure 1. Display of the sequence of constructive alignments.

For monitoring and management of the quality of study programs at all levels of study in the HEIs, CA of student workload expressed through European Credit Transfer and Accumulation System (ECTS), study programs (SPs), course and unit objectives, LOs, theoretical and practical contents, teaching and learning methodology, diagnostic, formative and summative assessment methods and effective acquisition of student generic and course-specific (professional) competencies are of crucial importance (Cullen et al., 2003; Vlăsceanu et al., 2004; Cincović et al., 2020). The currently valid standards and guidelines for quality assurance in the European Higher Education Area (ESG) were defined in a publication by the European Association for Quality Assurance in Higher Education (ENQA, 2015). The National Council of Higher Education of Serbia (NCHE) of the Republic of Serbia defined the Rulebook on Standards and Procedures for External Quality Control of Higher Education Institutions (2019). The National Entity for Accreditation and Quality Assurance in Higher Education (NEAQA) uses this rulebook, harmonized with the ESG, to assess study programs and HEIs in the Republic of Serbia. In essence, the quality of study programs should be ensured through monitoring and checking the goals, structure, and workload of students, as well as through updating the content and constant collection

of information about the quality of the program from appropriate social institutions (Vlăsceanu et al., 2004, Vukasović, 2006, Loughlin et al., 2021, Divjak et al., 2023).

Although the importance of CA has been clear for a long period, the work of Hamdoun (2023) emphasizes that the constructivist approach to learning and teaching is still missing in the educational practices of a reasonable number of HEIs. The paper by Silander & Stigmar (2023) examines the question of what university teachers need to know about subject content in higher education pedagogic courses. Special attention in the paper is focused on researching the relationship between theory and practice, disciplinary contents, and forms of pedagogical knowledge. The results showed that university teachers seek more practical, hands-on knowledge, while the government focuses on the theoretical content of pedagogical courses. It is also emphasized that all stakeholders included in this very important issue were unclear in their views on the content of pedagogical courses, which indicates that professional development cannot be seen as a strategic issue. Certainly, the participation of students as partners in assessment in higher education must be taken into account, as indicated by Chan & Chen (2023).

Bearing in mind the above, this paper aims to consider the most important elements

of constructive alignment in HE, i.e. student workload expressed through ECTS, study programs, course and unit objectives, LOs, theoretical and practical contents, teaching

and learning methodology, formative and summative assessment methods and effective acquisition of student generic and course-specific competencies.

THE EUROPEAN CREDIT TRANSFER AND ACCUMULATION SYSTEM

ECTS represents the basic tool of the European Higher Education Area (EHEA) for making the level of studies, SPs, and courses more transparent. This tool helps students to move between countries and to have their academic qualifications and study periods abroad recognized. Wagenaar (2006) described an introduction to the European Credit Transfer and Accumulation System (ECTS). Before that, the same author (Wagenaar, 2003) considered educational structures, LOs, workload and the calculation of ECTS. Key features of ECTS are given in the publication by the European Commission (2004). Kennedy (2006) and Kennedy & McCarthy (2016) have shown the connection between LOs and ECTS (2015). Very important questions are how ECTS is viewed by teachers (Gleeson et al., 2021) and how realistic are ECTS credits from the student's perspective (Salar et al., 2022).

The history of ECTS from 1989-2019 was described in detail by Wagenaar (2019). The development of ECTS had to start from scratch because worldwide there was no experience in setting up and running a national and/or international student workload-based transfer system that applied credit points. A Pilot Scheme (1989-1995) was set up to define ECTS. It involved five subject areas, and 145 HEIs in total, and set out to develop a sustainable, robust and reliable tool to facilitate international student mobility. Based on the notions of trust and confidence and the concept of "relative" student workload, this tool was unique. It opted for 60 credit points to represent one academic year. A detailed description of relevant aspects about key features of ECTS, ECTS and EHEA, ECTS for SPs design, delivery and monitoring, ECTS for mobility and credit recognition, ECTS and lifelong learning, ECTS and quality assurance and ECTS and supporting readers can find in documents the ECTS users' guide (European Commission, 2015).

The introduction of ECTS makes sense only if it is implemented at the end of the curriculum development cycle, i.e. if and only

if LOs have been properly defined by then, both for the entire SPs and for individual courses within the SPs, as well as if and only if, based on such defined LOs, methods have been adequately formulated for learning and teaching and assessment, i.e. evaluation of student achievements. Any introduction of the ECTS without satisfying the above conditions is a difficult mistake (Vukasović, 2006; European Commission, 2015).

The basis of the ECTS is the student workload. Therefore, ECTS is not a way to measure the quality of the teaching staff or their status, importance, or difficulty of the course itself. This needs to be especially emphasized since the experiences so far still bear witness to a wrong understanding of ECTS. In this sense, there are three important assumptions: 1. the regular working week of an average student should last 40 working hours; 2. the working week includes all student activities related to higher education, i.e. the working week includes the student's activity during the so-called contact classes, as well as his independent work (in terms of preparation for lectures, exercises, work in the laboratory, preparation for tests, exams, presentations, writing seminar papers and essays, reading literature, etc.) and 3. ECTS implies that one whole semester is worth 30 ECTS points (Vukasović, 2006; European Commission, 2015). Depending on the duration of the semester, i.e. from how many weeks the semester lasts, it is possible to determine how many hours one ECTS credit is worth. In this sense, the following can be distinguished: the relative value of ECTS (in the sense that one semester is worth 30 ECTS or one academic year is worth 60 ECTS) and the absolute value of ECTS, which is calculated by determining the number of hours that make up the workload during the academic year. In most cases, this number is between 1500 and 1800 hours per year, so one point represents 25–30 working hours (Wagenaar, 2003; Vukasović, 2006; European Commission, 2015; Wagenaar, 2019a).

Consequently, considering the defined

LOs and methods of teaching and evaluation, the estimation of student workload for each course is approached. During this process, it is necessary to take into account the independent work of students, and it is most often estimated that for one “contact class” there is one and a half to two hours of independent work, but this should be understood only as a general rule because such a proportion cannot be applied automatically since it represents an average ratio at the level of an average study program as a whole. It is possible to use other measures to assess the workload of students in independent work, such as the number of pages that can be read in an hour, estimating the duration of other learning or research activities, etc. The application of any measures should not be approached superficially, and it is also necessary to check the adequacy of those measures continuously during all semesters. It should be borne in mind that ECTS credits once determined are not determined forever. Precisely because the students’ workload includes their independent work, it is necessary to continuously check whether the workload related to the student’s independent work

is determined adequately. This implies that consultation with students is mandatory in this process. The participation of students in the bodies dealing with the introduction of ECTS is necessary, which is guaranteed by the Law on Higher Education, the relevant regulations and standards for accreditation, and the self-evaluation of study programs and HEIs as a whole. It should be noted that ECTS does not replace a grade in any case. Every student who has completed a certain course acquires the number of ECTS that are provided for that course, regardless of whether his/her grade is 6 or 10. In the supplement to the diploma, both ECTS and individual grades are listed, if they are provided for listing at all (Bekhradnia, 2004; Wagenaar, 2003; Vukasović, 2006; European Commission, 2015; Wagenaar, 2019a). In essence, by using LOs and workloads in curriculum creation and delivery, ECTS places the student at the centre of the educational process. Furthermore, the use of credit facilitates the creation and documentation of flexible learning paths, thus allowing students greater autonomy and responsibility (European Commission, 2015).

LEARNING OUTCOMES

Learning outcomes (LOs) are one of the basic elements of curriculum development, but their formulation in practice often comes down to simply satisfying the form prescribed by the Bologna Process, the Law on Higher Education, and standards for accreditation and self-evaluation of study programs and HEIs. However, the LOs hide within themselves the potential for a fundamental change in the HE processes (Allan, 1996; Maher, 2004). The potential of LOs stems primarily from the new philosophy of education, which implies a change in the focus of education from the teaching process to the learning and teaching process (Kennedy, 2006; Kennedy & McCarthy, 2016).

Using learning outcomes in the European Qualifications Framework and the National Qualifications Framework of Serbia (NQFS) are described in detail in relevant publications (European Commission, 2011, and Ministry of Science and Education of the Republic of Serbia, 2017, respectively). A very significant connection

between LOs and ECTS was mentioned earlier, which teaching staff should be aware of (Kennedy, 2006; Kennedy & McCarthy, 2016). Learning outcomes in HE have significant implications for curriculum design and student learning (Allan, 2004; Maher, 2004). There is a significant connection between LOs and student achievement assessment (Gosling & Moon, 2002; Moon, 2004; Moon, 2006; Nusche, 2008; Liu et al., 2012; Kennedy & McCarthy, 2016; Zlatkin-Troitschanskaia et al., 2016). A review of the literature regarding student LOs assessment in higher education and academic libraries was given by Goss (2022). A guide to formulating degree study program profiles including the program competencies and the program LOs is available (Lockhoff et al., 2011). Triangular relationship between learning outcomes, learning and teaching activities and outcome-based assessments in constructive alignments is shown in figure 2.

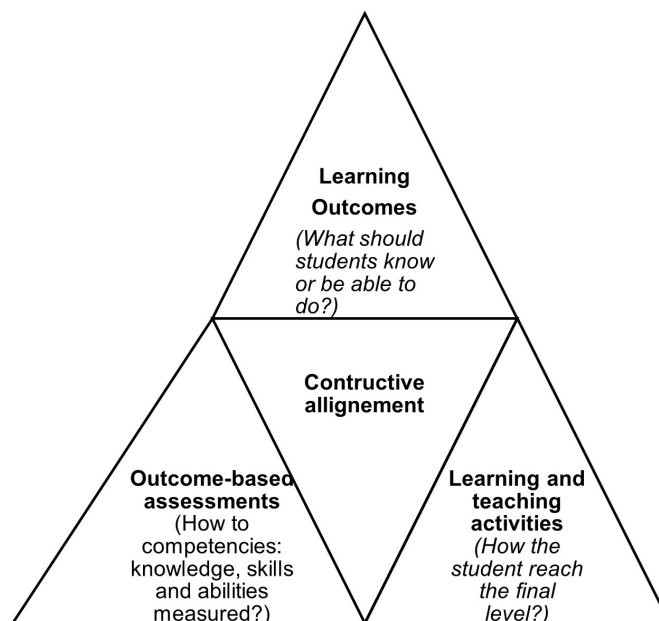


Figure 2. Triangular relationship in constructive alignment.

In essence, the focus of constructive alignment between the elements shown in figure 2 is on what students learn and how they do it with teacher facilitation. The learning outcomes should be determined before the start of the course and they should enable determining which learning activities students should undertake. The learning outcomes also determine the basis for conducting formative and summative assessments. These assessments should show to what extent students have achieved the learning outcomes.

It is a well-known fact that LOs are explicit statements about the result of learning, they describe the learner's capabilities, they are indicators of the area the learner knows, what the learner can do under certain circumstances, for which activity the learner is qualified and how the learner behaves concerning what learner does (what attitudes the learner exhibits). In education, the outcome is a minimum and compulsory learning outcome. The LOs enable confirmation of achievement and are aligned with the developmental characteristics of the learner. LOs are classified into cognitive, psychomotor, and affective domains, and are formulated at the level of knowledge, skills, and attitudes (Kennedy, 2006; Vukasović, 2006; Kennedy & McCarthy, 2016).

A learning outcome is a precisely written statement about what a student should know, understand, or be able to demonstrate at the end of a specific learning unit (a class, course, or upon obtaining a qualification, i.e. at the end of the study program) (Vukasović, 2006; Kennedy & McCarthy, 2016). In essence, the LOs are a clear definition of competencies, skills, and attitudes that the student should acquire during a certain period of study (Kennedy, 2006). LOs, therefore, do not refer to the content or teaching methods, but to what the student is expected to acquire or develop during learning, but the content and methods are defined based on them. LOs can be defined both for the entire study program and for an individual subject or course, and, if necessary, even for smaller units. LOs are most often written keeping in mind the minimum competencies and skills that an average student should acquire to complete a given learning unit, but it is also possible to write them keeping in mind the most successful students and the maximum of acquired competencies and skills. Therefore, it is always necessary to emphasize whether it is the expected LOs (those that correspond to the minimum of competencies and skills) or the desired LOs (those that correspond to the maximum of competencies and skills).

Regarding this comprehensive definition, the authors emphasize the following: it is important that the learning outcome is in written form (this achieves transparency and avoids problems in interpretation) and the “should” part indicates a possibility and not a guarantee, since the lecturer and the HEIs as a whole can only create the conditions for learning, but essentially cannot guarantee that the student will learn it. Also, the authors highlight that a well-written learning outcome should include the following sections: a verb that states what the student is expected to be able to do at the end of the learning period, a word that refers to what the

student has (in case the outcome refers to a skill, the word can describe how the skill is applied) and a word that refers to the nature of the performance required as proof that the subject has been mastered. Therefore, it is necessary to clearly define which competencies the student should have to be considered to have achieved the learning outcome. In addition, the learning outcome includes the obligation to demonstrate the disposition of those competencies (Gosling & Moon, 2002; Moon, 2004; Moon, 2006; Vukasović, 2006; Nusche, 2008; Liu et al., 2012; Kennedy & McCarthy, 2016; Zlatkin-Troitschanskaia et al., 2016).

GENERAL AND SUBJECT-SPECIFIC COMPETENCIES

It is of great importance that teaching staff and students understand the relationship between learning outcomes and competencies. According to NFQS (Ministry of Science and Education of the Republic of Serbia, 2017), competence is an integrated set of knowledge, skills, abilities and attitudes that enable an individual to effectively perform activities at work, following the expected standard. A framework for defining and comparing generic competencies in higher education was described by Kallioinen (2010). The term “competence” implies expertise in a particular field. Generic competence includes the knowledge, skills and abilities that an individual should possess at a certain level of education, regardless of the profession or scientific field he/she is engaged in. Professional competencies imply knowledge, skills and abilities related to a certain profession and/or a certain scientific field. Braun & Mishra (2016) presented a combination of five approaches for assessing higher education graduates’ competencies, focusing primarily on employment-related competencies (both cognitive and non-cognitive skills, such as personal and social skills, leadership, and communication skills). A detailed description of a proposal for the assessment of generic competencies in competency-based learning is given by Sanchez & Ruiz (2008). The development of generic competencies of graduates in HE must be aligned with labour market requirements (Pukelis & Pileickiene, 2012). Today, the development of digital competencies in HE is considered a core skill for teachers, supporting them in managing several

technological, curricular, and pedagogical aspects. Digital competence of teachers refers to digital skills, pedagogical-didactic awareness and understanding of the impact of learning strategies on student learning. As facilitators of the learning process, the teachers also support the development of digital competencies of their students (Inamorato dos Santos et al., 2023). In all this, the impact of pedagogical training perceived by teachers is very important for teacher professional development in higher education and the development of students’ competencies (Fernandes et al., 2023).

It can be said that competencies are the most important part of the formulation of learning outcomes. It is impossible to define the learning outcome if we do not have clearly defined competencies that we want to develop through a specific course or study program. The difference between the list of competencies and the list of learning outcomes is that the learning outcome also defines how it will be demonstrated, i.e. check whether a given competence has been acquired or not. In this connection, from the formulation of the learning outcomes comes the method of evaluating students, i.e. checks of acquired or developed knowledge, skills and abilities. It should be said that competencies and LOs in a large number of countries also have a normative role, which is primarily reflected in their use as a benchmark for certain study programs in quality assurance and accreditation processes (Vukasović, 2006; Kennedy & McCarthy, 2016).

If we agree that the student and the learning process (and not the teaching process)

are the focus of education and curriculum development, the first step in the creation of a new type of curriculum is starting from the end, i.e. defining the competencies that the student should have upon completion of the study program. And here we come to a problem related to the very concept of competence. One would think that this is nothing new and that both old and existing programs had final competencies in mind, and at the same time, one usually thinks of the goals of the programs, usually defined as a list of occupations that a graduate student will be able to perform or as a list of materials that student to know after graduation (Vukasović, 2006).

The term competence usually refers to knowledge, abilities and skills that a student acquires or should develop, which make them capable of doing something. Student-centred education aims, among other things, for students to be competent at the end of the educational process, that is, to bring students to possess certain knowledge, skills and abilities. Competencies in this sense represent a dynamic combination of knowledge and its application, attitudes and responsibilities that describe the learning outcomes of the educational program (Sanchez & Ruiz, 2008).

Of course, there is not, nor can there be, absolute agreement about what should be the knowledge, abilities and skills that should be set as the goal of education. Different partners in the education process (academic staff, employers, graduates, students still in education, etc.) usually have different views on what the final competencies should be, as well as on which competencies are most important. This, of course, does not mean that the dialogue should not continue, nor that the process of harmonizing between these partners about what should be the learning outcome expressed in the form of competencies is pointless. On the contrary, only if we begin to look at curriculum development and the educational process in general from its projected end and if we begin to define and discuss competencies, we will achieve greater transparency of the entire education process. This is precisely the often-mentioned new language that is being developed, namely the language of competencies that arises from the belief that this language provides the possibility to express comparability in terms of what

people who graduate can do. Also, the language of competencies enables the expression of common reference points for different academic disciplines, thus offering a non-prescriptive frame of reference for the academic community. In addition to the importance, they have for the educational process itself, teachers and students, competencies make it easier to communicate with parties interested in education - primarily employers - who otherwise have difficulty interpreting educational goals, as well as understanding what graduate students are, i.e. their potential employees know and can (Wagenaar, 2014; Wagenaar, 2019a; Wagenaar, 2019b).

There are several divisions of competence. Here we list the divisions created within the Tuning project (<http://tuning.unideusto.org/tuningeu/>). This division, first of all, divides competencies into generic and professional:

- Generic competencies should be possessed by everyone who completes a certain level of education, regardless of the science or profession they are engaged in (such as knowledge of a foreign language, application of knowledge in practice, or electronic literacy) and
- Professional competencies are identified for each profession or field of study and in this sense they are narrower.

Within the framework of the Tuning project, three groups of generic competencies have been defined as instrumental, interpersonal, and systemic.

Instrumental generic competencies include: 1. the ability to analyze and synthesize, 2. the ability to plan and organize, 3. basic general knowledge, 4. grounding in basic professional knowledge, 5. oral and written communication in the mother language, 6. knowledge of a foreign language, 7. elementary computer knowledge, 8. information management skills (ability to obtain and analyze information from various sources), 9. problem-solving, and 10. decision making.

Interpersonal generic competencies include: 1. ability to criticize and self-criticize, 2. teamwork, 3. interpersonal skills, 4. ability to work in interdisciplinary teams, 5. ability to communicate with non-experts from other fields, 6. an understanding of diversity and multiculturalism, 7. ability to work in an

international environment, and 8. ethical commitment.

Systemic generic competencies include 1. ability to apply knowledge in practice, 2. research skills, 3. ability to learn, 4. ability to adapt to new situations, 5. ability to create new ideas (creativity), 6. leadership, 7. understanding of cultures and customs of other countries, 8. ability to work independently, 9. creation and management of projects, 10. initiative and entrepreneurial spirit, 11. concern for quality and 12. a desire for success.

This, of course, does not mean that this division is the only possible one, nor that the list of competencies that Tuning and this text mention is final, and in particular it should be taken into account that each curriculum is

created at a specific HEIs and within a society that may need special general and specific competencies that are not listed here. If, for example, the transitional nature of society and the recent past of the region are taken into account, competencies such as social responsibility, critical thinking and reading, understanding gender and gender relations, etc. can be considered remarkably important. As for professional competencies, it should be emphasized that in this area dialogue within a specific profession is necessary both for the sake of mutual recognizability of the curriculum and for the recognition of parts of studies or final qualifications in the case of student mobility (Vukasović, 2006; Pantić, 2008).

METHODS OF LEARNING AND TEACHING AND EVALUATION OF STUDENT ACHIEVEMENTS

After the LOes have been properly defined, it is necessary to approach the development of teaching and assessment methods that will enable the realization of those LOs. Here, first of all, the relationship between teaching objectives and LOs should be considered. These two terms are not the same: LOs refer to students and what they learn, while learning objectives refer to teacher activities that enable the achievement of LOs (Kenedy, 2006; Kennedy & McCarthy, 2016). Previously realized curricula and SPs primarily talk about educational goals. Teaching objectives are primarily related to the content of teaching. This means that the content of the lessons, i.e. the description of the topics to be covered in one course should be defined after defining the LOs for that course. Otherwise, the course may be burdened with redundant material, the mastery of which does not lead to the acquisition of competencies expressed through the LOs (Vukasović, 2006).

Today, there is increasing interest in the concept of active learning and teaching (ALT) in higher education. In essence, ALT is a concept, which most often refers to focused on student teaching and learning methods, activating and motivating methods and activities led by the teacher (Bonwell & Eison, 1991; Prince, 2004; Felder & Brent, 2009; Mitchell et al., 2017). Often because of this, ALT is generally considered not a learning concept but an instructional concept. Student learning outcomes have been largely

positive in numerous studies of ALT (Prince, 2004; Freeman et al., 2014; Michael, 2006; Prince & Felder, 2006; Smith, 2005). Accordingly, ALT is a superior approach when compared to traditional, more content-focused approaches such as lectures. There are many barriers to student active learning in higher education (Børte, et al., 2023).

Regarding teaching methods, it is important to note that the success of students in achieving learning outcomes depends on the attitude of the teaching staff towards learning and teaching and the methods of learning and teaching (Marbach-Ad et al., 200; Latchanna, & Dagnew, 2009). Quality achievement of well-defined learning outcomes implies that students understand learning as a process in which they review their understanding of concepts and processes and/or create new concepts and understand the connections between them. For this to be possible, it is necessary that the teaching staff, for their teaching goal, do not simply go over the material, but try to achieve as much as possible the so-called "interactive" teaching, that is, to apply the active learning and teaching (ALT) methodology which implies the purposeful application of numerous strategies, methods and techniques of ALT in the classroom and during an independent study of students at home, in the library, etc. Interactive teaching implies active participation of students during lectures, exercises, laboratory work, etc., and

through discussions, presentations, asking questions, processing part of the material by the students themselves, etc., and a variety of methods used. This is especially important if it is taken into account that the learning outcomes imply the achievement of a diverse set of competencies that cannot be developed if the teaching methods are limited to *ex-cathedra* lectures or only to work in groups or only to presentations, etc. (Ivić et al., 2002).

As for the connection between learning outcomes and assessment methods and criteria, it is important to emphasize the following (Vukasović, 2006):

1. The difference between expected and desired LOs should be kept in mind. Concerning that difference, it is possible to define: a. that it is necessary to fulfil the expected learning outcomes for the transitional assessment and b. that for the highest grade it is necessary to fulfil the desired learning outcomes. In the "space" between the lower and upper criteria, criteria for other grades can be clearly defined;
2. The assessment method must correspond to the defined LOs, or one learning outcome and
3. It is possible that one assessment method evaluates the achievement of several learning outcomes and vice versa - that several different assessment methods are needed to assess the achievement of one learning outcome.

Bearing in mind that the LOs are expressed through generic and specific competencies, the question arises which teaching methods enable the acquisition or development of generic competencies, and which ones enable the acquisition or development of professional competencies? It was found that research studies and active learning promote professional competencies for the 21st century in Finnish teacher education (Niemi & Nevgi, 2014). It is similar in other HE, for example, biology (Armbruster et al., 2009) and information and communications technology engineering

(Llorens et al., 2017). It should be borne in mind that a part of generic competencies is already developed to some extent at lower levels of education, i.e. that the role of higher education is to deepen and/or expand these competencies. It also implies that the role of HE must not stop the development of given competencies or even redirect it, although there are courses in which students are discouraged from demonstrating certain competencies, such as the ability to think critically if students' opinions are not sought or devalued, or the ability to analyse and synthesize if the lecture is reduced to an *ex-cathedra* reading of the material, which is designated as my subject syndrome. Additionally, if the given competencies were not developed during previous education, the role of higher education must be to correct the mistakes made during previous education (Vukasović, 2006, Pérez Martínez et al., 2010).

Furthermore, it is important to understand that there are generic competencies that can be developed within one teaching unit (in the sense of an individual lesson, but also the whole course), i.e. a certain teaching unit is explicitly dedicated to the acquisition of certain generic competencies (or one of them), as well as generic competencies that can be developed within teaching units dedicated to specific competencies, but with the use of adequate learning and teaching methods. As for learning and teaching methods intended for the acquisition and development of professional competencies, the only thing that can be supposed at this level of generality is that the choice of learning and teaching methods must be approached responsibly, bearing in mind the learning outcomes related to the acquisition of professional competences. All this further emphasizes the importance of well-formulated learning outcomes. A well-formulated learning outcome, in essence, contains, more or less explicitly, the methods of learning and teaching as well as an indication of the methods of evaluating student achievements (Vukasović, 2006).

CONSTRUCTIVE ALIGNMENT

The paper under the title *Reclaiming Constructive Alignment* by Loughlin et al. (2021) gave many relevant aspects about the history of CA, adoption and adaptation of Outcomes-Based Education (OBE) by HE policymakers: the European context and the national context of UK and Sweden, a vitiated theory: CA in educational practice, alignment: curricula overwhelmed with policy requirements, LOs: a tension between internal and external quality demands, constructivism: disappears from the theory as practised, reaction and resistance: contested understandings, de-professionalisation, academic development, conflating theory with practice, critical appraisal of learning objectives and outcomes, criticism of constructivism and reclaiming CA. It states that CA and particularly LOs are often vilified among academic staff as a pernicious influence on learning and teaching. It is also emphasized that the mechanistic use of alignment and learning outcomes for validation and audit purposes can create an illusion of quality control which bears little relation to the reality of teaching practice and student learning.

In essence, before the definition of CA by John Biggs (1996), curriculum design in HEIs was largely an individual responsibility with teachers using their personal experiences to decide what students should learn. The idea that course teams might work collectively on a course design was only accepted in settings focused on distance education where a more industrial mode of developing course materials was required. CA is now explicitly named as a principle used by many HEIs when designing courses or programs. Part of its appeal is the apparent simplicity of the concept. Nevertheless, it is an idea that also has the potential to be misunderstood (Kandlbinder, 2014).

In the paper by Maffei et al. (2022) described the design of the constructively aligned educational unit. Students' perspectives on how different elements of CA support active learning are studied in the paper by Hailikari et al. (2022). It was found that different elements of CA had a clear role in guiding student learning and study. Factors related to teaching and assessment seem to play a large role. In the course that applied ALT, almost all students had a deeper approach to studying the

material. Teaching that sufficiently challenges students requires active student participation throughout the course, provides opportunities for peer support, and uses high-quality teaching materials, appears to support students to adopt a deep approach to studying. On the other hand, traditionally organized courses with lectures and final exams, and without engaged activities, had significantly more students who adopted a non-reflective or mixed approach.

In the Rulebook of the National Council for Higher Education, 2019, on standards and procedures for external quality assurance of higher education institutions and Standards and guidelines for quality assurance in the European Higher Education Area (ESG), European Association for Quality Assurance in Higher Education (ENQA) (2015) it is emphasized that it is necessary that the HEI regularly and systematically checks and, if necessary, re-determines the goals of the study programs and their compliance with its mission and goals, as well as the structure and content of the study programs in terms of the relationship between general-educational, theoretical-methodological, scientific-professional and professional-applied disciplines, the workload of students measured by the number of ECTS credits, the outcomes and expertise that students acquire when they complete their studies, as well as opportunities for employment and further education. At the same time, the HEIs should ensure that the LOs are based on the descriptors of qualifications of a certain cycle of education in a given scientific field and on the corresponding European and national framework of qualifications, including the requirements of international and national professional associations. To achieve this, the HEIs should have established procedures for approving, monitoring, and controlling study programs. In addition, the HEIs should regularly obtain feedback from employers, representatives of the National Employment Service, and other relevant organizations about the quality of studies in the degree programs. The HEIs should also provide students with participation in the assessment and quality assurance of study programs. The higher education institution must ensure continuous updating of the content of the curriculum

and its comparability with the curricula of corresponding foreign higher education institutions. Curricula of study programs should encourage students to think creatively, to a deductive way of research, as well as to apply that knowledge and skills for practical purposes. The conditions and procedures that are necessary for completing studies and obtaining a diploma of a certain level of education should be defined and available to the public, especially in electronic form, and should be aligned with the goals, contents, and scope of accredited study programs. A HEIs should have mechanisms for monitoring the quality of study programs (e.g. a formal legal procedure for approving study programs, procedures for monitoring the success of studies in a study program and establishing responsibility for their improvement, as well as regular and periodic evaluation of study programs). Within those procedures, the HEIs should regularly review the educational outcomes within the accredited study programs it runs, based on learning outcomes. It is of great importance that the HEIs regularly review how it has established harmony between teaching methods, LOs, and evaluation criteria, especially the teaching method oriented towards student learning and the evaluation system based on the measurement of learning outcomes. Also, the HEI should consider how the learning outcomes determine the content of the study program and its organization, teaching methods, strategies and techniques, and proposed courses and procedures for knowledge verification and assessment. Finally, it is also of great importance that the HEI looks at the subject mapping table as an aid to gaining insight into how the learning outcomes of the study programs are covered within the compulsory subjects defined by the study programme. The mentioned aspects of quality assurance are also emphasized by Biggs (2001), Biggs & Tang (2007), Biggs & Tang (2011a), and Cincović et al. (2020).

Within the framework of internal control, self-evaluation, and accreditation, the HEIs must consider in detail the evaluations of students' achievements in realizing the intended learning outcomes. In addition, the HEIs should have a strategy and methods used to check the achievement of learning outcomes for each study program. Also, the HEI should

assess to what extent the achieved results on study success match the planned learning outcomes. Reviewing the share of learning activities required to achieve the expected learning outcomes (time spent on activities directly led by teaching staff, time spent on independent work by students, on mandatory professional practice, time needed to prepare for the knowledge test, and time included in the knowledge test itself) in the total value ECTS for each subject or module is of crucial importance (Vukasović, 2006; Cincović et al., 2020).

Assessment of student workload necessary to achieve the set learning outcomes and compliance with ECTS should be subject to regular review, through monitoring and gathering feedback from students. The HEI should use a method of exact measurement, not estimation, of the student load for each ECTS, thereby documenting that the allocation of points to parts of the program is based on the student load necessary to achieve the learning outcomes in a formal sense. The institution submits, if it has such data, the results of a survey obtained from students in the process of regular workload monitoring. Also, HEIs should adopt measures and activities to reduce the rate of failure in exams and harmonize student workloads with the values of ECTS points (Gleeson, 2013; National Council for Higher Education, 2019; Cincović et al., 2020).

The improvement and continuous modernization of the existing study programs should be based on the development of science and the new requirements that are placed before the given educational profile. Teachers and students should be familiar with the defined requirements that the graduate work (thesis) should fulfil, especially in terms of academic methodology, formal aspects, practical orientation, and evaluation criteria. The HEI should establish an appropriate way to keep in touch with its graduates. It should evaluate the relevance of the study program for the labour market (local, national, international), as well as the achievements of the graduated students in their later professional development (ENQA, 2015).

Following the Rulebook of the National Council for Higher Education, 2019, on standards and procedures for external quality control of HEIs by using the SWOT analysis method, the

HEI should analyse and quantitatively evaluate the following elements:

- objectives of the study program and their compliance with learning outcomes,
- teaching methods oriented towards learning outcomes,
- an evaluation system based on the measurement of learning outcomes,
- compliance of the ECTS load with the learning activities required to achieve the expected learning outcomes,
- mutual conformity of learning outcomes and expected competencies based on descriptors of qualifications of a certain

cycle of education,

- the ability to functionally integrate knowledge and skills,
- procedures for monitoring the quality of study programs,
- feedback from practice about graduated students and their competencies,
- continuous modernization of study programs,
- availability of information about graduate work and professional practice and
- availability of information about study programs and learning outcomes.

CONCLUDING REMARKS

Based on the literature review of the most significant data related to CA between objectives, teaching and LOs, student competencies, and assessment methods in HE, it can be concluded:

- CA between objectives, teaching and LOs, student competencies, and assessment methods in HE is still not accepted in many HEIs, that is, learning and teaching practices in them are still based on the traditional approach using teacher-centred strategies, where the teachers impart knowledge and students are passive learners and therefore are not allowed to have autonomy over their learning. In this way, students are not experiencing enough chances to interact and communicate to achieve the necessary generic and professional competencies;
- Teachers in HEIs must become familiar with the essence of CA between student workload expressed through ECTS, SPs, course and unit objectives, LOs, theoretical and practical contents, teaching and

learning methodology, formative and summative assessment methods, and effective acquisition of student generic and course-specific competencies;

- Accordingly, this paper outlines the specifications of the CA approach which could be used to put an end to this daunting situation in HEIs by designing and developing the existing SPs to meet the 21st-century cognitive skills perspectives;
- CA framework could enable teachers to create and develop SPs based on the actual needs of the target learners in different contexts;
- Also, the involved staff could plan learning and teaching activities by applying this approach in the given daily lessons and
- Finally, it can be concluded that CA improves the quality of students' learning and enables the acquisition of appropriate competencies that trace the way for their lifelong learning.

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КОНСТРУКТИВНО УСОГЛАСУВАЊЕ МЕЃУ ЦЕЛИТЕ, НАСТАВАТА И УЧЕЊЕТО, КОМПЕТЕНЦИИТЕ НА СТУДЕНТИТЕ И МЕТОДИТЕ НА ОЦЕНУВАЊЕ ВО ВИСОКОТО ОБРАЗОВАНИЕ

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Резиме

Висококвалитетниот процес на учење во високото образование подразбира правилно и конструктивно усогласување на суштинските елементи, како студиските програми, целите на курсот, содржината на предметните програми, резултатите од учењето, обемот на работа на студентите, активностите во текот на наставата и учењето, методите на оценување и стекнувањето на соодветни компетенции, што во литературата е познато како конструктивно подредување (СА). Во суштина, СА ги усогласува однапред определените компетенции, активностите во текот на учењето и наставата, како и начините на оценување. СА е пристап заснован на резултатите од наставата, при што резултатите од учењето кои учениците треба да ги постигнат се дефинираат пред да се одржи наставата.

Успехот на студентите на сите нивоа на студирање првенствено зависи од конструктивното усогласување како еден од најзначајните и највлијателните принципи во високото образование. Деталното познавање на овој принцип и неговата доследна примена е основна обврска на наставничкиот кадар во високото образование. За да се развие „конструктивно усогласена“ предметна програма, наставникот треба да започне од предвидените компетенции специфични за курсот, потоа избор на најсоодветните активности за учење, поучни и наставни активности, како и методите за оценување за овие специфични компетенции.

Трудот подетално ја објаснува суштината на СА меѓу обемот на работа на студентите изразен преку ЕКТС, студиските програми, целите на наставните програми и курсот, резултатите од учењето, теоретските и практичните содржини, методологијата на наставата и учењето, методите на формативно и сумативно оценување и ефективно стекнување на генерички и компетенции специфични за курсот.

Клучни зборови: конструктивно усогласување, оптоварување на учениците, цели, резултати од учењето, активности за учење и настава, оценување, компетенции.



MACRONUTRIENT AVAILABILITY OF DIFFERENT ORIENTAL TOBACCO VARIETIES GROWN UNDER THE SAME AGROTECHNICAL AND ECOLOGICAL CONDITIONS

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Abstract

Macronutrients are essential for the growth and development of all plants, including oriental tobacco. Their availability determines the overall health and growth of tobacco plants and it is in the focus of sustainable tobacco production. Proper management of soil macronutrients is necessary for high and quality crop yield.

In order to evaluate the macronutrient content and factors influencing their availability, different varieties of oriental tobacco were selected for a field experiment. Plants were grown under the same agrotechnical conditions, on two locations, on a colluvial-delluvial and fluvisol soil type. Available forms and total content of nitrogen, phosphorus, potassium, magnesium and calcium as important macronutrients were determined in soil samples, while only total content was analyzed in leaf samples. Availability ratios were presented by available and total content as a direct measure of the potential effectiveness of the selected macronutrient in soil. Macronutrients Ca and P have greater availability as compared to N, K and Mg. Analyzation of the available and total content of macronutrients in soil and leaf samples gives helpful insight in application of certain cultivation practices for specific agricultural and environmental management purposes.

Key words: tobacco, nutrients, macronutrients, growth factors

INTRODUCTION

Macronutrients have a multilevel and vital role for plants with wide range of chemical activities within their cells. In order to reach optimal plant growth and overall plant health, it is important to understand macronutrients availability and to make certain that plants receive an adequate supply of them.

In agriculture, plants are usually supplied with nutrients from fertilizers. The impact of a certain nutrient varies depending on the plant type, and for oriental tobacco, it has direct reflection on the quality of the raw material (Adamu et al., 1989; Pelivanoska & Jordanoska, 2013, 2016, 2018). Nitrogen is essential element for tobacco plants and its availability determines the growth rate of the plant. The influence of nitrogen fertilizers is directly related to the content of nitrogen in the leaves; therefore, it highly effects the ripening, drying, yield

and quality of oriental tobaccos (Pelivanoska, 2009; Gholizadeh et al., 2012). It was shown that tobacco constantly accumulates nitrogen and phosphorus during the vegetation period and their content in the leaves is constantly increasing (Hawks & Collins, 1987). Phosphorus is essential for the formation of strong roots and stems, flower development, and seed production. It contributes to the maturity and development of tobacco as prerequisite for quality dried leaves that have good chemical properties (Filiposki, 1990; Mitreski & Korubin-Aleksoska, 2005; Filiposki et al., 2010). Potassium significantly contributes to the storage of carbohydrates, plays an important role in maintaining the ionic status of the plant, shortens the growing season, improves tobacco burning qualities (Pelivanoska, 2009; Filiposki et al., 2010; Pelivanoska et al. 2012, 2017;

Zaprijanova & Hristova, 2018). Magnesium is an integral part of the chlorophyll molecule and has a catalytic role in protein synthesis within plants (Campbell, 2000). Calcium plays a major role in cell maintenance, growth and activation of numerous enzymes in the tobacco plant. Additionally, it acts as an antagonist to various toxic metals such as Cu, Zn, Mn, Fe and protects plants from the toxic reaction (Tiffin, 1977).

The main factors that affect availability and uptake of macronutrients are their content and form in the soil, soil characteristics, plant biology, fertilization and utilization of

soil amendments (Tso, 1990; Bell et al., 1992; Rodgman & Perfetti, 2009; Golia et al., 2009; Khan et al., 1992; Zaprijanova et al., 2010; Subhashini, 2016; Jordanoska et al., 2018; Kumaresan et al., 2019; Lisuma et al., 2020). This study provides an analysis on the relationships between factors that have a crucial role in the availability and uptake of P, N, K, Ca and Mg. The main purpose was to determine the availability of selected nutrients for four oriental tobacco varieties, considering soil characteristics and different fertilization rates.

MATERIAL AND METHODS

Methodology of the field experiment

For the purpose of the research, two experiments were set in two different locations. One experimental field was on a colluvial-diluvial soil type at the experimental field at Scientific Tobacco Institute – Prilep and the other field was on fluvisol soil type in locality Dobrushevo. The experimental fields were set up in complete randomized block design with three replications and with four oriental tobacco varieties (P-23, P-79-94, Dzhebel Basma 1 and Elenski 817) (Pelivanoska & Jordanoska Shishkoska, 2019). The same agrotechnical practices were applied for all varieties at both locations. Availability of studied macronutrients was examined through application of different nitrogen rates (0, 20, and 30 kg/ha) and application of phosphorus (60 kg/ha) and potassium (40 kg/ha) for both experimental fields (Pelivanoska & Jordanoska Shishkoska, 2019).

Soil and plant analyses

Pretreatment of soil samples for physico-chemical analysis was done in accordance to ISO 11464 (2006). Soil samples were collected before planting and after harvest from both locations and analyzed for soil texture, pH, total nitrogen content, organic matter content,

carbon content, available phosphorus and available potassium content (Pelivanoska, 2012). The total macronutrient content in soil samples was determined according to ISO 14869-1 (2001), analyzed by the application of atomic emission spectrometry with inductively coupled plasma, ICP-AES (Varian, 715-ES). Available nitrogen, calcium and magnesium content was determined according to Kim (2005).

The harvest of the mature tobacco leaves was done manually in 5 rounds. Leaves were washed carefully to remove any adhering soil particles and rinsed with redistilled water. The plant material was dried and homogenized to a constant weight after drying at 75 °C for 12 hours. Total nitrogen in leaves was analyzed by modified Kjeldahl method (Srbinoska, 2012). For the analyses of total P, K, Ca and Mg, plant samples (0.5000 g) were digested in Teflon vessels with HNO₃ and H₂O₂ using the Mars microwave system (CEM, USA) and analyzed by ICP-AES with ultrasonic nebulizer CETAC (ICP/U-5000 AT), (US-EPA Method 3050B, 2007).

The reference standard materials JSAC 0401 (soil) as well moss samples M-2 and M-3 (Steinnes et al., 1997) were used for quality control.

RESULTS AND DISCUSSION

Soil properties

According to the results from soil samples, it is obvious that soils from the two experimental locations have different properties (Table 1). According to agrochemical parameters, soil sample from experimental field in Prilep has low humus content, poorly acidic soil pH and medium content of available phosphorus

and potassium. Soil sample from Dobrushevo has an average humus content, low content of available phosphorus, optimal content of available potassium and neutral to poorly acidic pH reaction. The results from soil analysis after the harvest and at the end of the vegetation are presented in Table 2.

Table 1. Soil properties of experimental fields in Prilep and Dobrushevo before planting.

	Prilep	Dobrushevo
Clay (%)	24.50	25.70
pH (H ₂ O)	6.64	7.15
pH (KCl)	5.98	6.18
Humus (%)	0.81	1.77
Total N (%)	0.04	0.09
Total P (%)	0.05	0.03
Total K (%)	0.21	0.36
Total Ca (%)	0.24	0.18
Total Mg (%)	0.19	0.39
Available N (mg/kg)	6.10	7.10
Available P (mg/kg)	68.4	28.8
Available K (mg/kg)	110	180
Available Ca (mg/kg)	656	812
Available Mg (mg/kg)	114	190

Table 2. Soil properties of different experimental plots in Prilep and Dobrushevo.

Studied element	Prilep			Dobrushevo		
	Ø	N ₂₀ P ₆₀ K ₄₀	N ₃₀ P ₆₀ K ₄₀	Ø	N ₂₀ P ₆₀ K ₄₀	N ₃₀ P ₆₀ K ₄₀
pH (H ₂ O)	6.12	6.02	6.02	6.58	6.70	6.70
pH (KCl)	5.96	5.86	5.86	6.10	6.12	6.12
Humus, %	0.62	0.60	0.60	0.96	0.92	0.92
Total N (%)	0.02	0.03	0.03	0.07	0.08	0.08
Total P (%)	0.04	0.04	0.04	0.02	0.02	0.02
Total K (%)	0.20	0.19	0.20	0.32	0.31	0.31
Total Ca (%)	0.23	0.24	0.25	0.19	0.20	0.20
Total Mg (%)	0.18	0.17	0.17	0.40	0.41	0.41
Available N (mg/kg)	5.18	6.70	6.70	7.20	8.20	8.20
Available P (mg/kg)	58.12	62.13	62.13	30.12	40.15	40.15
Available K (mg/kg)	120	150	150	220	210	210
Available Ca (mg/kg)	750	700	700	950	895	895
Available Mg (mg/kg)	112	110	110	179	192	192

*Ø- unfertilized control, N₂₀P₆₀K₄₀, N₃₀P₆₀K₄₀ (the index indicates applied nitrogen, phosphorus and potassium doses in kg/ha).

Tobacco leaf samples

The content of the analyzed macronutrients in tobacco leaves from both locations are given in Table 3 and Table 4. As it can be seen, highest content of nitrogen is detected in the treatments with highest nitrogen dosage of 30 kg/ha for all tobacco varieties and on both locations. According to Pelivanoska (2009), Pelivanoska et al. (2012) and Pelivanoska & Jordanoska (2016,

2018), the influence of nitrogen fertilizers is directly related to the ripening, drying, yield and quality of oriental tobaccos. The nitrogen content in tobacco leaves (1.4 % - 3.6 %) are in the limits to those observed in the oriental tobacco samples (Jordanoska et al., 2018). Similar findings are observed in the same tobacco varieties by Zaprijanova & Hristozova, (2018).

Table 3. Macronutrient content in tobacco leaves from experimental field in Prilep.

Treatment	Tobacco variety	N (%)	K (%)	P (%)	Ca (%)	Mg (%)
Ø	P-23	1.60	2.80	0.36	6.72	0.72
N ₂₀ P ₆₀ K ₄₀		2.10	3.20	0.36	7.33	0.78
N ₃₀ P ₆₀ K ₄₀		2.23	2.60	0.32	7.69	0.70
Ø	P-79-94	1.71	2.99	0.38	6.82	0.67
N ₂₀ P ₆₀ K ₄₀		2.46	2.92	0.32	9.32	0.82
N ₃₀ P ₆₀ K ₄₀		2.57	2.74	0.35	8.49	0.70
Ø	Dzhebel Basma 1	1.45	2.27	0.31	6.96	0.54
N ₂₀ P ₆₀ K ₄₀		1.89	2.25	0.27	8.32	0.58
N ₃₀ P ₆₀ K ₄₀		2.02	2.40	0.32	8.84	0.69
Ø	Elenski 817	1.68	2.21	0.30	7.36	0.66
N ₂₀ P ₆₀ K ₄₀		2.01	2.25	0.28	8.02	0.74
N ₃₀ P ₆₀ K ₄₀		2.23	2.52	0.35	7.61	0.73

*Ø- unfertilized control, N₂₀P₆₀K₄₀, N₃₀P₆₀K₄₀ (the index indicates applied nitrogen, phosphorus and potassium doses in kg/ha).

According to Campbell (2000), potassium content in dry plant material ranges from 1.0 to 5.0% and the required amount for normal development of the leaf is from 1.5 to 3.0% potassium. In our study, potassium content in all tested tobacco varieties is within these limits. Oriental tobacco P-66 is the most

cultivated variety in North Macedonia with average potassium content from 0.26% to 1.33% (Jordanoska et al., 2018). According to our findings, four tested oriental varieties (P-23, P-79-94, Dzhebel Basma 1 and Elenski 817), from both locations (Tabs. 3 and 4) have higher potassium content compared to variety P-66.

Table 4. Macronutrient content in tobacco leaves from experimental field in Dobrushevo.

Treatment	Tobacco variety	N (%)	K (%)	P (%)	Ca (%)	Mg (%)
Ø	P-23	1.78	3.24	0.33	5.19	1.41
N ₂₀ P ₆₀ K ₄₀		1.94	3.10	0.37	5.07	1.36
N ₃₀ P ₆₀ K ₄₀		2.19	3.53	0.35	5.36	1.42
Ø	P-79-94	1.8	2.91	0.35	4.79	1.16
N ₂₀ P ₆₀ K ₄₀		2.03	3.55	0.36	5.96	1.44
N ₃₀ P ₆₀ K ₄₀		2.39	3.79	0.36	5.91	1.51
Ø	Dzhebel Basma 1	1.46	2.79	0.31	5.23	1.36
N ₂₀ P ₆₀ K ₄₀		1.65	3.10	0.33	6.05	1.36
N ₃₀ P ₆₀ K ₄₀		1.9	2.68	0.29	5.44	1.22
Ø	Elenski 817	1.66	2.89	0.36	5.08	1.21
N ₂₀ P ₆₀ K ₄₀		1.94	2.45	0.30	4.67	1.18
N ₃₀ P ₆₀ K ₄₀		2.05	3.03	0.32	5.32	1.32

*Ø- unfertilized control, N₂₀P₆₀K₄₀, N₃₀P₆₀K₄₀ (the index indicates applied nitrogen, phosphorus and potassium doses in kg/ha).

Campbell (2000) found that phosphorus content in technically mature tobacco leaves from the upper, middle and lower harvest belt are 0.14-0.3 %, 0.13-0.3 %, 0.12-0.3 %, respectively. Similar content of phosphorus

was found in the most of the tested samples. The results are higher as compared to oriental tobacco P-66, that has phosphorus content from 0.04% to 0.2 % (Jordanoska et al., 2018; Zaprijanova & Hristozova, 2018).

Calcium content for all varieties is higher than the average 1.5% found in oriental tobacco P-66 and the ranges from 1.0 to 2.1% (Jones et al., 1991; Campbell, 2000; Jordanoska et al., 2018; Zaprijanova & Hristozova, 2018). Several studies found that calcium content in tobacco leaves depends on the calcium content of the soil and soil pH (Mylonas, 1984; Apostolova, 1990).

All tested varieties from experimental field in Prilep have lower magnesium content compared from the samples from Dobrushevo (Table 3 and Table 4). A range of magnesium content from 0.07-0.18% was observed in oriental variety P-66 (Jordanoska et al., 2018). The magnesium content in our samples was higher as compared to findings of Zaprijanova

& Hristozova (2018), who found magnesium content in narrow limit of 0.38-0.56%. Comparison of the results from both locations shows that tobacco leaf samples from location Dobrushevo are richer in macronutrients, which is accordingly macronutrients content in soil samples from same location.

Based on the available and total content of the studied elements in soil samples, we calculated the availability ratio (Figure 2). Availability ratios are presented as direct measure of the potential effectiveness of the examined macronutrient in soil. Based on the results from both locations, nutrients availability percentage sequence the following order: Ca>P>Mg>K>N.

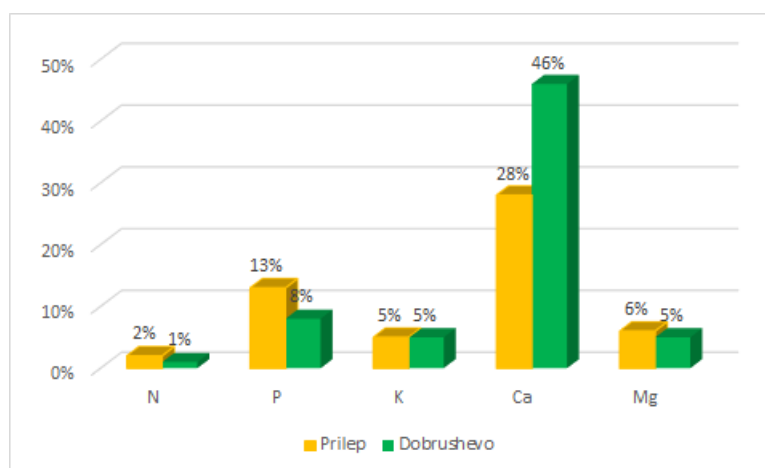


Figure 1. Availability of studied macronutrients calculated as a ratio of available content and total content of each macronutrient in soil before planting.

CONCLUDING REMARKS

According to our findings, there was no difference in the content of available and total nitrogen in the analyzed soil samples from the treatments with increased amounts of nitrogen (20 kg/ha and 30 kg/ha). The content of most of the studied elements in soil samples corresponds respectively with those from the oriental tobacco leaves from the particular location. Increasing doses of nitrogen fertilizer leads to

increasing content of nitrogen in the leaves. For any tested tobacco variety, absorption capacity differs respectively for individual macronutrient. Calcium and magnesium content for all tested varieties from both locations were higher than most of the findings for oriental tobacco. It was found that Ca has greater availability compared to other observed nutrients.

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ДОСТАПНОСТ НА МАКРОНУТРИЕНТИТЕ ЗА РАЗЛИЧНИ ВИДОВИ ОРИЕНТАЛЕН ТУТУН ОДГЛЕДАН ВО ИСТИ АГРОТЕХНИЧКИ И ЕКОЛОШКИ УСЛОВИ

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Резиме

Макронутриентите се неопходни за растот и развојот на сите растенија, вклучувајќи го и ориенталниот тутун. Нивната достапност го одредува целокупното здравје и растот на тутунските растенија и е во фокусот на одржливото тутунско производство. Правилно управување со макронутриентите во почвата е неопходно за одржување на висок и квалитетен принос.

Со цел да се оцени содржината на макронутриенти и факторите кои влијаат на нивната достапност, направен е опит со четири различни сорти на ориентален тутун. Растенијата се одгледани со исти агротехнички услови, на две локации на колувијално-делувијален тип почва и на флувисол. Вкупната и достапна содржина на азот, фосфор, калиум, магнезиум и калциум, како најважни макронутриенти е одредена во примероците почва, додека вкупната содржина е одредена во примероците од растителен материјал. Достапноста на испитуваните елементи е претставена како сооднос на одредената достапна и вкупна содржина во почвените примероци и служи како директна мерка за потенцијалната ефективност на избраниот макронутриент. Калциумот и фосфорот имаат поголема достапност во споредба со магнезиумот, калиумот и фосфорот. Анализирањето на соодносите меѓу својствата на почвата и достапноста на хранливите материји дава корисен увид за примената на одредени мерки за специфични цели во земјоделството и управување со животната средина.

Клучни зборови: тутунски растенија, нутриенти, макронутриенти, фактори на раст.



EVALUATION OF YIELD AND YIELD RELATED COMPONENTS OF SPRING BARLEY VARIETIES BASED ON MULTIVARIATE ANALYSES

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Abstract

The aim of this study was to evaluate the yield and yield components of spring barley varieties cultivated in the Republic of North Macedonia using multivariate analyses. Five spring barley varieties (Makedo, Xanadu, Josefin Variety, Gladys Variety and Scarlet Variety) were used as an experimental material. Makedo is Macedonian variety and the other barley cultivars were introduced. The experiment design was randomized complete block with three replications, carried out on the field areas in Probistip, Republic of North Macedonia. Makedo variety showed the highest value for grain yield (6 844 kg/ha), number of spikes per m² (668), number of grains per spike (22) and hectoliter weight (68.5 kg/hl). Using Principal Component Analysis two main components were extracted, accounted for 82.46% of the entire variability among the barley varieties for all investigated traits. The first principal component explained 59.92% of the variance and factor loading for number of spikes per m² (0.49), grain yield (0.48) and number of grains per spike (0.47) were the most important traits positively contributing to the PC1. The second PC accounted 22.53% of the variation and the factor loading for 1 000 grain weight (0.84) was the main highly positively trait contributing to the PC2. From all spring barley varieties only Makedo had positive values by both major components. Grain yield was positively correlated with the number of spikes per m² ($r = 0.795$), from the one side and from the other side, with the number of grains per spike ($r = 0.632$).

Key words: barley, grain yield, principal component analysis, correlation

INTRODUCTION

As one of the oldest plant species, barley has undergone substantial genetic change during its domestication. Over many thousand years of cultivation, the intended use of barley has moved from staple food to an important feed. Among the cereal crop barley is a species with the greatest adaptability to a wide range of environments. Barley is cultivated from arctic latitudes to tropical areas, grown at the highest altitudes and adapted to specific sets of agro-ecological areas (Alemayehu & Parlevliet, 1997). Most of the world's barley is used for feed, followed by malting, 2-3% as food and about 5% as a seed commodity (Ullrich, 2011). In Republic of North Macedonia wheat and barley are the major cereal crops. According to the data from the Statistical Office of the Republic

of North Macedonia (2023), barley production in Republic of North Macedonia in the last ten years tends to increase. On the National variety list in the Republic of North Macedonia, besides autumn forms of barley, only one variety (Makedo) is registered as a spring domestic variety (MAFWE, 2008).

A priority for global food security is insuring the yield potential and stability of small-grain cereals, such as wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.) (Zoltán et al., 2020). Successful growing of spring barley depends on many factors. Productivity is the final result of the effect and interaction of several yield related traits, which are basically polygenetic (Madić et al., 2014). Grain yield in barley is a trait that is affected by an interaction

of many factors like environment, physiology and morphology of the genotype (Mousavi et al., 2012). The most basic components of yield are grain weight, plant height, number of grains per spike and grain/kernel weight (Madić et al., 2009). Grain yield is dependent on combined and well-balanced effects of these yield components (Turk et al., 2003). Environmental factors like drought affect the yield components and consequently the grain yield of a genotype (Soleymani & Shahrajabian, 2013). Also, many authors studied the environmental effect and the influence of variety on barley grain yield (Helm, 1992; Paynter and Young, 2004; Petkovski et al., 2018). The most important traits in barley plants are grain yield and quality of product, number of spikes per unit area,

number of grains per spike and grain weight (Turk et al., 2003; Kavitha et al., 2009; Sukram et al., 2010). To be able to breed for a high yielding genotype, the relationship between yield and yield components and the correlation among yield components need to be well studied and understood (Dofing et al., 1992). Coefficient analysis and Principal Component Analysis are widely applied in the breeding researches of many authors (Dyulgerova, 2012; Gocheva, 2014; Markova Ruzdik et al., 2015) and those tools report the interdependencies between the yield formation elements.

The objective in this study was to evaluate the yield and yield formation elements of spring barley varieties cultivated in the Republic of North Macedonia, using multivariate analyses.

MATERIAL AND METHODS

Plant material and field trial

Five spring barley varieties (Makedo, Xanadu, Josefin Variety, Gladys Variety and Scarlet Variety) were used as an experimental material for this study. Only Makedo was

domestic variety and the other cultivars were introduced (Tab. 1). Macedo variety is registered as a Macedonian variety on the National variety list in the Republic of North Macedonia (MAFWE, 2008).

Table 1. The origin of spring barley varieties used in the experiment.

No.	Spring barley varieties	Breeder/Maintainer
1	Makedo	North Macedonia
2	Xanadu	Germany
3	Josefin Variety	France
4	Gladys Variety	Czech Republic
5	Scarlet Variety	Germany

The field trial was carried out on the experimental field area in Probištip, Republic of North Macedonia, during two growing seasons. Probištip is a town nestled in the

southwest corner of the Osogovo Mountains, in northeastern Macedonia, located at 42°00'N and 22°09'E, with an elevation above sea level of 589 m (Fig. 1).

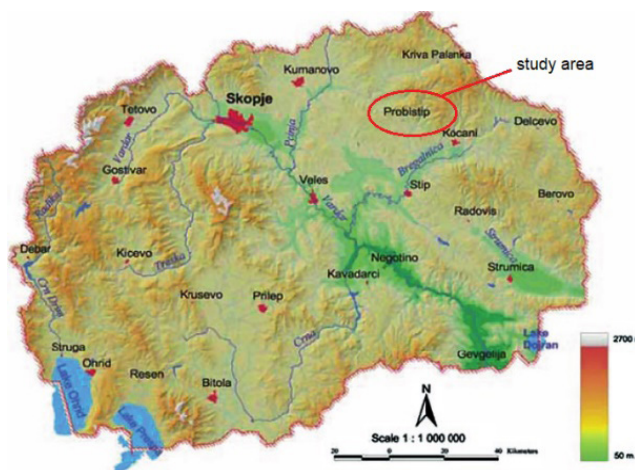


Figure 1. The investigated area on the territory of the Republic of North Macedonia.

Two row spring barley cultivars were arranged in a randomized complete block design with three replications. The size of experimental plot for each variety and replication was 5 m². Row to row distance was maintained at 20 cm and all recommended agronomic practices were followed for growing the crop. The sowing and the harvest were done manual.

Data collection

The number of spikes per m² was determinate by counting the plants from m² of each repetition. In maturity period, grain yield and its components were measured. According to descriptors for barley (IPGRI, 1994), 30 plants were randomly were sampled from each plot to determine plant height (cm). Plant height was measured from the base of the plant to the tip of the spike. Grains per spike were manually counted and the 1 000-grain weight has been

determinate to measure 1 000 grains of each repetition and variety. For hectolitre weight was used hectolitre measuring system. Grain yield was obtained from 5 m² and was calculated in kg/ha.

Statistical data analysis

To evaluate the yield and yielding components of tested barley varieties and to analyse the contribution of each trait on total variation, multivariate analyses were applied. Least significant differences were calculated using Statistical analysis software JMP (2002). Principal Component Analysis was done by StatGraph 2.1 (Mohammadi & Prasanna, 2003) and components with Eigen values greater than one were considered. Linear correlation was calculated according Singh & Chaudhary (1985), with significance levels of 0.05 and 0.01, using SPSS 19 (2010) software.

RESULTS AND DISCUSSION

In Table 1 the average values for grain yield and yield components of tested barley varieties are given. Number of spikes per m² is one of the important traits and play significant role in yield formation. LSD test show that all tested varieties significantly differ. Makedo variety showed the largest number of spikes per m² (668) and the lowest was notes by Josefin Variety (517). The tallest plants were registered by Josefin Variety (76.3 cm), followed by Scarlet Variety (69.8 cm). Number of grains per spike is also one of the main yield related components. According to LSD test, there were no significant differences between tested barley varieties except Makedo variety. Makedo variety had the highest value for number of grains per spike (22). 1 000 grain weight is a quality trait and it is indicator for the seed size. This

property depends of genetic variety but also of environmental conditions. In our study, this trait ranges from 43.5 g to 50.1 g, with average value of 47.1 g. Hectolitre weight (or weight per unit volume) is the weight of 100 litres of the cultivar and is the simplest criteria of grain quality. It gives us a rough index of flour yield. Higher hectolitre weight, means better flour yield. The factors affecting the hectolitre weight are kernel shape and uniformity of kernel size, orientation of kernels in container when it is filled, density of the grain influenced by structure of grain and its chemical composition. In our research, the highest value for hectolitre weight was observed by Makedo variety (68.5 hg/hl). Macedonian variety also showed the highest value for grain yield (6 844 kg/ha), followed by Xanadu Variety (6 638 kg/ha).

Table 2. Average values for yield and yield components of tested barley varieties.

Variety	Number of spikes per m ²	Plant height (cm)	Number of grains per spike	1 000 grain weight (g)	Hectolitre weight (kg/hl)	Grain yield (kg/ha)
Makedo	668a	64.8c	22a	49.4a	68.5a	6 844a
Xanadu	617c	64.4c	20b	43.5c	66.0c	6 638a
Gladys Variety	576d	68.8b	20b	50.1a	67.7ab	5 841a
Scarlet Variety	638b	69.8b	20b	46.3b	67.0b	4 949b
Josefin Variety	517e	76.3a	20b	45.8b	64.7d	5 279b
Mean	604	68.8	21	41.1	66.8	6 544
LSD_{0.05}	20.32	1.32	1.05	1.81	0.93	1 922.3

Principal Component Analysis (PCA) reflects the importance of the largest contributor to the total variation at each axis of differentiation (Sharma, 1998).

In this study, Principal Component Analysis was utilized to examine the variation and to estimate the relative contribution of tested traits for total variability. Principal Component Analysis was carried out by using five spring barley varieties and six traits. Table 3 presented the results of PCA, viz., percentage, cumulative variances, and Eigen value of the first two principal components. This multivariate tool usually is used to identify the properties that were the main source of variability. The importance of analysed traits to the different principal components can be seen from the corresponding loading factors.

In this research, two main components were extracted with Eigen value greater than one (Tab. 3). The first two principal components

accounted for 82.46% of the entire variability among the barley varieties for all investigated traits.

The first principal component (PC) explained 59.92% of the variance. The importance of traits to the different PC can be seen from the corresponding factor loading, presented in Table 3. Factor loading for number of spikes per m² (0.49), grain yield (0.48) and number of grains per spike (0.47) were the most important traits positively contributing to the first main component. This comment relates to fact that spike number per m² had a high significant correlation with grain yield. On the other hand, the plant height was negative correlated to PC1 (-0.51), which mean that higher plants should have lower grain yield. The second PC accounted 22.53% of the variation and the factor loading for 1 000 grain weight was the main highly positively trait of PC2 (Tab. 3).

Table 3. Principal Component Analysis and factor loading of all tested traits.

Parameter	PC1	PC2
Eigen value	2.99	1.12
Percentage of variance (%)	59.92	22.53
Cumulative percentage (%)	59.92	82.46
Trait	Factor loading of tested traits	
Number of spikes per m ²	0.49	-0.14
Plant height	-0.51	0.34
Number of grains per spike	0.47	0.35
1 000 grain weight	0.12	0.84
Hectolitre weight	0.15	0.25
Grain yield	0.48	-0.20

The similar results were reported by Žáková & Benková (2006). According Žáková & Benková (2006) two main components were extracted, which accounted 72.8% of variability and also, the grain yield had the highest positive factor loading but plant height was negatively correlated by first main component.

Abdullah et al., (2018), also used PC analysis in their experiment, and they reported

four main components with 85.5% cumulative variability.

In Table 4 are given the factor loading of tested barley varieties by main components. From all spring barley varieties only Makedo had positive value by both main components. This means that the most suitable for breeding, according to the tested traits and environmental conditions was Makedo variety.

Table 4. Factor loading of tested barley varieties by main components.

Variety	PC1	PC2
Makedo	2.54	0.71
Xanadu	0.54	-1.76
Gladys Variety	-0.25	0.87
Scarlet Variety	-0.64	-0.17
Josefin Variety	-2.19	0.34

Correlation explained the degree of association between two traits. The interrelationship of characters determines the response to selection in breeding programs. It also helps to apply indirect selection to improve the target traits of interest (Joshi & Okuno, 2010).

Manu researches had been used linear correlation to determine the interaction between grain yield and yield related components in barley (Žáková & Benková, 2006; Dorostkar et al., 2015; Markova Ruzdik et al., 2015; Abdullah et al., 2018; Kaur et al., 2018; Al-Sayaydeh et al., 2019; Tsige, 2020).

The values of phenotypic correlations of yield and yield components are depicted in Table 5. Grain yield was highly and significantly in positive correlation with the number of spikes per m² ($r = 0.795$), at level of significance $p < 0.01$. Dorostkar et al., (2015), Abdullah et al., (2018)

and Madić et al., (2019) also reported positive interaction between spike number per m² and grain yield.

Grain number per spike is an important yield component and is usually used as selection trait in barley breeding programmes. In our research, also, significant positive correlation was established between number of grains per spike and grain yield ($r = 0.632$, $P < 0.05$). This correlation was also reported by other researches such as Garcia del Moral et al., (1991), Doting & Knight (1992), Saed-Moucheshi et al., (2013), Dorostkar et al., (2015), Madić et al., (2019).

From the other side, grain yield and 1 000 grain weight had no significant correlation (Tab. 5). Same results were reported in Dorostkar et al., (2015) study.

Table 5. Linear phenotypic correlation coefficients between yield and yield related components.

	Plant height	Number of grains per spike	1 000 grain weight	Hectolitre weight	Grain yield
Number of spikes per m ²	-0.823	-0.467	-0.075	-0.660	0.795**
Plant height		0.636	0.172	0.760	0.492
Number of grains per spike			0.490	0.649	0.632*
1 000 grain weight				0.748	0.101
Hectolitre weight					0.424

*Statistical significance of differences at $P < 0.05$;

**Statistical significance of differences at $P < 0.01$

CONCLUDING REMARKS

From the performed research it can be conclude that the highest yield was obtained by Makedo variety. Also, Makedo showed the largest number of spikes per m², number of grains per spike and hectolitre weight. The used PCA extracted two major components that accounted 82.46% of the total variability among the barley varieties for all investigated traits. Using linear correlation, grain yield showed highly and significantly positive relationship with the number of spikes per m² and with the number of grains per spike. From all spring

barley varieties only Makedo had positive value by both main components, which means that this variety is suitable for cultivation and should be more present in barley production. Also, the other varieties can be included in barley production because they showed optimal values for the tested yield related traits and a suitable breeding strategy required to combine most, if not all, of the desired traits into a single genotype in order to come up with superior genotype.

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ЕВАЛУАЦИЈА НА ПРИНОС И КОМПОНЕНТИ НА ПРИНОСОТ КАЈ ПРОЛЕТНИ СОРТИ НА ЈАЧМЕН СО КОРИСТЕЊЕ НА МУЛТИВАРИЈАНТНИ АНАЛИЗИ

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Резиме

Целта на овој труд е да се направи евалуација на приносот и компонентите на принос кај пролетни сорти јачмен одгледувани во Република Северна Македонија со примена на мултиваријантни анализи. Како материјал за работа во овој експеримент беа употребени пет пролетни сорти на јачмен (Makedo, Xanadu, Josefin Variety, Gladys Variety и Scarlet Variety). Makedo е македонска сорта, а останатите се со странско потекло. Опитот беше поставен во рандомизиран блок систем, со три повторувања, спроведен на површините во Пробиштип, Република Северна Македонија. Сортата Makedo покажа највисока вредност за принос на зрно (6.844 kg/ha), број на класови на m² (668), број на зрна во клас (22) и хектолитарска маса (68.5 kg/hl). Со помош на компонентна векторска анализа издвоени се две главни компоненти, кои претставуваа 82.46% од целата варијабилност помеѓу сортите и испитуваните својства. Првата главна компонента (ПЦ1) претставува 59.92% од варијабилноста, а вредностите на факторите на оптоварување за бројот на класови на m² (0.49), принос на зрно (0.48) и број на зрна во клас (0.47) позитивно влијаат по првата главна компонента. Втората главна компонента (ПЦ2) претставува 22.53% од варијабилноста, а вредноста на факторот на оптоварување за маса на 1.000 зрна позитивно влијае по втората главна компонента. Од сите пролетни сорти кои беа предмет на истражување, само сортата Makedo покажа позитивни вредности по двете главни компоненти. Приносот на зрно покажа позитивна корелација со бројот на класови на m² (r = 0.795) од една страна и со бројот на зрна во клас (r = 0.632) од друга страна.

Клучни зборови: јачмен, принос на зрно, компонентна векторска анализа, корелација.



IMPLEMENTATION AND FUNCTIONING OF HACCP PRINCIPLES IN THE MACEDONIAN WINERIES: A MULTIPLE CASE STUDY

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Abstract

Wine is considered as a food and must be produced under conditions that will ensure the safety of the product for human consumption. The HACCP system is the internationally recognized as a food safety tool, and must be applied during the entire food production process. The implementation of a food safety management system based on the principles of HACCP in the wineries should minimise the number and extent of exposures to hazards and improve overall safety of the wine.

In this study, an exploratory multiple case study approach was adopted in order to provide better understanding of the process of implementation and functioning of the HACCP system in the Macedonian wineries. Twelve Macedonian wineries (cases) of varying size and annual production participated in the study. Two methods for collecting empirical data have been applied: survey by applying a questionnaire sent via e-mail and face-to-face interview.

Using survey data, the key factors affecting the functioning of the HACCP system in the Macedonian wine industry were analysed. The findings provide evidence that the most common motives for the implementation of HACCP system in the wineries are: enhanced compliance with regulation, improved communication and control, and implemented traceability system. Also, the results showed that the main factors that hinder the implementation and functioning of HACCP system are: excessive documentation, lack of time, and lack of knowledge relevant to food safety (especially among micro sized wineries).

Key words: *food safety management systems, wine*

INTRODUCTION

Wine holds a profound significance within the rich history and cultural heritage of the Macedonian people. In present times, it has emerged as a crucial agricultural asset for the country. Specifically, as outlined in the 2014-2020 National Strategy for Agriculture and Rural Development, viticulture and wine production contribute approximately 17% to 20% of the agricultural Gross Domestic Product (GDP). Demonstrating its economic influence, in 2021, the Republic of North Macedonia earned the 29th spot among global wine exporters based on financial impact. (<http://www.worldstopexports.com/wine-exports-country/>).

In the journey from vineyard to consumer, the winery serves as a vital link in the wine production process. As a producer in the food industry, the winery assumes responsibility for overseeing the entire food chain. Considering the extensive nature of this chain, which encompasses the grape as the raw material, each stage involved in obtaining wine, including maturation, product storage, bottling, and often distribution and sale to the end consumer, it becomes evident that a comprehensive and meticulous analysis is necessary. This entails conducting risk assessments, identifying critical points, and exercising control throughout the

entire production process to ensure the quality, safety, and traceability of the final product (Marcotrigiano et al., 2020).

Given our country's strategic objective of attaining full membership in the European Union, R.N. Macedonia harmonises its national legislation, including legal frameworks pertaining to food safety in wine production, with European regulatory standards. Wine producers, as well as other food business operators, are obliged to introduce, implement and maintain a permanent procedure or procedures based on Hazard Analysis Critical Control Point (HACCP) principles, together with the application of good hygiene practices, i.e., good production practices (Law on Food Safety of RNM) in order to minimise the number and extent of exposures to hazards and improve overall safety of the wine. The HACCP system is the backbone of any Food Safety System and its application in the winery should provide a

systematic preventive approach to food safety from biological, chemical, and physical hazards in production processes that can cause the wine to be unsafe, and designs measurements to reduce these risks to a safe level (Culler & Conklin, 2015).

Given that Macedonian wine has the potential to be the most significant export brand of the country's agricultural and food sector and bearing in mind that the quality and safety of wine directly affect the competitiveness of the product, our research was aimed at understanding the current situation, as well as analysis of the weaknesses, challenges and benefits of establishing quality and food safety systems in the wine sector in the Republic of North Macedonia. This paper showcases the results related to the process of implementation and functioning of the HACCP system in the Macedonian wineries.

MATERIAL AND METHODS

Qualitative research strategy - Case study

In this study, an exploratory multiple case study approach was adopted in order to provide better understanding of the process of implementation and functioning of the HACCP system in the Macedonian wineries. A case study is a research strategy that involves an empirical investigation of a specific contemporary phenomenon, conducted within its real-life context (Yin, 2009). This approach involves leveraging multiple sources of evidence and is considered appropriate for investigating organizations and companies. It is particularly useful when exploring subjects such as best practices, policy implementation and evaluation, industrial relations, management and organizational issues, organizational cultures, and processes of change and adaptation (Robson, 2016).

The research used non random sampling technique. Twelve Macedonian wineries (cases) of varying size and annual production were selected to participate in the study. To facilitate a comprehensive analysis, the selection criteria for the chosen cases encompassed both wineries that had implemented a voluntary food safety standard, as well as those that had not. Furthermore, to ensure heterogeneity and enhance the richness of the database for

analysis, the selection process considered the year of establishment of the wineries and their export activities. Care was taken to include representatives from both older wineries that were formerly state-owned, before the dissolution of the Yugoslav federation in 1991, as well as relatively new wineries established within the last two decades. The selection also included wineries with a strong focus on export markets, alongside cases that exclusively target the domestic market.

Data collection

Two methods for collecting empirical data have been applied: survey by applying a questionnaire sent via e-mail and face-to-face interview. The survey questionnaire contained open-ended and closed-ended questions. For the majority of questions, extra space was provided for additional comments by the respondents, which in term made it possible to avoid limitations on answers, and the respondents had the opportunity to provide a broader overview of the questions of interest for the research. The interviews were semi-structured, mostly with open-ended questions. All the chosen wineries, which had been contacted and agreed to take part in the study, completed the survey questionnaire. Subsequently, to delve deeper into the subject

of our research and gain a more comprehensive understanding of the voluntary food safety and quality systems in Macedonian wineries, interviews were conducted in five wineries. Each interview involved two company representatives. A representative from the management and a person responsible for managing quality and food safety systems in the winery were interviewed, as persons who are directly involved in the decision-making process and implementation of quality and food safety systems. The names of the interviewees were protected, and they were identified by their job positions. The interviews were prearranged and conducted in person, that is, face to face between the researcher (interviewer) and the surveyed (interviewed) person. During the interview, spontaneous questions that lead one to the next were also asked. The results of the survey questionnaire and the interview were used to produce a case study for each winery and as such were prepared for analysis.

In addition to the primary data provided through the survey questionnaire and interviews, the research also used secondary data like reports from relevant organizations, winery websites, as well as other published materials in order to obtain a more diverse information basis for analysis. The implementation of a multi-source data collection strategy was intended to facilitate the utilization of triangulation during data analysis. This scientific research approach, implemented throughout various stages of our investigation, aimed to uphold the credibility of the qualitative research.

Data analysis

Using survey data, the key factors affecting the functioning of the HACCP system in the Macedonian wine industry were analysed. In the process of analysis and conclusion in the research, the inductive-deductive process was applied, as an appropriate methodological

approach in case studies (Perry, 1998). The analysis of the data from our qualitative research was conducted in three phases: (i) In the first phase of the analysis, the qualitative data obtained from the survey questionnaire and the interviews were recorded and summarized. To ensure anonymity, as well as to organize the data in further analysis, each winery was assigned a code consisting of one letter and one number. The letters refer to the size of the company, and the classification was made according to the number of employees. In accordance with the recommendations of the European Commission (96/280/EC), regarding the definition of the size of enterprises, in our research, when coding, an enterprise with less than 10 employees is ranked in the Micro group; with more than 10, but less than 50 employees is ranked in the Small group and with more than 50 and less than 250 employees – Medium enterprise (winery). Hence, we have “C” for medium, “M” for small and “Mi” for micro-wineries. The number next to the corresponding letter in the code represents the average annual production level of the winery in the last 5 years, and the lowest number (1) refers to the lowest average annual production. The results of the survey questionnaire, interviews, and data from other sources were categorized with codes, recorded, and compiled. This process allowed for condensing the data into relevant information for the research and generating twelve case studies based on it. (ii) In the second stage of the analysis, in order to identify the similarities or differences between the cases, the obtained data were further selected and organized in tables according to the research questions. (iii) In the third stage, building upon the previous analysis, the data underwent further examination, incorporating insights from the reviewed existing literature on the topic, which in turn lead to the deriving and confirming research conclusions.

RESULTS AND DISCUSSION

Sample characteristics

Based on the applied coding criteria, five micro-wineries (Mi1, Mi2, Mi3, Mi4 and Mi5), three small wineries (M1, M2 and M3) and four medium-sized wineries (C1, C2, C3 and C4) were included in our research. Five of the wineries (M2, M3, C2, C3 and C4) have an average annual

production of more than 1,000,000 litres of wine, while the remaining seven wineries are characterized by an average annual wine production of less than 1,000,000 litres. The characteristics of the selected wineries on bases on the main criteria are presented in the Table 1.

Table 1. Winery characteristics.

Winery code	Year of establishment	Employees	Average annual production (L)	Export orientation (% of the total amount of wine sold annually)
Ми1	2008	4	10 000 (60% bulk, 40% bottled)	70%
Ми2	2010	2	12 500 (60% bulk, 40% bottled)	0%
Ми3	2006	3	50 000 (50% bulk, 50% bottled)	10%
Ми4	1997	8	160 000 (70% bulk, 30% bottled)	0%
Ми5	2003	9	203 000 (100% bottled)	70%
M1	2006	40	56 231 (5% bulk, 95% bottled)	2%
M2	1928 (last change of ownership-2016)	35	2 214 500 (>99% bulk)	91%
M3	2008	44	3 000 000 (80% bulk, 20% bottled)	75%
C1	1998	54	412 225 (100% bottled)	45%
C2	2002	100	3 690 440 (10% bulk, 90% bottled)	80%
C3	1979 (last change of ownership-2019)	92	7 800 000 (80% bulk, 20% bottled)	85%
C4	1883 (last change of ownership-2004)	195	14 000 000 (100% bottled)	50%

Of the five micro wineries covered in our research, three (Ми1, Ми2, Ми4) are focused more on the production of bulk wine, one winery (Ми3) produces an equal amount of bottled wine and bulk wine, and the Ми5 winery produces solely bottled wine. In small and medium-sized wineries, with the exception of wineries M2 and C3, bottled wine is the dominant product. Considering that both wineries that are an exception (M2, C3) were going through a transitional period in the research period - changes in ownership and financial challenges, it is plausible that these factors contributed to the witnessed state of affairs. From the analysis of data, it was determined that bottled wine convincingly dominates the export of wine, and with the exception of wineries (Ми1, C3)

all other wineries exporting wine to foreign markets, almost exclusively market bottled wine.

Table 2 presents the situation concerning the implementation of the HACCP system in wineries. From the data shown, it is clear that all 12 selected wineries as of 2020 have implemented and operate according to the HACCP principles. But when conducting the questionnaire and initial data processing, at the beginning of 2018, two micro wineries (Ми2, Ми4) had not implemented the HACCP system. The Ми2 winery was not ready to introduce it, while in the Ми4 winery the implementation was underway, and the system was implemented in November 2018.

Table 2. presents the situation concerning the implementation of the HACCP system in wineries.

Winery	Implementation of the HACCP system		Engagement of External Expertise in Implementation
	2018	2020	
Ми1	+	+	yes
Ми2	-	+	yes
Ми3	+	+	without external expertise
Ми4	on-going	+	yes
Ми5	+	+	yes
M1	+	+	yes
M2	+	+	yes
M3	+	+	yes
C1	+	+	yes
C2	+	+	yes
C3	+	+	yes
C4	+	+	without external expertise

(+) implemented HACCP system

In Table 2 it can also be seen that the implementation of the HACCP system in 10 wineries (82%) was carried out with the help of an external expert, and 2 wineries (18%) independently implemented the system (C4, Ми3). It is interesting to comment that the two wineries that have independently implemented the system belong to different groups of wineries according to the criteria we adopted in the research. Namely, winery C4 is a medium-sized winery with 195 employees, including an employee in the position of Food Safety and Quality Control Manager, while the other, Ми3, is a micro winery with only three employees, among which there is no expert with knowledge relevant to food safety. The winery Ми3 stated that it was more convenient for them to invest in their own education and learning for the implementation of the system, than to pay an external expert. Unlike these two wineries, most of the surveyed managers and employees in the quality and safety sector in the other wineries stated that it is much better to hire an external expert because they have

too many other obligations. The results of our study confirm the position of Mensah & Julien (2011), who consider that enterprise size has no significant effect in terms of motivation, benefits and challenges to compliance with food safety regulation. In accordance with the results obtained in our research, it seems that although Macedonian wineries most often decide to seek external expert assistance for the implementation of the system, the subsequent stages of the application of the system and the management of all activities are conducted independently.

Critical Control Points (CCPs) determination when conducting wine safety risk analysis is essential in the HACCP system. CCPs are the steps in the process where a control measure is applied and is essential to prevent, eliminate or reduce to an acceptable level the identified food safety hazard(s). Table 3 shows the number of CCPs determined per winery. The obtained data showed that winery Ми1 has the most (6) CCPs, and winery C1 has the least (1).

Table 3. Identified Critical Control Points (CCPs) in the wineries.

Winery	CCP1*	CCP2	CCP3	CCP4	CCP5	CCP6	Total CCPs
Ми1	x	x	x	x	x	x	6
Ми2		x	x			x	3
Ми3			x	x			2
Ми4	x	x					2
Ми5	x		x	x			3
M1		x		x		x	3
M2	x	x		x			3
M3	x		x				2
C1				x			1
C2			x	x		x	3
C3			x			x	2
C4	x			x	x	x	4
Total	6	5	7	8	2	6	

CCP1 - adding oenological agents

CCP2 - grape receival

CCP3 - filtration

CCP4 - bottling

CCP5 - stabilization

CCP6 - storage

* the number in the CCP mark is not related to the order of occurrence of CCPs in the production process

Literature sources do not provide a definitive stance on the exact number of Critical Control Points (CCPs) that wineries should identify. This determination is made by the hazard analysis team responsible for developing the HACCP plan. As a result, each winery independently establishes the number of CCPs based on their specific requirements. Kourtis & Arvanitoyannis (2001) consider seven critical control points in the wine-making process. It is also interesting to comment on the publication by the New Zealand Food Safety Agency, Code of Practice for grape wine WSMP (2011), which does not establish any CCPs in the wine-making process. The Agency believes that the control of hazards can be adequately achieved through

the Code of Practice, that is, by observing good manufacturing or hygiene practice.

When comparing the findings of our research with the data presented in the publication "Macedonian Wine Industry Performance Survey 2009" (Velkov, 2010), it becomes evident that a great improvement has been achieved regarding the implementation of the mandatory HACCP system, as well as voluntary standards in the Macedonian wineries. In contrast to the situation determined in our research – implemented HACCP system in all 12 wineries in 2020, in the survey conducted in 2009 - out of 57 wineries included in the research, only 26 had implemented the HACCP system (less than half).

Cross case analysis

The analysis of the obtained data among wineries (Cross case analysis) made it possible to determine the motives and aggravating factors for implementation and functioning of the HACCP system in Macedonian wineries:

MOTIVES for the implementation of HACCP system in the wineries

- Enhanced compliance with regulation
- Improved communication
- Improved control (e.g. implemented traceability system)

HINDERING FACTORS for implementation and functioning of HACCP system

- Excessive documentation
- Lack of time
- Lack of knowledge relevant to food safety (especially among micro sized wineries).

The survey data obtained showed that wineries (Ми1, Ми2, Ми3, M2) feel that the implementation of the system is more help in terms of monitoring documentation and procedures but are not convinced that the implementation of the system is a guarantee of product safety. The larger wineries (M3, C1, C3, C4), which have implemented voluntary standards in wineries, believe that the

implementation and functioning of the system guarantees product safety and is an excellent basis for the implementation of voluntary standards (IFS, ISO 22000, ISO 9001, ISO 14001, etc.) and international trade. Fairman & Yapp (2004) point out that micro and small businesses typically show insufficient knowledge and skills regarding food safety, hence are not aware of the risks posed by business and are not motivated to improve the food safety system. Therefore, for the implementation of such systems in these companies, the role of state bodies (inspectorate) that would encourage them in the process of compliance with legal provisions is of particular importance.

Regarding the engagement of professional staff for food safety management, survey results showed that the lack of staff is more pronounced in smaller wineries. Namely, in most micro wineries (three out of five) and one small-sized winery (M1) there is no expert employee with formal knowledge relevant to food safety, but this is done by an employee who is assigned this task and performs it in parallel with other work tasks. In the other small and all medium-sized wineries, it was stated that the winery has an employee with formal knowledge relevant to food safety who takes care of the management of the HACCP system. Often these persons are also engaged in the production process (oenologists, technologists), and only wineries C3 and C4 stated that they have a person employed in the position of Food Safety and Quality Control Manager. In the comment on this situation, it was indicated that this is not required by law, or rather they say: *"...no one requires us legally to have a person employed in the position, so we manage in a way that is easier for us, we do not pay plus, and the work is done"*. The findings of Aggelogiannopoulos et al. (2007) align with the results obtained in this study. Their research examined the implementation of the voluntary quality management standard ISO 9001 and concluded that smaller companies within

the wine sector face challenges in allocating a dedicated employee solely responsible for system management. Instead, these companies often distribute system-related tasks among existing employees, imposing additional responsibilities on them and thereby making implementation more challenging.

Record keeping in compliance with standard requirements was highlighted as a challenging aspect of implementing and operating the HACCP system in the majority of wineries. This was attributed to the time-consuming nature of documentation tasks and the need for trained staff to carry them out. In this context, it is interesting to bear in mind that barriers, such as time and finance, can conceal deeper and more complex attitudes in enterprises, including distrust of food safety legislation, lack of motivation in dealing with requirements regarding food safety legislation, and lack of knowledge and education (Yapp & Fairman, 2006).

The results of our analysis showed that all wineries have the full support of the management to create an environment conducive to improving the quality and safety of the product. Regarding the support of the HACCP system by the other employees in the wineries, it can be concluded that employees support the system once it is established and begins to function, but at the beginning of the implementation most wineries encounter resistance from the employees, who found it harder to get used to the new way of operating and keeping records. The wineries applied different strategies to overcome the problem: training, regrouping of working groups, additional education, and, if necessary, sanctions. After the conducted trainings and years of operation, today in the wineries the system is accepted, and the procedures are perceived as a common integral part of the overall process.

CONCLUDING REMARKS

The requirements that ensure quality during the entire winemaking process and safeguard against relevant hazards have become important to consumers and mandatory for protecting human health. When it comes to wine, the risks associated with quality typically

involve sensory aspects such as taste, colour, clarity, aroma, and the presence of specific components that make it an appealing and desirable product for consumers. Conversely, safety hazards can be categorized into physical risks (e.g., metal or glass fragments),

chemical hazards (such as pesticide residues, sulphur dioxide, heavy metals, or urea), and microbiological threats (e.g., the presence of pathogenic organisms), all of which pose potential health risks to consumers. The risk associated with microbial contamination is generally considered to be relatively low in the final product, given that the presence of alcohol and polyphenols, as well as the pH value of wine make the product an unsuitable environment for the existence of pathogenic microorganisms, but nevertheless, identification of all potential hazards and retrieval of appropriate preventive

and corrective actions is of primary importance to minimize the outbreaks of incidents in the wine sector that are hazardous for human health (Kourtis & Arvanitoyannis, 2001). Considering that wine is a strategic product in the agri-food sector of the country, the improvements of the food quality and safety management systems in the Macedonian wineries will contribute to increasing the competitiveness of Macedonian wine through increased consumer confidence and improved product image, and this will also provide an economic benefit to both the company and the state.

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ИМПЛЕМЕНТАЦИЈА И ФУНКЦИОНИРАЊЕ НА НАССР ПРИНЦИПИТЕ ВО МАКЕДОНСКИТЕ ВИНАРИИ: СТУДИЈА НА ПОВЕЌЕ СЛУЧАИ

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Резиме

Виното како прехранбена намирница мора да се произведува во услови кои ќе обезбедат безбедност на производот за човечка употреба. HACCP (Hazard Analysis Critical Control Point) системот е меѓународно признат систем за безбедност на храна, кој задолжително се применува во текот на целиот процес на производство. Имплементацијата на систем за управување со безбедноста на храната заснована на принципите на HACCP во винариите треба да го минимизира бројот и степенот на изложеност на опасности и да ја подобри севкупната безбедност на виното.

Во ова истражување беше примената студија на повеќе случаи (multiple case study), како методолошки пристап со цел да се обезбеди подобро разбирање на процесот на имплементација и функционирање на HACCP системот во македонските винарии. Во истражувањето учествуваа 12 македонски винарии (случаи) со различна големина и годишно производство. Применети се две методи за собирање емпириски податоци: анкета со примена на прашалник испратен преку е-пошта и интервју лице в лице.

Анализата на добиените податоци овозможи да се извлечат заклучоци во однос на факторите кои влијаат на функционирањето на HACCP системот во македонската винска индустрија. Наодите даваат доказ дека најчестите мотиви за имплементација на HACCP системот во винариите се: усогласеност со законската регулатива, подобрена комуникација и контрола и имплементиран систем за следливост. Исто така, анализата покажа дека главни фактори кои ги попречуваат имплементацијата и функционирањето на HACCP системот се: обемот на документација, недостаток на време и недостаток на знаење релевантно за безбедноста на храната (особено кај винариите со микро големина).

Клучни зборови: системи за управување со безбедноста на храната, вино.



CHEMICAL COMPOSITION OF SOME AUTOCHTHONOUS FRUIT SPECIES FROM MACEDONIA

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Abstract

The scope of this paper is determination of the content of soluble dry matters, total sugar, total acids, invert sugar and citric, malic, tartaric and lactic acid in some autochthonous fruit species: pomegranate (Zumnarija, Bejnarija, Valandovska kisela, Valandovska kiselo-slatka, Hidjas, Kisela, Lifanka, Ropkavec), apple (Ubavo cvetka, Shareno blago, Prespanka, Tetovka, Karapasha, Kozharka, Bela Tetovka) and cherry (Ohridska brza, Ohridska rana, Ohridska crna, Dolga shishka, Dalbazlija, Ohridska bela). Comparative studies were performed in relation to the standard varieties (Karamustafa-pomegranate, Idared-apple and Bigaro burlat-cherry).

Pomegranate fruits have the highest content of soluble dry matters (15.6%), total sugars (149 g/L) and total acids (20.1 g/L). The highest content of invert sugar was observed in pomegranate (113 g/L which means 76.3% of total sugar). In apple and cherry fruits, malic acid dominates (above 5 g/L), while in pomegranate fruits, citric and malic acids are almost equally represented. Tartaric acid is present only in apple fruits (0.383 g/L). Lactic acid was not found in all fruit species.

A strong positive correlation was found between soluble dry matter and total sugar with coefficient of determination $R^2 = 60\%$. A moderate positive correlation exists between the contents of total and invert sugar with coefficient of determination $R^2 = 20\%$, between total acids and invert sugar with coefficient of determination $R^2 = 22\%$ and between the content of malic and citric acid with coefficient of determination $R^2 = 30\%$. A strong negative correlation was found between the content of total acid and malic acid with coefficient of determination $R^2 = 37\%$ and very strong negative correlation between malic acid and invert sugar with coefficient of determination $R^2 = 77\%$.

Key words: pomegranate, apple, cherry, soluble dry matters, sugars, organic acids

INTRODUCTION

Fruit taste is an important trait majorly controlled by organic acids and together with aromatic volatile compounds and soluble sugars, they strongly facilitate overall organoleptic quality and fruit stability. The chemical composition of fruits depends on several factors as: genotype, climatic and pedological conditions, the way of growing the fruit trees, the applied agrotechnical measures, the degree of maturity and the way of keeping and storing the fruits.

Organic acids and soluble sugars play important roles, in fruit flavour and nutrition, thus, can affect fruit flavour and indirectly exert advantageous and disadvantageous effects on commercial fruit quality. Sugars are an important ingredient in fruits. Fresh fruits usually contain 2-15% sugars (glucose, fructose and sucrose) which are the significantly important for nutrition. Invert sugar (a mixture of equal parts of fructose and glucose) is the most prevalent. These are easy digestible sugars

for the body, necessary for the normal function of the muscles, they refresh the body, have a calming effect on the brain, remove fatigue and increase the level of serotonin (Selamovska & Miskoska-Milevska, 2021). According Li et al. (2020) sucrose is the most abundant sugar in peach juice (72.5% of the total sugar content). In blueberry, sweet cherry juice, glucose and fructose are the predominant sugar (Li et al., 2020; Sokol-Letowska et al., 2020), in sour cherry fruits glucose is dominant sugar (Gündoğdu & Bilge, 2012.). The taste in pomegranate fruits is governed mainly by the presence of sugars (glucose and fructose) (Mayuoni-Kirshinbaum & Porat, 2014). Apple, peach, apricot and banana contain almost equal amount of glucose, fructose and sucrose.

In fresh fruits, organic acids primarily include malic, citric, quinic and tartaric acid, in smaller quantities succinic, formic, salicylic, oxalic, benzoic and other acids. In pome fruits and stone fruits, malic acids predominate, berry fruits and citrus fruits mostly contain citric acid, less malic acid. Citric acid and quinic acid are the main organic acids in kiwifruit (Nishyama et al., 2008) and blueberry (Zhang et al., 2020). The presence of shikimic acid has been detected in gooseberries. In strawberry and raspberry there is the presence of salicylic acid (0.0011-0.0028 g/L) and small amounts of formic acid, in blackberries isocitric acid is detected, in unripe blackcurrant fruits there is succinic acid (Selamovska & Miskoska-Milevska, 2021). Tartaric acid is detected only in grape juice, malic acid is the predominant organic acid in apple (82.9% of the total acid content) and sweet cherry juice (98.2% of the total acid content), citric acid is the predominant organic acid in strawberry (75.3%) and blueberry juice (80.2% of the total acid content) (Li et al., 2020). Malic acid contributes to the pleasant sour taste of the fruit. It has a significant role in biochemical processes, controls blood sugar levels, high blood pressure and cholesterol, acts against allergies, chronic fatigue, problems with digestive tract, various inflammations, infections etc. Citric acid is natural antioxidant.

Citric and tartaric acids are naturally occurring weak organic acids. They play an

important role in product characteristics like taste and aroma. Their presence determines the tartness and flavour of fruits as well as fruit beverages. Both acids are used extensively as food acidulants. Together with fumaric and phosphoric acids, citric and tartaric acids are used in the food industry to enhance beverage flavour. Some studies indicate that citric acid and tartaric acid can have unwanted effects on human health, high concentrations of citric acid can affect tooth enamel, reduced enamel's hardness by 84% (Ren et al., 2009), high doses of tartaric acid can give rise to acute kidney injury, gastrointestinal symptoms and cardiovascular collapse (Naqvi, 2017; Rusyniak et al., 2012), therefore, regulatory organizations, such as Commission regulation of EU (2011), set up a maximum concentration of citric acid at 5.0 g/L (in fruit nectar) and 3.0 g/L (in fruit juice) and for tartaric acid is set at quantum satis, an amount not higher than necessary to achieve the purpose in accordance with good manufacturing practice.

The research is a part of the scientific project "Antioxidant activity of fruits of some indigenous varieties of fruits species, vegetables and grapes" where the content of specific biologically active substances (antioxidants) in fruits and vegetables are detected for each autochthonous variety and population of fruit species (pomegranate, apple and cherry), grapes and vegetables (tomato and pepper). As expected, confirmation of the quality and high content of biologically active substances in the fruits of our autochthonous varieties of fruit species, grown in agro-ecological conditions of R. N. Macedonia, was obtained. This research will show that the quality does not deviate from the world-famous international, introduced species and varieties and even surpass them. According to the values obtained from the examination of the parameters, most of the autochthonous varieties show higher values compared to the proposed standard varieties, which indicates the fact that the autochthonous varieties are characterized by high antioxidant activity of the fruits and have a strong positive impact on the human organism.

MATERIAL AND METHODS

a) Fruit samples

Eight autochthonous varieties of pomegranate (Zumnarija, Bejnarija, Valandovska kisela, Valandovska kiselo-slatka, Hidjas, Kisela, Lifanka, Ropkavec), seven autochthonous varieties of apple (Ubavo cvetka, Shareno blago, Prespanka, Tetovka, Karapasha, Kozharka, Bela Tetovka), and six autochthonous varieties of cherry (Ohridska brza, Ohridska rana, Ohridska crna, Dolga shishka, Dalbazlija, Ohridska bela) were taken as material for analysis. All fruits are from the harvest year 2021, collected in harvest maturity. Pomegranate fruits are from Valandovo region, cherry fruit from Ohrid and apple fruit from Resen region. Comparative studies were performed in relation to the standard varieties (karamustafa-pomegranate, idared-apple and burlat-cherry).

b) Chemical analyses

The laboratory analyses were performed in the oenological laboratory at the Institute of Agriculture in Skopje. The content of soluble dry matters (%), total sugar (g/L), total acids (g/L), invert sugar (g/L) and some organic acids as citric (g/L), malic (g/L), tartaric (g/L) and lactic (g/L) were examined. The content of soluble dry matters and total sugars was determined refractometrically. The determination of total acids was performed potentiometrically, using

titration with NaOH (0.25 N) to pH=7, according to the ISO 750:1998. Organic acids and the content of invert sugar were determined spectrophotometrically by appropriate enzymatic tests (Poyrazoğlu et al., 2002).

c) Statistical analyses

Correlation analysis (r.) between determined variables was applied using XLSTAT 2014 software. Data matrix has been introduced using descriptive statistical analysis: minimum, maximum, mean value and standard deviation.

Correlation coefficient (r.) description (LaMorte, 2021):

+1.0	Perfect positive
+0.8 to 1.0	Very strong positive
+0.6 to 0.8	Strong positive
+0.4 to 0.6	Moderate positive
+0.2 to 0.4	Weak positive
0.0 to +0.2	Very weak positive or no association
0.0 to -0.2	Very weak negative or no association
-0.2 to -0.4	Weak negative
-0.4 to -0.6	Moderate negative
-0.6 to -0.8	Strong negative
-0.8 to -1.0	Very strong negative
-1.0	Perfect negative

RESULTS AND DISCUSSION

The obtained results from the analysis are shown in Tab. 1. The examined fruit species have approximately the same values of the content of soluble dry matters. From the analysed fruit species, pomegranate has the highest content of soluble dry matters (15.6%), total sugar (149 g/L), invert sugar (113 g/L) and total acids (20.1 g/L). Compared to the standard varieties, Zumnaria, Lifanka and Ropkavec variety (pomegranate) and all varieties of apple and cherries have a higher content of soluble dry matter.

All varieties of apples, Zumnarija and Lifanka pomegranate variety and Ohridska

brza, Ohridska crna, Dalbazlija and Ohridska bela cherry variety, contain more sugar than the standard variety. Zumnarija (pomegranate), Ubavo cvetka (apple) and Ohridska crna (cherry) have the highest content of soluble dry matter and total sugar. The highest content of invert sugar (sum of glucose and fructose) has pomegranate fruits (113 g/L which means 76.3% from the total sugars), it has almost equal representation of glucose and fructose. The content of invert sugar is lower in apple and cherry (61.4 g/L means 54.9% from total sugars and 64.3 g/L which means 44.7% from total sugars, respectively).

Table 1. Results for the content of soluble dry matters (%), total sugar (g/L), total acid (g/L), invert sugar (g/L) and some organic acids: citric (g/L), malic (g/L), tartaric (g/L) in some autochthonous fruit species.

Fruit kind	Variety	Soluble dry matters /%	Total sugars /g/L	Total acids /g/L	Citric acid /g/L	Malic acid /g/L	Tartaric acid /g/L	Sum of glucose and fructose /g/L
Pomegranate	Zumnarija	19.0	194	7.00	0.898	0.800	Tr	112
	Bejnarija	15.4	134	4.10	0.974	1.891	Tr	110
	Valandovska kisela	11.2	131	35.2	0.983	0.182	Tr	102
	Valandovska kiselo-slatka	14.7	149	7.30	1.028	0.653	Tr	118
	Higjas	14.8	125	43.3	1.486	0.577	Tr	104
	Kisela	15.0	129	52.6	0.425	0.031	Tr	116
	Lifanka	18.2	169	23.3	0.687	0.920	Tr	99.4
	Ropkavec	16.5	150	4.00	1.103	0.865	Tr	129
	Karamustafa	15.6	159	4.20	0.497	0.817	Tr	129
Apple	Ubavo cvetka	20.3	151	5.80	0.971	5.21	0.187	71.8
	Shareno blago	13.2	106	6.10	0.950	6.00	0.336	61.7
	Prespanka	16.2	125	1.65	0.932	5.230	0.487	55.9
	Tetovka	12.0	104	3.00	0.947	3.951	0.381	58.6
	Karapasha	14.0	106	2.40	0.961	4.206	0.444	62.3
	Kozarka	13.2	114	7.00	0.973	6.122	0.285	57.4
	Bela tetovka	15.2	123	7.00	0.936	5.553	0.692	65.5
	Aidared	8.00	63.5	7.30	0.971	3.931	0.250	58.3
Cherry	Ohridska brza	16.8	130	4.60	1.81	5.58	Tr	57.50
	Ohridska rana	12.9	104	5.20	1.12	6.77	Tr	50.47
	Ohridska crna	18.2	156	4.60	1.84	5.98	Tr	73.22
	Dolga shishka	14.5	123	8.10	1.85	7.18	Tr	52.32
	Dalbazlija	16.5	193	5.60	1.13	6.37	Tr	81.57
	Ohridska bela	16.0	172	7.90	2.58	6.46	Tr	76.77
	Burlat	11.9	125	4.90	1.69	6.78	Tr	58.56

A strong positive correlation was found between soluble dry matters and total sugars. The coefficient of determination $R^2=60\%$ dependence of the variations in the content of soluble dry matters with the content of total sugars (Tab. 2). A moderate positive correlation was observed between total sugars and the

content of sum of glucose and fructose with coefficient of determination $R^2 = 20\%$, as well as between the content of total acids and the content of sum of glucose and fructose with coefficient of determination $R^2= 22\%$. Summery statistic is given in Tab. 3.

Table 2. Matrix of correlation (Pearson correlation).

Variables	Soluble dry matters /%	Total sugars /g/L	Total acids /g/L	Citric acid /g/L	Malic acid /g/L	Tartaric acid /g/L	Sum of glucose and fructose /g/L
Sol. dry maters /%	1	0.7768	-0.0662	0.1008	-0.0189	0.0514	0.2459
Total sugars /g/L	0.7768	1	0.0218	0.1763	-0.1314	0.0551	0.4477
Total acids /g/L	-0.0662	0.0218	1	-0.2224	-0.6101	-0.0213	0.4638
Citric acid /g/L	0.1008	0.1763	-0.2224	1	0.5446	-0.0143	-0.3611
Malic acid /g/L	-0.0189	-0.1314	-0.6101	0.5446	1	0.0168	-0.8776
Tartaric acid /g/L	0.0514	0.0551	-0.0213	-0.0143	0.0168	1	-0.0088
Glucose and fructose/g/L	0.2459	0.4477	0.4638	-0.3611	-0.8776	-0.0088	1

Values in bold are different from 0 with a significance level $\alpha=0.05$

Table 3. Summary statistics.

Variable	Observations	Minimum	Maximum	Mean	Standard deviation
Soluble dry matters /%	29	8.00	20.3	14.96	2.47
Total sugars /g/L	29	63.5	194	135	27.9
Total acids /g/L	29	1.65	52.6	10.8	12.4
Citric acid /g/L	29	0.42	2.58	1.17	0.45
Malic acid /g/L	29	0.03	7.18	3.98	2.40
Tartaric acid /g/L	29	0.18	0.69	0.38	0.07
Glucose and fructose/g/L	29	50.4	129	80.5	25.0

The content of these parameters depends on the type, variety, climatic conditions, degree of maturity etc. The determined results match the available literature data. Self-sprouting pomegranate varieties contain 9.5-13.8% dry matters (Markovski et al., 2017). Apples contain 6.6-15.5% total sugars and cherries contain 4.7-11.5% (Niketic-Aleksic according to Seelamovska & Miskoska-Milevska, 2021), while self-sprouting pomegranate varieties contain 110-152 g/L total sugar (Markovski et al., 2017). Cherry cultivars contents 119.94-215.90 g/kg glucose, 25.24-61.44 g/kg fructose and 1.73-7.60 g/kg sucrose (Gündoğdu & Bilge, 2012). The sum of sugars (glucose, fructose, sucrose and sorbitol) in sweet cherry fruit is ranged from 125 to 265 g/kg fresh weight (FW). In different cherry cultivars glucose content is range between 61.8-123 g/kg, fructose content between 47.6-101 g/kg and sucrose content between 3.57-12.5 g/kg (Usenik et al., 2008). Total sugars in pomegranate are ranged between 139-160 g/L (average 148 g/L) (Poyrazoğlu et al., 2002). The taste in pomegranate fruits is governed content mainly by the presence of sugars (glucose and fructose) and organic acids (Mayuoni-Kirshinbaum & Porat, 2014). According Sokol-Letowska et al. (2020) in sour cherries the main sugars are glucose (2.81-5.68 g/100gFW) and fructose (2.74-4.88 g/100g FW).

The autochthonous varieties of pomegranate have the highest content of total organic acids (about 20%). In pomegranate fruits, citric and malic acid are almost equally represented, with a slight predominance of citric acid, whose content is most pronounced in the Valandovska kiselo-slatka, Higjas and Ropkavec (more than 1.0 g/L). Compared to the standard variety, almost all pomegranate varieties have a higher number of total acids

(with the exception of Ropkavec and Bejnaija).

Apple and cherry fruits contain fewer total acids (about 5%). They are dominated by malic acid (above 5 g/L). Cherry fruits on average contain a higher citric acid content (1.72 g/L) than pomegranate and apple (0.898 g/L and 0.955 g/L, respectively). All autochthonous varieties of apples have a lower content of total acids compared to the standard variety, while autochthonous varieties of cherries, with exception of Ohridska crna and Ohridska brza, have a higher content of total acids than the standard variety. The highest content of citric acid was found in the fruits of the Ohridska bela variety (2.58 g/L).

A very small amount of tartaric acid was found in apple fruits, an average of 0.383 g/L, while in pomegranates and cherries, tartaric acid was not found. The presence of lactic acid was not found in all fruit species.

A statistically strong negative correlation was observed between the contents of total acids and malic acid, as well as weak negative correlation dependence with the content of citric acid. This means that if, for example, the content of total acids in the tested fruits increases, the part of citric and malic acids in the total acids decreases, so the increase of total acids is due to some other untested acids. The coefficient of determination R^2 shows a weak dependence of 37% between the contents of total acids and malic acid and only 5% between the content of total acids and citric acids. A moderate positive correlation was found between the content of malic and citric acid with coefficient of determination $R^2=30\%$. The very strong negative correlation between the content of malic acid and the sum of glucose and fructose in the fruits is particularly emphasized. The coefficient of determination R^2

shows as much as 77% dependence between the content of malic and the sum of glucose and fructose (Tab. 2).

Ma et al. (2018) noticed a strong positive correlation was detected between fruit total organic acid with malic acid and citric acid content. In contrast to malic acid, citric acid was predominantly detected in partial wild apples, while extremely low to undetectable concentrations of citric acid were observed in cultivated apple fruits.

The results obtained from the examination of the content of total acids, as well as some organic acids, match the available literature data. Total titratable acidity in pomegranate is ranged between 4.58-17.30 g/L (average 9.82 g/L), citric acid is predominant acid with a range of 0.33-8.96 g/L, malic acid is the second most abundant with a range of 0.56-6.86 g/L and tartaric acid respectively 0.28-2.83 g/L (Poyrazoğlu et al., 2002). Self-sprouting pomegranate varieties contain 4.9-34.7 g/L total acids (Markovski et al., 2017). According Mayuoni-Kirshinbaum and Porat (2014) pomegranate fruits content primarily citric and malic acids. The aroma evolves from the presence of dozens of volatiles, including alcohols, aldehydes, ketones and terpenes which provide a mixture of various "green", "woody", "earthy", "fruity", "floral", "sweet" and "musty" notes.

The sum of organic acids (malic, citric, shikimic and fumaric) according Usenik et al.

(2008) in sweet cherry ranged from 3.67 to 8.66 g/kg FW. According Sokol-Letowska et al. (2020) the content of total acids in sour cherries is 1294.4-2300.5 mg/100g FW. The main organic acids in sour cherries are malic and malonic acids. Apples contain 0.4-0.8% total acids, while cherries 0.3-0.6% (Niketic-Aleksic, cit. Selamovska & Miskoska-Milevska, 2021). Malic acid is the primary acid detected in apple (Ma et al., 2015, 2019), cherry (Gundogdu and Bilge, 2012; Serra et al., 2011), sweet cherry (Hayaloglu & Demir, 2015; Usenik et al., 2008), sour cherry (Sokol-Letowska et al., 2020), pear (Wu et al., 2022). The level of citric and tartaric acids in fruits nectars ranged from 1.26 to 4.42 g/L⁻¹ and 0.68 to 0.86 g/L⁻¹ and in fruits juices ranged from 3.03 to 7.67 g/L⁻¹ and 3.09 to 4.68 g/L⁻¹. The level of tartaric acid in fruit juices is higher than in fruit nectars. In general, the level of both acids is higher in fruit juices than in fruit nectars (Inić et al., 2020).

The results of this study indicate that the level of citric acid (0.898-1.719 g/L) and tartaric acid (0.38 g/L only in apple fruits) in fruit available on the market is below the concentration allowed by EU regulations and do not pose a threat to human health, confirming good manufacturing practices (GMP). The monitoring of the level of citric and tartaric acids in fruit beverages is needed in order to protect human health.

CONCLUDING REMARKS

According to the data obtained from determination of chemical composition of the fruits of autochthonous varieties of fruit species, the following conclusions can be extracted.

Pomegranate fruits have the highest content of soluble dry matters, total sugar, invert sugar and total acids. In the fruits of apple and cherry varieties, malic acid dominates, while in pomegranate varieties, the contents of citric and malic acid are almost equally represented. A very low content of tartaric acid is observed only in apple fruits. The presence of lactic acid was not found in the fruits of all fruit species.

A strong positive correlation was found between soluble dry matters and total sugars. A moderate positive correlation was established between the content of total sugars and the

sum of glucose and fructose in fruits, between the content of total acids and the sum of glucose and fructose, as well as between the content of malic and citric acid. Strong negative correlation exists between the content of total acids and malic acid, and very strong correlation between the content of malic acid and the sum of glucose and fructose.

According to the results obtained from the investigated parameters, most of the autochthonous apple, pomegranate and cherry varieties compared to the proposed standard varieties show higher values, which indicates the fact that the autochthonous varieties are characterized by the quality that does not deviate from the world-famous international introduced species and varieties.

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ХЕМИСКИ СОСТАВ НА НЕКОИ АВТОХТОНИ ОВОШНИ ВИДОВИ ОД МАКЕДОНИЈА

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Резиме

Целта на научниот труд е определување на содржината на растворливи суви материи, вкупни шеќери, вкупни киселини, инвертен шеќер и лимонска, јабољкова, винска и млечна киселина во некои автохтони овошни видови: калинка (зумнарија, бејнарија, валандовска кисела, валандовска кисело-слатка, хиџас, кисела, лифанка, ропкавец), јабољко (убаво цветка, шарено благо, преспанка, тетовка, карапаша, кожарка, бела тетовка) и цреша (охридска брза, охридска рана, охридска црна, долга шишка, далбазлија, охридска бела). Извршена е споредба со стандардните сорти *карамустафа* - калинка, *ајдаред* - јабољко и *бигаро бурлат* - цреша.

Плодовите од калинка имаат највисока содржина на растворливи суви материи (15.6%), вкупни шеќери (149 g/L) и вкупни киселини (20.1 g/L). Содржината на инвертен шеќер е најголема во плодовите од калинка (113 g/L односно 76.3% од вкупните шеќери). Во плодовите од јабољко и цреша доминира јабољковата киселина (над 5 g/L), додека во калинката речиси подеднакво се застапени лимонската и јабољковата киселина. Присуство на винска киселина има само во плодовите од јабољко (0.383 g/L). Нема присуство на млечна киселина во сите овошни видови.

Констатирана е силна позитивна корелација меѓу растворливите суви материи и вкупните шеќери (коефициент на детерминација $R^2=60\%$). Умерена позитивна корелација постои меѓу вкупниот и инвертниот шеќер со коефициент на детерминација $R^2=20\%$, меѓу вкупните киселини и инвертниот шеќер со коефициент на детерминација $R^2=22\%$ и меѓу содржината на јабољковата и лимонската киселина со коефициент на детерминација $R^2=30\%$. Висока негативна корелација постои меѓу содржината на вкупните киселини и јабољковата киселина со коефициент на детерминација $R^2=37\%$ и многу висока негативна корелација меѓу јабољковата киселина и инвертниот шеќер со коефициент на детерминација $R^2=77\%$.

Клучни зборови: *калинка, јабољко, цреша, растворливи суви материи, шеќери, органски киселини.*



OLEOGELS – AN ALTERNATIVE TO REPLACE ANIMAL FATS IN MEAT PRODUCTS

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Abstract

The changes in lifestyle of modern consumers have increased demand for healthier meat products. Animal fat, which is an integral part of meat products, is directly related to the occurrence of chronic diseases and overweight. Polyunsaturated vegetable oils are healthier for human consumption, but their liquid consistency can pose a problem when directly applied in the technological process. Application of oleogels, obtained with innovative technology for gelling vegetable oils using gelators, is a new approach to create healthier meat products with an improved fatty acid profile. This paper aims to review the different approaches for obtaining oleogels and the latest trends for their use in meat products.

Key words: *oleogels, gelators, vegetable oils, meat products*

INTRODUCTION

Fats can be present from 30% to 50% in some types of meat products, thus directly affecting their technological (Badar et al., 2021; Oliveira et al., 2021) and sensory characteristics (Totosaus-Sanchez, 2008; Zampouni et al., 2022). Although meat products contain a high percentage of fat in their composition, they are still highly respected and consumed mainly because of their sensory attributes (Agregán et al., 2018). However, a higher intake of saturated and trans-fatty acids is associated with the prevalence of various diseases, including diabetes, obesity and especially cardiovascular diseases (de Souza et al., 2015; Hooper et al., 2020).

In the past, reducing total fat intake was recommended as a preventive measure for

cardiovascular disease (Mozaffarian et al., 2018), but today, the focus is shifting to improving the quality of dietary fat (Schwingshackl et al., 2022). The trends for the creation of new formulations, in which complete or partial replacement of saturated and trans-fatty acids with unsaturated fatty acids, present a challenge for the food industry (Hooper et al., 2020).

By applying oleogels there is an opportunity to create a wide range of semi-solid and solid meat products with an improved lipid profile suitable for consumption (Wang et al., 2023). The market for healthier processed meats is expected to grow. Current generation of consumers are health conscious and choose 'health', regardless of the price of meat products (Badar et al., 2021).

OLEOGELS AS ALTERNATIVES TO ANIMAL FATS

Incorporating unsaturated fats into meat products is difficult due to maintaining the consistency of the product and obtaining a final product with undesirable softness, lower sensory acceptability and greater

chances of oxidative degradation (Pintado & Cofrades, 2020). Lima et al., (2022) consider that the incorporation of vegetable oils into meat products is a viable alternative, and oleogelation, as the technological strategy for

the restructuring of vegetable oils into oleogels and their incorporation into meat processing, will meet the demands of the industry and consumers.

The ideal fat should have the chemical composition of liquid oil (high fraction of mono and polyunsaturated fats) and all the functional properties of solid fat (Pehlivanoğlu et al., 2016). Oleogels have the characteristics of solid fats (providing unique taste, texture and plasticity) without changing the health and nutritional composition of vegetable oil (Ferro et al., 2021; Li et al., 2021). In fact, oleogels are solids with a three-dimensional network structure obtained by dissolving a gelator in an organic solvent under the influence of various interactions (Perta-Crisan et al., 2023). To obtain oleogels, direct and indirect methods are used (Feichtinger & Scholten, 2020), but the most commonly investigated are: straightforward dispersion and indirect approaches such as foam or emulsion-templated processes (Wang et al., 2023).

Direct dispersion involves: adding the gelator to oil, heating to a point above the melting point of the gelator, and natural or forced cooling. As a result of the cooling process, nucleation, crystallization and self-assembly occur (Aguilar-Záratea et al., 2019). The most commonly investigated gelators used in the direct dispersion method are: glycerol esters of fatty acids such as mono- and diacylglycerols, natural waxes, hydroxylated fatty acids such as 12-hydroxy stearic acid and its 12-hydroxy derivatives, trans-9-Octadecenoic acid (ricinelaidic acid), sphingolipids and mixed systems such as a combination of phytosterols and sterol esters in particular, β -sitosterol + γ -oryzanol and ethylcellulose (Draper & Adams, 2017).

The emulsion approach involves several steps: adding the gelator to the oil and stirring at low speed to achieve dispersion, adding water ($T < 100\text{ }^{\circ}\text{C}$) and homogenizing at 16 500 rpm. The resulting emulsion is transferred to a mould and dried by forced convection. The dried samples are processed at a high number of revolutions until the formation of an oleogel (Espert et al., 2021). In the foam approach, the gelator is dissolved in water, homogenized at 13 000 rpm to obtain a foam that is dried by lyophilisation to obtain a cryogel, which is ground and oil is added to it, after which it is processed by homogenization at 10 000 rpm in order to obtain an oleogel (Abdollahi et al., 2019). The most commonly used gelators for indirect methods are polysaccharides, proteins, polymers (Kavya et al., 2022).

The technological challenge is to design oleogels that exhibit the desired structural and physicochemical properties (Feichtinger & Scholten, 2020). The different approaches, the type and content of oil, as well as the type of gelator allow obtaining oleogels with different properties and functions (Wang et al., 2023). By using various types of gelators to structure oil, oleogels with distinct textural and thermorheological characteristics can be produced (Lim et al., 2017). Oleogel properties are closely related to solvent structure and processing conditions (Patel et al., 2015). By adding larger amounts of gelator, oleogels with higher viscosity are obtained, but as the temperature of the oleogel increases, its viscosity decreases (Ruiz Martinez et al., 2003). Oleogels that have a higher proportion of polyunsaturated fatty acids in their oil composition exhibit faster gelation rates, more extensive microstructures under varying cooling rates, and stronger Van der Waals forces (Han et al., 2022).

POSITIVE PROPERTIES OF OLEOGELS

Oleogels excel in the ability to encapsulate and aid in the controlled release of lipid-soluble nutrients (Manzoor et al., 2022). The solid-phase gelator can modulate lipid digestion, that is, alter the release of lipids into the bloodstream as a result of the complex network structure, protecting the sites for triacylglycerol digestion by the enzyme lipase (Hwang, 2020). During digestion, an oil-water interface is formed,

this interface allows the binding of surfactant components in the small intestine, such as bile salts and colipase/lipase complexes. Unlike classical lipids, the structuring networks of gelators act as a physical barrier preventing digestive enzymes from coming into close proximity to lipids and causing a delay in digestion (Tan et al., 2023). After 4 weeks of feeding rats an oleogel formed from rice bran

and rice bran oil, compared to margarine and beef tallow, it was confirmed that the levels of triacylglycerol decreased by about 30% in the serum and liver and increased the levels of excreted triacylglycerol by 30% in feces, compared to rats fed margarine and beef tallow (Limpimwong et al., 2017).

In their study, Ghosh et al. (2017) evaluated the nutritional properties of oleo-gels made from a blend of palm stearin with cetyl laurate and palm stearin with cetyl caprylate as gelators at 15% (w/w) for a blend of linseed oil and rice bran in rats. The analysis of the blood lipid profile revealed that rats fed with oleogels had lower cholesterol levels compared to those fed only the oil mixture. In a separate study, Tan et al. (2017) examined the effects of coconut oil in liquid or oleogel form on triglycerides, glucose, insulin, and hunger when consumed with a high-carbohydrate meal. The results indicated significant changes in glucose, insulin, triglycerides, and hunger. Gelled coconut oil also reduced the peak glucose response and increased the incremental area under the curve for postprandial triglycerides.

The absorption rate of plant sterols in the gastrointestinal tract is much lower, about 5% compared to cholesterol absorption which is about 55% - 60% (Scharfe & Flöter, 2020). Phytosterols such as β -sitosterol and γ -oryzanol, which are used as gelators, can affect the inhibition of intestinal absorption of cholesterol, thereby reducing the level of LDL cholesterol in the blood. For example, phytosterols in doses of 2-3 g/day reduce LDL-cholesterol levels by 6%-15% (NCEP 2002). Three different gelators were investigated for the gelation of canola oil: ethyl cellulose, mono- and di-glycerides, and a mixture of β -sitosterol + γ -oryzanol. Simulated intestinal lipolysis revealed a significantly different pattern of lipolysis for the different gelation mechanisms. Ethylcellulose-based oleogels are more susceptible to lipolysis compared to the β -sitosterol + γ -oryzanol mixture, while glycerides showed a high level of lipolysis up to 90% due to the hydrolysis of the gelator itself (Ashkar et al., 2019). However, it is challenging to incorporate these oleogels into water-containing systems, because the presence of water can interfere with the ability

of the gelator to assemble into tubules or cause recrystallization of the network (Duffy et al., 2009). Applications are successful where water activity is controlled through the interaction of other ingredients such as proteins and hydrocolloids (Matheson et al., 2018).

Another positive property of oleogels is the delivery of bioactive molecules, as a result of their lipid medium which is well suited to prevent deposition of bioactive substances, slow lipolysis and release of nutrients from crystalline and fibrillar networks (O'Sullivan et al., 2016; Martins et al., 2020). Two types of gelators, beeswax and a mixture of β -sitosterol + γ -oryzanol, were used for gelation of long-chain triglycerides. In both cases, the gelator was added in an amount of 8% (w/w) and enriched with 0.1% (w/w) β -carotene. In vitro digestion indicated a bioavailability of 26% of β -carotene in sterol-based oleogels and approximately 20% in beeswax-based oleogels (Martins et al., 2018). In vitro lipolysis and transfer of β -carotene was also investigated by Chloe et al., (2017) in canola oil gelled with ethylcellulose, results indicated increased stability of β -carotene in the oleogel and antioxidative behavior. Curcumin, a natural polyphenolic compound, possesses a spectrum of health benefits, including anticancer, anti-inflammatory, antioxidant, antiviral, and cytoprotective properties. However, its limited solubility in water, susceptibility to degradation from light, heat, and physiological pH conditions result in an exceptionally low bioavailability (Pérez et al., 2019). To address this challenge, curcumin was incorporated into a corn oil oleogel structured by β -sitosterol and lecithin. The oleogel demonstrated heightened oxidative stability and improved bioavailability compared to the control. Specifically, crosslinking curcumin in the oleogel increased its bioavailability in the fasting state to 67.66% (Li et al., 2019)."

A positive feature of oleogels is that they are obtained by gelation without hydrolysis, while sterols and vitamin E remain stable during gel formation (Xu et al., 2022). However, the effect of processing, cooking, freezing storage as well as the type of fat used can affect the stability and content of vitamin E in meat products (Wan Rosli et al., 2006).

APPLICATION OF OLEOGELS IN MEAT PRODUCTS

Oleogels have been recognized as a very promising alternative to replace trans and saturated fats, but no oleogels have yet been used on a commercial basis in food production (Hwang, 2020). By applying oleogels there is an opportunity to create a wide range of semi-solid and solid meat products with an improved lipid profile suitable for consumption (Wang et al., 2023). The use of low-quality oleogels can cause technological problems, such as improper drying, greasy appearance, delamination, etc. (López-Pedrouso et al., 2021).

By replacing 50% of the animal fat with an oleogel structured from sunflower oil using monoglycerides of phytosterols in a ratio of 15:5 (w/w) in sausages of the "Frankfurt" type, a product with an improved fatty acid profile is obtained without significantly compromising the physical, chemical, textural and sensory characteristics (Kouzounis et al., 2017). 12-fold reduction of the ratio of polyunsaturated fatty acids, n-6/n-3 compared to control samples, in fermented sausages of the "Fuet" type using oleogels based on 80% olive oil and 20% chia seed oil, obtained by direct dispersion using beeswax or emulsion approach using isolated soy protein and gelatin (Pintado & Cofrades, 2020). By completely or partially replacing the animal fat in foie gras with oleogel obtained from olive oil (44.39%), linseed oil (37.87%) and fish oil (17.74%) with gelators ethyl cellulose and beeswax, an optimal fatty acid profile was obtained, a high PUFA/SFA ratio and a low n-6/n-3 ratio. Lipid oxidation was increased while, the substitution insignificantly affected the emulsion stability, color and texture, compared to the control. Sensory characteristics were insignificantly affected using beeswax, while a negative effect on sensory properties was confirmed when using ethyl cellulose, depending on the level of substitution (Gómez-Estaca et al., 2019). A different degree of lipid oxidation depending on the oleogel used for fat replacement has been reported (Wolfer et al., 2018). Increased lipid oxidation compared to the control sausage was reported in samples using the rice bran wax oleogel (10%) during 0, 42, 70 and 84 days of storage, but the thiobarbituric acid TBA value never exceeded 0.201 mg/kg for any day of analysis. Curcumin was added to oleogels gelled with beeswax and ethylcellulose.

The resulting oleogels are incorporated into pork burgers. Curcumin effectively reduced the oxidation process, burgers with beeswax oleogel showed adequate technological properties, but with the addition of curcumin, a yellow color was obtained, which reduced the sensory acceptance (Gómez-Estaca et al., 2020). Incorporated into paté, oleogels obtained in optimal conditions 9.12% beeswax and 0.54% curcumin. The oleogels showed a mechanical strength similar to porcine hard adipose tissue and a high oil binding capacity of over 90%. The addition of added curcumin attenuated lipid oxidation during cold storage (Ramírez-Carrasco et al., 2020). Jeong et al. (2021) found that applying an oleogel with added β -carotene as an antioxidant resulted in lower peroxide number values in muffins. However, further research is needed to investigate the application of β -carotene or other antioxidants in oleogels and their use in meat products.

Replacing 50% of animal fat with an oleogel of glyceryl-monostearate with sunflower oil did not impact the firmness of sausages, while sensory evaluations showed that they were more acceptable than the control (Ferro et al., 2021). For good retention of the oil phase in foie gras, Barbut et al. (2020) recommend replacing 60% of the animal fat with an oleogel based on canola oil gelled with 12% ethyl cellulose and 3% glycerol monostearate. Also, Barbut et al. (2021) recommend replacing 60% of lard with an oleogel based on canola fat, ethyl cellulose and glycerol monostearate in foie gras to retain oil and maintain textural properties without affecting sensory properties and colour. Contrary to this, Martins et al. (2020), report a decrease in hardness and increased stickiness in foie gras by replacing animal fat by 60% with oleogel from linseed oil and beeswax, but also an increase in the percentage of polyunsaturated fats and a decreased ratio of n6/n3 by 90%. Tarté et al. (2020), obtained a softer product and an improved fatty acid profile by replacing 41.9% of the animal fat with soybean oil oleogel with rice bran wax in sausages, linseed oil-based oleogel, structured with a mixture of γ -oryzanol and β -sitosterol and beeswax, and incorporated into fermented sausages in an amount of 20% and 40% resulted in products with reduced sensory quality, which decreased with an increase in the

percentage representation of oleogel (Franco et al., 2020). Incorporation of oleogel obtained by gelling cannabis oil using gelators such as rice bran wax (5%; 7%) or candelilla wax (3%; 7%)

in meat patties improved their fatty acid and oxidative stability but sensory acceptability was low (Hamidioglu et al., 2022).

CONCLUSION

The attractiveness and actuality of oleogels obtained by gelling vegetable oils with the help of gelators is growing daily. The use of different vegetable oils and their combinations, as well as the use of different gelators offer a wide range and design of oleogels with different characteristics. However, as a common positive characteristic of meat products in which animal fats have been replaced with oleogels, it is an improvement in the fatty acid profile of meat

products, which makes the products more nutritionally acceptable for consumers. With the suitable choice of: technique, oil, gelator and selection of the optimal amount of replacement, a product will be obtained that will satisfy the sensory and technological qualities of the conventional product. The acceptability of a product by consumers is important assessment in creating a quality product with a successful replacement of the fats.

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ОЛЕОГЕЛОВИ – АЛТЕРНАТИВА ЗА ЗАМЕНА НА ЖИВОТИНСКИТЕ МАСТИ ВО ПРОИЗВОДИТЕ ОД МЕСО

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Резиме

Промената на животниот стил на современите потрошувачи ја зголеми побарувачката на поздрави производи од месо. Животинската маст која е составен дел од месни производи е директно поврзана со појавата на хронични заболувања и прекумерна телесна тежина. Полинезаситените растителни масла се поздрави за човечка исхрана, но нивната течна конзистенција може да претставува проблем при директно аплицирање во технолошкиот процес. Олеогеловите добиени со иновативна

технологија за желирање на растителните масла со помош на желатори и нивната апликација во производите од месо е нов пристап за креирање на поздрави месни производи со подобрен масно-киселински профил. Овој преглед има за цел да ги разгледа различните пристапи за добивање на олеогелови и најновите трендови за нивна употреба во производите од месо.

Клучни зборови: олеогелови, желатори, растително масло, производи од месо.



SENSORY CHARACTERISTICS OF COLD PRESSED SUNFLOWER OIL WITH THE ADDITION OF AROMATIC HERBS

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Abstract

Cold pressed sunflower oil is an unrefined oil with great biological value and good sensory properties. Due to its chemical composition, this oil easily oxidizes, creating harmful products for human health. Various artificial and natural stabilizers are added to prevent oxidation. Essential oils or aromatic herbs are used as natural stabilizers, which also change the sensory properties of the oils.

The oil analysed in this paper is cold pressed sunflower oil to which fresh aromatic herbs have been added, such as rosemary, garlic, parsley and mint. One aromatic herb is added to each bottle of oil, and one bottle of oil is without additives (control). The oils are stored for 6 weeks in the dark, and then the sensory characteristics of these oils are assessed through validated questionnaires and sensory tests. The following characteristics of the oils were determined through a series of hedonistic and visual tests: aroma, colour, clarity, taste, crunchiness, and chewiness.

The aromatic herbs that are added do not affect the crispness and chewiness, but they do affect the smell, taste, colour, and clarity. The oil without additive and the oil with the addition of rosemary have the most acceptable smell and taste and the highest grades for colour and clarity. Apart from these two oils (without additive and with the addition of rosemary), there is also the oil with the addition of garlic.

Key words: *cold pressed sunflower oil, rosemary, garlic, parsley, mint*

INTRODUCTION

Cold-pressed sunflower oil is highly valued for its pleasant sensory properties and high biological value. Unrefined sunflower oil has a light-yellow colour and the typical smell of sunflower seeds. It is rich in unsaturated fatty acids, vitamin E, provitamins, lecithin, phytosterols, minerals, and other ingredients. extremely useful for human health. (Dimić, 2005; Dimić et al., 2015; Konuskan et al., 2019; Bendini et al., 2011). Since oils oxidize easily, it is important to prevent their oxidation. The oxidative stability of oils is increased by antioxidant fractions from Lamiaceae herbs (Babovic et al., 2010; Mousavi et al., 2012; Niamat et al., 2016). Many herbs such as rosemary, mint, parsley, garlic, sage, thyme, etc. contain antioxidants that stabilize

lipids and lipid-containing foods (Eftinzijoska & Pavlovska, 2019; Yanishlieva et al., 2006; Bravi et al., 2016; Mousavi et al., 2012; Niamat et al., 2016; Temelkovska & Pavlovska, 2021).

Regarding the natural antioxidants, rosemary is widely accepted as one of the spices with the greatest antioxidant effect. (Yanishlieva et al., 2006; Olmedo & Grosso 2019; Aguilar et al., 2008). The antioxidant activity of rosemary extract is related to the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, rosmarinic acid, and other steroids and triterpenes (Genena et al., 2008; Nieto et al., 2018). The use of rosemary as a natural antioxidant is highly accepted by consumers

due to its beneficial sensory properties (Olmedo & Grosso, 2019; Ghafoor et al., 2020; Ali et al., 2021).

The most important active components of parsley are flavonoids, phenols, coumarins, vitamin C, and the high content of beta-carotene, which is an important liposoluble antioxidant (Trifunski & Ardelean, 2012). The characteristic aroma and taste of parsley come from the volatile components (Sitarek et al., 2015; Ulrich et al., 2011). Mint has a high content of monoterpenes ketones, aldehydes, natural phenols, and flavonoids that have a high antioxidant effect. The specific smell of menthol is due to α -cyteral, menthofuran, isomenthone, menthol, carvone, and linalool. (Zhang et al., 2022).

Garlic contains two types of antioxidant compounds, flavonoids and sulfur-containing compounds: allyl-cysteine, diallyl sulfide, and allyl trisulfide. Derivatives of amino acids that contain sulfur allin (S-allyl-L-cystein sulfoxide) can be converted into allicin (diallyldisulfide-S-oxide), a compound responsible for the smell of garlic, under the action of the enzyme allinase. (Pardo et al., 2007).

Rosemary, parsley, garlic, and mint improve human health due to their high antioxidant,

antimicrobial, anti-inflammatory, and anti-cancer potential and reduce the risk of chronic diseases such as diabetes, cardiovascular diseases, neurological disorders, etc. Food that is enriched with spices and herbs has functional, nutritional, and health benefits. (Labban et al., 2014; Rahbardar & Hosseinzadeh, 2020; Lesnik et al., 2021; Agyare et al., 2017; Nayak et al., 2020; Ansary et al., 2020).

Spices and herbs are widely used as food additives, increasing oxidative and microbiological stability and thus extending the shelf life of food (Przygodzka et al., 2016; Al Soudy et al., 2020; Rababah et al., 2012; El-Sayed & Youssef, 2019). Spices and herbs affect the sensory properties of the food to which they are added, improving the taste and smell, thereby making them acceptable for consumption (Habib et al., 2017; Tawfek & Ali, 2022; Amer & Rizk, 2022; Hamad et al., 2017; Issaoui et al., 2016).

One of the most important parameters of edible oils is the sensory evaluation, because with the addition of spices to improve the stability of the oils, there is a change in the sensory characteristics. (Olmedo & Grosso, 2019; Akçar & Gümüskesen, 2011).

MATERIAL AND METHODS

Cold-pressed "Fila" sunflower oils with a volume of 750 mL (Agrofila DOOEL Shtip, Republic of North Macedonia) were used for analysis. Oil without addition (control) and oils with additions of aromatic herbs and spices were used. 20 g of fresh rosemary (*Rosmarinus officinalis* L.), garlic (*Allium sativum* L.), parsley (*Petroselinum crispum* Mill.), or mint (*Mentha piperita* L.) originating from N. Macedonia were added to the oils. The herbs in the oils were added at a concentration of 2.8%. The oil without the addition was opened immediately before the analysis, while the oils with the addition of aromatic herbs and spices were stored in the dark for a period of six weeks.

Sensory analyses were performed at the Department of Nutrition, Faculty of Technology and Technical Sciences Veles. In the research, validated questionnaires were used to assess the sensory characteristics of oils by a selected group of 20 panellists (students), all of whom had previous experience and training in the

sensory analysis of different food products. All respondents were familiar with the activities that would follow. Before the beginning of each sensory test, the requirements in the questionnaire, the method of rinsing the oral cavity after each tasting, and the method of cleaning the nasal cavity after each analysis are clarified. Each sensory analysis was followed by a short break in order to rest the senses.

The following sensory tests were used for the sensory evaluation: hedonic smell test, preference and ranking test, colour and clarity test, hedonic colour and clarity test, taste test, the hedonic taste test, crispiness test, the hedonic crispness test, chewiness test, and the hedonic chewiness test (Carabante & Prinyawawatkul, 2018; Yang & Lee, 2019). To perform the hedonic smell test, colour and clarity test, and hedonic colour and clarity test, one sample of all cold-pressed sunflower oils (the oil without additives and the oils with additives) was used for these tests. In order not to mix the smells, the samples

were placed in transparent cups covered with a piece of paper, and between smelling the samples, it was necessary to smell the upper part of the palm. Each panellist received 5 samples

for analysis, blindly labelled with a three-digit code. The cups with the oil samples were placed on a white sheet of paper to observe the colour shade and clarity of the oil (Figure 1).

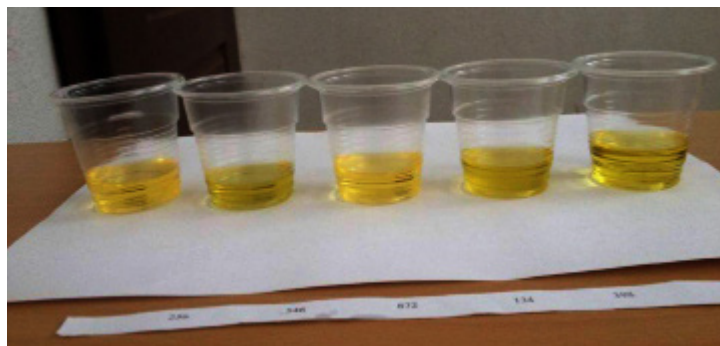


Figure 1. Oil's samples for sensory analysis.

To perform the crispiness test, the hedonic crispness test, the chewiness test, and the hedonic chewiness test, a few drops of the oils were placed on a piece of cucumber as a neutral sample. Samples of cucumber and a two-pack with oil drops were blindly marked with a three-digit code. The taste test and the hedonic taste test were determined by placing a few drops of oil on a piece of biscuit as a neutral sample. The panellists tried the samples in order from left to right and determined the required parameters according to the questionnaire requirements. Between each trial, the panellists cleaned the

oral cavity with water and a piece of biscuit.

For the hedonic tests, a 9-point hedonic scale (1 = disliked extremely, 2 = disliked very much, 3 = disliked moderately, 4 = disliked slightly, 5 = neither liked nor disliked, 6 = liked slightly, 7 = liked moderately, 8 = liked very much, and 9 = liked extremely) is used to determine the acceptability of the oils (Wichchukit & O'Mohony, 2014).

Statistical analysis was performed with descriptive statistics using Microsoft Office Excel data analysis (Levine et al., 2008).

RESULTS AND DISCUSSION

The results obtained for sensory characteristics of the oils from the hedonistic tests are shown in Table 1 and Figure 2.

Table 1. Mean sensory scores and standard deviation from the hedonistic tests.

Sample	OWA	OWR	OWG	OWP	OWM
	$\bar{x} \pm SD$				
Smell	6.90 ± 1.18	6.75 ± 1.83	3.30 ± 2.54	2.50 ± 1.57	3.15 ± 1.90
Taste	5.95 ± 1.64	5.90 ± 1.99	3.40 ± 2.74	3.85 ± 1.73	4.40 ± 2.01
Colour	7.45 ± 1.10	6.05 ± 1.93	6.65 ± 1.57	4.40 ± 1.79	5.25 ± 1.97
Clarity	7.67 ± 0.86	6.95 ± 1.50	6.80 ± 1.36	2.95 ± 1.70	3.50 ± 2.06
Crunchiness	7.75 ± 0.91	7.60 ± 0.99	6.85 ± 0.99	7.35 ± 0.75	7.10 ± 1.02
Chewability	7.85 ± 0.75	7.65 ± 0.67	6.85 ± 0.75	6.90 ± 0.85	6.75 ± 0.91
Overall acceptance	7.22 ± 0.81	6.83 ± 0.83	6.11 ± 1.52	5.09 ± 1.94	5.40 ± 1.53

*n = 20; $\bar{x} \pm SD$ mean value \pm standard deviation; OWA-oil without additive; OWR-oil with rosemary; OWG - oil with garlic; OWP -oil with parsley; OWM-oil with mint.

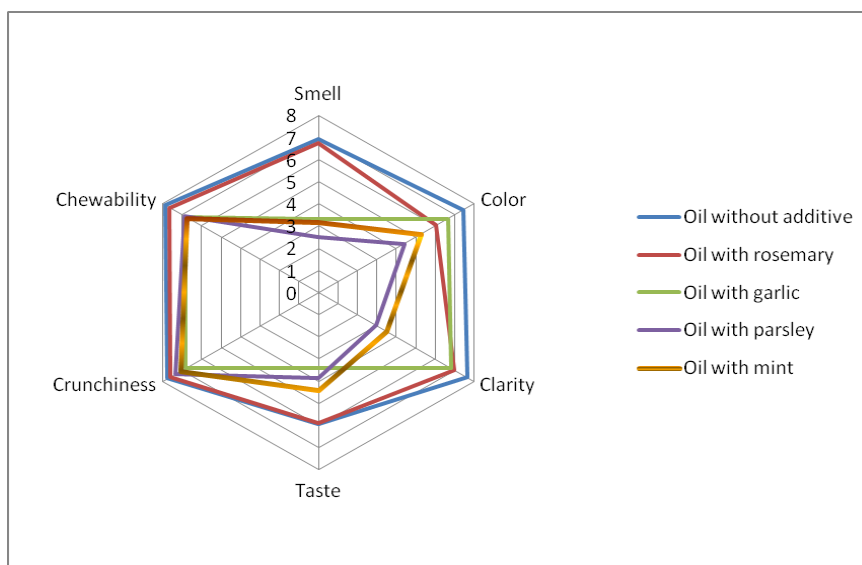


Figure 2. Sensory profile of the oils from the results obtained from hedonistic tests.

The biggest differences between the oils are in the ratings for smell, clarity, and taste (Table 1, Figure 2). The panellists gave high marks for smell to the oil without the addition (6.9 points) and to the oil with the addition of rosemary (6.75 points), while the oils with garlic, parsley, and mint have very low marks (3.30, 2.50, and 3.15 points, respectively). In terms of colour and clarity, the most acceptable are oils without additives (7.45 and 7.65 points, respectively), garlic oil (6.65 and 6.8 points, respectively), and rosemary oil (6.05 and 6.95 points, respectively), while parsley and mint oils are less acceptable (4.4, 2.95, 5.25, and 3.5 points, respectively). The

oil without addition and the oil with addition of rosemary have an acceptable taste (5.95 and 5.9 points, respectively), the oil with mint is less acceptable (4.4 points), at least the oils with addition of parsley (3.85 points) and garlic (3.4 points). Crunchiness and chewiness are acceptable for all oils by the panellists. The oils with the highest scores for overall acceptability are the oil without addition and the oil with rosemary (7.22 and 6.83 points, respectively). The oil with the addition of parsley has the lowest score for the hedonic tests (5.09 points).

A visual test is performed to determine the colour of the oils (Figure 3)

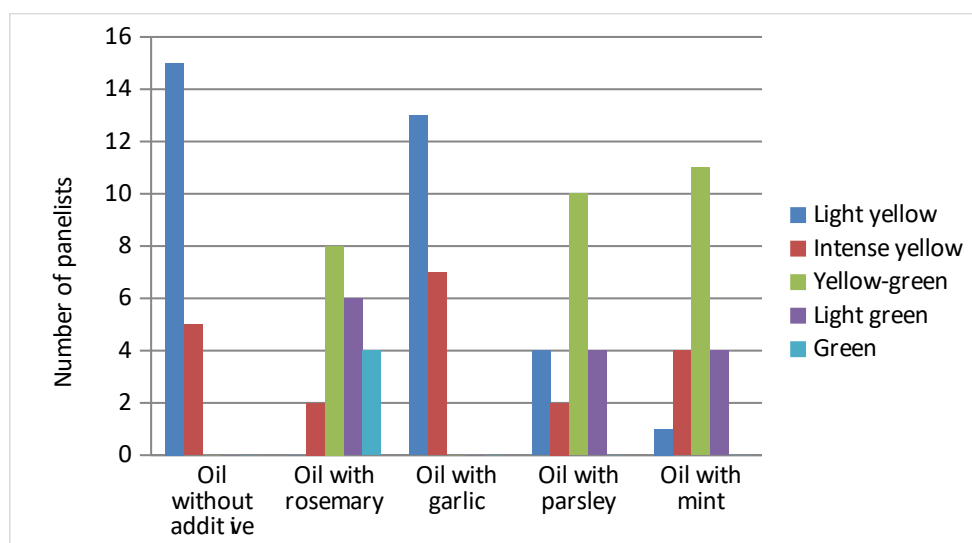


Figure 3. Sensory analysis for color of oils without and with additives.

According to the panellists, the oil without the additive has a light yellow to intense yellow colour (15 and 5 points, respectively), the oil with the addition of rosemary has a yellow-green to light green colour (8 and 6 points, respectively), the oil with the addition of garlic has a light yellow to intense yellow colour (13 and 7 points, respectively), and the oils

with the addition of parsley and mint have a mainly yellow-green colour (10 and 11 points, respectively) (Figure 3). The green pigments in the oil are chlorophyll compounds from herbs that have been added to the oil, chlorophyll also has a high antioxidant effect and reduces the oxidation process in cold pressed oils (Solymosi & Mysliwa-Kurdziel, 2017).

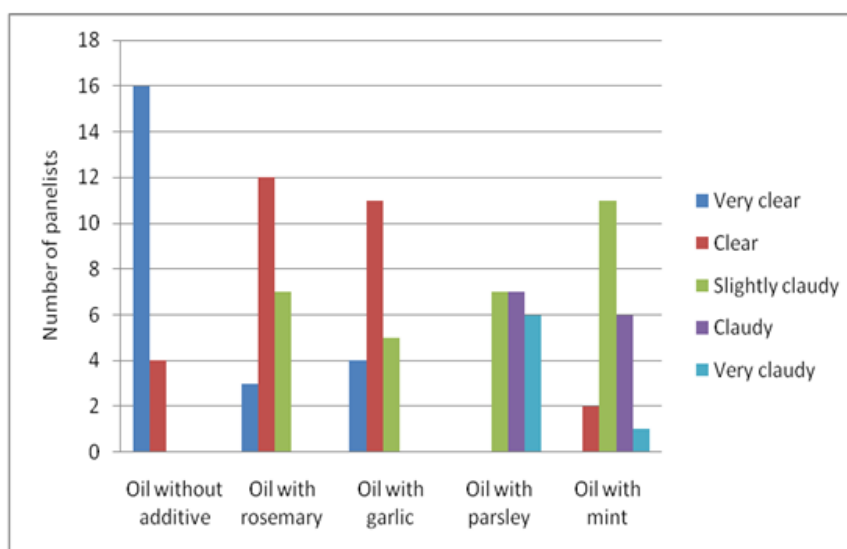


Figure 4. Sensory analysis for clarity of oils without and with additives.

The visual sensory test for the clarity of the oils shows that the clearest is the oil without additives, which is very clear (16.00 points), followed by the oils with the addition

of rosemary and garlic (12.00 and 11.00 points, respectively), which are clear. The oil with mint is slightly cloudy, and the oil with parsley is cloudy (Figure 4).

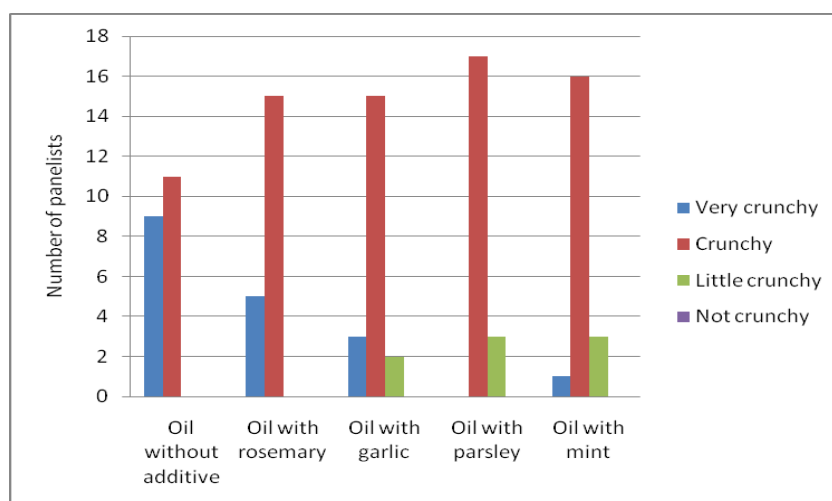


Figure 5. Sensory analysis for crispiness of oils without and with additives.

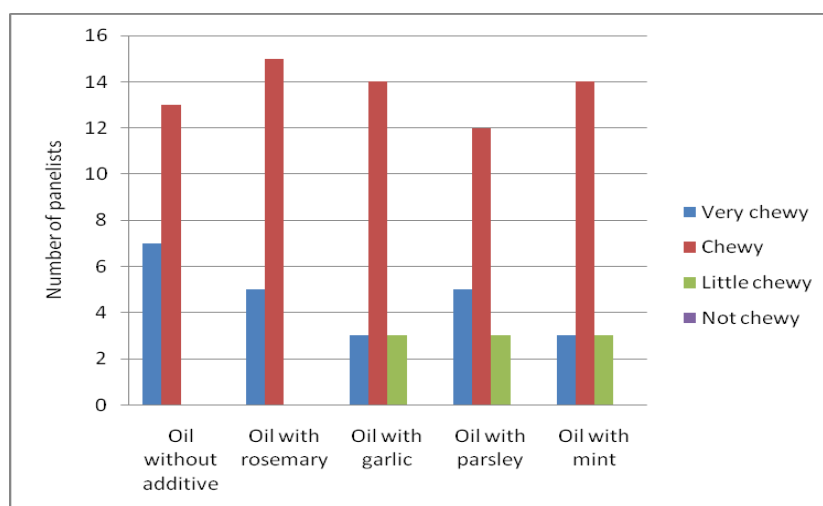


Figure 6. Sensory analysis for chewiness of oils without and with additives.

According to the sensory tests for the crunchiness and chewiness of the cucumbers with oil, there were no big variations between the obtained results, so all the cucumbers

with oil have good crunchiness and chewiness (Figures 5 and 6). This means that the additives in the oils do not affect the crunchiness and chewiness of the food.

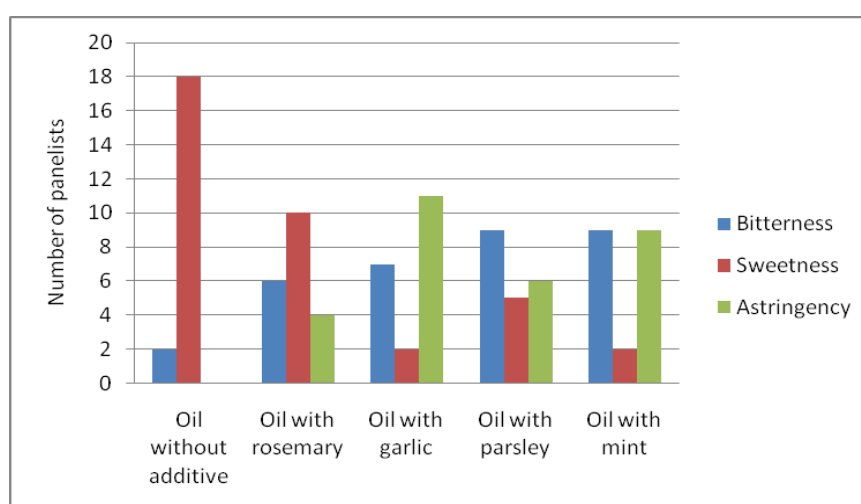


Figure 7. Sensory analysis for taste of oils without and with additives.

The taste of the oil was determined on a sample of a few drops of the analysed oil on a baking sheet as a neutral sample, and the results of these analyses are given in figure 7. The oil without addition and the oil with addition of rosemary have the highest sweetness (18.00 and 10.00 points, respectively). Oils with the addition of rosemary and garlic have a slightly bitter taste (6.00 and 7.00 points, respectively), and oils with the addition of parsley and mint have a more intense bitter taste (9.00 points, both). Oils with the addition of garlic and mint have the highest degree of astringency (11.00 and 9.00 points, respectively). According to the

oil taste preference test, the oil without the additive, the oil with the addition of rosemary, and the oil with the addition of garlic have a taste with greater acceptability than the oils with the addition of mint or parsley. The panellists mentioned that after consuming the samples of oils with the addition of parsley and oil with the addition of mint, they felt an unpleasant odour retronasally. Changes in the taste of oils with additives are due to the components from the herbs that have passed into the oil, and most often these are the phenolic components that have strong antioxidant activity.

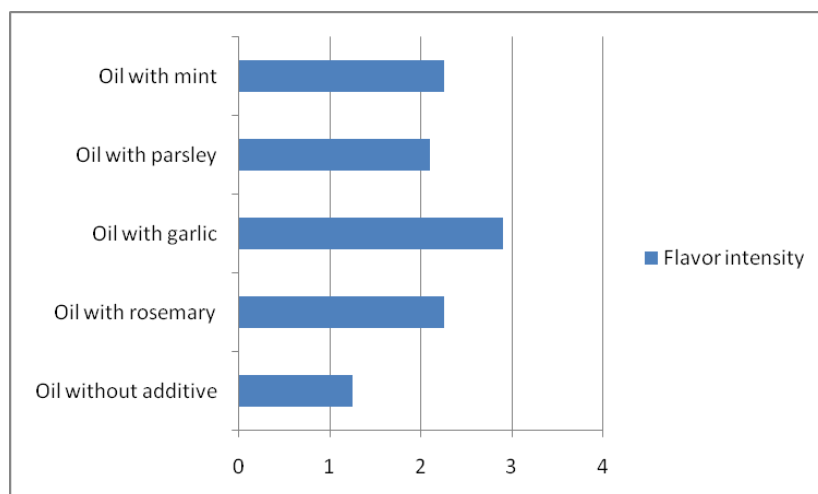


Figure 8. Sensory analysis for flavour intensity of oils without and with additives.

The flavour intensity test was made for the oils on a scale from 1 to 5, where 5 represents the most intense flavour and 1 represents the least intense flavour. Figure 8 shows that the taste of the oil with the addition of garlic is the most intense (2.90 points), while the taste of the oil without the addition is the least intense (1.25 points).

The majority of the respondents answered that the oil without addition and the oil

with addition of rosemary have the highest acceptability, while the oils with addition of garlic, parsley, and mint have an unacceptable smell. The panellists answered that the oil with the addition of garlic has the most intense smell, the oils with the addition of mint and parsley have an intense and unpleasant smell, the oil with the addition of rosemary also has an intense but pleasant smell and the oil without the addition has the least intensity smell.

CONCLUDING REMARKS

From the sensory analysis, it can be seen that the oils without addition and the oils with the addition of rosemary are more acceptable than the oils with the addition of garlic, parsley, and mint, in terms of their smell and taste. Oils with the addition of garlic have an intense smell of garlic, oils with the addition of parsley and mint have an unpleasant smell and taste.

Additives in oils do not affect the crunchiness and chewiness of food, and they are the same as oils without additives. The oils with the addition of parsley and mint are cloudier than the others. Oils with the addition of rosemary are the most acceptable for consumption because they have the most acceptable sensory properties.

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СЕНЗОРНИ КАРАКТЕРИСТИКИ НА ЛАДНО ЦЕДЕНО СОНЧОГЛЕДОВО МАСЛО СО ДОДАТОК НА АРОМАТИЧНИ БИЛКИ

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Резиме

Ладно цеденото сончогледово масло е нерафинирано масло со голема биолошка вредност и добри сензорни карактеристики. Поради својот хемиски состав ова масло лесно оксидира, при што се создаваат штетни продукти за здравјето на човекот. За спречување на оксидацијата се додаваат разни вештачки и природни стабилизатори. Како природни стабилизатори се користат етерични масла или ароматични билки кои ги променуваат и сензорните карактеристики на маслата.

Маслото кое е анализирано во овој труд е ладно цедено сончогледово во кое се додадени свежи ароматични билки: рузмарин, лук, магдонос и нане. Во секое шише масло се додава по една ароматична билка, а едно шише масло е без додаток (контрола). Маслата се чуваат 6 недели на темно, а потоа се врши процена на сензорните карактеристики на овие масла преку валидирани прашалници и сензорни тестови. Преку низа од хедонистички и визуелни тестови беа определени следниве карактеристики на маслата: мирис, боја, бистрина, вкус, крцкавост и џвакливост.

Ароматичните билки кои се додаваат не влијаат на крцкавоста и џвакливоста, но имаат влијание на мирисот, вкусот, бојата и бистрината. Маслото без додаток и маслото со додаток на рузмарин имаат најприфатлив мирис и вкус, а највисоки оценки за боја и бистрина освен овие две масла (без додаток и со додаток на рузмарин) има и маслото со додаток на лук.

Клучни зборови: ладно цедено сончогледово масло, рузмарин, лук, магдонос, нане.

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