

Sugar and Polyphenolic Diversity in Floral **29** Nectar of Cherry

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Abstract

Cherries (*Prunus avium* L. and *Prunus cerasus* L.) are economically important fruit species in the temperate region. Both are entomophilous fruit species, thus need pollinators to give high yields. Since cherry's flower is easy-to-reach, bees and other pollinators can smoothly collect nectar as a reward for doing transfer of pollen to receptive stigma. Nectar in cherry is usually attractive for insects,

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especially to honey bee (Apis mellifera) who is the most common pollinator. Nectar is predominantly an aqueous solution of sugars, proteins, and free amino acids among which sugars are the most dominant. Trace amounts of lipids, organic acids, iridoid glycosides, minerals, vitamins, alkaloids, plant hormones, non-protein amino, terpenoids, glucosinolates, and cardenolides can be found in nectar too. Cherry flower may secrete nectar for 2-4 days and, depending on the cultivar, produces up to 10 mg nectar with sugar concentration from 28% to 55%. Detailed chemical analysis of cherry nectar described in this chapter is focused on sugar and phenolic profile in sour cherry. The most abounded sugars in cherry nectar was fructose, glucose, and sucrose, while arabinose, rhamnose, maltose, isomaltose, trehalose, gentiobiose, turanose, panose, melezitose, maltotriose, isomaltotriose, as well as the sugar alcohols glycerol, erythritol, arabitol, galactitol, and mannitol are present as minor constituents. Regarding polyphenolics, rutin was the most abundant phenolic compound followed by naringenin and chrysin. Cherry cultivars showed different chemical composition of nectar which implies that its content is cultivar dependent.

Keywords

Prunus avium L. · *Prunus cerasus* L. · Flower · LC/MS · HPAEC · Polyphenolic profile · Sugars

1 Introduction

Cherry is the common name of several species of the genus *Prunus* originated from the common ancestor in area between the Black Sea and Caspian Sea in Asia Minor [1]. Among cherries, the sweet cherry, sour cherry, flowering ornamental cherry species, and a few other *Prunus* species used as rootstocks for cherries are considered important [2]. Cherries are members of the *Rosaceae* family, *Prunoideae* subfamily, and genus *Prunus* and are further placed within two subgenera *Cerasus* Pers. and *Padus* (Moench) Koehne [3]. The *Cerasus* Pers. subgenus and *Cerasus* Koehne section contain the diploid sweet cherry (2n = 2x = 16), and the tetraploid (2n = 4x = 32) sour cherry and ground cherry.

Cherries are one of the oldest fruit crops known to mankind. It is believed that Theophrastus has mentioned cherries roughly 300 years BC [4]. Another earlier writing suggests that Lucullus brought cherries back to Italy when he returned from the Pontu region (in present day Turkey). Archaeologists have discovered fossilized cherry pits in Stone Age caves and dwellings of western Switzerland, Bourget (France), and Parma (Italy) [5] that places cherry into the Neolithic Period (about 4000–5000 years ago).

Both sweet and sour cherry production, as the most economically important among cherries, has increased significantly during the past two decades in the traditional leading cherry-producing countries. The annual global sweet cherry production (average 2014–2016) is about 1.7 million tons and shows a slightly increasing tendency. The leading sweet cherry-producing country is Turkey, followed by the USA, Iran, Italy, Spain, Chile, and Ukraine. Sour cherry is often called the fruit species of Eastern Europe because the most important producing countries are located in this part of the world. Global production is about 1.3 million tons (average 2014–2016). In countries where there is a keen interest in sour cherry-based products, such as the eastern European countries, production is usually machine harvested and is increasing slightly. The world's leading sour cherry-producing country is Turkey, followed by the Russian Federation, Poland, Ukraine, Iran, the USA, Serbia, and Hungary [6].

Cherries are a deciduous fruit tree, having an attractive appearance during bloom time. The cherry fruit is a nutrient dense food with relatively low caloric content and significant amounts of important nutrients and bioactive food components including fiber, polyphenols, carotenoids, vitamin C, and potassium [7]. Sweet and sour (syn. tart) cherry ripen first among stone fruits, followed by apricot, peach, and plum. Because sweet cherry is first on the fresh market, it is in high demand in the late spring and early summer. The majority of sweet cherries are consumed fresh with the remaining 20–25% processed as brined, canned, frozen, dried, or juiced. In contrast, 97% of tart cherries are processed primarily for cooking and baking and the confectionary industries [7, 8].

Pollination is a crucial part of growing quality cherries because most of the cultivated varieties of sweet cherry are self-incompatible. To set fruits, they require pollen from suitable pollinating cultivars. Thus for the commercial production of sweet cherry, a good orchard design, with enough pollinizers have to be planted [9]. Besides, pollinating insects should be present for adequate transfer of compatible pollen to the stigma. Among sour cherry cultivars, there are more and more self-compatible ones; however, foreign pollination can improve quality even at these cultivars.

According to recent research, cherry flowers are very attractive to various insects [10]. They observed activity of 31 species of insects belonging to 5 orders and 13 families of class. Honey bees (*Apis mellifera*) have been assumed to be the main pollinators in cherry [11], due to their high demand for pollen and nectar and their hairy body, which collects and disperses the pollen [12–16]. However, honey bee is not active on temperature below 12 °C or in rainy weather conditions. In that case, pollination can be also successful since other insect species belonging to the *Bombus*, *Andrena*, and *Osmia* spp. could maintain their activities on lower temperatures and during rainy days [17]. Landscapes with wild bee habitats enhance pollination, fruit set, and yield of sweet cherry, presumably due to their higher pollination efficiency [18]. Therefore, it is very important to attract honey and wild bees to proper pollination of these crops, especially commercial crop production.

2 Role of Nectar

Plant species that depend on insect (or other animal) pollinators for their reproduction have put lots of effort in many floral traits such as floral display, flower architecture, color, scent, and nectar [19]. To attract pollinators, plants offer different types of rewards, where floral nectar represents the main plant reward for many pollinators [20]. Floral nectar composition, its quality, and chemical and physical features varies widely between species and type of nectary and most probably are related to different consumers and ecological factors (abiotic and biotic).

Flowers often have specialized structures that make the nectar accessible only for animals possessing appropriate morphological structures, and there are numerous examples of coevolution between nectarivores and the flowers they pollinate. The main function of nectar compounds is related to the attraction of pollinating insects. It is well-known that honey bee chemoreceptor can detect volatile substances, contained in the nectar of crop plants at distance of about 2 km [21]. In this way, pollinators are unintentionally mediating the transfer of pollen to receptive stigma, becoming a key attribute for increasing cross-pollination [22, 23]. Although floral nectar production represents a high cost for the plant, it ensures a higher possibility of fruit/seed set, higher reproductive success and gene transfer into next generation. The production of nectar (when starch granules in the parenchyma are broken down) often peaks when anthers start to shade pollen and when the stigma is the most receptive. Generally flowers secreting more nectar show more successful pollination events [24].

It is proved that secreted nectar volume correlates with flower size, which is probably due to the pleiotropic effects, where larger flowers have larger nectaries and more space for nectar [25, 26]. The amount of nectar reward is positively correlated with the number of pollinator visits, the number of flowers visited within a plant, and the duration of the visit within a flower [27]. Generally, energy received from nectar per insect (or other pollinator) must be enough to attract pollinators, but still need to encourage movement of the pollinator from flower/plant to another one. This means that nectar volume is correlating with the body size of the pollinator [28].

The attractiveness of nectar to pollinators depends on taste [29], but odor and color play an important role too [30, 31]. Characteristics such as volume, concentration, color, and taste may be related to the concentration and composition of dissolved sugar (especially glucose, fructose, and sucrose). But also other components, including minerals, phenolic compounds, and amino acids, may make a cardinal contribution to its attractiveness to honey bees [22, 32–34]. Bees prefer bright flowers, while visual and chemical associations are pushing it to navigate within the field [35].

Nectar concentration is highly influenced by geographical distribution, thus environmental factors, especially light, water, nutrients, CO₂ concentration, temperature, humidity, soil moisture, and wind [36]. Besides, nectar composition can vary between the two sexual phases of a given hermaphrodite flower [37], phenology phase, among flowers on different plants [38] and individuals, populations, cultivars, or subspecies of the same species [39]. Physiological factors such as flower age, health of plants, and damage to floral parts also affect the quality and composition of nectar.

3 Nectar Productions in Cherries

Cherry flowers are allogamous, actinomorphic, and are arranged in racemose clusters of 2–5 flowers. The sweet and sour cherry flowers are from 2.5 to 4.0 cm in

diameter, white, hermaphroditic, and are attractive for pollinators [40]. The cherry flower structure is usually characterized by stamina standing far from the pistil, thus insects can touch the stigma only during nectar collection, passing along the pistil [41]. In cherries like in most of the temperate fruit trees, sieve tubes more or less directly supply secretory parenchyma cells called the "nectariferous tissue" with prenectar prior to nectar secretion. The nectary is receptacular, covering the whole surface of the receptacle [42].

For the protogyn, sour cherry varieties is very important to know the periodicity of nectar production and the synchronization of the endogen rhythm with stigma receptivity and anther dehiscence. As it was stated in [43], dichogam flowers produce nectar periodically by 12th hours, the homogam ones by 6th hours, and the time of maximum production is synchronized by the stigma receptivity and anther dehiscence. In the hybrids of sweet and sour cherries, 3-h gaps can be observed in nectar production [43]. Additionally, the change of pollination strategy for the protogyn sour varieties: (i) stigma exerted – wind pollination, (ii) state of pollination chamber – beetle pollination, (iii) opening of anthers – pollination by bees and other insects was observed [44].

A sour cherry flower may secrete nectar for 2–4 days and, depending on the cultivar, produces 0.2–9.0 mg nectar. Generally, autochthonous landraces like "Cigánymeggy" or "Oblačinska sour cherry" type yield less but more concentrated nectar, with sugar values of 0.1–1.8 mg/flower/day, while cultivated varieties produce more but rather dilute nectar [45]. Among sour cherry cultivars, "Meteor korai" and "Debreceni bötermö" are one of the best nectar producers giving 10.27 μ l and 7.21 μ l of nectar respectively; with 13.96% and 16.6% of sugar, respectively [46].

The nectar of early blooming fruit trees such are cherries is important for honeybees in the brood rearing season, but rarely can provide unifloral honey, as well [45]. Sweet cherry blossom is more attractive for bees than sour cherry blossoms primarily because the nectar of sweet cherries is much richer in sugar (55%) than that of sour cherries (28%). But, sour cherry cultivars produce a significant amount of nectar at night [47], thus attracting night insects.

If a successful fertilization should be achieved even at self-incompatible cherry and sour cherry cultivars, all details of their pollination biology should be known, including the sugar and polyphenolic composition of nectar, as ones of the primary attractants [46, 48].

4 Nectar Compositions

The number of papers related to the examination of the chemical composition of the floral nectar is not large, although it is much more available for floral nectars than for extrafloral nectars. Mainly studies have focused on the qualitative aspect. The main components of the nectar, sugars and amino acids, were the most examined, while other solutes were not subjected to the research to that extent. This is rationale since the nectar is predominantly an aqueous solution of sugars. Also, sampling is not easy considering the duration of secretion (few hours to several days), and the amount of

nectar produced (less than 1 μL to few ml proportional to the nectary parenchyma volume).

Sugars, proteins, and free amino acids are the three major components of floral nectar among which sugars are the most dominant [49]. Nectar is highly variable at any taxonomic scale indicating that plant phylogeny can be a stronger determinant of nectar composition [50]. But, pollinator type can also shape the composition of nectar because different pollinators show preferences for solutions of different viscosity and/or sugar composition [51]. In general, insect pollinated flowers, like in cherry, produce relatively concentrated nectar.

Secondary metabolites and volatile compounds in flower nectar are appearing in low level. Compounds belonging to the secondary metabolism are either synthesized in the nectaries themselves or can also be derived directly from the phloem. They can have a range of effects on pollinator preference and performance, from fully negative to positive. More often, these compounds are usually regarded as "toxic compounds" and are involved in antimicrobial defensive functions, protection from nectar robbers, and pollinator attraction [29, 52]. On the other hand, secondary metabolites can significantly stimulate bees to feed, while indirectly, pollinators can have benefits from them by reducing gut pathogen loads [53, 54].

The phenolic compounds in nectar have several roles in attraction and/or repelling honey bees (phenolic compounds can give an astringent taste, thus inhibiting herbivores) in nutrition of pollinators, in oxidation prevention of other nectar substances, and in providing an aggregate value to honey commercialization by the certification of the botanical origin [29, 55]. In some cases, nectar constituents may also help defend the flower against invaders, which allows flower to promote out crossing and achieve its ultimate goal, and that is to set a fruit/seed [52]. Besides polyphenolic compounds, some amount of abscisic acid (ABA) can be found. The role of this plant hormone is the protection of plants in conditions of environmental stress, especially in reducing the penetration of UV-B ultraviolet radiation [56]. Also, jasmonic acid, its precursors and its derivatives, have been identified as a hormone that affects the secretion of floral nectar and defense responses [57, 58].

Volatile compounds, important cues that help insects locate flowers, mediate plant response to pathogen infection, plant-parasitoid signaling in response to herbivory, and plant-pollinator communication during flowering. Most of the floral fragrance compounds are terpenoids (most common monoterpenes), simple aromatics, amines, and hydrocarbons [59].

Amino acids are contributing to the taste of nectar and are important source of nutrients for animals, especially for those that are exclusively dependent on nectar for their nutrition, such as butterflies [60]. As it was stated in [34], phenylalanine is the most abounded one in nectar (which generally has a strong phagostimulatory effect on honeybees), followed by tyrosine, threonine, histidine, and aspartic acid. Also, it seems that some amino acids, like asparagines, are avoided by all guilds and bee families, while glycine-threonine, H-serine, serine, β -alanine, valine, leucine are bypassed by most bee families. Besides, some level of non-protein amino acids can be detected in nectar. Those compounds can be toxic and found in seeds which serve as deterrents to insect feeding. However, β -alanine, ornithine, homoserine, and γ -

aminobutyric acid (GABA) are also accumulated in nectar but are non-toxic [49]. According to the mineral analysis of nectar ion composition, concentration of K^+ is the highest, following by Na⁺. Some levels of Ca²⁺ and Mg²⁺ have been also detected. According to [61], potassium and sodium chloride deter honey bees. Proteins/enzymes, or so called "nectarin" in floral nectar includes invertase, transglucosidase, transfructosidase, phosphatase, tyrosinase, alliinase, nectarin, I-super-oxide dismutase, and others, playing important role in hydrolysis of sucrose, polymerization of glucose and fructose molecules, possibly defense and many more. Trace amounts of lipids, organic acids, iridoid glycosides (catalpol), vitamins, alkaloids (anabasine, gelsemine, nicotine, and caffeine), terpenoids (thymol), glucosinolates, and cardenolides can be found in nectar too.

Recently, a gene that encodes an apoplastic invertase of Arabidopsis has been discovered. This gene represents the first gene whose function is required for floral nectar secretion [62].

Chemical screening is usually done by standard chromatographic techniques hyphenated to spectral methods. New technologies and advanced techniques conquer difficulties in analyzing small fluid volumes, enabling more detail identification and quantification of nectar components.

Most of the individual studies on nectaries, nectar, and nectar consumers were included in a comprehensive book review [63]. Cherry nectar properties and chemistry were not examined to a great extent, and just a few papers discussing the composition of sour cherry floral nectar were published so far. As for the sweet cherry, no available data could be found. Therefore, presented results on cherry nectar included in this chapter rely on just a few published papers [46, 64] where nectar sugar profiles of sour cherry cultivars were reported. Most of the data on phenolics were drawn from the study carried out on "Oblačinska" sour cherry clones [64].

4.1 Nectar Carbohydrate Profile

Nectar carbohydrate profile is prevailed by three sugars, the disaccharide sucrose and its monosaccharide units, fructose, and glucose. Nectar components are believed to originate from phloem sap that is enzymatically processed and transformed within nectaries [65]. Since the phloem sap contains mostly sucrose, chemical reactions must occur to produce glucose and fructose in the nectar. The relative amounts of each are determined by hydrolyses of sucrose catalyzed by transglucosidases and transfructosidases localized in the nectaries which occur before or during nectar secretion [66].

The total sugar concentration in floral nectar can range from 5% (w/v) to 80% (w/v) [67] and may differ among individuals, populations, cultivars, or subspecies of the same species [38, 39, 68, 69]. Also, amounts and relative concentrations of the major constituents, glucose, fructose, and sucrose may vary among species from almost all sucrose to all hexose. According to [61], sucrose, maltose, glucose, fructose, trehalose, and melezitose are sweet for bees; while lactose, melibiose, raffinose, xylose,

and arabinose are tasteless; mannose and galactose are toxic to bees; where, gentiobiose and cellobiose and repellent to bees.

The nectar composition can vary greatly depending on plant species and environmental conditions [38], as well as on floral sexual phases [70], and flower position within inflorescences [71]. According to [72], between-plant variability of nectar sugar composition can be due to a casual selection of flowers of different ages, because in some cases, sucrose breakdown in nectar can be related to flower age. But this cannot be applied on the results of the recent investigation reported on sour cherry [64] were the flowers were in the same phenophase code [65], BBCH scale [73]. On the other hand, some authors considered nectar sugar composition as it is conservative taxonomic character [26, 74].

So far, sugar composition of sour cherry nectar was only explored by the two research groups. One research group was investigating sour cherry cultivars in Újfehértó, in the eastern Hungary in the period 1997–2000 [41, 46, 47]. The following sour cherry cultivars were examined: "Újfehértói fürtös," "Pándy 48," "Érdi jubileum," "Meteor USA," "Montmorency," "Debreceni bőtermő," "Nefris," "Sárándi S/Gy," "Korai pipacs," "Mej Djuk," "Kőrösi korai," "Érdi nagygyümölcsű," "Kántorjánosi 3," "Oblacsinszka," "Érdi bőtermő," "Cigány 404." Three sugar components (glucose, fructose, and sucrose) were determined by thin layer chromatography and quantitative evaluation was carried out by densitometry (CAMAG TLC Scanner II). The cultivars "Újfehértói fürtös," "Pándy 48," "Érdi jubileum," and "Érdi bőtermő" yielded nectar with high sucrose content in each season, even under varying climatic conditions, and are valued from an apicultural point of view [46].

Subsequently, in order to determine the floral insect attraction, the floral secretory product of the two cultivars, an autofertile cultivar (":Újfehértói fürtös") and an autosterile cultivar ("Pándy 48") were studied [41]. The nectar of both studied cultivars contained all three major sugar components: sucrose, glucose, and fructose. The nectar sugar composition of "Újfehértói fürtös" varied to a great extent according to the seasons. The phenomenon was explained by the great fluctuation in air temperatures, which influenced the sugar production of the cultivar to a great degree. "Pándy 48" yielded nectar with quite stable concentration and composition in the studied four seasons.

The other research group studied nectar of the most planted sour cherry cultivar in Serbian orchards, "Oblačinska" sour cherry, an autochthonous cultivar [64]. Investigation included 16 nectar samples of "Oblačinska" sour cherry clones. Both the content of sugars and sugar alcohols were studied using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC/PAD). Carbohydrates were separated on a CarboPac® PA10 pellicular anion-exchange column. Total of 14 sugars and 6 sugar alcohols were determined, showing great variability in carbohydrate profiles among studied genotypes. Nectars from several sour cherry clones stood out based on the notably different concentrations of the individual sugars and sugar alcohols. Such an unequaled nectar composition was related to the assumption that Oblačinska sour cherry is not a cultivar but mixture of many different genotypes.

As reported in [41], during 4-year study sucrose level in cultivars "Újfehértói fürtös "and "Pándy 48 "ranged from 18–59 mg/mL and from 27–59 mg/mL,

respectively. As expected, fructose, glucose, and sucrose were found to be the major constituents of all investigated "Oblačinska" sour cherry nectar samples [64]. Based on the total sugar content found in nectar, certain clones have been singled out as the most concentrated (up to 97.6 mg/mL), while the others had dilute nectars (23 mg/mL of sugars). Averagely fructose, glucose, and sucrose amounted 36.8%, 28.9%, and 30.9% of the total content of all carbohydrates, respectively, and this was in line with the other results [47]. The ranking based on fructose content takes into consideration human sensation of taste. Also, fructose in the concentration range from 15 mg/mL to 60 mg/mL in 14 sour cherry cultivars was reported [41], while in "Oblačinska" sour cherry nectars fructose content was up to 34 mg/mL [64].

On the basis of the sucrose/(glucose + fructose) quotient [20], the nectar of "Újfehértói fürtös" belonged to the sucrose-rich group each year, like the majority of sour cherry cultivars, whereas the secretory product of "Pándy 48" could be classified into the sucrose-dominant category in one of the seasons [46]. According to the proposed quotient, one "Oblačinska" sour cherry clone was hexose dominant [S/(G + F) < 0.1], four clones were hexose rich [S/(G + F) = 0.1–0.49], while other 11 were sucrose rich [S/(G + F) = 0.5–0.99] [64]. These results are in accordance with some previous results [46]. Proportions of sucrose over fructose and glucose have been linked with different classes of pollinators and found to be important in plant-mutualism interactions [75].

The sucrose-dominant nectar composition of 45 species belonging to tribe Sinningieae (*Gesneriaceae*) was also documented [76]. Several authors have suggested that sucrose-rich nectar is mostly found in flowers pollinated by insects with long mouth parts, whereas hexose-rich nectar has been found in flowers pollinated by short-tonged insects [20, 32, 77–79]. On contrary, an analysis in *Antirrhinum* and *Lycium* has revealed constant sugar composition despite a large variety of pollinators [78, 80].

According to [64], other carbohydrates, including the monosaccharides (arabinose and rhamnose), the disaccharides (maltose, isomaltose, trehalose, gentiobiose, turanose), the trisaccharides (panose, melezitose, maltotriose, isomaltotriose), as well as the sugar alcohols (glycerol, erythritol, arabitol, galactitol, and mannitol) were present as minor constituents in sour cherry nectars. The presence of mannitol, melezitose, panose, and maltotriose was not confirmed in some of the studied nectars. Minor sugars identified in nectars of some flowers, such as arabinose, galactose, mannose, gentiobiose, lactose, maltose, melibiose, trehalose, melezitose, raffinose, and stachyose can be toxic to potential pollinators [68, 81–84].

Among minor constituents, isomaltose, maltose, and sorbitol were the dominant in comparison to other components. For the sorbitol (a polyol with low molecular weight, highly soluble, and non-reducing compounds), results were fully expected, because this sugar alcohol is the main photosynthetic product and the primary translocated carbohydrate in *Rosaceae* [85]. Although has no influence to insects' preference, sorbitol is a frequent constituent of Mediterranean nectars [22]. Also, if it can be found in the fruit, it improves the sweet taste and texture of the mesocarp [86]. But also, its accumulation is considered as an adaptive response of plants to drought, salinity, or chilling stress [87]. Maltose is pretty rare or absent in nectars, and although it tastes sweet to honeybees, it is usually less attractive for them than sucrose [49, 88]. Earlier it was believed that maltose is synthesized in nectar itself [89], but its presence in nectar is due to the fact that maltose is a degradation product of starch (obtained from chloroplasts during starch degradation in night), while nectar secretion in flower starts with starch degradation [90]. Also, maltose is a major product of catabolism of starch in guard cells which can be found within the flower [91]. In regard to disaccharide isomaltose, it is more related to honeydew then to nectar, so that is the reason why honeydew honeys had significantly higher mean values of this sugar than the blossom honeys. In unifloral cherry honey, the concentration of the isomaltose was around 0.80% [92].

4.2 Phenolic Compounds in Cherry Nectars

Phenolic compounds are widespread natural constituents and their main function is to protect plants against various biotic and abiotic stresses. Their multiple roles in floral nectars and the relationship with pollinators were outlined in the literature [28]. Mainly, phenolics are associated with functions such as attracting pollinators or repelling nectar thieves, maintaining nectar in a microbe-free state, being important components of floral scents. Their role in cherry pollination could be the same, although yet not proved. Some phenolic compounds together with some other constituents may accumulate in floral nectar due to passive absorption by the nectar [30].

Although numerous flavonoids have been described in literature, their presence in floral nectar was not studied extensively. The same applies for the composition of cherry nectar topic. Often phenolic composition was reported only qualitatively [93], where floral nectar chemical compositions of 29 species native to Argentinian Patagonia and phenolic composition measured on qualitative scale were reported. A scarce number of papers show that nectar phenolic profile is characterized both by various phenolic aglycones and their derivatives. In rosemary nectar, kaemferol-3-sophoroside and quercetin-3-sophoroside were identified as the most abundant among 15 different flavonoids [94], while in Portuguese heather nectar (*Erica* sp.) flavonol aglycones quercetin, kaempferol, myricetin, and isorhamnetin were identified [95]. The occurrence of flavonols in higher plants was associated with lignification in cell walls and with UV absorption of flowers, as nectar guide [96]. Also, functional roles of flavonols as developmental regulators and/or signaling molecules in plants were discussed [97].

Although nectar composition of various sour cherry cultivars was examined [46], studies on floral nectar in terms of detailed phenolic characterization were not performed until the investigation of phenolic diversity in floral nectar of different "Oblačinska" sour cherry clones [64]. The phenolic complexity of sour cherry nectar was apparent and the qualitative phenolic profile was shown to be characterized mostly with flavonol glycosides. All identified glycosides were derivatives of kaempferol, quercetin, and isorhamnetin [64] (Table 1).

Table 1 Quantification of flavonol glycosides identified in floral nectars of "Oblačinska" sour cherry clones. The relative content of flavonol glycosides (in this table) was expressed as rutin equivalents (RE) per mL of nectar ($\mu g \text{ RE/mL}$)

Name of identified compound	Relative content (µg RE/mL)	
Kaempferol 3-O-(2"-O-hexosyl)hexoside-7-O-rhamnoside 1	0.002-0.197	
Quercetin 3,7-di-O-hexoside	0.002-0.029	
Quercetin 3-O-(2"-O-hexosyl)hexoside-7-O-rhamnoside 1	0.022-1.465	
Isorhamnetin 3-O-(2"-O-hexosyl)hexoside 1	3.285-4.066	
Quercetin 3-O-(2"-O-hexosyl)hexoside	0.001-0.245	
Kaempferol 3-O-(2"-O-hexosyl)hexoside-7-O-rhamnoside 2	0.002-0.170	
Isorhamnetin 3-O-(2"-O-hexosyl)hexoside 2	0.089-6.247	
Quercetin 3-O-(2"-O-hexosyl)hexoside-7-O-rhamnoside 2	0.001-0.038	
Quercetin 3-O-hexoside	0.002-0.225	
Kaempferol 3-O-(6"-O-rhamnosyl)hexoside	0.024-6.084	
Isorhamnetin 3-O-(6"-O-rhamnosyl)hexoside	0.067-6.104	
Quercetin 3-O-pentoside 1	0.001-0.022	
Kaempferol 3-O-hexoside	0.001-1.382	
Isorhamnetin 3-O-hexoside	0.001-1.639	
Kaempferol 3-O-(6"-O-acetyl)hexoside	0.001-0.177	
Isorhamnetin 3-O-(6"-O-acetyl)hexoside	0.004-2.331	
Quercetin 3-O-pentoside 2	0.001-0.503	

Further, sour cherry nectar phenolic profile was characterized with the presence of rutin, pinocembrin, and galangin, detected in all nectar samples, while gallic acid, hesperetin, and naringin were found in some samples. In earlier work, rutin was shown to act as a feeding stimulant for some insects [98]. Also, recently was proved that rutin has the highest antimicrobial (especially antibacterial) activity in honey [99] so there is a possibility that its function in nectar is the same. Pinobanksin, naringenin, and chrysin were detected in variable amounts in sour cherry nectar. Table 2 shows the content of phenolic compounds (average values). Naringenin plays an important role in plant development and it was reported to show bactericidal and/or bacteriostatic activity [100], and antimicrobial effects against yeasts [101], but shows low activity as feeding stimulant in insect-plant interaction [102]. Moreover, naringenin influenced bee foraging behavior as deterrent [103], but no relationship could be underlined between its level and yield efficiency (yield per trunk cross sectional area), of "Oblačisnka sour cherry" clones that were studied by. As a matter of fact, group of clones that stored high content of nagingenin is showing all kind of yield effectiveness, which stands the same for the group of clones with very low level of this flavanone [64, 104].

The positive influence of abscisic acid in nectar to the immune response of worker honeybees and larvae after being parasitized with *Varroa destructor* was described previously [105]. In plants abscisic acid regulates fundamental physiological functions and accumulates in response to different environmental stresses [106, 107] and can be found in phloem and xylem sap and in nectar [95, 108]. In honey, this

Table 2 Quantification	Compound name	Content (µg/mL)
of phenolic acids and flavonoids in nectars of	Gallic acid	0.005-0.010
Oblačinska" sour cherry lones (µg/mL)	Caffeic acid	0.003-0.015
	Rutin	0.096-6.472
	Naringin	0.026-0.092
	(-)-cis,trans-abscisic acid	0.005-0.331
	Naringenin	0.009-4.076
	Pinobanksin	0.005-0.128
	Hesperetin	0.002-0.006
	Chrysin	0.030-1.597
	Pinocembrin	0.010-0.764
	Galangin	0.014-0.719

phytohormone comes mainly from nectar [109]. The content of this phytohormone in "Oblačinska" sour cherry clones varied from 0.005 to 0.331 μ g/mL [64].

Regardless of the similar chemical structure, only certain flavonoids are capable to absorb light in the visible region of spectra, thus rendering color. Flavone glycosides and flavonol glycosides absorb near 350 nm, but their role in the floral pigmentation is not predominant, as they are weakly colored. Usually flavonoids accompany carotenoids which are dominant in yellow pigmentation. The early work on the flower *Rudbeckia hirta* indicated flavonols as pigments responsible for ultraviolet absorption in nectar guides for bees and other insects, and it was the first interpretation of ultraviolet absorption in a nectar guide in chemical terms [96]. Due to chemical modifications at the C-8 and C-6 position on A-ring, flavonols become yellow hydroxyflavonols [110]. Also, *O*-glycosylation at the 7,4′-positions or *O*-methylation at the 3′- or 3′,5′-positions may contribute to the yellow color [110]. Also, other authors reported flavonols importance for nectar guides, such in [111] who isolated the pigment from the petals of *Brassica rapa* and identified it on the basis of MS and NMR spectroscopic data as isorhamnetin 3,7-*O*-di-beta-D-glucopyranoside.

Although some species use colored nectar as a signal for pollinators [112, 113], we assume that this could not be the case with the nectar of the "Oblačinska" sour cherry. However, based on identified phenolic compounds, certain conclusions can be made. Namely, the presence of various flavonols in nectar of "Oblačinska" sour cherry could be the reason for its pale yellow color. Several derivatives with O-glycosylation at 7-position were identified (Table 1). Also, isorhamnetin which is 3'-methoxylated derivative of quercetin was typical flavonol in all nectars. Of all the quantified flavonols, the largest amount of izorhamnetin 3-O-(2"-O-hexosyl) hexoside 2 was found in nectars along with rutin and therefore this specific compound could be the one that contributes to the nectar color the most.

Finally, comparison of polyphenolic profiles of "Oblačinska" sour cherry fruits [114] and nectar of the same sour cherry clones revealed some disagreements. The fruit clones stored some of the phenolics not found in the corresponding nectar, such as gallic acid, naringin, and naringenin. The opposite was found for hesperetin,

where some quantity of this flavanone was found in nectar clones but not in the fruits. Finally, rutin was one of the most abundant compounds determined in the fruits of the same "Oblačinska" sour cherry clones and its content was highest in clone II/2, in both nectar and fruit [64, 114]. As it was stressed out, no matter that floral nectar is secreted through intrafloral nectaries as a phloem solution [115] and cherry fruit is formed form ovary within the flower, it seems that those two processes are quite different and fully independent. In fact, this result is expected, because deciduous fruit trees, to which sour cherry belongs, have accumulated necessary minerals and organic compounds by the end of the previous growing season and use these reserve nutrients to support initial growth and development in the following spring. Thus, during flowering time (when leaves are just started to expand and are without photosynthetic competence), reproductive development is under total reliance on reserves stored within the tree [116]. On the contrary, during sour cherry fruit development (which occurs ≈ 55 days from pollination to fully ripe fruit), leaves are fully developed and are having the main role as the main source of photo assimilates [117].

5 Conclusion

Except few studies, not much was done in the analysis of cherry nectar. Sweet cherry was not an object of any study so far, so the results of this chapter are based on sour cherry nectar. According to the chemical analysis of our model plant's ("Oblačinska sour cherry") floral nectar, it can be concluded that selected clones of this cultivar showed different sugar and polyphenolic profile, where constituents showed big variation. In sugars, fructose, glucose, and sucrose were the most abounded, while arabitol, rhamnose, arabinose, turanose, gentiobiose, panose, melezitose, and matotriose, together with galactitol and mannitol, were in minor quantities. Regarding polyphenols rutin, naringenin and chrysin were found in the highest levels. Only rutin, pinocembrin, and galangin, together with ()-cis, trans-ABA were detected in all nectar samples. Probably the cause of unequaled nectar composition (both for polyphenolics and sugars) in sour cherry is its hybrid origin (segmental allotetraploid between *Prunus cerasus* and *Prunus fruticosa*) and unstable inheritance.

In the future, nectar chemical composition, could be a breeding aim for creating a cultivar that will attract pollinators the most, and thus ensuring high yields, or have components that can protect plant from economically important bacteria/ viruses/fungi. Besides, this chapter would like to support and encourage scientists to analyze nectar for all other components and connect it with the pollenizer preference.

Also in the following years, nectar of sweet cherry, and other minor cherry species like European dwarf cherry (*Prunus fruticosa* Pall.), mahaleb cherry (*Prunus mahaleb* L.), and duke cherry (*Prunus \times gondouinii* Rehd.), and/or other agricultural plants, should be analyzed in details and connect it with honey quality.

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