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w Szczecinie

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Rozmnażanie oraz jakość owoców borówki wysokiej (*Vaccinium corymbosum* L.)

Propagation and fruit quality of highbush blueberry (*Vaccinium corymbosum* L.)

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STRESZCZENIE

Wysoka świadomość społeczeństwa bezpośrednio przekłada się na decyzje żywieniowe i wielkość spożycia produktów prozdrowotnych. Do takich produktów niewątpliwie należą owoce borówki wysokiej. Jagody tej rośliny zawierają dużą ilość fitochemikaliów, a zwłaszcza antocyjanów. Wraz z rosnącym popytem na spożycie, w parze musi iść równocześnie szybka produkcja tych owoców i sadzonek. Chcąc wyprodukować dużą ilość dobrej jakości roślin należy stosować sprawdzone metody i technologie, które pozwalają na przewidywalne, pewne wyniki. Jedną z takich metod są roślinne kultury *in vitro*, które pozwalają na masowe mnożenie genetycznie identycznych roślin na szeroką skalę. Odpowiednia agrotechnika wpływa również na wzrost niektórych pożądanych przez konsumentów substancji zawartych w owocach, jakimi są między innymi polifenole. Istotne jest by zastosowane rozwiązania były pochodzenia organicznego, a także miały korzystny, bądź przynajmniej obojętny wpływ na środowisko.

Duża zmienność genetyczna w obrębie gatunku *Vaccinium corymbosum*, powoduje, że nie istnieją uniwersalne dodatki, które w jednakowy sposób będą wpływały stymulująco na pożądane cechy roślin.

Celem naukowym pracy doktorskiej było opracowanie efektywnej metody mikrorozmnażania trzech odmian borówki wysokiej oraz zbadanie wpływu czynników fizykochemicznych oraz dodatków funkcyjnych pochodzenia naturalnego na wzrost i rozwój roślin w warunkach fitotronu.

Mając na uwadze powyższe cele, przeprowadzono cykl badań, które obejmowały opracowanie optymalnego podłoża dla inicjacji, namnażania oraz ukorzeniania roślin borówki wysokiej. Na etapie mikrorozmnażania przetestowano także oddziaływanie preparatu krzemowego (Hydroplus™ Actisil), dodatków organicznych i pH podłoża na rozwój roślin *Vaccinium corymbosum*. Kolejny etap badań stanowiła aklimatyzacja roślin do warunków *ex vitro*.

Ponadto, badaniom poddano także owoce borówki wysokiej. Przebadano wpływ oprysków opartych na chitozanie na jakość owoców borówki wysokiej oraz sprawdzono wpływ nawadniania, wielkości jagód i technologii przygotowania owoców na liofilizację i ich rehydratację.

Mając na uwadze istotny w produkcji roślinnej aspekt ekonomiczny, przeprowadzono kalkulację kosztów zastosowania różnych rodzajów światła w namnażaniu roślin borówki wysokiej w warunkach fitotronu. Niezbędne są dalsze doświadczenia, które pozwolą

na globalną ocenę możliwości wprowadzenia oszczędności do produkcji, uzyskując najlepszej jakości sadzonki roślin do hodowli przemysłowej.

Podsumowując, zrealizowany cykl badań umożliwił opracowanie protokołu rozmnażania w kulturach *in vitro* wybranych odmian borówki wysokiej oraz pozwolił zgłębić wiedzę nad jej biologią. Protokół ten może być stosowany do produkcji certyfikowanego materiału wegetatywnego lub różnych celów biotechnologicznych.

Słowa kluczowe: borówka wysoka, mikrorozmnażanie, kultury *in vitro*, zeatyna, chitozan, światło LED, polifenole, liofilizacja.

ABSTRACT

High public awareness directly translates into dietary decisions and the amount of consumption of health-promoting products. Such products undoubtedly include highbush blueberries. The berries of this plant contain a large amount of phytochemicals, especially anthocyanins. With the growing demand for consumption, the rapid production of these fruits and seedlings must go hand in hand at the same time. In order to produce a large quantity of good quality plants, it is necessary to use proven methods and technologies that allow for predictable, reliable results. One such method is plant *in vitro* cultures, which allow the mass multiplication of genetically identical plants on a large scale. Proper agrotechnology also influences the growth of certain consumer-desired substances contained in fruit, which include polyphenols. It is important that the solutions used are of organic origin and have a beneficial, or at least neutral, effect on the environment.

The high genetic variability within the species *Vaccinium corymbosum*, means that there are no universal additives that will have a uniformly stimulating effect on desirable plant traits.

The scientific objective of the dissertation was to develop an effective method for micropropagation of three cultivars of highbush blueberry and to study the effects of physicochemical factors and functional additives of natural origin on plant growth and development under phytotron conditions.

With these objectives in mind, a series of studies was conducted, which included the development of an optimal medium for the initiation, multiplication and rooting of highbush blueberry plants. At the micropropagation stage, the effects of the silicon preparation (Hydroplus™ Actisil), organic additives and pH of the substrate on the development of *Vaccinium corymbosum* plants were also tested. The next stage of the research was the acclimatization of plants to *ex vitro* conditions.

In addition, highbush blueberry fruits were also tested. The effect of chitosan-based sprays on the quality of highbush blueberry fruit was studied, and the effect of irrigation, berry size and fruit preparation technology on freeze-drying and rehydration was checked.

Taking into account the economic aspect, which is important in crop production, a cost calculation of the application of different types of light in the propagation of highbush blueberry plants under phytotron conditions was carried out. Further experiments are needed to globally assess the possibility of introducing savings into production, obtaining the best quality plant cuttings for industrial breeding.

In conclusion, the completed research cycle has made it possible to develop an *in vitro* culture propagation protocol for selected varieties of highbush blueberry and to explore its biology. This protocol can be used for the production of certified vegetative material or various biotechnological purposes.

Key words: highbush blueberry, micropropagation, *in vitro* culture, zeatin, chitosan, LED light, polyphenols, freeze-drying.

DOROBEK NAUKOWY STANOWIĄCY ROZPRAWĘ DOKTORSKĄ

Rozmnażanie oraz jakość owoców borówki wysokiej (*Vaccinium corymbosum* L.)

Lp.	Tytuł publikacji:	Pkt.*	IF**
P1	**Figiel-Kroczyńska, M.,** Krupa-Małkiewicz, M., Ochmian, I. (2022). Efficient micropropagation protocol of three cultivars of highbush blueberry (*Vaccinium corymbosum* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 50(4).	**40**	**1.249**
P2	**Figiel-Kroczyńska, M.**, Krupa-Małkiewicz, M., Ochmian, I. (2022). Effect of Actisil (Hydroplus™), organic supplements, and pH of the medium on the micropropagation of *Vaccinium corymbosum*. Acta Sci. Pol. Hortorum Cultus, 21(5).	**100**	**0.730**
P3	**Figiel-Kroczyńska, M.,** Ochmian, I., Krupa-Małkiewicz, M., Lachowicz, S. (2022). Influence of various types of light on growth and physicochemical composition of blueberry (*Vaccinium corymbosum* L.) leaves. Acta Sci. Pol. Hortorum Cultus, 21(2).	**100**	**0.730**
P4	**Figiel-Kroczyńska, M.,** Ochmian, I., Krupa-Małkiewicz, M. (2022). Effect of chitosan-based spraying on fruit quality of highbush blueberry cv. Sunrise. Progress on Chemistry and Application of Chitin and its Derivatives, Volume XXVII.	**70**	**-**
P5	Ochmian, I., **Figiel-Kroczyńska, M.**, Lachowicz, S. (2020). The quality of freeze-dried and rehydrated blueberries depending on their size and preparation for freeze-drying. Acta Universitatis Cinbinesis, Series E: Food Technology, 24(1).	**140**	**-**
Suma		**450**	**2.709**

*Liczba punktów według listy MNiSW zgodna z rokiem ukazania się pracy

**Sumaryczny Impact Factor (IF) według bazy Journal Citation Reports (JCR) z roku wydania

1. WSTĘP TEORETYCZNY

Borówka wysoka (*Vaccinium corymbosum* L.) należy do rodziny wrzosowatych (*Ericaceae*). Rodzaj *Vaccinium* obejmuje około 450 gatunków. Dzika borówka wysoka jest autochtoniczna dla klimatu subtropikalnego i umiarkowanego we wschodniej części północy Ameryki (Boches i in., 2006). Rośliny borówki wysokiej są tworzącymi korony krzewami i zazwyczaj osiągają 1,8-2,5 m wysokości (Song i Hancock, 2011). Hodowla borówki wysokiej prowadzona jest na szeroką skalę dopiero od 100 lat i można powiedzieć, że ma bardzo krótką historię, biorąc pod uwagę jej długi czas generowania jako uprawy krzewów (Nishiyama i in., 2021). Pomimo tego, owoce dzikich jadalnych gatunków *Vaccinium* są zbierane i spożywane przez ludzi od tysięcy lat w Europie, Azji i Ameryce Północnej (Song i Hancock 2011). Wielkość uprawy i spożycie owoców borówki wysokiej na świecie wykazuje tendencję wzrostową (Prodorutti i in., 2007; Brazelton, 2013; Podymniak, 2015; Brazelton i Young, 2017; Ochmian i in., 2020). Największymi producentami, według skali produkcji, są odpowiednio USA, Chile i Kanada. W Europie produkcja skoncentrowana jest w Hiszpanii, Polsce i Niemczech (Brazelton, 2013; Brazelton i Young, 2017). Polska zajmuje drugie miejsce w Europie i siódme na świecie pod względem produkcji owoców borówki amerykańskiej (Brazelton i Young, 2017).

Następstwem wysokiej i wciąż rosnącej świadomości społeczeństwa oraz dbałości o zdrowie jest wzrost zainteresowania owocami o walorach nie tylko smakowych, ale także prozdrowotnych. Wiedza na temat korzyści płynących ze spożycia jagód borówki dla zdrowia ludzkiego jest poparta rosnącą liczbą pozytywnych dowodów naukowych z badań obserwacyjnych i klinicznych na ludziach, a także badań mechanistycznych z wykorzystaniem modeli zwierzęcych i *in vitro* (Kalt i in., 2020). Ciemnoniebieskie słodkie owoce borówki ze względu na prozdrowotne właściwości biologiczne zaliczane są do grupy tzw. „superowoców" (z ang. „superfruit") (Yuan i in., 2020) i są spożywane w dużej ilości, głównie w formie deserowej (Prodorutti i in., 2007; Podymniak, 2015; Ochmian i in., 2000). „Superowoc" to termin marketingowy, a nie naukowy. Nazwa ta nie ma poparcia Komisji Kodeksu Żywnościowego FAO/WHO ani krajowych agencji regulacji żywności. Niemniej jednak, termin i koncepcja są luźno oparte na danych naukowych, a siłą marketingu jest potencjalna wartość zdrowotna dla konsumentów. Zgodnie z rozumieniem zdrowia jako odżywiania, o zdrowotności produktu spożywczego decyduje jego wartość odżywcza.

Owoce borówki wysokiej są powszechnie spożywane między innymi ze względu na wysoką zawartość związków bioaktywnych, a mianowicie polifenolowej klasy antocyjanów (Spinardi i in., 2019). Bogate w polifenole owoce borówki wysokiej wykazują liczne właściwości

zdrowotne, poprawiają wzrok, mają działanie antyoksydacyjne, przeciwzapalne, przeciwnowotworowe i przeciwbakteryjne (Schuchovski i Biasi, 2019). Jagody borówki wysokiej są jednym z najbogatszych źródeł antocyjanów wśród pospolitych spożywanych na świecie owoców, co sprawia, że są polecane zarówno jako żywność funkcjonalna, jak i suplementacja diety (Kalt i in., 2020; Yuan i in., 2020). Zawartością antocyjanów ustępują jedynie borówce niskiej (*Vaccinium angustifolium*). Spośród różnych fitochemikaliów, antocyjany mają prawdopodobnie największy wpływ na zdrowotną funkcjonalność borówki (Kalt i in., 2020). Oprócz owoców borówki wysokiej, cennym surowcem są również liście. Przygotowywane i spożywane jako napar są bogatym źródłem silnych przeciwutleniaczy fenolowych i mają bardzo dużą zawartość polifenoli i znaczną zdolność redukującą zgodnie z testem FRAP (Piljac-Žegarac i in., 2009). Napój z liści borówki wspomaga trawienie i jest szczególnie przydatny po spożyciu tłustych i ciężkostrawnych potraw. Ma on również właściwości obniżające poziom glukozy we krwi, dlatego polecany jest we wczesnych stadiach cukrzycy. Stosuje się go również w stanach zapalnych układu moczowego i pokarmowego. Napar jest aromatyczny i ma gorzki, taniczny smak wynikający z dużej ilości polifenoli (Wang i in., 2015).

W warunkach stresu rośliny wytwarzają i akumulują różne substancje. Aktywność antyoksydacyjna ekstraktów roślinnych wiążących się z obecnością fitochemikaliów, takich jak: antocyjany, kwasy fenolowe, flawonoidy i garbniki, nasila się w sytuacjach stresowych (Ochmian i in., 2015). Źródłem stresu dla rośliny może być także światło. Rośliny w różny sposób manifestują wpływ stresu. Może to być np. osłabiony wzrost, rozwój, zmiana koloru swoich tkanek związana z wytwarzaniem i akumulacją barwników roślinnych. Związki fenolowe mogą chronić organizmy przed stresem oksydacyjnym wywołanym przez wolne rodniki (Scalbert i in. 2005). Flawonoidy (w tym flawonole, antocyjany) i karotenoidy odgrywają główną rolę w ochronie roślin przed szkodliwym działaniem krótkofalowego światła nadfioletowego (UV 280–400 nm) (Pilarski i Kocurek, 2014, Vidović i in., 2015, Ochmian i in., 2019).

Wzrost krzewów borówki wysokiej jest ograniczony do stanowisk o niskim pH, wilgotnych, dobrze napowietrzonych, o stabilnym poziomie wód gruntowych i dużej zawartości materii organicznej (Finn i in., 1991; Ochmian i Kozos, 2014; Ochmian i in., 2020). Bardzo ważnym aspektem jest zapewnienie odpowiednich warunków wodnych (Treder, 2007; Ochmian i in., 2018). Pomimo światowego wzrostu upraw borówki wysokiej, dostępność odpowiednich gleb dla tego gatunku pozostaje problemem (Ochmian i in., 2018; Ochmian i in., 2020).

Ze względu na szybki wzrost powierzchni upraw borówki wysokiej, sadzonki powinny być produkowane szybko i w dużych ilościach. Tradycyjnie borówka jest rozmnażana przez sadzonki zielne, półzdrewniałe i zdrewniałe (Lyrene, 1981; Miller i in., 2004). Problemy wynikające z tego typu produkcji to bardzo niski procent ukorzenienia wielu genotypów, szczególnie w przypadku sadzonek pochodzących ze starych, zdrewniałych roślin (Miller i in., 2004), długi czas rozmnażania i komercjalizacji nowo pozyskanych roślin, a także bezpieczeństwo fitosanitarne. Jedną z metodą rozmnażania wegetatywnego roślin jest mikrorozmnażanie, znane również rozmnażaniem *in vitro* lub kulturą tkankową. Mikrorozmnażanie dotyczy rozmnażania klonalnego roślin wykorzystując tkanki, komórki lub całe organy. Jest jednym z najbardziej powszechnych terminów używanych do klonalnego rozmnażania roślin za pomocą różnych metod hodowli tkanek, komórek i organów (Loberant i Altman, 2010). Zastosowanie kultur *in vitro* eliminuje ograniczenia związane z tradycyjnym rozmnażaniem sadzonek, stanowiąc alternatywę dla szybszego wzrostu roślin przez cały rok, co zwiększa wydajność i opłacalność produkcji (Marino i in., 2014). Techniki rozmnażania kultur tkankowych można stosować jako system do efektywnej produkcji roślin wolnych od wirusów, które są genetycznie identyczne. W literaturze istnieje wiele doniesień na temat rozmnażania borówki *in vitro* (Sedlák i Paprštein, 2009; Ružić i in., 2012; Cüce i Sökmen, 2017; Kruczek i in., 2021). Jednakże wynika z nich duże zróżnicowanie pod względem doboru podłoża do namnażania, roślinnych regulatorów wzrostu, warunków wzrostu, typów eksplantatów, sposobu pobierania próbek i stanu fizjologicznego eksplantatów (Ružić i in., 2012; Krupa-Małkiewicz i in., 2017), co związane jest z dużą zmiennością w obrębie rodzaju *Vaccinium*. Niektóre gatunki i odmiany nadal wymagają dalszych badań w celu optymalizacji pożywek do namnażania (Vescan i in., 2012; Fan i in., 2017).

Na wzrost i rozwój eksplantatów w kulturach *in vitro* ma wpływ również odpowiednie pH podłoża oraz źródło i natężenie światła. Zbyt niskie pH (poniżej 4,5) lub zbyt wysokie pH (powyżej 7,0) hamuje wzrost i rozwój eksplantów *in vitro*. Jak sugeruje wielu autorów (Tetsumura i in., 2008; Ružić i in, 2012; Mohamed i in., 2018; Schuchovski i Biasi, 2019), najczęściej stosowanymi podłożami do namnażania borówki wysokiej *in vitro* są AN (Anderson's Rhododendron) i WPM (McCown Woody Plant Medium), podczas gdy Cappelletti i in. (2016) zasugerowali pożywkę według składu MS (Murashige i Skoog) jako bardzo skuteczne podłoże dla gatunków *Vaccinium*. Proces inicjacji wzrostu eksplantatów borówki wysokiej jest często etapem ograniczającym w kulturach *in vitro* (Reed i Abdelnour-Esquivel, 1991). Wzrost i proliferacja pędów pachowych w kulturach pędowych jest zwykle wspomagana przez włączenie roślinnych regulatorów wzrostu (zwykle cytokinin) do pożywki wzrostowej

(George i in., 2008). Cytokininy są regulatorami wzrostu niezbędnymi do zainicjowania merystemów pędowych w kulturach *in vitro*. Do tej grupy hormonów zaliczamy m.in. naturalną cytokininę – zeatynę (Ružić i Vujović, 2008). Stosowanie naturalnej zeatyny jako dodatku do pożywek hodowlanych jest jednak dość kosztowne, dlatego poszukuje się różnych rozwiązań, aby uzyskać podobny efekt przy niższych kosztach. Taką alternatywą może być woda kokosowa lub mleko kokosowe, które są bogatym źródłem fitohormonów roślinnych (głównie cytokinin) (Ma i in., 2008). Ponadto, u wielu gatunków roślin zaobserwowano korzystny wpływ krzemu na wzrost, rozwój, plonowanie i odporność na choroby. Krzem jest drugim najbardziej wszechobecnym pierwiastkiem występującym w glebach na całym świecie (Epstein, 1999; Richmond i Sussman, 2003; Currie i Perry, 2007; Sivanesan i Park, 2014).

Oprócz związków chemicznych na stymulację wzrostu i rozwoju roślin *in vitro* duży wpływ ma światło (Bourget, 2008; Massa i in., 2008; Morrow, 2008; Hogewoning i in., 2010). Kontrola natężenia światła i długości fali jest coraz częściej stosowanym narzędziem w produkcji roślinnej (Zoratti i in., 2014; Demotes-Mainard i in., 2016). Rośliny wykrywają i reagują na zmiany długości fal świetlnych z różnymi typami fotoreceptorów, w tym fitochromami, modulując w ten sposób wzrost i rozwój (Kami i in., 2010; Burgie i in., 2014; Galvão i Fankhauser, 2015; Demotes-Mainard i in., 2016). Przez lata najczęściej używanym źródłem światła do uprawy roślin *in vitro* były wysokociśnieniowe lampy sodowe, żarówki żarowe, świetlówki, oraz lampy metalohalogenkowe (Hahn i in., 2000; Kim i in., 2005; Gupta i Jatothu, 2013). Niedostosowana długość fali, czy źródło światła może niekorzystnie wpływać na wzrost i rozwój roślin oraz zwiększać koszty produkcji (Kim i in., 2005). Coraz częściej zastosowanie znajdują diody elektroluminescencyjne (LED) jako źródło światła dla roślin w ograniczonych przestrzennie uprawach (Hogewoning i in., 2010; Mengxi i in., 2010; Gupta i Jatothu 2013). Diody LED znalazły swoje zastosowanie ze względu na ich funkcjonalność. Są bezpieczniejsze w obsłudze niż obecne lampy, ponieważ nie mają szklanych osłon, wysokiej temperatury dotykowej i nie zawierają rtęci. Zalety półprzewodnikowego źródła światła (lampy LED) nad technologią fluorescencyjnych świetlówek obejmują możliwość zapewnienia wysokich natężeń światła z niskim promieniowaniem cieplnym, regulowane spektrum (długość fali), która pozwala na poprawę optymalizacji wydajność fotosyntezy, a co za tym idzie kondycji roślin. Lampy LED charakteryzują się dużym bezpieczeństwem i niskim zapotrzebowaniem na energię, co znacznie obniża koszty (Morrow, 2008).

Wielu autorów (Piwowarczyk i in., 2016; Krupa-Małkiewicz i Calomme, 2021) przedstawiło negatywny wpływ licznych czynników środowiskowych na zawartość pigmentu fotosyntetycznego. Według Krupy-Małkiewicz i Calomme (2021) zawartość barwnika

fotosyntetycznego w liściach jest ściśle skorelowana z ich barwą. Wartość parametru *L** (osiągająca odpowiednio od 0 do 100, odpowiednio dla czerni i bieli) jest zwykle wykorzystywana do śledzenia zmian intensywności koloru (Ochmian i in., 2012). Według Krupy-Małkiewicz i in. (2019) zmiana parametru *L** jest zwykle związana z fizjologicznymi atrybutami wizualnego wyglądu jasności.

Konsumenci z krajów wysoce rozwiniętych oczekują najwyższej jakości owoców. Jakość świeżych owoców oceniają na podstawie ich wyglądu na półce sklepowej (Kader, 2002). Chcąc spełnić wysokie oczekiwania odbiorców, producenci muszą dokładać wszelkich starań by trafiający do sprzedaży produkt był satysfakcjonujący. W celu uzyskania owoców najwyższej jakości stosowane są różne technologie i preparaty, wydłużające okres świeżości owoców, a także poprawiające wygląd ich skórki. W dobie szczególnej dbałości o zdrowe żywienie istotne jest, aby nie ograniczać korzystnych dla zdrowia człowieka naturalnych właściwości owoców, a stosować jedynie ekologiczne i naturalne rozwiązania. Optymalnym rozwiązaniem wydaje się być bezpieczny dla środowiska chitozan, pozyskiwany najczęściej z krewetek i innych skorupiaków (Petriccione i in., 2015). Chitozan jest drugim po celulozie najbardziej rozpowszechnionym biopolimerem na ziemi (Negm i in., 2020). Tworzy przezroczysty, nietoksyczny, biokompatybilny, biofunkcjonalny i biodegradowalny film (Dias i in., 2013; Ban i in., 2018; Krupa-Małkiewicz i Fornal, 2018). Chitozan odgrywa ważną rolę w mechanizmach odporności i obronie roślin przed patogenami (Krupa-Małkiewicz i Fornal, 2018; Shiekh i in., 2013; Malerba i Cerana, 2016). Opryskiwanie owoców i warzyw jadalną powłoką chitozanową wydłuża ich okres przydatności do spożycia, minimalizując tempo oddychania i zmniejszając utratę wody (Shiekh i in., 2013).

Jednym z istotnych problemów w uprawie borówki wysokiej jest jej ręczny zbiór. Zatrudnienie wystarczającej liczby pracowników sezonowych do zbioru owoców stanowi dużą trudność. Wynagrodzenie dla osób zbierających często jest rozliczane na podstawie kilogramów zebranych owoców, co sprawia, że w wyniku tego na krzewach pozostaje znaczna ilość małych, niekomercyjnych owoców. Owoce te nadają się głównie do przemysłu przetwórczego (Ochmian i Kozos, 2015). Każdego roku około 15% zbiorów owoców borówki wysokiej przeznaczane jest do przetworzenia (Brazelton, 2013). Owoce borówki wysokiej o najwyższej konsumenckiej jakości powinny mieć średnicę większą niż 10-12 mm. Jest to uzależnione od odmiany i wymagań odbiorcy (Wach, 2012). Jagody, które nie nadają się do sprzedaży ze względu na ich małe rozmiar można zamrozić lub wysuszyć. Tradycyjny sposób suszenia zmniejsza zawartość polifenoli i zdolność antyoksydacyjną w owocach borówki aż o 69% (Zielińska i Michalska, 2016). Długotrwała ekspozycja na wysokie temperatury powoduje

znaczne pogorszenie jakości produktu (Zielińska i in., 2013). Dobrym rozwiązaniem na zachowanie wysokiej jakości suszonych owoców jest liofilizacja (z ang. freeze-drying). Uzyskiwane w tym procesie owoce charakteryzują się stabilną strukturą i kolorem oraz niską zawartością wody, co gwarantuje długoterminowe przechowywanie oraz sprawia, że owoce pozostają atrakcyjne dla konsumenta. Ponadto proces ten, w przeciwieństwie do tradycyjnego suszenia, pozwala na zachowanie związków bioaktywnych w owocach (Ochwanowska i in., 2017; Sadowska i in., 2017). Owoce o mniejszej masie wymagają również krótszego czasu liofilizacji (Kozos i in., 2016). Proces ten odbywa się w niskich temperaturach (−40°C), redukując procesy enzymatyczne i chemiczne – co umożliwia zachowanie większości składników odżywczych. Jakość owoców uzyskanych w takim procesie suszenia jest bardzo wysoka (Rząca i Witrowa-Rajchert, 2007). Liofilizacja przedłuża żywotność produktu (Khin i in., 2007) i pozwala na zachowanie oryginalnego koloru owocu w znacznie lepszym stopniu niż inne metody suszenia (Piotrowski i in., 2008). Ważną cechą takiego produktu jest jego zdolność do szybkiego i całkowitego nawodnienia (Ciurzyńska i Lenart, 2009; Krokida i Marinos-Kouris, 2003). Suszone tą metodą owoce cieszą się coraz większą popularnością.

Mając na uwadze powyższe rozważania na temat wciąż rosnącego zainteresowania tematem borówki wysokiej, uznano za właściwe przeprowadzenie cyklu badań pozwalających na zgłębienie biologii tego gatunku.

2. CEL PRACY

Uwzględniając specyfikę badanego gatunku oraz dużą zmienność między odmianami warunkującą konieczność optymalizacji procesu mikrorozmnażania oraz uwzględniając aspekty ekonomiczne i ekologiczne wykorzystania owoców niekomercyjnych podjęto badania, które miały następujące zasadnicze cele:

- opracowanie protokołu mikrorozmnażania borówki wysokiej dla wybranych odmian;
- opracowanie optymalnego składu pożywki do rozmnażania wybranych odmian borówki wysokiej w kulturach *in vitro*;
- określenie wpływ Actisilu (Hydroplus™), dodatków organicznych i pH podłoża na mikrorozmnażanie *Vaccinium corymbosum*;
- ocena skuteczności ukorzeniania roślin/pędów w warunkach *in vitro* oraz ich aklimatyzację do warunków *ex vitro*;
- analiza ekonomicznych aspektów produkcji (źródło światła, możliwość zastąpienia drogich dodatków tańszymi odpowiednikami, wykorzystanie owoców niekomercyjnych) w celu uzyskania zadowalającego zwrotu z inwestycji;
- ocena możliwości użycia naturalnych i ekologicznych dodatków w produkcji roślin i owoców borówki wysokiej;
- ocena wpływu różnych rodzajów światła na wzrost i skład fizykochemiczny borówki wysokiej;
- porównanie skuteczności dwóch metod przechowywania owoców borówki wysokiej;
- określenie wpływu oprysku chitozanem na jakość owoców borówki wysokiej;
- porównanie wpływu zależności wielkości owoców oraz metody przygotowania borówki wysokiej na jakość liofilizowanych owoców.

3. MATERIAŁ I METODY BADAŃ

A. DOŚWIADCZENIA W KULTURACH *IN VITRO*

Mikrorozmnażanie borówki wysokiej przeprowadzono w Katedrze Genetyki, Hodowli i Biotechnologii Roślin Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie. Materiał roślinny stanowiły młode, zdrowe, intensywnie rosnące pędy trzech odmian borówki wysokiej (Elizabeth, Liberty i Meader), pochodzące z krzewów uprawianych na ekologicznej plantacji położonej około 60 km na wschód od Szczecina.

Dezynfekcja pędów i inicjacja kultur tkankowych

Po defoliacji, pędy płukano przez 1 godzinę pod bieżącą wodą z użyciem środka powierzchniowo czynnego (płyn do mycia naczyń Ludwik), a następnie sterylizowano powierzchniowo 70% (v/v) etanolem przez 30 sekund. Po tym czasie, eksplantaty moczono w 7,5% (v/v) podchlorynie sodu (NaOCl) przez 10 minut i 0,2% (v/v) siarczanie rtęci ($HgSO_4$) przez 10 minut. Eksplantaty przepłukano trzykrotnie sterylną wodą dejonizowaną pod komorą z przepływem laminarnym. Pędy o długości 1-2 cm inicjowano na pożywce WPM (Woody Plant Medium) (Lloyd i McCown, 1980) z dodatkiem 0,1 mg L^{-1} zeatyny. Kultury przechowywano przez 35 dni w fitotronie o stałej temperaturze 24±1 °C, długości światła 16/8 (dzień/noc), natężeniu światła białego 40 µM m^{-2} s^{-1} oraz wilgotności względnej 55-60%.
Pożywkę namnażającą wybrano na podstawie wstępnych doświadczeń (dane niepublikowane) (Ryc. 2a).

Namnażanie

Zainicjowane do wzrostu eksplantaty pędowe borówki wysokiej zawierające dwa pąki pachwinowe (pachwinowe), pasażowano na pożywki: MW (mieszanina równych części pożywek MS – Murashige i Skoog (1962) oraz WPM) z dodatkiem IBA (kwas indolilo-3-masłowy) w stężeniu 0,2 i 0,4 mg L^{-1} (odpowiednio MW 1 i MW 2); WPM z dodatkiem IBA w stężeniu 0,2 i 0,4 mg L^{-1} (odpowiednio WPM 1 i WPM 2) oraz WPM + 0,2 mg L^{-1} IBA + 0,4 mg L^{-1} GA_3 (kwas giberelinowy) - WPM 3; AN (Anderson's Rhododendron) (Anderson, 1984) z dodatkiem IBA w stężeniu 0,2 i 0,4 mg L^{-1} (odpowiednio AN 1 i AN 2) oraz AN + 0,4 mg L^{-1} IBA + 0,4 mg L^{-1} GA_3 (AN 3). Każda kombinacja pożywek zawierała również dodatek 0,1 mg L^{-1} zeatyny. Po 35 dniach w fitotronie eksplantaty usunięto ze słoików i przemyto dejonizowaną wodą sterylną w celu dokonania pomiarów. Zmierzono długość pędów, liczbę nowych pędów, świeżą i suchą masę oraz określono procent regeneracji pędów (%). Suchą

masę oznaczano po suszeniu w piecu z gorącym powietrzem w temperaturze 70 ºC przez 24 godziny.

Ukorzenianie i aklimatyzacja do warunków ex vivo

Namnożone pędy borówki wysokiej, zawierające po dwa pąki pachwinowe ukorzeniano na pożywce AN (AN A i AN B) i WPM (WPM A i WPM B), każda z dodatkiem 0,1 mg L^{-1} zeatyny i IBA lub IAA (kwas indolilo-3-octowy) w stężeniu 0,5 lub 1,0 mg L^{-1}. Po 35 dniach inkubacji w fitotronie określono procent ukorzenienia (%), długość pędów i korzeni, liczbę nowych pędów, korzeni i liści oraz świeżą i suchą masę.

Ukorzenione pędy adaptowano do warunków szklarniowych w maju 2020 roku. Rośliny z dobrze rozwiniętymi pędami i korzeniami przeniesiono do okrągłych czarnych doniczek o średnicy 7 cm, wypełnionych mieszanką sporządzoną z 4 l torfu, 1 l perlitu, 1 l wody i 1 ml ogólnoustrojowego fungicydu o wielomiejscowej aktywności (Previcur Energy 840 SL, Bayer). Zastosowany optymalny dla borówki wysokiej torf zawierał 66,9% materii organicznej, EC 0,24 mS/cm, masa objętościowa 0,35 kg dm^{-1}, pełna pojemność wodna 85,2% vv i pH w KCl 3,44. Doniczki umieszczono na stołach o wysokości 60 cm w szklarni w cyklach temperatury 20 °C/15 °C dzień/noc i natężeniu światła 110 μmol m^{-2} s^{-1} w okresie doświadczalnym. Temperatura wewnątrz szklarni była kontrolowana przez automatycznie otwierane otwory wentylacyjne. Po sześciu tygodniach rośliny przeniesiono do większych doniczek (jedna roślina na doniczkę) o średnicy 10 cm i pojemności 400 ml i wypełniono tym samym podłożem co poprzednio. Policzono liczbę roślin zaaklimatyzowanych oraz obliczono stopień aklimatyzacji (%). Rośliny na każdym etapie aklimatyzacji podlewano i opryskiwano dwa razy w tygodniu fungicydem (Switch 62,5 WG; Syngenta).

Po dwóch miesiącach rośliny zostały przeniesione (lipiec 2020) na plantację borówki, gdzie w aktualnie panujących warunkach pogodowych hodowano je na otwartej przestrzeni.

B. DOŚWIADCZENIA W FITOTRONIE

Doświadczenie przeprowadzono w gospodarstwie towarowym produkującym sadzonki borówki wysokiej w gminie Dobrzany (woj. zachodniopomorskie).

Charakterystyka źródeł światła

1) Lampa LED T8 4 stopy (1212 mm) biała zimna, 18 W, Toshiba (zwana dalej białą diodą LED), długość fali 410–780 nm, światło przy 52 μmol s^{-1} m^{-2} fotosyntetycznej gęstości strumienia fotonów (PPFD);

2) Lampa LED T8 Flora dla roślin (1200 mm), 18 W, kolor różowy, Greenie (zwany dalej fioletowym LED), długość fali 410–510 i 580–710 nm, światło przy 60 μmol s^{-1} m^{-2} PPFD;
3) Lampa rtęciowa z luminoforem L 36W/84 T8 Zimna biel, Lightech (zwana dalej białą fluorescencyjną świetlówką), długość fali 400–500, 530–550, i 580–630 nm, światło 36 μmol s^{-1} m^{-2} PPFD;
4) Lampa rtęciowa z luminoforem TLD 36W/16, CZERWONA, Philips (zwana dalej czerwoną fluorescencyjną świetlówką), długość fali 600–700 nm, światło 13 μmol s^{-1} m^{-1} PPFD.

C. LIOFILIZOWANIE OWOCÓW

Badania przeprowadzono w Katedrze Ogrodnictwa Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie. Owoce pochodziły z dwóch gospodarstw (plantacja A i B) specjalizujących się w uprawie borówki wysokiej, położonych około 25 km na wschód od Szczecina, w Puszczy Goleniowskiej. Do określenia ilości i jakości pozostawionych owoców na krzewach, co roku zbierano wszystkie owoce od 50 losowo wybranych krzewów. Owoce ze względu na wielkość podzielono na trzy grupy: <6 mm, 6-8 mm, >8 mm.
Plantacja A - nawadniana kropelkowo, wielkość pola 4,9 ha. Gleba mineralna - piasek gliniasty, zawartość organicznej materii 2,21%, węgiel organiczny 0,56%, EC 0,29 mS/m, pH 4,0-4,2.
Plantacja B - bez nawadniania, powierzchnia pola 4,3 ha. Gleba mineralna - glina piaszczysta, zawartość materii organicznej 2,94%, węgiel organiczny 0,67%, EC 0,21 mS/m, pH 4.4-4.6.

Proces liofilizacji i rehydratacji

Owoce przed suszeniem zostały wstępnie zamrożone w temperaturze -35°C przez około 1-2 godziny. Następnie jagody poddano liofilizacji. Użyto do tego celu liofilizatora Christ model Alpha 1-2. Owoce rozdrobniono w zamkniętym młynku laboratoryjnym. W celu skrócenia czasu suszenia, owoce były wcześniej:

a. nakłuwane igłą o średnicy 1 mm na głębokość ok 3-4 mm - wykonano 6 nakłuć na obwodzie owoców małych (<6 mm), większych (6>) 8 nakłuć,
b. przecięte na pół wzdłuż osi średnicy,
c. cały owoc - kontrola.

Tak przygotowane owoce zostały poddane proces suszenia przez 14, 16, 18, 20, 22 i 24 godziny. Po pewnym czasie zawartość wody w owocu była testowany zgodnie z Polską Normą (PN-90/A- 75101/03).
Suszone owoce poddano rehydratacji w wodzie destylowanej w temperaturze 20°C przez 6 godzin. Po określonym czasie (0,25-6 h) uwodnioną próbkę oddzielono od wody, osuszono

bibułą i zważono z dokładnością 0,01g. Względny przyrost masy uwodnionego owocu wyznaczono jako stosunek masy aktualnej próbki do masy początkowej. Oznaczenie przeprowadzono w trzech powtórzeniach.

D. ANALIZY WŁAŚCIWOŚCI FIZYCZNYCH I CHEMICZNYCH OWOCÓW, LIŚCI ORAZ PĘDÓW BORÓWKI WYSOKIEJ

Podstawowe parametry jakości owoców

Masę owoców borówki określono za pomocą wagi elektronicznej WPX 4500 z dokładnością ± 0,01 g (RADWAG, Polska).

Zawartość rozpuszczalnych substancji stałych oznaczono elektronicznym refraktometrem (PAL-1, Atago, Japonia).

Kwasowość oznaczano przez miareczkowanie ekstraktu wodnego 0,1 N wodorotlenkiem sodu (NaOH) do pH 8,1 (Elmetron CX-732, Polska), zgodnie z normą PN-90/A-75101/04. Zawartość kwasu *L*-askorbinowego i azotanów mierzono rekwantometrem RQflex 10 (Merck, Niemcy).

Jędrność i odporność na przebicie skórki borówki mierzono aparatem FirmTech2 (BioWorks, USA) na 100 losowo wybranych jagodach z trzech powtórzeń. Nakłucia wykonano stemplem o średnicy 3 mm. Wartość wyrażono jako gram-siła powodująca wygięcie powierzchni borówki o 1 mm.

Polifenole

Związki fenolowe oznaczono przy użyciu systemu UPLC-PDA-MS/MS Waters ACQUITY (Waters, Milford, MA, USA) składającego się z binarnego menedżera pompy, menedżera próbek, menedżera kolumny, detektora z matrycą fotodiodową (PDA) i tandemowego kwadrupolowego spektrometru masowego (TQD) z jonizacją przez elektrorozpylanie (ESI).

Aktywność antyoksydacyjna

ABTS (2,2'-azobis(3-etulobenzotiazolino-6-sulfonian) oznaczono zgodnie z metodą Arnao i in. (2001). Aktywność antyoksydacyjną oznaczano zgodnie z metodą Brand-Williams i in. (1995), z użyciem rodnika DPPH (1,1-difenylo-2-pikrylohydrazyl), pojemność antyoksydacyjną wyrażono w μmol Trolox g^{-1} dw. Pomiary $ABTS^{\cdot+}$ i FRAP wykonano za pomocą UV-Spektrofotometr 2401 PC (Shimadzu, Japonia). Zawartość kwasu *L*-askorbinowego mierzono rekwantometrem RQflex 10 (Merck, Niemcy).

Prolina, dialdehyd malonowy (MDA) i aktywności katalazy (CAT)

Stężenie proliny oznaczono według metody Batesa i in. (1973) przy użyciu spektrofotometru przy 520 nm w świeżych liściach borówki i obliczono jako μmol g^{-1} świeżej masy. Zawartość dialdehydu malonowego (MDA, produktu peroksydacji lipidów) w tkance roślinnej oznaczono metodą opisaną przez Sudhakara i in. (2001). Stężenie MDA obliczono z absorbancji przy 600, 532 i 450 nm, a zawartość MDA oszacowano za pomocą następujących równań:

MDA (nmol·g^{-1} fw) = [6,45 × (A532 – A600) – 0,56 A450] × V/fw.

gdzie: V – objętość próbki, A – absorbancja, fw – masa świeża.

Aktywność katalazy (CAT) oznaczono według procedury Lücka (1963). Spadek absorbancji spowodowany rozkładem H_2O_2 był monitorowany w sposób ciągły przy 240 nm przez 90 sekund. Jedna jednostka enzymu to ilość niezbędna do rozkładu 1 μM H_2O_2 g^{-1} śr. min^{-2}.

Składniki mineralne

Zawartość pierwiastków N, P, K i Ca w liściach oznaczono po mineralizacji na mokro w H_2SO_4 (96%, Chempur, Polska) i $HClO_4$ (70%, Chempur, Polska), natomiast Cu, Zn, Mn i Fe oznaczono po mineralizacji w HNO_3 (65%) i $HClO_4$ (70%) w stosunku 3:1 (IUNG 1972). Stężenie azotu całkowitego oznaczono metodą destylacji Kjeldahla (Lityński i in., 1976). Zawartość K zmierzono za pomocą atomowej spektrometrii emisyjnej natomiast zawartość Mg, Ca, Cu, Zn, Mn i Fe mierzono metodą atomowej spektrometrii absorpcyjnej z atomizacją w płomieniu. Zawartość P oszacowano metodą kolorymetryczną.

Testy mikrobiologiczne

Analizę stopnia porażenia owoców przez grzyby (drożdże i pleśnie) oparto na normie europejskiej ISO 70. Po wyhodowaniu inokulacji grzybów tworzących zarodniki próbki poddano ocenie taksonomicznej tradycyjną metodą obserwacji makroskopowej kolonii oraz obserwacji mikroskopowej zarodników i włókien.

Mikotoksyny oznaczano za pomocą wysokosprawnej chromatografii cieczowej – tandemowej spektrometrii masowej (HPLC-MS/MS). Próbkę oczyszczono na kolumnach powinowactwa immunologicznego AflaTest firmy Vicam (USA) dla aflatoksyn i OchraPrep firmy R-Biorharm AG (Niemcy) dla ochratoksyny A, zgodnie z procedurami określonymi przez producentów. Patulinę, deoksyniwalenol, T2, toksynę HT2 i zearalenon analizowano metodą HPLC-MS/MS. Próbki oczyszczono na kolumnach Bond Elut® Mycottoxin firmy Agilent (USA). Każda próbka została przebadana w trzech powtórzeniach.

Barwa

Pigment (kolor) liści (ze środkowej części pędu) mierzono w trybie transmisji metodą fotokolorymetryczną w systemie CIE *L*a*b** przy użyciu spektrofotometru CM-700d (Konica Minolta, Osaka, Japonia) (Hunterlab, 2012). Średnica otworu pomiarowego wynosiła 3 mm, typ obserwatora 10º, a oświetlacz D65. Wartość *a** mieściła się w zakresie od zielonego (-*a**) do czerwonego (+*a**). Parametr *b** opisał kolor w zakresie od żółtego (+*b**) do niebieskiego (*b**). Wartość parametru *L** oznacza monochromatyczność w zakresie od 0 (czarny) do 100 (biały).

E. ANALIZA STATYSTYCZNA

Wszystkie analizy statystyczne przeprowadzono przy użyciu programu Statistica 12.5 i 13.0 (StatSoft Polska, Kraków, Polska). Istotność statystyczną różnic między średnimi określono testując jednorodność wariancji i normalności rozkładu, a następnie ANOVA z testem post hoc Tukeya. Istotność ustalono na $p<0,05$.

4. OMÓWIENIE WYNIKÓW BADAŃ

Rozprawa doktorska składa się z cyklu publikacji spójnych tematycznie, opublikowanych w czasopismach naukowych listy MNiSW. Opracowanie zawiera opis badań dotyczących dobrania optymalnych warunków rozmnażania w kulturach *in vitro* trzech odmian borówki wysokiej uprawianych w Polsce. W prowadzonych doświadczeniach zbadano dodatkowo wpływ krzemu, wody kokosowej oraz mleczka kokosowego na łagodzenie skutków stresu wywołanego zmiennym pH w kulturach *in vitro* borówki wysokiej. Ocenie poddano także wpływ różnych długości światła na wzrost roślin i skład fizykochemiczny liści borówki wysokiej. Przebadano również jakość owoców borówki wysokiej poddanych procesowi liofilizacji i rehydratacji w zależności od ich wielkości i sposobu przygotowania.

P1. Figiel-Kroczyńska, M., Krupa-Małkiewicz, M., Ochmian, I. (2022.). Efficient micropropagation protocol of three cultivars of highbush blueberry (*Vaccinium corymbosum* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 50 (4).

Skutkiem wysokiego spożycia owoców borówki wysokiej jest stale powiększający się areał upraw tego gatunku. Następstwem tego jest rosnące zapotrzebowanie na szybką produkcję sadzonek tej rośliny. Najbardziej efektywnym systemem rozmnażania nowych odmian borówki wysokiej jest mikrorozmnażanie. Ze względu na dużą zmienność w obrębie rodzaju *Vaccinium* niektóre gatunki i odmiany nadal wymagają dalszych badań w celu optymalizacji warunków namnażania i składu pożywek . Celem przeprowadzonych badań było opracowanie protokołu mikrorozmnażania trzech odmian borówki wysokiej. Na podstawie danych literaturowych, do porównania wybrano pożywki MS, WPM oraz MW (mieszanina równych części pożywek MS i WPM).

Wyniki badań wykazały istotne różnice w wartości analizowanych parametrów roślin borówki wysokiej w zależności od zastosowanego do namnażania pędów podłoża oraz jego modyfikacji (Ryc. 1). Najwyższą wartość współczynnika regeneracji pędów między 80 a 100% uzyskano dla różnych kombinacji pożywek WPM i MW. Dowiedziono, że pożywka WPM 3 z dodatkiem GA_3 była najlepsza do namnażania odmiany Liberty (Ryc. 1a, b). W przypadku odmian Elizabeth i Meader najlepsze wyniki namnażania osiągnięto stosując odpowiednio pożywki MW 2 i MW 1. Istotnie niższy współczynnik namnażania pędów borówki wysokiej obserwowano na modyfikowanych pożywkach AN. Zaobserwowano, że pędy borówki na tym podłożu miały czerwone zabarwienie liści i łodyg, a ponadto liście te były małe, ale występowały liczniej. Natomiast pędy borówki odmian Elizabeth i Meader namnażane

na podłożach MW i WPM były jasnozielone i miały duże zielone liście. Ponadto, rośliny odmiany Liberty regenerowały się o ponad 20% lepiej w porównaniu do odmiany Elizabeth i Meader (Tab. 1)

Pędy 'Liberty' były również wyższe (odpowiednio o 21 i 37%) i było ich o 53% więcej w porównaniu do pędów ‘Elizabeth’ i ‘Meader’. Nie zaobserwowano istotnych różnic między liczbą pędów u 'Elizabeth' i 'Meader'. Lepsze rozkrzewienie roślin 'Liberty' przełożyło się na ich zwiększoną masę. Rośliny miały odpowiednio o 19,60% i 27,33% wyższą suchą masę w porównaniu do 'Elizabeth' i 'Meader'.

Najważniejszymi cechami świadczącymi o sukcesie ukorzeniania roślin w kulturach *in vitro* są liczba i długość korzeni oraz ich jakość. Maksymalną średnią liczbę i długości korzeni (odpowiednio 2,14; 0,77 cm) otrzymano u roślin ukorzenianych na pożywce WPM z dodatkiem 0,1 mg L^{-1} zeatyny i 1,0 mg L^{-1} IAA (WPM B). Skuteczność procesu ryzogenezy była skorelowana z wartością średniej świeżej masy roślin. Najlepszą ryzogenezę miały rośliny o najwyższej średniej świeżej masie (60,33 mg). Ponadto eksplantaty 'Liberty' wykształciły ponad 2,5 razy dłuższe korzenie w porównaniu z innymi testowanymi odmianami (Ryc. 1c). Natomiast, eksplantaty 'Elizabeth' wytworzyły odpowiednio o 259 i 143% mniej korzeni w porównaniu do odmiany Meader i Liberty. Jednak ich długość nie różniła się istotnie statystycznie od długości korzeni odmiany Meader. Zaobserwowano, że rośliny odmiany Meader i Liberty nie wytworzyły korzeni przy obu modyfikacjach pożywki AN. Dużą zdolność do ryzogenezy zaobserwowano tylko w przypadku eksplantatów ‘Elizabeth’. Najwyższy wskaźnik ukorzenienia *in vitro* (85%) uzyskano dla 'Liberty' na pożywce WPM A (WPM + 0,5 mg L^{-1} IAA + 0,1 mg L^{-1} zeatyny) oraz pożywce WPM B (WPM + 1,0 mg L^{-1} IAA + 0,1 mg L^{-1} zeatyny), najniższy (33%) - dla obu 'Elizabeth' i 'Meader' ukorzenianych na pożywce WPM A. Przy zastosowaniu pożywki WPM A i WPM B struktura korzeni była dobrze rozwinięta – korzenie były dłuższe, grubsze i lepiej rozgałęzione. Dlatego też, do aklimatyzacji do warunków szklarniowych wybrano tylko sadzonki o dobrze rozwiniętym systemie korzeniowym.

Procent roślin zaaklimatyzowanych do warunków szklarniowych (Ryc. 1d, e) wynosił od 70% (dla sadzonek odmiany Liberty namnażanych na pożywce WPM B) do 30% (dla sadzonek odmiany Meader namnażanych na pożywce WPM A). Rośliny na pożywce WPM B z dodatkiem IAA w stężeniu 1,0 mg L^{-1} wykazywały wyższą o 10% przeżywalność w porównaniu do zastosowania dwukrotnie niższego stężenia tego hormonu (pożywka WPM A). Ponadto, zaobserwowano dodatnią korelację między szybkością ukorzeniania w warunkach *in vitro* oraz szybkością aklimatyzacji do warunków *ex vitro*. Wśród testowanych

odmian borówki wysokiej, rośliny 'Liberty' wykazały najwyższy procent zaadaptowanych roślin (70%) z najlepiej rozwiniętym systemem korzeniowym.

Na podstawie uzyskanych wyników badań wynika, że zdolność ukorzeniania pędów była bardzo zróżnicowana pomiędzy badanymi odmianami borówki. Pożywka WPM z dodatkiem IAA i 0,1 mg L^{-1} zeatyny była najskuteczniejsza w indukcji korzeni. Najwyższy procent ukorzenionych sadzonek zanotowano dla odmiany Liberty (85%) i był on istotnie wyższy w porównaniu do odmiany Meader i Elizabeth. Ponadto, zaobserwowano że rośliny wszystkich badanych odmian ukorzenianych na podłożu AN nie wykształciły korzeni.

Przedstawione wyniki potwierdzają, że proces ryzogenezy *in vitro Vaccinium corymbosum* zależy od odmiany oraz warunków wzrostu rośliny .

Opracowany protokół regeneracji *in vitro* borówki wysokiej może pomóc w optymalizacji metodyki rozmnażania innych odmian borówki wysokiej oraz roślin z gatunku *Vaccinium* i może być przydatny np. do produkcji certyfikowanego materiału wegetatywnego lub do celów biotechnologicznych.

(a) (b) (c) (d) (e)

Ryc. 1. Rośliny borówki wysokiej ‘Liberty’ (a) inicjacja, (b) namnażanie, (c) i (d) aklimatyzacja, (e) owocowanie.

Tabela 1. Wpływ różnych podłoży na cechy morfologiczne i tempo regeneracji *in vitro* *V. corymbosum* 'Elizabeth', 'Liberty' i 'Meader' po 35 dniach hodowli

Odmiana	Pożywka								
	Długość pędów (cm)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	**ŚREDNIA**
Elizabeth	1.41b-f*	1.49b-g	1.83f-j	1.27a-d	1.59c-h	1.78f-i	1.80f-i	1.91g-k	**1.62B**
Meader	0.86a	0.98ab	1.23a-c	1.34b-e	1.56c-h	1.81f-j	1.89g-k	1.56c-h	**1.43A**
Liberty	1.67d-h	1.81f-i	1.71e-i	2.10i-k	2.23jk	2.27k	1.91g-k	1.94h-k	**1.96C**
ŚREDNIA	**1.34A**	**1.54AB**	**1.60B**	**1.57B**	**1.83C**	**1.98C**	**1.87C**	**1.82C**	
	Liczba nowych pędów								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	**ŚREDNIA**
Elizabeth	1.00a	1.13a	1.75a-c	1.80a-c	2.25b-e	2.38c-f	2.63c-g	2.75c-h	**1.95A**
Meader	1.13ab	1.25a-c	1.13ab	1.90a-c	2.13a-e	2.75c-h	2.88c-h	2.00a-d	**1.94A**
Liberty	2.00a-d	2.20a-e	1.80a-c	3.20e-h	3.50f-h	3.80gh	3.10d-h	3.90h	**2.94B**
ŚREDNIA	**1.42A**	**1.64A**	**1.58A**	**2.30B**	**2.69BC**	**3.04C**	**2.88C**	**2.96C**	
	Świeża masa (mg)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	**ŚREDNIA**
Elizabeth	40.90bc	41.40b-d	48.69c-g	43.20c-e	49.00c-g	52.70fg	50.80e-g	54.30f-i	**48.56B**
Meader	30.70a	32.70ab	35.89ab	45.78c-g	48.10c-g	51.41fg	55.95g-i	50.10c-g	**43.89A**
Liberty	50.24d-g	52.28fg	49.00c-g	67.24j	68.10j	70.12j	61.22hi	64.98ij	**60.40C**
ŚREDNIA	**43.82A**	**43.89A**	**45.51A**	**53.24B**	**59.41C**	**56.36C**	**57.73C**	**59.30C**	
	Sucha masa (mg)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	**ŚREDNIA**
Elizabeth	13.30a-c	12.90a-c	10.98ab	10.30ab	11.05a-c	15.05a-c	15.25a-c	15.40a-c	**12.22A**
Meader	9.65a	9.75a	9.95a	1.68a-c	10.75ab	14.83a-c	15.70a-c	14.95a-c	**12.84A**
Liberty	15.14a-c	16,74bc	14.54a-c	18.22c	18.34c	18.58c	16.78bc	17.86c	**17.03B**
ŚREDNIA	**13.51AB**	**14.33AB**	**11.43A**	**13.77AB**	**15.03AB**	**15.80B**	**16.20B**	**16.67B**	
	Współczynnik regeneracji korzeni (%)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	**ŚREDNIA**
Elizabeth	0	12.50	62.50	80.00	100	87.50	100	100	**67.81**
Meader	12.50	25.00	12.50	90.00	100	87.50	100	87.50	**64.38**
Liberty	70.00	80.00	70.00	100	100	100	90	100	**88.75**
ŚREDNIA	**27.50**	**39.17**	**48.33**	**90.00**	**100.00**	**91.67**	**96.67**	**95.83**	

*Średnie w kolumnach oznaczone tymi samymi literami alfabetu nie różnią się według testu Tukey'a na poziomie istotności $\alpha < 0.05$

P2. Figiel-Kroczyńska, M., Krupa-Małkiewicz, M., Ochmian, I. (2022). Effect of Actisil (Hydroplus™), organic supplements, and pH of the medium on the micropropagation of *Vaccinium corymbosum*. Acta Sci. Pol. Hortorum Cultus, 21(5).

Doświadczenie obejmowało trzy niezależnie przeprowadzone eksperymenty. Zbadano wpływ komercyjnego roztworu krzemu Hydroplus™ Actisil w stężeniach 50,100 i 500 mg dm^{-3}, wody kokosowej i mleka kokosowego w stężeniach 10 % i 15 % na cechy morfologiczne borówki wysokiej odmiany Liberty rozmnażanej *in vitro*, a także ich wpływ w łagodzeniu stresu związanego ze zmiennym pH podłoża WPM (pH 5,0; 5,5 i 6,0) W pierwszym etapie doświadczenia wybrano stężenie roztworu krzemu (Hydroplus™ Actisil) o najkorzystniejszym działaniu na cechy morfologiczne (wysokość rośliny, liczbę nowych pędów, świeżą masę) oraz

barwę liści borówki wysokiej rozmnażanej *in vitro*. Najwyższe eksplantaty (2,02 cm) z największą liczbą nowych pędów (1,91) i świeżą masą (55,16 g) uzyskano na podłożu WPM z dodatkiem 200 mg dm^{-3}, co było zgodne z zaleceniami producenta. Na podstawie uzyskanych wyników z pierwszego doświadczenia do dalszych badań wybrano roztwór Hydroplus™ Actisil w koncentracji 200 mg dm^{-3}.

W drugim eksperymencie do pożywki WPM dodano wodę kokosową oraz mleczko kokosowe jako naturalny dodatek organiczny, który mógłby zastąpić drogą zeatynę. Podobne do zeatyny działanie stymulujące wzrost roślin uzyskano, gdy do pożywki WPM dodano 15% wodę kokosową (CW), którą użyto do dalszych badań. Równocześnie otrzymane wyniki wykluczyły zastosowanie do mikrorozmnażania borówki wysokiej dodatku mleka kokosowego (CM). Dodanie CM do pożywki WPM całkowicie zahamowało wzrost i rozwoju pędów borówki *in vitro*.

W trzecim doświadczeniu ustalono wpływ komercyjnego roztworu krzemu w stężeniu 200 mg dm^{-3} i 15% stężenia wody kokosowej w łagodzeniu negatywnego wpływu pH na wzrost i rozwój *V. corymbosum in vitro*. Eksplantaty borówki wysokiej 'Liberty' wykazywały znaczne różnice w badanych cechach morfologicznych (wysokości, liczbie nowych pędów, świeżej masie) (Tab. 2) oraz parametrach biochemicznych (prolina, MDA i aktywność CAT) w zależności od zastosowanej pożywki. Wysokość roślin odmiany Liberty wynosiła 1,90 cm niezależnie od pH podłoża. Dodatek do pożywki WPM roztworu krzemu wpłynął na wzrost wysokości roślin o 10%, natomiast dodatek CW spowodował spadek wysokości roślin o 7% w porównaniu z kontrolą. Podobnie, dodatek krzemu spowodował wzrost średniej świeżej masy roślin o 26%, a dodatek CW – spadek o 48% w porównaniu z kontrolą (74,1 mg). Ponadto, dodatek Hydroplus™ Actisil, jak i CW do pożywki spowodował zmniejszenie liczby nowych pędów odpowiednio o 40% i 28%. Natomiast nie zanotowano znaczących różnic wartości dla takich cech, jak średnia wysokość rośliny i średnia liczba pędów na roślinę w przypadku zastosowania różnych pH pożywki WPM. Jednakże, rośliny rosnące na pożywce o wyższym pH miały mniejsze liście w porównaniu do liści roślin z pożywek o niskim pH. Średnia świeża masa roślin była znacznie niższa przy pH równym 6 na pożywce WPM w porównaniu z innym zastosowanymi wartościami pH (Tab. 2).

W niniejszym badaniu zaobserwowano, że wraz ze wzrostem pH pożywki wzrósł poziom stresu w roślinach grupy kontrolnej, na co jednoznacznie wskazywały wyższe paramerty wartości stężenia proliny, MDA i aktywności katalazy. Dodatek Hydroplus™ Actisil do pożywki WPM spowodował spadek stężenia proliny w porównaniu z kontrolą. Jednocześnie przy pH pożywki równym 5, nastąpił wzrost stężenia MDA i aktywności katalazy, natomiast dla pH 5,5 i 6,0

nastąpił spadek wartości tych parametrów w porównaniu z kontrolą. Dezaktywacja CAT była powiązana ze spadkiem stężenia MDA, co mogłoby sugerować sugeruje, że uszkodzenia oksydacyjne można zmniejszyć poprzez dodanie do pożywki produktu Hydroplus™ Actisil. W wyniku dodatku 15% wody kokosowej do pożywki WPM nastąpił wzrost wszystkich trzech parametrów (proliny, MDA i CAT). Stężenie proliny wzrosło średnio o około 38%, średnia wartość MDA zwiększyła się o około 33%, natomiast średnia wartość aktywności CAT wzrosła o 18%, odpowiednio w porównaniu z kontrolą.

Ponadto, zaobserwowano istotne różnice w barwie liści badanych roślin. Liście borówki wysokiej z podłoża WPM o pH 6,0 były najciemniejsze o czym świadczyła wartość parametru L^* (barwa od ciemnej do jasnej) wynosząca 34,19. Wartość parametru *a** (barwa od zielonej do czerwonej) oznaczona na powierzchni liścia mieściła się w przedziele od -4,71 (kontrola, pH 5,0) do 11,90 (WPM + 200 mg dm^{-3} Hydroplus™ Actisil, pH 5,5). Zaobserwowano, że wraz ze wzrostem pH pożywki wzrastała wartość parametru *a**. Odwrotne wyniki zaobserwowano dla parametru *b** (barwa od żółtej do niebieskiej), która wynosiła od 25,98 (kontrola, pH 5,0) do 8,52 (WPM + 200 mg dm^{-3} Hydroplus™ Actisil, pH 6,0). Dodatek Hydroplus™ Actisil lub wody kokosowej do pożywki WPM wpłynął pozytywnie na wybarwienie liści borówki wysokiej w porównaniu z grupą kontrolną. Przy czym, liście borówki wysokiej z pożywki WPM z dodatkiem 15% CW były bardziej zielone niż liście roślin z pożywki WPM z dodatkiem 200 mg dm^{-3} Hydroplus™ Actisil.

Niniejsze badania wykazały, że efektywność mikrorozmnażania *V. corymbosum* odmiany Liberty można poprawić poprzez dodatek do pożywki roztworu Hydroplus™ Actisil w stężeniu 200 mg dm^{-3}. Zastosowanie wody kokosowej jako naturalnego kompleksu organicznego wpływa stymulująco na wzrost i rozwój roślin borówki *in vitro* i może być stosowana jako zamiennik zeatyny. Najwyższą efektywność mikrorozmnażania uzyskano na podłożu WPM o pH 5. Spośród zastosowanych związków, tylko roztwór krzemu (Hydroplus™ Actisil) wykazał łagodzące działanie negatywnych skutków wywołanych podwyższonym pH pożywki.

Tabela 2. Wpływ dodatku 200 mg dm^{-3} Hydroplus™ Actisil i 15% wody kokosowej (CW) do pożywki WPM o różnym pH na cechy morfologiczne *V. corymbosum* 'Liberty' w warunkach *in vitro* po 53 dniach hodowli (n=32 pędy na powtórzenie)

		Pożywka WPM			
pH		kontrola	200 mg dm^{-3} Actisil	15% CW	**ŚREDNIA**
Wysokość	5.0	1.93b*	2.07c	1.91b	**1.97A**
roślin	5.5	1.86b	2.33d	1.51a	**1.90A**
[cm]	6.0	1.91b	1.83b	1.88b	**1.87A**
ŚREDNIA		**1.90B**	**2.08C**	**1.77A**	
Liczba	5.0	2.9f	1.3a	1.9cd	**2.0A**
nowych	5.5	2.2de	1.7bc	1.8bc	**1.9A**
pędów	6.0	2.5e	1.6b	1.8bc	**2.0A**
ŚREDNIA		**2.5C**	**1.5A**	**1.8B**	
Świeża masa	5.0	95.7d	75.2c	40.0a	**70.3B**
roślin	5.5	71.4c	123.4e	34.6a	**76.5B**
[mg]	6.0	55.1b	81.3c	40.9a	**59.1A**
ŚREDNIA		**74.1B**	**93.3C**	**38.5A**	

*Średnie w kolumnach oznaczone tymi samymi literami alfabetu nie różnią się według testu Tukey'a na poziomie istotności $\alpha < 0.05$

P3. Figiel-Kroczyńska, M., Ochmian, I., Krupa-Małkiewicz, M., Lachowicz, S. (2022). Influence of various types of light on growth and physicochemical composition of blueberry (*Vaccinium corymbosum* L.) leaves. Acta Sci. Pol. Hortorum Cultus, 21(2).

Światło ma kluczowe znaczenie dla roślin, ponieważ rośliny są zależne od niego w kwestii wytwarzania energii, a tym samym przetrwania. Ważne jest, aby w produkcji roślinnej stosować źródło światła, które ma pozytywny wpływ na rośliny. Dla producentów sadzonek nadrzędne znaczenie ma natomiast osiągnięcie jak najniższych kosztów produkcji. Istotę tej kwestii stanowi osiągnięcie kompromisu między efektywnością wzrostu i rozwoju roślin, czyli jakości produktu końcowego, a ekonomiką produkcji.

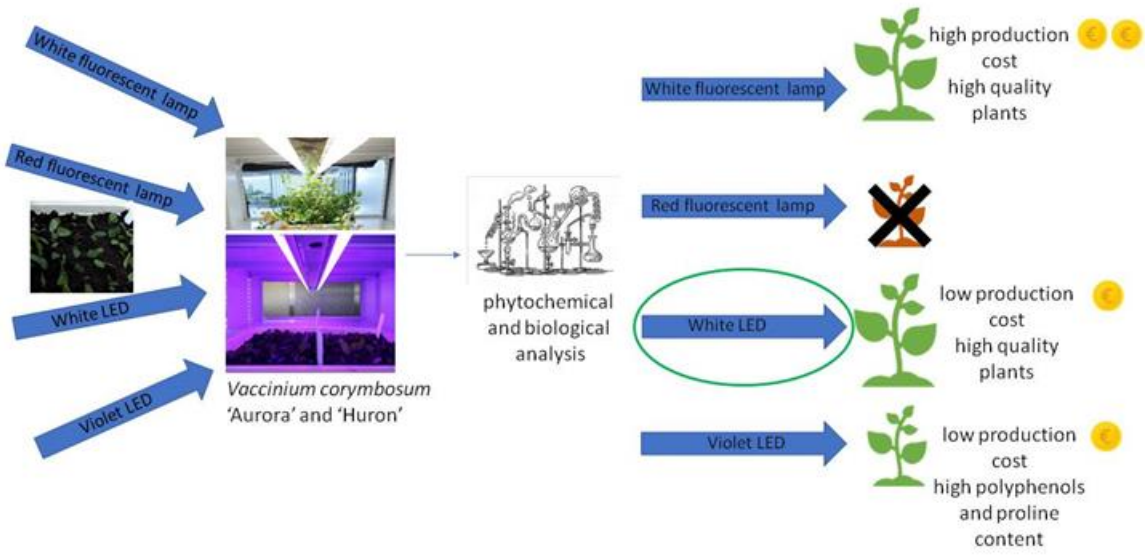
Niezależnie od rodzaju światła zastosowanego w fitotronie, rośliny borówki wysokiej odmiany Aurora były wyższe, miały jaśniejsze liście o wyższej zawartości polifenoli oraz niższej zawartość proliny w porównaniu do liści odmiany Huron. Doświetlanie roślin obu odmian fioletową diodą LED, spowodowało, że były one niższe i miały mniejsze liście. Dowiedziono, że źródło światła jest czynnikiem stresowym dla rośliny. Świadczy o tym niska wartość parametru CIE *a** i *b** oraz wysoka zawartość proliny całkowitej i polifenoli. Rośliny uprawiane w warunkach białego fluorescencyjnego światła miały o 22,7% mniejszą zawartość polifenoli, natomiast rośliny rosnące w warunkach światła białego LED miały o 19,2% niższą zawartość polifenoli, w porównaniu do uprawy pod fioletową diodą LED. Rodzaj zastosowanego światła wpłynął również na wielkość powierzchni liścia - największą średnią

powierzchnię liścia (7,29 cm^2) miały rośliny doświetlane białym światłem LED, natomiast najmniejsze - fioletowym światłem LED (6,63 cm^2).

Wybierając lampy LED osiągnięto około 50% oszczędności w porównaniu do stosowania świetlówek. Ekonomika produkcji wykazała, że do wzrostu roślin w fitotronie lepiej jest stosować źródła LED, niż inne źródła światła (Ryc. 2 i Tab. 3). W wielkotowarowej produkcji roślin borówki wysokiej, wskazane jest stosowanie białego światła LED, które ma pozytywny wpływ ekonomiczny na produkcję roślinną ze względu na niskie zużycie energii elektrycznej i korzyści dla środowiska poprzez eliminację rtęci ze źródła światła. Uzyskana pod tym źródłem światła jakość roślin jest zbliżona do tej białego światła fluorescencyjnego.

Tabela 3. Wynik ekonomiczny rocznego utrzymania szklarni w zależności od rodzaju światła (1 Euro = 1,08 USD)

Koszt użytkowania lampy	Rodzaj światła		
	biała fluorescencyjna świetlówka	biała lampa LED	fioletowa lampa LED
Cena 1 świetlówki (Euro)	8.84	3.78	15.93
Zużycie energii elektrycznej (W)	36	18	18
Żywotność świetlówki (h)	15 000	40 000	50 000
Żywotność lampy w latach	2.57	6.85	8.56
Roczne zużycie energii przez 1 świetlówkę (16 h*365 dni*W) = kWh	210	105	105
Cena 1 kWh (EUR)	0.14	0.14	0.14
Koszt zużycia energii dla 1 lampy rocznie (EUR)	29.34	14.67	14.67
Roczny koszt użycia 1 świetlówki (EUR) koszt energii + cena świetlówki	32.78	15.22	16.53
Roczny koszt pracy fitotronu (EUR)	6555.29	3044.00	3305.60



Ryc. 2. Wzrost roślin borówki wysokiej w zależności od zastosowanego światła; od góry – biała fluorescencyjna świetlówka, czerwona fluorescencyjna świetlówka, biała dioda LED, fioletowa dioda LED.

P4. Figiel-Kroczyńska, M., Ochmian, I., Krupa-Małkiewicz, M. (2022). Effect of chitosan-based spraying on fruit quality of highbush blueberry cv. Sunrise. Progress on Chemistry and Application of Chitin and its Derivatives, Volume XXVII.

Jakość owoców ma duży wpływ na decyzje konsumenckie. Stąd też, ważne jest, aby stosowane metody uprawy były naturalne i ekologiczne. Chcąc poprawić jakość owoców borówki wysokiej oraz wydłużyć ich świeżość podczas przechowywania zastosowano oprysk chitozanem (CH). Powłoka chitozanowa tworzy na powierzchni owoców półprzepuszczalny film, prowadząc do opóźnienia tempa oddychania, zmniejszenia utraty wagi, zachowując jednocześnie jakość i przedłużając okres przydatności do spożycia owoców. W badaniu zastosowano chitozan o różnej masie cząsteczkowej CH (5, 12, 21, 50, 125, 500 kDa).

Masa cząsteczkowa chitozanu ma decydujący wpływ na jego właściwości, zarówno fizyczne, chemiczne, jak i biologiczne. Zastosowanie chitozanu o wyższej masie cząsteczkowej 500 kDa, a zwłaszcza 125 kDa zwiększyło średnią masę owoców, odporność na przebicia skórki, jędrność owoców. Zwiększyła się również zawartości kwasu *L*-askorbinowego i szkodliwych $N\text{-}NO_3$ porównaniu z grupą kontrolną (Tab. 4). Najmniejsze owoce zebrano z krzewów opryskiwanych chitozanem o masie cząsteczkowej 5 kDa. Owoce poddane opryskiem CH 21 kDa charakteryzowały się najniższą zawartością $N\text{-}NO_3$ i $N\text{-}NO_2$.

W badanych owocach stwierdzono obecność 36 polifenoli, zakwalifikowanych do czterech grup. Najliczniej występującymi były antocyjany, następnie kwasy fenolowe, a najmniej liczne

flawonole i flawan-3-ole. Oznaczono łącznie 9 różnych antocyjanów. Najwyższą zawartość antocyjanów miały owoce opryskiwane chitozanem 500 kDa, ponad 2 razy więcej niż w owocach opryskiwanych CH 12 kDa. Dominującym związkiem zaliczonym do polifenoli był kwas chlorogenowy, należący do grupy kwasów fenolowych. Stanowił on średnio od 29,57 do 36,49% całkowitej zawartości polifenoli w owocach borówki wysokiej.

W zależności od masy cząsteczkowej zastosowanego CH owoce zmieniały odcień z czerwonego na niebieski, na co wskazują istotne zmiany parametrów barwy CIE *a** i *b**. Najciemniejszy i najbardziej intensywny niebieski odcień zaobserwowano w owocach po zastosowaniu CH 500 i 125 kDa, co jest niewątpliwie związane z wysoką zawartością antocyjanów. Natomiast najjaśniejszymi, o fioletowo-różowym zabarwieniu, były owoce opryskiwane chitozanem o masie cząsteczkowej 50 kDa.

Po zbiorze z owoców borówki wyizolowano grzyby należące do siedmiu różnych rodzajów (*Acremonium, Alternaria, Aspergillus, Aureobasidium, Cladosporium, Fusarium, Penicillium*). Najwięcej było grzybów z rodzaju *Fusarium* (44-56%). Równie licznie występowały grzyby z rodzaju *Penicillium* (16-80%) i *Aspergillus* (35-37%). Najmniej licznie występowały grzyby z rodzaju *Acremonium*. Pojawiły się one tylko w grupie kontrolnej i owocach opryskiwanych chitozanem o najniższej masie cząsteczkowej 5 kDa w ilości odpowiednio 5 i 7%. W grupie owoców opryskiwanych chitozanem o masie cząsteczkowej 125 kDa występowały bardzo licznie grzyby z rodzaju *Penicillium* (80%), a po zastosowaniu CH 21 kDa grzyby z rodzaju *Cladosporium* (73%).

W badanych próbkach wykryto cztery różne typy mykotoksyn: patulinę, aflatoksynę, deoksyniwalenol i zearalenon. Zidentyfikowane mykotoksyny występowały na niskim poziomie. Zawartość patuliny (1,03-3,04 μg kg^{-1}) była istotnie niższa niż maksymalne dopuszczalne stężenie patuliny w soku jabłkowym (50 μg kg^{-1}) ustalone przez WHO. Mykotoksyny pojawiły się tylko w grupie kontrolnej i dwóch grupach o najniższej masie cząsteczkowej chitozanu (5 i 12 kDa). Brak mykotoksyn w próbkach mógł być związany z występowaniem w próbkach grzybów *Aureobasidium*. Mogą one rozkładać mykotoksyny poprzez szlaki mikrobiologiczne. Wykazują silny antagonizm w stosunku do pleśni zanieczyszczających owoce i są w stanie zapewnić skuteczną ochronę biologiczną. Można również spekulować, że chitozan o wyższych masach cząsteczkowych hamował wzrost mykotoksyn.

Przeprowadzone badania dowodzą, że opryskiwanie owoców chitozanem może być zalecane w ekologicznej uprawie borówki wysokiej w celu uzyskania jędrniejszych owoców o zwiększonych właściwościach prozdrowotnych.

Tabela 4. Jakość i zdolność antyoksydacyjna borówki amerykańskiej w zależności od użytego chitozanu

	Molekularna masa chitozanu (kDa)						
	Kontrola	5	12	21	50	125	500
Masa 100 owoców (g)	274ab*	262a	288bc	302c	335d	342d	291bc
Przebicie (G mm^{-1})	124bcd	130cde	108a	117ab	121bc	142e	133e
Jędrność (G mm^{-1})	358bc	366bc	317a	359bc	352b	428d	375c
Rozpuszczalne substancje stałe (%)	15.3b	15.8c	15.6c	15.2b	14.5a	14.4a	15.1b
Kwasowość (g 100 g^{-1})	0.88d	0.81bc	0.85cd	0.87d	0.74a	0.77ab	0.80b
L-askorbinowy kwas (mg 100 g^{-1})	26.5b	22.4a	20.7a	27.8b	35.7c	36.2c	39.6c
N-NO_3 (mg 1000 g^{-1})	37.3bc	41.5c	33.4ab	30.2a	36.1b	55.9d	52.3d
N-NO_2 (mg 1000 g^{-1})	0.11ab	0.13bc	0.14c	0.09a	0.15cd	0.15cd	0.17d
$ABTS^{\cdot+}$ (µmol TE g^{-1})	14.7a	17.8b	20.2c	21.3c	18.4b	17.7b	15.5a
FRAP (µmol TE g^{-1})	8.11bc	7.36b	9.55d	8.78cd	5.77a	10.12e	9.84d
DPPH (µmol TE g^{-1})	22.2b	15.7a	31.0d	28.4c	21.6b	15.5a	16.3a

*Średnie w kolumnach oznaczone tymi samymi literami alfabetu nie różnią się według testu Tukey'a na poziomie istotności $\alpha < 0.05$

P5. Ochmian, I., **Figiel-Kroczyńska, M.**, Lachowicz, S. (2020). The quality of freeze-dried and rehydrated blueberries depending on their size and preparation for freeze-drying. Acta Universitatis Cinbinesis, Series E: Food Technology, 24(1).

Owoce borówki wysokiej, które ze względu na swoją małą średnicę nie nadają się do sprzedaży w formie świeżej, wykorzystywane są w przetwórstwie. Jedną z metod zagospodarowania takich owoców jest suszenie. Celem przeprowadzonego doświadczenia była ocena wpływu sposobu cięcia lub nakłuwania niekomercyjnych owoców borówki wysokiej na czas trwania procesu suszenia sublimacyjnego – liofilizacji. Ocenie poddano również wartość biologiczną świeżych, liofilizowanych i uwodnionych owoców.

Ilość owoców pozostawionych na krzewach zależała od pogody panującej w okresie wegetacji, zwłaszcza w okresie zbiorów. Masa owoców pozostawiona na krzewach wynosiła od około 1 do 2 t/ha, co stanowi do 5% całkowitego plonu dla 'Brigitta Blue'.

Pomimo małych rozmiarów owoców, charakteryzowała je wysoka wartość biologiczna. zwłaszcza z krzewów nienawadnianych. Mogło to być spowodowane wyższym stężeniem

związków mineralnych i organicznych, na co wskazywało wyższe stężenie ekstraktu oraz wyższa sucha masa. Jednocześnie owoce te były znacznie mniejsze.

Z jednego kilograma świeżych jagód uzyskano około 120 g suszonych owoców. Zawartość polifenoli wzrosła jedynie o 3-4 razy w zależności od wielkości owoców. Wskazuje to na rozkład tych związków w procesie liofilizacji. Degradacja polifenoli zachodziła w większym stopniu w owocach dużych. Wpływ na to mógł mieć wydłużony do 46 godzin czas suszenia. W celu uzyskania podobnego poziomu wilgotności przez małe owoce, wystarczyło suszyć je przez 32 godziny.

Właściwości fizykochemiczne liofilizowanych i ponownie uwodnionych owoców zależały od metody ich wcześniejszego przygotowania. Wcześniejsze przekrojenie na pół lub nakłucie na obwodzie owoców pozwoliło znacznie skrócić czas procesu liofilizacji. Przekrojone owoce zostały wysuszone najszybciej – najmniejsze po 16 godzinach, a duże (>8 mm) po 22 godzinach. Owoce nakłuwane na obwodzie, również znacznie skrócił czas tego procesu.

W owocach moczonych w wodzie przez 6 godzin, poziom polifenoli był porównywalny do poziomu w świeżych owocach. Owoce przecięte, zwłaszcza małe, wykazywały największy względny przyrost masy podczas nawadniania. Podczas pierwszych 15 minut kontaktu całego owocu z wodą nastąpił bardzo niewielki przyrost masy, co wskazuje na niski stopień uwodnienia owocu. Po 30 minutach względny przyrost masy wyniósł 0,5 natomiast w przypadku owoców krojonych – średnio 1.

W trakcie ponownego uwodnienia owoców zaobserwowano zmianę ich barwy. Owoce liofilizowane były ciemniejsze niż owoce świeże. Bez względu na ich wielkość oraz sposób przygotowania do suszenia, ich barwa stawała się jaśniejsza, o czym świadczą zmiany parametrów a^* i b^* oraz parametru koloru L^*. Największe zmiany barwy zaobserwowano dla owoców nakłuwanych. Po liofilizacji były najciemniejsze (L^* 32,5), a po 6 godzinach nawadniania parametr L^* osiągnął najwyższą wartość 54.

Wyniki przeprowadzonego doświadczenia potwierdzają, że owoce borówki wysokiej odmiany Brigitta Blue polecane są do dłuższego przechowywania. Charakteryzują się grubą skórką i intensywnym nalotem woskowym, który chroni owoce przed więdnięciem. Owoce, które nie nadają się do sprzedaży ze względu na ich małe rozmiar można zamrozić lub wysuszyć. Liofilizacja jest dobrą metodą konserwacji owoców borówki wysokiej, pozwalającą na utrzymanie właściwości prozdrowotnych na wysokim poziomie.

5. WNIOSKI

Osiągnięto założone cele badawcze, które zamieszczono w cyklu publikacji stanowiącym podstawę do ubiegania się o stopień naukowy doktora.

1. W celu uzyskania przez producenta zadowalającego zwrotu z inwestycji, konieczna jest analiza każdego etapu produkcji. Począwszy od wyboru materiału roślinnego, użytej pożywki do namnażania kultur *in vitro*, aplikowanych dodatków (Hydroplus™ Actisil, chitozan, woda kokosowa), źródła światła zastosowanego w fitotronie, oraz zagospodarowanie niekomercyjnych owoców (liofilizacja).
2. Ze względu na dużą zmienność genetyczną w obrębie gatunku *V. corymbosum*, nie można zastosować uniwersalnej pożywki do namnażania tych roślin. Opracowano protokół mikrorozmnażania borówki wysokiej dla wybranych odmian (Elizabeth, Liberty, Meader). Najwyższy współczynniki regeneracji pędów uzyskano na pożywce WPM oraz MW z dodatkiem zeatyny 0,1 mg L^{-1} (80 do 100%).
3. Zastosowanie substancji biologicznie czynnych dało pozytywne efekty zarówno w *in vitro* i w uprawie polowej borówki, a także wpisało się w ekologiczne trendy produktów do spożycia. Woda kokosowa jako naturalny kompleks substancji organicznych, może stanowić zamiennik dla komercyjnie stosowanej zeatyny. Komercyjny roztwór Si (Hydroplus™ Actisil) może być z powodzeniem stosowany w kulturach *in vitro* borówki wysokiej jako środek łagodzący stres wywołanym podwyższonym pH pożywki. Zastosowanie chitozanu w formie oprysku w okresie intensywnego wzrostu owoców, pozytywnie wpłynęło na ich jakość (ograniczanie wzrost patogenów grzybowych, wzrost masy owoców, poprawa jędrności i składu chemicznego).
4. Zastosowanie białego źródła światła LED w procesie mnożenia roślin borówki wysokiej w fitotronie wpłynęło na najlepszy wzrost roślin. Użycie tego źródła światła jest ekonomicznie uzasadnione, pod względem niskich kosztów rocznego utrzymania fitotronu. LED przynosi również korzyści ekologiczne. Pozwala na eliminację rtęci ze źródła światła oraz wpływa na ograniczenie zużycia energii elektrycznej.
5. Liofilizowanie, spowodowało niewielki spadek zawartości związków bioaktywnych w porównaniu do świeżych owoców. Skrócenie czasu suszenia owoców borówki wysokiej pozwoliło na zmniejszenie kosztów produkcji suszonych owoców i miało wpływ na zachowanie większej ilości związków polifenolowych, a co za tym idzie na ich wyższą

wartość biologiczną. Warto zatem wykorzystywać do tego celu małe niekomercyjne owoce borówki, które wykazują znacznie krótszy czas suszenia.

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KOPIE ARTYKUŁÓW STANOWIĄCYCH JEDNOTEMATYCZNY CYKL PUBLIKACJI

I OŚWIADCZENIA WSPÓŁAUTORÓW

Figiel-Kroczyńska M *et al.* (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 4, Article number 12856
DOI:10.15835/nbha50412856
Research Article

Efficient micropropagation protocol of three cultivars of highbush blueberry (*Vaccinium corymbosum* L.)

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Abstract

Highbush blueberry (*Vaccinium corymbosum* L.) is increasingly farmed for its nutritional and health benefits, but high yield and fruit quality require proper planting material. Modified Murashige and Skoog (MW), Anderson's Rhododendron (AN), and Woody Plant Medium (WPM) were compared for *in vitro* organogenesis and rooting of three highbush blueberries 'Elizabeth', 'Meader', and 'Liberty'. All media contained 0.1 mg L^{-1} zeatin applied with a combination of IBA, IAA, and GA_3. The results showed that MW medium is more suitable for *in vitro* multiplication of 'Elizabeth' and 'Meader', and WPM medium for 'Liberty'. However, medium supplemented with a low concentration of IBA (≤ 0.4 mg L^{-1}) and 0.1 mg L^{-1} zeatin increased the shoot regeneration rate of highbush blueberries multiplied *in vitro*. The rooting capability was studied by using WPM and AN medium with IBA and IAA with zeatin. The highest rooting rate (85%) and acclimatization (70%) were achieved in 'Liberty', and the lowest was in 'Elizabeth' (33.3% and 50%, respectively) and 'Meader' (33.3% and 43.8%, respectively). Rooted plantlets developed good quality roots and were transplanted into peat:perlite (4:1) substrates and acclimatized in a greenhouse under controlled conditions. We developed a complete micropropagation protocol for cvs. 'Meader', 'Elizabeth' and 'Liberty' blueberry. This protocol can be used for the production of certified vegetative material or different biotechnological purposes.

Keywords: acclimatization; highbush blueberry; *in vitro*; plant growth regulators; rooting

Abbreviations: AN, Anderson's Rhododendron medium; GA_3, Gibberellic Acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; MS, Murashige and Skoog medium; WPM, The McCown Woody Plant medium; MW, mixture of equal parts of MS and WPM media; TDZ, tidiazuron, 2iP, 2-isopentenyladenine.

Introduction

The highbush blueberry (*Vaccinium corymbosum* L.) belongs to the Ericaceae family. Highbush blueberry is a crop with an increasing trend in global consumption and cultivation volume (Podymiak, 2015;

Received: 22 Aug 2022. Received in revised form: 04 Dec 2022. Accepted: 08 Dec 2022. Published online: 12 Dec 2022.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Brazelton and Young, 2017; Ochmian *et al*., 2020). World production of highbush blueberry fruit was 655.0 metric tonnes (MT), an increase of about 14% compared to 2014. Poland ranks second in Europe and seventh in the world in terms of highbush blueberry fruit production (Brazelton and Young, 2017). The largest producers are the USA, Chile and Canada. In Europe, production is concentrated in Spain, Poland and Germany (Brazelton, 2013; Brazelton and Young, 2017). Blueberry fruits are valued for their high content of health-promoting components such as polyphenols, low calorie content, taste and nutritional value (Brazelton, 2013; Brazelton and Young, 2017). Highbush blueberry is a plant that requires acidic soils (pH 3.8-5.5), well aerated, with stable groundwater levels and high humus content (Ochmian *et al*., 2020; Figiel-Kroczyńska *et al*., 2022). Despite the worldwide increase in blueberry cultivation, the availability of suitable soils for this species remains a problem (Ochmian *et al*., 2018).

Due to the rapid increase in blueberry growing area, blueberry seedlings should be produced quickly and in large quantities. Traditionally, blueberry is propagated by coniferous, semi-coniferous and deciduous cuttings, by rhizomatous or rhizomatous cuttings of selected clones. Problems in this production are the very low rooting percentage for many genotypes, the long time to propagation and commercialization of newly obtained plants, and phytosanitary.

Tissue culture propagation techniques can be used as a system for effective plant production of virus-free plants which are genetically identical. There are many reports in the literature on *in vitro* propagation of blueberry (Sedlák and Paprštein, 2009; Ružić *et al*., 2012; Cüce and Sökmen, 2017; Kruczek *et al*., 2021). However, all aforesaid attempts showed great variations in terms of basal media as well as plant growth hormons, growth conditions, explant types, sampling, and physiological condition of the explants (Ružić *et al*., 2012; Krupa-Małkiewicz *et al*., 2017; Kruczek *et al*., 2020). Therefore, the results presented in current study are not broadly applicable, because the effectiveness of the medium and morphogenesis of *Vaccinium in vitro* plants dependent on genotype or even cultivar (Sedlák and Paprštein, 2009).

The aim of the presented study was to determine the best media for *in vitro* shoot proliferation and rooting of three highbush blueberry cultivars grown in Poland.

Materials and Methods

Characteristics of the area of research and characteristics of cultivars

Actively growing young nodal steam (about 10 cm) were collected from five-year-old *Vaccinium corymbosum* L. plants, from the bushes grown on organic farm specializing in the cultivation of the highbush blueberry, located about 60 km east of Szczecin (the North-Western part of Poland) between April and May 2018. The first three steps of propagation (initiation, multiplication, rooting) were conducted in the laboratory of the Department of Plant Genetics, Breeding and Biotechnology at the West Pomeranian University of Technology in Szczecin, Poland. The last stage – acclimatisation, was conducted on the same production plantation from which the research material originated and lasted from 2020 to 2021.

Plant material

The plants material consisted three cultivars of highbush blueberry: 'Elizabeth', 'Liberty' and 'Meader'. After defoliation, stems were washed with running tap water with a detergent (Ludwik washing-up liquid) for 1 h and then surface-sterilized with 70% (v/v) ethanol for 30 s. Next explants were incubated in 7.5% (v/v) sodium hypochlorite (NaOCl) for 10 minutes and 0.2% (v/v) mercury sulphate ($HgSO_4$) for 10 minutes. Explants were rinsed with sterile deionized water three times under laminar flow hood. The stems were further cut as two-node stem explants (about 1-2 cm) and cultured on initiation medium WPM (Lloyd and McCown, 1980) with the addition of 0.1 mg L^{-1} zeatin. Initial medium was selected based on preliminary experiments (unpublished data).

Multiplication and culture conditions

An axillaries bud of highbush blueberry was taken from initial *in vitro* culture. The shoot explants were transferred to MW medium (mixture of equal parts of MS – Murashige and Skoog (1962) and WPM media) with the addition of IBA in a concentration of 0.2 and 0.4 mg L^{-1} (MW 1 and MW 2, respectively); WPM medium with the addition of IBA in a concentration of 0.2 and 0.4 mg L^{-1} (WPM 1 and WPM 2, respectively) and WPM + 0.2 mg L^{-1} IBA + 0.4 mg L^{-1} GA_3 (WPM 3); AN medium (Anderson, 1984) with the addition of IBA in a concentration of 0.2 and 0.4 mg L^{-1} (AN 1 and AN 2, respectively), and AN + 0.4 mg L^{-1} IBA + 0.4 mg L^{-1} GA_3 (AN 3). Each medium combination also contained the addition of 0.1 mg L^{-1} zeatin (Table 1). After 35 days, explants were removed and washed with deionized distilled water, shoot length, number of new shoots, fresh and dry mass were measured as well as shoot regeneration rate (%). Dry mass of explants was determined after drying in the hot-air oven at 70 °C for 24 hours.

Table 1. Multiplication mediums with different plant growth regulators

Medium	mg L^{-1} IBA	mg L^{-1} GA_3	mg L^{-1} zeatin
AN* 1	0.2	-	0.1
AN 2	0.4	-	0.1
AN 3	0.4	0.4	0.1
WPM 1	0.2	-	0.1
WPM 2	0.4	-	0.1
WPM 3	0.4	0.4	0.1
MW 1	0.2	-	0.1
MW 2	0.4	-	0.1

AN* – Anderson Rhododendron; WPM – Woody Plant Media; MW – a mixture of equal parts of MS – Murashige and Skoog and WPM media

In vitro and ex vitro rooting

Axillary shoots height 2.5 cm were rooted in AN medium (AN A and AN B) and WPM medium (WPM A and WPM B), each supplemented with 0.1 mg L^{-1} zeatin and IBA or IAA at concentration of 0.5 or 1.0 mg L^{-1} (Table 2). After 35 days of culture, rooting percentage (%), the length of the shoots and roots, the number of new shoots, roots and leaves, as well as fresh and dry mass were recorded.

Table 2. Rooting medium with different plant growth regulators

Medium	mg L^{-1} IBA	mg L^{-1} zeatin	mg L^{-1} IAA
AN* A	0.5	0.1	-
AN B	1.0	0.1	-
WPM A	-	0.1	0.5
WPM B	-	0.1	1.0

AN* – Anderson Rhododendron; WPM – Woody Plant Media

Explants were initially selected under an *in vitro* culture, and then made adaptive to greenhouse conditions in May 2020. The plantlets with well-developed shoots and roots were transferred into round black pots of diameter 7 cm, filled with a mixture of 4 L peat, 1 L perlite, 1 L water and 1 mL systemic multi-site fungicide (Previcur Energy 840 SL, Bayer). Peat consisted 66.9% organic matter, EC 0.24 mS/cm, volume weight 0.35 kg dm^{-1}, full water capacity 85.2% vv and pH in KCl 3.44. It was characterized by optimal content for blueberry (Komosa, 2007). The pots were placed on 60-cm high tables in the greenhouse at 20 °C/15 °C day/night temperature cycles and light intensity of 110 mol m^{-2} s^{-1} during the experimental period. The temperature inside the greenhouse was controlled by vents that opened automatically. After six weeks, the plants were transferred to the biggest pots (one plant per pot) diameter of 10 cm and 400 mL capacity and filled with the same substrate as previously. The number of plants that were successfully acclimatised was counted

and the acclimatisation rate (%) was calculated. The plants at any stage of acclimatisation were watered and sprayed twice a week with a fungicide (Switch 62,5 WG; Syngenta).

After two-month, plants were transferred (July 2020) to a blueberry plantation, where they were kept in the currently prevailing weather conditions in an open space.

Plant culture conditions

In vitro culture was conducted in 300 mL flask filled with 30 mL of medium. All media were supplemented with 30 g L^{-1} sucrose (Chempur, Poland) and 100 mg L^{-1} myo-inositol (Duchefa, The Netherlands) and were solidified with 8 g L^{-1} agar (Biocorp, Poland). pH of all the media was adjusted to 5.8. prior to autoclaving at 121 °C and 0.1 Mpa. All cultures were incubated in a growth room at a temperature of 25 ± 2 °C under 16 hours photoperiod with a photosynthetic flux density (PPFD) of 40 μmol m^{-2} s^{-1} provided by Narva (Germany) emitting daylight cool white and 60-70% humidity. Each combination included 32 shoots (4 explants per flask in eight replications).

Statistical analysis

All statistical analyses were performed using Statistica 13.0 (StatSoft, Cracow, Poland). Statistical significance of the differences between means was determined by testing the homogeneity of variance and normality of distribution, followed by ANOVA with Tukey's post hoc test. The significance was set at $p<0.05$.

Results

Multiplication

Significant differences in multiplication parameters were observed between media with different hormonal compositions (Table 3). In our study, among the combinations of multiplication medium tested, WPM media and MW media yielded the best shoot regeneration rate from 80 to 100% (Table 3). It was found that the WPM 3 medium for multiplication with the addition of GA_3 was the best for cultivar 'Liberty'. In case of cultivars 'Elizabeth' and 'Meader' the best results in multiplication were achieved when MW 2 and MW 1, respectively were used. Significantly worse *in vitro* multiplication results of selected cultivars of blueberry were found on different modifications of AN medium. Moreover, it was observed that shoots multiplied on AN medium was showed red colouration of leaves and stems and their leaves were small and more abundant, whereas shoots multiplied on MW and WPM medium were light green and had large and green coloured leaves as observed for 'Elizabeth' and 'Meader' (Figure 1). Furthermore, 'Liberty' plantlets regenerated more than 20% better than 'Elizabeth' and 'Meader'.

Among the cultivars tested, the 'Liberty' multiplied much better than 'Elizabeth' and 'Meader'. The plantlets of the 'Liberty' were 21 and 37% higher than 'Elizabeth' and 'Meader', respectively. No significant differences were observed between the number of shoots for 'Elizabeth' and 'Meader'. While 'Liberty' developed 53% more new shoot per plant in comparison to the other cultivars tested. The greater height and bushiness of 'Liberty' plantlets translated into their higher mass. The plants had 19.60 and 27.33% higher dry mass, compared to 'Elizabeth' and 'Meader', respectively.

Table 3. The influence of the various medium on the morphological traits and regeneration rate *in vitro* of *V. corymbosum* 'Elizabeth', 'Liberty' and 'Meader' after 35 days of cultivating

Cultivar	Medium								
	Shoot length (cm)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	MEAN
Elizabeth	1.41b-f*	1.49b-g	1.83f-j	1.27a-d	1.59c-h	1.78f-i	1.80f-i	1.91g-k	1.62B
Meader	0.86a	0.98ab	1.23a-c	1.34b-e	1.56c-h	1.81f-j	1.89g-k	1.56c-h	1.43A
Liberty	1.67d-h	1.81f-i	1.71e-i	2.10i-k	2.23jk	2.27k	1.91g-k	1.94h-k	1.96C
MEAN	1.34A	1.54AB	1.60B	1.57B	1.83C	1.98C	1.87C	1.82C	
	Number of new shoots								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	MEAN
Elizabeth	1.00a	1.13a	1.75a-c	1.80a-c	2.25b-e	2.38c-f	2.63c-g	2.75c-h	1.95A
Meader	1.13ab	1.25a-c	1.13ab	1.90a-c	2.13a-e	2.75c-h	2.88c-h	2.00a-d	1.94A
Liberty	2.00a-d	2.20a-e	1.80a-c	3.20e-h	3.50f-h	3.80gh	3.10d-h	3.90h	2.94B
MEAN	1.42A	1.64A	1.58A	2.30B	2.69BC	3.04C	2.88C	2.96C	
	Fresh mass (mg)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	MEAN
Elizabeth	40.90bc	41.40b-d	48.69c-g	43.20c-e	49.00c-g	52.70fg	50.80e-g	54.30f-i	48.56B
Meader	30.70a	32.70ab	35.89ab	45.78c-g	48.10c-g	51.41fg	55.95g-i	50.10c-g	43.89A
Liberty	50.24d-g	52.28fg	49.00c-g	67.24j	68.10j	70.12j	61.22hi	64.98ij	60.40C
MEAN	43.82A	43.89A	45.51A	53.24B	59.41C	56.36C	57.73C	59.30C	
	Dry mass (mg)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	MEAN
Elizabeth	13.30a-c	12.90a-c	10.98ab	10.30ab	11.05a-c	15.05a-c	15.25a-c	15.40a-c	12.22A
Meader	9.65a	9.75a	9.95a	1.68a-c	10.75ab	14.83a-c	15.70a-c	14.95a-c	12.84A
Liberty	15.14a-c	16,74bc	14.54a-c	18.22c	18.34c	18.58c	16.78bc	17.86c	17.03B
MEAN	13.51AB	14.33AB	11.43A	13.77AB	15.03AB	15.80B	16.20B	16.67B	
	Shoot regeneration rate (%)								
	AN1	AN2	AN3	WPM1	WPM2	WPM3	MW1	MW2	MEAN
Elizabeth	0	12.50	62.50	80.00	100	87.50	100	100	67.81
Meader	12.50	25.00	12.50	90.00	100	87.50	100	87.50	64.38
Liberty	70.00	80.00	70.00	100	100	100	90	100	88.75
MEAN	27.50	39.17	48.33	90.00	100.00	91.67	96.67	95.83	

*Means followed by different letters in columns are significantly different at the 5% level according to Tukey's multiple ranges

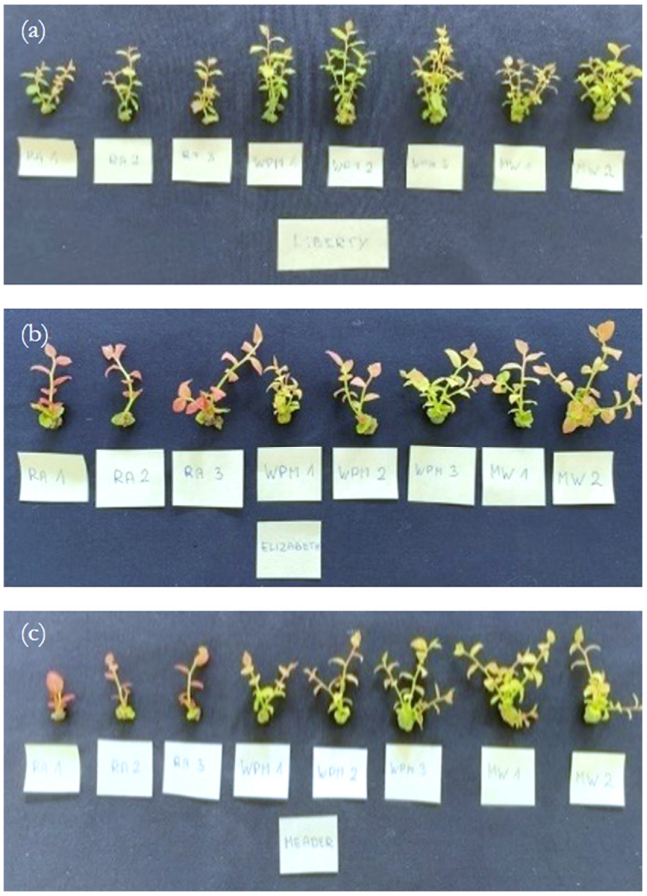

Figure 1. *In vitro* multiplication of highbush blueberry 'Elizabeth' (a), 'Liberty' (b), and 'Meader' (c) on different multiplying medium

In vitro and ex vitro rooting

The most important characteristics indicating the success of *in vitro* rooted plants are the number of roots, root length, and their quality as well as *ex vitro* rooting rate. The maximum mean number of roots (2.14) with the longest root (0.77 cm) was observed on the WPM B medium supplemented with 0.1 mg L^{-1} zeatin and 1.0 mg L^{-1} IAA (Table 4). Good rhizogenesis was correlated with the highest mean fresh mass of plantlets (60.33 mg). Moreover, 'Liberty' explants developed more then 2,5 times longer roots in comparison to other cultivars tested (Table 4 and Figure 2). Conversely, the highest shoots length of these plantlets (1.77 cm) was lower than 'Elizabeth' and 'Meader' by 12 and 11%, respectively. It was observed, that 'Elizabeth' explants had 259 and 143% a smaller number of roots than cvs. Meader and Liberty, respectively. However, their length was not statistically different from that of the roots of the cultivar 'Meader'. In addition, plantlets of cultivar 'Meader' and 'Liberty' did not produce roots at both modifications of AN medium. Very weak rhizogenesis was observed only in case of 'Elizabeth' explants (Figure 3). The highest *in vitro* rooting rate (85%) was obtained for cv. Liberty on WPM A (WPM+0.5 mg L^{-1} IAA+0.1 mg L^{-1} zeatin) and WPM B medium (WPM+1.0 mg L^{-1} IAA+0.1 mg L^{-1} zeatin), the lowest - for both 'Elizabeth' and 'Meader' rooted on WPM A medium (Figure 3). When WPM A and WPM B medium were used, the roots' structure was well developed – the roots were longer, thicker and better branched (Figure 2). Therefore, for acclimatization to greenhouse conditions only plantlets with well-developed roots and the best rhizogenesis rooted on WPM A and WPM B medium were selected.

Table 4. *In vitro* rooting capacity of *V. corymbosum* 'Elizabeth', 'Liberty', and 'Meader'

Cultivar	Medium				
	Shoot length (cm)				
	AN A	AN B	WPM A	WPM B	MEAN
Elizabeth	2.01d*	1.56a-c	1.90b-d	1.27a	1.70A
Meader	1.98c-d	1.72a-d	1.85b-d	1.52a-c	1.74A
Liberty	1.69a-d	1.77a-d	1.53a-c	1.43ab	1.61A
MEAN	1.89B	1.67AB	1.76AB	1.43A	
	Number of roots				
	AN A	AN B	WPM A	WPM B	Mean
Elizabeth	0.06	0.18	0.83	0.82	0.41A
Meader	-	-	2.05	2.95	1.49B
Liberty	-	-	2.60	2.00	1.17B
MEAN	0.02A	0.07A	1.91B	2.14B	
	Root length (cm)				
	AN A	AN B	WPM A	WPM B	MEAN
Elizabeth	0.20	0.35	0.33	0.60b	0.43A
Meader	-	-	0.31	0.55	0.44A
Liberty	-	-	0.84b	1.43c	1.08B
MEAN	0.20A	0.35A	0.49A	0.77B	
	Fresh mass (mg)				
	AN A	AN B	WPM A	WPM B	MEAN
Elizabeth	30.01a	45.48	36.92a	63.51b	42.77A
Meader	33.10a	27.73a	39.33a	62.57b	42.47A
Liberty	38.12a	40.07ab	42.61ab	50.39ab	42.80A
MEAN	32.84A	37.50A	39.26A	60.33B	
	Dry mass (mg)				
	AN A	AN B	WPM A	WPM B	MEAN
Elizabeth	8.20a	10.20a	8.98a	15.80b	10.79A
Meader	8.70a	7.78a	9.50a	15.93b	10.48A
Liberty	9.26a	9.64a	10.04a	10.63a	9.98A
MEAN	8.86A	9.31A	9.18A	13.24B	

*Means followed by different letters in columns are significantly different at the 5% level according to Tukey's multiple ranges

Figure 2. *In vitro* rooting of highbush blueberry 'Elizabeth' (a), 'Liberty' (b) and 'Meader' (c) on WPM B medium

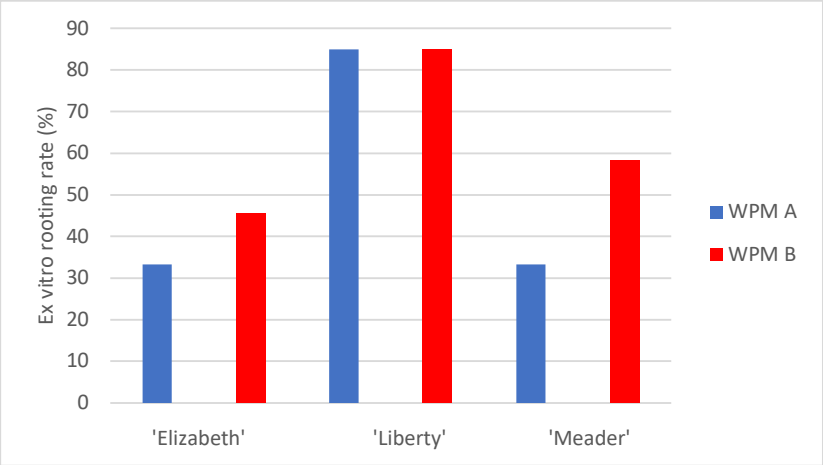

Figure 3. *Ex vitro* rooting rate (%) of *V. corymbosum* 'Elizabeth', 'Liberty' and 'Meader'

The percentage of plants acclimatized to the greenhouse conditions varied from 70% (for plantlets of cv. 'Liberty' from WPM B medium) to 30% (for plantlets of cv. 'Meader' from WPM A medium) (Figure 4). In the case of IAA concentrations used, plant survivability was lower by a 15 – 20% when 0.1 mg L^{-1} IAA (WPM A) was applied. Moreover, a positive correlation was observed between the *in vitro* rooting rate and the acclimatization rate. However, among the tested cultivars highbush berry 'Liberty' showed the highest percent of adapted plantlets (70%) with the best developed roots (Figure 5).

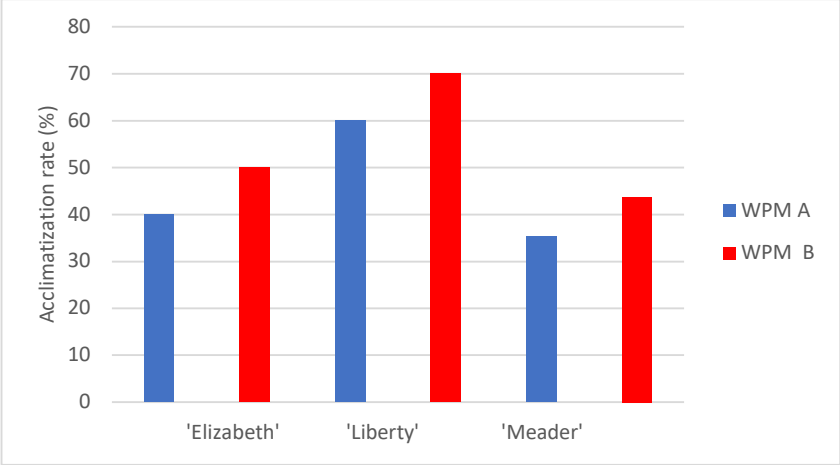

Figure 4. Acclimatization rate (%) of *V. corymbosum* 'Elizabeth', 'Liberty', and 'Meader'

Figure 5. Acclimatization of highbush blueberry 'Elizabeth' (a), 'Liberty' (b and d) and 'Meader' (c) at different stages

Discussion

Due to the great variability within genus *Vaccinium*, some species and cultivars still require further research to optimize multiplication media (Vescan *et al*., 2012; Fan *et al*., 2017). The addition of zeatin, in preliminary experiments to develop the optimum composition of medium for the initiation of highbush blueberry (data unpublish), showed a positive effect on plant growth. Zeatin at concentration 0.1 mg L^{-1} was the most favourable for shoot regeneration in *V. corymbosum* cultivars. As a result, it was decided to add to the medium zeatin at each stage of micropropagation. As suggested by many authors (Tetsumura *et al*., 2008; Ružic *et al*., 2012; Mohamed *et al*., 2018; Schuchovski and Biasi, 2019) the most frequently media used for *in vitro*

multiplication of highbush blueberry are AN and WPM, while MS was suggested as very effective medium for *Vaccinium* species by Cappelletti *et al.* (2016). This study also confirmed that WPM and MW (mixture of equal parts of MS and WPM media) achieves the most efficient multiplication of blueberry plants. However, multiplication rate depends not only on the medium but also on the response of individual species, cultivars, and even the original position of the explant from which nodal segments were excised for micropropagation (Cüce and Sökmen, 2017). Positive results in terms of blueberry regeneration efficiency were obtained culturing 'Elizabeth' and 'Meader' cultivars in a MW medium supplemented with IBA 0.4 mg L^{-1} or 0.2 mg L^{-1} (respectively) and zeatin 0.1 mg L^{-1}. Best regeneration efficiency was obtained culturing 'Liberty' shoots in a WPM medium supplemented with IBA 0.4 mg L^{-1} with GA_3 0.4 mg L^{-1} and zeatin 0.1 mg L^{-1}. The same tendency in positive influences of 0.5 mg L^{-1} zeatin combined with low concentration of IBA on length of axial shoots in highbush blueberry cultivars was observed by Litwińczuk and Wadas (2008), and Ružić *et al.* (2012). A much higher concentration of zeatin (2 mg L^{-1}) for the regeneration of shoots of highbush blueberry cv. 'Berkley' was successfully applied by Ostrolucká *et al.* (2007). Other responses of the growth regulators were observed by Vescan *et al.* (2012), indicated that 5 mg L^{-1} of 2iP (2-isopentenyladenine) was very good alternative to expensive zeatin for increasing efficiency and lowering cost *in vitro* culture establishment for highbush blueberry cv. 'Elliot'. Also, Cappalletti *et al.* (2016) reported that TDZ (tidiazuron) is more efficient in shoot regeneration of blueberry 'Duke' compared to zeatin.

Based on the results obtained, it can be concluded that the propagation medium is important for rooting of blueberry shoots. On the well-developed blueberry shoots, the roots also developed well. According to Mohamed *et al.* (2018) for *Vaccinium* species L. IBA and IAA are frequently used for *in vitro* root initiation and increasing root number and length even alone or in combination with each other. The process of rhizogenesis stimulated by the presence of auxin in the medium supports the elongation of root hair cells by importing auxin into non-root forming epidermal cells. Cellular auxin levels regulate the expression of genes that determine root formation. In our study, rooting capacity of shoots varied greatly among tested blueberry cultivars. WPM medium with the addition of IAA and 0.1 mg L^{-1} zeatin was the most effective for root induction. The rooting rate in cultivar Liberty (85%), along with the others rooting parameters, was significantly higher than in those obtained for 'Meader' and 'Elizabeth'. However, all cultivars tested in the study did not developed root on AN medium. Our findings are in agreement with previous report. Meiners *et al.* (2007) observed that the addition of 0.5 mg L^{-1} with activated charcoal (AC) gave the highest *in vitro* rooting success of different *Vaccinium* species. Also, Cüce and Sökmen (2017) concluded that as far as the rooting proces was concerned, IBA and IAA concentrations increased the root formation, whereas NAA treatments did not give positive response. In contrast, Ostrolucká *et al.* (2009) used AN medium supplemented with IBA and AC for *in vitro* rooting of *V. corymbosum* and *V. vitis-idaea* microshoots with the highest rooting success (85-95%). Sedlák and Paprštein (2009) achieved 70% rooting rate for cv. Berkeley, 61% for 'Bluecrop' and only 9% for cv. 'Spartan'.

Conclusions

The presented results confirm that the process of *in vitro* rhizogenesis of *Vaccinium* sp. depending on the plant species, the growth conditions of the mother plant and the physiological conditions of the explant. The *in vitro* regeneration protocol of highbush blueberry developed in this paper could help optimize shoot propagation system for other *Vaccinium* species and can be useful, for example, to produce certified vegetative material or biotechnological purposes.

Authors' Contributions

Conceptualization: M K-M; Data curation M F-K, M K-M; Formal analysis: M F-K, M K-M, I O; Funding acquisition: M F-K, M K-M, I O; Investigation: M F-K, M K-M; Methodology: M F-K, M K-M; Supervision: I O; Writing original draft: M F-K, M K-M, I O; Writing review and editing: M K-M; All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research was supported by the West Pomeranian University of Technology Grant No. 503-07-081-09/4.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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Notes:

ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 21(5) 2022, 25–37
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2022.5.3
ORIGINAL PAPER
Accepted: 11.03.2022

EFFECT OF ACTISIL (HYDROPLUS™), ORGANIC SUPPLEMENTS, AND PH OF THE MEDIUM ON THE MICROPROPAGATION OF *Vaccinium corymbosum*

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ABSTRACT

The effect of commercial Hydroplus™ Actisil, coconut water, coconut milk, and pH of the WPM medium on the micropropagation of *V. corymbosum* 'Liberty' was studied. Three experiments were performed with different concentrations of silicon Hydroplus™ Actisil (Si), coconut water (CW), coconut milk (CM), and different pH as a stress factor. Si was applied at a concentration of 50, 100, 200 and 500 mg dm^{-3}. The highest explant (2.02 cm) with the highest number of new shoots (1.91) and fresh weight (55.16 g) was obtained on WPM medium with the addition of Si in concentration 200 mg dm^{-3}. In experiment 2, similar to 0.1 mg dm^{-3} zeatin explant growth was achieved when 15% CW was added to the WPM medium (2.13 cm). The use of CM did not have a positive effect on blueberry growth *in vitro*. The results of experiment 3 indicated that explants of blueberry better developed when pH was lower (5.0) with the highest number of new shoots (2.85) and fresh weight (95.67g). However, there were no significant differences in plant height between pH used. The application of 200 mg dm^{-3} Actisil benefits the negative effect of higher pH of the WPM medium on micropropagation of blueberry in case of plant height, fresh weight, and biochemical parameters (proline, malondialdehyde – MDA and catalase – CAT activity).

Key words: blueberry, *in vitro*, zeatin, silicon, Woody Plant Medium, coconut water and milk

INTRODUCTION

Highbush blueberry (*Vaccinium corymbosum*) is increasingly important in fruit production due to its high content of biologically active substances, mainly flavonoids (anthocyanins, flavonols, and flavanols), procyanidins, and phenolic acids [Brazelton 2013, Schuchovski and Biasi 2019, Ochmian et al. 2020]. Rich in polyphenols, blueberry fruits show numerous health properties, improve vision, have antioxidant, anti-inflammatory, anticarcinogenic, and antibacterial effects [Schuchovski and Biasi 2019]. Recently, there has been a trend of renewing old varieties of highbush blueberry, which is closely related to global industrialization. Currently, China has become the world's fastest-growing highbush blueberry producer [Guo et al. 2019]. With the rapid expansion of blueberry cultivation scale and variety renewal, the increase in demand for healthy blueberry seedlings worldwide is inevitable. The growth of highbush blueberry bushes is limited to sites with low pH and moist soils with high organic matter content [Fin et al. 1991]. In addition, a major challenge for commercial growers is the low rooting percentage and the time required to propagate

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and market newly obtained cultivars [Schuchovski and Biasi 2019].

The use of *in vitro* cultures eliminates the limitations associated with traditional seedling propagation, providing an alternative to faster plant growth throughout the year, which increases production efficiency and profitability [Marino et al. 2014]. The best plant growth under *in vitro* conditions can be achieved by using the right composition and pH of the medium for the species and even the cultivar. Too low a pH (below 4.5) or too high a pH (above 7.0) inhibits the growth and development of *in vitro* explants [Pierik 1997]. For the initial phase of *in vitro* culture, a combination of cytokinins – mostly zeatin can usually be used [Ružić et al. 2012]. The use of natural zeatin as an additive to the culture media is expensive, therefore different solutions are being sought to give a similar effect at a lower cost. Such alternatives can be coconut water and coconut milk, which are rich sources of plant phytohormones (mainly cytokinins) [Ma et al. 2008]. Moreover, in numerous plant species, the beneficial effects of silicon on growth, development, yield, and disease resistance have been observed. Silicon is the second most ubiquitous element found in soils worldwide [Epstein 1999, Richmond and Sussman 2003, Currie and Perry 2007, Sivanesan and Park 2014]. Silicon content in soils can vary considerably from 1 to 45% of dry weight [Sommer et al. 2006], while in plants from 0.1% to 10% of dry weight [Ma and Takahashi 2002]. Hence, plants were classified into accumulating plants e.g. blueberry [Morikawa and Saigusa 2004, Figiel-Kroczyńska and Ochmian 2019], and non-accumulating plants e.g. petunia [Krupa-Małkiewicz and Calomme 2021]. Numerous authors described a positive role of Si in improving crop growth and yield under abiotic and biotic stress conditions by increasing the activity of antioxidant enzymes like CAT, increased accumulation of proline and malondialdehyde concentration (MDA), the secretion of endogenous hormones, and the production of lignins, chitinase, phenolic compounds, phytoalexin, and glucanases [Sahebi et al. 2016, Luyckx et al. 2017, Mandlik et al. 2020].

The experiment aimed in study the effect of silicon in the form of a commercial Hydroplus™ Actisil solution (Yara, Poland) and coconut water and milk on mitigating the effects of changing the pH of the medium used for the propagation of highbush blueberry. An appropriate concentration of silicon solution was selected for the micropropagation of highbush blueberry and the effectiveness of using coconut water and milk in the propagation medium as a substitute for the more expensive plant growth regulator – zeatin, was determined. Morphological (plant height, number of new shoots, fresh weight) and biochemical (proline, malondialdehyde MDA, catalase CAT activity) traits as well as leaf colour (CIE *L*a*b**) of highbush blueberry propagated on media of different composition and pH were observed.

MATERIAL AND METHODS

Characteristics of the area of research and plant material. Three separate experiments were conducted in the laboratory of the Department of Plant Genetics, Breeding and Biotechnology at the West Pomeranian University of Technology in Szczecin, Poland in 2020.

Silicon. In the first stage, the experiment on selection of silicon solution concentration with the most favourable effect on the morphological characters of the tested blueberry plants was performed. The plant material consisted of 17–20 mm shoots of *V. corymbosum* 'Liberty' obtained from sterile stabilized *in vitro* culture cultivated on the McCown Woody Plant medium – WPM [Lloyd and McCown 1981, Duchefa Biochemie B.V, The Netherlands]. The shoot explants were transferred to WPM medium with the addition of commercial silicon solution (Hydroplus™ Actisil, Yara Poland) in concentrations of 50, 100, 200, and 500 mg dm^{-3}. The silicon (0.6% Si) contained in Hydroplus™ Actisil was in the form of orthosilicic acid (H_4SiO_4), stabilized with choline. The pH of all media was adjusted to 5.8.

Coconut water (CW) and coconut milk (CM). The second experiment was conducted to establish the effects of the optimal concentration and source of promoting growth substance (coconut water and coconut milk) on the morphological growth parameters of *V. corymbosum* 'Liberty' as a cheaper substitute for plant growth hormone – zeatin. Explants (17–20 mm) were multiplied on the WPM medium supplemented with CW and CM in the concentration of 10% and 15% both. The control was WPM medium with the addition of 0.1 mg dm^{-3} zeatin. The pH of the medium

was adjusted to 5.8. Both coconut milk and water were added to the WPM medium before autoclaving.

Based on the two initial experiments, the concentration of Hydroplus™ Actisil equal to 200 mg dm^{-3} and coconut water 15% which gave the most beneficial effects on the morphological characteristics of the *V. corymbosum* 'Liberty' explants were used in the third (target) experiment.

pH, silicon, coconut water. The third experiment was conducted to establish the effect of commercial silicon solution and coconut water in alleviating the negative influences of pH on *V. corymbosum* growth and development. The explants (17–20 mm) were transferred to the WPM medium with different pH (5.0, 5.5, and 6.0) with or without 200 mg dm^{-3} Hydroplus™ Actisil, Yara or 15% coconut water.

General culture conditions. All media were supplemented with 3% (w/v) sucrose (Chempur, Poland), 0.8% (w/v) agar (Biocorp, Poland), 100 mg dm^{-3} myo-inositol (Duchefa Biochemie B.V, The Netherlands), heated and 30 ml were poured into a 450 ml flask. Next, they were autoclaved at 121°C (0.1 MPa) during the time required according to the volume of medium in the vessel. Cultures were incubated in a growth room at a temperature of 24 ±2°C under 16 hours photoperiod with a photosynthetic photon flux density (PPFD) of 40 µmol $m^{-2}s^{-1}$ provided by Narva (Germany) emitting daylight cool white. Each combination included 32 shoots (8 replications with 4 explants per flask). After 35 days, explants were removed and wash with deionized distilled water, and shoot and root length (cm), number of new shoots, and determination of colour were measured. The explants were weighed for estimation of plant fresh mass (g).

Determination of colour. The pigment (colour) of leaves (from the middle part of the shoot) was measured in transmission mode by photocolorimetric method in CIE *L*a*b** system [Hunterlab 2012] using spectrophotometr CM-700d (Konica Minolta, Japan). The diameter of the measurement hole was 3 mm, the observer type 10°, and the illuminant D65. The value of *a** was range from green (–*a**) to red (+*a**). The parameter *b** described the colour in the range from yellow (+*b**) to blue (–*b**). The value of parameter *L** means monochromaticity in the range from 0 (black) to 100 (white).

Analysis of proline, malondialdehyde (MDA), and determination of catalase (CAT) activity (EC 1.11.1.6). The concentration of proline was determined according to the method of Bates et al. [1973] using spectrophotometer at 520 nm in blueberry fresh leaves and calculated as µmol g^{-1} FW. The content of the malondialdehyde (MDA, a product of lipid peroxidation) in plant tissue was determined by the method described by Sudhakar et al. [2001]. The concentration of MDA was calculated from the absorbance at 600, 532, and 450 nm, and MDA contents were estimated using the following equations:

$$\text{MDA (nmol g}^{-1}\text{ FW)} =$$
$$= [6.45 \times (A532 - A600) - 0.56\ A450] \times V/FW,$$

where: V – volume of the sample, A – absorbance, FW – fresh weight.

The catalase (CAT) activity was determined according to the method by Lück [1963]. The decrease in absorbance, caused by the decomposition of H_2O_2, was monitored continuously at 240 nm for 90 s. One unit of enzyme is the amount necessary to decompose 1 µM H_2O_2 g^{-1} FW min^{-2}.

Statistical analysis. All statistical analyses were performed using Statistica 13.0 (StatSoft Polska, Cracow, Poland).

Statistical significance of the differences between means was determined by testing the homogeneity of variance and normality of distribution, followed by ANOVA with Tukey's post hoc test. The significance was set at $p < 0.05$. The relationship between morphological (plant height, number of new shoots, and fresh weight) and biochemical (proline, MDA, CAT activity) traits of highbush blueberry grown under *in vitro* conditions was illustrated.

RESULTS AND DISCUSSION

Application of Hydroplus™ Actisil in the WPM medium

Plant tissue culture media contain both non-organic and organic nutrients that support healthy plant growth. To improve growth and morphogenesis *in vitro*, the culture medium is often optimized and the extent of modification depends largely on the species

or even the cultivar [Sahebi et al. 2015]. The effect of Si in the nutrient solution was studied by several authors. Rodrigues et al. [2017] describe that silicon as a sodium silicate at a concentration of 1–2 mg dm^{-3} promotes better yam (*Dioscorea* spp.) development *in vitro*. Gallegos-Cedill et al. [2018] showed that the inclusion of Si (Siliforte®) to the nutrient solution of *V. corymbosum* L. cv. Ventura benefits from its vegetative growth. However, Costa et al. [2020] have used potassium silicate (K_2SiO_3) at a concentration of 1 mg mg dm^{-3} in liquid MS medium to propagate 'Dwarf Cavendish' banana in bioreactors. Their study demonstrated that silicon solution improved plantlet growth. However, the mechanism of the supplementation effect of Si on plant height is unclear [Kamenidou et al. 2008]. According to Morikawa and Saigusa [2004] Si is not known to be an essential element for the growth of blueberries, rather it was the element that accumulated mostly in the leaves. In addition, Si is not included in any commercial basal medium for plant tissue culture, may only be present in trace amounts.

In this study, the effect of commercial silicon solution (Hydroplus™ Actisil) supplementation on morphological traits (plant height, number of new shoots, fresh weight) and colour of the blueberries leaves propagated *in vitro* depended on its concentrations (Tab. 1, Fig. 1). It was observed that concentrations of 50, 100, and 500 mg dm^{-3} statistically decrease plant height from 50 to 41%, in comparison to the concentration of 200 mg dm^{-3} (2.02 cm). Also, the number

Table 1. Effect of different concentrations of silicon (Hydroplus™ Actisil) in the WPM medium on morphological traits and leaves colour (using CIE *L*a*b** system) of *V. corymbosum* 'Liberty' under *in vitro* condition comparison to the WPM medium with 0.1 mg L^{-1} zeatin, after 53 days of culture (n = 32 shoots per treatment)

		WPM medium				
		Zeatin (mg dm^{-3})	Hydroplus™ Actisil (mg dm^{-3})			
		0.1	50	100	200	500
Color parameters CIE leaves	*L**	36.40c*	29.25a	35.04bc	35.45c	31.62ab
	*a**	10.55a	10.22a	10.25a	12.64a	10.26a
	*b**	19.66a	15.76a	16.18a	21.00b	15.20a
Plant height (cm)		2.15b	1.19a	1.02a	2.02b	1.02a
Number of new shoots		2.75c	1.41a	1.38a	1.91b	1.13a
Fresh weight (mg)		36.36a	37.47a	50.61ab	55.16b	38.83a

*Means followed by different letters in columns are significantly different at 5% level according to Tukey's multiple range

WPM + 0.1 mg dm^{-3} zeatin, WPM + 50 mg dm^{-3} Actisil, WPM + 100 mg dm^{-3} Actisil, WPM + 200 mg dm^{-3} Actisil, WPM + 500 mg dm^{-3} Actisil

Fig. 1. *In vitro* grown *V. corymbosum* 'Liberty' plantlets after 35 days of culture on WPM medium with the addition of 0.1 mg dm^{-3} zeatin and different concentrations of Hydroplus™ Actisil

of new shoots per plant and plant fresh weight statistically decreased (from 41 to 26% and from 32 to 8%, respectively) when the concentration of 50, 100, and 500 mg dm^{-3} was used in WPM medium (Tab. 1).

Our results and the reported literature [Sivanesan and Park 2014, Rodrigues et al. 2017, Costa et al. 2020] indicated the positive role of silicon on the growth and development of several species, including an increase in the number of leaves and fresh weight of plants grown *in vitro*. The positive effect of Si on plants growth may be due to increased nutrient uptake and photosynthetic activity.

In our study, it was confirmed that according to the producer recommendation the commercial silicon solution (Hydroplus™ Actisil) at a concentration of 200 mg dm^{-3} is optimal for blueberry cv. Liberty *in vitro* cultivation.

The obtained results of the colour determination of the leaves analyzed in the transmitted mode using the photocolorimetric method in the CIE *L*a*b** system [Krupa-Małkiewicz et al. 2019], showed that the addition of 200 mg dm^{-3} Hydroplus™ Actisil to the WPM medium affected the parameter *L** of the highbush blueberry leaves as compared to the three other concentrations used in this study (Tab. 1). The value of which ranged from 29.25 (50 mg dm^{-3} Actisil) to 35.45 (200 mg dm^{-3} Actisil). It was observed that silicon solution did not affect the intensity of the green colour (parameter *a**). There were no significant differences among the examined four groups of plants. However, the colour of highbush blueberry leaves with an addition 200 mg dm^{-3} Hydroplus™ Actisil, was from 23 to 28% more yellow than the other three groups (Tab. 1).

Application of coconut water (CW) and coconut milk (CM) in the WPM medium

Coconut water and coconut milk were used to enhance axillary shoot growth compared to the more expensive zeatin, to precondition potential explants that were later used as a source of shoot explants in a further experiment. To stimulate *in vitro* responses, CW is usually added to the medium at concentration ranging from 5 to 30%. However, the optimum concentration varies among plant species and must be empirically determined [Al-Khayri 2010]. A number of authors suggested that the addition of 10% or 15% CW improved the number of shoots per explant, promoted callus induction and somatic embryogenesis, for instance in *V. corymbosum* [Fira et al. 2008], leaftea *Melothria maderaspatana* [Baskaran et al. 2009], lemon grass *Cymbopogon pendulus* [Bhattacharya et al. 2010]. Based on empirical experimentation involving a range of concentrations of CW, these studies have determined the optimum concentration of CW which would allow a similar effect to that of 0.1 mg dm^{-3} zeatin and would be a less expensive alternative.

The addition of 0.1 mg dm^{-3} zeatin or 10% and 15% CW to the WPM medium had a statistically significant effect on subsequent growth, as shown by the increased mean values for all shoot parameters in comparison to the CM (Tab. 2, Fig. 2). The plant height and number of shoots grown on WPM medium with 15% CW were similar compared to the WPM medium with the addition of 0.1 mg dm^{-3} zeatin. Whereas, the addition of CM to the WPM medium inhibited growth and shoot development of blueberry. On this medium plants grow slowly and no new shoots per plant were developed. Also, fresh weight (FW) in this group was from 49% to 54% lower than for plants grown on medium containing 0.1 mg dm^{-3} zeatin. The use of CM in the WPM medium did not have a positive effect on blueberry growth *in vitro*. The reason for this may have been that the high fat content of coconut milk can have a damaging effect on cell structure [Harkacz et al. 1997]. The fat may have disrupted osmosis and flow in the micropropagated blueberries explant. Therefore, coconut water was chosen for later studies.

In this study, it was observed that the addition of coconut water and zeatin to the WPM medium affected the colour of the highbush blueberry leaves as compared to the medium with coconut milk (Tab. 2). The leaves of plants on medium with CW and zeatin had the significantly brightest color and it was evidenced by parameter *L**. It was observed that the addition of CM and CW to the WPM medium, regardless of concentration, had no significant influence on the intensity of the green colour (parameter *a**). Measured on the blueberry leaf surface, that valus ranged from –1.83 (15% CM) to 1.34 (10% CW), in comparison to the 0.1 mg dm^{-3} zeatin (–6.55). However, it was observed that addition to the WPM medium CM significantly reduced the parameter *b** (indicated the location along the axis between yellow and blue). Blueberry leaves

Table 2. Effect of different concentrations of coconut water and coconut milk in the WPM medium on morphological traits and leaves colour (using CIE *L*a*b** system) of *V. corymbosum* 'Liberty' under *in vitro* condition comparison to the WPM medium with 0.1 mg L^{-1} zeatin, after 53 days of culture (n = 32 shoots per treatment)

		WPM medium				
		0.1 mg dm^{-3} zeatin	coconut water 10%	coconut water 15%	coconut milk 10%	coconut milk 15%
Color parameters CIE leaves	*L**	46.40c	42.36bc*	46.71c	34.08a	34.80ab
	*a**	–6.55b	2.12a	1.18a	2.21a	2.28a
	*b**	29.66c	26.33c	24.82c	20.03a	20.88ab
Plant height (cm)		2.15b	1.98b	2.13b	1.27a	1.29a
Number of new shoots		2.75b	1.13a	2.52b	1.00a	1.00a
Fresh weight (mg)		36.36b	31.33b	30.13b	16.96a	19.00a

*Means followed by different letters in columns are significantly different at 5% level according to Tukey's multiple range

WPM + 0.1 mg dm^{-3} zeatin WPM + 10% CM WPM + 15% CM WPM + 10% CW WPM + 15% CW

Fig. 2. *In vitro* grown *V. corymbosum* 'Liberty' plantlets after 35 days of culture on WPM medium with the addition of 0.1 mg dm^{-3} zeatin and different concentrations of coconut milk (CM) and coconut water (CW)

growing in the WPM medium with the addition of zeatin or CW (independence of its concentrations) indicated a higher value of parameter *b** (25.80–29.66) as compared to the other explants (Tab. 2).

Many authors [Peixe et al. 2007, Yong et al. 2009, Al-Khayri 2010] reported that coconut water contains mainly water (94%) and growth-promoting substances that influence *in vitro* cultures including inorganic ions, amino acids, nitrogenous compounds. CW and CM also appear to have growth regulatory properties, e.g., auxin, various cytokinins, GAs and ABA which are a class of phytohormones [Boase et al. 1993, Ma et al. 2008, Yong et al. 2009]. According to Yong et al. [2009] the cytokinin found in coconut water support cell division, and thus promote rapid growth. Other than in our study results described Boase et al. [1993], who indicated that the addition to the BAP-based MS medium 10% of coconut milk can improve shoot responses of *in vitro* grown kiwifruit, both males and females. As Peixe et al. [2007] and Al-Khayri [2010] points out, the use of natural organic compounds to replace expensive chemical sources of plant growth regulators can reduce the cost of micropropagation and consequently lower the market price for seedlings.

Effect of variable pH, silicon, and coconut water on in vitro development and growth of highbush blueberry

The effect of stress on plant growth is often determined by measuring shoot and root length [Krupa-Małkiewicz et al. 2019]. Low pH (high H^+ activity) can directly inhibit plant growth and development

[Kidd and Proctor 2001, Pavlovkin et al. 2009], possibly through adverse effects at the root plasma level, as well as through reduced uptake and translocation of mineral nutrients [Martins et al. 2011]. One of the limiting factors for the development and production of blueberry is the pH of the soil. It is well known that blueberries develop well in acidic soil with a pH of 4.5 to 5.5 and with low levels of fertility [Gallegos-Cedillo et al. 2018]. According to Fira et al. [2008] the pH recommended for the media used for Blue Crop *in vitro* propagation is 5.

When the blueberry 'Liberty' explants were cultured on media with different pH levels and with the addition of Hydroplus™ Actisil or coconut water, showed significant differences in plant height, a number of new shoots, FW, and biochemical traits tested (proline, MDA, and CAT activity) (Tab. 3 and 4, Fig. 3). In cultivar Liberty the mean plant height was 1.90 cm, regardless of the pH of the medium. The addition to the WPM medium 200 mg dm^{-3} of Hydroplus™ Actisil has increased in the plant height of 10%, and CW caused a reduction in shoot growth of 7%, in comparison to the control. Similarly for the mean FW, Si solution caused an increased of 26% of mean FW, but CW – decreased of 48% of mean FW compared to the control (74.1 mg). In contrast, both Actisil and CW reduced the number of new shoots per plant by 40% and 28%, respectively (Tab. 3). While no significant differences were observed for different pH of the WPM medium for such traits as mean plant height and mean number of shoots per plant, agreed with the visual observations that plants grown at higher pH were about the same height but had smaller leaves and were less vigorous when compared with the plants at low pH (Tab. 3). Although the mean plant FW was significantly lower on pH 6 of WPM medium compared to the other pH used. Similar results were obtained by Finn et al. [1991] when the tolerance of higher pH in the seedlings of *V. angustifolium* and *V. corymbosum in vitro* was studied. They have proposed that *V. augustifolium* could be a source of genes for tolerance to high pH. In other hand, Tsuda et al. [2014] showed a varied response of highbush blueberry to different pH levels (pH from 4 to 7) of culture medium. Moreover, they indicated that *in vitro* screening method could become a very useful tool for the selection of germplasm with tolerance to higher pH with very small planting within a short time.

Table 3. Effect of 200 mg dm^{-3} Hydroplus™ Actisil and 15% coconut water (CW) in the WPM medium with different pH on morphological traits of *V. corymbosum* 'Liberty' under *in vitro* condition after 53 days of culture (n = 32 shoots per treatment)

		WPM medium			
	pH	control	200 mg dm^{-3} Actisil	15% CW	mean
Plant height (cm)	5.0	1.93b*	2.07c	1.91b	1.97A
	5.5	1.86b	2.33d	1.51a	1.90A
	6.0	1.91b	1.83b	1.88b	1.87A
Mean		1.90B	2.08C	1.77A	
Number of new shoots	5.0	2.9f	1.3a	1.9cd	2.0A
	5.5	2.2de	1.7bc	1.8bc	1.9A
	6.0	2.5e	1.6b	1.8bc	2.0A
Mean		2.5C	1.5A	1.8B	
FW plant (mg)	5.0	95.7d	75.2c	40.0a	70.3B
	5.5	71.4c	123.4e	34.6a	76.5B
	6.0	55.1b	81.3c	40.9a	59.1A
Mean		74.1B	93.3C	38.5A	

*Means followed by different letters in columns are significantly different at 5% level according to Tukey's multiple range

Table 4. Effect of 200 mg dm^{-3} Hydroplus™ Actisil and 15% coconut water (CW) in the WPM medium with different pH on biochemical traits of *V. corymbosum* 'Liberty' under *in vitro* condition after 53 days of culture (n = 32 shoots per treatment)

		WPM medium			
	pH	control	200 mg dm^{-3} Actisil	15% CW	mean
Proline (μmol g^{-1})	5.0	15.3b*	12.2a	27.8e	19.6A
	5.5	19.8cd	15.7b	33.1f	21.7AB
	6.0	22.4d	17.8bc	31.7e	24.0B
Mean		19.2B	15.2A	30.9C	
MDA (nmol g^{-1})	5.0	18.8a	22.3b	43.0f	28.0A
	5.5	26.0c	17.5a	39.3e	27.6A
	6.0	35.5d	19.8ab	37.8de	31.0A
Mean		26.8B	19.9A	40.0C	
Catalze (μM H_2O_2 g^{-1} min^{-2})	5.0	61.1a	70.4bcd	76.8de	69.4A
	5.5	69.0bc	63.8ab	87.3f	73.4A
	6.0	72.3cd	67.9abc	83.2ef	74.5A
Mean		67.5A	67.4A	82.4B	

*Means followed by different letters in columns are significantly different at 5% level according to Tukey's multiple range

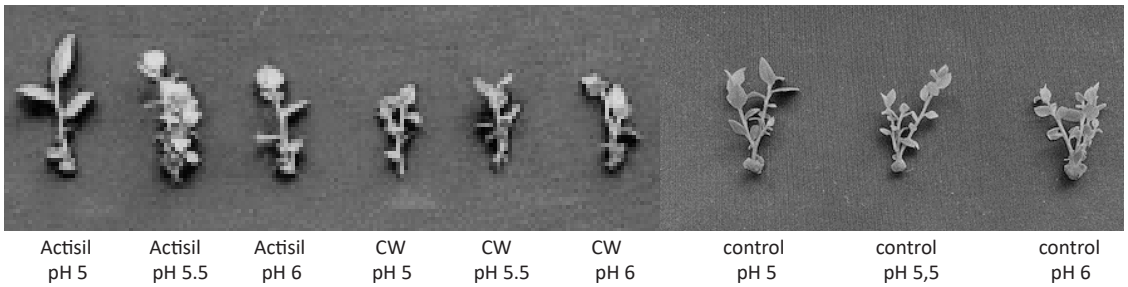

Fig. 3. *In vitro* grown *V. corymbosum* 'Liberty' plantlets after 35 days of culture on WPM medium with/without the addition of 200 mg dm^{-3} Hydroplus™ Actisil (Si) or 15% coconut water (CW) and different pH (5.0; 5.5; 6.0) as a control

Krupa-Małkiewicz and Calomme [2021] investigated the effects of different concentrations of Hydroplus™ Actisil on the morphological and biochemical traits of petunia under *in vitro* and greenhouse conditions. It was found that the addition of commercial silicon solution to the MS medium decreased plant height and root length but increased proline, MDA, total polyphenol and CAT activity, which may indicate that silicon provokes a stress response of the *in vitro* plants. In contrast, Gallegos-Cedillo et al. [2018] indicated that application of 1.2 mM of silicon (Siliforte®) benefits significantly the vegetative growth of blueberry plants (*V. corymbosum* L. cv. Ventura). They observed that increase the pH level to 6.25 decreased the dry weight of stem and leaves of blueberry. Maximum vegetative development was obtained at pH values ranging from 4.00 to 5.50 in coir fiber. In other hand, Fira et al. [2008] confirmed that it is possible to use CW to the medium instead of zeatin but a low number of new shoots were obtained and the explants grow slowly.

Biochemical parameters of the stress response

According to many authors [Barbosa et al. 2015, Krupa-Małkiewicz and Smolik 2019] elevated proline and MDA levels in plant tissues are quite a good indicator of the negative effects of various stress factors on a plant. Under the influence of stress factors, the production and accumulation of proline occurs, which provides protection to the cells by lowering the osmotic potential and drives water uptake. Many studies demonstrate that plants tolerant to different environmental stress factors accumulate more proline, than sensitive plants. An increased catalase activity may also indicate that a stress factor is acting on the plant. According to Sędzik et al. [2019], CAT is the major antioxidant enzyme associated with scavenging oxygen species (ROS).

In the present study, it was observed that as the pH of the medium increased, stress levels in plants increase, as indicated by increasing concentrations of proline, MDA and catalase activity (Tab. 4). The addition of Hydroplus™ Actisil to the WPM medium increased the concentrations of the tested biochemical parameters only when pH 5.0 of the medium was used, and decreased when the pH of the medium was 5.5 or 6.0, compared to the control. Deactivation of CAT is linked with decreasing concentration of the MDA, suggesting that oxidative damage may be reduced by the addition of Actisil. In contrast, proline concentration increased from 42 to 82% compared to the control, when the addition of 15% coconut water was used. Similarly, the addition of 15% CW to the WPM, regardless of the pH, increased concentrations of MDA (from 6 to 129%) and CAT activity (from 16 to 27%), compared to the control. The results of this study are in line with previous findings which showed Si as a plant-beneficial element associated with the mitigation of abiotic stresses [Barbosa et al. 2015, Gallegos-Cedillo et al. 2018].

Leaf colour

Many authors [Piwowarczyk et al. 2016, Krupa-Małkiewicz and Calomme 2021] suggested that environmental stress factors have a negative effect on the photosynthetic pigment content. According to Krupa-Małkiewicz and Calomme [2021] contents of photosynthetic pigment in leaves are closely correlated to their colour. In this study, it was observed that leaves of highbush blueberry differed significantly in colour (Fig. 4). The value of parameter *L** (reaching from 0 to 100, black and white, respectively) is usually used

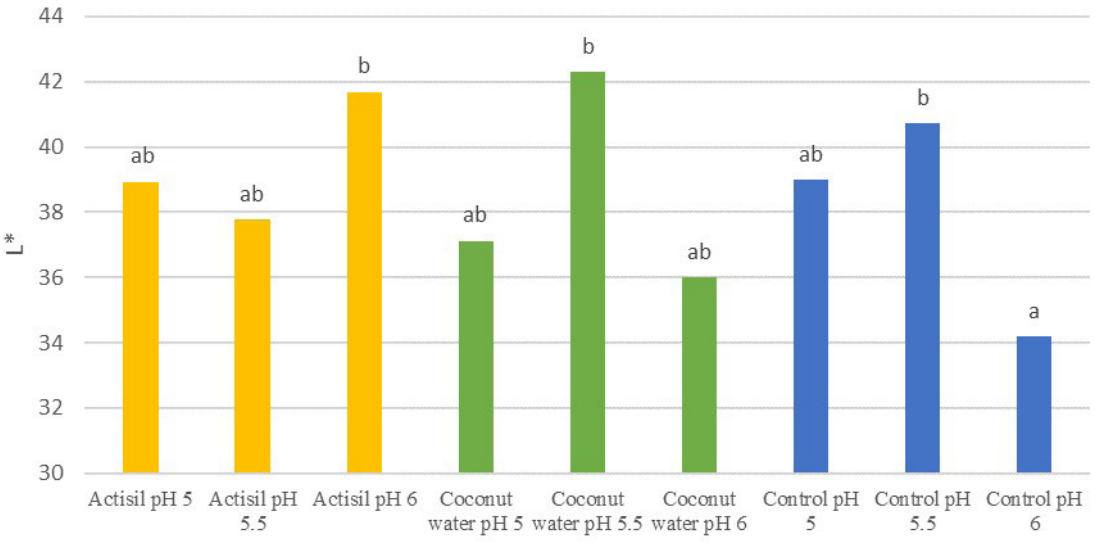

Fig. 4. Effect of 200 mg dm^{-3} Hydroplus™ Actisil and 15% coconut water (CW) in the WPM medium with different pH as a control on leaves colour using CIE *L*a*b** system, *L** the lightness coefficient at the end experiment. The figure: indicate x-axis – combinations of WPM medium, n = 32 shoots per treatments

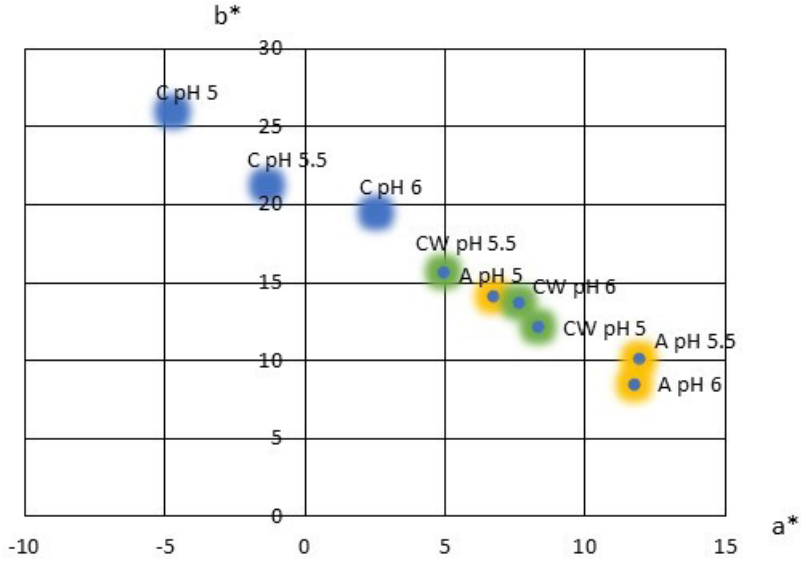

Fig. 5. Effect of 200 mg dm^{-3} Hydroplus™ Actisil (A) and 15% coconut water (CW) in the WPM medium with different pH as a control (C) on leaves colour using CIE *L*a*b** system, *a** (green colour) and *b** (yellow colour) at the end experiment, n = 32 shoots per treatments

for tracking the colour changes [Ochmian et al. 2010]. According to Krupa-Małkiewicz et al. [2019], the change in the parameter *L** is usually related to physiological attributes of the visual appearance of brightness. However, the highbush blueberry leaves grown on WPM medium pH 6.0 were the darkest (34.19). In the case of leaf colouration from the other combinations of the experiment, the differences were slight. The value of *a** parameter (colour ranging from green to red) determined on the surface of leaf ranges from –4.71 (control, pH 5.0) to 11.90 (WPM + 200 mg dm^{-3} Actisil, pH 5.5) (Fig. 5). It was observed that as the pH of the medium increased, the value of the parameter *a** increased. Opposite results were observed for the parameter *b** (colour ranging from yellow to blue), ranging from 25.98 (control, pH 5.0) to 8.52 (WPM + 200 mg dm^{-3} Actisil, pH 6.0) (Fig. 5). The addition of Actisil solution or coconut water to the WPM medium affected the colour of the highbush blueberry leaves as compared to the control group. Whereby, the leaves of highbush blueberry grown on WPM medium with addition of 15% CW were greener and less yellow than leaves grown on WPM medium with Actisil.

In the studies of Krupa-Małkiewicz and Calomme [2021], the application of Actisil in MS medium, regardless of concentration, had a positive influence on the intensity of the green and yellow colours of petunia leaves *in vitro*. According to Barbosa et al. [2015], increasing chlorophyll concentration in leaves can improve light interception and better performance of photosynthetic parameters.

CONCLUSIONS

In conclusion, this study has shown that micropropagation of *V. corymbosum* cv. Liberty can be enhanced by supplementing the culture medium with 200 mg dm^{-3} Hydroplus™ Actisil solution as recommended by the producer. Moreover, results have shown that using coconut water as a natural complex of organic substances influences plant height and number of new shoots and can replace zeatin. Maximum vegetative development of blueberry explants was obtained at pH 5. Commercial Si solution (Hydroplus™ Actisil) can be successfully used as an agent in blueberry *in vitro* culture, strongly stimulating plant growth and

reducing stress at higher pH of the medium. Coconut water did not alleviate the negative effects of the higher pH of the WPM medium in micropropagation of highbush blueberry.

SOURCE OF FUNDING

This research was supported by the West Pomeranian University of Technology Grant No. 503-07-081-09/4.

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ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 21(2) 2022, 87–101
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2022.2.8
ORIGINAL PAPER
Accepted: 6.10.2021

INFLUENCE OF VARIOUS TYPES OF LIGHT ON GROWTH AND PHYSICOCHEMICAL COMPOSITION OF BLUEBERRY (*Vaccinium corymbosum* L.) LEAVES

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ABSTRACT

It is important to use light that has a positive effect on plants. For plant growers, achieving the lowest possible cost of shrub production is crucial. We investigated the influence of light (white and violet LEDs as well as fluorescent white and red light) on the rooting and growth of blueberry cuttings (*V. corymbosum* L.) 'Aurora' and 'Huron'. Blueberry cuttings (4 cm tall) were planted into boxes with peat, which were placed in a phytotron at 22°C and illuminated for 16 hours a day. The plants died under the red fluorescent light source and, therefore, we discontinued its use. The other three light sources had a positive effect on plant growth and development. The light source had little effect on the content of macroelements in the leaves. Plants grown under white fluorescent and white LED light did not significantly differ in the height (22.0–25.8 cm), proline (4.67–7.23 μmol g^{-1}), and polyphenol content (4987–5212 mg 100 g^{-1}). In both cultivars, the violet LED light reduced plant growth and increased the content of polyphenols (6,448 mg 100 g^{-1}) and proline (8.11–9.06 μmol g^{-1}) in the leaves, which may indicate abiotic stress.
During the rooting of highbush blueberry cuttings, it is advisable to use white LED light. It has a positive economic impact on crop production due to low electricity consumption and it benefits the environment by eliminating mercury. The plant quality is similar to that of fluorescent white light.

Key words: phytotron, LED light, polyphenols, proline, macro- and microelements

INTRODUCTION

The genus *Vaccinium*, which belongs to the family Ericaceae, comprises approximately 400 species. The largest producers of blueberry are the USA, Chile, and Canada [Brazelton and Young 2017]. In Europe, blueberry production is concentrated in Poland, Germany, and Spain [Brazelton 2013]. In recent years, there has been an increasing consumer interest in blueberry fruit mainly because of its taste and health-promoting properties. The average annual growth rate of blueberry production worldwide is 8.2% (FAO UN 2018). Despite the increase in blueberry cultivation, the selling price of blueberries still remains very high [Podymniak 2015].

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Also the leaves are a very valuable material besides the fruit. Blueberry leaves, prepared and consumed as tea, are a rich source of potent phenolic antioxidants, and have very high TP content and significant reducing capacity according to the FRAP assay [Piljac-Žegarac et al. 2009]. Blueberry leaf tea supports digestion and is especially useful after eating fatty and hard to digest foods. This tea also has blood glucose lowering properties, hence it is recommended in the early stages of diabetes. It is also used in inflammatory conditions of the urinary and digestive systems. The infusion is aromatic and has a bitter, tannic taste due to the high amount of polyphenols [Wang et al. 2015].

Light is the primary environmental factor stimulating plant growth and development [Bourget 2008, Massa et al. 2008, Morrow 2008, Hogewoning et al. 2010], and its control is an increasingly used tool in production [Zoratti et al. 2014, Demotes-Mainard et al. 2016]. The intensity and composition of the radiation reaching the plants has a very strong influence on the formation of the aboveground plant parts and is a key factor in morphological development and leaf formation of plants [Pilarski and Kocurek 2014]. Plants detect and react to changes in light wavelengths with different types of photoreceptors, including phytochromes, thereby modulating their growth and development [Kami et al. 2010, Burgie et al. 2014, Galvão and Fankhauser 2015, Demotes-Mainard et al. 2016]. Phytochromes can be inactive or active, and the balance between these two forms changes dynamically with changes in the light spectrum composition in the range 300–800 nm, and it is strongly correlated with the red/far red (R/FR) ratio similar as for blue light [Holmes and Smith 1977, Sager et al. 1988, Kong et al. 2018]. For years, the most commonly used light sources for *in vitro* plant cultivation were high-pressure sodium lamps, incandescent lamps, fluorescent, and metal halide lamps [Hahn et al. 2000, Kim et al. 2005, Gupta and Jatothu 2013]; the wavelength of these light sources may not always be appropriate for plants, thereby adversely affecting plant growth and increasing production costs [Kim et al. 2005]. In recent years, light-emitting diodes (LEDs) have increasingly been used as a light source for plants in limited space cultivation [Morrow 2008, Hogewoning et al. 2010, Mengxi et al. 2010, Gupta and Jatothu 2013]. LEDs have found their application because of their good cost efficiency, relatively high power to light conversion rate, different colors (spectrum), relatively low surface temperature, long life, gas-free semiconductor construction, etc. [Bourget 2008, Morrow 2008, Olle and Viršile 2013, Kozai 2016]. Of the various narrow-spectrum lights, the blue and red LEDs are most commonly used for plant growth. The wavelengths of blue and red LED lights (460 and 660 nm, respectively) are highly effective in absorbing chlorophyll, resulting in optimal photosynthetic performance of plants [Massa et.al. 2008, Johkan et.al. 2010, Gupta and Jatothu 2013]. The results of numerous experiments have shown a variable response of plants to LED light, depending on the species and *in vitro* conditions [Hahn et al. 2000, Głowacka 2002, Kurilčik et al. 2008, Hung et.al. 2016].

Phenolic compounds can protect organisms from oxidative stress caused by free radicals [Scalbert et al. 2005]. The antioxidant activity of plant extracts is related to the presence of phytochemicals such as anthocyanins, phenolic acids, flavonoids, and tannins, which increase in stressful situations [Cao et al. 1996, Ochmian et al. 2015]. In addition to glycine betaine (GB), proline is one of the main organic osmolites that accumulate in plants in response to environmental stress, such as drought, salinity, extreme temperatures, UV radiation, and heavy metals [Rzepka-Plevneš et al. 2009, Ashrafa and Foolad 2016, Krupa-Małkiewicz et al. 2018]. Excessive proline accumulation is a common physiological reaction of plants under biotic and abiotic stress conditions [Verbruggen and Hermans 2008, Shevyakova et al. 2009, Liang et al. 2013, Krupa-Małkiewicz et al. 2018].

The aim of the experiment was to study the effect of different types of light on the growth and physico-chemical parameters of blueberry cuttings of Aurora and Huron cultivars.

MATERIALS AND METHODS

Study area and plant material

The experiment was carried out in a commodity farm that produces highbush blueberry plantlets in Dobrzany Municipality (Zachodniopomorskie Voivodeship, Poland) and in the lab of the Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Poland.

Shoot cultures were incubated in a growth chamber for 5 months at 22°C under four light sources with a 16 h photoperiod and photosynthetic photon flux density (PPFD) uniformly maintained at around 50–60 μmol s^{-1} m^{-2}. The research material included Aurora and Huron cultivars of highbush blueberry (*Vaccinium corymbosum* L.).

Characteristics of the light sources

In the experiment, the following linear LED lamps were used:

1) T8 4 ft (1212 mm) LED tube cool white, 18 W, Toshiba (hereafter referred to as white LED), wavelength 410–780 nm, light at 52 μmol s^{-1} m^{-2} photosynthetic photon flux density (PPFD);

2) T8 Flora LED light source for plants (1200 mm), 18 W, pink color, Greenie (hereafter referred to as violet LED), wavelength 410–510 and 580–710 nm, light at 60 μmol s^{-1} m^{-2} PPFD;

as well as the following linear fluorescent lamps:

3) mercury lamp with a luminophore L 36W/84 T8 Cool White, Lightech (hereafter referred to as white fluorescent lamp), wavelength 400–500, 530–550, and 580–630 nm, light at 36 μmol s^{-1} m^{-2} PPFD;

4) mercury lamp with TLD luminophore 36W/16, RED, Philips (hereafter referred to as red fluorescent lamp), wavelength 600–700 nm, light at 13 μmol s^{-1} m^{-2} PPFD.

Characteristics of the cultivars

'Aurora' (U.S. Plant Patent 15,185). Bushes have moderate vigor, are stocky, and with a spreading growth habit, especially when young. It begins to yield fruit at the end of July and continues to yield until the first days of September [Strik et al. 2014].

Berries are medium to large sized (1.5–2.5 g), dark blue, firm, with a small scar, mild flavored.he fruit needs to be let to hang on the plant to fully ripen and sweeten, and it hangs for much longer time, without shriveling, than Elliott cultivar does.

'Huron' (U.S. Plant Patent 21,777). The plants of this cultivar are vigorous and upright. Canes are numerous and moderately branched, and the fruits are well exposed. Yield is medium (performs better with cross-pollination) [Strik et al. 2014]. Plants possess excellent winter hardiness, and late flowering.

Its berries are moderately large (1.7–2.5 g), with small, dry picking scars, medium blue colored, with excellent firmness and superior flavor if allowed to fully ripen [Hancock 2011].

It is a productive, early ripening cultivar with very high fresh market quality and a long storage life.

Cuttings and substrate characteristics

Shoots were taken from mother plants that grew in the phytotron. The cuttings were cut into 3 cm fragments with two internodes (3 leaves). The first leaf has been removed. The prepared material was planted into boxes measuring 35 cm × 25 cm × 7 cm, and substrate height was 5 cm. In each box, there were 2 × 25 plantlets of a given cultivar arranged in five rows of five plantlets (Fig. 1). Four boxes were prepared for each combination. One repetition consisted of 25 plants. The substrate used in the experiment was a mixture 20 mL of Previcur Energy 840 SL (systemic multi-site fungicide), 20 L water and 90 L peat. Peat had 66.9% organic matter, EC 0.24 mS/cm, volume weight 0.35 kg dm^{-1}, full water capacity 85.2 %vv and pH in KCl 3.44. It was characterized by optimal content for blueberry [Komosa 2007] $N\text{-}NO_3$ + $N\text{-}NO_4$ – 2.64 mg 100 g^{-1}, P – 3.3 mg 100 g^{-1}, K – 5.1 mg 100 g^{-1}, high Ca 38.9 mg 100 g^{-1} and Mg – 7.0 mg 100 g^{-1}.

Measurements and observations

Leaf sampling. For analyses, 2 leaves were collected after 8 weeks from the middle part of all shoots from 20 plants from each box. All measurements (leaf area and colour) and analyses (proline, phenolic and mineral contents) were performed on these leaves. All chemical determinations were performed in triplicate.

Plant size. Plant height was measured four times at monthly intervals using a measuring tape with an accuracy of 1 mm. At the end of the experiment (after 5 months), the leaves were taken and stored for further analyses. On a representative sample of 100 leaves, the area of each leaf was measured with a DIAS 4 scanner (DIAS Infrared GmbH, Germany) [Ochmian et al. 2012].

Color measurement. Color parameters by model Commission Internationale de l'Eclairage (CIE) were: L^* (L^* = 100 indicated white; L^* = 0 indicated black), a^* (+a^* indicated redness; −a^* indicated greenness), and b^* (+b^* indicated yellow; −b^* indicated blue). Color coordinates were determined in the CIE $L^*a^*b^*$ space for the 10° standard observer and the D 65

Fig. 1. Blueberry cuttings

standard illuminant. CIE *L*a*b** was measured was measured using the CM700d spectrophotometer on a representative sample of 100 leaves from each combination (Konica Minolta, Osaka, Japan) [Hunterlab 2012, Ochmian et al. 2013].

Determination of proline content. The concentration of free proline was measured three times in each blueberry leaf, and proline accumulation was determined according to Bates et al. [1973].

Identification of phenolic compounds. Polyphenolic compounds were analyzed using UPLC-PDA--MS/MS Waters ACQUITY system (Waters, Milford, MA, USA) consisting of a binary pump manager, sample manager, column manager, photo diode array (PDA) detector, and tandem quadrupole mass spectrometer (TQD) with electrospray ionization (ESI) [Mijowska et al. 2016].

Identification of mineral contents. The contents of elements in leaves were determined after mineralization: N, P, K, and Ca were determined after wet mineralization in H_2SO_4 (96%, Chempur, Poland) and $HClO_4$ (70%, Chempur, Poland), whereas Cu, Zn, Mn, and Fe were determined after mineralization in HNO_3 (65%) and $HClO_4$ (70%) in the ratio of 3 : 1 [IUNG 1972]. The total N concentration was determined by the Kjeldahl distillation method, and $N\text{-}NO_3$ and $N\text{-}NH_4$ was determined potentiometrically [Lityński et al. 1976]. The K content was measured using atomic emission spectrometry, whereas the content of Mg, Ca, Cu, Zn, Mn, and Fe was measured using flame atomic absorption spectroscopy. The P content was assessed by the colorimetric method.

Statistical analysis

All statistical analyses were performed using Statistica 12.5 (StatSoft Polska, Cracow, Poland). Non-parametric methods (Kruskal–Wallis test) were used if neither the homogeneity of variance nor the normality of distribution was established previously. Statistical significance of the differences between means was determined by testing the homogeneity of variance and normality of distribution, followed by multifactor ANOVA with the Tukey's post hoc test. The significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Plant growth

In our experiment, white and violet LED light and white fluorescent light had a positive effect on the growth of blueberry plants, whereas red fluorescent light did not have the predicted effect on plant growth. Although the light spectrum of red fluorescent lamps (λ 600–700 nm) is supposed to increase photosynthetic activity, plant growth was not observed in either

blueberry cultivar since the beginning of the experiment. Moreover, doubling the number of fluorescent lamps in order to increase the luminous flux did not have a positive effect on plant growth efficiency. Plant growth may have been negatively affected by the low photosynthetic photon flux density of these lamps. They also emit wavelengths in the very limited 600–700 nm range. Studies have shown that blue light has a significant effect on chlorophyll biosynthesis [Kamiya et al. 1981, Shin et al. 2008, Klamkowski et al. 2012]. It was found that the low concentrations of Chl *a* and *b*, which in plants have been treated with monochromatic red light, have even led to photooxidative stress in plants due to an increase of O_2^- and H_2O_2. radicals [Bae and Choi 2008, Hogewoning et al. 2010]. A minimum of 20−30 µmol s^{-1} m^{-2} of blue light is necessary to reach natural-like growth and plant morphologies [Barnes and Bugbee 1992]. Other experience shows that the most effective lighting is red and blue in the correct ratio for the species [Ohtake et al. 2018, Pennisi et al. 2019, Chiang et al. 2020]. Therefore, we decided to stop the experiments with red fluorescent light. The plants did not develop new shoots, leaves, or root systems, and they started to dry out. The leaves were green and firm, but gradually turned brown and dried up. On the other hand, the blueberry plants illuminated with the other three light types (white fluorescent, white LED, and violet LED) showed undisturbed growth. The plants looked typical, had a vivid green color, and similar internode lengths. Under phytotron conditions, we found that plants of the Aurora cultivar were 7.5% higher than the plants of Huron cultivar regardless of the light source (Tab. 1). However, the analysis of the type of light showed that violet LED light had the worst impact on plant growth. Despite having a similar photosynthetically active radiation (PAR) value, the plants of both cultivars had the lowest height compared to the fluorescent and LED white light (Tab. 1, Fig. 2).

Table 1. Characteristics of the highbush blueberry 'Aurora' and 'Huron' depending on the type of light

Plant parameters		Cultivars	Type of light			
			fluorescent white lamp	white LED	violet LED	mean
Height of plants (cm)		Aurora	25.8 ±1.7d	24.1 ±1.2c	20.5 ±1.9a	23.5B
		Huron	22.9 ±1.3b	22.0 ±1.5b	20.6 ±1.6a	21.8A
		mean	24.4B	23.1B	20.6A	
Leaf area (cm^2)		Aurora	6.62 ±0.42ab	7.63 ±0.49b	7.15 ±0.39ab	7.13A
		Huron	7.12 ±0.39ab	6.95 ±0.44ab	6.11 ±0.35a	6.73A
		mean	6.87AB	7.29B	6.63A	
Proline (µmol g^{-1})		Aurora	4.67 ±0.17a	5.85 ±0.22b	8.11 ±0.27d	6.21A
		Huron	7.23 ±0.24cd	6.71 ±0.29c	9.06 ±0.25e	7.67B
		mean	5.95A	6.28A	8.59B	
Colours parameters CIE	*L**	Aurora	46.49 ±3.17d	42.59 ±2.58bc	41.28 ±3.37b	43.45B
		Huron	39.96 ±1.97a	39.69 ±1.84a	43.06 ±2.99c	40.90A
		mean	43.23A	41.14A	42.17A	
	*a**	Aurora	–32.29 ±1.73bc	–33.03 ±1.45c	–29.58 ±1.33a	-31.63A
		Huron	–29.14 ±1.40a	–31.85 ±0.99b	–28.51 ±1.17a	-29.83A
		mean	–30.72AB	–32.44B	–29.05A	
	*b**	Aurora	24.42 ±1.82d	21.4 ±0.98c	18.16 ±1.06a	21.33A
		Huron	20.83 ±1.38bc	20.89 ±1.15bc	20.11 ±1.30b	20.61A
		mean	22.63B	21.15B	19.14A	

Mean values denoted by the same letter do not differ statistically significantly at 0.05 according to the Tukey's test. Small letters indicate the interaction between factors (cultivars and light), large letters indicate the main factors

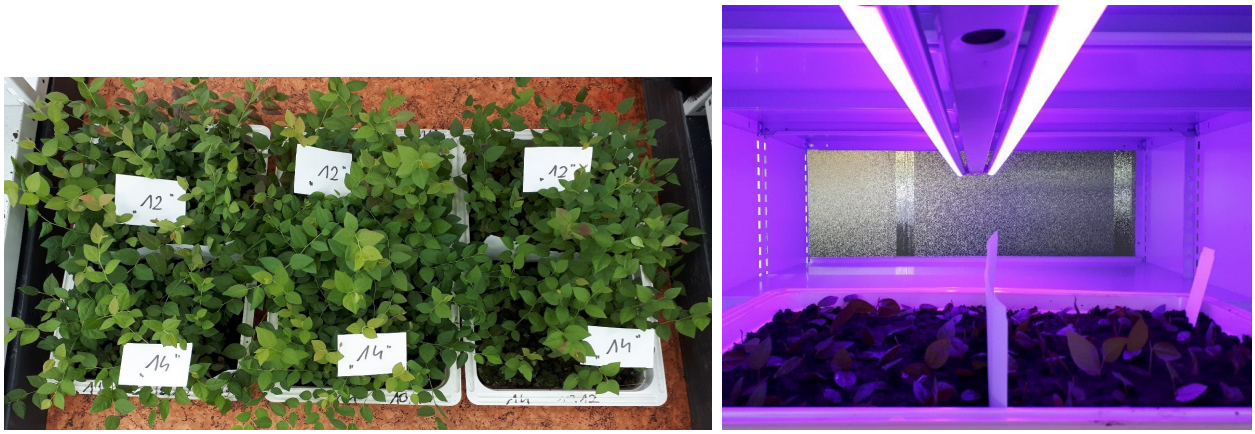

Fig. 2. Plants of the highbush blueberry 'Aurora' and 'Huron' depending on the light used; from the left – white LED, white fluorescent lamps, violet LED

Analysis of the light types showed that the plants had the largest area of one leaf (7.29 cm^2) under white LED light and the smallest one under violet LED light (6.63 cm^2) (Tab. 1). Plants of both cultivars illuminated with violet LED light were also less green – it is indicated by a^* and b^* parameters. This suggests that the plants illuminated with this light had less amount of chlorophyll. The leaves of the Aurora cultivar were lighter than those of Huron, as evidenced by the CIE parameters L^* (Tab. 1). The brightest leaves of Aurora cultivar were measured on plants that were illuminated with a fluorescent white lamp. The value of the L^* parameter (reaching from 0 to 100, black to white, respectively) is usually used for tracking color changes [Ochmian et al. 2019a]. The leaf color determined by the CIE L^* parameter was typical for highbush blueberry and similar to that of plants in other studies [Ochmian 2012].

According to several authors [Yang and Zhang 2011, Krupa-Małkiewicz et al. 2019], elevated proline levels in plant tissues are fairly good indicators of the negative effects of various stress factors on plants. In the present study, 23.5% higher proline level was observed in 'Huron' plants than in 'Aurora' plants. This may indicate that compared to 'Huron' plants, 'Aurora' plants have higher sensitivity to light stress. Moreover, higher proline values were observed in plants of both cultivars growing under the influence of violet LED light (Tab. 1). Similar results were described by Chen et al. [2012] in the highbush blueberry cultivar Sharpblue, which had a higher resistance to elevated temperatures but also a higher proline content than the other 3 cultivars – Duke, Brigitta, and Misty.

Macro- and microelements

Compared to the other light sources, the violet LED has increased the accumulation of N, K, and Ca in the 'Aurora' leaves and the opposite reaction of N and Mg uptake was observed in 'Huron' (Tab. 2). Depending on the standards for highbush blueberry developed by different authors [Eck 1988, Bal 1997, Komosa 2007, Ochmian et al. 2021], most of the investigated macroelements in the leaves were at an optimal level. The exception was calcium; although it was at a high level in the substrate 38.9 mg 100 g^{-1}, optimum 10–30 mg 100 g^{-1} (Tab. 2), we found that it was significantly below the recommendations for blueberry leaves; 0.40–0.80 mg 100 g^{-1}. The content of macroelements in the leaves of both cultivars was similar to that in the leaves of plants grown in peat under field conditions [Ochmian et al. 2019a]. However, in field conditions (loamy sand), low nitrogen content was recorded in the leaves even after the application of the optimal doses of nitrogen [Ochmian et al. 2018]. The nutrient content in the leaves is a good indicator of plant nutritional status. However, in the case of blueberry bushes, this can be difficult to determine because different authors had different specifications of the optimal abundance of certain elements (for example N) in blueberry leaves 1.7%–2.1% [Hanson 2006], 2.10% [Smolarz and

Table 2. The content of macroelements and microelements in leaves of highbush blueberry 'Aurora' and 'Huron' depending on the type of light

Mineral element	Cultivars	Type of light			
		fluorescent white lamp	white LED	violet LED	mean
		g 100 g^{-1}			
N	Aurora	1.78 ±0.06*a*	1.89 ±0.05ab	1.95 ±0.07b	1.87A
	Huron	2.24 ±0.0.07c	2.35 ±0.08c	1.97 ±0.06b	2.19B
	mean	2.01A	2.12A	1.96A	
P	Aurora	0.23 ±0.01a	0.25 ±0.01ab	0.22 ±0.00a	0.23A
	Huron	0.29 ±0.01c	0.28 ±0.01bc	0.31 ±0.01c	0.29A
	mean	0.26A	0.27A	0.27A	
K	Aurora	0.41 ±0.02b	0.37 ±0.01a	0.46 ±0.02c	0.41A
	Huron	0.50 ±0.02cd	0.52 ±0.02de	0.56 ±0.02e	0.53B
	mean	0.46A	0.45A	0.51A	
Ca	Aurora	0.23 ±0.00a	0.24 ±0.01ab	0.27 ±0.01bc	0.25A
	Huron	0.28 ±0.01c	0.26 ±0.01ac	0.29 ±0.01c	0.28A
	mean	0.26A	0.25A	0.28A	
Mg	Aurora	0.15 ±0.01a	0.17 ±0.01ac	0.16 ±0.01ab	0.16A
	Huron	0.18 ±0.01bc	0.19 ±0.01c	0.15 ±0.01a	0.17A
	mean	0.17A	0.18A	0.16A	
		mg 1000 g^{-1}			
Fe	Aurora	47.3 ±2.7a	53.2 ±3.4ab	58.9 ±3.2b	53.1A
	Huron	84.4 ±5.4e	67.8 ±4.3c	77.5 ±4.9d	76.6B
	mean	65.8AB	60.5A	68.2B	
Zn	Aurora	5.23 ±0.19a	5.74 ±0.23b	7.05 ±0.28c	6.01A
	Huron	7.11 ±0.21c	7.85 ±0.24d	8.36 ±0.20e	7.77B
	mean	6.17A	6.80B	7.71C	
Mn	Aurora	72.1 ±0.3ab	65.8 ±0.2a	83.4 ±0.2c	73.8A
	Huron	94.2 ±0.4d	99.4 ±0.3d	80.6 ±0.3bc	91.4B
	mean	83.2A	82.6A	82.0A	
Cu	Aurora	1.77 ±0.04bc	1.82 ±0.05c	1.64 ±0.03a	1.74A
	Huron	1.73 ±0.03b	1.94 ±0.04d	1.79 ±0.03bc	1.82A
	mean	1.75A	1.88B	1.72A	

Explanations in Tab. 1

Mercik 1993], 1.52%–2.17% [Glonek and Komosa 2006], 1.8–2.1% [Eck 1988], 2.25%–2.75% [Pliszka 2002], 1.8%–2.0% [Sagoo et al. 2016].

The nitrogen content in the leaves of the 'Aurora' and 'Huron' plants had 1.87 and 2.19 mg 100 g^{-1}, respectively (Tab. 2). The high nitrogen content in the leaves of the 'Huron' plants grown under white LED light was also confirmed by their intensive color (Tab. 1). The type of light also influenced the iron content in the leaves of the two cultivars. The average iron content in the plants exposed to violet LED light was 11.3% higher than in plants exposed to white LED light. 'Huron' plants had on average 30.7% more iron than 'Aurora' plants (Tab. 2). Despite the lower amount of Fe in the leaves of 'Aurora' shrubs than that in the recommendations by Eck [1988], the plants did not show any external signs of Fe deficiency. Similar to N, the standards for Fe content differ among studies by different authors (43–61 mg 1000 g^{-1} in Glonek and Komosa 2006; 60–200 mg 1000 g^{-1} in Eck 1988]. In both cultivars, Zn and Cu contents were lower than those in the standards (respectively; optimum 8–30 mg 1000 g^{-1} and 2–20 mg 1000 g^{-1}), and low copper content in peat soils is common [Ochmian et al. 2019a]. However, in other field experiments, the iron content in the leaves was at a higher level [Ochmian et al. 2019b] compared to the plants grown in a phytotron. Glonek and Komosa [2006] found similar Fe content in blueberry leaves (53.9–57.7 mg kg) as those in the leaves of 'Aurora', and higher Mn content (107.6–128.0 mg kg) than those in the leaves of both cultivars from the present study. Mn is an essential element that is bound to a number of essential enzymes; for example, the activity of superoxide dismutase is suppressed by low Mn status [Li and Zhou 2011]. The optimum Mn content in leaves should be 0–350 mg 1000 g^{-1} [Eck 1988]. Rivera et al. [2015] concluded that the Fe content of the soil depends on how it is used.

Polyphenols

Studies [Oszmiański et al. 2011, Li et al. 2013, Değirmencioğlu et. al. 2017] have shown that highbush blueberry leaves are a valuable source of polyphenols. In the leaves of the two investigated blueberry cultivars, we identified 12 phenolic acids, eight flavonols, and six flavan-3-ols. High polyphenol content may indicate the effect of a stress factor, in this case a light source (namely the range of light wavelengths). Flavonoids (including flavonols, anthocyanins) and carotenoids play a major role in protecting plants against the harmful effects of short-wave super-violet light (UV 280–400 nm) [Pilarski and Kocurek 2014, Vidović et al. 2015, Ochmian et al. 2019b]. The observed high content of different polyphenols in blueberry leaves depended mainly on the type of light, but also on the cultivar. Plants illuminated with violet LED light had the highest polyphenol content; they were also the lowest and had the smallest leaves. This may explain the increased accumulation of Fe and Zn in leaves treated with violet light. These elements are important for the activity of the enzymes, catalase (Fe) and superoxide dismutase (Fe, Zn) [Bhoomika et al. 2013]. This suggested that this type of light was a stress factor for blueberry plants. Plants illuminated with white fluorescent light had the lowest level of polyphenols in their leaves; in addition, they were the tallest and had the largest leaves. There was also a significant difference in polyphenol content between the cultivars. The average content of the determined polyphenols in 'Aurora' was 6705.27 mg 100 g^{-1}, and it was 34.5% higher than that in 'Huron' (Tab. 3). The polyphenol content in the leaves of 'Aurora' was similar to that in the leaves of *Vaccinium myrtillus* [Değirmencioğlu et al. 2017]. Li et al. [2013] and Oszmiański et al. [2011] indicated that the leaves of highbush blueberry are richer in polyphenols compared to the leaves of other fruit plants, including blackberry, raspberry, honeyberry, and strawberry.

A positive relationship was observed between the content of two main groups of polyphenols, phenolic acids and flavonols, in plants grown under violet LED light. The content of these polyphenols was very high in these plants. On average, the results for both cultivars (Tab. 3) showed that compared to white fluorescent light and white LED light, violet LED light caused an increase in leaf polyphenols by as much as 29% and 23%, respectively. Additionally, plants illuminated with violet LED light had high proline content, which led us to conclude that violet light caused stress in the examined plants. The highest total polyphenol content (7440.44 mg 100 g^{-1}) was recorded in Aurora cultivars illuminated with violet LED light, whereas the lowest was recorded in Huron cultivars illuminated with white fluorescent light (54.5% lower than that in

Table 3. The content of polyphenols in leaves of highbush blueberry 'Aurora' and 'Huron' depending on the type of light

Cultivars	Aurora			Huron			Aurora	Huron
Type of light	fluorescent white lamp	white LED	violet LED	fluorescent white lamp	white LED	violet LED	mean	mean
1	2	3	4	5	6	7	8	9
Compounds (mg 100 g^{-1} DW)	Phenolic acid							
1-*O*-Caffeoylquinic acid	30.89 ±0.06e[a]	10.19 ±0.02b	11.65 ±0.02c	14.23 ±0.03d	8.26 ±0.02a	9.91 ±0.02b	17.58B	10.80A
trans-3-*O*-Caffeoylquinic acid	538.37 ±1.08c	476.26 ±0.95b	1030.20 ±2.06e	327.89 ±0.66a	480.60 ±0.96b	933.40 ±1.87d	681.61B	580.63A
cis-3-*O*-Caffeoylquinic acid	17.06 ±0.03e	7.86 ±0.02b	14.53 ±0.03d	10.46 ±0.02c	10.64 ±0.02c	5.49 ±0.01a	13.15B	8.86A
3-*O*-*p*-Coumaroylquinic acid	72.46 ±0.11d	23.82 ±0.05b	40.39 ±0.08c	8.76 ±0.02a	11.50 ±0.02a	206.36 ±0.41e	45.56A	75.54B
5-*O*-*p*-Coumaroylquinic acid	2704.32 ±4.33d	2624.76 ±5.25d	2964.60 ±5.93e	894.97 ±1.79a	1072.78 ±2.15b	1483.00 ±2.97c	2764.56B	1150.25A
trans-5-*O*-Caffeoylquinic acid	20.01 ±0.04b	13.16 ±0.03a	23.55 ±0.05c	22.69 ±0.05c	28.04 ±0.06d	27.90 ±0.06d	18.91A	26.21B
5-*O*-Caffeoylquinic acid	269.52 ±0.53b	372.83 ±0.75d	688.64 ±1.38f	183.31 ±0.37a	328.33 ±0.66c	529.83 ±1.06e	443.66B	347.16A
4-*O*-Caffeoylquinic acid	31.72 ±0.06d	7.44 ±0.01c	5.42 ±0.01b	4.91 ±0.01b	4.94 ±0.01b	2.19 ±0.00a	14.86B	4.01A
3-*p*-coumaroyl-5-feruloy-lquinic	18.1 ±0.04f	0.39 ±0.00a	1.32 ±0.00b	9.52 ±0.02e	2.15 ±0.00c	3.03 ±0.01d	6.60B	4.90A
3,5-Di-*O*-caffeoylquinic acid	6.28 ±0.01a	41.88 ±0.08e	21.21 ±0.04d	7.28 ±0.01b	9.62 ±0.02c	65.15 ±0.13f	23.12A	27.35B
4,5-Di-*O*-caffeoylquinic acid	6.28 ±0.01a	7.01 ±0.01b	8.12 ±0.02c	8.76 ±0.02d	15.24 ±0.03f	13.89 ±0.03e	7.14A	12.63B
cis-5-*O*-*p*-Coumaroylquinic acid	3.65 ±0.01b	3.98 ±0.01b	6.25 ±0.01c	3.59 ±0.01b	2.81 ±0.01a	2.51 ±0.01a	4.63B	2.97A
Total	3718.66 ±7.65d	3589.58 ±7.18d	4815.89 ±9.63e	1496.37 ±2.99a	1974.92 ±3.95b	3282.66 ±6.57c	4041.37B	2251.31A
	Flavonols							
Myricetin 3-*O*-galactoside	13.09 ±0.03c	16.58 ±0.03d	31.89 ±0.06e	1.83 ±0.00a	6.71 ±0.01b	13.35 ±0.03c	20.52B	7.30A
Myricetin 3-*O*-glucoside	8.10 ±0.02d	0.68 ±0.00bc	0.97 ±0.00c	0.11 ±0.00a	0.30 ±0.00ab	0.64 ±0.00bc	3.25B	0.35A
Myricetin hexoside-acetate	1.19 ±0.00b	0.92 ±0.00b	2.34 ±0.00d	0.40 ±0.00a	1.55 ±0.00c	2.39 ±0.00d	1.48A	1.45A
Quercetin 3-*O*-diglucoside	0.33 ±0.00a	0.37 ±0.00a	1.92 ±0.00c	0.41 ±0.00a	0.64 ±0.00b	3.21 ±0.01d	0.87A	1.42B
Quercetin 3-*O*-rutinoside	26.59 ±0.05c	29.14 ±0.06d	82.59 ±0.17	14.99 ±0.03a	19.17 ±0.04b	34.98 ±0.07e	46.11B	23.05A
Quercetin 3-*O*-rutinoside	1.81 ±0.00c	1.77 ±0.00c	5.55 ±0.01d	0.25 ±0.00a	1.05 ±0.00b	0.95 ±0.00b	3.04B	0.75A

'Aurora'). Very high contents of trans-3-*O*-caffeoylquinic acid (1030.20 mg 100 g^{-1}) and 5-*O*-p-coumaroylquinic acid (2964.60 mg 100 g^{-1}) were also recorded in Aurora cultivars grown under violet LED light. Generally, among the 35 examined polyphenols, 5-*O*-p-coumaroylquinic acid occurred in the highest amount in the investigated plants. Degree of polymerization is an important structural feature determining the bitterness and astringency of taste and the biological activity of proanthocyanidins [Waterhouse and Ebeler 1998]. Thus, in measured samples were noted the statistical differences between fluorescent white lamp and LED (white and violet). The highest DP was found in the sample after fluorescent white lamp used and was 17% higher than in the sample after LED.

Production economics

When choosing a particular light source, manufacturers are primarily guided by economic profit. They expect a good quality product that is also energy-efficient at a low price. Violet LED lamps used in this experiment were more expensive than white LED lamps and white fluorescent lamps. In economic calculations (Tab. 4), we noticed the scale of savings that can be achieved with LEDs solution. The phytotron, in which we have conducted the experiment, allows us to produce 20.000 plantlets in one cycle. This requires 200 fluorescent lamps with a length of about 120 cm. In the present study, a white fluorescent lamp was 76.26% cheaper than a violet LED lamp and 44.51% cheaper than a white LED lamp. Taking into account the purchase price of fluorescent lamps, the price of electricity, energy consumption, photoperiods, bulb life, and the annual cost of bulb maintenance, we calculated the annual cost of phytotron maintenance (Tab. 4). Considering all costs generated in the annual cycle in our phytotron, we found that the cheapest solution were white LED lights (3044 euros a piece). Despite the high price for a single violet LED lamp, the cost of maintaining the phytotron was slightly higher (3305.60 euros). This was because of the longer service life of fluorescent lamps, which by the manufacturer is 50.000 h. White fluorescent lamps were the most expensive to maintain. Despite the low price of this light source, because of its very short service life (15.000 h), its maintenance cost was 6.555 euros. However, according to the manufacturer, the photosynthetic efficiency of these fluorescent lamps decreases remarkably after 12.000 h. In practice, after 2 years, the fluorescent lamps in phytotrons are usually replaced even if they are still working.

By choosing LED lamps, we achieved about 50% savings compared to using fluorescent lamps. Production economics showed that it is better to use LED sources than other light sources for plant production in phytotrons. Fluorescent lamps are economically disadvantageous, and the mercury contained in them is also dangerous for the environment. LEDs are safe

Table 4. Economic effects of the annual greenhouse maintenance depending on the type of light

Cost of using lamp	Type of light		
	fluorescent white lamp	white LED	violet LED
Price of 1 lamp (EUR)	8.84	3.78	15.93
Electricity consumption (W)	36	18	18
Uptime (h)	15 000	40 000	50 000
Uptime in years	2.57	6.85	8.56
Annual power consumption 1 lamp (16 h/day × 365 days = year × lamp power kW) = kW/year	210	105	105
Price 1 kWh (EUR)	0.14	0.14	0.14
Power consumption cost for 1 lamp per year (EUR)	29.34	14.67	14.67
Annual cost of using 1 lamp (EUR) energy + lamp price	32.78	15.22	16.53
Annual cost of phytotron work (EUR)	6555.29	3044.00	3305.60

for the user and the environment because they are not made of brittle glass, do not heat up, and do not contain any dangerous materials such as mercury [Olle and Viršile 2013].

The results of research on production economics showed that the use of a white LED light source is a good solution for blueberry production. Its maintenance was significantly cheaper than fluorescent lamps, and plants grown under this light source had the largest leaf surface area among all investigated light sources. In addition, on average, the plants grown under white LEDs were vivid green and did not differ in height from the plants grown under white fluorescent light. However, according to the results of our experiment, in order to obtain polyphenol-rich plants for commercial purposes, we recommend the use of violet LED light sources (Tab. 3). The leaves of plants grown under this light source could be used as infusions to provide antioxidant substances. In their *in vitro* studies, Piljac-Žegarac et al. [2009] confirmed that blueberry leaf-based tea is a very good source of strong antioxidants.

CONCLUSIONS

Regardless of the type of light used in the phytotron, the plants of the Aurora cultivar were higher, and they had lighter leaves with higher polyphenol contents and lower proline contents compared to those of the Huron cultivar. When grown under violet LED lights, the plants of both cultivars were lower and had smaller leaves. This light source is a stress factor for the plants, which was evidenced by low CIE *a** and *b** leaf color as well as high contents of total proline and polyphenols. The plants grown under fluorescent white light had a 22.7% lower total of polyphenols, and the plants grown under LED white light had a 19.2% lower total than those grown under violet LED light. In large-scale production of highbush blueberry plants, it is advisable to use white LED light owing to the high quality of the plants grown under this light as well as their beneficial effect on production economics.

SOURCE OF FUNDING

This research was supported by the Polish Ministry of Science and Higher Education grant No. N N310205737 and West Pomeranian University of Technology of grants nr 518-07-014-3171-03/18.

Presented here research received external funding in project “ZUT 2.0 - Modern Integrated University” grant number POWR.03.05.00-00-Z205/17.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTION

Conceptualization: M.F-K. I.O., M.K-M. S.L., Material: M.F-K. Methodology: M.F-K. I.O., M.K-M. S.L. Formal analysis and investigation: M.F-K. I.O., M.K-M. S.L. Writing – original draft preparation: M.F-K. I.O., M.K-M. S.L. Writing – review and editing: M.F-K. I.O., Funding acquisition: I.O. Supervision: I.O.

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EFFECT OF CHITOSAN-BASED SPRAYING ON THE QUALITY OF HIGHBUSH BLUEBERRIES (SUNRISE CULTIVAR)

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Abstract

The consumption of highbush blueberries has been growing rapidly in recent years due to their taste and health-promoting qualities. Various solutions have been sought to obtain the highest quality fruit after harvest. In the era of eco-friendly products, it is important that the methods used are natural and ecological. For this purpose, chitosan (CH) was sprayed five times on highbush blueberry bushes before harvesting. Different molecular weights of CH (5, 12, 21, 50, 125, and 500 kDa) were used in this study. The physical and biochemical characteristics of the fruit were investigated. The antioxidant activity, microbial contaminants, and mycotoxins in fruit were also analysed. Application of CH affected the quality of highbush blueberries after harvest. The molecular weight of CH had a significant effect on the studied traits. The application of high-molecular-weight CH improved physical characteristics such as the average weight of 100 blueberries, firmness, and puncture. Furthermore, the blueberries had a more intense blue colour; were characterised by a higher content of L-ascorbic acid and polyphenols, especially anthocyanins; and did not contain mycotoxins. Spraying with CH can be recommended in the organic cultivation of highbush blueberries to obtain robust fruit with health-promoting qualities.

Keywords**:** *chitosan, highbush blueberry, fruit quality, mycotoxins, polyphenols, fungi*

Received: 24.02.2022
Accepted: 18.05.2022

1. Introduction

In recent years, there has been marked growth in blueberry acreage and yield [1-5]. Recent global data indicate yearly blueberry production of nearly 1.0 million ton [4]. The largest global producers of highbush blueberries are the United States; Chile; Canada; and European countries such as Spain, Poland, and Germany [2, 4]. Dark blue and sweet blueberries are classified as a so-called 'superfood' due to their health-promoting biological properties [6] and are highly consumed among the public, mainly in dessert form [1, 3, 5].

Blueberries are a rich source of valuable nutrients such as minerals, vitamins, flavones, and polyphenols [7, 8]. Numerous studies indicate that blueberries are a source of natural antioxidants. Many of the health-promoting properties of blueberries are attributed to phenolic acids and various flavonoids, especially anthocyanins [7, 9]. Among fruit, blueberries have the highest anthocyanin content; hence, they are recommended as a functional food as well as a dietary supplement [6].

Consumers judge the quality of fresh fruit based on their appearance and freshness on the store shelf [10]. Various measures and technologies are used to achieve the highest quality fruit. It is important not to reduce natural properties of fruit and their benefits for human health by using chemicals – ecological solutions should be favoured. The optimal solution seems to be environmentally safe chitosan (CH), extracted most often from the shells of shrimp and other crustaceans [11]. CH is the second most abundant biopolymer on earth after cellulose [12]. It forms a transparent, non-toxic, biocompatible, biofunctional, and biodegradable film [13-15]. In addition, it exhibits haemostatic and antimicrobial activity, mucoadhesive properties, and excellent processability (including film-forming capabilities) [13]. CH shows activity against multidrug-resistant bacteria and fungi, which pose a challenge to modern medicine [16]. CH plays an important role in plant pathogen resistance and defence mechanisms [15, 17, 18]. Coating fruit and vegetables with edible CH extends their shelf life by minimising respiration rates and reducing water loss [17].

Physicochemical characteristics such as the degree of deacetylation and molecular weight play a key role in the quality of CH in its diverse applications. CH can be divided into four groups according to the degree of deacetylation: low (55%-70%), medium (70%-85%), high (85%-95%), and ultra-high (95%-100%) [19]. Moreover, there is low-molecular-weight CH (LMC) and high-molecular-weight CH (HMC). Molecular weight affects the biological activity of CH [20]. LMC usually exhibits more significant biological properties than HMC [21]. HMC (500-1,000 kDa) is readily soluble in dilute acids but insoluble in water [20].

This study aimed to investigate the effect of CH of different molecular weights on physicochemical changes in highbush blueberries of the Sunrise cultivar after harvest. The type and amount of microbial contaminants and mycotoxins in the fruit were also analysed.

2. Materials and Methods

2.1. Characteristics of the Research Area and Plant Material

The experiment was conducted in the Department of Horticulture at the West Pomeranian University of Technology in Szczecin from 2018 to 2020. Highbush blueberries of the Sunrise cultivar were harvested from bushes grown on a specialised highbush blueberry farm, located about 25 km east of Szczecin, in the Goleniowska Forest. The plantation contains a 4.9 ha field irrigated by droplets and has mineral soil: loamy sand, organic matter 2.21%, organic carbon 0.56%, electrical conductivity 0.29 mS/m, and pH 4.0-4.2 [22].

2.2. Characteristics of the CH and Treatment Application

Depolymerised CH obtained by controlled free-radical degradation was used in the experiment. Chitosan was produced by the Center for Bioimmobilization and Innovative Packaging Materials of the West Pomeranian University of Technology in Szczecin. Different molecular weights of CH (5, 12, 21, 50, 125, 500 kDa) with a degree of deacetylation of 85% were used in the study.

CH was sprayed five times on highbush blueberry bushes: three times before harvesting, and after the second and third harvests. The control group consisted of bushes sprayed with water.

2.3. General Fruit Parameters

Every year, the blueberry yield and weight were measured (using a WPX 4500 instrument, with precision of ± 0.01 g; RADWAG, Poland). The content of soluble solids was determined by an electronic refractometer (PAL-1, Atago, Japan). Acidity was determined by titration of the aqueous extract with 0.1 N sodium hydroxide (NaOH) to an endpoint of pH 8.1 (Elmetron CX-732, Poland), according to the PN-90/A-75101/04 standard [23]. The L-ascorbic acid and nitrate contents were measured with an RQflex 10 requantometer (Merck, Germany) [22].

2.4. Colour

The CIE L^*, a^*, and b^* parameters defined by the Colour Measurement Committee of the Society of Dyers and Colourists (CMC) were measured using a Konica Minolta CM-700d spectrophotometer (Japan). The colour parameters and indices were averaged over 35 measurements [24].

2.5. Firmness

Firmness and puncture resistance of the blueberry skin was measured with a FirmTech2 apparatus (BioWorks, USA) on 100 randomly selected blueberries from three replicates. Punctures were made using a stamp with a diameter of 3 mm [24]. It is expressed as a gram-force causing the blueberry surface to bend 1 mm.

2.6. Extraction and Identification of Polyphenols

Three replicates of 1000 g of randomly chosen blueberries were kept frozen in polyethylene bags at -65°C until analysis, then prepared according to the methodology described by Lachowicz *et al.* [25]. Compounds were extracted with methanol acidified with 2.0% formic acid. The separation was conducted twice by incubation for 20 min under sonication (Sonic 6D, Polsonic, Poland) followed by shaking from time to time (a few times or rarely). Subsequently, the suspension was centrifuged in an MPW-251 centrifuge (MPW MED. INSTRUMENTS, Poland) at 19000 *g* for 10 min. Prior to analysis, the supernatant was purified with a Hydrophilic PTFE 0.20 µm membrane (Millex Samplicity Filter, Merck).

In blueberry extracts, polyphenols were identified by using an ACQUITY Ultra Performance LC system with a binary solvent manager, a photodiode array detector (Waters Corporation, USA), and a G2 Q-TOF micro mass spectrometer (Waters, UK) equipped with an electrospray ionisation (ESI) source operating in both negative and positive modes (UPLC-PDA/QTof-MS/MS) [25].

2.7. Antioxidant Activity

For the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, the procedure followed the method of Arnao *et al.* [26]. The ferric ion reducing antioxidant

power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays were conducted according to the method of Brand-Williams *et al.* [27]. The antioxidant capacity is expressed as µmol Trolox equivalent (TE) g^{-1} dry weight (dw). Measurements for the $ABTS^{\cdot+}$ and FRAP assay involved the UV-2401 PC spectrophotometer (Shimadzu, Japan). The L-ascorbic acid content was measured with an RQflex 10 quantometer (Merck, Germany) [24].

2.8. Fungal Infestation and Mycotoxin Content in Blueberries

Analysis of the degree of fruit infestation by fungi (yeasts and moulds) was based on the European standard ISO 70 [28]. After cultivation of spore-forming fungal inoculates, samples were subjected to taxonomic evaluation using the traditional method of macroscopic observation of colonies and microscopic observation of spores and filaments.

Mycotoxins were determined by using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) [29]. The sample was purified on the AflaTest immunological affinity columns from Vicam (USA) for aflatoxins and OchraPrep from R-Biopharm AG (Germany) for ochratoxin A, according to the procedures specified by the manufacturers. Patulin, deoxynivalenol, T2, HT2 toxin, and zearalenone were analysed by HPLC-MS/MS. The samples were purified on Bond Elut® Mycotoxin columns from Agilent (USA). Each sample was run in triplicate.

2.9. Statistical Analysis

All statistical analyses were performed with Statistica 12.5 (StatSoft Polska, Poland). The data were subjected to one-way analysis of variance (ANOVA). Group comparisons were performed using Tukey's least significant difference (LSD) test; significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Blueberry Quality

The mean weight of 100 blueberries, puncture, firmness, soluble solids (SS), titratable acidity (TA), nitrate nitrogen ($N\text{-}NO_3$), and nitrite nitrogen ($N\text{-}NO_2$) were determined in fresh blueberries soon after harvest. Blueberries from bushes sprayed with CH 125 kDa had the highest weight (342 g/100 fruit), while fruit sprayed with CH 5 kDa had the lowest weight (262 g/100 fruit). The average weight of 100 blueberries was much higher than in the study by Ochmian *et al.* [30] on the highbush blueberry Patriot cultivar, where it was 120-140 g on average. The heaviest fruit was also the firmest (428 G mm^{-1}) and had the highest puncture value (142 G mm^{-1}). Fruit that had been sprayed with CH 21 kDa had the lowest $N\text{-}NO_3$ and $N\text{-}NO_2$ contents (30.2 and 0.09 mg 1000 g^{-1}, respectively), while fruit sprayed with CH 125 and 500 kDa had the highest $N\text{-}NO_3$ and $N\text{-}NO_2$ contents ($N\text{-}NO_3$: 55.9 and 0.15 mg 1000 g^{-1}, respectively; $N\text{-}NO_2$: 52.3 and 0.17 mg 1000 g^{-1}, respectively). Ochmian *et al.* [30] obtained lower $N\text{-}NO_3$ (19.3-31.4 mg 1000 g^{-1}) and higher $N\text{-}NO_2$ (0.75-0.95 mg 1000 g^{-1}) (Table 1).

3.2. L-Ascorbic Acid and Antioxidant Activity

The L-ascorbic acid content ranged from 20.7 to 39.6 mg 100 g^{-1}. Ochmian *et al.* [30] reported a vitamin C content in highbush blueberries of 23.4-26.2 mg 100 g^{-1} for the Patriot cultivar, depending on the substrate. Zia and Alibas [31] reported a vitamin C content for mighty fresh blueberry of 40.29 mg 100 g^{-1} dw. Kalt *et al.* [32] reported that the L-ascorbic acid content of fresh blueberries was 7-20 mg 100 g^{-1}. However, López *et al.* [33] reported that the vitamin C content of fresh blueberries was 20.97 mg 100 g^{-1}. Blueberries sprayed with CH 500 kDa had the highest L-ascorbic acid content; it was

almost twice as high as the content in the blueberries sprayed with CH 12 kDa. Compared with the control group, spraying with CH 50, 125, and 500 kDa increased the L-ascorbic acid content, while spraying with CH 5 and 12 kDa decreased the L-ascorbic acid content (Table 1).

The FRAP method was used to determine the antioxidant capacity of the blueberries. The antioxidant capacity varied due to the molecular weight of CH (5.77-10.12 μmol TE g^{-1}). The blueberries treated with CH 125 kDa had the highest value, even higher than in the study by Ochmian *et al.* [5], where the FRAP value was 4.33-7.23 μmol TE g^{-1}. Among the treatments, the blueberries treated with CH 12 kDa had the highest antioxidant activity, denoted by DPPH and $ABTS^{\cdot+}$ radical scavenging. The antioxidant activity, determined by DPPH, was as much as twice as high (31.0 μmol TE g^{-1}) as for the 125 kDa sample (15.5 μmol TE g^{-1}) (Table 1).

Table 1. The quality and antioxidant capacity of highbush blueberries depending on the molecular weight of the applied chitosan.

	Molecular weight of chitosan (kDa)						
	Control*	**5**	**12**	**21**	**50**	**125**	**500**
Weight of 100 blueberries (g)	274ab**	262a	288bc	302c	335d	342d	291bc
Puncture (G mm^{-1})	124bcd	130cde	108a	117ab	121bc	142e	133e
Firmness (G mm^{-1})	358bc	366bc	317a	359bc	352b	428d	375c
Soluble solid (%)	15.3b	15.8c	15.6c	15.2b	14.5a	14.4a	15.1b
Titratable acidity (g 100 g^{-1})	0.88d	0.81bc	0.85cd	0.87d	0.74a	0.77ab	0.80b
L-ascorbic acid (mg 100 g^{-1})	26.5b	22.4a	20.7a	27.8b	35.7c	36.2c	39.6c
$N\text{-}NO_3$ (mg 1000 g^{-1})	37.3bc	41.5c	33.4ab	30.2a	36.1b	55.9d	52.3d
$N\text{-}NO_2$ (mg 1000 g^{-1})	0.11ab	0.13bc	0.14c	0.09a	0.15cd	0.15cd	0.17d
$ABTS^{\cdot+}$ (μmol TE g^{-1})	14.7a	17.8b	20.2c	21.3c	18.4b	17.7b	15.5a
FRAP (μmol TE g^{-1})	8.11bc	7.36b	9.55d	8.78cd	5.77a	10.12e	9.84d
DPPH (μmol TE g^{-1})	22.2b	15.7a	31.0d	28.4c	21.6b	15.5a	16.3a

Note. *Control – not spraying; **Means with the same letter do not differ significantly according to Tukey's test ($p > 0.05$).

3.3. Blueberry Colour

One of the key parameters affecting consumer acceptability of fruit is the surface colour. Highbush blueberries have a blue-black skin but due to the waxy coating, they appear light blue [34]. The colour of the studied blueberries was determined by the L^*, a^*, and b^* parameters. A change in the L^* parameter indicates blueberry darkening [35].

Depending on the molecular weight of the applied CH, the blueberries changed from pink to blue, as indicated by significant changes in the *a** and *b** parameters. The darkest and most intense blue shade was observed in blueberries treated with CH 500 and 125 kDa (Figure 1), which is undoubtedly related to the high anthocyanin content. The darkest blueberries had the highest anthocyanin content (Figure 1 and Table 2). On the other hand, the blueberries treated with CH 50 kDa exhibited the lightest purple-pink colouration. Ochmian *et al.* [35] reported similar *L** (26.2 to 32.6) and *b** (-28.3 to - 25.9) values.

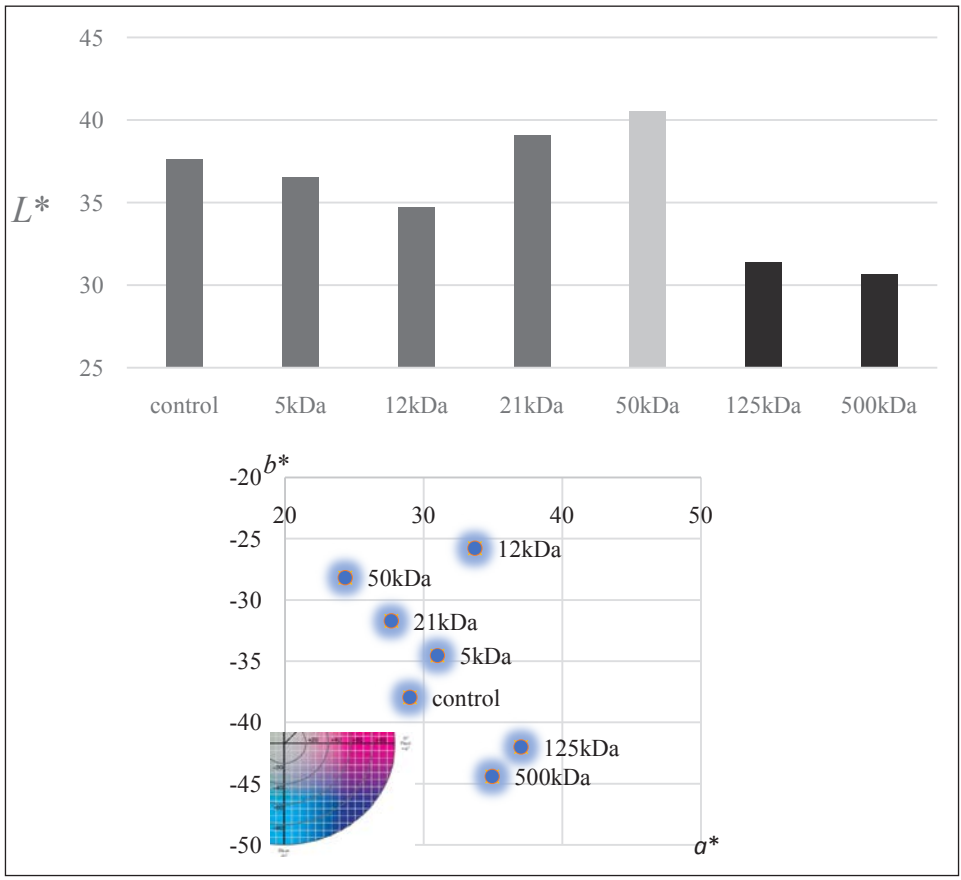

Figure 1. Change in highbush blueberry colour depending on the molecular weight of the applied chitosan.

3.4. Polyphenols

Highbush blueberries are a rich source of polyphenols such as phenolic acids, anthocyanins, and other flavonoids [36]. The studied highbush blueberries contained 36 polyphenols (Table 2). The most abundant were anthocyanins followed by phenolic acids; the least abundant were flavonols and flavan-3-ols. The polyphenol content depended to a large extent on the molecular weight of the applied CH (Table 2). There were nine anthocyanins detected in the blueberries (Table 2). Anthocyanins are the most important polyphenols in blueberries [8] and are responsible for their black, blue, and red pigments. Blueberries treated with CH 500 kDa had the highest anthocyanin content (499.70 mg

100 g^{-1}); it was 2.05 times greater than the anthocyanin content of blueberries treated with CH 12 kDa. Similarly to the studies by Su *et al.* [35] and Ochmian *et al.* [5], chlorogenic acid (a phenolic acid) was the dominant polyphenol. On average, it accounted for between 29.57% and 36.49% of the total polyphenol content in highbush blueberries (Table 2).

Table 2. Polyphenol content in blueberries [mg 100 g^{-1}].

Compounds (mg 100 g^{-1} DW)	Molecular weight of chitosan (kDa)						
	Control	**5**	**12**	**21**	**50**	**125**	**500**
Caf-glu*	1.71	1.13	0.55	6.28	0.57	0.33	0.82
Caf-glu	4.59	5.15	5.71	1.32	7.33	4.63	10.03
Caf-glu	0.60	0.85	1.10	2.54	2.20	2.05	2.35
Neochl acid	6.08	4.38	2.68	3.56	3.72	3.88	3.56
Chlo acid	271.92	242.03	212.14	217.83	238.49	254.21	269.58
Crychlo acid	1.64	1.13	0.62	2.69	2.22	3.07	1.37
Phenolic acid	**286.53d****	**254.67bc**	**222.80a**	**234.21ab**	**254.53bc**	**268.16cd**	**287.70d**
Myr 3-gal	3.79	3.57	3.35	3.80	10.96	9.99	11.92
Que diglu	1.16	0.78	0.40	0.99	0.85	0.96	0.74
Que 3-rha-hex	6.27	5.46	4.66	4.38	1.44	1.16	1.72
Que 3-rut	3.44	3.29	3.15	2.72	1.67	0.70	2.63
Que 3-gal	4.62	7.63	10.65	5.59	16.22	13.48	18.97
Que 3-methex	3.01	4.48	5.94	3.41	4.22	4.12	4.33
Que 3-glu	1.03	1.45	1.87	2.42	1.10	0.73	1.47
Que 3-ara	4.11	5.52	6.93	2.55	2.99	2.38	3.60
Que 3-cafgal	1.19	1.13	1.06	0.80	0.49	0.32	0.67
Que 3-cafglu	0.68	0.61	0.54	0.45	0.28	0.25	0.32
Que 3-oxapen	6.18	10.79	15.41	4.23	1.85	0.13	3.57
Que 3-rha	0.55	0.89	1.23	1.18	1.40	2.19	0.61
Que 3-dimethoxyrha	1.04	2.53	4.02	0.21	0.35	0.63	0.07
Que 3-(6'-acetyl) gal	0.08	0.32	0.55	0.51	0.15	0.15	0.14
Que 3-(6'-acetyl) gal	0.40	0.51	0.62	0.26	0.03	0.02	0.04
Flavonols	**37.55a**	**48.96bc**	**60.37d**	**33.50a**	**43.99b**	**37.19a**	**50.79c**
Procyanidin dim	5.84	6.70	7.56	9.63	6.11	6.19	6.03
Procyanidin dim	5.34	6.01	6.68	6.71	5.60	4.59	6.61
Procyanidin dim	13.06	40.34	67.63	19.80	23.27	30.50	16.03
Cat	13.01	9.80	6.60	9.22	17.99	32.00	3.99
(-)Epicat	4.25	7.94	11.64	8.45	8.77	6.38	11.16
Procyanidin trim - B3	0.89	2.63	4.38	2.23	3.76	3.03	4.49
Flavan-3-ols	**42.38a**	**73.43de**	**104.48f**	**56.04bc**	**65.50cd**	**82.68e**	**48.31ab**
Del-3-*O*-glu	91.75	71.34	50.92	61.18	87.16	55.54	118.78
Del 3-ara	2.28	2.11	1.95	1.27	2.39	2.36	2.41
Pet-3-*O*-glu	68.61	53.66	38.72	39.34	69.79	103.94	91.21
Cya-3-*O*-glu	47.04	43.43	39.82	44.49	51.96	71.58	66.38
Cya 3-ara	8.33	11.96	15.58	10.45	20.98	26.39	15.57
Pet 3-ara	27.19	24.99	22.79	20.47	34.34	49.14	42.24
Mal 3-gal	79.98	63.51	47.04	49.97	59.56	86.48	98.63

Compounds (mg 100 g^{-1} DW)	Molecular weight of chitosan (kDa)						
	Control	**5**	**12**	**21**	**50**	**125**	**500**
Mal 3-ara	4.75	5.08	5.41	8.40	7.70	8.53	6.87
Mal-3-*O*-glu	32.77	26.99	21.20	40.42	49.06	40.51	57.62
Anthocyanins	**362.69c**	**303.07b**	**243.44a**	**275.99ab**	**382.93c**	**444.46d**	**499.70e**
TOTAL	**745.20c**	**693.25b**	**641.30a**	**611.28a**	**765.48c**	**853.25d**	**911.72e**

*Caf-glu - Caffeoyl-glucose; Neochl acid - Neochlorogenic acid; Chlo acid - Chlorogenic acid; Crychlo acid - Cryptochlorogenic acid; Myr 3-gal - Myricetin 3-galactoside; Que diglu - Quercetin diglucoside; Que 3-rha-hex - Quercetin 3-rhamno-hexoside; Que 3-rut - Quercetin 3-rutinoside; Que 3-gal - Quercetin 3-galactoside; Que 3-methex - Quercetin 3-methoxyhexoside; Que 3-glu - Quercetin 3-glucoside; Que 3-ara - Quercetin 3-arabinoside; Que 3-cafgal - Quercetin 3-caffeoylgalactoside; Que 3-cafglu - Quercetin 3-caffeoylglucoside; Que 3-oxapen - Quercetin 3-oxalylpentoside; Que 3-rha - Quercetin 3-rhamnoside; Que 3-dimethoxyrha - Quercetin 3-dimethoxyrhamnoside; Que 3-(6'-acetyl)gal - Quercetin 3-(6'-acetyl)galactoside; Procyanidin dim - Procyanidin dimer; Cat - Catechin; (-)Epicat - (-)Epicatechin; Procyanidin trim - B3 - Procyanidin trimer - B3; Del-3-O-glu - Delphinidin-3-O-glucoside; Del 3-ara - Delphinidin 3-arabinoside; Pet-3-O-glu - Petunidin-3-O-glucoside; Cya-3-O-glu - Cyanidin-3-O-glucoside; Cya 3-ara - Cyanidin 3-arabinoside; Pet 3-ara - Petunidin 3-arabinoside; Mal 3-gal - Malvidin 3-galactoside; Mal 3-ara - Malvidin 3-arabinoside; Mal-3-O-glu - Malvidin-3-O-glucoside.

**_Note._ Means with the same letter are not significantly different according to Tukey's test ($p > 0.05$).

3.5. Fungi and Mycotoxins

Filamentous fungi are widely distributed throughout the world [37, 38]. They are found in soil, water, and materials of organic origin, and their spores are found in the air and on the surfaces of all kinds of materials. Some species of *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium* produce toxic secondary metabolites called mycotoxins, which threaten human and animal health [39]. Fresh fruit, including blueberries, are susceptible to fungal infections that occur both on plantations and at harvest [40]. Toxigenic species of *Fusarium* and *Alternaria* are often classified as field fungi because they require very high substrate moisture for growth and mycotoxin synthesis [41]. Fungi belonging to seven different genera (*Acremonium*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Fusarium*, and *Penicillium*) were isolated from blueberries after harvest. *Fusarium* spp. were dominant in the samples (44%-56%) (Table 3). *Penicillium* spp. (16%-80%) and *Aspergillus* spp. (35%-37%) were also very abundant. *Acremonium* spp. were the least abundant: they appeared only in the control group and blueberries that had been sprayed with CH 5 kDa (5% and 7%, respectively). *Penicillium* spp. were highly abundant (80%) in blueberries that had been sprayed with CH 125 kDa, and *Cladosporium* spp. (73%) were highly abundant in blueberries that had been sprayed with CH 21 kDa.

Four different types of mycotoxins were detected in the tested samples: patulin, aflatoxin, deoxynivalenol, and zearalenone. Mycotoxins are secondary metabolites of filamentous fungi and therefore occur naturally in food [37]. One fungus can produce different mycotoxins, and one mycotoxin can be produced by several different fungi [42]. The mycotoxins were found at low levels (Table 3). The patulin content (1.03-3.04 μg kg^{-1}) (Table 3) was significantly lower than the maximum allowable patulin concentration in apple juice (50 μg kg^{-1}) [43] set by the World Health Organization. Mycotoxins appeared only in the control group and the two groups that had been sprayed with the lowest molecular weights of CH (5 and 12 kDa). The absence of mycotoxins in the samples may have been related to the occurrence of *Aureobasidium* spp. in the samples. They can degrade mycotoxins through microbial pathways [44]. They show strong antagonism

to fruit-contaminating moulds and can provide effective bioprotection [45]. It can also be speculated that HMC inhibited the growth of mycotoxins. According to Gutierrez-Martinez *et al.* [40], CH could be an environmentally friendly alternative to the use of chemical fungicides in controlling postharvest diseases of fruit.

Table 3. Fungal genera and mycotoxins identified in the blueberries

Chitosan molecular weight (kDa)	Percentage of fungal genera (%)		Mycotoxins (µg kg^{-1})	
Control	*Fusarium*	44	Patulin	1.03
	Aspergillus	35	Deoxynivalenol	0.88
	Penicillium	16		
	Acremonium	5		
5 kDa	*Aspergillus*	36	Aflatoxin	1.79
	Penicillium	32	Patulin	0.65
	Alternaria	22		
	Acremonium	7		
	Cladosporium	3		
12 kDa	*Fusarium*	54	Patulin	3.04
	Aspergillus	37	Deoxynivalenol	0.59
	Cladosporium	9	Zearalenone	0.10
21 kDa	*Cladosporium*	73		
	Penicillium	19		
	Aureobasidium	8		
50 kDa	*Penicillium*	56		
	Aureobasidium	27		
	Alternaria	13		
	Cladosporium	4		
125 kDa	*Penicillium*	80		
	Aureobasidium	13		
	Cladosporium	7		
500 kDa	*Fusarium*	51		
	Penicillium	44		
	Alternaria	5		

4. Conclusion

The results of the study indicate that spraying highbush blueberries with CH improved their quality after harvest. The molecular weight and high deacetylation degree of CH have a decisive influence on its physical, chemical, and biological properties. The application of HMC (125 and 500 kDa) improved blueberry physical parameters such as mean weight of 100 fruit, puncture, and firmness, and they had the highest L-ascorbic acid, N-NO3, and FRAP content relative to the control group. These blueberries also had the most intense blue colour and high polyphenol contents, especially anthocyanins, and were not contaminated with mycotoxins. Based on the trend of improvement of the studied traits,

it can be assumed that using higher molecular weight CH than that used in the experiment would produce even better results.

5. References

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10.2478/aucft-2020-0006

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Series E: Food technology

THE QUALITY OF FREEZE-DRIED AND REHYDRATED BLUEBERRIES DEPENDING ON THEIR SIZE AND PREPARATION FOR FREEZE-DRYING

– Research paper –

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ABSTRACT: A significant increase in highbush blueberry plantings has been observed annually. However, there is a problem with the handling of fruit that does not meet the requirements for dessert berries. One of the methods to use the fruit is drying. The research was conducted in the Department of Horticulture at the West Pomeranian University of Technology in Szczecin. The fruit was harvested at a plantation specialising in the production of highbush blueberry from irrigated and non-irrigated plots. This study aimed to assess how cutting or pricking highbush blueberry fruit affects the duration of the sublimation drying process – freeze-drying. The biological value of fresh, freeze-dried, and rehydrated fruit was assessed. The amount of fruit left on bushes depended on the weather prevailing in the growing season, especially during the harvest period. The mass of fruit left on the bushes was approx. 1 to 2 t/ha. Although these fruits were small, they had a very high biological value. After freeze-drying and rehydration, the blueberry fruit retained their colour, high content of polyphenols and showed high antidiabetic activity and antioxidant capacity. The large fruit reached the moisture content of approx. 12%, which ensures safe storage and the appropriate texture, after 46 hours of drying, and the smaller fruit - after 32 hours. Fruit cutting or pricking reduced the drying time by half. Reducing this time decreased drying costs and increased biological value.

Key words: *Vaccinium corymbosum*, colour, polyphenol, bioactive compounds, Brigitta Blue

INTRODUCTION

Recently a lot of new farms have specialized in the cultivation of highbush blueberry (*Vaccinium corymbosum* L.). Poland ranks first in Europe in terms of area planted with bushes exceeding 19.000 hectares, as well as quantity of harvested fruits (Mystkowska et al., 2017), ahead of Germany and Spain (Brazelton, 2013). Planting new bushes is primarily associated with favorable climate and soil conditions (Ochmian et al., 2019a). The production of these fruits is very profitable for farmers. In recent years the price per 1 kg of the fruit of late cultivars exported to Western Europe is 4-7.5 euros and is quite stable. One of the major issues in the cultivation of highbush blueberry is its manual harvesting, which requires a large number of people to conduct (30-40 people for a few hectares of the plantation). Often a significant number of small, non-commercial fruits remain on the bushes. Those fruits are mainly suitable for the processing industry (Ochmian and Kozos, 2015). An alternative solution is the usage of harvesting combines, however, the fruits harvested in such a way can be mainly dedicated for processing (Mitek et al., 2006). Every year, approximately 15% of the highbush blueberries have been intended for processing (Brazelton, 2013). Top-quality, high blueberry fruit should have a diameter greater than 10-12 mm. Depending on the cultivar and customer requirements (Wach, 2012).

Counterparties require that the fruit not have defects have a wax coating. Therefore, the plantations have a certain amount of fruit left on bushes. Most are small fruits, which do not satisfy the requirements of the customers. A collection of such fruit is

Received: 23.01.2020
Accepted in revised form: 16.04.2020

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cumbersome, employees must spend more time on it. Combine harvesters can be used, but the fruit must then be sorted. They are often damaged and mature unequally (Mitek et al., 2006). Studies carried out on different types of sorting have shown that this technological process has a negative impact on fruit quality (Zerbini, 2006; Harasymowicz-Boggio, 2012). Fruits largely lost wax coating and firmness (Bednarek et al. 2017).

Positive effects on the size and quality of the fruit affect organic soils, rich in humus (Ochmian and Kozos, 2014). A very important aspect is the provision of adequate water conditions (Treder, 2007; Ochmian et al. 2018). The shortage of atmospheric precipitation, but also their inadequate distribution during the growing season limits the growth of bushes, reduces yield and quality deterioration (Koszański et al., 2011). The biggest impact on the quality of the fruit has a drought that occurs in a period of strong growth of the fruit (Perrier et al., 2000). Especially in recent years where climatic conditions are highly variable, irrigation of blueberries is of crucial importance (Tryngiel-Gac et al., 2013).

It is worth collecting and managing fruit that does not meet the requirements for commercial fruit. Blueberries are rich in many compounds, which have a health-promoting effect (Gu et al., 2002), e.g. anthocyanins (Wu et al., 2006; Ochmian et al., 2018) polyphenols (Okan and Yayli, 2018) and anti-diabetic activity (Roopchand et al., 2013). In smaller fruits, there is a greater concentration of bioactive compounds (Ochmian and Kozos, 2014). Especially since most of the fruit left on the bushes are small.

Fruits that are unsuitable for sale due to their small size can be frozen or dried. Long-term exposure to high temperatures causes significant deterioration of the product quality. Such drying reduces the content of polyphenols and antioxidant capacity in blueberry fruit by up to 69% (Zielińska and Michalska, 2016). Prolonged exposure to elevated temperature, result in substantial degradation of product quality (Zielińska et al., 2013). A good solution development such fruit may be lyophilization (freeze-drying). A fruit with a stable structure and color is obtained, and the low water content promotes long-term storage. However, the high speed and degree of rehydration make freeze-dried fruit attractive to the consumer. Also, this process, unlike traditional drying, allows the preservation of bioactive compounds in fruit (Ochwanowska et al., 2017; Sadowska et al., 2017). Lower weight fruit also requires shorter freeze-drying time (Kozos et al., 2016). Therefore a better solution is freeze-drying - lyophilisation. This process is carried out at low temperatures (−40°C), reducing the enzymatic and chemical processes - which maintains most of the nutrients. The quality of the fruits obtained during such drying process is very high (Rząca and Witrowa-Rajchert, 2007). Drying prolongs the life of the product (Khin et al., 2007) and allows you to keep the original color of the fruit in a much better degree than other methods by drying (Piotrowski et al., 2008). Dried fruits of this type are becoming more and more popular. An important feature of such a product is its ability to rehydrate quickly and completely (Ciurzyńska and Lenart, 2009; Lewicki, 1998a; Krokida and Marinos-Kouris, 2003).

With the constantly increasing crop area and observed climate changes, I have attempted to assess the amount of fruit that remains on highbush blueberry bushes grown on plantations. Determined if irrigation affects the quantity and quality of non-commercial fruit. In the second part of the publication, there will be presented the results of the development of these fruits by freeze-drying - lyophilizing. The test results may affect the higher profitability of the plantations.

MATERIAL AND METHODS

Characteristics of the research area and plant material

The experiment was conducted in the Department of Horticulture at the West Pomeranian University of Technology in Szczecin in the 2013-2018 growing season. The fruits were harvested from bushes grown on two farms specializing in the cultivation of the highbush blueberry, located about 25 km east of Szczecin, in the Goleniowska Forest. There were studied bushes of Brigitta Blue cultivar. Characteristics of plantation:

Plantation A - irrigated by droplets, the size of field 4.9 ha. Mineral soil - loamy sand, content of organic matter 2.21%, organic carbon 0.56%, EC 0.29 mS/m, pH 4.0-4.2.

Plantation B - no irrigation, size of field 4.3 ha. Mineral soil - sandy loam, content of organic matter 2.94%, organic carbon 0.67%, EC 0.21 mS/m, pH 4.4-4.6.

As recommended for highbush blueberries, mineral fertilizers were applied annually. Fruit intended for research were collected after the commercial harvest. They were mostly small fruits, non-commercial - the manual collection would be uneconomic. Left on the bushes they would be

destroyed. For determining the quantity and quality of the fruit on bushes left, every year from 50 randomly selected bushes all the fruits were collected, weighed and divided into three groups: <6 mm, 6-8 mm, >8 mm. From these fruits, an aggregate sample was prepared. Measurements were made on the fruits taken from it.

Weather conditions during the experiment

Significant differences in weather have been observed between 2013 and 2018. During this period the weather was significantly different from that typical for this region. The average temperature from April to October was higher than 0.1 to 1.5°C compared to the average temperature over a long period (1951-2012). In the years 1950-1989, the average temperature in the vegetation period was 13°C (Schernewski, 2011), but in some years it has not even reached 12.5°C. Between 1990 and 2006 the average temperature in the growing season increased to 13.8°C, but also never fell below 13.5°C. Precipitation varied over the years and in individual months. Extremely low precipitation occurred in 2018, especially during the fruit ripening period - in August and September (Table 1). In July, the precipitation was 92.8 mm, but as much as 79 mm of rainfall fell within 3 days and this water mostly flowed down the field surface.

General fruits parameters

Every year yield and fruit weight were measured (RADWAG WPX 4500 ±0.01 g). The content of soluble solids was determined by an electronic refractometer (PAL-1, Atago, Japan). Acidity was determined by titration of the aqueous extract with 0.1 N NaOH to an end point with pH 8.1 (Elmetron CX-732), according to the PN-90/A-75101/04 standard. Dry matter content was determined according to the relevant Polish Standard (PN-90/A-75101/03, 1990).

Determination of colour

The pigment (colour) of fruits was measured in transmission mode by photocolorimetric method in CIE L**a***b** system (Hunterlab, 2012; Chełpiński et al. 2019). The diameter of the measurement hole was 3 mm, the observer type 10º and the illuminant D65. The value of *a** indicates the surface colour of dried fruits of analysed genotypes in the range from green (-*a**) to red (+*a**). The parameter *b** described the colour in the range from yellow (+*b**) to blue (-*b**). The value of parameter L* means monochromaticity in the range from 0 (black) to 100 (white).

Extraction procedure and identification of phenolic compounds, antioxidant and antidiabetic activity

Three replicates of 100 g randomly chosen blueberry (separately for each group; <6 mm, 6-8 mm, >8 mm) were kept frozen at −65°C until analysis. Fruits then prepared and analyses according to the methodology of Oszmiański et al. (2018). The fruits were extracted with methanol acidified with 2.0% formic acid. The separation was conducted twice by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland). The sample was shaken several times. Subsequently, the suspension was centrifuged (MPW-251, MPW MED. INSTRUMENTS, Warsaw, Poland) at 19.000×g for 10 min. Before analysis, the supernatant was additionally purified with a Hydrophilic PTFE 0.20 µm membrane (Millex Samplicity Filter, Merck). In blueberry extracts', polyphenol identification was executed by using an ACQUITY Ultra Performance LC system appointed with a binary solvent manager, a photodiode array detector (Waters Corporation, Milford, MA, USA) and a G2 Q-TOF micro mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionisation (ESI) source operating in both negative and positive modes.

For ABTS•+ (2,2'-azobis(3-etylobenzotiazolino-6-sulfonian) assay, the procedure followed the method of Arnao et al. (2001). The FRAP (Ferric-Reducing Antioxidant Power) assay was done according to Benzie and Strain (1996). The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was done according to the method of Yen and Chen 1995. The antioxidant capacity was expressed as millimoles of Trolox per 100 g D.W. Measurements using ABTS•+ and FRAP were done with UV-2401 PC spectrophotometer.

The activity of the fruit extracts was assayed according to the procedure described previously by Podsędek et al. (*α*-glucosidase) and Nickavar and Yousefian 2011 (*α*-amylase). The amount of the inhibitor (expressed as mg of fruit per 1 mL of the reaction mixture under assay conditions) required to inhibit 50% of the enzyme activity is defined as the IC_{50} value. The IC_{50} of the fruits tested was obtained from the line of the plot of the fruit concentration in 1 mL of reaction mixture versus the % inhibition. All extractions were carried out in triplicate.

treatment, the berries were subjected to freeze-drying. Was used for this purpose lyophilizer of Christ model Alpha 1-2 LD + settings:

Freeze-drying(Lyophilizing) process

Before the drying process, the fruit was pre-frozen at −35°C in approx. 2 hours. Immediately after this

1) pre-drying was performed at temperature −56°C, pressure 0.018 Mbar
2) a final drying step carried out for the last 4 hours of drying temperature −76°C, pressure of 0.001 Mbar.
The fruit was crushed using a closed laboratory mill. To reduce the drying time, the fruits were:
1) punctured with a needle with a diameter of 1 mm to a depth of about 3-4 mm - 6 punctures were made on the circumference of small (<6 mm) fruit, larger (6>) 8 punctures,
2) cut in half along the diameter axis,
3) whole - control.
Fruit prepared in this way was subjected to the drying process for 14, 16, 18, 20, 22 and 24 hours. After a certain time, the fruit's water content was tested according to Polish Norm (PN-90/A-75101/03).

Rehydration process
Dried fruits were subjected to rehydration in distilled water at 20°C for 6 h. After a specified time (0.25-6 h), the rehydrated sample was separated from water, dried with blotting paper and weighed with an accuracy of 0.01 g. The relative weight gain of rehydrated fruit was determined - the ratio of the current sample weight to the initial weight. The determination was performed in three repetitions.

Statistical Analysis
All statistical analyses were performed using Statistica 13.0 (StatSoft Polska, Cracow, Poland). Non-parametric methods (Kruskal–Wallis test) were used if neither the homogeneity of variance nor the normality of distribution was established previously. Statistical significance of the differences between means was determined by testing the homogeneity of variance and normality of distribution, followed by ANOVA with Tukey's post hoc test. The significance was set at $p<0.05$. Multivariate analysis was performed by applying principal component analysis (PCA). The data were auto-scaled during pre-processing.

Table 1. Temperature and rainfall in the period from April to October (vegetation season) in 2013-2018 compared to the multiannual period (1951-2012) in Szczecin

	Month							
	IV	V	VI	VII	VIII	IX	X	Mean
Year	average temperature (C°)							
2018	12.3	16.6	18.5	20.2	20.1	15.4	10.3	**16.2**
2017	6.8	13.5	16.8	17.2	17.9	13.3	11.0	**13.8**
2016	8.8	15.7	18.5	19.0	17.8	16.8	8.6	**15.0**
2015	8.7	12.5	15.6	18.6	21.1	14.1	13.7	**14.9**
2014	10.8	13.4	16.3	21.3	17.5	15.4	11.8	**15.2**
2013	8.4	14.4	16.9	19.3	18.7	13.0	10.9	**14.5**
1951–2012	8.0	13.0	16.4	18.2	17.6	13.8	9.2	**13.7**
	rainfall (mm)							Total
2018	26.8	22.5	15.0	**92.8 79/in 3 days**	21.4	16.3	20.2	**215.0**
2017	42.3	99.2	118.1	182.4	145.4	31.6	95.1	**714.1**
2016	20.2	18.9	69	50.1	47.8	18.3	55.3	**279.6**
2015	29	48	32.8	62	14.7	34.4	22.1	**243.0**
2014	47.5	85.3	26.5	70.8	104.6	80.9	32.8	**448.4**
2013	20.8	88.1	112.5	50.4	35.9	43.9	45.8	**397.4**
1951–2012	39.7	62.9	48.2	69.6	74.2	58.7	37.3	**390.6**

RESULTS AND DISCUSSION

Fruit yield
The tests were performed on two commercial plantations located in similar conditions (Table 1). On plantation A, drip irrigation was used, which maintained the soil moisture at an optimum level. This had an impact on the amount of fruit that was left on the bushes. In the period 2013-2018, several times less fruit was harvested from the bushes on the irrigated plantation A. The amount of fruit left on the bushes was highly diversified in different years of the study (Table 2). This was influenced by temperature and rainfall during the growing season; April-October (Table 1). In the years of low precipitation (2018, 2016 and 2015), the amount of fruit left on the non-irrigated bushes was much higher compared with the years of typical weather (2013-2014). In 2017, in the growing season, rain fell almost twice as much as in the years typical for

this region. Therefore, the amount of fruit left on both plantations was high. Rain hindered the harvesting of fruit that was of poor quality. Due to extensive and unpredictable weather changes, the irrigation of plantations, as well as the use of roofs facilitating fruit harvesting, are becoming important.

The irrigation of bushes resulted in the amount of non-commercial fruit being on average almost twofold as low as non-irrigated bushes. On both plantations, the highest amount of fruit was left on bushes in the years of unusual weather. On the irrigated plantation, there was on average 0.33 kg of fruit per bush, which produces 943 kg of fruit per hectare. This is 4-5% of the total yield for the 'Brigitta Blue' grown on this plantation. On average, 0.64 kg of fruit was harvested from non-irrigated bushes, which amounts to 1.868 kg per hectare of plantation. With an average yield of about 15 tonnes from that plantation, this already represents approx. 12% of the yield. These are small quantities of fruit, especially since several kilograms of blueberry can be harvested from a bush, depending on the cultivar (Glonek and Komosa, 2013; Wach, 2012, Ochmian et al., 2019a). This amount of fruit left on bushes may have been influenced by the weather. The year 2015 was exceptionally unfavorable for the highbush blueberry crops. The temperature during the ripening and harvesting period (August) was 3.5°C higher compared with the multiannual period. It was found that there had been as many as 11 days when the air temperature exceeded 30°C. Besides, the rainfall only amounted to 14.7 mm, while it is about 74 mm in a typical year. Much of the fruit was also damaged in 2018. From June to September, the temperature was extremely high, with almost no rainfall. Although rainfall in July amounted to 92.8 mm, as much as 79 mm fell in three days (10-12 July). In 2013, the weather conditions were similar to the multi-year conditions, which is why the amount of fruit that was not harvested was the lowest one.

In all the years of research conducted on irrigated bushes, a significant share of the yield was made up of larger fruit with a diameter of 6-8 mm (on average 41% of the yield). As far as bushes grown on non-irrigated plantations are concerned, the highest amount of harvested small fruit was <6 mm in diameter (on average 47%). This fruit, in each size class, had a much smaller mass per fruit (Table 3). In 2017, fruit with a diameter of >8 mm harvested on both plantations constituted the highest amount. Very heavy rain (July - 182.4 mm, August - 145.4 mm) hindered fruit harvesting. Less fruit was also found on the irrigated plantation, which constituted waste – mechanically damaged or contaminated by pathogens (Table 2). The large variation in fruit size and the predominance of small fruit suggest their use in the processing industry. They can be dried, especially the smallest ones, because the size of the fruit is a feature that significantly affects the time of this process (Kozos et al., 2016).

Table 2. Mass of non-commercial fruit harvested from the 'Brigitta Blue' bush

	Year		Fruit size				Waste
			<6 mm	6-8 mm	>8 mm	Sum	
			Harvested fruit weight (g)				
Fruits left on the bushes - yield (g/bush)	irrigatied	2018	195	168	64	**427**	91
		2017	135	277	223	**635**	123
		2016	77	110	74	**261**	23
		2015	73	135	82	**290**	42
		2014	44	71	115	**230**	11
		2013	37	42	60	**139**	20
	no irrigation	2018	377	389	26	**792**	85
		2017	168	420	168	**756**	137
		2016	345	228	43	**616**	55
		2015	422	336	62	**820**	62
		2014	310	171	54	**535**	38
		2013	174	140	95	**409**	40
	irrigatied	Mean	**94a***	**134a**	**103a**	**330A**	**52**
	no irrigation	Mean	**299b**	**264b**	**75a**	**654B**	**70**
Estimated yield (kg/ha)	irrigatied		269	383	294	**943**	
	no irrigation		854	754	214	**1868**	

*Mean values denoted by the same letter do not differ statistically significantly at 0.05 according to Tukey's test

Fruit quality

Fruit left on the bushes was classified as non-commercial due to its low weight. These fruits, on the other hand, have high biological value and are rich in substances with pro-healthy effects. Their quantity is comparable and sometimes higher than in fruit intended for sale - at least 10 mm in diameter, depending on the recipient. In general, fruit harvested from non-irrigated bushes had a higher biological value. This may be due to a higher concentration of mineral and organic compounds, as indicated by a higher extract concentration and higher dry matter. This fruit was also much smaller (Table 3). The higher content of these substances was found in fruit with a minimum diameter of <6 mm. Regardless of the method of farming, the smallest fruit had 23% more polyphenols (701 mg/100 g) than fruit larger than 8 mm in diameter (571 mg/100 g) (Table 3). In another experiment, the content of polyphenols in the fruit of this cultivar was much lower (228 mg/100 g), but their size, determined by the mass of 100 pieces of fruit, was almost threefold higher and amounted to 299 g (Ochmian and Kozos, 2015). The size of the fruit and the method they are grown influenced the content of polyphenols (Ochmian et al., 2015). In the experiment by Scalzo et al. (2015), the total polyphenol content in Brigitta Blue fruit was even lower and was only 138 mg/100 g. In other cultivars tested, the average content of polyphenols in fruit was 178 mg/100 g.

Small fruit, especially those harvested from non-irrigated bushes, also had the highest antioxidant activity. They had the highest antioxidant activity both in terms of DPPH and ABTS•+ radicals and in terms of the ability to reduce iron ions (FRAP). Small fruit had several dozen percent's higher antioxidant activity compared with the fruit with a diameter of >8 mm. Antioxidant activity determined by DPPH, despite the large variation due to the size of the fruit and the method of farming (1.11-2.11 μmol TE/g), was similar to the fruit of other cultivars. However, the tested fruit of the 'Brigitta Blue' had a significantly higher antioxidant activity ABTS•+ (6.88-15.56 μmol TE/g) compared with other cultivars (0.09-2.76 μmol TE/g) (Guiné et al., 2018).

Table 3. Quality of fresh highbush blueberry 'Brigitta Blue' fruit depending on the cultivation method and fruit size

	Fruit size:	<6 mm	6-8 mm	>8 mm	Mean
Weight of 100 fruits (g)	irrigated	42a*	56b	131c	76B
	no irrigation	35a	47ab	89b	57A
	mean	39A	52B	110C	
Soluble solids (%)	irrigated	15.9a	15.6a	15.2a	15.6A
	no irrigation	17.1b	17.0b	16.8b	17.0B
	mean	16.5A	16.3A	16.0A	
Titratable acidity (g/100 g)	irrigated	0.62a	0.65a	0.59a	0.62A
	no irrigation	0.78b	0.73ab	0.76b	0.76A
	mean	0.70A	0.69A	0.68A	
Dry mass (%)	irrigated	16.7b	16.1ab	15.4a	16.1A
	no irrigation	19.7c	19.5c	17.9b	19.0B
	mean	18.2B	17.8AB	16.6A	
Polyphenols (mg/100 g)	irrigated	664d	611bc	559a	611A
	no irrigation	738e	639cd	583ab	653A
	mean	701C	625B	571A	
ABTS•+ (μmol TE/g)	irrigated	13.21c	11.37b	6.88a	10.49A
	no irrigation	15.56d	14.67d	10.35b	13.53B
	mean	14.39B	13.02B	8.62A	
FRAP (μmol TE/g)	irrigatied	6.41c	4.78a	4.33a	5.17A
	no irrigation	7.23d	7.11d	5.39b	6.58A
	mean	6.82B	5.95AB	4.86A	
DPPH (μmol TE/g)	irrigated	1.77c	1.38b	1.34b	1.50A
	no irrigation	2.11d	1.60c	1.11a	1.61A
	mean	1.94B	1.49AB	1.23A	
α-amylase IC_{50} (mg/mL)	irrigated	8.24b	11.34d	14.01f	11.20A
	no irrigation	7.13a	9.54c	12.35e	9.67B
	mean	7.69C	10.44B	13.18A	
α-glucosidase IC_{50} (mg/mL)	irrigated	21.37bc	21.92c	26.78e	23.36A
	no irrigation	19.82a	21.03b	23.10d	21.32B
	mean	20.60B	21.48B	24.94A	

*Mean values denoted by the same letter do not differ statistically significantly at 0.05 according to Tukey's test. Small letters indicate the interaction between factors (fruit size and irrigation), large letters indicate the main factors.

Larger Brigitta Blue fruits had much lower FRAP value compared to small ones. Regardless of their size, these fruits had significantly lower iron ion reduction capacity (6.82 μmol TE/g on average) than in the study conducted by Zhang et al. (2016) - 11.72 μmol TE/g and Scalzo et al. (2015) - 12.21 μmol TE/g. Zhang et al. (2016) found that the fruit of the *Vaccinium corymbosum* cultivar had a comparable ability to reduce iron ions, while much lower than the fruit of cultivars of other species of *Vaccinium*, e.g. Gardenblue - 33.97 μmol TE/g. ABTS•+ , DPPH or FRAP are used to determine the antioxidant capacity of plant products (Nenadis and Tsimidou, 2002).

Many authors claim that strong radical scavenging activity is associated with high polyphenol content (Tural and Koca, 2008; Dinda et al., 2016; Moldovan et al., 2016). The polyphenol content also influences the activity of inhibitors. The type of polyphenols is more important for the inhibition of digestive enzymes than their total content (Girones-Vilaplana et al., 2014). Wang et al., (2015) claim that inhibition of *α*-glucosidase is associated with a higher content of phenolic acids. The inhibition of these enzymes may be effective in regulating type 2 diabetes by controlling glucose absorption (Podsędek et al., 2014). Studies have confirmed that fruit with a higher content of polyphenols (<6 mm) had the highest activity of inhibitors. However, this effect was weaker compared with the fruit of other species. It averaged 7.60 for *α*-amylase IC_{50} and 20.60 for *α*-glucosidase IC_{50}. For dogwood tree fruit, it was at the level of 4.51 for *α*-amylase IC_{50} and 13.18 for *α*-glucosidase IC_{50} (Ochmian et al., 2019b). For cherry, *α*-amylase activity was IC_{50} 3.46 mg/mL, while the inhibition capacity of *α*-glucosidase was IC_{50} 11.64 mg/mL (Kirakosyan et al., 2018).

Polyphenolic compounds

According to the experiments of the polyphenols fraction by UPLC-PDA/QTof-MS/MS, the presence of 38 components present in fresh and dried blueberry was detected (Table 4). All components were tested in negative and positive ion mode and identified by UV spectrum, evidence MS and MS/MS and also data were collated with data in the publication (Giovanelli and Buratti, 2009; He et al., 2016; Sellappan et al., 2002; Wang et al., 2019). In the fractions of hydroxybenzoic acid identified three caffeoyl-glucose with *m/z*=341 and retention time 3.22, 3.39 and 3.95 min. In the fractions of hydroxycinnamic acid was determined three components as 3-*O*- (Rt=3.28min), 5-*O*- (Rt=3.57min), and 4-*O*-caffeoylquinic acids (Rt=4.08min). These compounds presented $[M-H]^-$ at *m/z*=353 with fragment *m/z*=191 which portray quinic acid and is created by the loss of -162Da. These components were defined by available standards and data (Giovanelli and Buratti, 2009; Sellappan et al., 2002). The next group consisted of 16 derivatives of flavonols. These were isomers of 15 quercetin and 1 medicine with MS/MS at *m/z*=301 and 317, respectively. Detected of sugar substituents was evaluated by selecting them such as pentose (-132Da), rhamnose (-146Da) and/or hexose (-162Da) and identified also loss of deoxyhexose (-308Da). Furthermore, these components existed in the form of mono, diglycosides, connection with caffeic acid, and as methoxylated and acylated isomers. The information about fresh and dried blueberry flavonols was described earlier by Giovanelli and Buratti (2009). The flavanols profile demonstrated in the blueberry was represented by 2 monomers as (+)-catechin, (-)-epicatechin with *m/z*=289 and 4 procyanidins as B2 and B3 with *m/z*=577 (He et al., 2016). Ten components including the fraction of anthocyanins were tested in the blueberry. They were represented by one peonidin, two petunidin, two delphinidin, three malvidin and two cyanidin derivatives with *m/z*=301, 317, 303, 331 and 287 (as characteristic fragment ion), respectively. These isomers existed in the form of glucosides, galactosides with losses of 162Da and arabinosides with losses of 132Da. The information about blueberry anthocyanins was confirmed in the literature (Wang et al., 2019; He et al., 2016).

The content of polyphenols in fresh fruit largely depended on their size (Table 5). The smallest fruits were the most abundant in these compounds, by 21% compared with >8 mm fruit. The largest group of determined compounds were anthocyanins ranging from 44 to 58%. Ten compounds belonging to this group were identified, delphinidin-3-O-glucoside being in the highest amount in the fruit. Anthocyanins, classified as glycosides, are colour compounds that give the fruit its characteristic blue colour. Phenolic acid (207.32-224.92 mg/100 g), especially chlorogenic acid (193.15-207.37 mg/100 g), was also a large group in blueberry fruit. Regardless of the size of the fruit, flavonols and flavan-3-ols were also found. The content of polyphenols in fruit is influenced, among others, by the method of farming, but also by the size of the fruit (Ochmian et al., 2015). Small blueberry fruit is more abundant in polyphenolic compounds (Ochmian and Kozos, 2014). Significant differences may also occur in individual years, which may be influenced by weather conditions (Spinardi et al., 2019).

Table 4. Identyfication of polyphenolic compounds in 'Brigitta Blue' blueberry fruits

Compounds	$[M–H]^-$ MS (*m/z*)	$[M–H]^-$ MS/MS (*m/z*)	λ_{max} (nm)	Rt (min)
Phenolic acids				
Hydroxybenzoic acid				
Caffeoyl-glucose	341		313	3.22
Caffeoyl-glucose	341		313	3.39
Caffeoyl-glucose	341		313	3.95
Hydroxycinnamic acids				
3-*O*-caffeoylquinic acid	353	191	324	3.28
5-*O*-caffeoylquinic acid	353	191	325	3.57
4-*O*-caffeoylquinic acid	353	191	318	4.08
Flavonols				
Myricetin 3-*O*-galactoside	479	317	340	5.10
Quercetin 3-*O*-diglucoside	625	463/301	354/254	5.83
Quercetin 3-*O*-rhamnoside-hexoside	609	463/301	352/254	5.91
Quercetin 3-*O*-rutinoside	609	301	351/207	6.01
Quercetin 3-*O*-galactoside	463	301	359/209	6.07
Quercetin 3-*O*-methoxyhexoside	493	463/301	345/275	6.18
Quercetin 3-*O*-glucoside	463	301	353/254	6.70
Quercetin 3-*O*-arabinoside	433	301	347/242	6.80
Quercetin 3-*O*-caffeoylgalactoside	623	447/301	340/277	6.89
Quercetin 3-*O*-caffeoylglucoside	623	447/301	340/277	7.00
Quercetin 3-*O*-oxalylpentoside	505	301	353/270	7.05
Quercetin 3-*O*-rhamnoside	447	301	353/270	7.11
Quercetin 3-*O*-dimethoxyrhamnoside	507	301	358/270	7.16
Quercetin 3-*O*-(acetyl)-galactoside	505	301	353/260	7.69
Quercetin 3-*O*-(acetyl)-galactoside	505	301	354/260	8.12
Quercetin	301		340	8.80
Flavan-3-ols				
Procyanidin B2	577	289	278	2.01
Procyanidin B2	577	289	277	2.63
(+)-Catechin	289		276	2.89
Procyanidin B2	577	289	277	3.96
(-)-Epicatechin	289		277	4.17
Procyanidin B3	863	577/289	277	6.62
Anthocyanins				
Cyanidin 3-*O*-glucoside	449^+	287	515/279	3.53
Delphinidin 3-*O*-glucoside	465^+	303	524/276	3.04
Malvidin 3-*O*-glucoside	493^+	331	526/347/276	4.99
Peonidin 3-*O*-glucoside	463^+	301	518/279	4.35
Petunidin 3-*O*-glucoside	479^+	317	525/278	3.82
Delphinidin 3-*O*-arabinoside	435^+	303	530/279	3.26
Cyanidin 3-*O*-arabinoside	419^+	287	522/283	4.02
Petunidin 3-*O*-arabinoside	449^+	317	530/280	4.31
Malvidin 3-*O*-galactoside	493^+	331	530/278	4.52
Malvidin 3-*O*-arabinoside	463^+	331	524/275	4.78

The biological value of freeze-dried fruit

Long-term storage of dried fruit depends on its low moisture content. However, removing water from the fruit to a very low level (2-3%) is not justified. This significantly prolongs the freeze-drying process and significantly increases energy consumption. The prolongation of this time also adversely affects the aroma of the final product (Lis et al., 2004). For dried fruits, this value should be from 18 to 23%, and for vegetables - from 8 to 12%. Such moisture content allows for the long-term storage of dried products in typical conditions (Gyurova and Enikova, 2014).

Table 5. Polyphenol content in the fresh and dried blueberry 'Brigitta Blue' fruit (mg/100 g) - average sample of fruits from irrigated (A) and non-irrigated (B) plantations

	Fresh			Lyophilized		
	<6 mm	6-8 mm	>8 mm	<6 mm	6-8 mm	>8 mm
Polyphenol sub-class: *Hydroxybenzoic acid*						
Caffeoyl-glucose	7.71b*	4.22a	7.01b	39.07*c*	22.85*b*	12.97*a*
Caffeoyl-glucose	1.81c	0.81a	1.04b	17.62*b*	14.42*a*	13.31*a*
Caffeoyl-glucose	0.63b	0.41a	2.40c	5.08*b*	2.96*a*	6.52*b*
Polyphenol sub-class: *Hydroxycinnamic acids*						
3-*O*-caffeoylquinic acid	2.73b	1.98a	2.65b	9.77*b*	7.83*a*	10.97*c*
4-*O*-caffeoylquinic acid	207.37ab	217.05b	193.15a	1241.71*b*	1224.80*b*	790.97*a*
5-*O*-caffeoylquinic acid	1.05b	0.46a	1.07b	5.73*b*	2.96*a*	2.85*a*
Phenolic acid	**221.31b/A**	**224.92b/A**	**207.32a/A**	**1318.97*b*/C**	**1275.83*b*/C**	**837.60*a*/B**
Myricetin 3-*O*-galactoside	9.17c	2.47a	7.19b	42.34*c*	15.66*a*	18.55*b*
Quercetin 3-*O*-diglucoside	0.57b	0.29a	0.56b	3.96*c*	1.11*a*	1.96*b*
Quercetin 3-*O*-rhamno-hexoside	1.32b	3.44c	0.31a	5.88*a*	17.53*c*	10.16*b*
Quercetin 3-*O*-rutinoside	2.03b	2.33c	1.61a	4.42*a*	5.97*c*	5.25*b*
Quercetin 3-*O*-galactoside	14.59c	7.86a	9.87b	67.26*b*	40.93*a*	38.78*a*
Quercetin 3-*O*-methoxyhexoside	3.33a	4.39b	2.95a	16.91*b*	22.64*c*	11.44*a*
Quercetin 3-*O*-glucoside	1.13a	1.39b	2.54c	17.75*a*	38.03*b*	16.73*a*
Quercetin 3-*O*-arabinoside	2.77b	5.11c	0.24a	1.41*a*	6.16*c*	2.03*b*
Quercetin 3-*O*-caffeoylgalactoside	0.52b	0.78c	0.40a	1.94*b*	2.93*c*	1.04*a*
Quercetin 3-*O*-caffeoylglucoside	0.24b	0.40c	0.18a	1.26*a*	5.92*c*	2.10*b*
Quercetin 3-*O*-oxalylpentoside	2.75b	11.38c	2.23a	4.27*b*	17.93*c*	1.84*a*
Quercetin 3-*O*-rhamnoside	0.46a	0.91b	0.39a	3.10*a*	7.48*b*	2.94*a*
Quercetin 3-*O*-dimethoxyrhamnoside	0.05a	0.12b	0.06a	1.58*b*	1.32*a*	1.88*c*
Quercetin 3-*O*-acetyl)-galactoside	0.11a	0.40b	0.13a	0.51*a*	2.01*c*	1.18*b*
Quercetin 3-*O*-acetyl)-galactoside	0.03a	0.46b	0.01a	0.99*a*	3.27*c*	1.16*b*
Quercetin	0.11b	0.05a	0.09b	3.39*c*	2.71*b*	1.12*a*
Flavonols	**39.19b/B**	**41.77b/B**	**28.76a/A**	**176.97*b*/D**	**191.59*c*/D**	**118.16*a*/C**
Procyanidin B2	4.63a	5.58b	14.69c	37.87*a*	47.72*b*	80.18*c*
Procyanidin B2	5.08a	4.93a	5.87b	89.85*c*	56.50*a*	68.78*a*
(+)-Catechin	12.33a	49.91b	12.60a	87.68*b*	140.14*c*	66.06*a*
Procyanidin dimer	3.07a	4.87b	3.18a	14.22*a*	14.97*a*	14.13*a*
(-)-Epicatechin	8.59a	8.59a	11.61b	12.22*a*	17.35*b*	28.25*c*
Procyanidin B2	3.45b	3.23b	2.68a	11.57*c*	9.05*b*	6.65*a*
Flavan-3-ols	**37.16a/A**	**77.13c/C**	**50.62b/B**	**253.41*a*/D**	**285.73*b*/E**	**264.05*a*/D**
Cyanidin-3-*O*-glucoside	73.86c	63.42b	51.49a	275.52*b*	304.01*c*	183.65*a*
Delphinidin-3-*O*-glucoside	98.24b	66.32a	72.21a	283.47*b*	197.75*a*	195.84*a*
Malvidin-3-*O*-glucoside	45.22b	33.24a	29.19a	112.82*b*	121.06*c*	89.91*a*
Peonidin-3-*O*-glucoside	0.00a	0.00a	0.00a	0.02a	0.01a	0.01a
Petunidin-3-*O*-glucoside	52.64c	30.31a	40.15b	113.40*b*	92.44*a*	90.66*a*
Delphinidin 3-*O*-arabinoside	1.87b	1.45a	3.61c	5.50*b*	5.31*b*	4.20*a*
Cyanidin 3-*O*-arabinoside	12.35b	11.86b	9.22a	21.54*a*	29.38*c*	24.47*b*
Petunidin 3-*O*-arabinoside	33.50b	25.98a	24.37a	56.32*b*	59.43*b*	35.38*a*
Malvidin 3-*O*-galactoside	80.71c	36.94a	58.65b	215.64*b*	112.32*a*	112.11*a*
Malvidin 3-*O*-arabinoside	5.39c	4.08b	3.57a	15.67*b*	14.37*b*	11.44*a*
Anthocyanins	**403.78c/B**	**273.60a/A**	**292.46b/A**	**1099.87*a*/E**	**936.07*b*/D**	**747.66*a*/C**
Total	**701.43b/B**	**617.41a/A**	**579.16a/A**	**2849.22*c*/D**	**2689.21*b*/D**	**1967.46*a*/C**

*Mean values denoted by the same letter do not differ statistically significantly at 0.05 according to Tukey's test. The lowercase letters are designated averages: for fresh - a, lyophilized - *a*. The capitals are denoted by averages.

In the first stage of the research, the whole fruit was freeze-dried to obtain moisture of <20%. Such a moisture content is provided for in the Polish Standards for dried apple fruit (PN-A-77608).

Studies have shown that, after 24 hours of drying, only small fruit (<6 mm) reached the assumed moisture content. The moisture content of large fruit was over 25%. Blueberries of the Brigitta Blue cultivar are recommended for longer storage. They are characterised by a thick skin and an intense wax deposit, which protects the fruit from withering. This may have hindered the collection of water from the fruit during the drying process. Fruit that achieved moisture in the range of 19-20% did not have a satisfactory appearance - they were soft, not crunchy. Due to the similar nature of the fruit, standards were adopted for blueberries. Dried fruit should have a moisture content of <15%. However, a moisture content up to 12% is the most recommended amount. This moisture content in the experiment was reached by large fruit only after further 22 drying hours (Table 6).

Blueberry fruit subjected to a very long sublimation drying process retained a high content of polyphenolic compounds (Table 5). This method of fruit preservation allowed the most biologically active compounds to be retained, as evidenced by the high antioxidant and anti-diabetic properties of this fruit. Compared to fresh fruit, freeze-dried fruit had a several times higher content of polyphenols, which may result from their dehydration. The mass of dried fruit, compared with fresh ones, decreased more than eightfold - from 1 kg of fresh blueberry, approximately 120 g of dried fruit was obtained. However, the content of polyphenols increased only 3-4 times, depending on the size of the fruit. This indicates the degradation of these compounds in the freeze-drying process. The degradation of these compounds occurred to a greater extent in large fruit. This may have been influenced by an extended drying time of up to 46 hours. After this period, the fruit had the assumed moisture content of 12.3%. For small fruit to reach this moisture content, it was enough to dry them for 32 hours. The length of the drying period may have been one of the reasons for lowering the polyphenol content. In fruit dried for 32 hours, the total polyphenol content increased 4 times and in those which were freeze-dried for the longest time (46 hours) 3.4 times as compared to fresh fruit. There was an increase in the content of flavan-3-ols (4-7 times) and phenolic acid (4-6 times) mostly in dried fruit, especially the small ones. However, a small increase in the content of anthocyanins in freeze-dried fruit was found. This may indicate the degradation of these compounds, especially during prolonged drying. It can also be confirmed by the results of antioxidants and antidiabetic properties of dried fruit. With the increase in drying time, the antioxidant activity, especially determined with ABTS•+, decreased significantly. It was also found that in large fruit (the longest dried), there was the smallest increase in the ability of the amylase enzyme to reduce. The increase in the ability to reduce glucose in dried fruits was at a similar level (about 2 times) regardless of their length of drying. Similar to the work of Su (2016), a dominant phenolic acid obtained in this study was chlorogenic acid. The anthocyanin content was only threefold higher compared with fresh fruit. Freeze-drying enables better preservation of nutrients, including polyphenols, aroma, and colour compared with fruit dried in a traditional method (Ciurzyńska and Lenart, 2010; Rutkowska et al., 2012). Blow-dried aronia fruit had an anthocyanin content 8 times lower than freeze-dried fruit (Bober and Oszmiański, 2004). Freeze-dried fruit also had a higher anthocyanin content compared with fruit dried conventionally with warm air (Bustos et al., 2018). However, these authors claim that drying berries at medium temperatures resulted in the fruit retaining a significant proportion of anthocyanins, and this process was much more cost-effective than in the case of freeze-drying. Boysenberry retained almost half of the total polyphenol content compared with freeze-dried samples, while other berries lost approx. 70% (Sablani et. al., 2011).

Table 6. Effect of sublimation drying time on dry matter of 'Brigitta Blue' highbush blueberry fruit and its antioxidant and antidiabetic properties

	Fruit size		
	<6 mm	6-8 mm	>8 mm
Freeze drying time (h)	**24**	**28**	**34**
Dry weight (%)	19.7a*	19.5a	19.8a
Freeze drying time (h)	**32**	**38**	**46**
Dry weight (%)	12.4a	12.1a	12.3a
ABTS•+ (µmol TE/g)	104.6a	95.3b	52.7c
FRAP (µmol TE/g)	27.9a	42.8b	24.5a
DPPH (µmol TE/g)	23.6a	19.8b	15.4c
α-amylase IC_{50} (mg/mL)	4.47a	5.18a	8.02b
α-glucosidase IC_{50} (mg/mL)	9.32a	9.89a	11.70b

*For explanation, see Table 2

It was also found that freeze-dried fruit was characterised by several times higher antioxidant value and activity inhibitors compared with fresh fruit. This is confirmed by Hernández-Alcántar's observations, (2016) that higher polyphenol content causes higher antioxidant activity. The ability to reduce the iron ions of the tested fruit was similar to the freeze-dried fruit of rabbit-eye blueberry genotypes. Like blueberry fruit, rhubarb blueberry fruit was also characterised by several times higher FRAP than frozen fruit (Vuthijumnok et al., 2013). Small fruit had much more beneficial health effects versus fresh fruit (Table 6).

Quality of freeze-dried and rehydrated fruit depends on the preparation method

To reduce the fruit drying time, successive portions of fruit were cut in half or pricked on the circumference. Once so prepared, the assumed moisture content was reached much faster than with the whole fruit (Figure 1). Cut fruit was dried the fastest to 12% – the smallest ones after 16 hours, and the large ones (>8 mm) after 22 hours. Fruit pricking on the circumference also significantly reduced the time of this process. Regardless of their size, whole fruit required an extended freeze-drying cycle. To dry small fruit to reach 12% moisture, the freeze-drying process took 32-46 hours. Red currant fruit also required a long sublimation drying time (Šumić et al., 2016). As the water content of the dried fruit decreases, the drying time and energy demand increase (Lis et al., 2004).

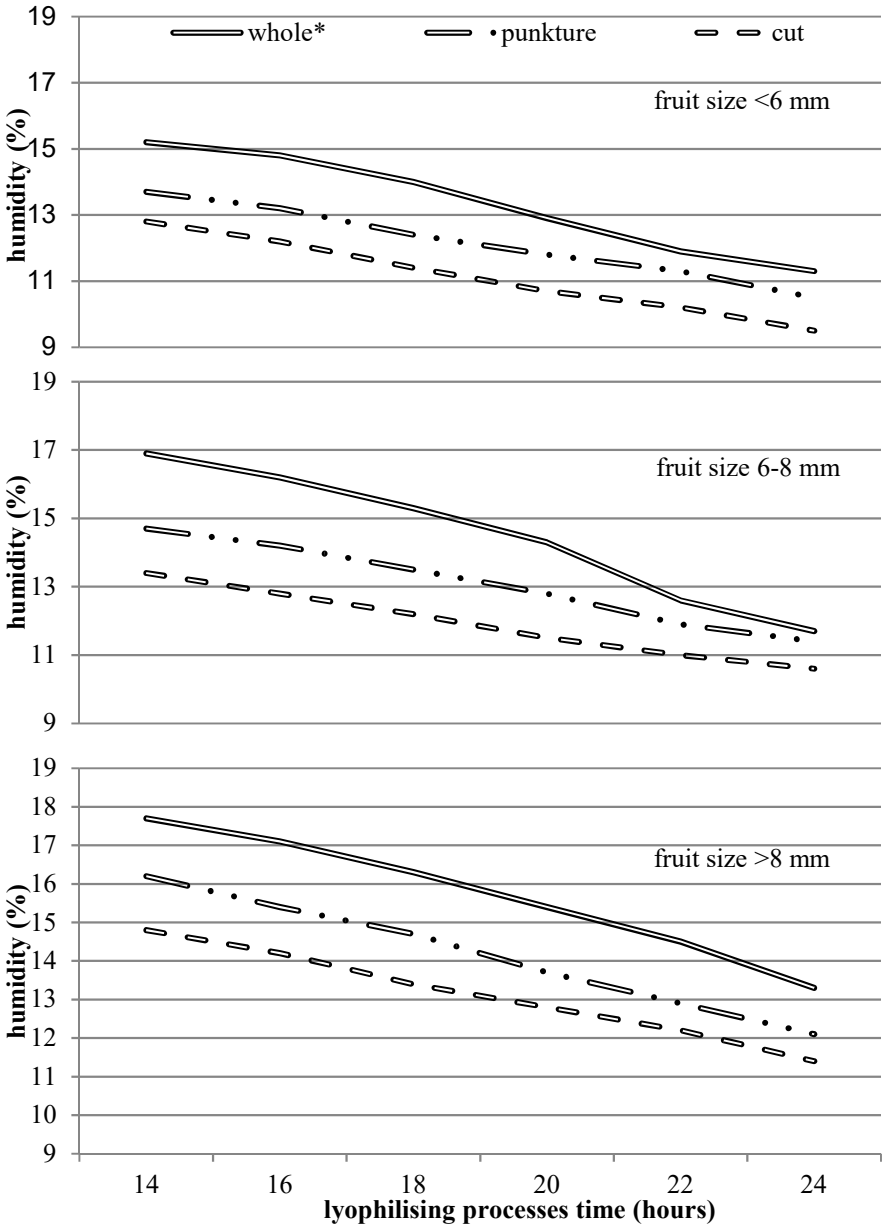

Figure 1. Influence of freeze-drying time and preparation of 'Brigitta Blue' highbush blueberry fruit on its humidity - the whole fruit was previously sublimation-dried for 24 hours

This method of fruit preparation, in addition to shortening the drying time, also significantly reduced the degradation of polyphenols (Table 5). In the cut fruit, there was approx. 6% more than in whole fruit. This may have been influenced by a significantly reduced drying time. During rehydration, a loss of polyphenolic compounds was found. This was associated with the hydration of fruit, after 6 hours of rehydration, as well as the significant washing-out of the compounds (Table 7). In fruit that had been soaked in water for 6 hours, the level of polyphenols was comparable to that of fresh fruit. During rehydration, there is an increase in the mass and volume of the dried fruit, while at the same time the content of soluble components in dry matter decreases (Lewicki, 1998b). The rate of tissue hydration increases with increasing water temperature (Choińska et al., 2014).

The ability to rehydrate dried fruit and its properties after this process was also studied. Changes in the content of polyphenols in fruits and changes in their colour were evaluated. Rehydration is a complex process aimed at restoring the properties of dried fruits to the state before drying. During the rehydration several processes take place simultaneously: water absorption by dried fruits, which increases their weight, volume and changes the concentration of substances contained in them (e.g. polyphenols). Water soluble substances (sugars, acids, minerals, and vitamins) are also washed out of the rehydrated material. The loss of soluble components during rehydration largely depends on the structure of the material. The greater the degree of fruit fragmentation is, the greater the ability to rehydrate will be (Kaleta et al., 2013). This is confirmed by the obtained results (Figure 2).

Table 7. Changes in the polyphenol content of 'Brigitta Blue' highbush blueberry fruit after rehydration

		Fruit size			Mean	
		<6 mm	6-8 mm	>8 mm		
		polyphenols (mg/100 g)				
Fresh	whole	701b*	617a	579a	632	**b****
Lyophilized	whole	2777ef	2608c	1893a	2426A	**d**
	puncture	2841fg	2689cd	1939a	2490AB	**de**
	cut	2929g	2741de	2070b	2580B	**e**
	mean	2849B	2679A	1967A		
After 15 minutes of rehydration	whole	2542c	2389b	1667a	2199A	**c**
	puncture	2549c	2374b	1706a	2210A	**c**
	cut	2504c	2401b	1623a	2176A	**c**
	mean	2532A	2388B	1665C		
After 6 hours of rehydration	whole	729g	658ef	634de	674B	**b**
	puncture	694fg	611de	587cd	631B	**b**
	cut	542bc	507b	449a	499A	**a**
	mean	655B	592A	557A		

*Mean values denoted by the same letter do not differ statistically significantly at 0.05 according to Tukey's test. Small letters indicate the interaction between factors (fruit size and preparation of the fruit), large letters indicate the main factors.
** Relationships between all the fruits tested

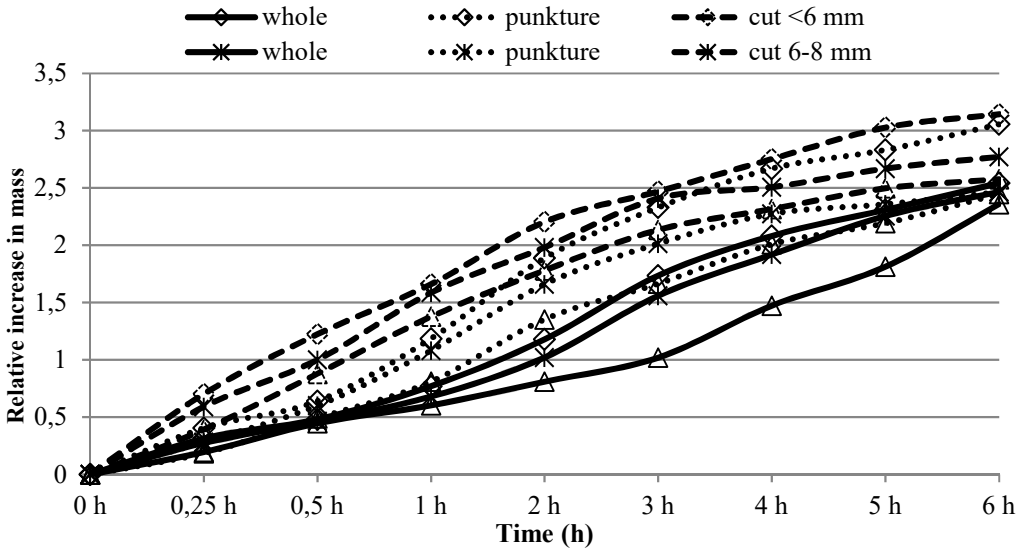

Figure 2. Relative weight gain of 'Brigitta Blue' highbush blueberry fruit during rehydration depending on fruit size and preparation for freeze-drying

Cut fruit, especially small ones, showed the largest relative mass gain during rehydration. During the first 15 minutes of contact between whole fruit and water, there was a very small mass gain, indicating a low degree of hydration of the fruit. From the consumer's point of view, it is a beneficial process if the fruit is to be crispy after contact with liquids. If they are, for example, added to muesli, it is unfavourable for them to soak in water quickly. After 30 minutes, the relative mass gain was 0.5, while in the case of cut fruit – it was on average 1.

During rehydration, a change in the colour of the fruit was also observed. Freeze-dried fruits were darker compared with fresh fruits. Regardless of their size or method of preparation for drying, they became brighter during rehydration, as evidenced by changes in parameters *a** and *b** (Figure 3) and colour parameter L* (Table 8). The greatest changes in colour were observed for the pricked fruit. After freeze-drying, they were the darkest ones (L* 32.5) and, after 6 hours of rehydration, parameter L reached the highest value of 54. These changes were independent of the size of the fruit. During the pricking of the fruit, it was observed that a significant amount of juice leaked out, which deposited on the surface of the skin to then be washed out by water.

Regardless of the size of the fruit and their method of preparation, their chromatic colour changed from blue to red (Figure 3). Again, the greatest changes were observed for the pricked fruit. Parameter *a** changed from –15.2 to −11.8 to 8.2-18.5, while parameter *b** from −31.9 to −26.8 to 16.9-21.4. Freeze-drying enables the better, more natural preservation of the colour of fruit compared with drying with warm air (Bustos et al., 2018). The colour and appearance of berries are important for the consumer, and the fruit of a darker colour indicates a higher anthocyanin content (Laaksonen et al., 2016). Based on colour, we can assess the condition of the plants and fruit (Antal et al., 2013).

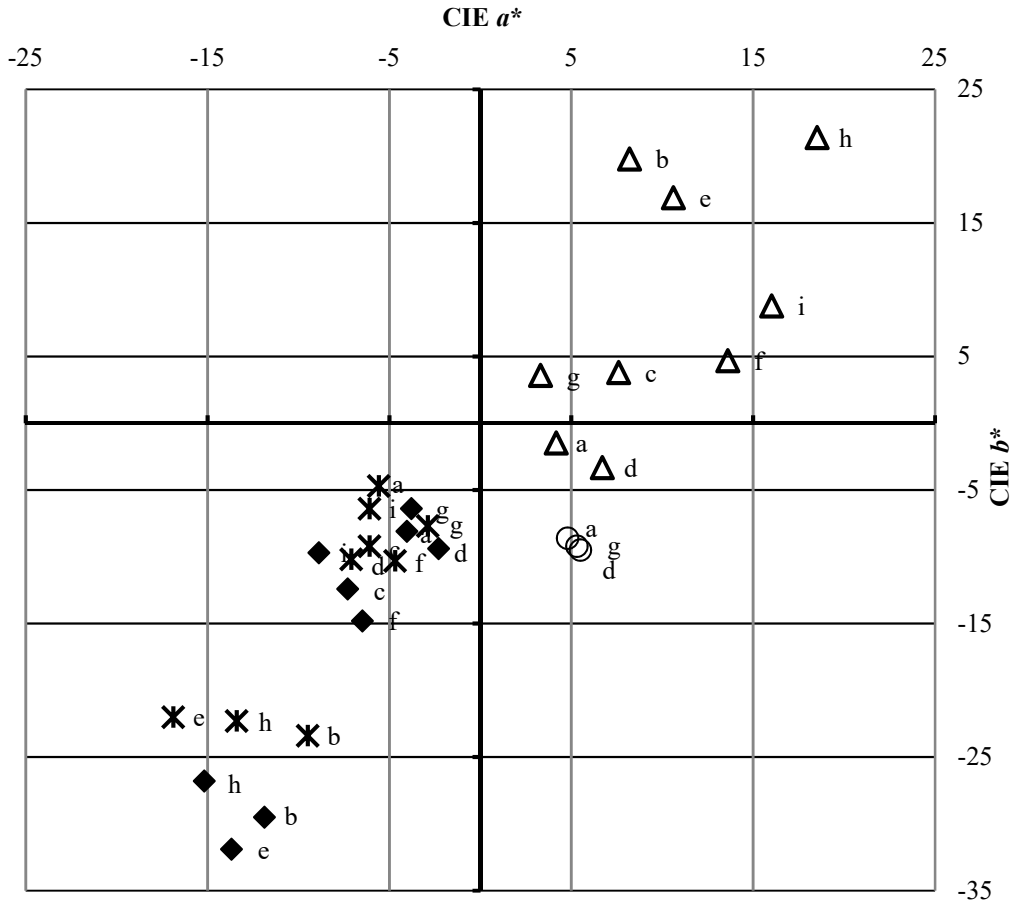

Figure 3. Changes in chromatic colour parameters (CIE *a** and *b**) of blueberries.
○ Fresh; ◆ Lyophilized; ✳ After 15 minutes of rehydration; △ After 6 hours of rehydration.

	Fruit size		
	<6 mm	6-8 mm	>8 mm
whole	a	d	g
puncture	b	e	h
cut	c	f	i

Table 8. Changes in the monochromatic parameter of the colour of 'Brigitta Blue' blueberry fruit

		Fruit size			Mean	
		<6 mm	6-8 mm	>8 mm		
		colour CIE L*				
Fresh	whole	51.33a*	52.05a	50.70a	51.36	**e****
Lyophilized	whole	46.4de	44.5cd	47.8e	46.2A	**c**
	puncture	33.2a	31.3a	32.9a	32.5C	**a**
	cut	42.7bc	43.8cd	40.6b	42.4B	**b**
	mean	40.8A	39.9A	40.4A		
After 15 minutes of rehydration	whole	43.5a	41.3a	42.8a	42.5A	**b**
	puncture	31.5b	31.7b	33.3b	32.2B	**a**
	cut	39.8a	41.6a	40.9a	40.8A	**b**
	mean	38.3A	38.2A	39.0A		
After 6 hours of rehydration	whole	47.4ab	47.9ab	49.6b	48.3B	**cd**
	puncture	52.6c	54.0cd	55.5d	54.0A	**f**
	cut	48.5b	46.4a	51.9bc	48.9B	**de**
	mean	49.5A	49.4A	52.3A		

*Mean values denoted by the same letter do not differ statistically significantly at 0.05 according to Tukey's test. Small letters indicate the interaction between factors (fruit size and preparation of the fruit), large letters indicate the main factors.

** Relationships between all the fruits tested

CONCLUSIONS

The amount of fruit left on bushes is affected by the weather, especially the temperature and rainfall in the growing season. In years of low rainfall, the number of fruits left on bushes is lower. The irrigation of bushes resulted in the number of non-commercial fruits being almost twice as low as on non-irrigated bushes. Blueberry fruit was characterised by its high biological value, especially the small ones. They should, therefore, be handled. Freeze-drying is a good method of preservation. After this process, the fruit darkens but has a colour similar to fresh fruit. Freeze-dried fruits are characterized by a much higher content of polyphenols, higher antidiabetic activity, and antioxidant capacity compared to fresh fruit. After rehydration, most of the compounds have a value similar to fresh fruit, which indicates that it is a good way to preserve the fruit and to keep pro-healthy substances in it. Moisture content, which ensures the safe storage and appropriate texture, should be approx. 12%. Larger fruit of the Brigitta Blue variation (>8 mm) required 46 hours of drying, and smaller ones - 32 hours, to reach the assumed moisture content. Fruit cutting or pricking significantly reduced the sublimation drying time. Large ones, once cut, reach the assumed moisture content after 22 hours of drying, and the pricked ones - after 24 hours. For small fruit, the freeze-drying period can be reduced by 4 hours. Reducing the drying time reduces costs and has an impact on the preservation of more polyphenolic compounds and that affects their higher biological value.

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OŚWIADCZENIA AUTORÓW O WKŁAD W PUBLIKACJĘ

Publikacja 1:

Figiel-Kroczyńska, M., Krupa-Małkiewicz, M., Ochmian, I. (2022). Efficient micropropagation protocol of three cultivars of highbush blueberry (*Vaccinium corymbosum* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 50 (4).

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, postawieniu hipotez badawczych, wyborze metodyki badań, założeniu doświadczenia, przeprowadzeniu doświadczenia i wykonaniu części analiz, opracowaniu wyników, przygotowaniu manustkryptu do druku, przeprowadzeniu korekty manuskryptu po recencji.

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Publikacja 2:

Figiel-Kroczyńska, M., Krupa-Małkiewicz, M., Ochmian, I. (2022). Effect of Actisil (Hydroplus™), organic supplements, and pH of the medium on the micropropagation of *Vaccinium corymbosum*. Acta Sci. Pol. Hortorum Cultus, 21(5).

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, postawieniu hipotez badawczych, wyborze metodyki badań, założeniu doświadczenia, przeprowadzeniu doświadczenia i wykonaniu części analiz, opracowaniu wyników, przygotowaniu manustkryptu do druku, przeprowadzeniu korekty manuskryptu po recencji.

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Publikacja 3:

Figiel-Kroczyńska, M., Ochmian, I., Krupa-Małkiewicz, M., Lachowicz, S. (2022). Influence of various types of light on growth and physicochemical composition of blueberry (*Vaccinium corymbosum* L.) leaves. Acta Sci. Pol. Hortorum Cultus, 21(2).

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, postawieniu hipotez badawczych, wyborze metodyki badań, założeniu doświadczenia, przeprowadzeniu doświadczenia i wykonaniu części analiz, opracowaniu wyników, przygotowaniu manuskrytu do druku, przeprowadzeniu korekty manuskryptu po recenzji.

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OŚWIADCZENIA AUTORÓW O WKŁAD W PUBLIKACJĘ

Publikacja 4:

Figiel-Kroczyńska, M., Ochmian, I., Krupa-Małkiewicz, M. (2022). Effect of chitosan-based spraying on fruit quality of highbush blueberry cv. Sunrise. Progress on Chemistry and Application of Chitin and its Derivatives. Volume XXVII.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, postawieniu hipotez badawczych, wyborze metodyki badań, przeprowadzeniu doświadczenia i wykonaniu części analiz, opracowaniu wyników, przygotowaniu manustkryptu do druku, przeprowadzeniu korekty manuskryptu po recencji.

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OŚWIADCZENIA AUTORÓW O WKŁAD W PUBLIKACJĘ

Publikacja 5:

Ochmian, I., **Figiel-Kroczyńska, M.**, Lachowicz, S. (2020). The quality of freeze-dried and rehydrated blueberries depending on their size and preparation for freeze-drying. Acta Universitatis Cinbinesis, Series E: Food Technology, 24(1).

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, postawieniu hipotez badawczych, wyborze metodyki badań, przeprowadzeniu doświadczenia i wykonaniu części analiz, opracowaniu wyników, przygotowaniu manuskrytu do druku, przeprowadzeniu korekty manuskryptu po recenzji.

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